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Original Research

The Occurence of Cyanobacteria Blooms in the Obrzyca River Catchment Area (Poland), a Source of Drinking Water

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Abstract

The issues presented in this study concern the important problem of the occurrence of cyanobacterial blooms in surface water used for water supply purposes. The objective of this study was to analyze the occurrence of cyanotoxic risk in the catchment area of the Obrzyca River, which is a source of drinking water for the inhabitants of Zielona Góra. At the points of Uście and at its tributary at Wojnowo, the river was more exposed to the blooms of toxigenic algae, whose active microcystins synthesize more here than in other places (Sadowo, Chwalim, Ostrzyce, Lubiatów). Throughout the study, conducted in 2008-12, we observed several times that the amounts of cyanobacteria exceeded $500 \cdot 10^3$ individuals per litre (ind.·dm^{-3}). The observed cyanobacterial blooms were dominated by the following species: *Dolichospermum flos-aquae* and *Planktothrix agardhii* in the Obrzyca River at Uście, and *D. spiroides, D. affinis,* and *Aphanizomenon flos-aquae* at its tributary in Wojnowo. The maximum value of intracellular MC-LR, totalling 21.4 µg·dm^{-3}, was found at Wojnowo in September 2011. The analysis of the physico-chemical water quality indicators showed that the places along the Obrzyca River where periodic cyanobacterial blooms took place were characterized by eutrophic conditions, and the total nitrogen to total phosphorus ratio (N/P) fell within the range of 10 to 16, and the water temperature exceeded 20ºC. In order to assess the toxic hazard caused by periodic cyanobacterial blooms in the catchment area of the Obrzyca River for drinking water for Zielona Góra residents, regular monitoring of the cyanobacteria and their toxins is required – especially at the points in Uście and Wojnowo as well as at the water intake at the water treatment plant (WTP) in Zawada (in central-western Poland).

Keywords: cyanobacterial bloom, water treatment plant, microcystins, toxigenic genotypes

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Introduction

In eutrophic waters, and especially where the concentrations of total phosphorus, ammoniacal nitrogen, and nitrate nitrogen exceed 0.1 mg·dm^{-3} and the total nitrogen exceeds the value of 1.5 mg·dm^{-3}, cyanobacteria can multiply at a fast rate, which results in the formation of bloom [1-2]. A bloom is the effect of a massive development of various species of phytoplankton groups in water: cyanobacteria, diatoms, chlorophyta. A visible effect of the bloom is the change in the colour of the water body (green, olive green, brown, red) and the formation of foam or scum. Massive growth of plankton in temperate climate waters is typical of the period from May to September-October [3]. However, in European water bodies cyanobacterial blooms can even persist in wintertime (under ice) [4]. In Poland the occurrence of cyanobacterial blooms able to synthesize microcystins throughout the year and dominated by *Planktothrix agardhii* (Gomont) Anagnostidis and Komarek have been observed [5]. This fact indicates the need for year-round monitoring of surface waters in the vicinity of water intakes in a temperate climate. Blooms occur when the amounts exceed 500·10^3 individuals (colonies, filaments, cells) in one dm^3 or 20-50 µg·dm^{-3} of chlorophyll-a concentration, which corresponds to biomass excceding 5 mm^3·dm^{-3} [6]. Apart from making water treatment more difficult (the choking of filters), cyanobacterial blooms deteriorate the quality of water – not only by degrading its organoleptic properties (unpleasant taste and smell) but also by contaminating water with cyanotoxins that are microcystins, which belong to the group of hepatotoxins. Consuming water containing these hepatotoxins produces such symptoms as: acute liver damage, skin rash, fever, vomiting, and diarrhoea [7]. According to the WHO guidelines, the permissible threshold value of microcystin (MC-LR) in drinking water equals 1 µg·dm^{-3} [8]. This threshold value has been adopted in legal regulations in many countries, including the Czech Republic, France, Norway, Spain, China, or Japan [6]. The threats resulting from the presence of cyanobacterial blooms and cyanotoxins in surface water constitute a global and important problem concerning the sources of drinking water [9-12].

Microcystins were also detected in treated drinking water in Poland (maximum 0.8 µg·dm^{-3}) [13]. The water was drawn from a lowland dam reservoir dominated by *Microcystis aeruginosa* (Kützing). The problem, however, was resolved in subsequent years by mixing surface water with groundwater [14].

Also in the Zawada WTP in Lubuskie Province (central-western Poland), one stage in the process of potential hepatotoxin elimination involves mixing surface water with groundwater. One of the sources of raw water for the Zielona Góra water supply system is the surface water drawn from the Obrzyca River, which is used for supplying the population (mainly of the town of Zielona Góra) with drinking water (Fig. 1). Therefore, the quality of water in the river is of vital importance, especially in the context of the potential presence of toxic cyanobacteria. This study was aimed at assessing the potential threat from cyanobacteria and determining the concentrations of microcystins at six points along the catchment area of the Obrzyca River from its start to the drinking water intake.

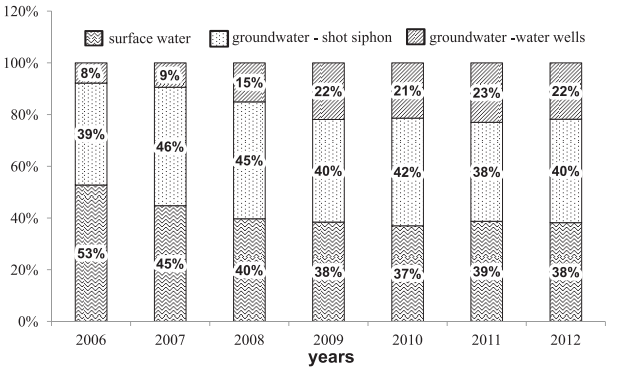

Fig. 1. The percentage of water used to supply the population of the town of Zielona Góra with particular intakes.

Material and Methods

Sampling

The samples were taken from the catchment area of the Obrzyca River from May to September 2008-11, and from May to October 2012. Altogether, we took 156 samples. The sampling points were located along the Obrzyca River at the following places: Lubiatów (the beginning of the river course), Uście, Chwalim, Sadowo (the end of the river course, surface water intake), and along its tributaries in Wojnowo and Ostrzyca (Fig. 2, Table 1). The above sampling points were selected for testing because they have been under permanent control concerning the physical and chemical water quality indicators. Samples for hydrobiological analysis were collected at 10 dm^3 with buckets and then filtered through a mesh plankton, diameter 10 mm (final volume 0.25 dm^3). The samples for other analyses were collected in plastic bottles with a capacity of 5 dm^3, which were transported in a thermos on ice. On the sampling site we determined temperature and dissolved oxygen content in water. After arrival at the laboratory the samples were separated into individual physical and chemical tests.

Hydrobiological Analysis

Hydrobiological analysis was conducted with the use of a Sedgwick Rafter counting chamber with a volume of 0.001 dm^3 in a given number of fields with the following parameters: height 1 mm, area 1 mm^2. An MN 358/A (OPTA-TECH, Poland) microscope was used for observation. The cyanobacteria cell count was carried out under 160x magnification and the interspecies cyanobacteria count was conducted under 640x

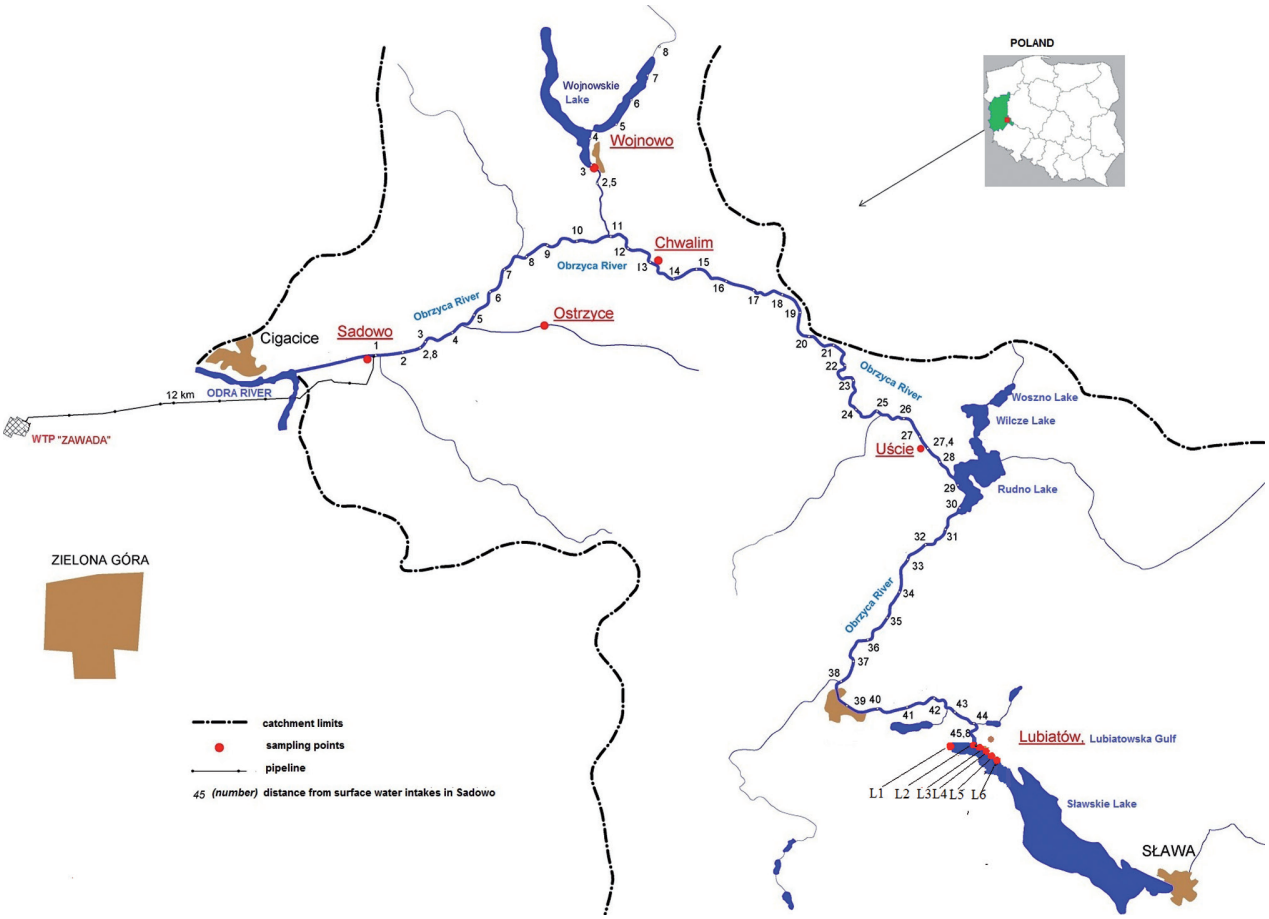

Fig. 2. Test area.

magnification. Samples for hydrobiological analyses were preserved with Lugol's iodine. The plankton count was calculated as follows:

Table 1. Sampling points.

Sampling point	Commune/ county	Geographic location	Distance from WTP in Zawada [km]*
Lubiatów	Sława/ Wschowa	N 51°55`14`` E 15°57`20``	57.8 (45.8)**
Uście	Kolsko/ Nowa Sól	N 52°00`40`` E 15°56`50``	39.4 (27.4)
Chwalim	Kargowa/ Zielona Góra	N 52°03`43`` E 15°49`13``	25.0 (13.0)
Wojnowo	Kargowa/ Zielona Góra	N 52°05`43`` E 15°47`14``	26.0 (14.0)
Ostrzyce	Trzebiechów/ Zielona Góra	N 52°02`51`` E 15°45`39``	19.5 (7.5)
Sadowo (surface water intake)	Sulechów/ Zielona Góra	N 51°02`18`` E 15°40`27``	12.0 (0.0)

* along the riverline ** in brackets - distance along the riverline to the surface water intake in Sadowo

$$N = \frac{XAd}{av}$$

…where:
N – number of units (colonies) in 0.001 dm^3
X – total count in a given number of fields
A – total volume of chamber (0.001 dm^3)
d – dilution ratio (at dilution ratio 1:1, d = 2)
a – number of fields counted
v – volume of one field ($1 \cdot 10^{-6}$ dm^3)

When converting the colony count from 0.001 dm^3 into 1 dm^3 sample concentration, the plankton net was taken into account [15]. For cyanobacteria with straight filaments 100 µm was set as one individual. Curved trichomes of *Dolichospermum* spp. and one colony of *Microcystis* spp. was indicated as individuals [16]. Species identification was done with identification keys for algae: [17-22].

Genetic Analysis

In order to detect toxigenic algae genotypes over the period June to October 2012, the *mcyE* gene was used in 10 water samples taken at Wojnowo and Uście. Analysis involved the identification of a fragment of a sequence of the *mcyE* (405 bp) gene, coding for the mcyE enzyme, which takes part in the biosynthesis of

microcystins. Gene identification was carried out using the PCR method (Eppendorf, Mostercycler) following the procedure described by Rantala et al. [23]. The analysis of the band intensity was conducted by means of the ImageI 1.40 program according to the procedure described by Mankiewicz-Boczek et al. [5]. Earlier research has shown that using densitometric semi-quantification is a simple, low-cost, quick, and repetitive screening method for the estimation of *mcyE* gene density related to the occurrence of toxigenic genotypes of microcystin-producing cyanobacteria [5].

Immunoenzymatic Analysis

To determine the intracellular microcystin we used an ELISA assay. The samples were filtered through GF/C Whatman glass microfibre. The final volume of the filtered sample was 0.50 dm^3. The collected suspension was then frozen at -20ºC [24]. Defrosted three times, the filter papers with the filtered suspension were dried, then filled with 75% methanol (c. 0.005 dm^3) and stored at 2-8ºC. After a 24-hour period the sample was homogenized by hand by grinding the filter paper and then centrifuged at 4,000 rpm for 10 minutes using the MPW-350e (MPW, Poland) laboratory centrifuge. The supernatant was then decanted and made up with water to its original volume before filtration [own method]. In order to determine the MC-LR equivalent we used ready-made Abraxis test kits. The absorbance values of the samples were read at two wavelengths – 450 and 605 nm – using a DR 5000 (HACH, Germany) spectrophotometer. After the spectrophotometric measurements, percentage of Bo was calculated (percentage of relative standard or sample inhibition in relation to the negative control). On the basis of the % Bo results and using the calibration curve we determined the MC-LR equivalent in test samples. The detection limit was 0.15 $\mu g \cdot dm^{-3}$ [25].

Physical and Chemical Water Quality Indicator Analysis

The total phosphorus and nitrogen content was determined by spectrophotometry using a cuvette test using a Pharo 300 spectrophotometer (Merck, Germany). The contents of ammoniacal, nitrate nitrogen, orthophosphates, and total iron were determined by spectrophotometry on the method of HACH. Absorbance measurement were made by a DR 5000 spectrometer (HACH, Germany). Examination of pH was carried out electrochemically using a 540 GLP pH-meter (WTW, Germany) and 81 SenTix electrode (WTW, Germany). The water temperature and dissolved oxygen concentration was determined using an on-site HQ 40d oxygen meter (HACH, Germany). Examination of the suspension was determined by gravimetric method using CP224S-OCE weight (Sartorius, his Göttingen). Turbidity was measured by nephelometry using a 2100 IS AN turbidimeter (HACH, Germany).

Statistical Analysis

Statistical analysis was performed using Statistica 10.0 by Statsoft and Excel 2010 by Microsoft. The calculations included the analysis of Pearson's r correlation at the significance level $\alpha = 0.05$.

Results and Discussion

Cyanobacterial Blooms and Microcystins Content

The monitoring of the amount of cyanobacteria in surface waters and especially drinking water sources is a fundamental element of a quality control system. In water exposed to the risk of cyanobacteria contamination another stage in the monitoring process is to increase its frequency. Many water treatment plants drawing surface water use hydrobiological analyses as a tool of early warning against cyanotoxin risk, for instance in Poland the Sulejowski reservoir [13, 26], the Dobczyce reservoir [27], and the Siemianówka reservoir [28]; in Europe the Kurtboğazi and Çamlidere reservoirs (Turkey) [29], Lake Zurich (Switzerland) [30], and the Garaši and Bukulja reservoirs (Serbia) [12]; and around the world Missisquoi Bay of Lake Champlain (Canada) [9] and Vaal Dam in South Africa [11]. The first warning level for treated water is given when the amount of cyanobacteria cell count in raw water exceeds 2000 per ml or biomass content exceeds 0.2 $mm^3 \cdot dm^{-3}$. At this warning level tests should be performed for the quantitative count of cyanotoxins in raw water [31-32].

In the analyzed samples from the Obrzyca River in Sadowo, Chwalim, Lubiatów, and in its tributary at Ostrzyce no cyanobacterial blooms were detected (colony count <100 thousands$\cdot dm^{-3}$). In the tested samples from the catchment area of the Obrzyca River cyanobacterial blooms occurred at Uście (June and September 2012) and in its tributary at Wojnowo (June 2009, September 2011, July 2012). During the massive occurrence of cyanobacteria the dominant species were the following: *Dolichospermum spiroide*s (Klebahn)*, Dolichospermum affinis* (Lemmermann)*, Dolichospermum flos-aquae* (Lyngbye) Brébison ex Bornet et Flahault*, Aphanizomenon flos-aquae* Ralfs ex Barnet et Flahault*, Planktothrix agardhii*, and their count ranged within $606 \div 882 \cdot 10^3$ ind.$\cdot dm^{-3}$ (Tables 2-3).

P. agardhii was abundant in the lakes of Wielkopolska, Lubuskie, and Lublin Province. The species is a known producer of demethylated microcystin variants. *Dolichospermum* genera is also common in Poland and causes cyanobacterial blooms, for example in Lake Orle and Lake Białe and Zemborzycki Reservoir with the genera *Aphanizomenon* and *Planktothrix*. *Dolichospermum* sp. can produce different types of toxins: hepatotoxic microcystins, cylindrospermopsin, neurotoxic anatoxin-a, and saxitoxin. *Aphanizomenon* sp. is one of the most commonly occurring cyanobacterium in Polish fresh

Table 2. The characteristics of cyanobacteria in the Obrzyca River at Uście.

Sampling months [month/year]	Cyanobacteria amount [thousand ind.·dm^{-3}]	Dominant species*	Intracellular microcystins (ELISA) [µg·dm^{-3}]
05/2008	38.5	*Limnothrix redeckei*	0.50
06/2008	41.6	*Limnothrix redeckei*	0.40
07/2008	11.5	*Dolichospermum flos-aquae*	0.30
08/2008	70.6	*Dolichospermum flos-aquae*	1.16
09/2008	176	*Microcystis aeruginosa*	4.26
05/2009	146	*Dolichospermum affinis*	0.78
06/2009	40.6	*Dolichospermum spiroides*	0.44
07/2009	311	*Dolichospermum spiroides*	0.51
08/2009	266	*Microcystis flos-aquae*	8.50
09/2009	173	*Microcystis flos-aquae*	4.09
05/2011	9.36	*Dolichospermum affinis*	<0.15
06/2011	129	*Dolichospermum flos-aquae*	1.80
07/2011	252	*Planktothrix agardhii*	15.7
08/2011	187	*Dolichospermum affinis*	3.97
09/2011	78.6	*Planktothrix agardii*	15.7
05/2012	80.1	*Limnothrix redeckei*	1.20
06/2012	606	*Dolichospermum flos-aquae*	4.30**
07/2012	290	*Aphanizomenon flos-aquae*	3.70**
08/2012	233	*Planktothrix agardhii*	4.80**
09/2012	**657**	*Planktothrix agardhii*	5.00**
10/2012	263	*Planktothrix agardhii*	5.45**

*in bold type: species dominant during blooms; ** *mcyE* gene detected,
maximum values of intrcellular microcystins in one month were underlined.

waters (Lake Świętokrzyskie, Lake Malta, Warta River) and tends to occur in association with *Dolichospermum* species. Some strains of *Aphanizomenon* produce cylindrospermopsin (*Aph. flos-aquae, Aphanizomeno gracile* Lemm.) and neurotoxic compounds such as saxitoxins (*Aph. gracile*), anatoxin-a (*Cuspidothrix, Aphanizomenon issatschenkoi* (Usač)) and homoanatoxin-a [33-34].

Other dominant species detected in the most likely to bloom sampling points in the Obrzyca River were: *Microcystis aeruginosa*, *Microcystis flos-aquae* (Wittrock) Kirchner, *Limnothrix redeckei* (van Goor) Meffert, and *Woronichinia naegeliana* (Unger) Elenkin (Tables 2-3).

The study shows that the amounts of cyanobacteria in the Obrzyca River, especially at Uście, grew from year to year. Also, high concentrations of intracellular microcystins were noted, produced by, among otherthings, the cyanobacteria of the *P. agardhii* species, whose growth and dominance was observed in 2011 and 2012. The *P. agardhii* species is particularly dangerous because it is biochemically capable of producing three times the amount of microcystins than other cyanobacteria, for example *Microcystis* sp. [33-34]. In the future this process may have a negative impact on water quality (also in sections of the river closer to water intake point for water supply purposes). Therefore, regular monitoring of cyanobacteria along the whole river course should be conducted, especially at inflow nodes of tributaries starting in lakes and at the surface water intake. When the threat of excessive cyanobacteria occurs in the drinking water intake, measures should be taken to reduce their negative impact. The amount of phytoplankton, including cyanobacteria, can be reduced in the processes of coagulation or flocculation as a result of aggregation of smaller particles into bigger ones. The efficiency in removing cyanobacteria during coagulation depends on the optimization of the coagulant dosage and the pH of the process. During coagulation some problems such as cell lysis may occur, and thus the release of intracellular toxins [9].

The amount of intracellular microcystins in the Obrzyca River at Uście ranged from <0.15 to 15.7 µg·dm^{-3}.

Table 3. Characteristics of cyanobacteria in the tributary of the Obrzyca River at Wojnowo.

Sampling months [month/year]	Cyanobacteria amount [thousand ind.·dm^{-3}]	Dominant species*	Intracellular microcystins (ELISA) [µg·dm^{-3}]
05/2008	0.62	*Dolichospermum affinis*	<0.15
06/2008	15.2	*Dolichospermum affinis*	<0.15
07/2008	27.8	*Dolichospermum affinis*	<0.15
08/2008	100	*Dolichospermum affinis*	2.50
09/2008	453	*Microcystis aeruginosa*	3.50
05/2009	1.96	*Dolichospermum affinis*	<0.15
06/2009	**882**	*Dolichospermum spiroides*	0.32
07/2009	103	*Dolichospermum spiroides*	0.99
08/2009	104	*Woronichinia naegeliana*	2.20
09/2009	103	*Dolichospermum spiroides*	3.50
05/2011	0.46	*Dolichospermum affinis*	<0.15
06/2011	75.4	*Dolichospermum flos-aquae*	<0.15
07/2011	22.5	*Planktothrix agardhii*	0.77
08/2011	**623**	*Dolichospermum affinis*	9.70
09/2011	338	*Planktothrix agardhii*	21.4
05/2012	0.08	*Limnothrix redeckei*	<0.15
06/2012	226	*Aphanizomenon flos-aquae*	<0.15**
07/2012	**650**	*Aphanizomenon flos-aquae*	<0.15 **
08/2012	77.2	*Planktothrix agardhii*	1.61 **
09/2012	221	*Planktothrix agardhii*	5.45 **
10/2012	336	*Planktothrix agardhii*	5.80 **

*in bold type: species dominant during blooms; ** *mcyE* gene detected,
maximum values of intrcellular microcystins in one month were underlined.

The maximum amounts of tested cyanobacteria occurred in the last sampling months of a given year. The maximum contents of intracellular microcystins in the catchment area of the Obrzyca River at Wojnowo were detected in September of particular study years and their values ranged from 3.50 to 21.4 µg·dm^{-3}.

Similarly, the highest microcystin concentrations in both lakes Lubosinskie and Bytynskie (Wielkopolska Region, western Poland) were detected in early autumn [35].

Maximum concentrations of intracellular microcystins detected in the Obrzyca River catchment repeatedly exceed the established by WHO permissible threshold value of microcystin (1 µg·dm^{-3}. MC-LR) in drinking water [8].

It is not always high numbers of cyanobacteria that resulted in high levels of intracellular microcystins. Despite the high content of cyanobacteria (May and July 2009 in the Obrzyca River at Uście or June 2009 and 2012 in a tributary of the Obrzyca River at Wojnowo), intracellular contents MC-LR determined by ELISA were below 1 µg·dm^{-3}. Such disproportionate results may be due to cell aging altering the production of toxic genotypes within the population of cyanobacteria [35], or the presence of conditions that do not stimulate the synthesis of microcystins in the cells (environmental factors, for example temperature, nutrient content) [36-37] or lack of strains able to synthesize hepatotoxins [38].

Studies presented by Neilan et al. [39] clearly show an effect of environmental conditions on cyanotoxin production in the laboratory, but it is still not completely known how these effects are regulated at a molecular level and how this translates into actual responses in the environment.

Toxic Genotypes of Cyanobacteria

Due to the massive periodic occurrence of cyanobacterial blooms at two sampling points located on the Obrzyca River (Uście) and its tributary (Wojnowo), in 2012 the monitoring included assessment of the occurrence of toxigenic genotypes of cyanobacteria

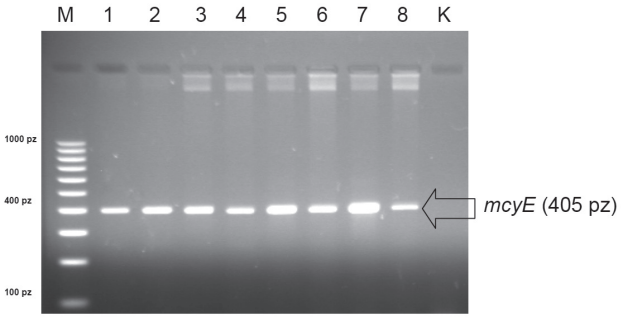

Fig. 3. The electrophoretic separation of the products of the amplification of the mcyE gene fragment in DNA samples from the Obrzyca River at Uście: 1 (30.06.2012) , 3 (18.07.2012). 5 (14.08.2012), 7 (07.09.2012) and in the tributary of the Obrzyca River at Wojnowo: 2 (30.06.2012), 4 (18.07.2012), 6 (14.08.2012), 8 (7.09.2012); M- DNA marker 100-1000 (Blirt SA, Poland); K-control with no DNA.

capable of producing microcystins. Toxigenic genotypes were detected in all 10 analyzed samples from Uście and Wojnowo (Tables 2-3, Fig. 3). On the basis of the band intensity of the *mcyE* gene it was determined that most toxigenic genotypes probably occurred at Uście in September, when *Planktothrix agardhii* was dominant (Table 2 and Fig. 3 (1, 3, 5, 7)).

The genetic studies performed in 2012 confirmed the results of hydrobiological analyses concerning the occurence of microcystin-producing cyanobacteria. Both in the Obrzyca River at Uście and in its tributary at Wojnowo, we detected toxigenic genotypes capable of synthesizing hepatotoxins. The largest amount of amplification product and the largest amount of cyanobacteria biomass were detected in samples taken in September, at the end of the vegetation period, in which the dominant species was *Planktothrix agardhii*.

Table 4. Values of correlation coefficients between the amount of cyanobacteria (CYA) and water quality indicators.

Water quality indicator	CYA
N_{total}	**0.22***
NH_4	0.02
NO_3	**-0.33***
PO_4	-0.03
P_{total}	-0.04
N/P	-0.04
Fe_{total}	**0.19***
Turbidity	**0.51***
pH	**0.40***
Total suspension	**0.39***
Water temperature	**0.22***
Dissolved oxygen	**0.34***

Statistically significant correlation coefficients ($p < 0.05$) were marked in bold and with *

Based on the presence of the *mcyE* gene Rantala et al., [23] proved that in Finnish lakes, where the dominant species is *Planktothrix agardhii*, 63% constitute clones capable of synthesizing microcystins. In this study the activity of genotypes of toxic cyanobacteria dominated by *P. agardhii* was confirmed by further immunoenzymatic tests, which determined the maximum concentrations of intracellular microcystins to be 21.4 µg·dm^{-3} in the tributary of the river in Wojnowo and 15.7 µg·dm^{-3} at Uście.

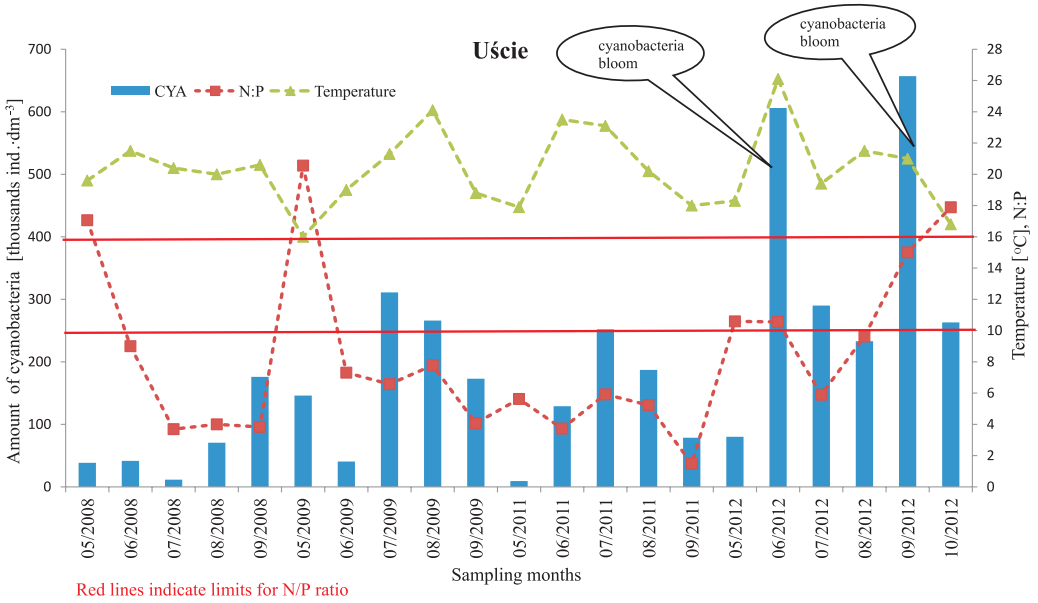

Fig 4. The amount of cyanobacteria vs water temperature and the N/P ratio in the Obrzyca River at Uście in 2008-2012.

The Impact of Physical and Chemical Parameters on the Formation of Cyanobacterial Blooms

In this study we analyzed 12 water quality indicators (ammoniacal nitrogen, nitrate nitrogen, orthophosphates, total phosphorus, N/P, total iron, the contents of dissolved oxygen, pH, water temperature, turbidity, and total suspension) in terms of their impact on the amount of cyanobacteria. The results of statistical analysis are presented in Table 4.

The following had a positive correlation with the amount of cyanobacteria in the catchment area of the Obrzyca River: total nitrogen, total iron, turbidity, total suspension, pH, water temperature, and the content of dissolved oxygen. There was a negative correlation between the amount of cyanobacteria and the content of nitrate nitrogen. There was no correlation between the amount of cyanobacteria and ammoniacal nitrogen, phosphorus compounds, and the N/P ratio.

In 2008-12 two cyanobacterial blooms occurred in the Obrzyca River at Uście (Fig. 4) in 2012. The first excessive amount of cyanobacteria ($606 \cdot 10^3$ ind.·dm^{-3}, or calculated 35.6 mm^3·dm^{-3} biomass) was observed in June, when the N/P ratio was 11 and water temperature 26.1ºC. The other bloom ($657 \cdot 10^3$ ind.·dm^{-3}, or calculated 45.1 mm^3·dm^{-3} biomass) in the Obrzyca River at Uście was noted in September of the same year, when the N/P ratio fell within the 10-16 range (value: 15) and water temperature exceeded 20ºC (21.0ºC). At the remaining sampling points no cyanobacterial bloom was observed as the N/P value remained outside the 10-16 range and water temperature was below 20ºC. The largest amount of cyanobacteria ($882 \cdot 10^3$ ind.·dm^{-3}, or calculated 31.5 mm^3·dm^{-3} biomass) in the tributary of the Obrzyca River at Wojnowo (Fig. 5) was observed in June 2009, when the N/P ratio was 12.5 and water temperature equaled 25ºC. In May 2012, when water temperature was 14.5ºC, the amount of cyanobacteria was at its minimum of $0.08 \cdot 10^3$ ind. dm^{-3}, despite the fact that the ratio value remained within the 10:16 range.

Dolman et al. [40] observed an abundance of cyanobacteria when the TN:TP ratio ranged from 12 to 20. The highest biomass of *P. agraphia* equaled 34 mm^3·dm^{-3} and was detected for TN:TP ratio ranging from 12 to 16. For the same TN:TP values a maximum amount of *Dolichospermum* sp. (near 4 mm^3·dm^{-3} biomass) and *Microcystis* sp. (13 mm^3·dm^{-3} biomass) was observed.

Also in Lake Nakamum the peak of cyanobacteria amount was observed for minimum value of TN:TP ratio (near 25:1), and total biomass of cyanobacteria equaled 36 mm^3·dm^{-3}, whereas the most abundant species, *Aphanizomenon gracile*, equaled 16 mm^3·dm^{-3} [41].

In summer nitrogen is the element limiting the amount of cyanobacteria when the N/P ratio ranges within 10-20. It can then be taken up in the form of ammoniacal, nitrate, and nitrite ions [33].

The maximum growth rate of cyanobacteria takes place when water temperature exceeds 25ºC. This temperature optimum is higher than for chlorophyta or diatoms; therefore, the positive correlation between the amount of cyanobacteria and water temperature is only natural [33-34, 36].

During detected cyanobacterial blooms in the Obrzyca River at Uście and Wojnowo the contents of total phosphorus ranged from 0.10 to 0.22 mg·dm^{-3}, and for total nitrogen from 1.90 to 3.00 mg·dm^{-3}. Such nutrient concentrations are sufficient to multiply cyanobacteria. In Germany, 102 lakes of Brandenburg saw the highest

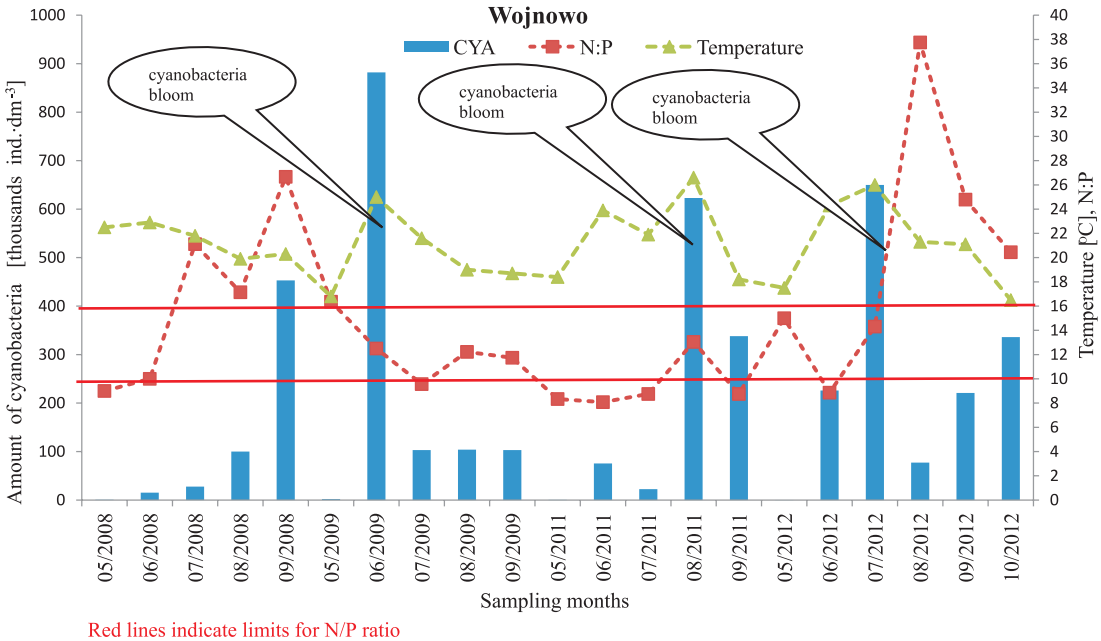

Fig. 5. The amount of cyanobacteria vs water temperature and the N/P ratio in the catchment area of the Obrzyca River at Wojnowo in 2008-2012.

cyanobacterial biovolume when total nitrogen content ranged from 1 to 2 mg·dm^{-3} and phosphorus from 0.05 to 0.25 mg·dm^{-3} [40] .

Orihel et al. [41] studied the impact of phosphorus, nitrogen, and total iron on the growth in phytoplankton biomass Total iron was shown to have a positive impact on the contents of cyanobacteria. Both phosphorus and iron were indispensable for the stimulation of biomass growth. Cyanobacteria have an especially high Fe demand for their physiological processes, including N_2 fixation. The catchment area of the Obrzyca River showed the existence of positive directly proportional correlations between total nitrogen and total iron and the amount of cyanobacteria (Table 4).

In the study by Peretyatko et al. [42] on 42 Belgian ponds, phytoplankton bloom with the dominance of cyanobacteria occurred at pH >8, whereas the blooms of other phytoplankton groups preferred pH to be relatively lower (pH<8). Other authors state that the maximum biomass growth of cyanobacteria takes place at pH = 9 [33]. The presented studies showed that there is positive correlation between the amount of cyanobacteria and the pH of water (Table 4).

In September 1994 at Zawada WTP, microstrainers were installed in order to reduce the amount of phytoplankton, including cyanobacteria, in raw water from the Obrzyca River. WTP is the only plant using microtrainers in the water treatment system in Poland [43]. The process of microstraining ensures significant reduction in the amounts of phytoplankton, including cyanobacteria. The efficiency of the microstraining process in the removing of cyanobacteria reaches up to 90%. The use of a non-reactive process of microstraining as a pre-treatment process of algae-rich water purification is justified because it reduces the amount of side-chain precursors of oxidation products and, indirectly, by reducing the amount of cyanobacteria – decreasing the contents of intracellular cyanotoxins [3, 44].

Water mixing supported by the use of microstrainers in the technological process at Zawada should eliminate microcystins from drinking water. An individual study carried out in 2009 showed that no microcystins were detected in drinking water for the inhabitants of Zielona Góra [3]. In order to eliminate any toxic threat from cyanobacteria to the Zielona Góra water supply system one should introduce constant MC-LR monitoring in drinking water.

Conclusions

1) Toxigenic cyanobacterial blooms occur in the catchment area of the Obrzyca River. It is therefore highly probable that cyanobacterial toxins may threaten the quality of drinking water intended for consumption by the inhabitants of Zielona Góra.
2) The Obrzyca River at Uście and in its tributary at Wojnowo were particularly exposed to cyanobacterial blooms, more than in other places. Amounts of cyanobacteria exceeding 500·10^3 ind.·dm^{-3} were observed several times. All determined cyanobacteria species were capable of synthesizing hepatotoxins. During the blooms the content of microcystins periodically reached the value of 21.4 μg·dm^{-3}.
3) In the tested waters the total nitrogen to total phosphorus ratio (N/P) in the observed cyanobacterial blooms was in the range 10-16, and water temperature exceeded 20ºC.
4) The use of very sensitive molecular methods for the detection of the genes of *mcyE* microcystin synthetase complements the microscopic analysis. These methods can be a tool of early warning against potential cyanobacterial threats, particularly in waters constituting drinking water sources.

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