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# THE INFLUENCE OF HEAVY METAL IONS ON NEUSTONIC BACTERIA

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## Abstract

This paper showed, that the samples recovered from the subsurface water always contained much fewer bacteria than those sampled from the surface microlayer. The neustonic bacteria are more resistant to the heavy metals ions than bacteria collected from subsurface water. The mean data obtained allow a statement that copper ions provoked the least response, while lead and cadmium ions - the greatest ones.

**Key words:** heavy metals, surface microlayer, neustonic bacteria

## INTRODUCTION

The surface microlayer, often called biofilm, is formed on the surface of water under the influence of physical and chemical processes. Those processes take place mainly between compounds (of water, sugars and fats) that gather on the water surface (Norkrans 1980). The compounds which compose the biofilm form a well-arranged layer that separates the water environment from the atmosphere (Horne 1972). Due to the process of floatation, many chemical substances which migrate from the atmosphere into the water, and reversly, from the bottom sediments into water and atmosphere are trapped in the surface film by organic compounds of heavy concentration.

One of such groups of chemical compounds which cumulate in the microfilm are salts and ions of heavy metals. The metal accumulation in the surface microfilm at particular periods of time depends on many factors, a.o.: the microfilm composition, physical and chemical form of metals, transport controlling processes, the film duration and the duration of metal occurrence in the film (Armstrong and Elzerman 1982). Metal occurrence time in film may last from a few minutes to a few hours.

Many metals are necessary for an organisms growth and development, yet some of them, especially when at elevated concentrations, are harmful for living organisms. Many heavy metals are deadly for microorganisms even at very low concentrations (Mills and Colwell 1977). Metal toxicity depends very much on their compounds' concentrations and solubility in water; and also on chemical reactivity, i.e. the ability to form complex components with organic matter fractions, inorganic compounds and to assimilate living organisms. Inorganic metals compounds, which solve well in water, are most toxic; whereas the organic ones do less harm. However, the existing possibility of biochemical releasing of metal from the organic compounds puts at great risk all organisms present in the environment.

## MATERIAL AND METHODS

### Study Area

The investigations were carried out in Lake Jeziorak Mały. The water body is located within the town of Iława and is part of the Iławskie Lake District. The lake has no tributaries nor outlets; only in its northern part it is linked with Lake Jeziorak by a narrow and shallow canal (1.5 m). The water body surface is 26 ha, and the maximum depth is 6.4 m.

### Sampling

The water was sampled in June (20th June), July (17th July) and August (21st Aug.) 1996 at three different sampling stations (Fig. 1). The surface microlayer was sampled by means of four techniques:

1) a glass plate skimming thickness a 100 μm water layer
2) a plexiglass plate skimming thickness a 150 μm water layer
3) Garrett's net 1 of 65 μm mesh size, skimming water layer of 250 μm thickness
4) Garrett's net 2 of 200 μm mesh size, skimming water layer of 300 μm thickness.

The subsurface water samples were taken from the depth of 10 cm by means of a sterile glass pipette with the use of a Pippet-boy device (De Ville). The water samples, from both microfilm and subsurface layer, were collected into sterile glass bottles. The samples were transported to the laboratory in a thermoinsulated container with ice at ± 7°C. The time between sampling and the analysis usually did not exceed 6 hours.

### Total Number of Bacteria Counting

The total bacteria number (TN) in the samples was determined by means of direct counting on membrane filters (Millipore) in an epifluorescence microscope, in specimens stained with acridine orange (Zimmermann 1977).

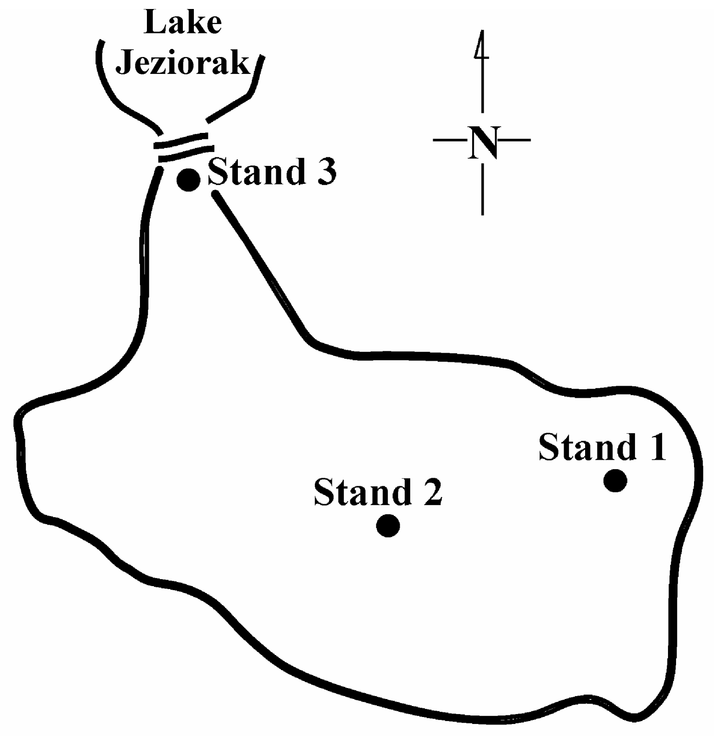


Fig. 1. Outline of the Lake Jeziorak Mały

**Number of Heterotrophic Bacteria**

The heterotrophic bacteria number (TVC) was determined by means of spread plate method. Sterile buffered water was used for solutions of samples (Daubner 1976). 0.1 ml samples were introduced onto Plate Count Agar (Difco) and spread over by a glass rubbing rod. After a 6 day cultivation at 20°C the colony heterotrophic bacteria number (CFU) was counted and then the colony strains were randomly picked and transferred onto a semi-solid medium (as above). The strains were kept at 4°C in a fridge for further study, and were transferred onto fresh semi-solid medium every two months.

**Determination of Heavy Metal Concentrations in Water Samples**

A spectrophotometric method was employed to analyse the water samples for the concentrations of the following heavy metals: copper, cobalt, cadmium and lead. In order to do this NANOCOLOR tests by Machery-Nagel AG were used, which have the ISO Certificate. For particular determinations a Marcel s330 PRO (Marcel) spectrophotometer was used.

### Examination of Heavy Metal Influence on Bacteria Growth

Bacteria strains were preincubated for 72 hours at 20°C on a mineral medium which was composed of the following: $K_2HPO_4$ - 1g; $KNO_3$ - 0.5 g; $MgSO_4$ x $7H_2O$ - 0.4 g; $CaCl_2$ - 0.2 g; NaCl - 0.1 g; $FeCl_3$ - 0.01 g; $H_2O$(distiled) - 1000 ml; pH - 6.8 - 7.2. Then, in form of 0.1 ml suspension of 0.2 optic density, (reached beforehand and at 560 nm wave length), they were transplanted into a fresh mineral medium, of the same composition as above, containing a specific concentration of a heavy metal under investigation. The investigations covered the influence of Cu, Co, Cd and Pb. Metal concentrations were determined on the basis of earlier studies on concentrations of the same metals in the lake water samples.

The culture containing Cu, Co, Cd and Pb was obtained by mixing with $CuSO_4$ , $CoCl_2$, $Cd(NO_3)_2$ x $4H_2O$ and $Pb(NO_3)_2$ respectively. The following concentrations were used: for copper: 0; 0.1; 0.2; 0.5; 1.0 mg/l; for cobalt: 0; 0.05; 0.1; 0.5; 1.0 mg/l; for cadmium and lead: 0; 0.02; 0.05; 0.1 mg/l.
After a 24 hours incubation period at 20° C, and at 560 nm wave length a spectrophotometric measurement of the optical density of the culture followed with the use of Marcel s330 PRO spectrophotometer.

Among 400 strains those were selected which evidently reacted to the metal presence by showing stimulation or decline in growth; and also those which did not demonstrate any response at all. Selected strains were inoculated onto a medium of higher heavy metal concentrations: 0; 0.1; 0.5; 1.0; 5.0; 10.0 mg/l (equal for all the metals). Bacterial protein concentration in cultures after 24 hours of incubation at 20°C was measured by means of the Bredford method. All the analyses (microbiological and chemical) were made in the three replications.

## RESULTS

Table 1 presents data on Total Bacteria Number (TN) and Heterotrophic Bacteria Number (TVC) found in the investigated water samples. It is quite clear that maximum TN in the surface microfilm occurred in July , whereas TVC in August. In June and August the TN data were slightly lower, yet very close. In the subsurface water the maximum TN was recorded in June, and TVC in July.

Table 1.

Bacteria number in surface microlayer and subsurface water of Lake Jeziorak Mały (numbers expressed in /1L)

| Sampling date | Sampling method | | | | | Average | | E |
|---|---|---|---|---|---|---|---|---|
| | a | b | c | d | e | SM | SUB | SM/SUB |
| June | 29,25*<br>33,0** | 24,75<br>171,0 | 21,33<br>30,0 | 18,22<br>38,8 | 14,10<br>43,3 | 23,38<br>68,2 | 14,10<br>43,3 | 1,65<br>1,57 |
| July | 38,31<br>69,0 | 38,04<br>159,6 | 19,93<br>142,0 | 35,04<br>183,1 | 11,80<br>59,3 | 32,83<br>138,42 | 11,80<br>59,3 | 2,78<br>2,33 |
| August | 32,52<br>58,7 | 33,91<br>317,0 | 21,25<br>288,6 | 28,32<br>95,5 | 10,01<br>17,8 | 29,0<br>189,9 | 10,01<br>17,8 | 2,89<br>10,67 |

Explanations: a - glass plate sampling; b - plexiglass plate sampling; c - Garrett net 1 sampling; d - Garrett net 2 sampling; e - subsurface layer water; SM - surface microlayer water; SUB - subsurface water; E - enrichment factor; * - Total Bacteria Number x 10; ** - Heterotrophic Bacteria Number x $10^3$.

Analysis of surface microfilm data obtained by means of different sampling techniques showed that similar bacteria number (TN) occurred in the water which had been sampled with the use of glass and plexiglass plates. Greater differences occurred in the case of Garrett nets 1 and 2. The samples recovered from the subsurface water always contained much fewer bacteria than those sampled from the surface microfilm.

Table 2 presents data on concentrations of the studied heavy metal ions in the surface microfilm (a-d) and in the subsurface water (e) of Lake Jeziorak Mały. Those data give evidence of the maximum heavy metal ion concentrations having occurred in June; slightly lower were recorded in July while in August they were significantly lower. The average highest concentrations of copper and cobalt were recorded in June and for the surface microfilm it was: 0.193 and 0.028 mg/l respectively; whereas for the subsurface water: 0.085 and 0.010 mg/l respectively. The average highest concentration of lead in the surface biofilm was recorded also in June in the amount of 0.114 mg/l, while in the subsurface water the maximum occurred in July amounting to 0.082 mg/l. The highest average concentration for cadmium in the surface microfilm was recorded in June amounting to 0.229 mg/l and in the subsurface water in July, in the amount 0.178 mg/l. A comparison of metal ions concentrations in both layers resulted in the findings of Cu and Co having had significantly higher concentrations in the surface microfilm, than in the subsurface water, whereas the concentrations of Pb and Cd were at a similar level in both layers or even slightly higher in the subsurface water.

Table 2

Heavy metal concentrations (mg/l) in surface microlayer and subsurface water of Lake Jeziorak Mały

| Sampling method | Copper (Cu) | | | | Cobalt (Co) | | | | Lead (Pb) | | | | Cadmium (Cd) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | June | July | August | Average | June | July | August | Average | June | July | August | Average | June | July | August | Average |
| a | 0.193 | 0.150 | 0.004 | 0.115 | 0.028 | 0.006 | 0.002 | 0.012 | 0.040 | 0.015 | 0.003 | 0.019 | 0.229 | 0.101 | 0.001 | 0.110 |
| b | 0.141 | 0.139 | 0.000 | 0.093 | 0.017 | 0.008 | 0.011 | 0.012 | 0.040 | 0.019 | 0.021 | 0.026 | 0.128 | 0.046 | 0.13 | 0.062 |
| c | 0.104 | 0.152 | 0.002 | 0.086 | 0.016 | 0.010 | 0.000 | 0.008 | 0.075 | 0.033 | 0.003 | 0.037 | 0.023 | 0.180 | 0.042 | 0.081 |
| d | 0.118 | 0.089 | 0.000 | 0.069 | 0.015 | 0.008 | 0.000 | 0.007 | 0.114 | 0.018 | 0.000 | 0.044 | 0.013 | 0.108 | 0.032 | 0.051 |
| e | 0.085 | 0.063 | 0.026 | 0.058 | 0.010 | 0.006 | 0.002 | 0.006 | 0.052 | 0.082 | 0.045 | 0.059 | 0.069 | 0.172 | 0.008 | 0.083 |

Explanations: a - glass plate sampling; b - plexiglass plate sampling; c - Garrett net 1 sampling; d - Garrett net 2 sampling; e - subsurface water.

Low concentrations of metal ions (0-1 mg/l) provoked four types of response bacteria: B - no response, S - growth stimulation, I - growth impediment, S/I - stimulation at lowest concentrations/impediment at increased concentrations. Table 3 presents a percentage pattern of strains number according to their response types. An average of 59,6% did not demonstrate any response to the metal ions presence; whereas 6,3% responded by growth increase. The Cu and Co ions revealed a growth impediment in around 11.5% of the strains, and in the case of Pb and Cd ions the phenomenon occurred in 30.2 % of the strains. With Cu and Co ions the S/I reaction type occurred in about 20.2 % of the strains; while with Pb and Cd ions it was recorded for 5.6 % of the strains. The percentage pattern of the bacteria groups demonstrating a particular response was subject to very clear changes depending on the seasons. Between June and August the strain number which did not show any feedback to the metal ions presence decreased by about 17%.

Table 3

Percentage of bacteria strains showing particular response types to low concentrations (0 – 1,0 mg/l) of heavy metals (values average).

| Metal type: Cu | | | | |
|---|---|---|---|---|
| Type of response | June | July | August | Average |
| I | 5,8 | 15,2 | 10,4 | 10,4 |
| B | 72 | 54,5 | 52,8 | 59,7 |
| S/I | 16,4 | 16,2 | 28,8 | 20,4 |
| S | 5,8 | 14,1 | 8,0 | 9,3 |
| **Metal type: Co** | | | | |
| Type of response | June | July | August | Average |
| I | 9,3 | 13,1 | 14,9 | 12,4 |
| B | 75,6 | 54,6 | 54,0 | 61,4 |
| S/I | 5,8 | 24,3 | 29,9 | 20,0 |
| S | 9,3 | 8,0 | 1,2 | 6,1 |
| **Metal type: Pb** | | | | |
| Type of response | June | July | August | Average |
| I | 16,3 | 34,4 | 32,3 | 27,3 |
| B | 77,9 | 55,6 | 55,1 | 62,8 |
| S/I | 2,3 | 4,0 | 4,6 | 3,6 |
| S | 3,5 | 6,0 | 8,0 | 5,8 |
| **Metal type: Cd** | | | | |
| Type of response | June | July | August | Average |
| I | 25,6 | 38,4 | 35,8 | 33,2 |
| B | 58,1 | 52,6 | 53,9 | 54,8 |
| S/I | 9,3 | 7,0 | 6,8 | 7,7 |
| S | 7,0 | 2,0 | 3,5 | 4,1 |

Explanations: I - growth impediment; B - no distinct response; S/I - stimulation at lowest concentrations and impediment at higher concentrations; S - growth stimulation.

At the same time, lead and cadmium bacteria strains which reacted with growth impediment increased by 13% on average. Bacteria strains showing a S/I reaction type to the presence of copper and cobalt ions increased by 18%. No clear differences were noted in the response to low concentrations of heavy metal ions presence between the surface microfilm and subsurface water strains.

Table 4 and Fig. 2-5 present data on heavy metal concentrations (1-10 mg/l) affecting the development of bacteria biomass development, expressed as strain percentage that react to the phenomenon by a decline in bacterial protein concentration. Beside the above mentioned response types, an additional B/I reaction type was identified where strains did not respond to the concentrations of 0.1 and 0.5 mg/l; whereas at higher concentrations (1.0; 5.0; 10.0 mg/l) growth impediment was recorded. The average data obtained allow a statement that copper ions provoked the least response, while lead and cadmium ions - the greatest ones. Besides, in a fairly great number of strains (SM - 31.2 %; SUB - 21.4%) cadmium provoked an S/I reaction type which might suggest that despite the normally occurring toxicity, when at very low concentrations, it may positively affect the microorganism development. In the case of cobalt ions, a stimulation of bacteria growth was noted in the strains originating from both the surface microfilm (3.2 %) and the subsurface water (4.1%).

## DISCUSSION

The increase in water ecosystem pollution is accompanied by changes and disturbances in the functioning of whole water bodies. The main reason for those changes is a disorder or even an interruption in the natural matter circulation, one of the main element being the bacterioneuston closely attached to the water surface microfilm. The pollution which significantly affects the growth rate, and indirectly the bacterioneuston number, is caused by heavy metals.

The available data on total bacteria number occurring in Lake Jeziorak Mały point to the fact that the water body surface microfilm contains much more bacteria than the subsurface water, which is in accordance with earlier investigations (Marumo et al. 1971; Crow et al. 1975; Kim 1983). The maximum number data obtained for July and August in Lake Jeziorak Mały confirmed those arrived at by Niewolak (1973). The heterotrophic bacteria number was also higher in the surface microfilm than in the subsurface water. This resulted from the occurrence of higher concentrations of many organic substances acting as nutrients (Carlson 1983) in the surface microfilm.

The data on heavy metal ions concentrations obtained in the course of the study indicate that their greatest concentrations both in the surface microfilm and in the subsurface water took place in June; whereas in August the metals occurred in trace amounts. This might have happened due to a gradual immobilization of metal ions having inflowed along with spring thaw water, which formed complexes with organic components. On the other hand, a further supply of the ions of the studied metals was lower than the complex forming processes and the bacterial accummulation. Due to different relationships of particular ions to complex forming with organic matter there happened clear differences in the metal ions distribution.

Table 4

Percentage of bacteria strains in different water layers showing particular response types to higher concentrations (1,0–10,0 mg/l) of heavy metals (values average).

| Metal type: Cu | | | | | | | |
|---|---|---|---|---|---|---|---|
| Type of response | Sampling method | | | | | Average | |
| | a | b | c | d | e | SM | SUB |
| I | 41,1* | 19,4 | 41,6 | 27,3 | 42,8 | 32,3 | 42,8 |
| B/I | 0,0 | 8,3 | 16,6 | 13,3 | 4,7 | 9,5 | 4,7 |
| B | 24,4 | 38,8 | 27,7 | 46,1 | 26,7 | 34,2 | 26,7 |
| S/I | 34,4 | 33,3 | 13,8 | 13,0 | 25,5 | 23,6 | 25,5 |
| S | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 |
| Metal type: Co | | | | | | | |
| Type of response | Sampling method | | | | | Average | |
| | a | b | c | d | e | SM | SUB |
| I | 43,3 | 48,3 | 25,0 | 44,5 | 62,5 | 40,2 | 62,5 |
| B/I | 33,3 | 35,0 | 16,6 | 16,1 | 12,5 | 25,2 | 12,5 |
| B | 6,6 | 16,6 | 27,7 | 17,8 | 8,3 | 17,1 | 8,3 |
| S/I | 16,6 | 0,0 | 22,2 | 16,6 | 12,5 | 13,8 | 12,5 |
| S | 0,0 | 0,0 | 8,3 | 4,7 | 4,1 | 3,2 | 4,1 |
| Metal type: Pb | | | | | | | |
| Type of response | Sampling method | | | | | Average | |
| | a | b | c | d | e | SM | SUB |
| I | 24,4 | 61,1 | 38,8 | 59,9 | 67,8 | 47,5 | 67,8 |
| B/I | 37,7 | 19,4 | 30,5 | 11,1 | 18,4 | 24,6 | 18,4 |
| B | 31,1 | 8,3 | 0,0 | 6,6 | 0,0 | 11,5 | 0,0 |
| S/I | 6,0 | 33,3 | 25,0 | 22,2 | 12,5 | 16,1 | 12,5 |
| S | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 |
| Metal type: Cd | | | | | | | |
| Type of response | Sampling method | | | | | Average | |
| | a | b | c | d | e | SM | SUB |
| I | 53,3 | 52,7 | 27,7 | 37,1 | 65,4 | 45,7 | 65,4 |
| B/I | 13,3 | 27,7 | 5,5 | 22,8 | 13,0 | 17,3 | 13,0 |
| B | 0,0 | 0,0 | 16,6 | 6,6 | 0,0 | 5,8 | 0,0 |
| S/I | 33,3 | 19,4 | 38,8 | 33,3 | 21,4 | 31,2 | 21,4 |
| S | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 |

Explanations: I - growth impediment; B/I - no response at lowest concentrations and impediment at increased concentrations; B - no response; S/I - stimulation at lowest concentrations and impediment at higher concentrations; S - growth stimulation; a - glass plate sampling; b - plexi-glass plate sampling; c - Garrett net 1 sampling; d - Garrett net 2 sampling; e - subsurface water; SM – surface microlayer water; SUB – subsurface water

The cobalt ions demonstrated a strong tendency towards forming complexes with organic matter, whereas copper compounds dissolved very well in water, hence the occurrence of significantly higher concentrations of both metals in the surface microfilm than in the subsurface water of the water body. On the other hand, lead and cadmium compounds, which dissolve hard and are slow in the migration from

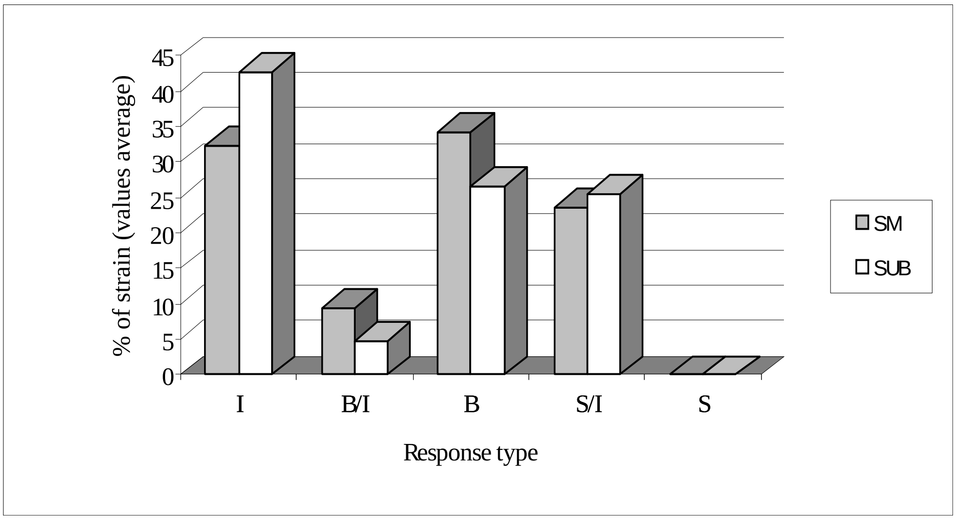


Fig. 2. Bacteria response types to higher concentrations of Cu ions

Explanations: I - growth impediment, B/I - no response at lowest concentrations and impediment at increased concentrations, B - no response, S/I - stimulation at lowest concentrations an impediment at higher concentrations, S - growth stimulations; SM – surface microlayer water; SUB – subsurface water

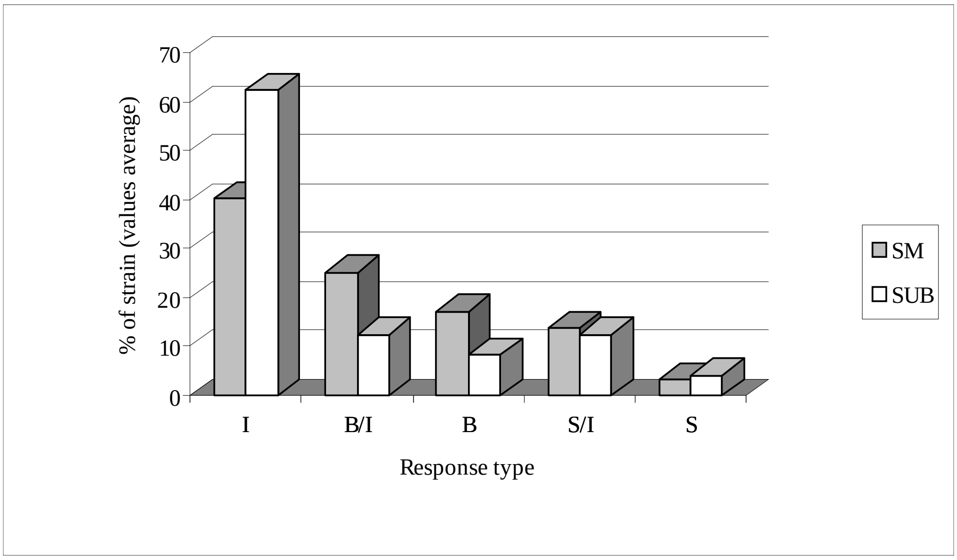


Fig. 3. Bacteria response types to higher concentrations of Co ions

Explanations as on Fig.2

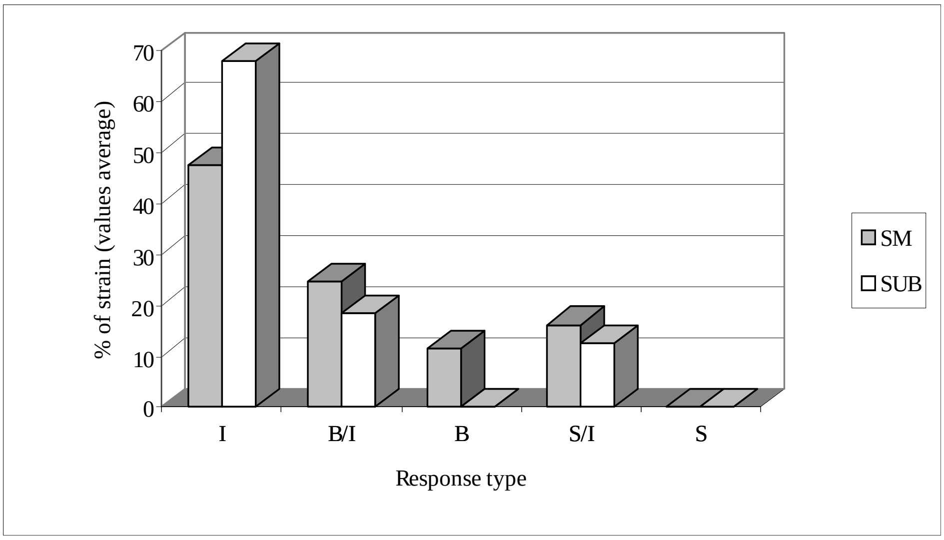


Fig. 4. Bacteria response types to higher concentrations of Pb ions
Explanations as on Fig.2

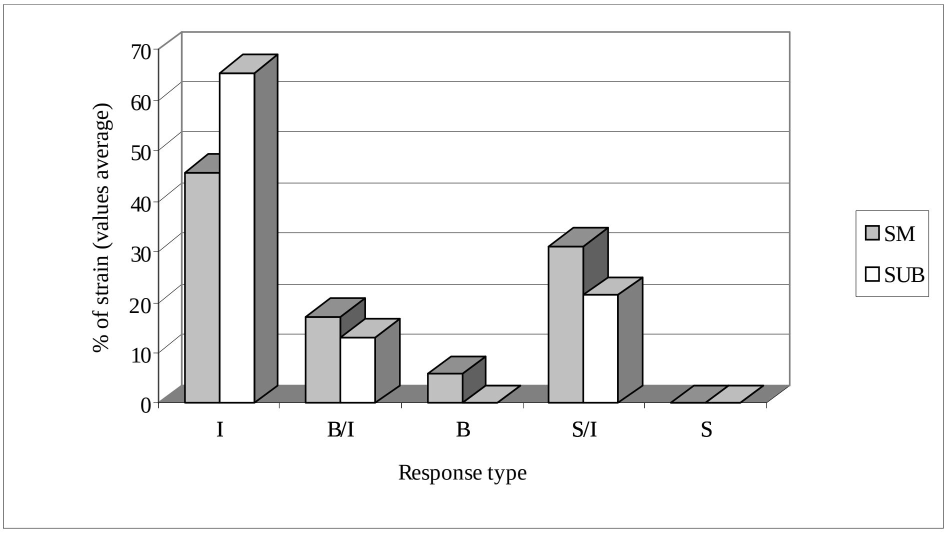


Fig. 5. Bacteria response types to higher concentrations of Cd ions
Explanations as on Fig.2

surface microfilm into the subsurface water, in July and August revealed higher concentrations of those metals ions in the deep water than in the microfilm.

In contrast to Kim (1985) no significant differences were recorded in the response of bacteria strains originating from particular water layers to metal concentrations occurring in the environment at that time. An average of 60% showed no feedback to the presence of natural concentrations heavy metals that occurred in Lake Jeziorak Mały. That result was fully justified by the fact that the optimum conditions of microorganism growth in laboratory cultures make them resistant to toxic factors, and also that the complex forming properties of inorganic and organic compounds (included in the cultures) might weaken the ions toxicity (Nriagu 1983). Neither did the microorganisms respond by a heavy growth impediment as they had been adapted to such concentrations in their habitat environment, in the water body. The fairly great differences between the copper and cobalt ions' impeding influence on bacteria growth must have resulted from their different influences on microorganisms in general. Lead and cadmium ions being toxic to microorganisms and influence for biologic processes make up group B; while copper and cobalt ions being microelements whose low concentrations are favorable for microorganism development make up "Borderline" group (Beveridge and Doyle 1989). This must also be the cause of differences occurring between the two pairs of metals with regard to S/I reaction types in the strains studied.

The data analysis enables a comment that seasonal changes in the percentage shares of the particular response types were correlated with the seasonal changes in heavy metal concentrations in the water of the lake investigated. In a June sample of the highest heavy metal concentration, an average of 71% of the strains proved resistant to the environmental concentrations of heavy metal ions, while in an August sample with the lowest concentration of heavy metals, the number of resistant strains diminished by 17%, but on the other hand, an increase in the number of strains sensitive to those metal ions' environmental concentrations was recorded. The data confirmed the ones by Mills and Colwell (1977), who maintained that bacteria originating from the water containing heavy metals ions revealed a better tolerance to heavy metals than those from unpolluted environments.

Data provided by experiments with higher concentrations of heavy metal ions indicated that strains in the subsurface water revealed growth impediment more frequently than bacteria in the surface microfilm. Copper ions had the least toxic effect, where the I response type occurred in 32.3 % microfilm strains on average, and in 42.8% subsurface water strains. The most toxic were lead ions, and in the case of cobalt a growth stimulation was recorded in a few percentage of strains which confirmed the thesis of this metal being one of microelements. In reference to cadmium ions, very interesting yet hard to interpret results were obtained. In both microfilm and subsurface water strains a fairly great percentage (31.2 and 21.4% respectively) demonstrated a S/I type response which suggested bacteria growth stimulation exerted by that metal ions at low concentrations with a simultaneous lack of ions of other metals. Another possible explanation of that phenomenon is that low concentration cadmium ions completely entered a complex with the compounds included in the medium.

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## WPŁYW JONÓW METALI CIĘŻKICH NA BAKTERIE NEUSTONOWE

### Streszczenie

Uzyskane wyniki badań wykazały, że liczebność bakterii w podpowierzchniowych warstwach wody była znacznie mniejsza niż w błonie powierzchniowej. Bakterie neustonowe były bardziej odporne na jony metali ciężkich niż bakterie wyizolowane z wody podpowierzchniowej. Najbardziej hamująco na wzrost bakterii wpływały jony ołowiu a w najmniejszym stopniu działanie inhibitorowe wykazywały jony miedzi.