

**Utilisation of macroalgae
from the Sopot beach
(Baltic Sea)***

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KEYWORDS

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ANNA FILIPKOWSKA¹
LUDWIK LUBECKI¹
MAŁGORZATA SZYMCZAK-ŻYŁA¹
GRAŻYNA KOWALEWSKA^{1,*}
RADOSŁAW ŻBIKOWSKI²
PIOTR SZEFER²

¹ Marine Pollution Laboratory,
Institute of Oceanology,
Polish Academy of Sciences,
Powstańców Warszawy 55, PL-81-712 Sopot, Poland;

e-mail: Kowalewska@iopan.gda.pl

*corresponding author

² Department of Food Sciences,
Medical University of Gdańsk,
Halleria 107, PL-80-416 Gdańsk, Poland

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Abstract

The aim of this work was to explore the possibilities of utilising the macroalgae accumulating on the Sopot beach, a part of the Gulf of Gdańsk (Baltic Sea). During this work (2004–2006) a range of activities were undertaken: monitoring

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the occurrence of macroalgae, collection of plant material, taxonomic identification, laboratory analyses of contamination (metals – Cd, Pb, Ni, Zn, Cu, Cr, Hg; radionuclides – ^{137}Cs , ^{40}K ; organic contaminants – polychlorinated biphenyls, polycyclic aromatic hydrocarbons), the stage of algal degradation and studying possible uses of the plant material collected on the beach. The most suitable way of utilising the plant material seems to be as a biofertiliser. Laboratory growth tests were carried out to assess this possible use; they were successful.

1. Introduction

The EU directive forbidding biodegradable waste going to landfill sites was passed some time ago (1999/31/EC). Another EU Directive concerning the quality of bathing water (2006/7/EC) obliges the authorities responsible for beach management to organise the removal of macroalgae accumulating on the shore. The implementation of this second Directive has started in Poland. According to these two Directives, the macroalgae collected on the beach may not be transferred to landfills but should be utilised. The Sopot beach is located on the southern Baltic Sea, on the coast of the Gulf of Gdańsk. Boasting fine white sand and descending gently to the sea, the beach adjoins the Sopot town centre. This location of the beach arises from the history of this place, which for a long time was just a fishing village, but later became a place of recreation, at first for the townsfolk of nearby Gdańsk, and later for people from other parts of the country and elsewhere. Now it has become a seaside resort well known both in Poland and abroad. Some four kilometres long, the beach is divided into two parts by a timber pier (Figure 1), the longest structure of its type in Europe. The beach and the adjacent streets cater for the commercial and cultural needs of the throngs of holidaymakers, especially in the summer. The Sopot Municipality is responsible for the management of the beach. Because of its location, all matters connected with this area are treated as priority problems by the authorities. One of the most pressing problems at present, not only for Sopot but also for many other coastal zones of the Baltic, not to mention other marine areas in the world, is eutrophication. At Sopot this is manifested, among other things, by intensive blooms of unicellular algae and the accumulation of macroalgae at the waterline at certain periods and locations in the summer (Figure 2). Up till now, the macroalgae have been removed from the beach by the municipal services and disposed of on land allocated for that purpose on the town's outskirts. In the future, they will have to be transported to the municipal refuse tip, which is situated a long way from the beach.

The aim of this work was to look into the possibilities of putting the macroalgae accumulating on the Sopot beach to some good use. A range of activities, such as monitoring the occurrence of macroalgae, collection



photo Jerzy Czarkowski

Figure 1. Bird's eye view of the Sopot beach



photo Anna Filipkowska

Figure 2. Macroalgae accumulating at the Sopot beach (site No. 1, 24.06.2004)

of plant material, taxonomic identification, laboratory analyses of algal contamination, the stage of algal decomposition using high performance liquid chromatography (HPLC), and studying possible uses of the plant material collected on the beach, were undertaken in 2004–2006.

2. Experimental

2.1. Sample collection

The Sopot beach is located on the western side of the Gulf of Gdańsk, which receives the waters of the River Wisła (Vistula), the largest Polish river flowing into the Gulf, along with those of some other, smaller watercourses.

The locations of the monitoring and sampling stations are shown on the map (Figure 3). Every June, July and August in each year of the project (2004, 2005 and 2006) at sites 1–5, and additionally at sites A–E, 2I, 2II and 3I when macroalgae abundance was at a maximum, plant material was collected on the beach. This was then sent to the laboratory for taxonomic identification and the following analyses: degree of degradation, content of metals, radionuclides and organic contaminants. Altogether, 29 samples were collected, which were later divided into sub-samples, treated in accordance with the demands of each separate analytical procedure. Additionally, in June 2005, grass from the park adjoining the shore was collected as comparative material.

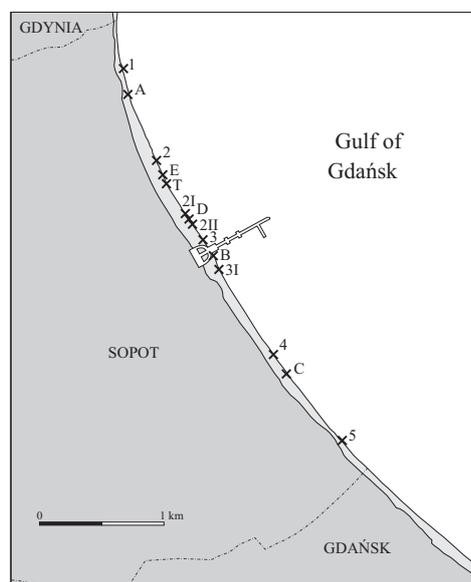


Figure 3. Sampling sites

2.2. Macroalgae species

After sampling, the fresh macroalgae and vascular plants collected were assessed qualitatively. The macrophytes were identified to species or genus level. Dominant species were identified and the taxon abundance was estimated on a three-point scale – sporadic occurrence, common, dominant.

2.3. Degree of degradation

The degree of degradation of the plant material was estimated from the pigment composition in the macroalgae samples as a percentage of chlorophyll *a* (Chl *a*) in the sum of chloropigments *a* (sum of chlorophyll *a* and its derivatives = \sum Chl_{ns} *a*). The following pigments were determined in all samples: chlorophyll *a*, chlorophyllide *a*, chlorophyll *a* allomer and epimer, phaeophytin *a* and its epimer, phaeophorbides *a*, pyropheophytin *a*, and the sum of steryl chlorins. Pigment extraction and macroalgae sample analysis were carried out according to the procedure for analysing pigments in marine sediment samples described by Kowalewska (2005). In brief, the plant material was sonication-extracted with acetone (2–3 min). The extraction was repeated (no more than three times) until the visual disappearance of colour from the supernatant. The combined acetone fractions were re-extracted in the acetone extract:benzene:water system (15:1:10 v/v/v). The benzene layer was transferred to a glass vial and evaporated to dryness in a stream of argon. The extracted material was dried and weighed. The pigment extract was then dissolved in acetone and injected into a chromatograph system equipped with a diode-array detector (DAD, Knauer, Germany) (mobile phase – acetone:water gradient system; elution rate – 1 ml min⁻¹). The pigment content was determined on the basis of the chromatogram and spectrophotometric absorption at 660 nm and calculated per weight of dry plant material.

2.4. Contamination

Metals

After delivery to the laboratory, the plant material was stored at –20°C. Prior to analysis the samples were thawed out at room temperature and rinsed in distilled water. Then the macroalgae were dried at 60°C to constant weight and crushed to powder with a pestle and mortar. Three replicate analytical samples were prepared from each sample. Approximately 1.0 g (weight accurate to four significant figures) of dried algal samples was digested in a Microwave Digestion System (MLS 1200 MEGA) with analytically ultrapure HNO₃. All the glassware was cleaned before use by soaking in 1 mol dm⁻³ HNO₃ for 72 h and rinsing with Milli-Q water. The

levels of Cd, Cu, Ni, Pb, Zn and Cr were determined by flame atomic absorption spectroscopy (FAAS) in a Philips PU 9100X spectrometer. The quality assurance of the analytical measurements was checked against an analysis of seaweed reference material (*BCR 279 – U. lactuca*). The agreement between the analytical results for the reference material and their certified values was satisfactory, i.e. the recoveries and the relative standard deviations (RSD) were $> 92\%$ and $< 10\%$ respectively. To check for contamination, blanks were analysed after every five samples using this procedure (Żbikowski et al. 2007). Hg was determined using atomic absorption spectrometry with cold vapour generation (CV-AAS). Samples were decomposed according to the mineralisation procedure described by Ciesielski et al. (2006). The quality assurance was checked using the same reference material as above (*BCR 279 – U. lactuca*) – the recovery was satisfactory (110%).

Radionuclides

After collection the plant material was dried at 105°C . The radionuclides ^{137}Cs and ^{40}K were determined by gamma spectrometry in 0.5 l Marinelli vessels according to the Official Method 973.67 (©2000 AOAC International), with the difference that an HP Ge detector was used instead of an NaI (Tl) instrument. Samples of IAEA-152 (^{137}Cs) and RGK-1 ^{40}K were used as reference materials (Knapinska-Skiba et al. 2001). Overall, the relative standard deviation was c. 8.5% for ^{137}Cs and 3% for ^{40}K .

Organic contaminants – polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs)

The relevant PCB and PAH extractions and clean-up procedures were used according to those described earlier (Kowalewska et al. 2003, Filipkowska et al. 2005). A frozen macroalgae sample was allowed to thaw and was then sonication-extracted five times with acetonitrile (15 min.). The acetonitrile fractions were combined and re-extracted in the acetonitrile extract : water : benzene system (10:10:1 v/v/v). The benzene extract was evaporated in a rotavapor and cleaned up by thin layer chromatography (TLC). The chromatogram was developed in the acetone : hexane system (20:35 v/v).

PCBs were extracted from the silica gel with acetonitrile and purified on three different types of micro-columns. The extract was initially cleaned up on an RP-18 column, then on a column with copper, and finally on a column with Florisil. The procedure for the samples collected in 2005 was slightly modified in that a glass column containing silica gel replaced the TLC plates. The sample was analysed in a gas chromatograph (GC 6000, Vega Series 2,

Carlo Erba Instruments) with an electron capture detector (ECD 80/800, Fisons Instruments). The GC system was fitted with a fused silica capillary column with a 5% phenyl-substituted dimethylpolysiloxane phase, 0.25 mm ID \times 60 m, 0.25 μ m film thickness (CP-Sil 8 CB Low Bleed/MS, Varian or HP-5, Hewlett Packard). Peak identification and determination of PCB concentrations were based on a comparison of retention times and individual peak areas in the sample chromatogram with the respective peak retention times and standard curves. The relative standard deviation for the sum of 12 PCBs was 5% ($n=4$). The following 12 PCBs were quantified: PCB 18, 28, 31, 44, 52, 101, 118, 138, 149, 153, 180, 194.

PAHs on the silica gel were detected under UV light, extracted with acetonitrile and purified of sulphur. The sample dissolved in isooctane was injected into a gas chromatographic system equipped with a mass spectrometer detector (Varian GC/MS, Saturn 2100T). A fused silica capillary column with a 5% phenyl-substituted dimethylpolysiloxane phase, 0.25 mm ID \times 60 m, 0.25 μ m film thickness (Varian, CP-Sil 8 CB Low Bleed/MS or VF-5ms) was used. The relative standard deviation for the sum of 15 PAHs was 7% ($n=4$). The following 15 PAHs were determined: acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, dibenzo(a,h)anthracene, benzo(ghi)perylene.

2.5. Growth test

Small (\sim 5–8 cm high) tomato plants were planted in three sets of containers (7 plants in each of the sets). The containers were filled with the same gardening soil of high humus content. Additionally, the first set of containers was supplemented with a layer of macroalgae dried at room temperature. During the nine-week long experiment the three sets were watered with tap water. The second set (without macroalgae) was watered with an aqueous solution of a commercial fertiliser produced specifically for tomato cultivation. The third set of plants, the control, neither contained macroalgae in the soil nor received liquid fertiliser. Plant growth was assessed by periodically measuring the height of each tomato plant (in cm).

3. Results and discussion

3.1. Macroalgae species and degree of degradation

The plant material collected on the beach during the three summer seasons contained mainly macroalgae, with a small proportion of sea grass *Zostera marina* and unicellular algae. The macroalgae were covered with

Table 1. Macroalgae species identified on the Sopot beach

Green algae	Brown algae	Red algae
<i>Cladophora</i> spp.	<i>Pilayella littoralis</i>	<i>Ceramium</i> spp.
<i>Enteromorpha</i> spp.	<i>Ectocarpus</i> spp.	<i>Polysiphonia</i> spp.
<i>Ulotrix</i> sp.		<i>Phyllophora brodiaei</i>
<i>Stigeoclonium</i> spp.		

a species-rich epiphytic flora and fauna. Three groups of macroalgae were identified – green, brown and red algae, with the dominant species being *Cladophora* spp., *Enteromorpha* spp., *Pilayella littoralis* and *Ceramium* spp. (Table 1). The majority were green (22–75%) and red algae (17–71%); only a small percentage were brown algae (0–50%) (Figure 4). Macroalgae have already been used as indices of the ecological and trophic state of the marine environment (e.g. Arévalo et al. 2007, Ballesteros et al. 2007).

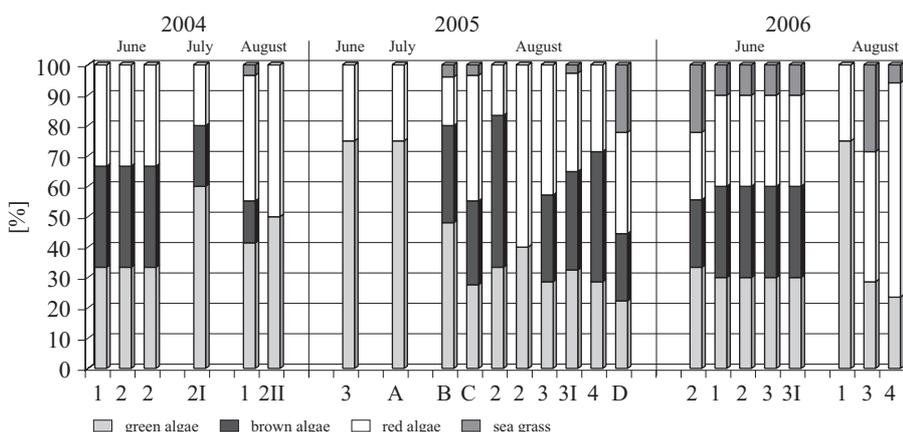
**Figure 4.** Macroalgae and sea grass from the Sopot beach – composition of the biomass

Figure 5a shows the total chlorophyll *a* content in the plant material. There was considerable variation in Chl *a*, from ~ 400 nmol g^{-1} to 3800 nmol g^{-1} , depending on the composition of the macroalgae and the freshness of the plant material. The degree of degradation of this material is best reflected by the percentage of chlorophyll *a* in the sum of chloropigments *a* (Figure 5b): the fresher the material, the more chlorophyll *a* it contains. The state of degradation of the plant material varied from fresh to decomposed, as can be deduced from the chlorophyll *a* content, which ranged from 85% to 1.5% of the sum of all chloropigments *a*.

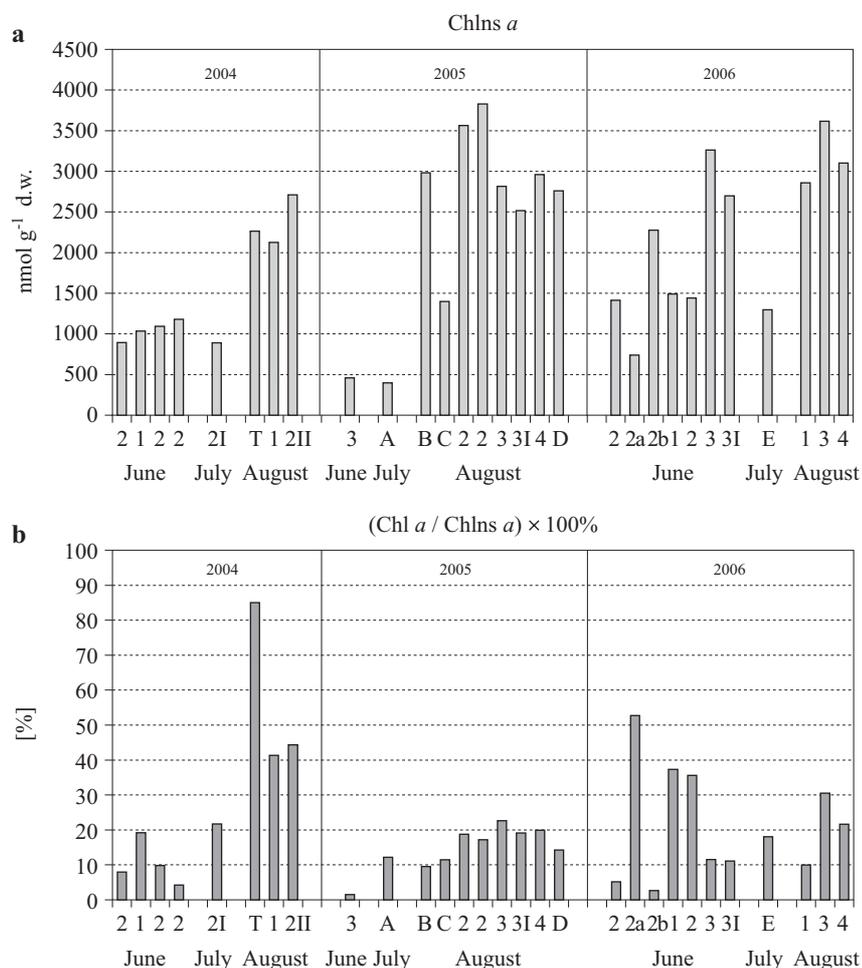


Figure 5. Pigment content in macroalgae from the Sopot beach: a) content of chlorophylls *a*; b) percentage of chlorophyll *a* in the sum of chlorophylls *a*

3.2. Contamination

Table 2 presents the contents of all the contaminants determined (metals: Hg, Cd, Pb, Ni, Zn, Cu, Cr; radionuclides: ¹³⁷Cs, ⁴⁰K; sum of 12 PCBs; sum of 15 PAHs) in the collected material. For comparison, the samples of grass collected from the park near the beach were also analysed.

There were only trace amounts of mercury in the macroalgae ($\leq 0.014 \mu\text{g g}^{-1}$), which is in agreement with the literature data from this and other Baltic regions (Struck et al. 1997). These values are well below those stated in the relevant regulations issued by the Polish Ministries of Agriculture and the Environment (DzU Nr 236, 165, 55)

Table 2. (continued)

1	2	3	4	5	6	7	8	9	10	11	12	13
2	28.06.2006	–	–	–	–	–	–	–	–	–	–	1396
3	28.06.2006	–	–	–	–	–	–	–	–	–	–	4099
3I	28.06.2006	–	–	–	–	–	–	–	–	–	–	2919
<i>E</i>	17.07.2006	–	–	–	–	–	–	–	–	–	–	181
1	21.08.2006	–	–	–	–	–	–	–	–	–	–	213
3	21.08.2006	–	–	–	–	–	–	–	–	–	–	1164
4	21.08.2006	–	–	–	–	–	–	–	–	–	–	3926
park grass	15.06.2005	–	0.27	6.79	1.11	86.0	11.85	3.39	–	–	3.64	710

T – sea grass only (*Zostera marina*); *2a* – green algae only (*Enteromorpha* spp., *Cladophora* spp.); *2b* – brown algae only (*Pilayella littoralis*); *E* – red algae only (*Ceramium* sp.); *)not determined.

concerning the quality of fertilisers, soil and dredged materials. That is why the mercury concentration was not determined in the macroalgae collected in 2005; in that year the mercury analysis was replaced by the chromium and nickel analyses mentioned in these regulations. The highest metal contents determined were: cadmium – $1.41 \mu\text{g g}^{-1}$, lead – $10.50 \mu\text{g g}^{-1}$, nickel – $5.13 \mu\text{g g}^{-1}$, zinc – $223 \mu\text{g g}^{-1}$, copper – $19.50 \mu\text{g g}^{-1}$ and chromium – $4.38 \mu\text{g g}^{-1}$ (Table 2). In each case the metal content in the collected material lay well below the thresholds permitted in the three sets of regulations and was comparable with that determined in the park grass (Figure 6). In 2006, therefore, the metal determinations were not carried out.

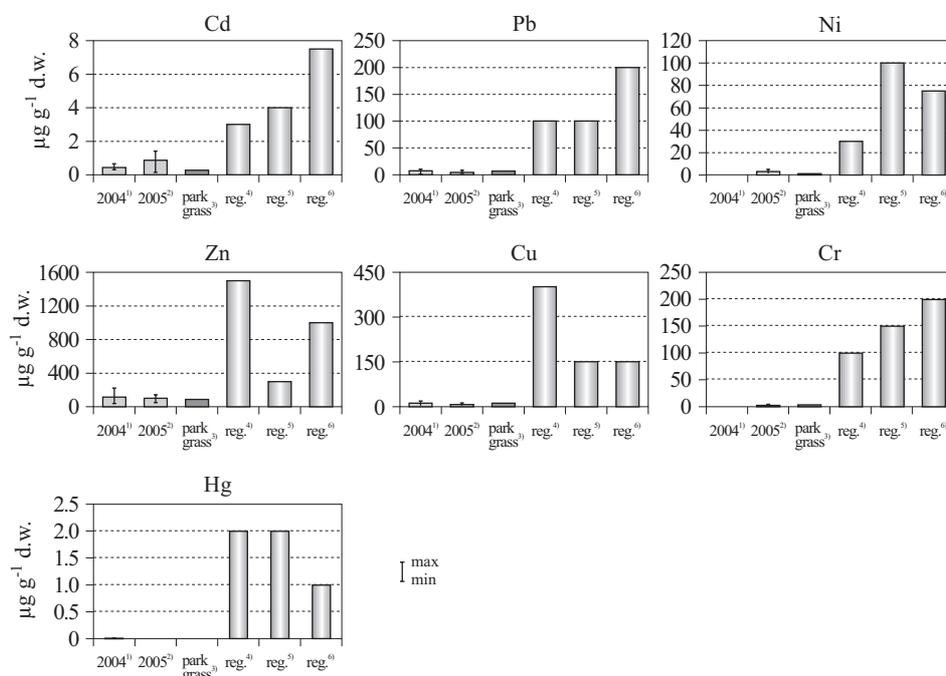


Figure 6. Average metal content in macroalgae from the Sopot beach, park grass; regulation limits. ¹⁾Sopot beach, $n = 8$; ²⁾Sopot beach, $n = 10$; ³⁾grass from the park adjoining the shore, $n = 2$; ⁴⁾Polish regulation – fertilisers (DzU Nr 236); ⁵⁾Polish regulation – soil (DzU Nr 165); ⁶⁾Polish regulation – dredged materials (DzU Nr 55)

Radioactivity determined on the basis of ^{137}Cs ($5\text{--}48 \text{ Bq kg}^{-1}$) and ^{40}K ($528\text{--}728 \text{ Bq kg}^{-1}$) was compared to the maximum values measured in different macroalgae species from other regions of the world, although admittedly there are not many data to compare with (Table 3). Nevertheless, it is

Table 3. Radionuclide contamination – comparison with maximum values in seaweeds from various regions of the world

	Region	Maximum value [Bq kg ⁻¹]	Reference
¹³⁷ Cs	Sopot	48	this paper
	Syrian Coast	< 1.2	Al-Masri et al. 2003
	Southern Spain	3.8	Manjón et al. 1995
	Aegean Sea	596	Sawidis et al. 2003
	Northern Scotland	~ 200 ^{a)} ~ 10 ^{b)}	Martin & Heaton 1989
	Norway Coast	~ 21	Yiou et al. 2002
	Commercially available edible algae	< 53	van Netten et al. 2000
⁴⁰ K	Sopot	728	this paper
	Syrian Coast	2500	Al-Masri et al. 2003
	Commercially available edible algae	4370	van Netten et al. 2000

^{a)}May 1986 – right after the Chernobyl accident, ^{b)}November 1987.

worth noting that ⁴⁰K concentrations in seaweeds from the Sopot beach were much lower than those determined in commercially available edible algae (van Netten et al. 2000); the ¹³⁷Cs contents were comparable.

The sum of 12 determined PCBs varied from 0.75 to 3.3 ng g⁻¹ in 2004, and from 0.09 to 1.24 ng g⁻¹ in 2005 (Table 2). The \sum 12 PCB content in macroalgae was much lower than the permitted values for soil (DzU Nr 165) and dredged materials (DzU Nr 55) and was comparable with the result obtained for the park grass (Figure 7). It was therefore decided to forego the PCB determinations in 2006.

The PAH contents in the plant material varied significantly in comparison to the other contaminants and were therefore determined throughout the period from 2004 to 2006. Concentrations of \sum 15 PAHs in macroalgae and sea grass ranged between 14 and 5418 ng g⁻¹ d.w. (Table 2). The lowest annual mean PAH content (423 ng g⁻¹) was reported in the samples collected in 2004, and the mean levels in the subsequent years were similar (1404 and 1484 ng g⁻¹ respectively) (Figure 8). All the samples analysed met the requirements established by Polish regulation for dredged materials (DzU Nr 55). The regulation for soil (DzU Nr 165) states the limits both for particular PAHs – naphthalene, phenanthrene, anthracene, fluoranthene, chrysene, benzo(a)anthracene, benzo(a)pyrene,

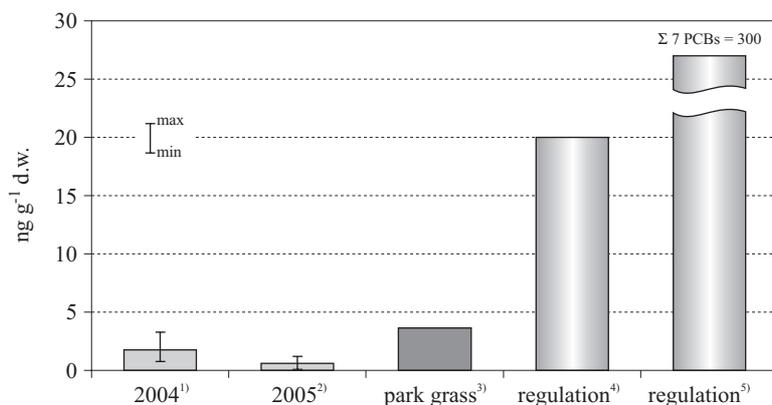


Figure 7. Average PCB content in macroalgae from the Sopot beach, park grass; regulation limits. ¹⁾Sopot beach, n = 7; ²⁾Sopot beach, n = 10; ³⁾grass from the park adjoining the shore, n = 2; ⁴⁾Polish regulation – soil (DzU Nr 165); ⁵⁾Polish regulation – dredged materials (DzU Nr 55)

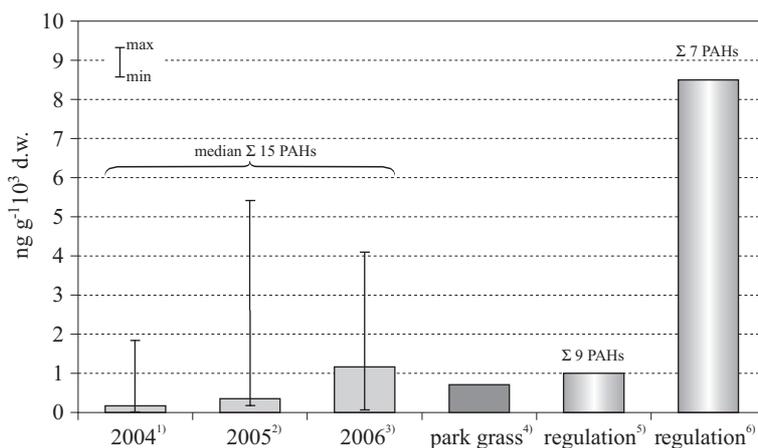


Figure 8. Average PAH content in macroalgae from the Sopot beach, park grass; regulation limits. ¹⁾Sopot beach, n = 7; ²⁾Sopot beach, n = 10; ³⁾Sopot beach, n = 11; ⁴⁾grass from the park adjoining the shore, n = 2; ⁵⁾Polish regulation – soil (DzU Nr 165); ⁶⁾Polish regulation – dredged materials (DzU Nr 55)

benzo(a)fluoranthene, benzo(ghi)perylene – as well as for the sum of these 9 PAHs. If the level of just one of these PAHs is higher than permitted, such a sample does not conform to this regulation. In view of this, 17 of the 28 samples were below the permissible PAH levels for soil. On the other hand, the majority of the samples (17) were less contaminated with PAHs than the park grass reference material.

3.3. Assessing the possible uses of the plant material

Different ways of utilising the material removed from the beach have been considered, taking into account the availability, species composition and contamination of the material, as well as the cost-effectiveness of the utilisation methods. Some macroalgal species are used as food for humans, especially in a number of Asian countries, and also in livestock feed (Munilla et al. 1995, Phaneuf et al. 1999, van Netten et al. 2000, Hong et al. 2007). Seaweed species, which are exceptionally rich in proteins, lipids, vitamins, pigments, minerals and other biologically active substances, are employed in traditional medicine, as functional food and in cosmetics in various parts of the world. Macroalgae can also be used as materials for industry and for the production of biofertilisers. Extracts obtained from seaweeds (e.g. *Sargassum* spp., *Turbinaria* spp., *Caulerpa chemnitzia*, *Enteromorpha intestinalis*) contain trace elements, vitamins, amino acids, cytokinins and growth-promoting hormones; moreover, research has shown that they are superior to other fertilisers, yielding economically satisfactory results (Sivasankari et al. 2006, Hong et al. 2007). Seaweed fertilisers are now commercially available.

The algae accumulating on the Sopot beach in summer make up a different material than that occurring on Atlantic or North Sea coasts. It is heterogeneous, at a different stage of decomposition, and its abundance is hard to predict. That is why the use of this material by the food, pharmaceutical or cosmetics industries seems rather improbable. Even if it were feasible to separate some of the biomass fractions in order to use them for production purposes, the whole procedure would be unprofitable. The inhomogeneous consistency and flexible structure of the biomass would also make it impossible to produce, say, construction materials for furniture, interior building and thermal insulation, such as are produced elsewhere from sea grass. On the other hand, the production of artistic materials like design products or letter paper is a possibility but is unlikely to be profitable given the present economic climate in Poland (Ransby 2004).

The next possibility considered was the use of this material for biogas production. In Poland, there are numerous active biogas installations, from large-scale ones to small ones fed with straw and green plant fuel that serve a few farms; so far, however, algae have not been used as such a fuel. Many projects have investigated seaweed crops for the production of compost and biogas (Chynoweth et al. 2001, Caliceti et al. 2002, Demirbas 2005). Some macroalgal species like *Macrocystis pyrifera*, and genera such as *Sargassum*, *Laminaria*, *Ascophyllum*, *Ulva*, *Cladophora*, *Chaetomorpha* and *Gracilaria*, have been explored as potential methane sources. But in spite of the large seaweed biomass in various regions of the world, anaerobic

digestion for biogas generation appears to be unsatisfactory and therefore uneconomic (Gunaseelan 1997, Caliceti et al. 2002). Moreover, such devices would have to operate non-stop throughout the year to be profitable, so in Sopot, additional fuel, besides macroalgae, would be necessary. And even in summer, the amounts of algae collected on the Sopot beach might well be insufficient, not to mention varying and unpredictable in different years.

3.4. Growth test

The preliminary tests with the macroalgae material collected on the Sopot beach were very promising. The growth rates of plants fertilised by the commercial fertiliser and those growing in the soil supplemented by macroalgae were comparable and conspicuously faster than in the control containers (Figure 9). But, as already mentioned, this was a preliminary test, during which not all the factors responsible for proper plant development were controlled. The very rapid decomposition of macroalgae when mixed with soil was also noted, a process that looks promising from the biofertiliser production point of view.

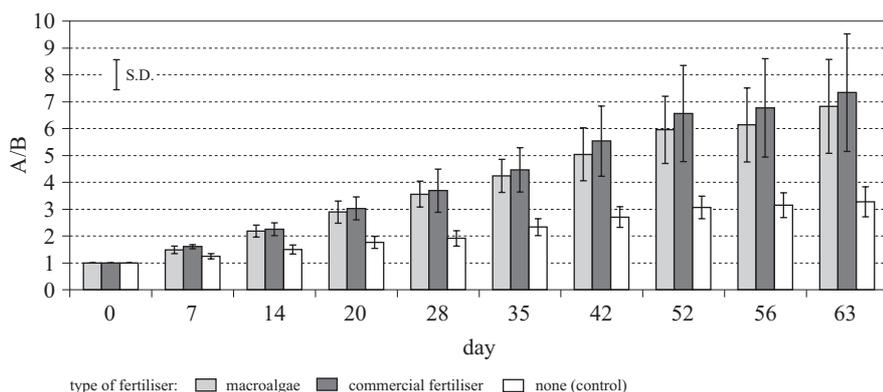


Figure 9. Growth tests of tomato plants – (number of plants $3 \times n = 7$) A – plant height on n-day of experiment; B – initial height of plant (0 day)

4. Conclusions

One realistic way of utilising algal material at present is as a natural fertiliser. Macroalgae and the products derived from them are valuable biofertilisers, rich in a variety of different unique components and well known among gardeners in other maritime countries. Such fertilisers are permitted in Poland (DzU Nr 77), but all the types applied so far have been imported. The quality of a commercial biofertiliser product must be controlled

in a laboratory accredited by the Polish Ministry of the Environment. According to Polish regulations, the fertiliser certificate should guarantee a stable and nutritious composition and limited contamination (DzU Nr 77); in the case of the macroalgal material from the Sopot beach, however, these conditions cannot be guaranteed. Such plant material is therefore more likely to be suitable as compost for city parks and green areas. As far as contaminants are concerned, such material could be treated as 'industrial waste': this assumes, of course, that such treatment will not cause the soil quality at the site of application, e.g. parks and gardens in the coastal zone, to deteriorate.

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