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Bioaccumulation of gamma emitting radionuclides in red algae from the Baltic Sea under laboratory conditions

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Radionuclides
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Abstract

The bioaccumulation ability of radionuclides ^{51}Cr, ^{54}Mn, ^{57}Co, ^{60}Co, ^{65}Zn, ^{85}Sr, ^{109}Cd, ^{110m}Ag, ^{113}Sn, ^{137}Cs and ^{241}Am in two red algae species from the southern Baltic Sea – *Polysiphonia fucoides* and *Furcellaria lumbricalis* – was determined under laboratory conditions. *P. fucoides* demonstrated better bioaccumulative properties towards most of the investigated radionuclides. As a result, *P. fucoides* can be recommended as a good bioindicator of radioactive environmental pollution. The bioaccumulation of radionuclides in *F. lumbricalis* was studied during an extended laboratory experiment. The initial extensive uptake of radioisotopes was followed by the rapid removal of cations; in general, concentrations tended to decrease with time. ^{137}Cs displayed a different behaviour, its concentration in the algae increasing over time mainly due to its large ion radius; this is a factor that could be responsible for the stronger mechanical and chemical bonding of Cs^+ and that could hamper the movement of ions in both directions.

1. Introduction

The bioaccumulative properties of marine organisms towards radionuclides may be very useful for potential application in monitoring and assessment procedures of the marine environment as such and especially in monitoring nuclear facility waste sites. Radionuclides can be used as

radiotracers in studies of heavy metal and organic pollutant behaviour (uptake, distribution and retention) in marine flora (Wolterbeek et al. 1995, Boisson et al. 1997, Malea & Haritonidis 2000, Kleinschmidt 2009, Strezov & Nonova 2009) and fauna (Warnau et al. 1999, Fowler et al. 2004, Kumblad et al. 2005).

It is to be anticipated that marine algae are the most suitable indicators of dissolved metal forms because, in contrast to animals, there is no dietary route involved in the uptake of the trace element (Szefer 2002a). Marine algae concentrate metals from seawater, and variations in metal concentrations in the thallus are often taken to reflect the metal concentration in the surrounding seawater (Szefer & Skwarzec 1988, Lobban & Harrison 1997). The other rationale for using macroalgae in basic investigations and for monitoring purposes is their widespread distribution, relatively easy accessibility and intensive physiological and growth processes, which take place within a relatively confined period of the year and which are accompanied by increased uptake and quick response to the contamination.

Because heavy metals can have different influences on marine algae, it is important to recognize bioaccumulation as a means of assessing the potential risk arising from the presence of heavy metals in the environment. From the environmental pollution point of view, heavy metals can be classified into three groups: non-critical, toxic but very insoluble or very rare, and very toxic and relatively accessible (Lobban & Harrison 1997). Some heavy metals from the last category, e.g. manganese, iron, copper and zinc, are essential micronutrients, and their ultimate influence depends strongly on their concentrations found in algal organisms. They may limit algal growth if their concentrations are too low, but at the same time they can be very toxic at higher concentrations (Lobban & Harrison 1997).

The concentration of heavy metals in algal thalli is a result of the efficiency of bioaccumulation, which in turn is a result of the combined bioaccumulation affinity of macroalgae for a particular metal, as well as the bioavailability and physical/chemical form of that metal. The tendency for macroalgae to bioaccumulate various substances depends strongly on their morphology and physiology, which in turn are closely related to the group of algae to which they belong. As shown for Baltic benthic plants, the concentrations of heavy metals (Bojanowski 1973, Szefer & Skwarzec 1988, Falandysz 1994) as well as radionuclides (Bojanowski & Pempkowiak 1977, Skwarzec & Bojanowski 1992) have changed over a wide range in species representing different divisions. Further toxic interaction (besides the elevated concentrations) may arise from the radiation if an unstable heavy metal isotope is accumulated. The radiation emitted can lead to

mutagenic interactions of various kinds, affecting growth and metabolic processes.

Metals are taken up by algae both passively and actively. Some, like strontium, are passively adsorbed by polysaccharides in the cell wall and intercellular matrix. Others, like Zn and Cd, are taken up actively against a large intracellular concentration gradient (Lobban & Harrison 1997). Metabolically controlled uptake mechanisms were proven in the case of ^{54}Mn, ^{65}Zn, ^{110m}Ag, ^{109}Cd and ^{60}Co by Boisson et al. (1997), who demonstrated the temperature-dependent uptake kinetics observed for these radionuclides.

An understanding of the bioaccumulation of radionuclides and heavy metals in macroalgae can assist the development of environmental monitoring programmes (Burger et al. 2006, HELCOM 2009). Such information is also indispensable in the development of models and methodologies for assessing the impact of radioactivity originating from nuclear facilities, especially with regard to radioactivity in the marine environment and marine life (Lepicard et al. 2004, Brown et al. 2006, Kumblad et al. 2006).

As far as applications based on monitoring systems are concerned, an essential step is to identify bioindicator organisms, among which marine plants play a very important role. This may be achieved by collecting basic information on the bioaccumulative properties of individual macroalgal species towards radionuclides or heavy metals.

Information based on investigations into bioaccumulation processes can also be useful in assessing the potential application of benthic plants as biofertilizers (Filipkowska et al. 2008), as bioadsorbents for metal removal in wastewater treatment (Radway et al. 2001) and in heavy metal detoxification (Cobbett 2000).

The present study aimed to evaluate the bioaccumulative properties of two red algae species – *Polysiphonia fucoides* and *Furcellaria lumbricalis* – towards gamma-emitting radionuclides. The reference solution used in the experiment contained eleven radionuclides: ^{51}Cr, ^{54}Mn, ^{57}Co, ^{60}Co, ^{65}Zn, ^{85}Sr, ^{109}Cd, ^{110m}Ag, ^{113}Sn, ^{137}Cs and ^{241}Am, all analogues of mainly very toxic and relatively accessible metals (Lobban & Harrison 1997), representing the most common radionuclides present in discharged radioactive waste.

P. fucoides and *F. lumbricalis* were selected on the basis of observations made during our previous studies (to be published), in which red algae demonstrated a greater bioaccumulation affinity for ^{137}Cs under natural conditions than green and brown algae species. The other reason was the relatively simple access to live organisms, owing to their widespread distribution in the southern Baltic Sea.

2. Material and methods

2.1. General description of the experiment

The bioaccumulation of gamma emitting radionuclides was examined in two species of red algae (*Polysiphonia fucoides* and *Furcellaria lumbricalis*) under laboratory conditions. Macrophytes were sampled in the area around the Kępa Redłowska, in the Gulf of Gdańsk (Figure 1), and were collected with the stony substrate by scuba divers in May 2009. Stones covered with red macroalgae were rinsed with seawater to remove sand, solid pollutants and organisms (e.g. *Gammarus*) inhabiting the thalli, and immersed in two aquaria with dimensions of $50 \times 80 \times 50$ cm equipped with aerating filters. *F. lumbricalis* and *P. fucoides* were put into separate aquaria filled with seawater previously passed through Whatman filters (GF/C). The water temperature was related to room temperature ($23 \pm 1°C$), and the water salinity was 7.0 (PSS'78). The experiment lasted from July to December 2009.

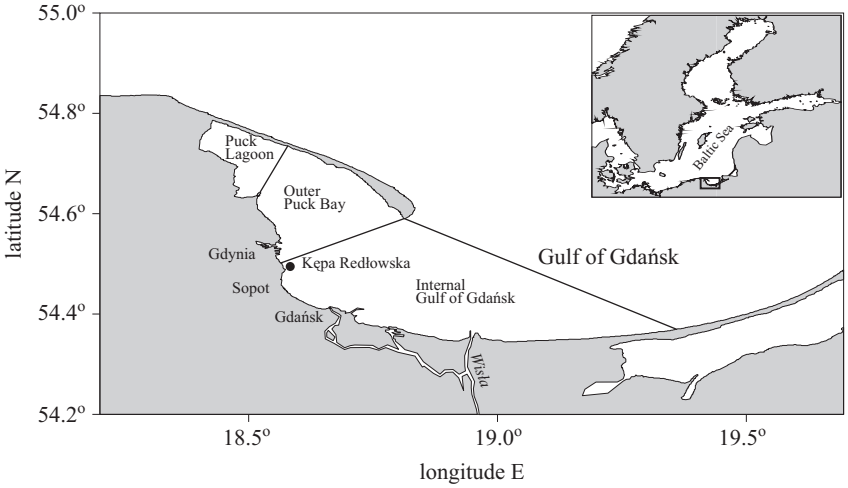

Figure 1. Location of the sampling region

The plants in the aquaria were left to equilibrate and on 20 July 2009 1 ml of mixed gamma standard solution (code BW/Z-62/27/07, total activity 72.67 kBq/15.06.2009, total weight 10.02732 g; produced by OBRI POLATOM, Świerk k/Otwocka, Poland) was added to each aquarium. The standard solution was a mixture of 11 radionuclides (^{51}Cr, ^{54}Mn, ^{57}Co, ^{60}Co, ^{65}Zn, ^{85}Sr, ^{109}Cd, ^{110m}Ag, ^{113}Sn, ^{137}Cs, ^{241}Am) (see Table 1). The initial concentrations of radionuclides in spiked seawater were calculated

Table 1. Assigned activities of isotopes in the mixed gamma standard solution, measured activities of isotopes in the samples, calculated accuracy, precision and initial concentrations in spiked seawaters

Radionuclide	Half-life [days]	Assigned activity ± uncertainty [kBq ml^{-1}] on 15.06.2009	Measured activity ± uncertainty [kBq ml^{-1}] on 15.06.2009	Accuracy [%]	Precision [%]	Initial concentration ± $1\sigma^*$ [Bq dm^{-3}] on 20.07.2009
^{51}Cr	28	1.579 ± 0.055	1.634 ± 0.082	3.5	6.1	7.74 ± 0.27
^{54}Mn	312	0.544 ± 0.019	0.591 ± 0.030	8.7	6.1	5.92 ± 0.21
^{57}Co	272	0.099 ± 0.003	0.108 ± 0.005	9.4	5.8	1.06 ± 0.03
^{60}Co	1924	0.500 ± 0.018	0.536 ± 0.027	7.2	6.2	5.80 ± 0.21
^{65}Zn	244	0.882 ± 0.031	0.957 ± 0.048	8.6	6.1	9.39 ± 0.33
^{85}Sr	65	0.266 ± 0.009	0.282 ± 0.014	5.9	6.0	2.16 ± 0.07
^{109}Cd	461	1.716 ± 0.060	1.601 ± 0.080	6.7	6.1	19.16 ± 0.67
^{110m}Ag	250	0.399 ± 0.014	0.349 ± 0.017	12.5	6.1	4.26 ± 0.15
^{113}Sn	115	0.356 ± 0.012	0.388 ± 0.019	8.9	6.0	3.39 ± 0.11
^{137}Cs	10968	0.394 ± 0.014	0.419 ± 0.021	6.4	6.1	4.62 ± 0.16
^{241}Am	157899	0.514 ± 0.018	0.573 ± 0.029	11.6	6.1	6.04 ± 0.21

$^*1\sigma$ – standard deviation.

using the activities in the standard solution and the volume of seawater in the aquaria. They are presented in Table 1.

The exposed macroalgae were first sampled after 20 days. Samples of *P. fucoides* and *F. lumbricalis* were collected for the analysis of their radionuclide content. As the total biomass of *P. fucoides* in the experimental aquarium was very small, all the material was used up in this first determination and the investigation of bioaccumulation was terminated in this species at this very early stage and continued solely with *F. lumbricalis*. Subsequent samplings were carried out after 25, 20, 6 and 78 days.

Initial radionuclide concentrations were determined in both macroalgae species in specially designated samples, which were collected at the same time as the plants later exposed during the experiment.

Seawater samples of 450 ml volume were taken in parallel with the plant samples, and radionuclide concentrations were measured in Marinelli geometry with the same gamma spectrometry method. The water was sampled together with the first sampling of the plants. Most of the radionuclide activities in seawater were below the limits of detection: ^{51}Cr – 0.82, ^{54}Mn – 0.08, ^{57}Co – 0.09, ^{60}Co – 0.11, ^{65}Zn – 0.15, ^{85}Sr – 0.9, ^{109}Cd – 2.04, ^{110m}Ag – 0.13, ^{113}Sn – 0.13, ^{137}Cs – 0.07, ^{241}Am – 0.28 [Bq dm^{-3}].

2.2. Analysis

The macroalgae samples taken from the aquaria were dried, weighed to determine dry mass content, ashed at 450°C and homogenized. They were then placed in 40 mm diameter cylindrical dishes, in which form they were ready for radioactivity measurements.

Gamma emitting radionuclide activity was measured with the gamma spectrometric method, using an HPGe detector, with a relative efficiency of 18% and a resolution of 1.8 keV for a ^{60}Co peak of 1332 keV. The detector was coupled to an 8192-channel computer analyser. The limits of detection (expressed in Bq kg^{-1} d.w.) of the radionuclides in the algae were as follows: ^{51}Cr – 64.6, ^{54}Mn – 7.3, ^{57}Co – 4.8, ^{60}Co – 7.9, ^{65}Zn – 15.2, ^{85}Sr – 7.9, ^{109}Cd – 93.0, ^{110m}Ag – 6.1, ^{113}Sn – 7.6, ^{137}Cs – 6.8, ^{241}Am – 22.8.

The reliability and accuracy of the method applied was validated by participation in the *HELCOM-MORS proficiency test determination of radionuclides in fish flesh samples* organized by IAEA-MEL Monaco (IAEA-414, Irish and North Sea Fish). Fish flesh can be regarded as a substitute for ashed macroalgae samples with almost the same density as the prepared samples. Results of the ^{137}Cs and ^{40}K determinations are presented in Table 2 (after IAEA 2010). In order to determine the accuracy

Table 2. Results of *HELCOM-MORS proficiency test determination of radionuclides in fish flesh* (IAEA 414 – certified reference material) – Laboratory No. 4, Institute of Meteorology and Water Management, Maritime Branch, Gdynia, Poland, Reference Date: 01 January 1997, after IAEA (2010)

Analyte	IAEA value	IAEA unc.	Lab. value	Lab. unc.	Lab. unc.	Rel. bias
	[Bq kg^{-1} d.w.]				[%]	
^{40}K	481	16	474.5	19.3	4.1	1.35
^{137}Cs	5.18	0.10	5.06	0.64	12.6	2.32
	Trueness	P	Precision	Final score		
	[%]			[%]		
^{40}K	passed	5.2	passed	acceptable		
^{137}Cs	passed	12.8	passed	acceptable		

and precision of the radionuclide determination, a water sample containing 1 ml of the mixed gamma standard solution (code BW/Z-62/27/07, applied in the experiment) was prepared and the isotope activities measured using the same geometry and gamma spectrometry method (Table 1).

3. Results and discussion

3.1. Comparison of the bioaccumulation properties of *Polysiphonia fucoides* and *Furcellaria lumbricalis*

The initial, radioactive concentrations (i.e. the concentrations prior to exposure) of the analysed radionuclides in plants were below the limit of detection of the method, except for ^{137}Cs. The levels of ^{137}Cs were 31.7 ± 1.2 Bq kg^{-1} d.w. in *P. fucoides* and 16.9 ± 0.8 Bq kg^{-1} d.w. in *F. lumbricalis*.

The radionuclide activity levels found in *P. fucoides* and *F. lumbricalis* after 20 days of exposure under laboratory conditions are presented in Figure 2. The concentration of zinc was the highest in both species: the activity of ^{65}Zn in *P. fucoides* was 25 847 Bq kg^{-1} d.w., a value that was over three times higher than that determined in *F. lumbricalis*. The concentration of ^{110m}Ag was also very high in *P. fucoides* (16 487 Bq kg^{-1} d.w.) in comparison with the other radionuclides (Table 3). The activity of ^{110m}Ag was much lower in *F. lumbricalis* – 2462 Bq kg^{-1} d.w. Apart from these high concentrations of ^{65}Zn and ^{110m}Ag, the activity levels of most of the other radionuclides were close to or less than 5000 Bq kg^{-1} d.w. Values close to 5000 Bq kg^{-1} d.w. were recorded for ^{54}Mn in *F. lumbricalis*, ^{60}Co in both species and ^{113}Sn in *P. fucoides*. ^{241}Am concentrations were very similar in both species – 3431 and 3259 Bq kg^{-1} d.w. in *P. fucoides* and

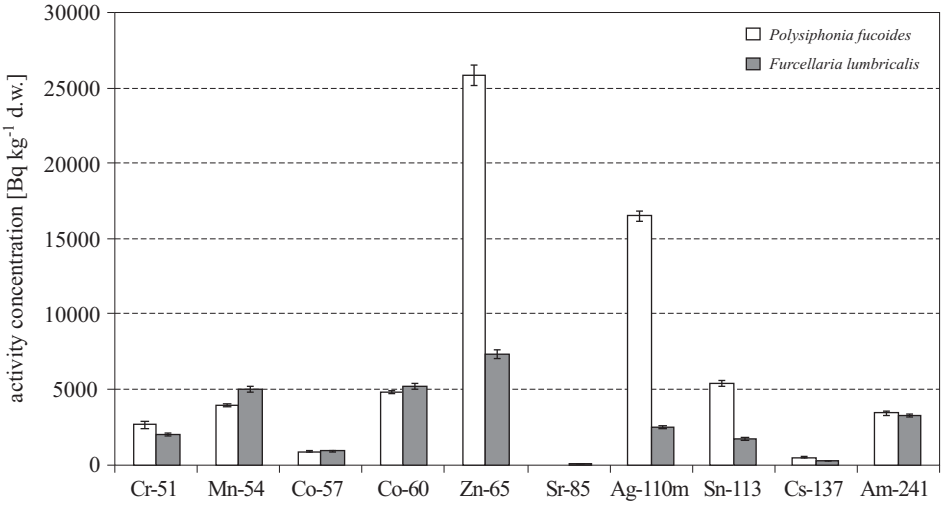

Figure 2. Radionuclide activity concentrations in *Polysiphonia fucoides* and *Furcellaria lumbricalis* after 20 days' exposure

F. lumbricalis, respectively. ^{57}Co also exhibited similar behaviour in both species of macroalgae, but the concentrations were much lower – 846 and 886 Bq kg^{-1} d.w., respectively.

The lowest activity concentration was determined for ^{85}Sr (58 Bq kg^{-1} d.w.) in *F. lumbricalis*, whereas in *P. fucoides* the level of this radionuclide was below the limit of detection. A possible explanation of this fact is the passive adsorption of strontium cations by negatively charged polysaccharides present in the cell wall, which in *F. lumbricalis* is much thicker. *F. lumbricalis* belongs to the coarsely branched group of macroalgae with a corticated internal anatomy, according to the Littler functional-form model (Littler & Littler 1980, Lobban & Harrison 1997), and its external walls form a type of skeleton in which strontium ions may be trapped more efficiently.

An index commonly used to compare the bioaccumulation properties of the species under scrutiny here is the concentration factor (CF), calculated as the ratio of the radionuclide concentration found in an organism to its concentration in seawater (Szefer 2002b). However, the concentration factor can only be related to the steady state conditions found in the natural environment. In the present study, it was not possible to calculate concentration factors, because a steady state was not attained during the experiment, and conditions changed, especially with regard to radionuclide concentrations in the algal thalli and in the seawater.

Hence, it seemed reasonable to suggest another factor, named the 'interspecific diversity factor' ($\mathrm{ISDF}_{P/F}$) for the purposes of this study. $\mathrm{ISDF}_{P/F}$ is defined as the ratio of the radionuclide concentration in one species (*P. fucoides*) to its corresponding concentration in another species (*F. lumbricalis*), as described by the following formula:

$$\mathrm{ISDF}_{P/F} = A_{Polysiphonia}/A_{Furcellaria}. \tag{1}$$

This factor enables the bioaccumulation abilities of two species towards a single radionuclide to be compared. In this case, the term 'bioaccumulation ability' should be understood as the relationship between the rate of bioaccumulation during a given time interval and the bioaccumulative capacity. However, the simple measurement of radionuclide concentrations does not suffice to distinguish which of these two components is the most influential on the final result.

The interspecific diversity factor can be used directly as a basis for comparing the bioaccumulative properties of the species under study, provided certain conditions are satisfied: i) competition between the plants in the bioaccumulation processes should be prevented by placing them in separate aquaria, ii) the initial concentrations of radionuclides should be the same in both aquaria, and iii) the bioaccumulation process should be related to the same time interval, thus precluding the effect of radionuclide concentration decrease as a result of radioactive decay. All of these requirements were met in our experiment.

The calculated $\mathrm{ISDF}_{P/F}$ values of nine radionuclides are illustrated graphically in Figure 3. The values close to 1.0, as determined in the cases of three radioisotopes – ^{57}Co, ^{60}Co and ^{241}Am (Table 3) – mean that no great diversity was observed between *F. lumbricalis* and *P. fucoides* and that the bioaccumulation of these radionuclides proceeded according to a very similar pattern in both species. A value slightly in excess of 1.0 was found in the case of ^{51}Cr, and only in one case – ^{54}Mn – was $\mathrm{ISDF}_{P/F}$ markedly < 1.0. This may indicate that bioaccumulation proceeds more easily and faster in *F. lumbricalis*.

Considerably higher values, exceeding 3.0, were calculated in the cases of zinc (^{65}Zn) and tin (^{113}Sn) isotopes, while the highest $\mathrm{ISDF}_{P/F}$ value of 6.7 was recorded for silver ($^{110\mathrm{m}}$Ag), indicating the preference of *P. fucoides* for the bioaccumulation of $^{110\mathrm{m}}$Ag.

The estimated value of $\mathrm{ISDF}_{P/F}$ for radioactive caesium isotopes, which showed the lowest concentrations in both species, was almost 2.0, again indicating that bioaccumulation was more effective in *P. fucoides*. It should be stressed that the interspecific diversity factor obtained for ^{137}Cs

accumulation under steady-state environmental conditions, calculated using concentration levels in plants prior to exposure (the black bar in Figure 3), was very close to this value (1.9). This could indicate that the bioaccumulative efficiencies in both red algae during the laboratory experiment remained in the same proportion to their efficiencies in the marine environment.

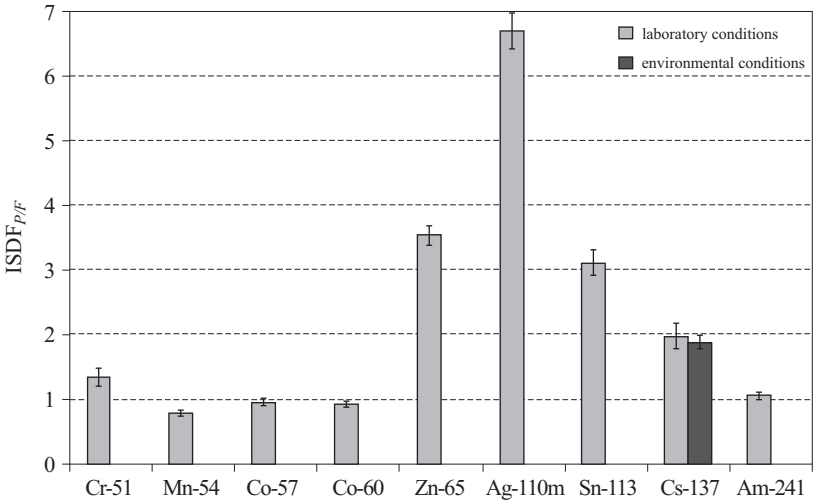

Figure 3. Interspecific diversity factor (ISDF$_{P/F}$) determined for the radionuclides

In both species, bioaccumulation was achieved by foliar uptake; the surface exchange area was therefore one of the most important parameters during this process (Lobban & Harrison 1997). For this reason, the higher concentrations of most of the radionuclides found in *P. fucoides* can be related primarily to the extensive surface exchange area specific to this species. According to the Littler functional-form group model (Littler & Littler 1980), in which those authors divide macroalgae into six different groups based upon external morphology and internal anatomy, *P. fucoides* belongs to the filamentous group. This group is characterized by a delicately-branched external morphology, uniseriate, multiseriate or lightly-corticated internal anatomy, and a soft texture that may also facilitate bioaccumulation. Additionally, the specific internal construction of the genus *Polysiphonia* consisting of a central axis, elongated cells, surrounded by pericentral cells of the same length to create a semi-pneumatic construction, may influence the bioaccumulative capacity to a large extent (Szweykowska & Szweykowski 1979).

Table 3. Activity concentrations of radionuclides in *Polysiphonia fucoides* and *Furcellaria lumbricalis* and values of interspecific diversity factors ($ISDF_{P/F}$)

	Activity concentration ± $1\sigma^*$ [Bq kg^{-1} d.w.]						$ISDF_{P/F}$
	Polysiphonia fucoides	*Furcellaria lumbricalis*					
Isotope \ Date	10.08.2009	10.08.2009	04.09.2009	23.09.2009	29.09.2009	16.12.2009	
^{51}Cr	2664 ± 237	1994 ± 124	850 ± 51	< LLD	< LLD	< LLD	1.34 ± 0.14
^{54}Mn	3908 ± 117	5002 ± 177	3059 ± 58	3339 ± 80	3206 ± 66	362 ± 166	0.78 ± 0.04
^{57}Co	846 ± 36	886 ± 39	692 ± 22	705 ± 27	704 ± 40	362 ± 11	0.95 ± 0.06
^{60}Co	4797 ± 128	5222 ± 182	3941 ± 72	4301 ± 87	4320 ± 72	2708 ± 20	0.92 ± 0.04
^{65}Zn	25847 ± 664	7320 ± 264	4349 ± 88	4464 ± 137	4602 ± 122	2989 ± 41	3.53 ± 0.16
^{85}Sr	< LLD	58 ± 7	< LLD	< LLD	< LLD	< LLD	–
^{110m}Ag	16487 ± 378	2462 ± 86	965 ± 18	1234 ± 31	1305 ± 28	599 ± 8	6.70 ± 0.28
^{113}Sn	5390 ± 215	1732 ± 81	1049 ± 38	909 ± 44	920 ± 40	367 ± 14	3.11 ± 0.19
^{137}Cs	468 ± 40	238 ± 12	210 ± 7	426 ± 23	464 ± 21	603 ± 10	1.97 ± 0.20
^{241}Am	3431 ± 135	3259 ± 117	1998 ± 39	1916 ± 50	2055 ± 53	< LLD	1.05 ± 0.06

*1σ – standard deviation.

3.2. Long-term bioaccumulation in *F. lumbricalis*

The activity changes of eight radionuclides in *F. lumbricalis* thalli during the time of exposure are presented in Figures 4–6. The curves enable five stages in the process of radionuclide accumulation by the macroalgae to be identified. The first two stages are common to all radioisotopes. The first one, observed twenty days after the addition of the standard radionuclide solution, indicates an increase in their concentration in the plant as a consequence of intensive bioaccumulation. In the second stage, the concentrations of all radionuclides declined. It should be noted that all the radionuclides reached their maximum and minimum values on the approximate curves within a short period of time.

The first stage can definitely be related to the initial rapid uptake of radionuclides from the medium. In the beginning, radionuclide uptake occurs spontaneously and independently of metabolism, requiring no energy; this was also observed for nutrient uptake (Lobban & Harrison 1997). Then, other mechanisms of adsorption and transportation, both passive and active, may play a more important role. To be adsorbed, each ion has to pass barriers such as the laminar layer, the cell wall and the plasmalemma, before finally reaching the cytoplasm (Lobban & Harrison 1997). The thickness of the laminar layer depends on the turbulence in the surrounding water. Under laboratory conditions, because of aeration, the effect of this layer can probably be ruled out, and the uptake will not be limited by the rate

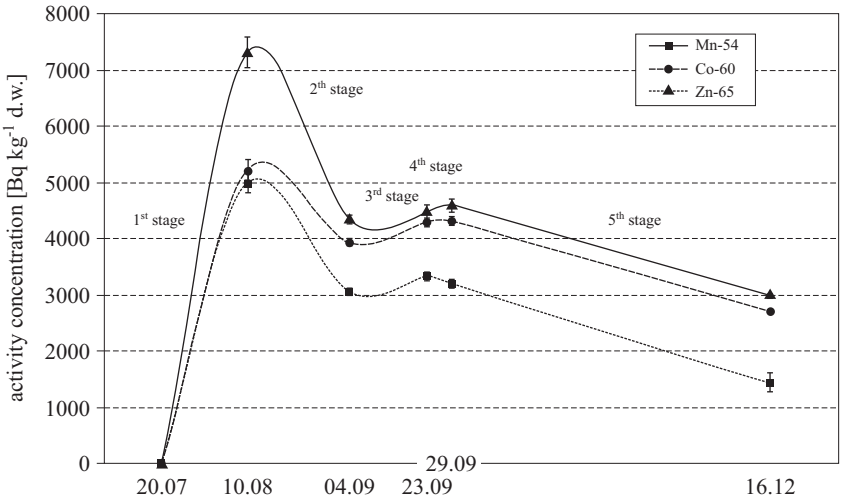

Figure 4. Plots showing the changes in concentrations of ^{54}Mn, ^{60}Co and ^{65}Zn in *Furcellaria lumbricalis* recorded during exposure from 20 July to 16 December

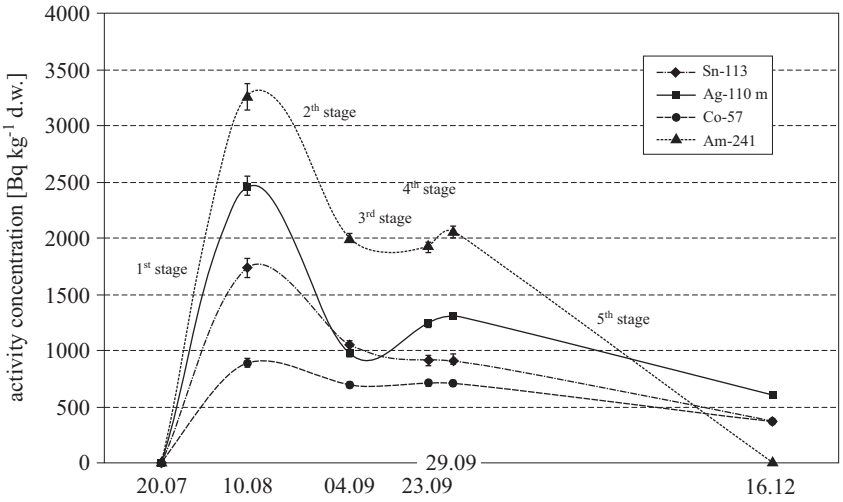

Figure 5. Plots showing the changes in concentrations of ^{113}Sn, ^{57}Co, ^{241}Am and ^{110m}Ag in *Furcellaria lumbricalis* recorded during exposure from 20 July to 16 December

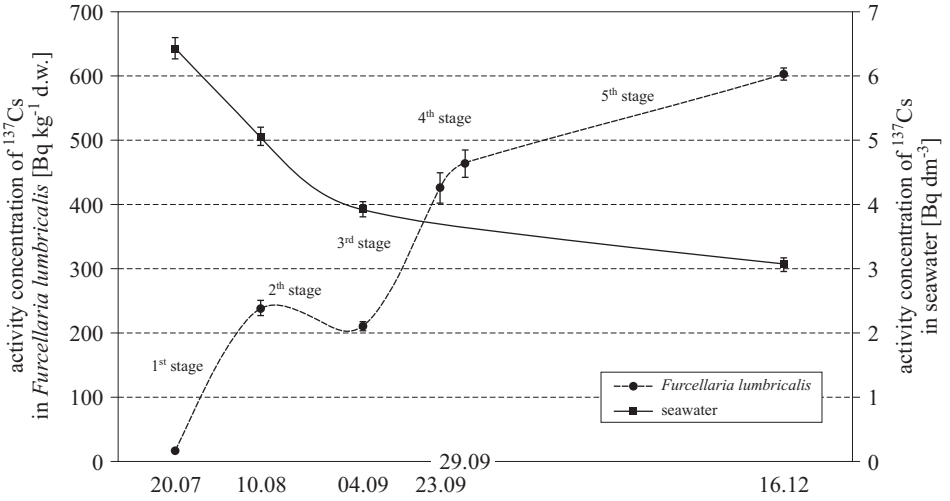

Figure 6. Plots showing the changes in concentrations of ^{137}Cs in *Furcellaria lumbricalis* and in seawater recorded during exposure from 20 July to 16 December

of diffusion across this layer. The cell wall does not generally present a barrier to ion entry, unlike the plasmalemma, which may be more difficult to penetrate (Lobban & Harrison 1997).

Generally, during the first stage, ions are introduced to the so-called apparent free space that, in seaweeds, includes the cell wall and all

intercellular spaces exterior to the plasmalemma (Lobban & Harrison 1997). The apparent free space consists of two parts: the first of these is called the water-free space, and the second one, which relates to the deeper parts of the thallus, is the Donnan free space. Ions introduced to the water-free space can be readily removed, as was observed in the second stage distinguished on the curves (Figures 4–6), when a decline in radionuclide concentrations in the plant occurred. The decrease in radionuclide concentrations is attributable mainly to release processes, as the concentrations in the seawater medium and in the plant tissue began to equilibrate, subsequent to intensive bioaccumulation.

^{90}Sr and ^{51}Cr were detected in algal thalli after 20 days of exposure; however, they were not found in samples taken after the second stage, following 45 days of exposure. The short half-lives of these radionuclides – 65 for ^{90}Sr and 28 days for ^{51}Cr – and their relatively low initial concentrations should be considered responsible for this absence. Additionally, as already mentioned, strontium cations were largely retained within the cell wall and did not reach deeper layers.

The rates of radionuclide bioaccumulation and excretion were determined at each stage of exposure (Table 4 and Figure 7). In the case of ^{113}Sn, ^{241}Am, ^{54}Mn and ^{65}Zn, the ratio of the bioaccumulation rate in the first stage to the excretion rate in the second (Figure 7) was close to 3, indicating that the maximum concentration reached in the first stage exerted little or no influence on the removal rate. In the case of the cobalt isotopes, the

Table 4. Average bioaccumulation and excretion rates determined for each stage

Stage	Date	Average bioaccumulation and excretion rate [Bq kg^{-1} d.w. per day]			
		Mn-54	Co-57	Co-60	Zn-65
1	20.07–10.08	250.1 ± 8.9	44.3 ± 2.0	261.0 ± 9.1	365.8 ± 13.2
2	10.08–04.09	−77.7 ± 1.5	−7.8 ± 0.3	−51.2 ± 0.9	−118.8 ± 2.4
3	04.09–23.09	14.7 ± 0.4	0.68 ± 0.03	19.0 ± 0.4	19.2 ± 0.6
4	23.09–29.09	−22.2 ± 0.5	−0.17 ± 0.01	3.2 ± 0.1	7.3 ± 0.2
5	29.09–16.12	−36.7 ± 4.2	−7.1 ± 0.2	−33.6 ± 0.3	−33.6 ± 0.5
		Ag-110m	Sn-113	Cs-137	Am-241
1	20.07–10.08	123.0 ± 4.3	86.5 ± 4.1	11.1 ± 0.6	162.8 ± 5.9
2	10.08–04.09	−59.9 ± 1.1	−27.3 ± 1.0	−1.1 ± 0.1	−50.4 ± 1.0
3	04.09–23.09	14.2 ± 0.4	−7.4 ± 0.4	11.4 ± 0.6	−4.3 ± 0.1
4	23.09–29.09	11.8 ± 0.3	1.8 ± 0.1	6.3 ± 0.3	23.2 ± 0.6
5	29.09–16.12	−14.7 ± 0.2	−11.5 ± 0.4	2.9 ± 0.1	−42.8 ± 2.1

(−) indicates the direction of ion flow, that is the process of excretion.

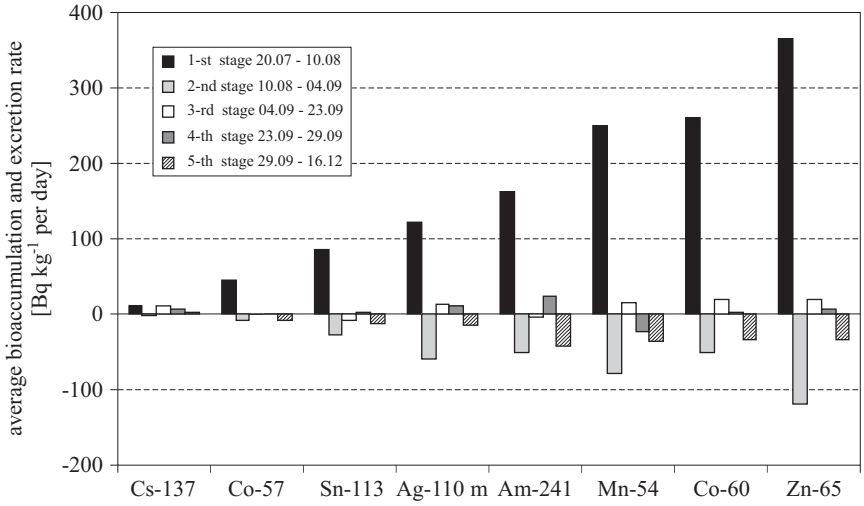

Figure 7. Bioaccumulation and excretion rates calculated for five exposure periods for ^{54}Mn, ^{60}Co, ^{65}Zn, ^{113}Sn, ^{57}Co, ^{241}Am, ^{110m}Ag and ^{137}Cs

respective ratios for ^{57}Co and ^{60}Co were 5.7 and 5.1. The highest ratio of bioaccumulation to excretion (9.9) was registered in the case of caesium, indicating obstructed removal of ions.

During the third stage, a second increase in radionuclide concentrations, indicating uptake, was observed in the cases of ^{65}Zn and ^{60}Co, with bioaccumulation rates close to 19 Bq kg^{-1} per day. Slightly lower values, $\sim$ 14 Bq kg^{-1} per day, were found for ^{54}Mn and ^{110m}Ag; the increase in the ^{57}Co concentration was negligible.

In some cases the fourth stage, lasting only 6 days, was a continuation of the preceding one. Further increases in concentration were observed in the cases of ^{65}Zn, ^{60}Co and ^{110m}Ag, although the slopes of the curves, reflecting the bioaccumulation rates, demonstrate a slowing down of uptake. ^{57}Co and ^{113}Sn concentrations tended to remain unchanged. With regard to americium, an increase in concentration was observed in the fourth stage, in contrast to the decrease noted during the third stage. Only ^{54}Mn showed the reverse behaviour: its concentrations decreased considerably during the fourth period, a trend that continued in the fifth and final stage. Generally, the concentrations of all the radionuclides except caesium decreased during the final stage of exposure. The rate of ion removal was the highest for ^{241}Am. This cannot be attributed solely to half-life and radioactive decay because ^{241}Am has the longest half-life (432.6 years) of all the studied isotopes. ^{65}Zn and ^{60}Co demonstrated very similar removal rates, which is illustrated by

the parallel, closely related removal curves (Figure 3). The removal of ^{57}Co was found to proceed at the slowest rate, and this may be related to the low initial concentration of the radionuclide found in *F. lumbricalis*, which could have limited the flow of ions in both directions.

The results obtained in the final stage of the experiment were applied to calculate the biological depuration rate constant (Table 5) from a single-component model described by the equation (Warnau et al. 1999):

$$A_t = A_0 e^{-\lambda t}, \tag{2}$$

where

A_t – activity of the radionuclide at the end of the experiment (after the 5th stage) [Bq kg^{-1} d.w.],

A_0 – activity of the radionuclide related to the maximum value reached after the 4th stage [Bq kg^{-1} d.w.],

λ – biological depuration rate constant [d^{-1}],

t – 78 days (the duration of the 5th stage).

The constant λ allows the radiotracer biological half-life to be calculated according to the equation:

$$T_{b1/2} = \ln 2/\lambda, \tag{3}$$

where $T_{b1/2}$ – biological half-life [d]. The fastest removal rates are recorded in the case of ^{241}Am; the other radionuclides have slower but quite similar depuration rates.

Table 5. Biological depuration rate and biological half-life calculated for the radionuclides

Radionuclide	^{57}Co	^{113}Sn	^{110m}Ag	^{241}Am	^{54}Mn	^{60}Co	^{65}Zn
λ [d^{-1}]	0.0085	0.0118	0.0100	0.0853	0.0102	0.0060	0.0055
$T_{b1/2}$ [d]	5.5	5.1	5.3	3.2	5.3	5.8	5.9

Besides ^{85}Sr, ^{137}Cs exhibited the lowest concentrations of all the studied radionuclides in *F. lumbricalis*; hence the curve depicting the changes in caesium concentration during the experiment differed from the others. Comparison of the shape of the curves illustrating the changes in ^{137}Cs concentrations in *F. lumbricalis* and seawater (Figure 6) shows that very intensive bioaccumulation of caesium occurred in the first stage, which corresponded to a decline in the seawater concentration of this element. In the second stage, even though the elimination of caesium from the plant had started, caesium ions were still being removed from the water at a considerable rate. The slope of the seawater curve then changed, showing

that the decrease in caesium concentration in the surrounding water was now proceeding at a much slower rate, tending to stabilize and reach a constant value, as was to be expected.

Following the decrease in caesium concentration during the second stage, as with the other radionuclides, its bioaccumulation continued during the third stage at the same rate as in the first stage, directly after the addition of the isotopes to the seawater. Then, the rate of caesium bioaccumulation started to decrease, but in contrast to the other isotopes, its uptake continued until the end of the experiment. The concentration factor calculated for the last sample in December was 196, whereas under environmental steady state conditions it is 280.

An interesting aspect is the fact that caesium ions were only eliminated from the body of the algae during the second stage. This may be attributed to the removal of cations found in the apparent free space and which were not bound in any other way.

The dissimilarity of ^{137}Cs bioaccumulation in *F. lumbricalis* in comparison with the other radionuclides may be related primarily to the radius of caesium ions, which at 0.165 nm is the largest radius of all the cations. The transport of Cs^+ ions from the laminar layer, through the cell and plasmalemma, to the intracellular space is therefore more difficult, and it is this that ultimately influences the rate of bioaccumulation.

4. Conclusions

- *Polysiphonia fucoides* demonstrated better bioaccumulative properties towards most of the investigated radionuclides than *Furcellaria lumbricalis*. This was especially noticeable in the cases of ^{65}Zn and ^{110m}Ag, their concentrations reaching about 25 000 and 16 000 Bq kg^{-1} d.w. respectively.

- The higher bioaccumulative affinity of *P. fucoides* was also expressed in the form of the interspecific diversity factors determined for these two radioisotopes. The $ISDF_{P/F}$ values of ^{113}Sn and ^{137}Cs were also noticeably higher. In the case of five radionuclides – ^{51}Cr, ^{54}Mn, ^{57}Co, ^{60}Co and ^{241}Am – the $ISDF_{P/F}$ values were all close to 1.0, suggesting that the abilities of macroalgae to bioaccumulate these elements are comparable.

- Based on the results of this study, *P. fucoides* can be recommended as a bioindicator of radioactive environmental pollution (caused by accidental and controlled releases), especially in view of its widespread distribution, large biomass and long growing season.

- The uptake of all the radionuclides by *F. lumbricalis* during the exposure experiment proceeded in a very similar way, especially in the first stage, when uptake was intensive, and in the second stage, when removal rates of unbound cations from the free space were high.
- ^{137}Cs displayed a different behaviour, its concentration in the algae increasing over time mainly because of its large ion radius; this is a factor that could be responsible for the stronger mechanical and chemical bonding of Cs^+ and that could hamper the movement of ions in both directions.

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