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Effect of Heavy Metal Ions on Neustonic and Planktonic Bacteria Isolated from Lake Jeziorak Mały

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Abstract

The object of study was the effect of metal ions Hg^{+2}, Cu^{+2}, Zn^{+2} on the development of neustonic and planktonic bacteria from Lake Jeziorak Mały. It was found that planktonic bacteria were more susceptible to the heavy metal ions used in the experiment than neustonic bacteria. The bacteria showed higher tolerance to various concentrations of heavy metals in spring and summer than in autumn and winter. Neustonic bacteria isolated from sites exposed to municipal and industrial pollution showed higher tolerance to increased concentrations of heavy metals. Bacteria cultured in trophically rich medium were less susceptible to various concentrations of heavy metals than those cultured in trophically poor medium. The effect of toxicity of heavy metal ions on bacteria increased with the increase in their concentration and decreased in the following succession: Hg>Cu>Zn.

Keywords: surface microlayer water, neustonic bacteria, planktonic bacteria, heavy metal

Introduction

At the boundary between the phases air - water in water basins there forms a surface film, often called biofilm, from several to several hundred μm in thickness, with increased surface tension. That microlayer shows considerable physico - chemical and biological distinctness from the subsurface water [1]. The abundance and activity of the microorganisms living in it, much higher than those found in subsurface water [2, 3, 4, 5], are maintained by the richness and diversity of chemical compounds: carbohydrates, lipids and proteins [6, 7, 8], in spite of the presence among them of heavy metals [9, 10, 11], usually occurring here at higher concentrations than in deeper water layers.

The effect of heavy metals on the microorganisms living in that extreme ecotope depends on a number of factors, and in particular on the pH of the environment and on the interactions of other ions and compounds, which may act synergistically or antagonistically [12]. In acid environment heavy metals can occur as free cations, whereas at pH changed to basic there form insoluble oxides and hydroxides [13]. At low pH of the environment there occurs an increase in bioaccessibility of heavy metal ions to organisms and in the possibility of their bioaccumulation, and at the same time in their toxicity, while higher pH reduces their toxic effect. The toxicity of heavy metals also depends on their chemical reactivity. They can form complexes with the suspension and organic compounds, such as nitriltriacetic acid, citrates or cysteine [14], and in the course of migration in surface waters a significant part is also played by humus compounds. Their function groups (carboxylic, ketone, amino and thionic) bind metals into complexes, thus immobilizing them temporarily [15, 16]. A serious problem is biomethylation of metals and their transportation in the environment related with it. It constitutes an essential mechanism, playing an important part in various processes occurring in the organism's cells, responsible for the capacity of heavy metal ions to move in the environment and for their bioaccumulation to a toxic level. Many heavy metal ions, including Hg^{+2}, Cu^{+2} and Zn^{+2}, tend to combine with proteins, particulary with their sulphhydryl groups. Those ions are responsible for the formation of harmful substances, causing disturbances in the functioning of enzymes and of the genetic apparatus in bacteria [17, 18].

Due to progressing industrialization, high concentrations of heavy metals occur more and more frequently in natural environment with toxic effect on the bacterial microflora. That has led to a situation where microorganisms have been forced to develop various protective mechanisms. Here belongs the ability of bacteria to change the valence of metals [19], to methylate or demethylate them, which leads to their inactivation and adoption of neutral or volatile form.

The ocurrence in water environment of higher concentrations of heavy metal ions can lead to their biosorption and accumulation in the bacterial cells. Biosorption is related to the formation of bonds of metals with active groups of chemical compounds occurring in the cell envelopes, walls and membranes of bacteria [20, 21]. As a result of the microorganisms metabolic processes, the heavy metals on the one hand cumulate and form complexes inside the cells, which causes their temporary inactivation and immobilization [22, 23], but on the other hand they are released from organic and mineral compounds, which makes their presence in the environment dangerous.

Considering that the ability to move about as well as the progress of the transportation and bioaccumulation of heavy metals are of high importance for the situation of the natural environment, the object of the present work is to estimate the effect of some heavy metals on the development of heterotrophic bacteria, living in the surface and subsurface water layer of the lake, depending on the concentration of the metal ions as well as on the site and time of year in which the bacteria were isolated.

Materials and Methods

Study Area

The studies were carried out in Lake Jeziorak Mały, which is a part of the Iława Lake District. It is a small mid-town lake, about 26 ha in area, with a mean depth of 5 m, strongly polluted, classified as a highly eutrophized lake [24]. Municipal sewage, industrial waste and rain-water from the town of Iława run into the lake. Water samples were taken from three stands (Fig. 1.).

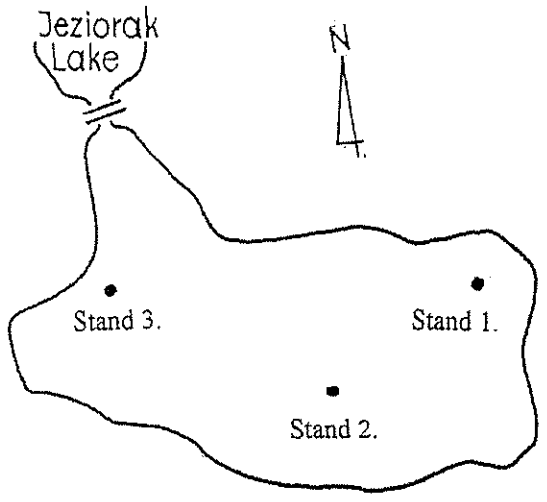


Fig. 1. Outline of Lake Jeziorak Mały

Stand 1 was situated off shore close to a built-up area. Stand 2 was in the central part of the lake. Stand 3 was situated near a lumber mill.

Sampling

The studies included the water surface microlayer and subsurface water 5 cm deep. Samples of subsurface water were taken with sterile glass pipettes. The surface water microlayer was collected by means of a 50x50 cm Garrett net with 50 μm mesh. The water was collected into sterile glass bottles and stored in an ice-box, where the temperature did not exceed 7°C, until it was taken for analysis.

Strain Isolation

In order to isolate neustonic and planktonic bacteria, the water samples were diluted with buffer water [25], then using the spread plates method, plated in 5 replicates on iron-peptone agar medium after Ferrer, Stapert and Sokolski [26]. Incubation was conducted for 6 days at 20°C. Next, 25 random colonies from each sample were picked up and transferred into a semi-liquid iron-peptone agar medium. After checking the colonies for purity, they were stored at 4°C for further study.

Before starting to study the effect of heavy metals on the development of bacteria, the isolated strains were proliferated on slants of the iron-peptone agar medium for 72 hours at 26°C. Then they were washed off with 2.5 ml of sterile buffer water. The suspension thus obtained was used as inoculum for study.

Heavy Metals

The following heavy metals in the form of their salts were used in the experiment: $HgCl_2$, $CuSO_4$, $ZnSO_4$. The mother liquors of the heavy metal salts were prepared in distilled water. The final concentrations of ions of particular metals in culture media were as follows:

Hg^{+2} - 0.5 ppm, 1.5 ppm, 5.0 ppm, 7.5 ppm
Cu^{+2} - 0.5 ppm, 2.0 ppm, 6.0 ppm, 10.0 ppm
Zn^{+2} - 50 ppm, 500 ppm, 1000 ppm, 2000 ppm

Microbiological Experiments

In the study of effect of heavy metals on the development of heterotrophic, neustonic and planktonic bacteria were used: a) the diffusion toxicity test after Różycki [27] and b) the spectrophotometric measurement of the bacteria's development in liquid culture. The cultures of bacteria in the presence of heavy metals were run in a trophically rich medium containing: casein hydrolyzate (Difco) - 5.0 g, yeast extract (Difco)

- 1.0 g, glucose - 2.0 g, NaCl - 1.0 g, $MgSO_4$ - 2.8 g, KCl - 0.3 g, distilled water 1000 cm^3, pH 7.0-7.2 and in a trophically poor Czapek medium, modified by Dox and Thom [28], composed of saccharose 3.0 g, $NaNO_3$ 2.0 g, K_2HPO_4 - 1.0 g, $MgSO_4$ - 1.0 g, KCl - 0.5 g, $FeSO_4$ x $7H_2O$ - 0.01 g, distilled water - 1000 cm^3, pH 7.0-7.2.

a) The diffusion toxicity test was carried out using two-layer plates, in which the lower layer was iron-peptone agar, and the upper layer consisted of 5 ml agarized medium, trophically rich or trophically poor. After being inoculated with 0.1 ml bacterial suspension prepared as described above, the media were immediately placed in a refrigerator at 4°C for 30 min for quick solidification and inhibition of possible growth of the tested bacteria. Then five cylindrical holes were cut out in the media (∅ 3.2 mm). Into four of them were poured 20 μl each of various concentrations of the heavy metals under study, and into the fifth (control) sterile distilled water. The plates with the inocula were incubated for 5 days at 26°C, after which the diameters of the bacterial growth inhibition round the holes were measured. All experiments were carried out in triplicate.

b) For the spectrophotometric measurement of bacterial development, the bacteria were incubated in liquid media, one trophically rich and one trophically poor, with various heavy metals concentrations added (as above). Test-tubes containing 4 ml medium were inoculated with a one loopful of the bacterial suspension, prepared as above. After 7 days of incubation at 26°C, light absorption in the bacterial cultures at 560 nm wavelength was measured using a Specol 221 spectrophotometr (Carl Zeiss Jena) relative to the control (blind) sample, which was uninoculated media.

Results and Discussion

The experiments demonstrated that all heavy metals used in them (Hg^{+2}, Cu^{+2}, Zn^{+2}) had a toxic effect on the neustonic and planktonic bacteria from Jeziorak Mały. Their effect depended on the kind of the metal, its concentration, the kind of medium as well as on the site and time of year when the bacteria were isolated. The experiments demonstrated that the toxicity of metals progressed in the following order: Hg>Cu>Zn (Figs. 4 and 5). The toxic effect of mercury was many times higher than that of copper and zinc. At 0.5 ppm concentration it allowed very good growth of only 10% of strains on trophically poor medium (Fig. 5) and of 26% of strains incubated on trophically rich medium (Fig. 4). Those data confirm the results obtained by Gadd and Griffiths [29] and Whitton [30]. According to Coogan [31], besides causing disturbances in the genetic apparatus of microorganisms, mercury in the form of competitive inhibitors can also substitute ions of other important metals, e.g. Mg^{+2}, in the active centres of enzymes.

The lowest susceptibility to the heavy metal ions used in the experiment was found in bacteria isolated from the stand situated in the vicinity of a lumber mill (Figs. 2 and 3). The toxic effect on the populations of bacteria isolated from the other stands was slightly higher. Bacterial susceptibility or insusceptibility to heavy metal ions seems to be directly related to the presence of those elements in the environment as constituents of environmental pollution. That is in line with the results of studies conducted by Mills [32] and Kim [12], which demonstrated a high level of adaptation in bacteria living in polluted environments. Tolerance of microorganisms to higher heavy metals concentrations in the environment is also manifested as the ability to form extracellular polymers. The results of studies conducted on media enriched to various degrees with organic substances, described in the present paper, point to a dependence of the toxicity of metals on the concentration of the organic complex (Figs. 2 and 3). In media richer in organic compounds there probably forms an organic complex with heavy metals, which causes temporary detoxication of the environment and a limitation of bioaccessibility of heavy metals. The inhibitory effect of Cu and Zn was considerably reduced when they occurred in a trophically rich medium. That is confirmed by the results obtained by Kim [33] and Niewolak [34]. According to those authors, the decrease in biological toxicity of heavy metal ions may be due to the effect of other ions, e.g. Mg^{+2}, whose concentration in the trophically rich medium used in the experiment was twice as high as in the trophically poor medium. It follows from the data obtained in the present work that this rule does not concern mercury, which shows high toxicity both in organic complexes and in inorganic compounds.

Differences in the effect of heavy metal ions were found between the planktonic strains isolated from 5-cm depth and the neustonic strains from the surface microlayer water of the lake. It follows from the data that the bacteria living in the surface film are less susceptible to the toxic effect of the metals under study (Figs. 2, 3, 4 and 5). Kim [33] has found that neustonic bacteria's tolerance to heavy metals is due to their adaptation to the higher concentrations occurring in the surface film than in subsurface water. That is in line with the results of Waldichuk [35], who found that the concentrations of pollutants, including heavy metals, is 100 to 1000 times higher in the surface microlayer than in the deeper water layers.

The most tolerant to heavy metals were strains isolated in spring and summer, the least those isolated in winter (Figs. 4 and 5). That difference is probably due to the phenomenon of adaptation of the neustonic and planktonic strains to higher levels of pollution in spring and summer, caused by the influx of tourists and more rainfall introducing atmospheric pollution from the catchment area into the lake.

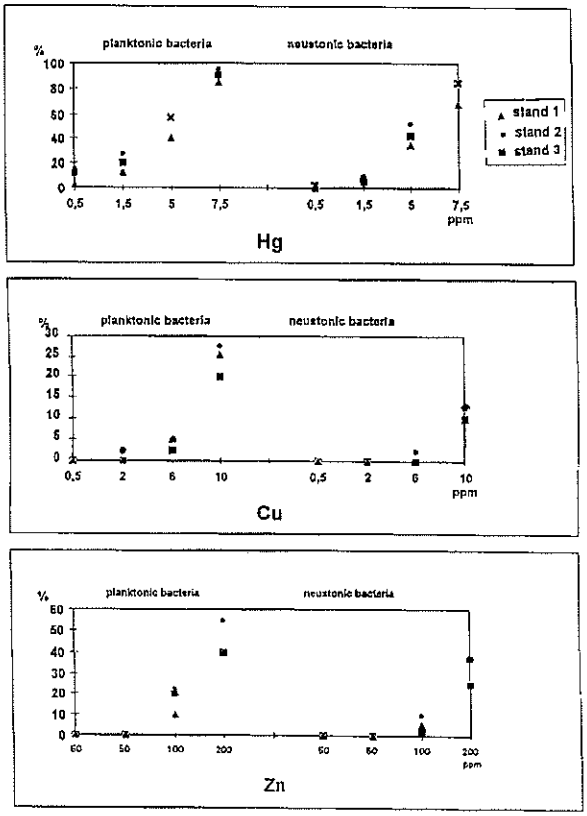


Fig. 2. Percentage of strain incubated in trophically rich medium, susceptible to particular concentrations of heavy metals (annual mean)

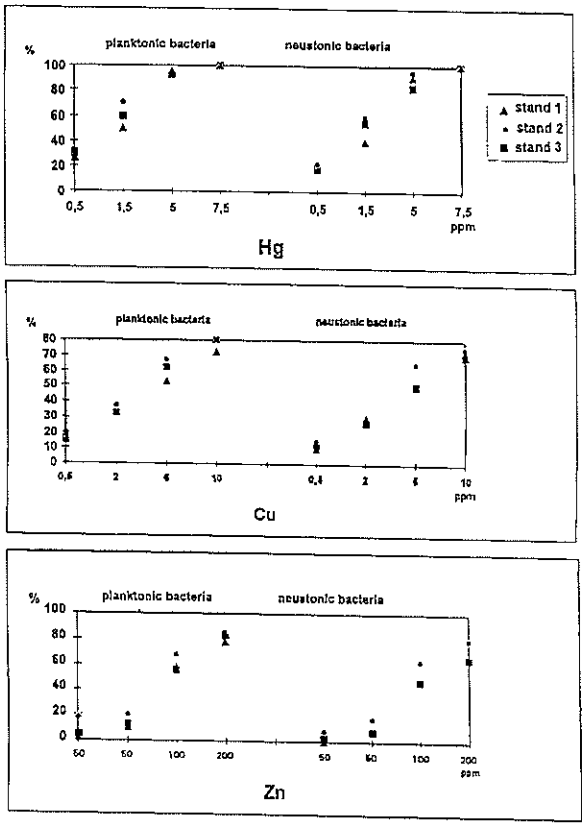


Fig. 3. Percentage of strain incubated in trophically poor medium, susceptible to particular concentrations of heavy metals (annual mean)

Data concering the tolerance of neustonic microorganisms of inland waters to the presence of heavy metals are very scarce. More attention has been focused on studies of microorganisms living in the surface layer of sea waters [1, 3, 5, 9, 31], which is why there is only limited possibility of a wider discussion concerning the neustonic microflora of inland basins. It is therefore necessary to continue intensive studies in order to obtain fuller data about neustonic bacteria and their role in the detoxication of heavy metals in inland waters. A better understanding of the physiological and biochemical properties of that group of bacteria can also help to understand better their role and importance in the processes of self-purification of waters.

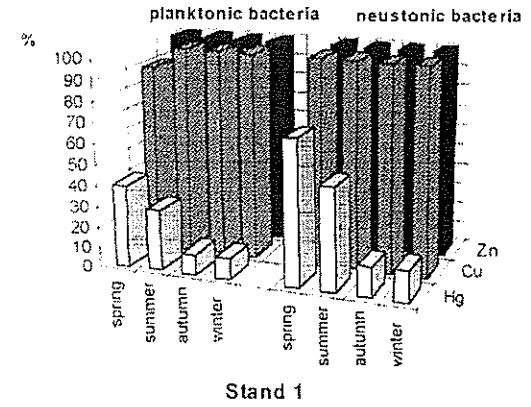
Conclusions

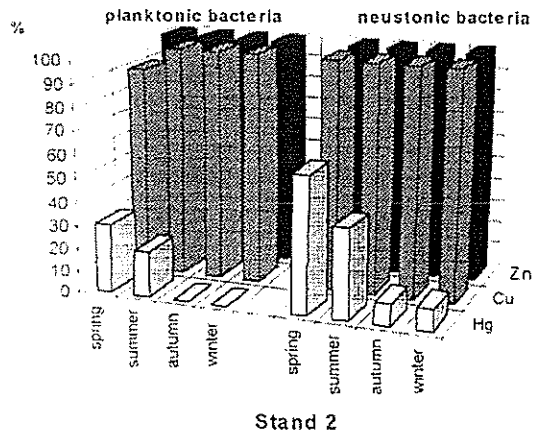
Summing up the results of the present work, the following conclusions can be drawn :

a) the ions of the heavy metals used in our work, viz. mercury, copper and zinc, had a toxic effect on most planktonic and neustonic bacteria of the lake under study
b) the effect of heavy metal ions on the microflora depended on the kind of metal, its concentration and the presence of organic substances in the culture medium
c) the toxicity effect of heavy metal ions increased with an increase in their concentration and decreased in the following order: Hg>Cu>Zn
d) bacteria isolated from stands exposed to municipal or industrial pollution were more tolerant of increased concentrations of heavy metals
e) planktonic bacteria were more susceptible to high concentrations of heavy metal ions than neustonic bacteria.

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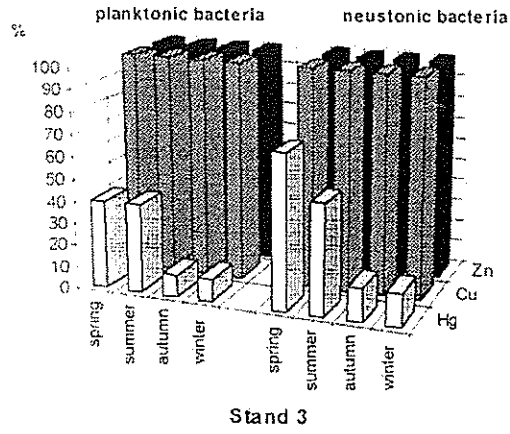
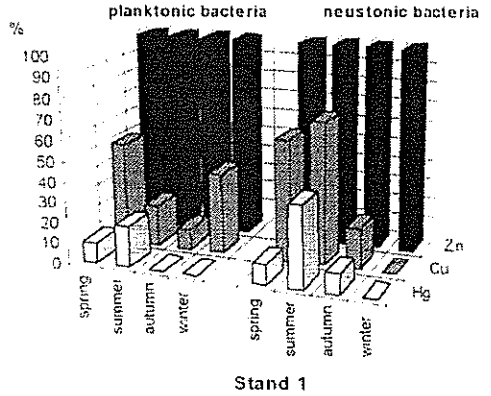
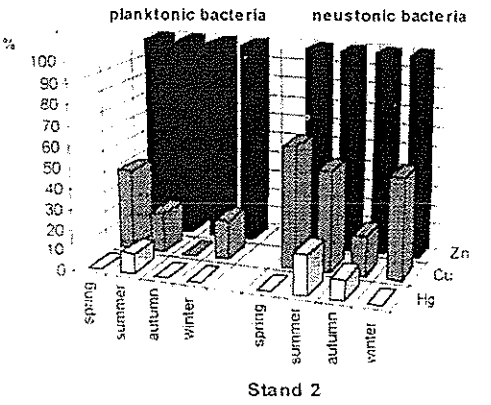


Fig. 4. Development of bacteria in trophically rich medium in the presence of heavy metal ions – percentage of strains developing very well. Hg - 0.5 ppm, Cu - 0.5 ppm, Zn - 50pm





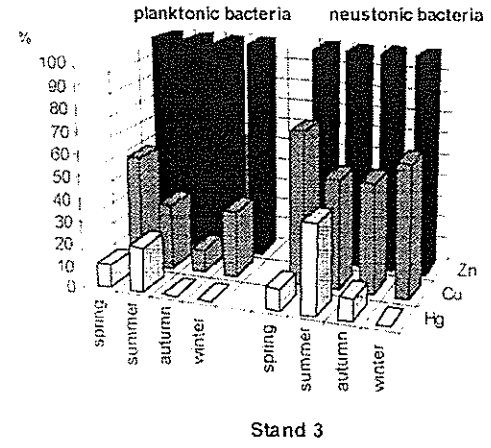


Fig. 5. Development of bacteria in trophically poor medium in the presence of heavy metal ions - percentage of strains developing very well. Hg - 0.5 ppm, Cu - 0.5 ppm, Zn - 50 ppm.

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