

Seasonal changes of ^{137}Cs in benthic plants from the southern Baltic Sea

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Abstract ^{137}Cs activity concentrations were determined in samples of macrophytes *Polysiphonia fucooides* (red algae) and *Zostera marina* (vascular plant) collected during the entire vegetation season in the Gulf of Gdańsk in the southern Baltic Sea. The measurements showed considerable seasonality of ^{137}Cs activity in both species; an increase of cesium concentrations was observed from spring to autumn with maximal levels $49.1 \pm 1.4 \text{ Bq kg}_{\text{d.w.}}^{-1}$ (*P. fucooides*) and $14.5 \pm 1.0 \text{ Bq kg}_{\text{d.w.}}^{-1}$ (*Z. marina*) in late autumn. ^{137}Cs concentrations observed in a given season are the result of a number of processes, the intensity of which can differ depending on external environmental conditions. The effects of these processes can differ and their directions can frequently be opposite to one another. The examined macrophytobenthic plant species could serve as bioindicators of radionuclide pollution for monitoring purposes on condition that the samples of plants are taken within a strictly defined period of the year to give comparable results and to supply realistic information about pollution levels.

Keywords Radioactive cesium · Marine phytobenthos · Seasonal bioaccumulation · Baltic Sea

Introduction

The use of benthic plants as bio-indicators for monitoring surveys of environmental pollutants is widely

recommended by many authors [1–5]. Macrophytobenthic organisms are also investigated as potential reference organisms to be used in models for assessing the radiological consequences of radioactive releases, arising both from routine operation of nuclear facilities [6] and from accidental situations [7]. The main reason for such applications is their well known ability to accumulate trace elements like heavy metals and radionuclides [5, 8–12]. The main advantage of using seaweeds and vascular plants as indicators of radionuclides is their way of exchanging elements with the environment [13]. In the case of macroalgae the exchange is accomplished through the entire surface of the thalli, so-called foliar uptake, while in the case of vascular plants it is accomplished mainly via the root system. Additionally, in the case of benthic plants, the response of a given bioindicator occurs relatively quickly after the pollution event [14]. In contrast to benthic fauna or fish, which accumulate hazardous substances as long as they live, the level of radionuclides in benthic plants can reflect changes of radionuclide concentrations observed in seawater during a single year of vegetation.

The rationale for using seaweeds as indicators of radionuclide contamination also includes the fact that contaminant concentrations in environmental solutions (e.g. seawater) are often close to the analytical detection limits and often vary with time. Seaweeds, accumulating metals from solution, can integrate short-term temporal fluctuations in concentration [13].

Bioaccumulative processes are determined by external and internal factors [13]. 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 [13].

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The life history of a plant, which may be defined as a continuous interaction between the plant and its biotic or abiotic environment, is a significant factor with regards to the bioaccumulation process as the life cycle also depends on both the internal and external factors. A more widespread mechanism of the life cycle is that of following circa-annual rhythms as a means of timing growth [13], but seaweeds can also (or instead) respond to environmental cues. The most important and obvious cues are temperature and light and the switch from vegetative growth to reproduction (which in most seaweeds involves very little growth) often depends on them [13]. Some plants are more sensitive to temperature and others to light conditions.

Besides the development phase and age of the studied plant, intensity of vital processes is another parameter directly related to seasonality. Changing thermal conditions, light conditions and concentrations of various substances (e.g. nutrients) all affect the bioaccumulation ability [13]. The seasonal variability of trace metal concentrations has been observed in *Zostera marina* and *Ulva rigida* [11] and, in the latter case the concentrations of Cu and Cd seemed at least partially to reflect growth dynamics [3, 15]. Low concentrations were found in spring, when the plant biomass reached its maximum. The increase in the biomass led to a decrease in the metal content due to dilution [16]. The diluting effect on radionuclide concentrations caused by growth could be an even more significant parameter than the biological loss of radionuclides [17].

The complexity of bioaccumulation in benthic plants and the multitude of factors affecting this process are the main causes of the wide range of radionuclide concentrations observed in the plants and, as a consequence, in concentration coefficients determined by various authors regarding specific radionuclides or plant species [6]. Therefore, the results obtained have to be interpreted with considerable caution and it must be noted that studies concerning the effects of particular factors on bioaccumulation process are in great demand, especially in an environmental context.

This paper presents the results of a study of radionuclide bioaccumulation in marine plants during an entire vegetation period, the aim of which is to examine seasonal changes in the activity of ^{137}Cs in selected plant species and to determine to what degree the biological activity and developmental phase influence the bioaccumulation process. The selection of radioactive ^{137}Cs was prompted by the fact that it is a nuclide of solely anthropogenic origin and its activities in marine matrices in the Baltic Sea are much higher than those of other artificial isotopes [18–22]; its half-life is relatively long at 30.05 years [23]. The majority of ^{137}Cs in the Baltic Sea was deposited as a result of the accident at the Chernobyl nuclear power plant in

1986. It is estimated that the overall load of ^{137}Cs that reached the sea from this source was 4.7 TBq, comprising 82% of the total amount of ^{137}Cs that has accumulated in the Baltic seawater [19, 24]. For this reason, the Baltic Sea is still considered the most polluted marine area in terms of ^{137}Cs [25].

Materials and methods

Sampling

The study was conducted on *Polysiphonia fucoides*, a species of the red algae division as well as *Z. marina*, representing vascular plants. The selected species are characteristic of the flora of the southern Baltic Sea and can be used as potential bioindicators of the marine environment pollution.

Benthic plants were collected in the Gulf of Gdańsk in the southern Baltic Sea, at one location called Kępa Redłowska at definite depth of 2–3 m (Fig. 1). Sampling was conducted by a scuba diver who collected the plants from the seafloor. At each depth the metal frame of 0.5×0.5 m was deployed three times and the ensuing subsamples were integrated, the results were then related to the stretch of 0.75 m^2 ($3 \times 0.25 \text{ m}^2$). Integrated samples immersed in plastic bags were transported to the laboratory, where further preparation was conducted. The sampling was performed to follow the seasonal cycle of plant development in November 2008, in January, in February, in March, in April, in May and in July 2009; the macrophyte community at this depth was comprised mainly of *P. fucoides* and *Z. marina*.

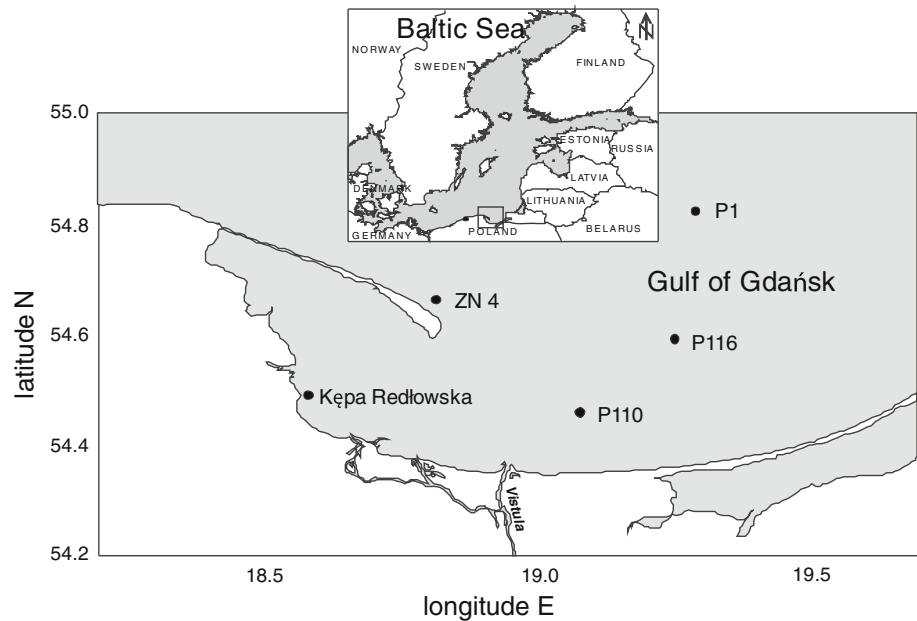
Seawater samples were taken during the monitoring cruises onboard R/V 'Baltica' in June 2008 and 2009. Sampling was carried out at stations located in the Gulf of Gdańsk area (Fig. 1) from the surface layer, bottom (2 m above the seabed) and down the water profile (at ZN4 and P1 stations) with the rosette sampler (General Oceanics, model 1015). Salinity and temperature were measured simultaneously along the sampling profile using sonda CTD MARK III (Neil Brown Instrument System Inc.).

Temperature and salinity data from 2006 to 2007 are the results of systematic monitoring in the area of Kępa Redłowska. They were measured with Valeport 602 sonda CTD (in 2006) and with Idronaut 304 sonda CTD (in 2007).

Sample preparation

The plant material was analyzed taxonomically, individual species occurring in the samples were identified and biomass of each species was determined gravimetrically;

Fig. 1 Sampling stations for macrophytobenthic plants and for seawater located in the southern Baltic Sea



taxonomic analyses and biomass determination were carried out according to the HELCOM COMBINE guidelines [26]. For the radionuclide activity determination, the selected plant species were dried, ashed at 450 °C and homogenized. Ashed samples were transferred in counting boxes of the appropriate shape and size.

^{137}Cs activity concentration in seawater samples was measured by two methods. In 2008, the radiochemical method was applied and preparation of samples was following a procedure described in Zalewska and Lipska [22]. Non-filtered seawater samples of water of approx. 30 dm³ were acidified to pH 1–2 with hydrochloric acid and 20 mg of a natural carrier of cesium was added. After equilibration, 10 g of ammonium phosphomolybdate (AMP) was added and the content was stirred for 20 min. After 24 h the liquid phase was decanted and separated AMP was dissolved in NaOH. Then cesium was re-precipitated on 1 g of AMP, re-dissolved in NaOH and purified in a column filled with Bio-Rex 40 cation-exchange resin. Following separation of the accompanying ions (NH_4^+ , Na^+ , K^+ and Rb^+), cesium was eluted with 3 M hydrochloric acid. The obtained eluate was evaporated to dryness, digested and dissolved in dilute hydrochloric acid. Finally cesium was precipitated as chloroplatinate and its recovery was estimated gravimetrically. β -Radiation of samples was measured using Low-Level Beta Counter FHT 7700T (ESM Eberline) with the background count rate of 0.01 counts s⁻¹ and the minimum detectable activity of 3 mBq per sample. The measurement time for beta radiation analysis was 7,200 s.

In 2009, ^{137}Cs activity was determined by gamma spectrometry method. As an initial step 20 mg Cs⁺ was added to each acidified seawater sample as a carrier. Cesium was absorbed on 10 g of AMP during 20 min stirring. The

separated (by decantation and filtration) AMP was dried and measured using gamma spectrometry system.

Analysis and measurement

^{137}Cs content in samples of biological material and in seawater samples taken in 2009 was measured by gamma spectrometry method using an HPGe detector with a relative efficiency of 18% and a resolution of 1.8 keV for peak of 1332 keV of ^{60}Co . The detector was coupled with an 8192-channel computer analyser and GENIE 2000 software. The measurement time for gamma spectrometry was 80,000 s.

The reference solution: “Standard solution of ^{137}Cs , code SRCs-23, IM/25/O/00”, activity 4.2 kBq, total uncertainty $\pm 1.5\%$, referenced date 9.11.2000, produced at the IBJ-Swierk k/Otwocka Poland, was used for preparing reference samples for the equipment calibration. Different measurements geometries were applied in isotope determination of seawater and plant samples. The respective reference samples were prepared in identical geometries as in the relevant matrix.

The reliability and accuracy of the applied method was verified by participation in the inter-comparison exercises organized by IAEA-MEL Monaco (IAEA-414, Irish and North Sea Fish) [27]. Repeated analysis gave a value equal to $5.06 \pm 0.64 \text{ Bq kg}_{\text{d.w.}}^{-1}$ while the estimated target value was equal to $5.18 \pm 0.10 \text{ Bq kg}_{\text{d.w.}}^{-1}$.

Results and discussion

Changes in the ^{137}Cs concentration of *P. fucoides* and *Z. marina*, collected systematically at depth of 2–3 m

between July 2008 and October 2009, are shown in Fig. 2. The changes show similar character although a significant difference in the level of isotope accumulation can be observed, with much higher ^{137}Cs concentrations determined in *P. fucoides* across the entire study period. Differences in ^{137}Cs activity in relation to time and sampling location were also observed in the case of red algae by Nonova and Strezov [28]. Comparatively lower concentrations of ^{137}Cs , observed in vascular plant *Z. marina* (Fig. 2), when compared with *P. fucoides*, may be linked to the method by which elements are exchanged between these benthic plants and their environment. The foliar uptake which is characteristic of algae makes bioaccumulation of cesium ions more effective taking into account the exchange surface area and additionally the water surrounding the plant provides a continuous source of the radionuclide. Vascular plants, on the other hand, can exchange elements with their environment both through root systems embedded in sand and through leaves. The contribution of these two exchange pathways in the general uptake depends strongly on the element availability, i.e. concentration. There is evidence that leaf tissues have higher nutrient uptake affinities than root tissues at low-element availability [29]. However, in general the pore water shows higher nutrients concentration, which could be also the case of cesium and for this reason it could be expected that the main way of this isotope exchange is through the root system.

An increase in the content of radioactive cesium was observed in both plant species from July until the end of the year, more intensive in the case of *P. fucoides*. The maximal concentrations measured in *P. fucoides* and *Z. marina*

were $49.1 \pm 1.4 \text{ Bq kg}_{\text{d.w.}}^{-1}$ and $14.5 \pm 1.0 \text{ Bq kg}_{\text{d.w.}}^{-1}$ in November 2008 and January 2009 respectively. Simultaneously, seawater temperature was decreasing and found its minimal level in February—Fig. 3. The data presented here for 2006 and 2007 show seasonal pattern of seawater temperature and salinity that is common for the investigated area. The subsequent warming up of the marine environment was not accompanied by an increase in ^{137}Cs content in the plants; on the contrary, its concentrations decreased further. The minimum ^{137}Cs concentration in *Z. marina* ($1.7 \pm 0.3 \text{ Bq kg}_{\text{d.w.}}^{-1}$) was measured in March (2009). In the case of *P. fucoides* the minimum concentration ($12.5 \pm 1.0 \text{ Bq kg}_{\text{d.w.}}^{-1}$) was found in April (2009). In May 2009 cesium activity increased again to 20.8 ± 2.5 and $4.5 \pm 1.9 \text{ Bq kg}_{\text{d.w.}}^{-1}$ in *P. fucoides* and *Z. marina* respectively. ^{137}Cs activity increased in the following months in *P. fucoides* and its activity level determined in July 2009 ($23.8 \pm 3.9 \text{ Bq kg}_{\text{d.w.}}^{-1}$) did not differ from that in July 2008 ($24.2 \pm 1.4 \text{ Bq kg}_{\text{d.w.}}^{-1}$). The similar patterns of seasonal changes observed in both plants suggests that bioaccumulation processes are mainly determined by the physiological activity connected directly with environmental factors. Similar observation have been made for the heavy metals Pb, Cu, Zn and Fe in *Enteromorpha linza*, concentrations of which followed the same pattern with the minimum observed in April and maximum values observed in October and January [30].

Radionuclide concentrations observed in the studied benthic plants were related solely to the plant morphology and physiology, those having close relation to environmental conditions like temperature and sunlight, and not to the changes of ^{137}Cs in seawater. Spatial distribution of

Fig. 2 ^{137}Cs activity concentration in *P. fucoides* and *Z. marina* at different vegetations periods, at the level of 2–3 m depth (errors bars are related to counting errors— 1σ)

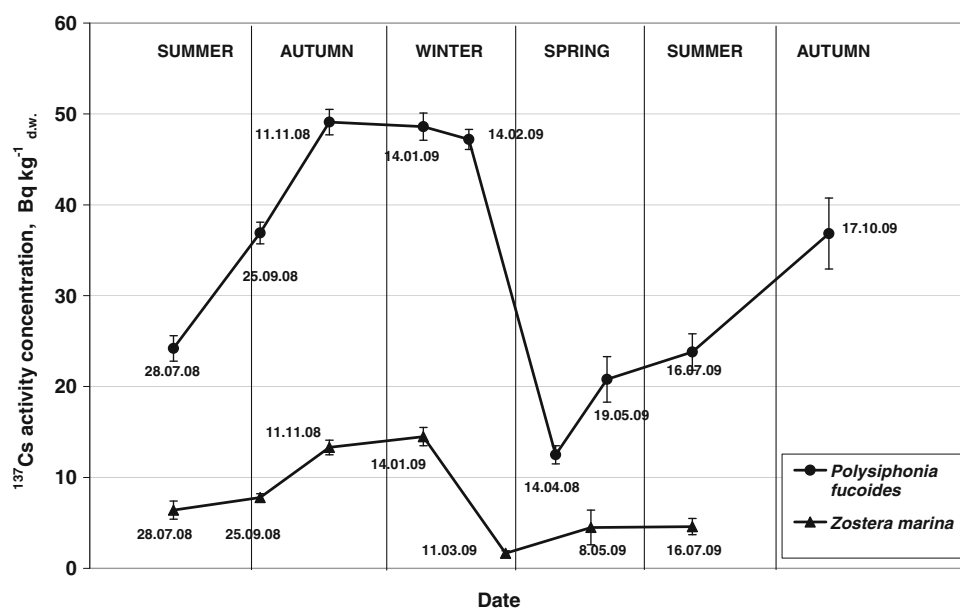
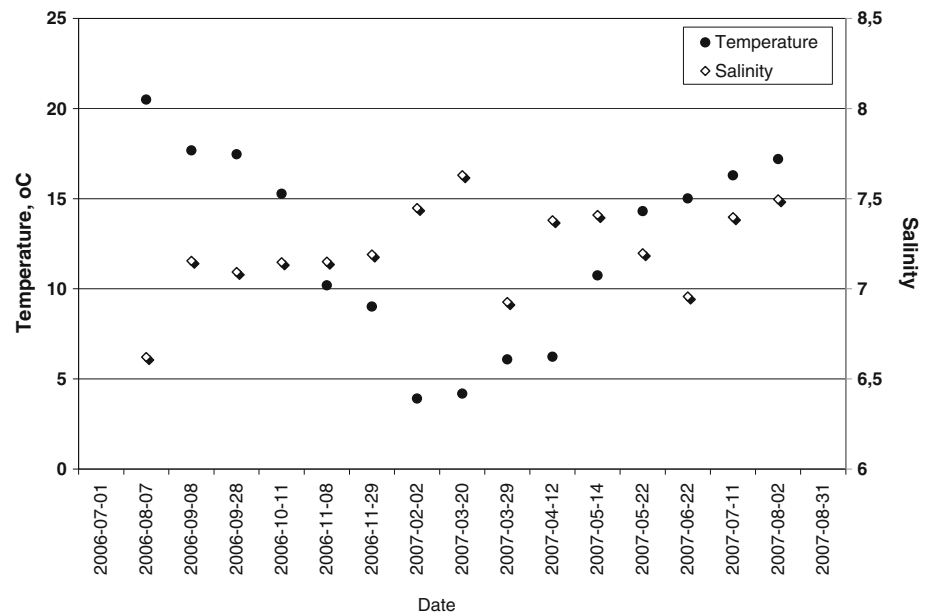


Fig. 3 Seasonal changes of salinity and temperature of surface water, at Kępa Redłowska (Gulf of Gdańsk), in period 2006–2007



^{137}Cs in the Gulf of Gdańsk, examined at four locations showed a homogenous pattern of radionuclide concentrations (Fig. 4). No significant differences were noted between 2008 and 2009, either. Monitoring measurements (in 2006 and 2007) of seawater temperature and salinity in the area close to the macrophyte sampling region showed strong seasonality in the surface seawater temperature changes (Fig. 3). Simultaneously, temperature remained quite uniform in the water column on given dates (Fig. 5). Salinity remained stable along the vertical profile (Fig. 6) and showed minor seasonal response (Fig. 3). The uniform vertical distribution of temperature and salinity provide evidence for the strong mixing through waves and advection, in the examined area and hence imply that ^{137}Cs concentrations will be uniform down the water column profile.

The life history of the *Polysiphonia* genus is quite a complicated process [31, 32]. In the vegetatively favourable period, during the spring and summer, an intense increase in biomass of *Polysiphonia* is being observed. In the winter time, the biomass is considerably reduced and *Polysiphonia* creates spore forms called tetraspore and delivered by tetrasporophyte. Small male and female plants, called early gametophyte, are formed from tetraspore. These forms start to grow during the spring to create gametophyte and tetrasporophyte, which are recognized as young forms of benthic plants. They mature during the summer and autumn [13]. From April to September, several phases of reproductive cycle can generally be observed but, as expected, the young plants dominate at the beginning of this cycle. It is a well known fact that the lowest concentrations of metals usually occur in the growing part

of a plant, and that higher, more constant values are recorded in the older tissue [11, 33]. It has been explained in the case of *Fucus vesiculosus* by relatively slow accumulation of metals in the young parts of plants, as well as by synthesis of a greater number of binding sites in older tissue [34]. It has also been shown in *Elodea canadensis* for example that element (^{241}Am) distribution varies depending on the age of the leaf blades and the state of the cells [33].

The curves which characterise seasonal changes of ^{137}Cs in *P. fucoides* and *Z. marina* tissues may indicate a relationship between external environmental factors, such temperature and light conditions, and the life cycle of the plants. In summer, under optimal growth conditions, intensive plant growth and fruiting result in an increase of biomass. These growth processes are directly related to metabolic processes: photosynthesis and respiration. Simultaneously, as the intensification of metabolic processes results in enhanced metal uptake, the augmentation of biomass causes a diluting effect. Therefore, the final cesium concentrations in the plants in summer can be seen to be the result of these two opposing processes (Fig. 7). In autumn, as in summer, there are two opposite tendencies which affect cesium content in the plant tissues. Metabolic processes are still rather intensive and enhance bioaccumulation but, on the other hand, the decline in temperature and sunlight conditions, acts against the metabolic activity and slows it down. This slowing down causes a decrease in the rate of bioaccumulation and is also seen in the biomass decline, although the latter effect can be considered as a concentration process (opposite to the diluting process connected with the increment of biomass). The final effect,

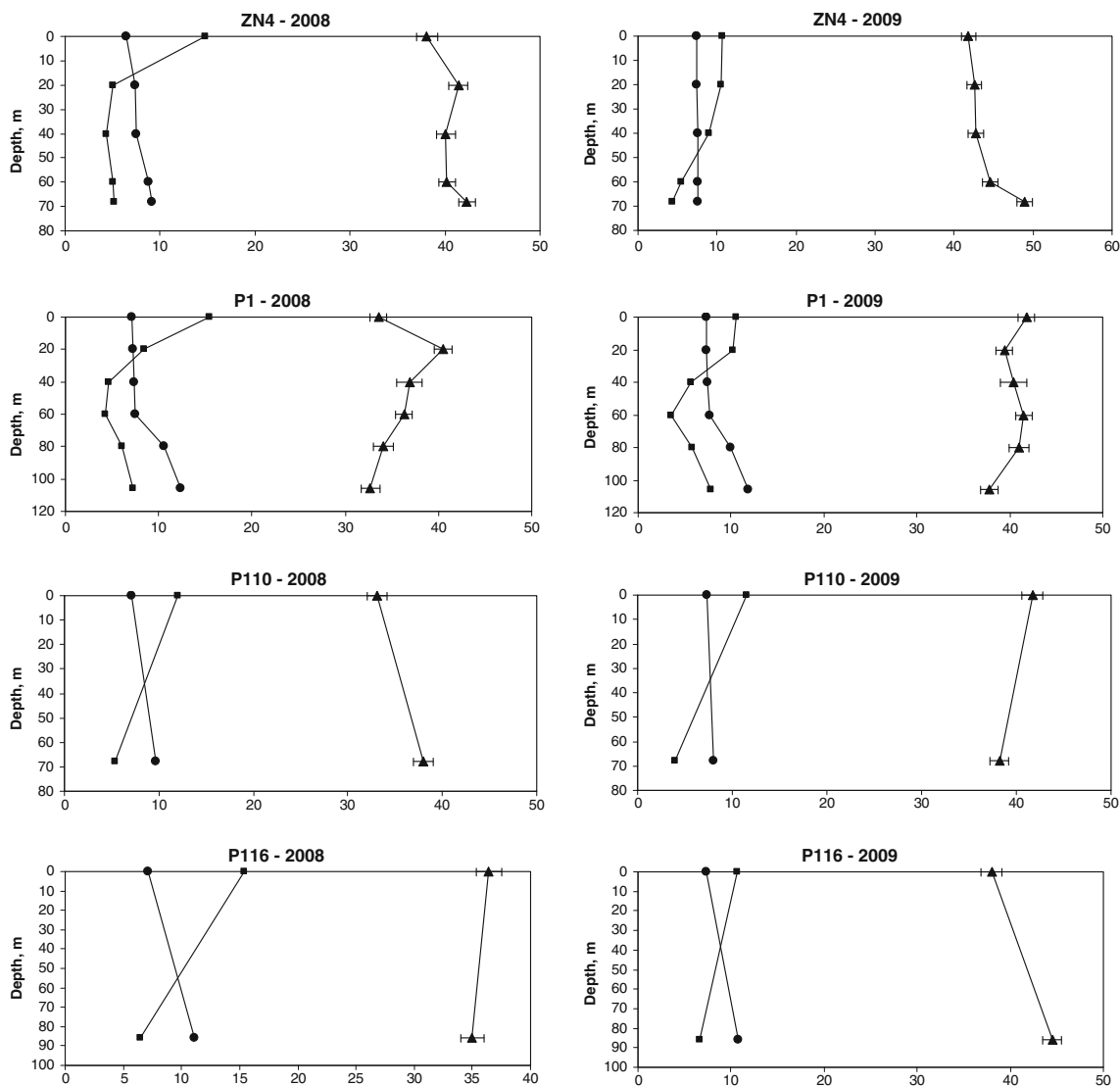


Fig. 4 Activity concentrations of ^{137}Cs , Bq m^{-3} (filled triangle) in seawater, salinity (filled circle) and temperature, $^{\circ}\text{C}$ (filled square) at four stations, in the Gulf of Gdansk, in 2008 and 2009 (errors bars are related to counting errors— 1σ)

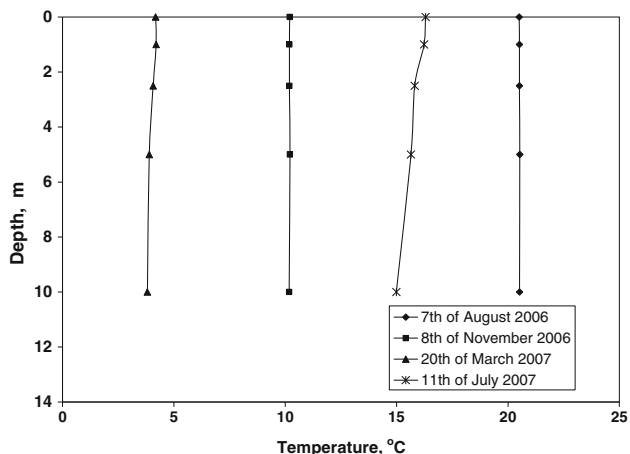


Fig. 5 Vertical distribution of temperature, at Kępa Redłowska, Gulf of Gdansk

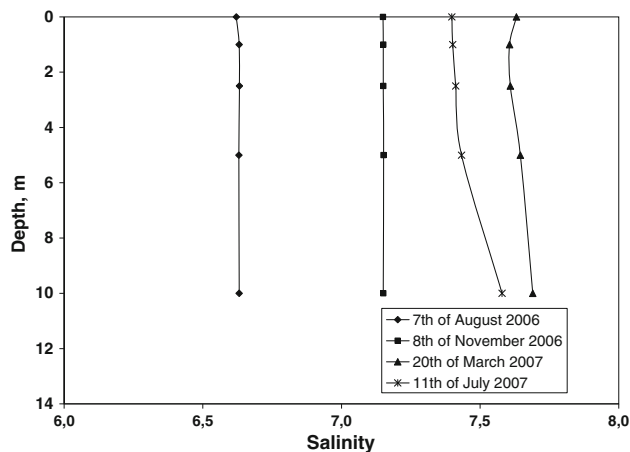


Fig. 6 Vertical distribution of salinity, at Kępa Redłowska, Gulf of Gdansk

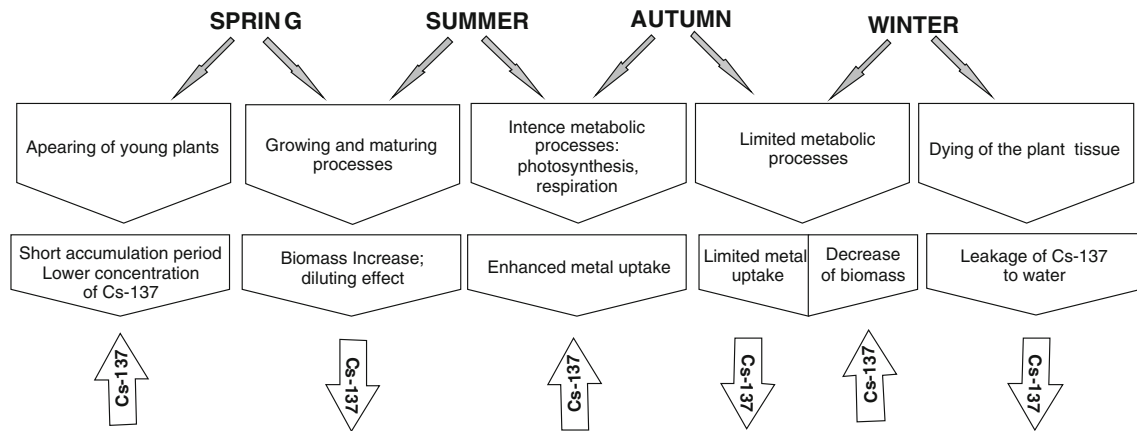


Fig. 7 Schematic presentation of processes at different vegetations periods and their influence on the ^{137}Cs level in plants

resulting from these opposed activities, is the continued increase of ^{137}Cs concentration in plant tissue in autumn. In winter, when the loss of biomass is very intensive due to plant fading, cesium ions can be released/resuspended in the water and hence their concentration in plant cells declines. This process generates an additional source of ^{137}Cs in the marine environment. Young plants that appear at the turn of the year and in spring contain low amounts of ^{137}Cs mainly because of the short time of bioaccumulation.

Conclusions

1. A strong seasonality in ^{137}Cs bioaccumulation in macrophyte species *P. fucooides* and *Z. marina* was detected. ^{137}Cs concentrations increased in both plants from spring to autumn, reaching their maximum values in late autumn.
2. The intensity of ^{37}Cs bioaccumulation varied depending on the physiological activity of the plants, i.e. intensity of synthesis and respiration processes which in turn were affected by environmental conditions. The final ^{37}Cs concentration in the plant tissue was a result of these processes which can have opposed influence.
3. *Z. marina*, a vascular plant showed lower ^{137}Cs concentrations as compared to *P. fucooides* indicating worse efficiency of the bioaccumulation process in vascular plant. However, the pattern of seasonal changes observed in both plants in the entire investigation period was very similar indicating that bioaccumulation processes are mainly determined by the physiological activity connected directly with environmental factors.
4. *P. fucooides* can be recommended as a good bioindicator for monitoring of ^{37}Cs pollution on account of the level of isotope concentrations during the study period, the available biomass along the depth profile

and the occurrence throughout the entire vegetation season. At the same time, however, the application of plant bioindicators for monitoring purposes has to be done with particular caution as the time of year exerts a strong influence on the analysed element concentrations.

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