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Toxicology

IMPACT OF BLUE-GREENS FROM GENERA OF *MICROCYSTIS* ON SOME AQUATIC ANIMALS

WPŁYW SINIC Z RODZAJ U*MICROCYSTIS* NA NIEKTÓRE ZWIERZĘTA WODNE

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Dynamics of mortality of chosen representatives of hydrofauna were examined according to changes of blue-greens (genera: *Microcystis*) algal concentrations. Algal concentrations that caused 50% (LC_{50}) and the total mortality of testing organisms were determinated. There was checked a possibility of the using pigment-cells' reactions and avlues of pH of blood of testing organism as indicators of toxic impact of blue-green algae.

INTRODUCTION

The intensive economic activity of man is the reason of growing trophia of lakes and reservoirs. Increasing and excessive input of nutrients (N and P) makes possible mass development of algae that quantity exceeds ecological norms and caused changes in water's ecosystems.

The Szczecin Bay is an example of still eutrophicated and polluting reservoir that is receiver of big amount of nutrients from Odra river. The compounds of phosphorus especially phosphates are crucial here. The Odra river through the Szczecin Bay transportes to the Baltic Sea 2740 tones of P–PO per year. So far 54.6% of total supply of phosphorus from territory of Poland.

Surplus of phosphorus loads have resulted in heavy mass algal blooms in waters of the Szczecin Bay. Mass development of phytoplankton was observed during warmer seasons of year and considered mostly blue-greens that become a predominated species of phytoplankton in this water region since 1984. Blooms of blue-green algae[1]

[1] 6.56. mln/dm^3 colonies of *Microcystis* in the Szczecin Bay in 1983 (Świerczyński and others 1986).

that occurred during summer dangerous impact on water's organisms of the Szczecin Bay. These algal blooms can cause limitations of development or even elimination other forms of life of this water region. As a result of the intensive photosynthesis process of *Microcystis* aeruginosa high levels of pH factor values were observed. Alkalization of water environment reaches extremely high level about 10.0 pH that was reported for the Szczecin Bay already in 1975 by Mutko (1979, 1986). Thus, probably, this kind of activity of this "toxic" blue-green algae either reduced quantity of population of *Dreissena polymorpha* Pall, and exerts an influence on radical changes in quantity and quality of phytoplankton, zooplankton, benthos, and ichtiofauna of the Szczecin Bay (Drzycimski 1986, Mutko 1986 /no published/, Kompowski and Pieńkowski 1986, Świerczyński 1986, Piesik and others /no published/). However big biomass of *Microcystis* being produced in this water region from June up to October of each year is negligible as item of diet of planktivorous fauna among other things, because of big sizes of cells of this blue-green algae species (Kadłubowska 1975, Kajak and others 1975, Opuszyński 1978, Kajak 1979).

Matter of *Microcystis* cells after their death is a source of the intensive putrefactive proecess in the water and on the bottom.

This process aggravates environmental conditions as a result of input of endotoxins and additional loads of nutrients (N and P)[2].

This blue-green algae species have a various influence on limitation of the living organisms development. Production and excretion of organic substances by blue-greens is known phenomenon (Spodniewska 1971). Blue-green algae of genera *Microcystic* produce endotoxins that contain hydroxylamine (Shelubsky 1951). These substances are excreted by living cells into water in high temperatures especially intensive if there is presence of sulphur compounds[3].

Endotoxins penetrate into water from dead cells during putrefactive processes. These compounds are extremely dangerous to fishes (Szerow 1974, Prost 1980 and others).

The other process of influence of blue-green algae on hydrobionts is production of species organic compounds that penetrates into organism of the animals e.g. fishes and increases of activity of thiamineses enzyme. This enzyme degrades vitamin B_1 then drives to avitaminosis. There is conjecture that thiamineses is produced and excreted by blue-green algae cells (Shelubsky 1951).

Taking into consideration either the data described above and author's own experience. The author of this paper tried to find out the least number of algae cells of

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[2] max 0.65 mg $P-PO_4^{3-}/dm^3$ in the Szczecin Bay was reported (Mutko 1986). 0.03 mg $P-PO_4^{3-}/dm^3$ was assumed as level that is enough to algal blooms occurrence (Vollenweider 1968, 1976).

[3] there is necessary to rate that the Szczecin Bay is the receiver of considerable loads of the sulphates in waste waters from Chemical Factory "Police".

genus *Microcystis* that causes the motality of 50% experimental animals and lethal dose of algae cells for these animals.

Moreover there was aimed at examine of possibilities of the using pigment cells of skin and blood reaction of tested fishes as the indicator of toxical effect of *Microcystis* and indirectly, as indicator of water's trophia as well.

METHODS

In the course of the realization of this work the method of algal cultivation in macroscale and controlling standard of tested organism were worked out.

Algae of *Microcystis sp.* for experimental purposes were cultivated in aquariums of total volumes from 30 to 100 dm^3. The liquid mineral-organic culture medium was used. Culture medium was formulated in the course of experiments. It base itself on the Lefevre's substrata. Surplus of the nitrates and phosphates were added and enrichment with micro-nutrients ($MnCl_2$, $CuCl_2$, H_2BO_3) and vitamins (A, B_1, B_2, B_6, C, D, E, PP) were applied.

According to the bacteriological rules the reaction of the culture medium was increased up to pH = 8.9 with $Ca(HCO_3)_2$. The reaction of these monocultures of algae were maintaining at level of pH = 9–10 by the means of constant illumination and stable temperature at 25°C (in accordance with Liebman 1960).

Level of macro and micro-nutrients were still controlled. Howeover fishes (*Carassius carassius, Carassius auratus*) and mollusc (*Dreissena polymorpha*) were put into each aquarium. Fishes were a biological agitator of algal cultures and preyed on zooplankton. Whereas *Dreissena polymorpha* eliminated all algae except big colonies of *Microcystis* – Fig. 1 (Kajak 1979, Stańczykowska 1977) so far *Dreissena* stabilizes development of blue-green algae. Fishes and mollusks accelerated nutrient cycle and supplied CO_2 that is limiting factor of algal growth (Odum 1982, Round 1981).

Material to experiments – *Microcystis* spp. were taken from culture with the planktonic conical net made with mill-gauze No. 25. Then so collected material was quantitative examined in order to obtain standard of concentration[4]. The notion of the "algal unit" was introduced. Number of 20 *Microcystis* cells were assumed as 1 algal unit. Because of high growth rate of blue-greens biomass (Rynolds, Walsby 1975) quantity of *Microcystis* was controlled in experimental vessels with applying algal units.

[4] Standard of concentration – such algal concentration in the water volume unit that there is possible to prepare phytoplankton natural concentrations with it under laboratory conditions.

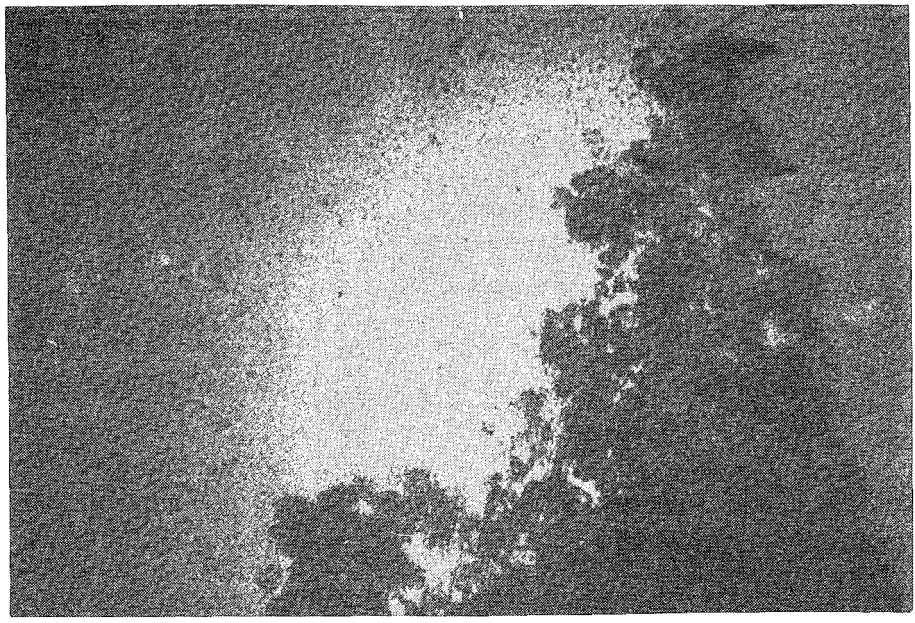


Fig. 1. Photography of *Microcystis* colony. This colony was isolated from algal monoculture (in accordance with methodic)

TESTING ORGANISMS[5]

The group of testing organisms taken into experiments consisted of: fry of crucian carp (Carassius carassius) and goldfish (Carassius auratus) (5–10 cm body length), fry of carp (Cyprinus carpio) of 80–100 g, adult specimens of European eel (100–300 g), and adult specimens of three-spined stickleback (*Gasterosteus aculeatus*) (8 cm body length). Fishes were of good state of health. State of health was controlled each time by examination of random chosen samples of fishes (minimum 5 specimens ineach sample). Total number of 2000 fishes was taken to the experiments. Fishes were adopted to aquarium conditions through mirimum 5 days.

However to group of testing organisms other water's animals were added: *Dreissena polymorpha, Chironomus* sp., and *Pallasea quadrispinosa.* These invertebrates were also acclimated to aquarium conditions through at least 5 days. All animals were fed during period of acclimation. One day before beginning of the experiment feeding of animals was discontinued.

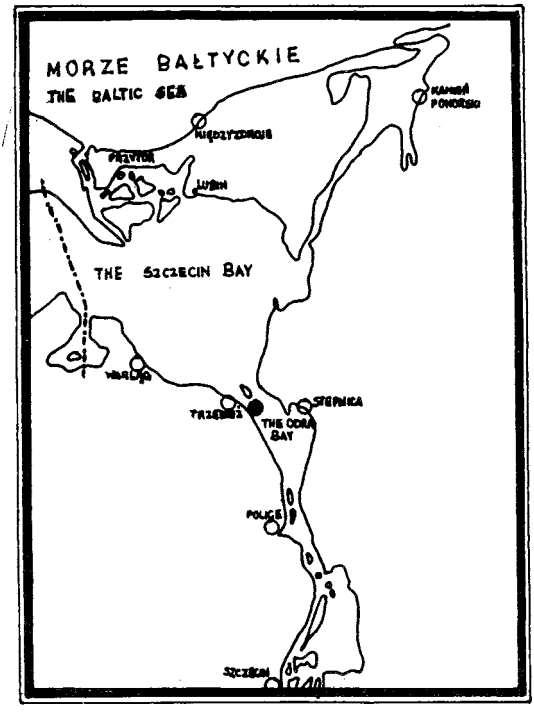
Sizes of the experimental aquarium dependent on size and quantity of tested organisms. Maximal total volume of aquarium for fishes was 50 dm^3. Minimal total volume

[5] animals taken to the experiment were collected in Ińsko Lake and neighboring fish pounds.

of aquarium for invertebrates was 0.5 dm^3.Standard controlling organisms were put into water without addition of algae. The tap water and water from Ińsko[6] lake were used. Only tap water was used to the experiments. The constant temperature (20°C)[7]and oxygenation were maintained. (There were some problems with ove-oxygenation because of intensive activity of *Microcystis*). The lethal dose LC_{50} was calculated on the basis of the results of bio-tests by the means the Reed's method (Kufel, Leonowicz-Babiak 1985).

Microscopic examination of pigment-cells fishes' skin

The pigment-cells of skin of the control and experimental fishes were examined by the microscope. Pigment cells of dorsal part fishes' skin (except for head and fins) were observed. Piece of fish skin for observations were taken from place situated close to back of fish head and processed according to the Burkowski's and Kulkin's preparation method with applying the modification of Lutnicka (1988)



• the test stand

Fig. 2

[6] for *Pallasea quadrispinosa* lower temperature was applied (15°C) because it is glacial relic species.

[7] most of animals were collected in mesotrophic Ińsko lake.

Collecting of blood samples to pH factor determination

The caudal part of captures fishes were immediately rinsed with distilled water and dried. The blood was sampled from the caudal artery and was collected into small glass bottles. The reaction of blood was measured with pH-meter. In order to conduction measurements of the osmotic resistance of blood (Ezell and others 1969) two drops of anti-coagulant were added and blood was stored in ice.

The field reconnaissance works were a supplement of the laboratory works. The biotests were conducted under the natural circumstances of test situated in the Szczecin Bay (Fig. 2). Fishes of two species were talen to these experiments: *Carassius carassius* and *Gasteroteus aculeatus*. Location of test stand was determined by hydrochemical and hydrobiological conditions of this water region such as, relatively high level of nutrients, sulphates, and blue- green algae concentrations. Fishes were put into small live-boxes that were placed on three different depths: 0.3 and 1 meter under surface and 0.3 meter above the bottom. The fishes behavior and mortality dynamics were followed respectively to the changes of the chosen factors such as: number of *Microcystis* expressed with the algal units, pH, temperature, oxygenation, PO_4^{3-} ions concentrations.

The field experiment because of its short duration (June 1990) can be only assumed as an attempt on transposition the results of the laboratory works onto natura l water conditions.

Same obtained results were checked with statistics methods by the means of parametric Duncan's test (significance level $\alpha = 0.05$), (Góralski A. 1974).

The results statistically confirmed are accented in further part of this paper.

RESULTS AND DISCUSSION

There was found out during the experimental works that the most sensitive to algal influence are respectively: three-stickleback (LC_{50} = 4.3 mln algal units per dm^3), carp (LC_{50} = 7.3 mln algal units per dm^3) and European eel (LC_{50} = 13.3 mln algal units per dm^3). Crucian carp was the most resistant species in terms of blue-greens impact (LC_{50} = 26.4 mln algal units per dm^3) – Tab. 1.

Moreover the lethal dose of algae concentration was determined for three-stickleback under laboratory circustames. The lethal dose was equal to 6 mln algal units per dm^3.

Increasing the luminous flux density up to level its natural parameters there was stated the toxic concentration of algae at 4 mln algal units per dm^3 level.

When such concentrations of algae were reached in the experimental aquariums pH values of the water oscillated close to 10 so much higher than the safe value.

Table 1

Mean values of LC_{50} examinated fishes – normal conditions
(mln algal units/dm^3)

Species	Number of series	LC_{50}	Standard deviation
Gasterosteus aculateatus	11	4.3	1.2
Cyprinus carpio	14	7.3	3.4
Anguilla anguilla	13	13.3	7.2
Carassius carassius	19	26.4	13.4

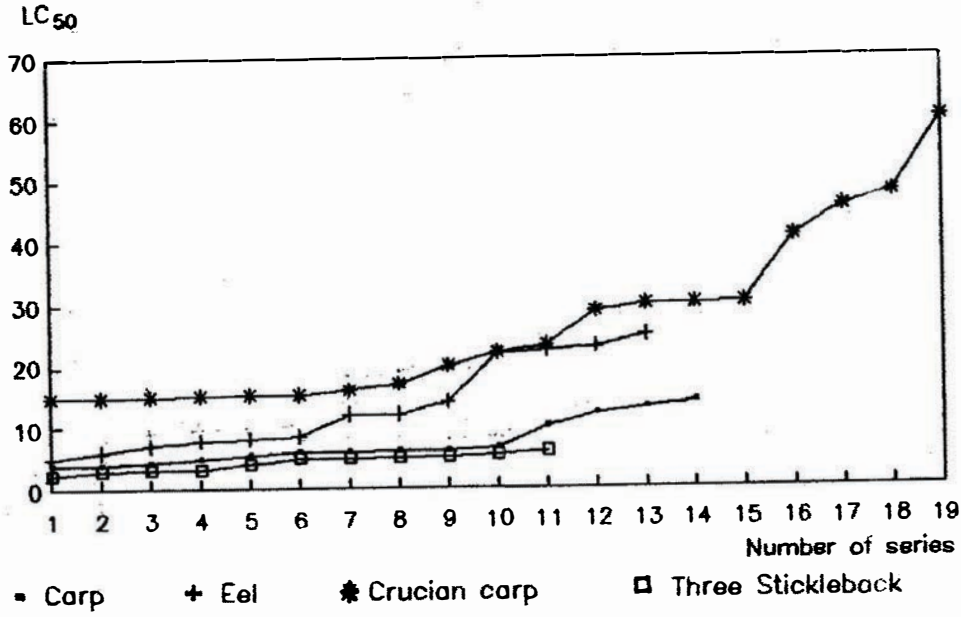


Fig. 3. Variety of LC_{50} values for each fishes' species obtained by the means of series of experiments – normaln conditions (values in ascended order)

The most favourable range of pH values of the water for fishes (between 6.5 and 8.5) was know since a long time. The safe values of pH as varied in range of 5.5 to 9.0 were reported by Sokołow, Winogradow 1977; Ishio 1965; Orsanco 1955; Schofield 1976. Algal resistance of the examined fishes considerably varied even in the case of one species. Thus there was very difficult to find the strict value of lethal dose. In Fig.3 were showed

high divergences of LC_{50} values that were obtained for examined fishes. For example LC_{50} varied in range 2.5 mln algal units per dm^3 to 6 mln algal units per dm^3 for three-stickleback and between 15 mln a.u./dm^3 and 60 mln a.u./dm^3 for crucian carp.

Values of LC_{50} obtained for species were varied Tab. 1 between each other too. There were noticed that the toxic properties of *Microcystis* were obtained at high concentrations of these blue-greens even if not lethal pH values were maintained.

Experiments conducted at excellent light showed that the toxic properties of *Microcystis* occurred already at concentration 1.5–2 mln a.u./dm^3 (so far values often lethal to three-stichleback and carp).

When harmful influence of high pH level was eliminated by intensive mixing of the water there still alive eels and carps were observed at algal concentrations of 500 mln a.u./dm^3. In the case of curcian carp that was got accustom to the increasing algal there was noted that concentrations value of 900 mln. a.u./dm^3 was still not lethal. So high algal concentrations were not toxic for the greater part of tested fishes during 96 hours of experiment (per acute toxicity). Analysis of the above described data seems to suggest that toxic properties of these blue-greens depends on activity of endotoxine and ability to change-over of the environment by fishes.

Probably the ability to excretion of endotoxines by Microcystis depends not only on temperature and sulphates occurence (Shelubsky 1951) but on pH of the surrounding water as well. The high variety of the resistance of fishes on toxic impact of blue-green algae was a necessity to work out the easy method of evaluation of fishes reaction on stress caused by algal blooms.

Changes of the intensity of coloration of skin according to values of algal concentrations and pH measurements of the blood were these methods.

Authors of this work noticed some changes of shape of melanophores caused by growing algal concentrations. The strong concentration of pigment-cells was observed at growing algal concentration. Al lethal algal concentrations melanophores assumed almost a circular shape.

Fig. 4a–4i is confirmation of above described observations (in accordance with data collected by Burkowski and Kulkina 1981) of fishes of polluted waters. Changes of melanophores were almost homogeneous in all experiments especially in the case carp and eel[8]. The pigment concentrations in pigment cells depended only on *Microcystis* concentration. (the pH influence on intensity of this phenomenon wasn't observed). However similar changes were observed for fishes kept in water with other algal cultures. The *Microcystis* was predominant and the *Scenedesmus* was subdominant in these algal cultures. The measurement of pH of blood of tested fishes was found as good method for evaluation of the stress caused by blue-green algae. As a result of the stress

[8] it is an indirect result of quite easy observations of melanophores those fishes skin.

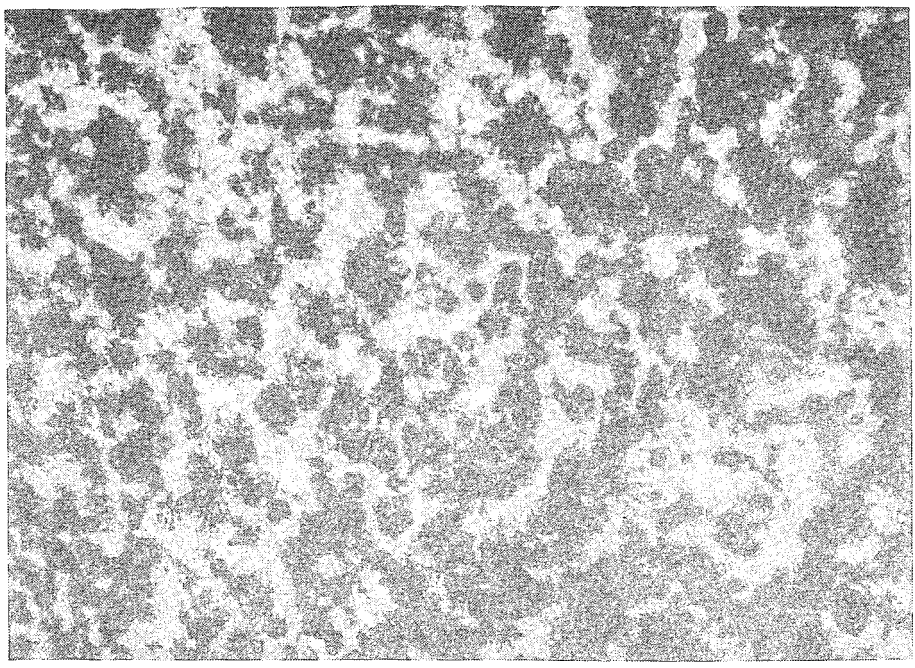


Fig. 4a. Numerous melanophores of the carp skin. A sample taken from back partof the body. The controling group

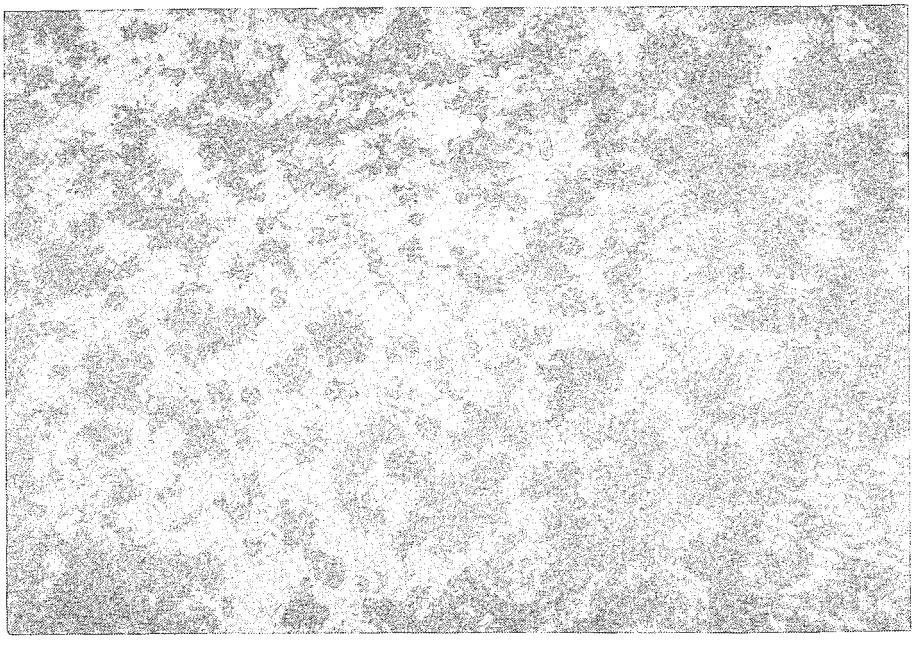


Fig. 4b. Variety of cell shapes of pigment-cells of carp caused by displecement of the melanina and concentration this pigment in cell centres. The experimental group algal concentration 8 mln a.u./dm^3

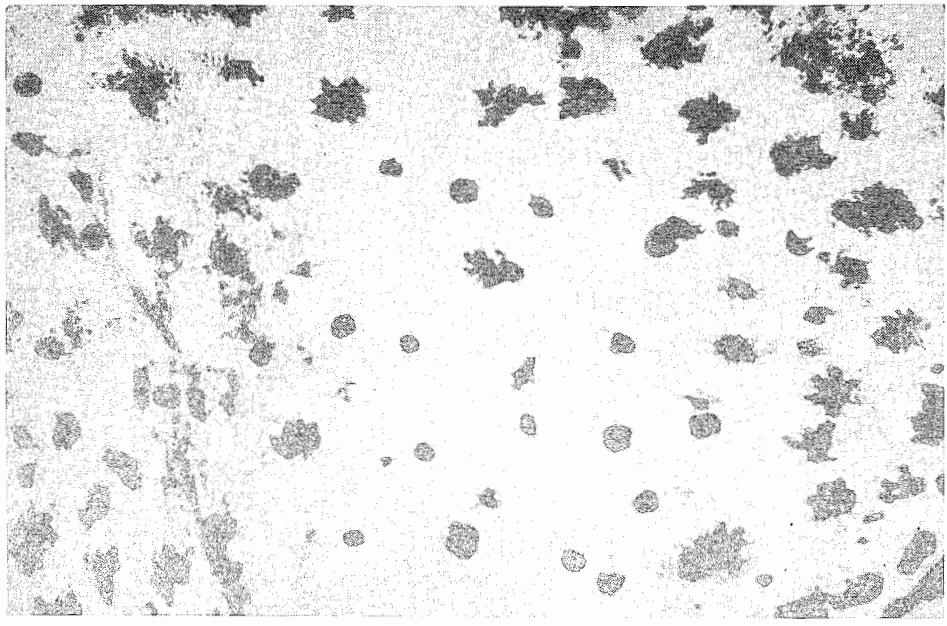


Fig. 4c. Strong concentration of pigment-cells of carp it sample taken from back part of the body. The experimental group, algal concentration 16 mln a.u./dm^3.

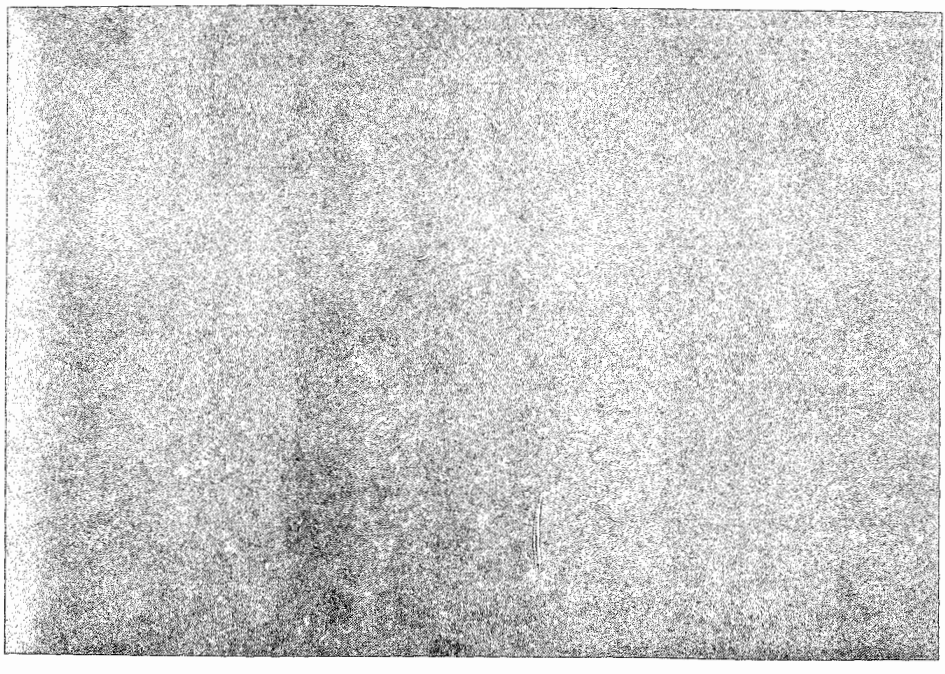


Fig. 4d. Numerous melanophores of eel skin taken from back part of the body. The controling group

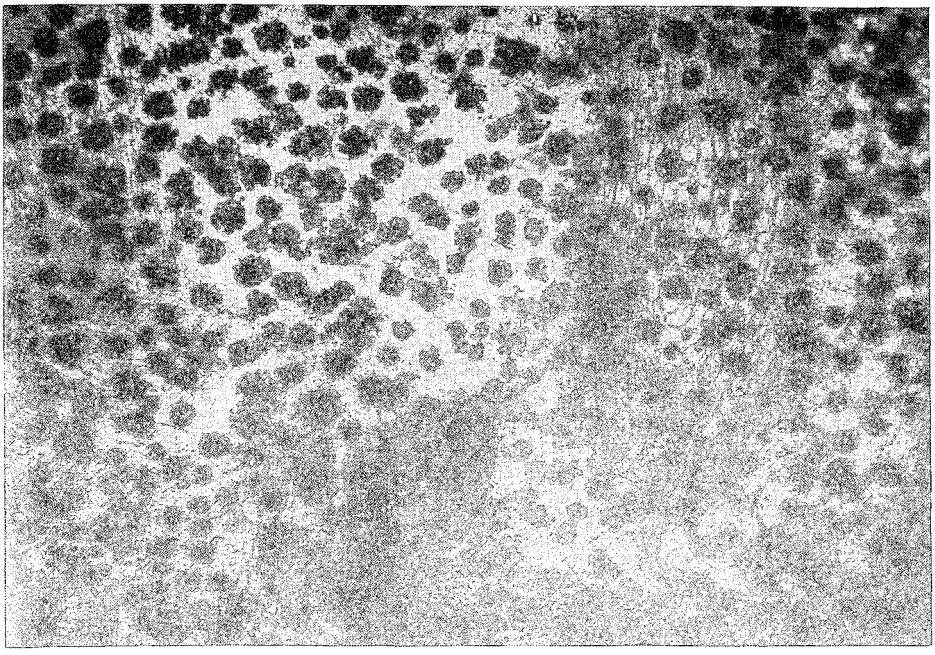


Fig. 4e. Variety of cell shapes of pigment-cells of eel caused by displacement of the melanina and concentration this pigment in centres eel. The experimental group – algal concentration

Fig. 4f. The decay of appendixes of malanophores caused by displacement of the pigment toward cell – centres. The experimental group – algal concentration

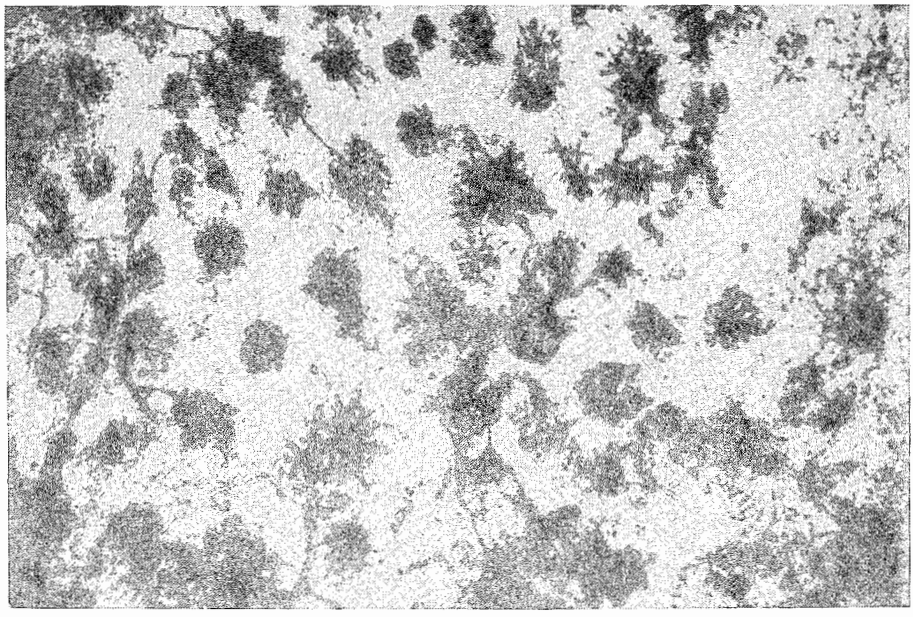


Fig. 4g. Numerous star-shaped melanophores of crucian carp skin. A sample taken from back part of the body. The controling group

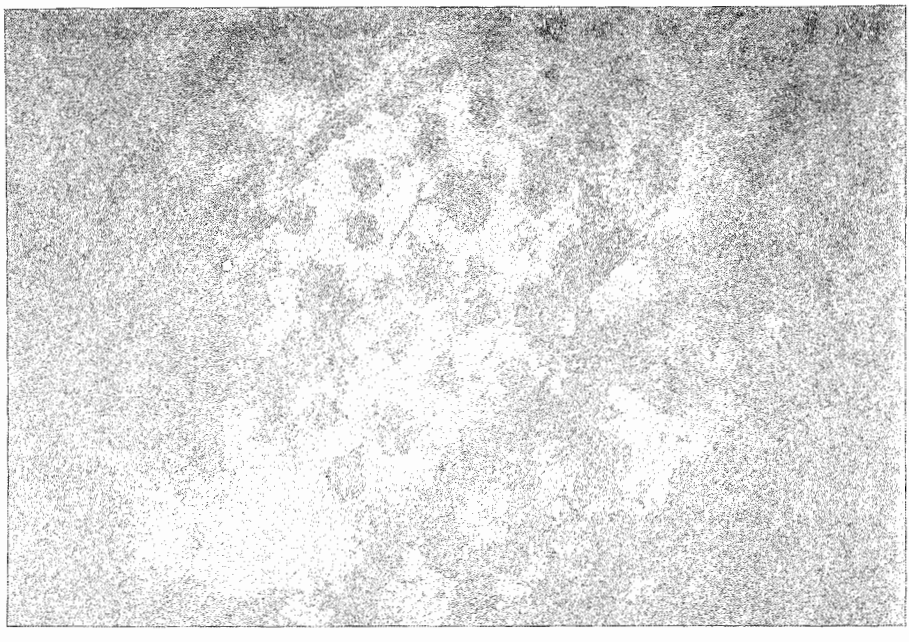


Fig. 4h. Lack of shape differences between melanophores of the crucian carp skin of the experimental group (algal concentration 64 mln a.u./dm^3 and 72 hours of the experiment duration

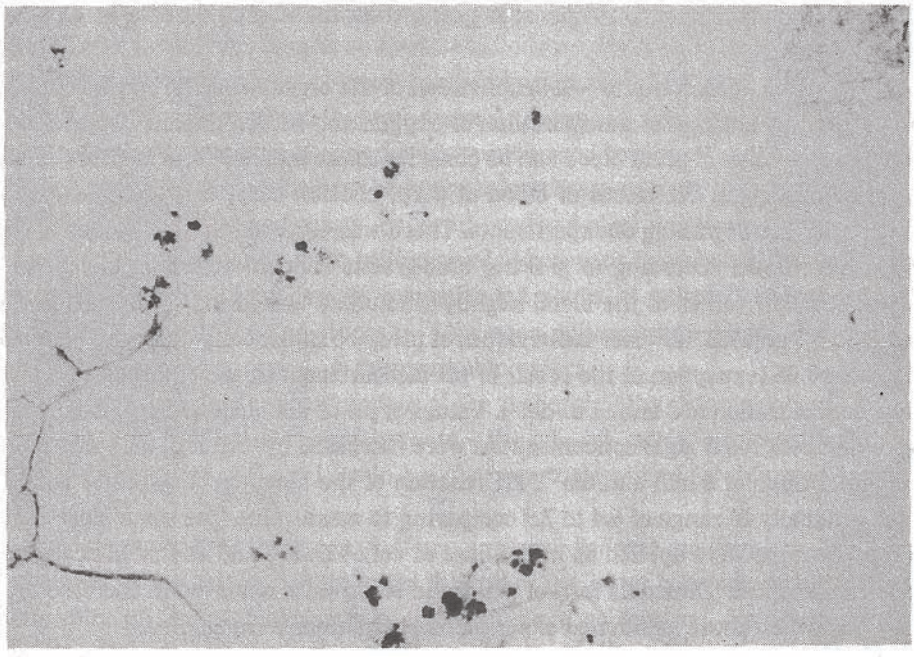


Fig. 4i. Very clear to see aggregations of few melanophores fo the crucian carp skin (2 manths of the experiment duration)

Table 2

Mean values of pH values of blood of examinated fishes according to changes algal of concentration (blood of live fishes)

Species	Algal concentration mln a.u./d^3	Time of experiment [h]	Number of animals	pH of blood	Standard deviation
Cyprinus carpio	kontrol	12	25	7.9	0.12
	8	12	50	7.5	0.11
	16	12	50	7.1	0.34
	32	12	50	6.4	0.43
Anguilla anguilla	kontrol	12	54	7.3	0.13
	8	12	54	7.2	0.12
	16	12	52	7.0	0.11
	32	12	54	6.7	0.49
	64	6	21	5.7	1.02
Carassius carassius	kontrol	12	28	7.3	0.09
	8	12	46	7.2	0.20
	16	12	33	7.2	0.13
	32	12	30	7.1	0.19
	64	6	35	6.9	0.38

the tissue concentration of lactic acid grows then the alkalic reserve of blood goes down.

This phenomenon drives to toal acidification of the organism disturbances of osmotic processes, and changes of transportation of oxygen and carbon dioxide (Głębocka 1981 and others). Value of pH of blood can be consider as the indicator and measure of above described changes. PH values of blood of carp, crucian carp, eel were measured 12 hours after the beginning of experiments. There was observed that pH values of fishes blood went down according to growing blue-greens concentrations (Table 2). In the case of eel, pH values of the blood slightly fluctuated comparing to, mean pH value, between 6.7 and 7.3. However mean values of pH were statistically different – Duncan's test $\alpha = 0.05$ (exception of the result of pH measurement in the controlling sample at algal concentration of 8 min. a.u./dm^3). Values of pH of the blood of carp (mean values) were different too if algal concentrations were increased by order of magnitude of the minimum value of 8 mln a.u./dm^3. PH reaction of the blood of this species decreases itself gradually in range of 6.4 to 7.9 comparing to mean value (the same algal concentration changes were applied as in the case of eel). However no any statistically significant differences (Duncan's test) of pH of the blood were observed in the case of crucian carp when above mentioned experimental conditions were applied.

Moreover, apart from the blood acidify the blood alkalic resistence were measured in a sample of random chosen fishes. Because of few repetitions of these measurements the results were not taken under consideration by authors of this paper. Although the value of the blood alcalic resistance seems to be a promising method of evaluation the toxic impact of *Microcystis*.

The results of field work confirmed fluctuation of toxic properties of blue-green algae observed under laboratory circumstances. Behavior of two species of fishes: three-stickleback and crucian carp were observed during two week lasting field observations. The death only of the three-stichleback was noted. Mortality of this species were observed to fishes placed close to the surface and the bottom. Total mortalities of fishes was comparable in the surface and the bottom zones.

This phenomenon can be explained by the highest changes of value of environmental conditions in those both zones during day – night cycle. The *Microcystis* concentration was high (during the experiment) – 2.0–2.5 mln a.u./dm^3. Moreover the highest number of dead fishes were found in the surface zone when the sunny weather occurred.

The variety of toxic properties of *Microcystis* was even more clear to see if some invertebrates such as *Dreissena polymorpha, Chironomus* sp., or *Pallasea quadrispinosa* were applied as testing organisms.

There was find out the high level of resistence of *Dreissena polymorpha* to toxic impact of *Mocrocystis*. Authors found alive specimens of *Dreissena polymorpha* in aquariums where algal concentrations were even 500 mln a.u./dm . The pH = 8 was un-

der control and maintained through this experiment. If the pH level was not controled the toxic influence of *Microcystis* on *Dreissena polêmorpha* occured already at 10 mln a.u./dm^2 of algal concentration. Moreover, the considerably drop in number of *Microcystis* in the water was observed that was probably caused by filtering activity of *Dreissena polymorpha* during time of experiment (minimum od 3 days).

Chironomus sp. appeared the most resistant invertebrate on toxic influence of *Microcystis.* Specimens of *Chironomus* sp. endured 1 miliard algal unit/dm^3 concentration. The level of the resistance to toxic influence of *Microcystis* is probably dependend on attachment of testing organisms to corresponded zones of polluted waters (Pantle, Buck 1955; Starmach 1960 and others): – *Dreissena polymoprha* – oligo-, β-mezosaprobic waters *Chironomus* sp. – α- mezosaprobic waters.

Unlike above described representatives of the zoobenthos the strict impact of *Microcystis* was stated for *Pallasea quadrispinosa* – the glacial relict, typical inhabitants of α-mezotrofic waters. LC_{50} = 1.9 mln a.u./dm^3 was determined for this species (temperature at 15°C).

In Fig. 5 is showed the influence of *Microcystis* on *Pallasea quadrispionsa.* There was observed in the case of invertebrates that pH of the water is an important factor if the mortality of testing organism was taken under consideration. Value of pH also indirectly influence on gas proportions (CO_2,O_2) in water's environment. Thus this phenomenon probably have an effect on the mortality of testing organisms too.

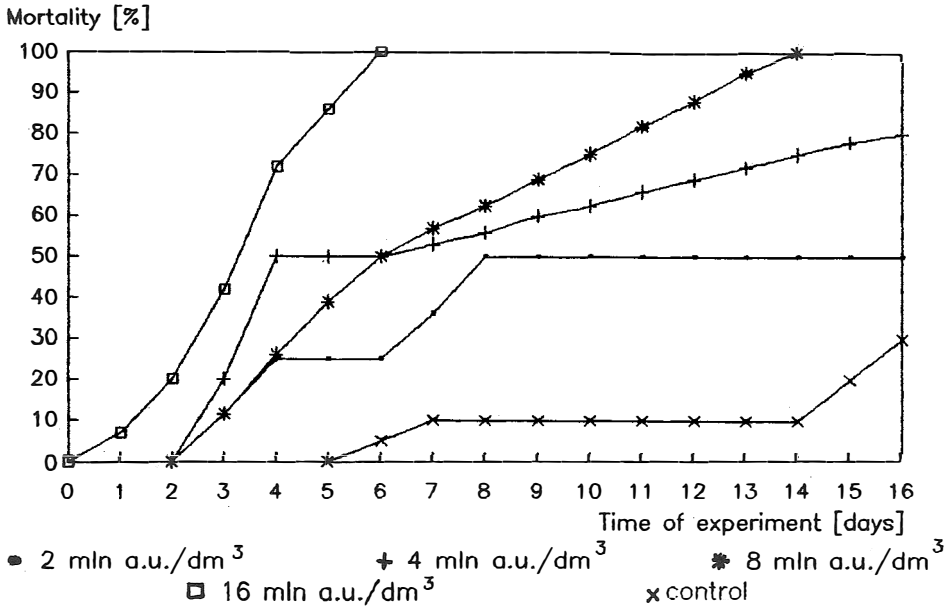


Fig. 5. Dynamics of mortality of Pallasea quadrispinosa according to changes of blue-greens (Microcystis) concentrations

Results of this work can have the importance as a method to foresseing of toxic influence of *Microcystis* on the water's fauna. The analysis of the results seem to indicate that endotoxins excreted by blue-green algae are more biological active at high values of pH of the water. Moreover there was stated that pigment-cells show clear to see and the same, reaction on algae occurrence in environment. There is possibility of the using the observation of melanophores as biotest because of easiness of these observations.

There was also determined that the most resistant fish species on the influence of *Microcystis* is crucian carp followed by (in order of resistance) ell, carp and three stickleback Table I and Fig. 3.4.

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WPŁYW SINIC Z RODZAJU *MICROCYSTIS* NA NIEKTÓRE ZWIERZĘTA WODNE

STRESZCZENIE

W okresie od maja 1990 roku do października 1991 roku badano szkodliwe oddziaływanie sinic z rodzaju *Microcystis* na hydrofaunę.

Materiałem do badań było około 2000 sztuk ryb należących do 5 gatunków: karaś (*Carassius carassius* i *C. auratus*), karp (*Cyprinus carpio*), węgorz (*Anguilla anguilla*), ciernik (*Gasterosteus aculeatus*) oraz bezkręgowce: małż – *Dreissena polymorpha*, larwy muchówki – *Chironomus* sp. i skorupiak – *Pallasea quadrispinosa*.

Określając stężenie glonów powodujące 50% śmiertelności (LC_{50}) i całkowitą śmiertelność testowanych zwierząt stwierdzono zróżnicowanie tych wielkości w obrębie gatunku i między gatunkami. W oparciu o LC_{50} ustalono, że najbardziej wrażliwe na działanie sinic z testowych ryb są cierniki (LC_{50} = 4.3 mln. jednostek glonowych/dm^3), a najmniej karasie (LC_{50} = 26.4 mln. j.g./dm^3). Natomiast wśród bezkręgowców najbardziej wrażliwy na działanie *Microcystis* był skorupiak *Pallasea quadrispinosa* (LC_{50} = 1.9 mln. j.g/dm^3), a najmniej muchówka *Chironomus* sp. (LC_{50} = 1 mld. j.g/dm^3).

Podczas doświadczeń zauważono zdolność zmiany własnego środowiska przez sinice (jego alkalizację) oraz negatywny wpływ tego środowiska na przeżywalność zwierząt. Stwierdzono również, że w miarę wzrostu stężenia glonów odczyn pH krwi ryb ulegał obniżeniu. U węgorza wykazano obniżenie odczynu od pH = 7,3 do 6,7,

a u karpia od pH = 7,0 do 6,4. Wzrost liczebności *Microcystis* powodował także koncentrację pigmentu wewnątrz melanoforów i zmianę ich kształtu od drzewkowato rozgałęzionych do niemal kulistych, zmieniając tym samym intensywność zabarwienia skóry ryb.

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