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Bioaccumulation of ^{137}Cs by benthic plants and macroinvertebrates

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Abstract

^{137}Cs activity concentrations were determined in macrophytes and macrozoobenthic organisms from the southern Baltic Sea. Cesium isotope content was analysed in macroalgae species (green, red and brown algae representatives) and in some species of vascular plants. The analyzed macroinvertebrate organisms included bivalves and a crustacean.

Concentration factors (CF) were calculated using the determined ^{137}Cs concentration in the flora and fauna organisms against that in seawater, and the bioaccumulative properties were compared. The study pointed out that the most important factors in the cesium bioaccumulation process occurring in plants are related to morphology. The highest CF values were obtained in algae *Polysiphonia fucoides*, *Ectocarpus siliculosus* and *Cladophora glomerata*. Decidedly lower CF values were observed in the vascular plants and macrozoobenthos representatives.

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INTRODUCTION

At present, the Baltic Sea continues to show the highest level of ^{137}Cs contamination of any part of the world ocean (HELCOM 2003, 2009; WOMARS 2005; Zalewska and Lipska 2006; Ikaheimonen et al. 2009). It was estimated that the average concentration of ^{137}Cs in seawater (representative for the whole Baltic Sea area) was equal to 60 Bq m^{-3} in 2000 (WOMARS 2005), while in 2004, specifically in the southern Baltic the average activity of ^{137}Cs was 45 Bq m^{-3} (Zalewska and Lipska 2006). The later value was still 3 times higher than prior to the Chernobyl disaster in 1986. The Chernobyl disaster was the main source of radiocesium isotopes in the Baltic. The direct input of ^{137}Cs from the Chernobyl nuclear power plant was estimated at 4.7 PBq (Nielsen et al. 1999; HELCOM 1995, 2003).

^{137}Cs, precipitated into seawater with atmospheric fallout as well as discharged with the riverine outflow, is distributed to other elements of the marine environment. The isotope can be bioaccumulated and/or absorbed by suspended particulate matter (SPM), which is mainly composed of plankton, together with biogenic detritus and mineral particles (Knapińska-Skiba et al. 1994, 1995, 2002, 2003). Cesium ions can also be bioaccumulated by higher flora (Boisson et al. 1997, Sawidis et al. 2003, Burger et al. 2006, Pinder et al. 2006) and fauna organisms (Smith et al. 2002, Malek 2004, Kryshev and Ryabov 2000). The bioaccumulation and absorption processes partake in diminishing ^{137}Cs content in the marine environment. The importance of biota in ^{137}Cs removal from seawater is rather low in comparison to other processes such as radioactive decay, sedimentation and water exchange (Ikaheimonen et al. 2009). And although the role of benthic plants in the food chain is rather limited, they are very important components of the marine

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environment as a habitat forming element. Benthic plants demonstrate properties such as large size of thalluses and widespread distribution, which can be regarded as very useful when taking into account the possible application of benthic plants as bio-indicators in marine pollution studies. The most important among those properties is their well-known ability to bioaccumulate stable heavy metals (Lobban and Harrison 1997; Szefer 2002 a,b; Stengel et al. 2004) as well as radionuclides (Szefer 2002 a,b; Skwarzec et al. 2003). Macrophytes uptake metal ions from seawater and metal concentration increases inside the plant, hence variations in the concentration of metals in the thallus are often taken to reflect the metal concentration in the surrounding water (Lobban and Harrison 1997) as the concentrations in seawater are far too low for accurate determination; the plants act as concentration agents.

The rationale for using seaweeds as indicators of stable metal and radioactive isotope contamination is based on three main factors:

1. Metal concentrations in environmental solutions are often close to the analytical detection limits and may vary with time. Seaweeds can concentrate metals from solution and integrate short-term temporal fluctuations in concentration (Lobban and Harrison 1997);
2. Accessibility of benthic plants;
3. Intensive physiological processes and growth take place in a relatively short period of the year and are followed by increased uptake and quick response to contamination (Lobban and Harrison 1997).

Bioaccumulative processes are determined by external and internal factors. The external factors are temperature, light conditions, salinity and the concentration of the investigated trace element, in both its physical and chemical form. Internal factors are connected with the morphology, physiology and habitat of the investigated organisms (Lobban and Harrison 1997).

The main aim of this study was to compare the bioaccumulative properties towards ^{137}Cs of various macrophyte species, representing different divisions, and also to compare the bioaccumulation of ^{137}Cs by the representatives of various benthic animals.

The final aim was to identify and recommend organisms, specific to the southern Baltic Sea region, which would fulfill all property requirements as reference organisms in the monitoring programs, in the impact assessment system (Brown et al. 2006) and in the detection of accidental release of radioactive substances (Valkovic 2000).

MATERIALS AND METHODS

Study area

Benthic plants were collected at a number of locations in the Polish section of the southern Baltic Sea (Fig. 1a) over several years (Table 1).

Samples of benthic fauna were taken during off-shore cruises in September of 2004, 2005 and 2006. The trawling transect is shown in Fig. 1b.

Biological material sampling and sample preparation

Macrophytes

The sampling of macrophytobenthos was done along a depth profile by a scuba diver. At each sampling depth the plants were collected from a sea floor plane enclosed in a metal frame of 0.5 x 0.5 m dimensions.

Samples of stranded bladderwrack (*Fucus vesiculosus*) were collected at the beaches along the shores of Puck Bay in Poland and in the Estonian part of the Gulf of Finland.

The plant material was analyzed taxonomically, individual species occurring in the samples were identified, the biomass of each species was determined gravimetrically and quantitatively; taxonomic analyses and biomass determinations were carried out according to the HELCOM COMBINE guidelines (HELCOM, 1997).

For the radionuclide activity determination, the plant samples were dried, ashed at 450°C and homogenized. The activity of ^{137}Cs in benthic plants was measured using gamma spectrometry (see section 2.3 Gamma spectrometry analysis).

Benthic macroinvertebrates

Samples of benthic organisms *Mytilus trossulus*, *Macoma baltica*, *Cardium glaucum*, and *Balanus improvisus* were collected using bottom trawls. Samples were frozen on board without any additional treatment and in such form they were transported to the laboratory. Every year a sample of blue mussels consisted of 100 individuals. The soft tissue of the mussels was separated, freeze-dried, ashed at 450°C

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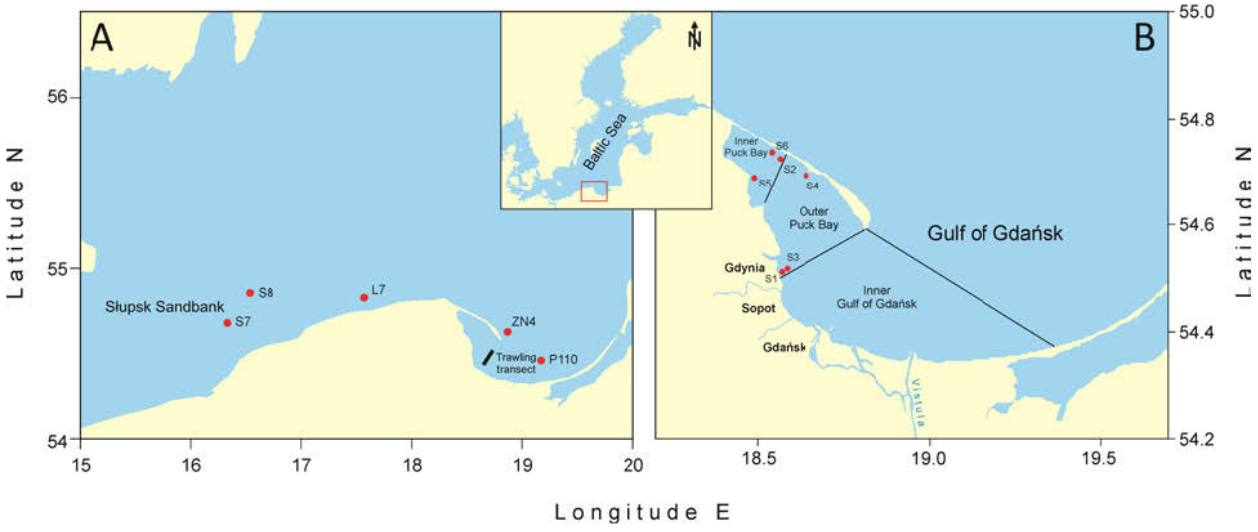


Fig. 1. Sampling stations located in the southern Baltic Sea: A - macrophytobenthic plants; B - macrophytobenthic plants and macrozoobenthic organisms.

Table 1

Sampling area, sampling date, identified species, and activity concentrations of ^{137}Cs in benthic plants and limit of detection values.

Sampling area	Sampling station	Sampling date	Identified species	Sampling depth (m)	Dry weight (g)	^{137}Cs ± 1σ (Bq kg^{-1} d.w.)	LOD (Bq kg^{-1} d.w.)
Kępa Redłowska	S1	5.09.2005	*Polysiphonia* spp.	5	16.6	27.5 ±0.9	0.85
				6	1.4	46.3 ±5.8	7.02
				7	0.49	49.1 ±12.5	15.0
Jama Kuźnicka	S2	8.10.2008	*Potamogeton pectinatus*	1	94.3	1.2 ±0.2	0.31
				2	18.2	3.9 ±0.5	0.69
				3	10.3	2.5 ±0.7	0.55
			Zostera marina	1	13.0	< LOD	1.38
Puck Bay	S3	19.05.2009	*Cladophora glomerata*	2 – 3	4.1	15.4 ±2.0	2.69
			Polysiphonia fucoides		3.5	20.8 ±2.5	3.33
		16.07.2009	*Cladophora glomerata*		7.6	15.4 ±1.4	2.03
			Enteromorpha spp.		15.0	2.9 ±0.5	0.90
			Polysiphonia fucoides		2.0	23.8 ±3.9	6.15
			Ectocarpus siliculosus		3.8	17.4 ±2.2	3.39
			Zostera marina		7.0	4.6 ±0.9	1.47
	S4	21.05.2009	*Pilayella litoralis*		19.4	4.2 ±0.5	0.57
			Zanichellia palustris		6.6	< LOD	1.96
			Zostera marina		13.5	< LOD	1.03
Puck Lagoon	S5	21.05.2009	*Ceramium diaphanum*	2 - 3	4.3	< LOD	3.04
	S6		*Pilayella litoralis*		16.4	3.3 ±0.5	0.93
			Potamogeton pectinatus		4.2	< LOD	3.34
Słupsk Sandbank	S7	9.10.2008	*Furcellaria lumbricalis*	15	17.3	10.8 ±0.7	0.64
	S8	20.10.2009	*Furcellaria lumbricalis*	19	44.2	5.9 ±0.3	0.35
			Cocotylus truncatus	19	2.2	< LOD	6.33
Puck Bay	-	1.09.2008	*Fucus vesiculosus*	from the beach	17.3	3.6 ±0.5	0.60
Gulf of Finland	-	19.09.2009	*Fucus vesiculosus*	from the beach	14.9	7.1 ±0.6	0.87

Table 2

Results of HELCOM-MORS Proficiency Test Determination of Radionuclides in Fish Flesh (IAEA 414 – certified reference material) – Laboratory N°4, Institute of Meteorology and Water Management, Maritime Branch, Gdynia, Poland, Reference Date: 01 January 1997 after IAEA (2010).

Analyte	IAEA Value (Bq kg^{-1} d.w.)	IAEA Unc. (Bq kg^{-1} d.w.)	Lab. Value (Bq kg^{-1} d.w.)	Lab. Unc. (Bq kg^{-1} d.w.)	Lab. Unc. (%)	Rel. Bias (%)	Trueness	P (%)	Precision	Final Score
^{40}K	481	16	474.5	19.3	4.1	1.35	Passed	5.2	Passed	Acceptable
^{137}Cs	5.18	0.10	5.06	0.64	12.6	2.32	Passed	12.8	Passed	Acceptable

and homogenized. In the case of the other bivalves and the crustacean, the whole body was used for cesium determination due to the relatively small mass of the soft tissue of these animals. Ashed samples were immersed in the counting boxes of appropriate shape and size.

Seawater

^{137}Cs concentration in seawater is analysed within the HELCOM MORS PRO program on an annual basis (IMGW 1987-2001). Seawater samples are taken in the southern Baltic Sea region at stations P110, ZN4, L7 (Fig. 1b). For the determination of ^{137}Cs activity concentration in aqueous samples, the method described in Zalewska and Lipska (2006) was applied.

Gamma-spectrometric analysis

^{137}Cs content in samples of biological material was measured by a gamma spectrometric method using an HPGe detector with a relative efficiency of 18% and a resolution of 1.8 keV for a peak of 1332 keV of ^{60}Co. The detector was coupled with an 8192-channel computer analyser.

The reliability and accuracy of the applied method was verified by participation in the HELCOM – MORS Proficiency Test Determination of Radionuclides in Fish Flesh Sample organized by IAEA-MEL Monaco (IAEA-414, Irish and North Sea Fish). Fish flesh material may be recognized as a substitute for ashed macroalgae samples for almost the same density of prepared samples. Results of ^{137}Cs and ^{40}K determination, as well as information on accuracy and precision, are presented in Table 2 after IAEA (2010).

RESULTS AND DISCUSSION

The results of ^{137}Cs activity concentration analyses in marine macrophytes are presented in Table 1.

In samples collected at stations S3 (Table 1) the representatives of green algae (*Cladophora glomerata*) and red algae (*Polysiphonia fucoides*) were found in May (2009). In July (2009), additional species *Enteromorpha* spp., *Ectocarpus siliculosus* and *Zostera marina* were also identified here as a result of intensive vital and growing processes occurring in the best period of the year regarding temperature and light conditions. At station S4, located close to the Hel Peninsula, the composition of macrophyte samples was different. Only brown algae *Pilayella litoralis* and vascular plants - *Zostera marina* and *Zanichellia palustris* - were present.

^{137}Cs activity determined in *C. glomerata* in May and July were equal and relatively high, 15.4 Bq kg^{-1} d.w. (dry weight), in comparison to the 2.9 Bq kg^{-1} d.w. obtained in the other green algae *Enteromorpha* spp..

The highest concentrations of ^{137}Cs, 46.3 Bq kg^{-1} d.w. and 49.1 Bq kg^{-1} d.w. were found in *Polysiphonia* spp. in September 2005, at 6 and 7 m depths, respectively. Lower ^{137}Cs level *P. fucoides* showed in May (20.8 Bq kg^{-1} d.w.) and in July (23.8 Bq kg^{-1} d.w.) in the Puck Bay region (Table 1).

In May, in vascular plants *Zanichellia palustris* and *Zostera marina*, ^{137}Cs concentrations were below the limit of detection (LOD) of the applied method while in July, the concentration of ^{137}Cs determined in *Zostera marina* reached 4.6 Bq kg^{-1} d.w. This may be explained by the increased rate of metabolic processes and therefore the increase in bioaccumulation and assimilation rates observed during the summer.

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An unexpectedly high concentration of the isotope was found in *E. siliculosus* - 17.4 Bq kg^{-1} d.w. as compared with the value measured in another other brown algae, *P. litoralis* - 4.2 Bq kg^{-1} d.w.

In October, at station S2 in the Jama Kuźnicka, a specific hollow in Puck Lagoon, solely vascular plant species were found. *P. pectinatus* was present at water depths from 1 to 3 m, and the highest ^{137}Cs concentration (4.0 Bq kg^{-1} d.w.) was found at 2 m, but the isotope content at different depths did not vary significantly. Cesium activity in *Z. marina* was below the LOD level.

In the sample taken at station S6 in Puck Lagoon in May (2009) the brown algae *Pilayella litoralis* dominated and the level of cesium content (3.3 Bq kg^{-1} d.w.) was similar to that observed in Outer Puck Bay (station S4). The mass of collected *Potamogeton pectinatus* at this station was too small for accurate measurement.

Only one representative of red algae - *Ceramium diaphanum* - was found at station S5 in Puck Lagoon in May, 2009, and the activity of ^{137}Cs in this plant did not exceed the LOD.

Generally, the composition of macrophyte samples from Puck Lagoon was similar to those from Puck Bay, indicating the importance of the time of macrophyte sampling as well as providing insight into the influence of the type of substratum.

The macrophyte sample taken in the Słupsk Sandbank (station S7) in 2008 consisted only of *Furcellaria lumbricalis*. The sampling depth was as much as 15 m, however the illumination conditions were still good for this red algae's growth. The concentration of ^{137}Cs measured in *F. lumbricalis* was 10.8 Bq kg^{-1} d.w.. The sampling in this area was repeated in 2009, and at a depth of 19 m another red algae, *Cocotylus truncatus,* was found in addition to *F. lumbricalis*. The ^{137}Cs activity in *F. lumbricalis* in 2009 was about 2 times lower than in the previous year whilst in *C. truncatus* it was below 6.3 Bq kg^{-1} d.w..

Fucus vesiculosus, recognized as a very good bio-indicator (Szefer 2002 a,b), almost vanished from the waters of the southern Baltic for many years, probably because of the increasing eutrophication of the area. Very small amounts of *F. vesiculosus* were found stranded and collected at beaches along Puck Bay's shores. The ^{137}Cs activity determined in it was 3.6 Bq kg^{-1} d.w. and was comparable with values obtained in another brown algae – *P. litoralis*. In *F. vesiculosus* from the beach in Tallinn (Estonia), in the Gulf of Finland region, the result was nearly two times higher. This may simply be the result of differences in salinity between these two regions since salinity in the Gulf of Finland is about 2 (PSS'78) while the average salinity in the Baltic Proper is about 7 (PSS'78). Hence, at lower salinity the bioaccumulation of cesium in *F. vesiculosus* is more intensive. Different cesium concentrations in seawater in the two regions cannot be regarded as responsible for the observed differences in caesium content in *F. vesiculosus* samples. This is because despite the fact that the Gulf of Finland was (after the Chernobyl accident in 1986) one of the most contaminated areas in the Baltic Sea region, the concentration of radioactive cesium in Baltic waters is now rather uniform, and ^{137}Cs is distributed more evenly across the whole Baltic area nowadays.

The results of ^{137}Cs activity determination in *Mytilus trossulus* over a three year period are presented in Table 3. In 2004 and 2005 the levels of ^{137}Cs activity were very similar, while in 2006 the obtained result was markedly higher. The main cause may be the age, i.e. the size range of the shells of the investigated organisms, which was extended in 2006. The total length of a mussel shell is directly related to the age and the effective time of exposure to hazardous substances which are taken up by filtering from water. The levels of ^{137}Cs activity found in *Cardium glaucum* (Table 4) were lower than observed in the blue mussels. In the case of *Macoma balthica* the obtained result was similar to that in blue mussels,

Table 3

Activity concentration of ^{137}Cs in the soft tissue of *Mytilus trossulus*.

Year	Numbers of organisms	Length range (cm)	^{137}Cs ±1σ (Bq kg^{-1}d.w.)
2004	100	3.6 – 5.6	4.96 ±0.49
2005	100	3.8 – 5.5	4.03 ±0.44
2006	100	3.9 – 6.6	6.97 ±0.38
		Mean value	5.32 ±1.50

Table 4

Activity concentration of ^{137}Cs in the whole body of *Balanus improvisus, Cardium glaucum* and *Macoma balthica*.

Sample	^{137}Cs ±1σ (Bq kg^{-1} d.w.)
Balanus improvisus	1.53 ±0.16
Cardium glaucum	1.05 ±0.17
Macoma balthica	3.08 ±0.24

especially when taking into account that the presence of shells, which may affect the final result by increasing the mass of a whole sample though they do not accumulate cesium ions.

For each phytobenthos and zoobenthos organism the concentration factor was calculated (Szefer 2002a) as a ratio between ^{137}Cs concentration in the studied organism and in seawater, based on data from 2009 (Fig. 2). In order to obtain normalized results, dry weight, which is a stable parameter, was applied to determine the ^{137}Cs concentration in organisms. In 2009, the levels of cesium activity in seawater were equal to 41.8, 41.7 and 39.2 Bq m^{-3}, respectively, at the three sampling points: ZN4, P110 and L7 (Fig. 1b).

The calculated concentration factors in the studied benthic organisms diminish in the following order:

$CF_{P.\ fucoides} > CF_{E.\ siliculosus} > CF_{C.\ glomerata} > CF_{F.\ lumbricalis} > CF_{M.\ trossulus} > CF_{Z.\ marina} > CF_{P.\ litoralis} > CF_{P.\ pectinatus} > CF_{F.\ vesiculous} > CF_{Enteromorpha\ spp.}$.

The obtained results explicitly indicate that *P. fucoides* shows the best bioaccumulative properties with regards to Cs^+ ions. *E. siliculosus*, from the brown algae division, and *C. glomerata*, representing the green algae division, are not far behind. The main feature linking these three benthic plants is the large external surface of their thalluses.

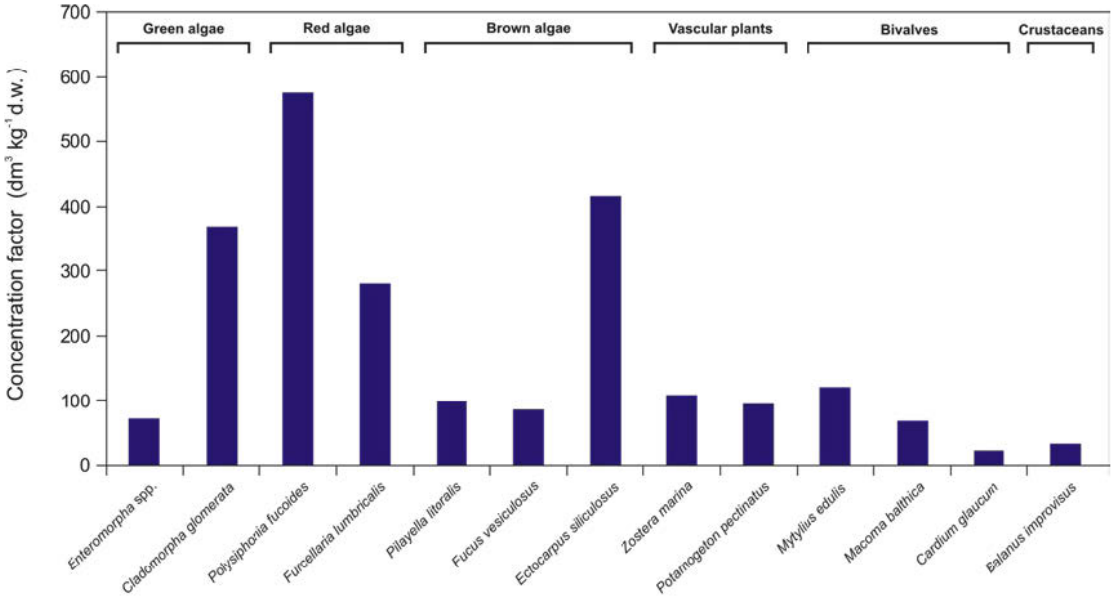


Fig. 2. Concentration factors (CF) for investigated organisms.

The concentration process is related to the dynamic steady state between the uptake and excretion processes. The concentration factor is the quantitative measure which allows the relative ability of biota representatives to absorb and/or take up the analyzed trace element to be assessed (Szefer 2002a). Changes of ^{137}Cs concentration in seawater are not so dynamic (a visible decrease takes more than one algal vegetative period) so we can assume that there were steady state conditions in the marine environment during the investigation period. As a result, the obtained concentration factors allow the bioaccumulative abilities of the investigated organisms towards ^{137}Cs to be compared.

Bioaccumulative processes are determined by external and internal factors. The internal factors are related to the morphology and physiology of the investigated organisms. One of the most important morphological parameters is the external surface exchange area (mentioned above), which remains in close relation to the method of exchange of elements with the environment. Vascular plants exchange elements with their environment by root systems embedded in sand, which is a limited source of ^{137}Cs since the amount of cesium accumulated in sand is low. On the other hand, algae exchange elements with the environment by foliar uptake which makes bioaccumulation of cesium ions much easier and the

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water surrounding the plant provides a continuous source of the radionuclide.

Besides that, the *P. fucoides*, *E. siliculosus* and *C. glomerata* possess very large surface exchange areas. These algae belong to the filamentous group, which is one of the groups within the functional-form model proposed by Littler (Littler and Littler 1980, Lobban and Harrison 1997). Littler's model assumes that functional characteristics (e.g. uptake of environmental elements) depends on the morphology and surface to volume ratios. The filamentous group is characterized by delicately-branched external morphology, uniseriate, multiseriate or lightly-corticated internal anatomy, and soft texture (Littler and Littler 1980, Lobban and Harrison 1997).

For *F. lumbricalis*, belonging to the coarsely-branched group with coarsely- branched, upright external morphology, corticated internal anatomy and fleshy wiry texture, a lower concentration factor value was observed which might indicate a slower and more laborious bioaccumulative process. Zn concentrations found in representatives of the coarsely-branched group were generally lower than those found in representatives of the filamentous group (Stengel et al. 2004) and this agrees with the result obtained for cesium in our study.

The results obtained in the case of green algae (*Enteromorpha* spp.), from the sheet macrophyte group, are surprising when taking into account that the thin, tubular and foliose external morphology, uncorticated internal anatomy and soft texture may have facilitated higher uptake process results obtained for zinc (Stengel et al. 2004). In this sense, *Enteromorpha* spp. demonstrated a much lower bioaccumulative ability towards cesium ions than expected. However, the much smaller surface exchange area in comparison with, for example, *P. fucoides* may be responsible for such a result.

Very low ^{137}Cs concentration was found in *F. vesiculosus*, a brown algae which represents the thick leathery group with thick blades and branches, differentiated, heavily-corticated, thick-walled internal anatomy and leathery, rubbery texture.

Considerably lower concentration factors, and at similar levels, were obtained in brown algae (*P. litoralis* and *F. vesiculosus*) and vascular plants (*Z. marina* and *P. pectinatus*). In the latter group, lower concentrations of ^{137}Cs are definitely linked to the method by which nutrients and trace elements are assimilated.

Among the macrophytobenthic organisms the weakest bioaccumulative properties towards cesium ions were shown by *Enteromorpha* spp..

With regard to zoobenthic species, the highest concentration factor was determined in *M. trossulus*. The value obtained was comparable to those in vascular plants and brown algae.

The comparison of the concentration factors calculated for macroalgae and zoobenthic organisms leads to the conclusion that phytobenthic plants may be regarded as much better bio-indicators when their bioaccumulative abilities are taken into account. It is especially important when considering that, in the case of macrophytobenthic organisms, the uptake processes are practically limited to one period of the year, when intensive living processes take place, whereas mussels accumulate pollutants over a longer time. The relatively quick response time with regard to pollution is also very important.

CONCLUSIONS

- ^{137}Cs activity concentrations in the analyzed macrophytes varied from about 1 Bq kg^{-1} d.w. found in the vascular plant *Potamogeton pectinatus* to 49 Bq kg^{-1} d.w. observed in the red algae *Polysiphonia fucoides*.
- ^{137}Cs activity concentrations in the analyzed benthic macroinvertebrate representatives varied from 1 Bq kg^{-1} d.w. found in *C. glaucum* to approximatley 7 Bq kg^{-1} d.w. observed in *M. trossulus*.
- Differences in ^{137}Cs concentration in macrophytes were observed regarding sampling in different parts of the vegetation period, suggesting a seasonal uptake of the radionuclide.
- The highest concentration factors were determined in red algae – *P. fucoides*, brown algae – *E. siliculosus* and green algae – *C. glomerata*. The common feature linking these plants is the external structure of their thalluses and large surface exchange area, which seems to be the most essential parameter for bioaccumulation ability.
- *P. fucoides* could be recommended as a nearly perfect bio-indicator of radioactive contamination in the sea since it showed the greatest bioaccumulative ability towards ^{137}Cs arising from the large surface exchange area, large intercellular volume related to the so called "pneumatic construction" influencing its capacity for

bioaccumulation, quick response to the polluting element, wide-spread distribution as well as high rate of biomass growth and long vegetative period
- Macrophytes turned out to be better bioindicators in relation to radioactive ^{137}Cs than the benthic animals.

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