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APPLICATION OF ALGAE IN ACTIVE BIOMONITORING OF THE SELECTED HOLDING RESERVOIRS IN SWIETOKRZYSKIE PROVINCE

ZASTOSOWANIE GLONÓW W BIOMONITORINGU AKTYWNYM WYBRANYCH ZBIORNIKÓW RETENCYJNYCH WOJEWÓDZTWA ŚWIĘTOKRZYSKIEGO

Abstract: During the years 2014-2015, biomonitoring studies were carried out at three holding reservoirs located in Swietokrzyskie Province (central Poland): Kielce artificial lake, Chancza reservoir and Sielpia reservoir. In sea water algae *Palmaria palmata* (Linnaeus) Weber & Mohr, exposed in the analysed waters, the increases of concentrations were determined by the atomic absorption spectrometry method (AAS), of the following: Mn, Fe, Cu, Zn, Cd and Pb. Conductivity and pH were also determined in the reservoirs waters. The differences between the increases of heavy metal concentrations in the samples of algae found along the coastline were indicated; they result from different distances from the pollution sources, such as resorts, communication routes and industrial plants.

Keywords: sea algae, *Palmaria palmata* (Linnaeus) Weber & Mohr, heavy metals, biomonitoring, retention reservoirs, atomic absorption spectrometry

Introduction

The algae [1, 2], which have good sorption characteristics [3-5] are most often used in active biomonitoring of surface waters.

Algae are thalloid plants. Apart from eukaryotic organisms (among others *Palmaria palmata* and *Spirogyra* sp.), there are also those with prokaryotic cell structure. This group has a diverse morphological structure of thallus and various shapes [6-8].

The studies carried out with the use of sea and fresh water algae confirm their practical application not only in biomonitoring of surface waters pollution [9-16] but also waters phytoremediation [17] and as biosorbents in the effluent treatment process [18].

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Some instrumental methods of water chemical composition, *eg* atomic absorption spectrometry (AAS), require concentrating of water samples in order to determine the trace quantities of heavy metals. Algae can be used for that purpose due to very good sorption characteristics. After mineralisation of algae samples, the solutions contain much higher concentrations of metals, compared to such concentrations in water [3]. Determining the correlation between concentrations of analytes in algae and water can be used to prepare a simple method for determination of heavy metals concentration in surface waters, on the basis of the chemical analysis of the algae thallus composition.

The objective of the studies was to apply active biomonitoring to determine the distribution of bioaccessible forms of heavy metals: Mn, Fe, Cu, Zn, Cd and Pb along the coastline of the retention reservoirs: Kielce artificial lake, Chancza and Sielpia reservoirs and demonstrate heterogeneity of the distribution of analytes concentrations in the studied reservoirs resulting, among others, from the distance from pollution sources. The sea algae *Palmaria palmata* were used in the study and exposed in the studied reservoirs.

Characteristics of the research field

The Kielce reservoir was created within Kielce municipal border, by closing the Silnica river valley with a weir, at its 8th kilometre. The areas in the reservoir vicinity have both recreation and economic function. The artificial lake water is supplied from a stream, which also collects communal waste from Maslow - a locality near Kielce. The amounts and diversity of both organic and non-organic compounds supplied to the reservoirs causes a considerable pollution level of its waters [19].

The Chancza reservoir is located on the Czarna Staszowska river, near the town of Staszow, in the south-eastern part of Swietokrzyskie province. The area belongs to the Nida Basin macroregion and Szydlow Upland and Orlowski Range mezoregions. The Czarna Staszowska river is a left tributary of the Vistula and its waters, as well as the waters of the Chancza reservoir, are in category II, as far as physical and chemical elements are concerned [20].

The Sielpia reservoir is an artificial lake with 60 hectares of surface, located in the north-western part of Swietokrzyskie province, between Kielce (approximately 35 km) and Konskie (approximately 10 km), in the locality of Sielpia Wielka. The Czarna Maleniecka river influences cleanliness of the reservoir waters; the river's chemical condition in 2014 was estimated as good (class II as far as physical and chemical elements are concerned). Rain waters from numerous resorts and woodlands surrounding feed its waters [20].

The bodies of water included in biomonitoring studies abound in numerous flora and rare fauna species. These reservoirs are the recreation spots for the inhabitants of Swietokrzyskie province [21].

Materials and methods

Palmaria palmata (Linnaeus) Weber & Mohr sea algae purchased from the company BogutynMłyn from Radzyn Podlaski (PL) were used for the research. The sea algae were purchased dried. In order to remove the salts released in consequence of destruction of cell membranes, the algae were rinsed with demineralised water and dried for 24 hours at the temperature of 323 K. The algae prepared in this way were stored for further analyses.

The concentrations of metals naturally accumulated in the dry mass (d.m.) of *Palmaria palmata* amounted to the following: $c_{Mn,0}$ = 615 ±13 mg/kg d.m.; $c_{Fe,0}$ = 966 ±18 mg/kg d.m.; $c_{Cu,0}$ = 4,32 ±0.08 mg/kg d.m.; $c_{Zn,0}$ = 32,7 ±0.1 mg/kg d.m.; $c_{Cd,0}$ = < 0,81 mg/kg d.m.; $c_{Pb,0}$ = < 4,38 mg/kg d.m.

The representative (averaged) algae samples with the mass of 1 g d.m. were placed in perforated polyethylene containers of capacity *ca* 15 cm^3. Prior to exposition, algae were immersed for 15 minutes in demineralised water, in order to activate sorption centres. Next, samples were exposed in waters of the studied reservoirs for 50 minutes. Biomonitoring of the Chancza reservoir included also the waters feeding the reservoirs, form the rivers: Czarna Staszowska and Lagowica. The samples were immersed in water approximately 1 meter from the shore, in the littoral zone. The study included five repetitions. Simultaneously, water samples were taken in the defined measuring spots, in order to determine physical and chemical parameters (pH and conductivity).

Figure 1 presents the map of measuring spots locations.

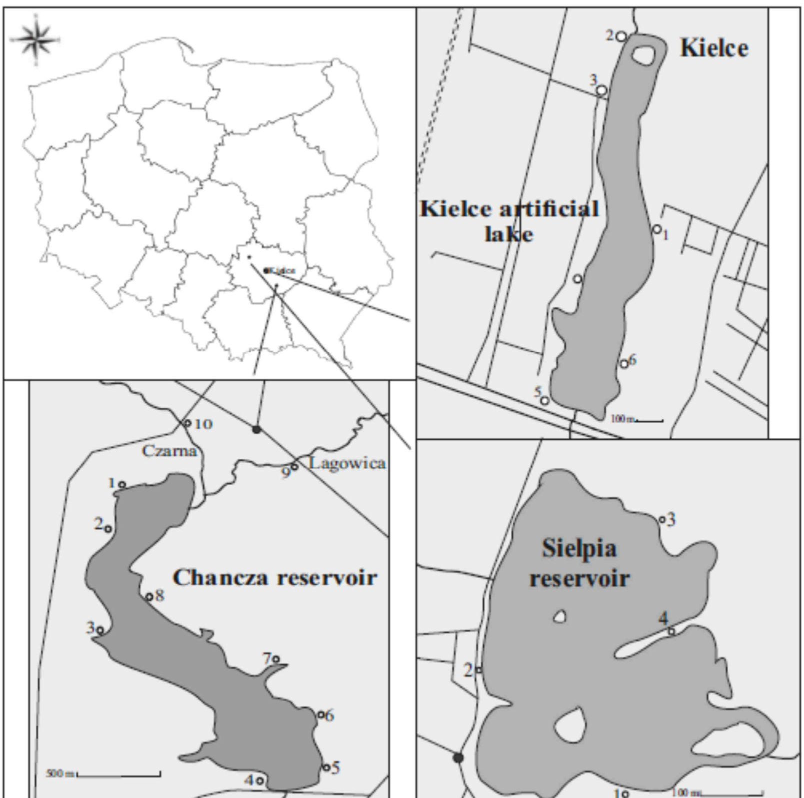


Fig. 1. Measuring spots locations

After exposition, algae samples were rinsed with demineralised water and dried at the temperature of 323 K. The algae samples (dry mass each of them was 0.400 ±0.001 g) were mineralised in the mixture of nitric(V) acid and hydrochloric acid (HNO_3 65% : HCl

37% = 1:3) using a Speedwave Four made by Berghof, DE microwave oven. The mineralization process temperature was 180°C. MERCK company reagents were used to prepare solutions. Heavy metals (Mn, Fe, Cu, Zn, Cd and Pb) in mineralised algae samples were determined by atomic absorption spectrometry method (AAS), using the equipment iCE 3000 made by Thermo Electron Corporation (USA).

The conductivity and pH of the water, in which algae were immersed, were measured with the equipment made by Elmetron Sp.j. from Zabrze (PL): pH meter CP 401, which absolute measurement error was ΔpH = 0.02 and Δκ = 0.1 μS/cm, respectively.

Quality and quality assurance

Table 1 presents the limits of detection and the limits of quantification of heavy metals for the spectrometer iCE 3500 [22]. Calibration of spectrometer was performed with a standard solution from ANALYTIKA Ltd. (Czech Republic). The values of the highest concentrations of the models used for calibration (2 mg/dm^3 for Cd, 5 mg/dm^3 for Cu, Zn, Pb, 7.5 mg/dm^3 for Mn and 10 mg/dm^3 for Fe) ware approved as linear limits of the signal dependence on the concentration.

Table 1

The instrumental detection limits (*IDL*) and instrumental quantification limits (*IQL*) for the spectrometer iCE 3500 [mg/dm^3] [22]

Metal	***IDL***	***IQL***
Mn	0.0016	0.020
Fe	0.0043	0.050
Cu	0.0045	0.033
Zn	0.0033	0.010
Cd	0.0028	0.013
Pb	0.0130	0.070

Table 2

Comparison of measured and certified concentrations in BCR-414 *plankton* and in BCR-482 *lichen*

Metal	**BCR-414 *plankton***		**AAS**		***Dev.*****
	Concentration	**±Uncertainty**	**Average**	**±*SD****	
	[mg/kg d.m.]				**[%]**
Mn	299	12	284	13	–5.0
Fe	1.85	0.19	1.79	0.20	–3.2
Cu	29.5	1.3	28.4	1.6	–3.7
Zn	112	3	107	3	–4.5
Cd	0.383	0.014	n.d.	n.d.	n.d.
Pb	3.97	0.19	3.75	0.21	–5.5
Metal	**BCR–482 *lichen***		**AAS**		***Dev.*****
	Concentration	**±Uncertainty**	**Average**	**±*SD****	
	[mg/kg d.m.]				**[%]**
Mn	33.0	0.5	31.7	0.68	–3.9
Fe	804	160	n.d.	n.d.	n.d.
Cu	7.03	0.19	6.63	0.17	–5.7
Zn	100.6	2.2	95.1	2.3	–5.5
Cd	0.56	0.02	0.53	0.03	–5.3
Pb	40.9	1.4	38.2	1.0	–6.6

* - standard deviation, ** - relative difference between the measured and certified concentration 100%·$(c_z – c_c)/c_c$, n.d. - not determined

Table 2 shows heavy metals concentrations, determined in the certified reference materials as BCR-414 *plankton* and BCR-482 *lichen*, prepared by the *Institute for Reference Materials and Measurements, Belgium*.

In order to determine uncertainty of the measurement method, algae samples were analysed five times, maintaining the whole cycle of the research method. For the samples of algae exposed in the defined measurement spots, the value of coefficient of variation $CV_{śr}$ (*coefficient of variation*), determined on the basis of the value of standard deviation *SD* ($CV_{śr.}$ [%] = 1/3 · ∑ ($SD_i/c_{x,śr.i}$) · 100%, where: $SD_i/c_{x,śr.i}$ is a standard deviation, calculated for the i-series (number of measuring spots in other reservoirs i = 6, 10 and 4), referred to the mean value from the series ($c_{x,śr.i}$)) is, for *Palmaria palmata*, within the range 11-15%.

Results interpretation method

The increases of analytes concentrations in algae, indicating waters pollution, were determined on the basis of the relative accumulation factor (*RAF* - *Relative Accumulation Factor*) [23]:

$$RAF = \frac{c_{(a,1)} - c_{(a,0)}}{c_{(a,0)}} \qquad (1)$$

where: $c_{(a,0)}$ - mean concentration of the analyte in algae before the exposition [mg/kg d.m.], $c_{(a,1)}$ - mean concentration of the analyte in algae after the exposition [mg/kg d.m.].

Results and discussion

Table 3 presents pH and conductivity values of the water samples from the studied reservoirs, in the designed algae exposition locations (Fig. 1).

Table 3

The results of measurements of physicochemical parameters of the water samples

Reservoir	Parameter	pH	Conductivity [μS/cm]
The Kielce artificial lake	**Mean**	**7.82**	**527**
	Minimum	7.69	499
	Maximum	7.89	624
	±*SD*	0.07	48
The Chancza reservoir	**Mean**	**8.30**	**342**
	Minimum	7.64	312
	Maximum	8.68	476
	±*SD*	0.36	48
The Sielpia reservoir	**Mean**	**6.14**	**145**
	Minimum	5.91	140
	Maximum	6.25	153
	±*SD*	0.16	5.7

The Sielpia reservoir waters have relatively low pH values (pH < 7) and low salinity, which results in low conductivity. Considering the pH value one may declare that the Chancza reservoir waters contain little CO_2, with domination of bicarbonates, created in consequence of binding carbon dioxide by calcium [24]. The Kielce artificial lake has the greatest salinity.

Palmaria palmata sea algae were used in order to assess the differences of concentrations of bioaccessible forms of the analysed metals.

Table 4 presents mean concentrations of heavy metals determined in the samples *Palmaria palmata*, exposed in the studied reservoirs.

Table 4

Concentrations of heavy metals in algae *Palmaria palmata* [mg/kg d.m.]

Spot no.	Mn	Fe	Cu	Zn	Cd	Pb
The Kielce artificial lake						
1	636	936	6.43	40.4	< 0.81	< 4.38
2	640	1021	8.292	40.4	< 0.81	< 4.38
3	618	935	7.73	33.2	< 0.81	< 4.38
4	622	1048	11.3	43.4	< 0.81	< 4.38
5	623	1049	10.7	46.4	< 0.81	< 4.38
6	640	1047	8.43	36.8	< 0.81	7.98
Mean	**630**	**1006**	**8.83**	**40.1**	**< 0.81**	**< 4.98**
±*SD*	10	55	1.86	4.7	-	-
The Chancza reservoir						
1	621	1030	10.7	54.0	< 0.81	6.99
2	692	1136	7.83	46.1	< 0.81	16.3
3	622	975	6.34	35.9	< 0.81	9.38
4	625	1048	10.4	40.6	< 0.81	9.68
5	625	1042	10.9	40.2	< 0.81	24.1
6	623	1005	7.55	30.4	< 0.81	20.8
7	624	1012	6.16	33.3	< 0.81	11.6
8	625	1014	4.84	34.5	< 0.81	< 4.38
9	626	1054	9.46	42.6	< 0.81	20.4
10	624	1045	6.97	29.7	< 0.81	19.4
Mean	**631**	**1036**	**8.12**	**38.7**	**< 0.81**	**< 14.3**
±*SD*	22	42	2.12	7.6	-	-
The Sielpia reservoir						
1	634	1102	6.93	35.2	< 0.81	13.9
2	632	1064	8.71	40.9	< 0.81	14.8
3	634	1081	8.68	39.0	< 0.81	10.5
4	631	1039	41.4	41.8	< 0.81	13.6
Mean	**633**	**1072**	**16.4**	**39.2**	< 0.81	**13.2**
±*SD*	2	27	16.7	2.9	-	1.9

Based on the carried out research it was stated that *Palmaria palmata* sea algae accumulated lead well. Lead concentrations in the majority of the algae samples after exposition were higher than the analytical determination method limit: *MQL* > 4.38 mg/kg d.m. The previous laboratory tests of sea algae showed that their sorption properties, compared to fresh water algae, were less influenced by the presence of other ions in solutions, within the conductivity changes range from 200 to 1000 μS/cm [25]. Large *SD* values, with reference to mean concentration values of the determined metals for each reservoir, confirm the various pollution levels of waters and signify the possibility that the determined metals originate from several different sources.

Figures 2-5 present the values of determined *RAF* coefficients (Relation 1) for Mn, Fe, Cu, Zn and Pb. Increases of lead concentrations were noticed in *Palmaria palmata* algae exposed in the Chancza and Sielpia reservoirs and in the measuring spot 6, located in the Kielce artificial lake.

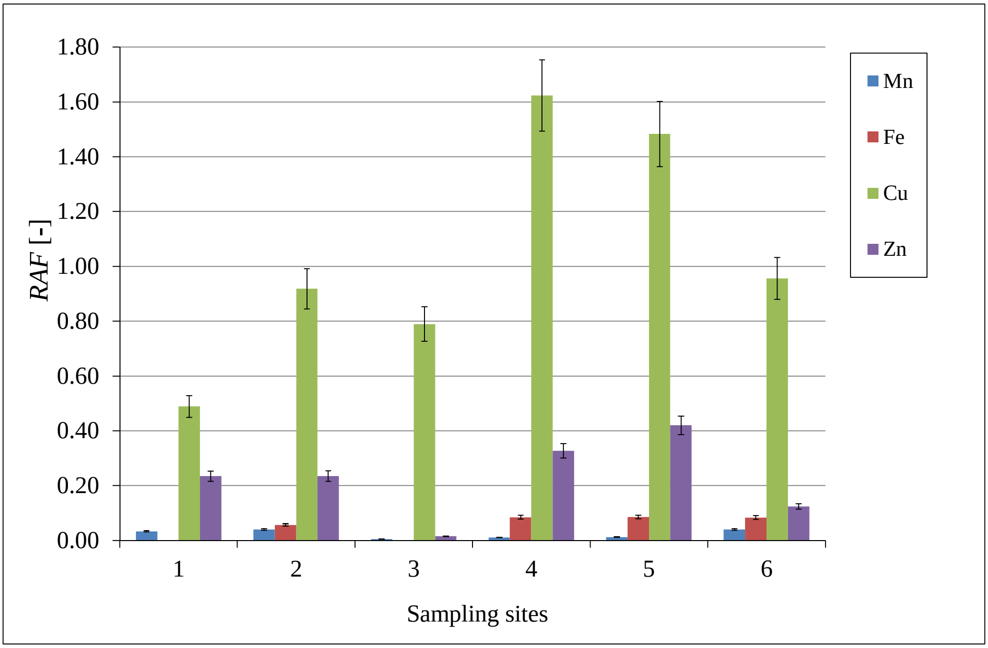


Fig. 2. Increases of heavy metals concentrations in *Palmaria palmata* algae exposed in the Kielce artificial lake waters

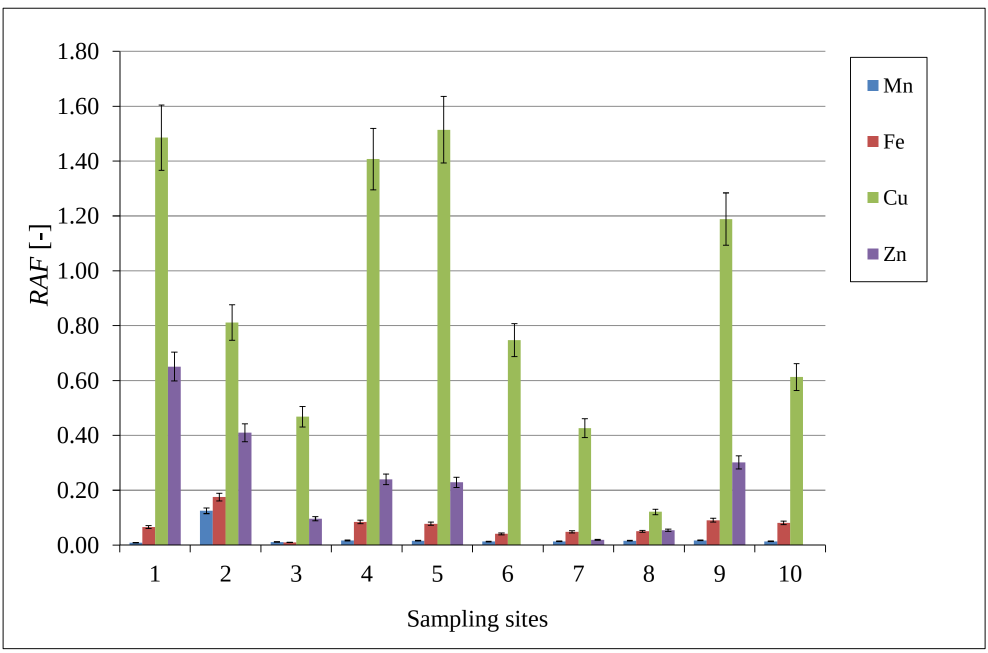


Fig. 3. Increases of heavy metals concentrations in *Palmaria palmata* algae exposed in the Chancza reservoir waters

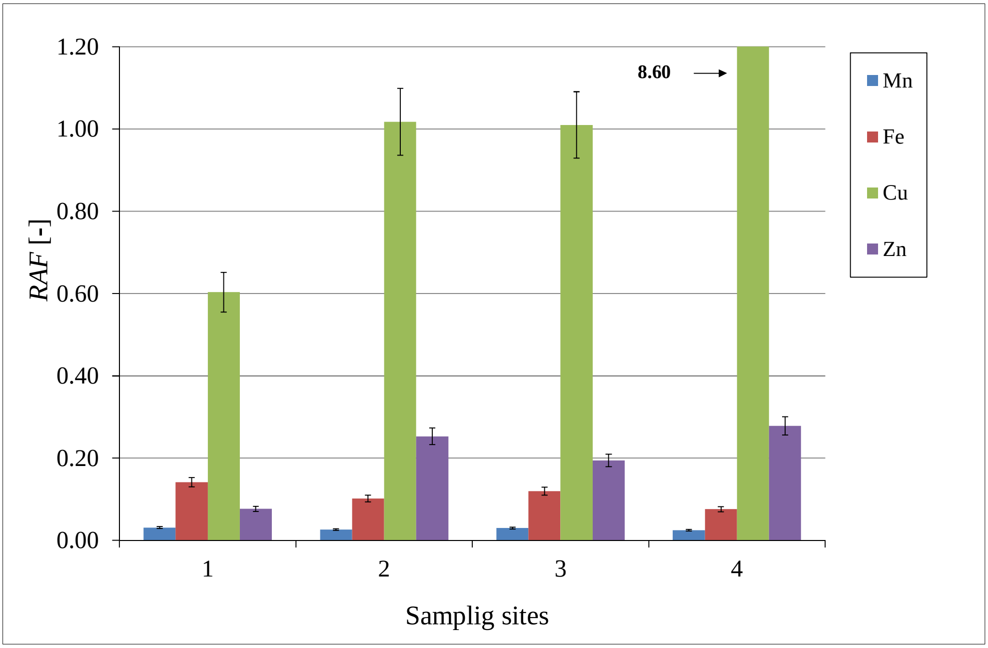
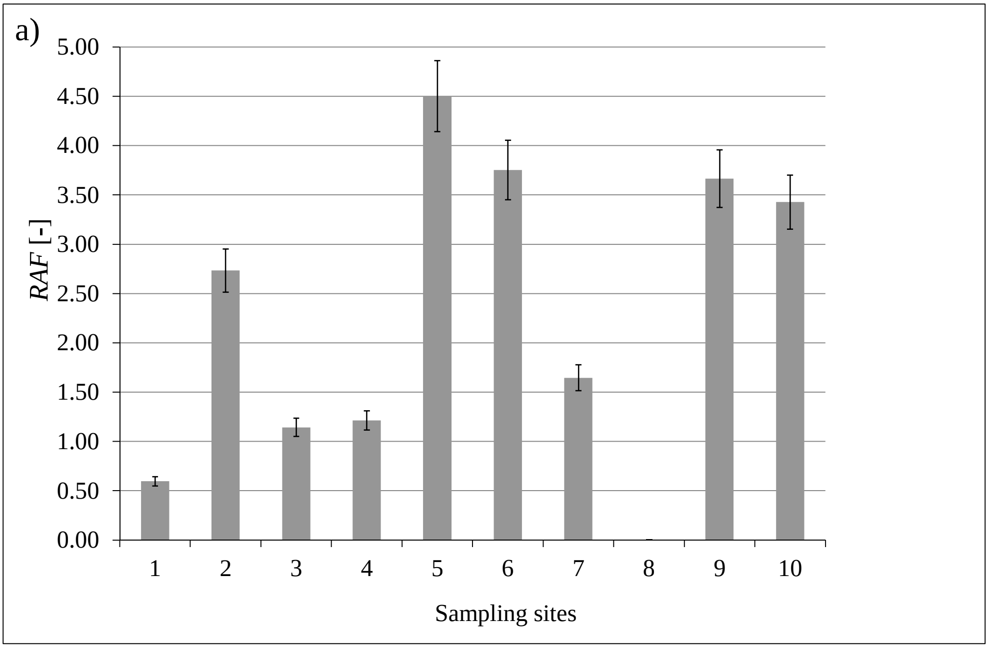


Fig. 4. Increases of heavy metals concentrations in *Palmaria palmata* algae exposed in the Sielpia reservoir waters

The results presented in the graphs in Figures 2-5 show a differentiated level of metals accumulation in individual reservoirs. Considering the method's unreliability, the *RAF* values < 0.26 are within the measurement error limits and do not signify concentration increases post-exposition. No statistically relevant differences in concentrations of Mn and Fe, in the algae exposed in various spots of the Kielce artificial lake, were identified. Waters of the reservoir are polluted mainly with copper and zinc, only in spots 4 and 5. The aggregate quarry in Wisniowka near Kielce may be the potential source of these metals. The quarry discharges mining waters to the Silnica river, which carries them on to the Kielce artificial lake. It is also significant that the lake is located close to the former copper ore mining and processing centres (Miedziana Gora).

According to the research, considerably high copper concentrations are present in the Chancza reservoirs as well as the Kielce artificial lake. The samples exposed in this reservoir had large increases of lead concentrations, particularly in exposition spots 5 and 6 and in the waters of the rivers flowing into the reservoir: Lagowica and Czarna Staszowska rivers (spots 9 and 10). This may signify that the potential lead pollution sources are the lake's tributaries and the increased lead concentrations in the samples exposed in spots 5 and 6 may result from the vicinity of bottom sediments accumulation, rich in pollutants. The spots 5 and 6 are located near the outlet of the Czarna river from the lake. The increases of zinc in the samples exposed in the lake (*RAF* > 0.26) were noted in spots 1 and 2.



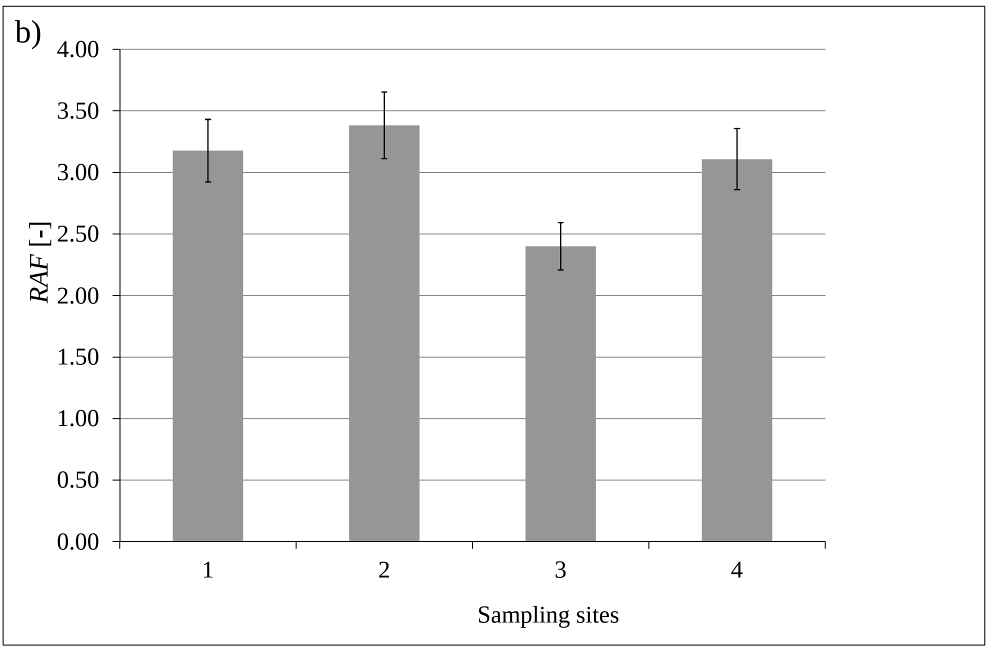


Fig. 5. Increases of lead concentrations in *Palmaria palmata* algae exposed in the waters of: a) Chancza, b) Sielpia

Large increases of Cu and Pb in algae after the exposition were noted in the Sielpia and Chancza reservoirs. The Sielpia reservoir is a popular resort in Swietokrzyskie province. There are resorts and summer houses along the whole coast of the reservoir. A district road passes close to the western coast of the reservoir. However, it seems that the largest water pollution source is the bottom sediment, accumulated in large amounts during the 1997 flood. Activities aiming at revitalisation of the reservoir have been carried out recently.

Summary and conclusions

Algae are perceived as one of the major bioindicators of heavy metal pollution in surface waters biomonitoring. Analysis of heavy metals concentrations captured in algae thallus provides much information regarding the pollution introduced to water environment, allows to asses changes in environment quality and to identify the sources of pollution.

On the basis of the carried out research it was demonstrated that *Palmaria palmata* sea algae can be successfully used as biomonitors of water ecosystems pollution with heavy metals. The biomass of *Palmaria palmata* proved to be a very good biomonitor in detecting spot sources of lead pollution.

Even though algae are not homogeneous material, they can be successfully used as biosensors in detecting heavy metals pollution of surface waters.

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