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ALGAE AS A SOURCE OF INFORMATION ON SURFACE WATERS CONTAMINATION WITH HEAVY METALS

GLONY JAKO ŹRÓDŁO INFORMACJI O ZANIECZYSZCZENIU METALAMI CIĘŻKIMI WÓD POWIERZCHNIOWYCH

Abstract: This paper is a synthetic set of information that relate to the goals and directions of research linked with the use of algae for the assessment of contamination of surface water by heavy metals. The presented examples concern the use of algae in biomonitoring of aquatic ecosystems, which in many countries, including Poland became a permanent part of an integrated environmental monitoring. The paper discuss the results of research carried out in situ, whose aim was to assess the contamination of selected aquatic ecosystems by heavy metals based on the chemical composition of thalluses, as well as the identification of potential sources of these analytes. The analysis of the research results referred to in the cited literature was supported by the conclusions of the research of their own.

Keywords: algae, heavy metals, surface waters, biomonitoring

The term *algae* (Gr. *Phykos,* Lat. *Algae*) is used to refer to a group of thallus plants, *ie* paraoza (morphological groups: *Protophyta* and *Thallophyta*). Among them, apart from eukaryotes (inter alia *Spirogyra* sp.), there are also organisms with prokaryotic cell structure, such as blue-green algae (*eg Nostoc commune*), also known as *Cyanobacteria*. The author of one of the pioneering works devoted entirely to this group of organisms is Samuel Gottlieb Gmelin (1744–1774). In his work *Historia Fucorum* (*fucus* – the term used to describe algae, introduced by the Romans and commonly used till the end of the 18th century), published in 1768, he described 99 species of algae. Many of the algae species described by Gmelin have disappeared by now. At present, the term *algae* covers over 27 thousands of species in various systematic groups of thallus plants [1]. In Poland, the term was introduced into the botanical terminology by Jozef Rostafinski (1850–1928) [2–5].

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Algae do not constitute a systematic unit; however, due to practical reasons, a certain taxonomic order was introduced, which is manifested, depending on the interpretations of individual authors, by a creation of some systems. Since 1931, the system of Adolf Pascher had been used, according to which the algae were divided into 9 phyla: *Cyanophyta*, *Glaucophyta*, *Euglenophyta*, *Pyrrophyta*, *Chrysophyta*, *Chlorophyta*, *Charophyta*, *Phaeophyta* and *Rhodophyta* [6]. Taxons differentiated in 1980 by H. Ettl are based on this system. Algae were divided into 9 phyla, and the tenth phylum, conditionally included to algae, consists of *Cyanophyta* [4]. In 1995, Christiaan van den Hoek introduced a new taxonomy of algae, differentiating 11 phyla [7, 8]. Among them, there are two prokaryotic phyla: blue-green algae (*Cyanophyta* = *Cyanobacteria*) and *Prochlorophyta*, as well as 9 eukaryotic phyla: glaucophytes (*Glaucophyta*), haptophytes (*Haptophyta*), cryptophytes (*Cryptophyta*), dinophytes (*Dinophyta*), euglenoids (*Euglenophyta*), red algae (*Rhodophyta*), yellow-green algae (*Heterokontophyta*), chlorarachniophytes (*Chlorarachniophyta*) and green algae (*Chlorophyta*) [9]. In 2000, James E. Graham and Lee W. Wiloox introduced the term "classification of blue-green algae and algae", which covered the orders and classes, but without differentiation into phyla [8, 10].

Algae are encountered in every place where water is present, at least periodically. They inhabit both the aqueous environment and land. They live both in saltwater (saline, salty lakes, seas and oceans) and in freshwater (springs, rivers, ponds, lakes and swamps). Some species are able to live on snow and ice, while others in hot springs (the highest temperature at which algae were found was 358.2 K). Due to different ecological requirements, algae can be divided into 11 groups. Table 1 shows the ecological groups of algae, their brief characteristics as well as some examples of algae species [4].

Algae display varied morphological structure of the thallus (single-celled species (*Ochromonas ludibunda*), species forming colonies (*Hydrurus foetidus*), and multi-celled organisms (*Ulva lactuca*), species with thread-like thallus (*Cladophora*) and ramified thallus (*Caulerpa prolifera*). They also vary in shape and size, from species as small as 1 μm to large, leaf-like species that fix to surfaces using rhizoids (thread-like processes). In the trophic chain, algae are mostly autotrophic, although heterotrophic algae and algae that enter symbiotic relations with other organisms are also encountered. For instance, the symbiotic relations between algae and fungi take the form of lichens [4, 5, 11, 12].

Studies carried out on algae concentrate on, among others, their chemical composition, biology, taxonomy, physiography and also their application for the assessment of water contamination with heavy metals, pesticides and radionuclides.

The main research issues realised in the process of aqueous ecosystems biomonitoring with the application of algae include the following: the assessment of contamination of the studied aqueous reservoirs (both saltwater and freshwater reservoirs), identification of contamination sources, a long-term monitoring and assessment of tendencies in changes in the chemical composition of contamination, as well as comparative studies.

Table 1

Classification of algae due to their ecological requirements [4]

Ecological group	Characteristics of ecological conditions	Algae species
Planktonic algae	Algae suspended in water and passively drifting with water currents	*Triposolenia longicornis*
Neuston	Algae living on the border of water and atmosphere (the surface film of a body of water)	*Chromulina rosanoffii*
Benhtos	Algae living on the bottom or the bank of water reservoirs	*Chaetomorpha aerea*
Algae of inland salty waters	Algae existing in saline and salty lakes; salinity level may be 60–80 ‰	*Dunaliella salina*
Algae on snow and ice	Algae existing on the surface of snow and ice; defined as cryophytes, cryoplankton, cryoseston,	*Chlamydomonas nivalis*
Hot springs algae	Algae existing in hot springs (thermal springs)	phylum *Chrysophyta*
Soil algae	Algae existing in the surface layer of soil (up to several dozen cm)	*Chlorhormidium flaccidum*
Terrestrial algae	Algae existing on a damp surface of soil; defined as geophytes	*Botrydium granulatum*
Aerophytic algae	Algae existing in places periodically damp, *eg* building roofs, walls, rocks	*Trentepohlia iolithus*
Epibiontic algae	Algae existing on the surface of living organisms – plants and animals	*Chlorangiopsis epizoica*
Algae in a living environment	Algae existing on another living organism (symbiosis, parasitism)	*Schmitziella endophloea*

Aqueous ecosystems are sensitive to physicochemical changes in biotope. In order to monitor some of these changes, such as heavy metal concentration, the bioindicators and biomonitors are applied, *ie* organisms which indicate the measurable morphological, anatomic and physiological changes [13]. These organisms, in order to be used as effective indicators of environmental pollution level, must fulfil certain criteria, for instance such as the following: relatively sedentary lifestyle, wide geographical prevalence, easiness to identify and sample, relatively high tolerance in relation to the studied pollution and the existence of statistically significant correlation between the analyte concentration, *eg* in water and in the organism that lives in this water [14]. Algae, due to their abundance in highly diversified environmental conditions, are becoming more and more popular biomonitors [15]. Additionally, they are very good sorbents of heavy metals. The author's own studies led to the conclusion that a sample of the alga *Parmalia palmata* with the mass of 1.0 g, after being placed in the 400 cm^3 of $CdCl_2$ solution with concentration of 0.5 mg/dm^3 accumulates almost 100 % of Cd^{2+} ions present in this solution.

The aim of this paper is to collect and systematise data regarding assumptions and trends in the studies carried out on different species and types of algae. The paper presents information regarding biomonitoring studies connected with the assessment of contamination levels of aqueous ecosystems with heavy metals on the basis of the analysis of thallus chemical composition of algae. Some practical applications of algae are suggested, for instance in water phytoremediation and in the process of sewage treatment.

Biomonitoring of freshwater ecosystems

There is a high diversity in freshwater ecosystems in nature. Small reservoirs, rock or ground hollows filled with water belong to periodical waters. Bigger reservoirs (ponds and lakes), as well as flowing waters (springs, brooks and rivers) belong to more or less permanent waters. The character of such ecosystems changes significantly depending on their morphometry as well as the type of catchment basin [11]. Numerous biomonitoring studies of the freshwater ecosystems have been carried out, in which the indicator organisms are algae. One of the examples is the study utilising algae such as *Spirogyra adnata* to determine the contamination of the waters of lake Naintial, situated at the foot of the Himalayas (India) with metals: Cr, Cu, Fe, Mn, Ni, Pb and Zn. As compared to other species of the same phylum *Chlorophyta, eg Oedogonium* sp., thalli of *Spirogyra adnata* contained large concentration of Pb (95 µg/g d.m.). Heavy metals in algae were determined by the atomic absorption spectrometry method (AAS). Potential sources of heavy metal contamination of this reservoir include the following: tourism development on the studied area, commuter traffic and illegal construction works. The authors concluded that algae, due to their very good sorption properties, may be applied in phytoremediation, *eg* to remove heavy metals from water [16]. This conclusion is confirmed by the results published in literature [17–19].

In India, the biomonitoring studies were carried out on lake Pulicat (South-Eastern India). By means of the AAS method, the following heavy metals were determined in the alga *Ulva lactuca*: Cd, Cr and Pb. Concentrations of metals accumulated in algae were compared with the concentrations in benthic sediments and the water of the lake. It was observed that in the alga *Ulva lactuca* and in the sediments, heavy metal concentrations were changing in accordance with the column: Cr > Pb > Cd, while in water the most abundant metal was Cr and the least abundant was Pb. The authors also observed seasonal changes in Cd and Pb concentration in the algae and sediment samples [20].

From April 2000 to December 2004, the contamination with such metals as Cd, Cr, Hg and Pb was monitored in the water of lake Sariyar in Turkey. Samples of water and phytoplankton containing algae of the phyla *Chlorophyta*, *Chrysophyta*, *Euglenophyta* and others were used for the study. Seasonal changes in concentrations of Cd, Cr, Hg and Pb were observed (determined by means of the AAS method) in the samples of water and phytoplankton. Significantly higher concentrations of some of the analysed metals were observed in phytoplankton (the heavy metal concentrations were changing in accordance with the column Pb > Cr > Cd > Hg) as compared with the water samples. For instance, Cd concentration in water, in summer, was app. 20 mg/dm^3 and in phytoplankton app. 75 mg/g, while in winter, in water the concentration was app 35 mg/dm^3, and in phytoplankton app. 105 mg/g. It was observed that the differences in heavy metal bioaccumulation result from the seasonal variability of algae species in phytoplankton, as well as different sorption properties of various algae species [21].

In Turkey, the biomonitoring studies of the Tigris River were carried out. On the basis of heavy metal concentrations (determined by means of the AAS method): Cd, Co, Cu, Fe, Mn, Ni, Pb and Zn, accumulated by the algae *Spirogyra* sp., the assessment of contamination of the studied ecosystem was conducted. It was observed that heavy -

metal concentrations in algae undergo seasonal changes. The highest concentrations: Co, Cu and Ni were determined in summer, Mn and Fe in autumn and Zn in spring [22].

From January 1992, water and algae collected from two streams near the city of Shillong in India were analysed: Wah Diengling, flowing through forested areas (the outskirts of the city) and Umkhrah, flowing through highly urban part of the city of Shillong. By means of the AAS method, the following heavy metals were determined: Cd, Cu, Mn, Pb and Zn. It was observed that the algae collected from the stream Umkhrah revealed app. twice higher Pb concentration, three-fold higher Zn concentration and five-fold higher concentration of Cd, Cu and Mn as compared to the algae samples collected from the stream Wah Diengling. It was shown that high heavy metal concentration in algae from the stream Umkhrah resulted from its localisation. This watercourse was fed with sewage from nearby shops, garages, households, as well as water coming from arable fields (crop protection chemicals contain high concentrations of Cu and Mn) [23].

Other examples of biomonitoring studies of freshwater ecosystems with the utilisation of algae are studies carried out in Poland (the Large Turawa Lake, the Odra River) [24, 25], the Czech Republic (the Thaya River) [26], Russia (12 rivers of the Kola Peninsula, The Yenisei River in Siberia, Lake Baikal) [27–29], Scotland (a lake in the range of the Lochnagar Mountains) [30], Greece (the Evros River delta) [31], Argentina (lakes in the Andes: Nahuel Huapi, Gutirrez and Moscardi) [32], Uganda (Lake George, a brook near the city of Kampala) [33, 34], Nigeria (the Niger River) [35], Malawi (watercourses near the city of Blantyre, Eastern Africa) [36], The USA (a watercourse near the city of Oak Ridge, the Missouri River) [37, 38]. Australia (4 rivers near Sydney: Hawkesbury, Parramatta, Cooks and Clyde) [39].

Literature data confirms that algae sorb heavy metals under natural conditions. Biomass collected from waters with high concentrations of heavy metals is characterised by higher concentrations of these analytes in their structures as compared with the biomass collected from uncontaminated waters. The authors indicate that algae are highly sensitive biosensors, sensitive to, for instance, seasonal changes in analyte concentrations in aqueous ecosystems. A correlation between heavy metal concentration in algae and in benthic sediments and water has still been investigated.

Biomonitoring of saltwater ecosystems

Saltwater ecosystems, due to their diversified physical environment (biotope), are also characterised by a high diversification of biocenoses. There are various categories of saltwater ecosystems. These include ecosystems of tide zone, littoral zone, offing and ocean depths. Also in the case of these ecosystems, various biomonitoring studies with algae have been carried out for many years. As an example, the studies in the Gulf of Aden (Yemen) can be quoted, in which the algae of phyla *Chlorophyta*, *Phaeophyta* and *Rhodophyta* were used. It was observed that concentrations of heavy metals accumulated in algae were several orders of magnitude higher than in water, while the highest heavy metal concentrations were found in the *Chlorophyta* phylum. It was also shown that algae, depending on their species, accumulated some metals selectively [40].

Another example in this field of research is the study in the Black Sea, The Bosphorus, and the Sea of Marmara. Heavy metals (Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn) were determined in the samples of benthic sediments, algae and fish. It was observed that during the experiment, between 1998 and 2000, the contamination level of the analysed water reservoirs with these analytes decreased [41].

In the Thermaikos Gulf (Greece), the accumulation of heavy metals (Cd, Cu, Fe, Pb and Zn) in the alga *Ulva rigida* and in benthic sediments was assessed. The samples were collected along the gulf coastline. Seasonal changes in the concentration of the analysed analytes in algae was observed, connected, for instance, with a different dynamics of biomass growth, depending on the season. The authors indicated positive correlations in concentrations of Pb and Cu, Fe and Cu as well as Cd and Pb determined in algae and benthic sediments. The concentration of the analysed analytes in water and in algae was decreasing in accordance with the column Fe > Zn > Pb > Cu > Cd. On the basis of the conducted studies the authors concluded that the alga *Ulva rigida* may be good biomonitors of aqueous environment contamination with metals: Pb, Zn and Cd [42, 43]. In the biomonitoring studies in the Gulf of Thermaikos the algae *Gracilaria verrucosa* [44] and *Enteromorpha linza* [45] were also used.

On the basis of the literature review presented above, the author of this paper marked on the map in Fig. 1 the saltwater ecosystems covered with biomonitoring in which different species of algae were used.

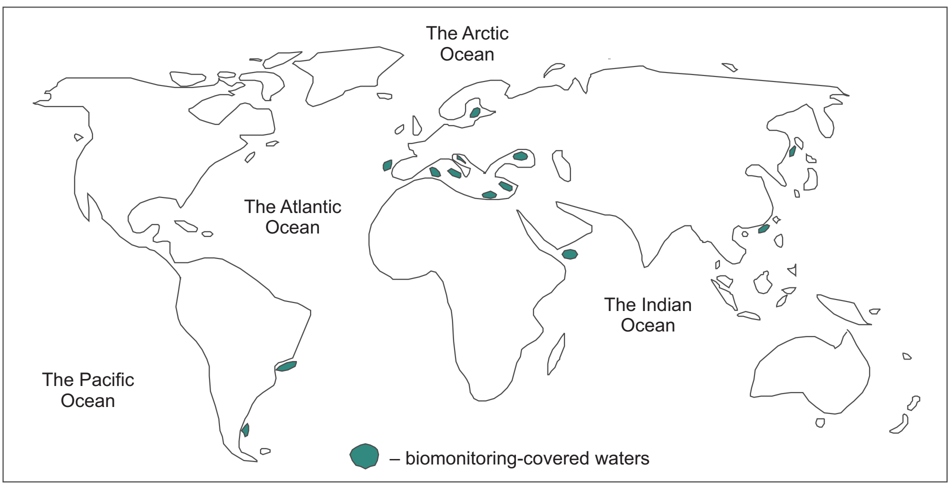

Fig. 1. Oceanic and sea waters covered with biomonitoring with algae

Biomonitoring studies with the application of algae were also conducted in Poland (the Baltic Sea) [46, 47], Russia (the Sea of Japan) [48], China (the waters of the South China Sea at the coast of Hong Kong were monitored) [49, 50], Portugal (the waters of the Ria de Aveiro lagoon) [51], Italy (the Mediterranean Sea, the Tyrrhenian Sea, the Adriatic Sea) [52–54], Bulgaria (the Black Sea) [55, 56], Greece (the Aegean Sea) [57], Algeria (the Mediterranean Sea) [58], Egypt (the Mediterranean Sea) [59], Turkey (the

Black Sea, the eastern coast of the Aegean Sea, the Marmara Sea) [60–62], Brazil (the waters of Sepetiba Bay, Guanabara Bay, Ribeira Bay and the Gulf of San Jorge were monitored) [63–67].

Literature analysis regarding the biomonitoring of saltwater ecosystems confirmed that algae may be used as bioindicators. On the basis of the analysis of chemical composition of thalli, it can be determined whether a given water reservoir is contaminated with a specific analyte. However, there is no reliable method, the application of which could facilitate the assessment of contamination of a given reservoir with heavy metals through the analysis of these metals concentrations in algae samples.

Aqueous ecosystems contamination with heavy metals – analytes bioaccumulation in situ

The algae, which live in aqueous ecosystems, that are diversified in terms if physicochemical parameters, are characterised by various concentrations of heavy metals in thalli. Table 2 presents average concentrations of heavy metals in thalli collected from different aqueous ecosystems.

Table 2

Heavy metal concentrations in algae living in different ecosystems [μg/g d.m.]

Mn	Fe	Cu	Zn	Cd	Pb	Alga	Location	Ref.
176	115	47	34	—	95	*Spirogyra adnata*	the Nainital Lake, India	[16]
—	—	—	—	38.1	11.6	*Ulva lactuca*	the Pulicat Lake, South East India	[20]
106.4	—	22.7	38.3	0.21	14.3	Algae	the Way Diengling stre-am	[23]
658.0	—	113.1	109.2	1.16	26.0	Algae	the Umkhrah stream	[23]
45.1	778	13.8	21.2	< 0.02	< 0.1	*Ulva lactuca*	the Black Sea – the Sile station	[41]
41.1	902	11.3	13.5	< 0.02	< 0.1	*Ulva lactuca*	the Black Sea – the Si-nop station	[41]
192.4	8821	18.2	43.2	< 0.02	< 0.1	*Enteromorpha linza*	the Black Sea – the Sile station	[41]
778.4	616.1	9.4	16.9	0.22	1.1	*Fucus vesiculosus*	the North Sea – Holy Is-land, England	[68]
105.2	2197	29.1	286.4	1.10	15.4	*Ulva lactuca*	the Marmara Sea – the Menekşe	[62]
—	4149	74.1	5.0	—	93.5	*Ulva lactuca*	the Tolo Harbour, Hong Kong	[69]

Concentrations of heavy metals accumulated in algae *in situ* depend, for instance, on the characteristic (different, frequently changeable) contamination of waters and benthic sediments with heavy metals, their bioaccessibility for the elements of biota, algae sorption properties and the concentration of other, outside analytes in the aqueous reservoir.

In order to assess the bioaccumulation capacity of heavy metals by the biota elements, *eg* algae, a bioconcentration factor (*BCF*) is determined [70].

$$BCF = c_{x,a} / c_{x,w} \quad (1)$$

where: x – heavy metal ions,
$c_{x,a}$ – heavy metal concentration in algae [mg/kg d.m.],
$c_{x,w}$ – heavy metal concentration dissolved in water collected from the site of algae sample collection [mg/dm^3].

The values of the factor *BCF* > 1000 reveal very good sorption properties of the biota elements (*eg* the aqueous plants: *Hydrocotyle umbellata* [71] and *Eichhornia crassipes* [102], zooplankton [70, 73], and algae [74]), and suggest the possibility of their application in biomonitoring and effective phytoremediation [71]. Table 3 shows the values of the *BCF* factor showing the accumulative properties of the analysed species of algae in relation to the analysed heavy metals.

Table 3

Values of the bioconcentration factor (*BCF*)

Mn	Fe	Ni	Cu	Zn	Cd	Pb	Ref.
Spirogyra sp.							
12590	9090	2568	> 4200	12531	> 1527	> 1372	[75]
Enteromorpha sp.							
—	—	—	2833	—	1125	4423	[76]
Bostrychia sp.							
90000	$> 18 \cdot 10^6$	5555	47778	93810	—	$> 7 \cdot 10^5$	[39]
Caloglossa leprieurii							
20000	$> 1 \cdot 10^7$	4444	37778	76429	—	$> 3 \cdot 10^5$	[39]

The determination of the values of bioconcentration factors reveals very good sorption properties of the analysed algae. A varied bioaccessibility of the determined heavy metals in relation to the algae thalli may be influenced by, for instance, the form of analyte existence, concentration and the time of exposure of a plant to its activity, cellular distribution of metal, forms of storing and detoxication of metal, interactions with other compounds present in the cell and the specific features (*eg* adaptive capacity) [77].

In order to assess the contamination of waters with heavy metals, the algae are used as biosensors in the passive biomonitoring (the analysis of the composition of algae naturally existing in the aqueous ecosystem). However, there are also studies within the active biomonitoring (transplanting/moving the algae from waters or cultures poorly contaminated with heavy metals to the ecosystems contaminated with the analyes). In France, the waters of the New Caledonia lagoon were studied. The algae *Lobophora variegata* were transplanted from waters with poor heavy metal contamination (the Maa Bay), with the concentration, *eg*, Cr < 0.02 μg/g d.m. to the waters contaminated with mining sewage (the Boulari Bay). In the three-month transplantation period, an increase in the analyte concentration in thalli was observed. Chrome concentration increased to app. 200 μg/g d.m. Transplantation of algae from the Boulari Bay to the area of the Maa Bay did not lead to a statistically significant desorption of analytes from the algae samples [78].

The authors of the publications on biomonitoring of aqueous ecosystems with the application of algae very rarely provide the assessment of uncertainty of the applied measurement methods. On the basis of the studies carried out by the author of this paper regarding the assessment of heavy metal contamination of the Large Turawa Lake (the south-western Poland), it was observed that the biomonitoring results may contain up to 26 % of the measurement uncertainty. For the samples of the alga *Spirogyra* sp. collected from three measurement sites of the studied reservoir, the value of the coefficient of variation CV_{mean} determined on the basis of the standard deviation value ranged between 20–26 %. The SD value differences for the samples collected from the same site result from heterogeneity of the studied material [25].

Summary and conclusions

Biomonitoring is gaining popularity as a method for assessing the pollution of various ecosystems, including the aquatic environments. It has the fundamental advantages of the inexpensive and straightforward sampling method which does not require any special personnel training, and of the natural ability of biomonitors to accumulate the accessible forms of pollutants. The analysis of concentrations of trace elements bound in thalli provides information regarding the pollutants introduced to the aqueous ecosystems. Studies with algae indicate a possibility of their application in the assessment of aqueous ecosystems contamination with heavy metals, in identification of contamination sources and in the long-term monitoring of the contamination level of the studied reservoirs. For the biomonitoring purposes the following algae are most frequently used: unicellular algae constituting phytoplankton and multicellular organisms, *eg Spirogyra adnata*, *Spirogyra species*, *Palmaria palmata*, *Ulva lactuca* and *Ulva rigida*. Whether a given species of algae can be used for water biomonitoring depends on its prevalence, which allows for collection of adequate amount of material. Also, in order to fulfil the requirement of representation in relation to the studied ecosystem, a given species of algae should manifest a relatively sedentary lifestyle.

One of the problems connected with biomonitoring is the validation of research procedures, which results from the multidimensionality of the algae-environment interactions. The sorption mechanisms and dynamic equilibria in the system algae-solution, including contamination bioaccumulation time have not yet been recognised sufficiently. Determining correlations between analyte concentrations in algae and in water, as well as identification of the abiotic factors influencing sorption may in the future be used for the purpose of a simple measurement of heavy metal concentrations in surface waters, which may be helpful in the development of a classification method of surface waters in which the algae will play the role of water quality biosensors.

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GLONY JAKO ŹRÓDŁO INFORMACJI O ZANIECZYSZCZENIU METALAMI CIĘŻKIMI WÓD POWIERZCHNIOWYCH

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Abstrakt: Publikacja jest syntetycznym zbiorem informacji, które dotyczą celów i kierunków badań związanych z wykorzystaniem glonów do oceny zanieczyszczenia wód powierzchniowych metalami ciężkimi. Zaprezentowane przykłady dotyczą wykorzystania glonów w biomonitoringu ekosystemów wodnych, który w wielu krajach, także w Polsce stał się trwałym elementem zintegrowanego monitoringu środowiskowego. Omówiono wyniki badań prowadzonych *in situ*, których celem była ocena zanieczyszczenia wybranych ekosystemów wodnych metalami ciężkimi na podstawie składu chemicznego plech, a także wskazanie potencjalnych źródeł tych analitów. Analizę wyników badań omówionych w cytowanej literaturze poparto wnioskami z badań własnych.

Słowa kluczowe: glony, metale ciężkie, wody powierzchniowe, biomonitoring