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Regular research paper

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INTERACTION OF ESTUARINE BACTERIONEUSTON AND BACTERIOPLANKTON WITH ELEVATED MERCURY CONCENTRATIONS

ABSTRACT: The effect of different concentrations of mercury on the number and respiratory activity of neustonic and planktonic bacteria derived from estuarine Lake Gardno (Baltic Coast, Poland) was studied. The laboratory experiments demonstrated that mercuric ions (Hg^{2+}) exhibited toxic effects on abundance and oxygen uptake of neustonic and planktonic bacteria. Bacterioneuston and bacterioplankton showed different responses to the mercuric ions. The neustonic bacteria showed higher levels of tolerance to various concentrations of Hg ($10\text{--}15\text{ mg dm}^{-3}$) in the culture medium than the planktonic ones ($<10\text{ mg dm}^{-3}$). It was found out that the bacteria isolated from freshwater zone of Lake Gardno were more tolerant to the increasing concentration of mercury ions ($10\text{--}20\text{ mg dm}^{-3}$) than bacteria isolated from the seawater and mixed zones of lakes. Non-pigmented bacteria from all layers and zones were more tolerant to the higher Hg^{2+} concentrations than pigmented ones. High concentrations (above $8\text{ }\mu\text{g cm}^{-3}$) of mercury chloride in the respiratory substrate blocked completely oxygen uptake by neustonic and planktonic bacteria.

KEY WORDS: estuaries, bacterioneuston, bacterioplankton, mercury, abundance, respiratory activity

1. INTRODUCTION

Interactions of bacteria with heavy-metal ions have aroused considerable interest in recent years. Studies of these interactions have focused especially on conversions of mercury in aquatic ecosystems (Barkay *et al.* 1990, Farrell *et al.* 1993, Cursino *et al.* 1999, Reyes *et al.* 1999). Mercury is an extremely hazardous chemical element because of its volatility in the metal state and ability to form numerous toxic volatile organic compounds under the action of bacteria present in aquatic ecosystems (Ostrovskii *et al.* 2000). Mercuric ions (Hg^{2+}) and organic forms (org-Hg) are cytotoxic to bacteria because they are liposoluble and strongly bind sulphhydryl groups with membrane proteins and in effect then inhibit bacterial macromolecule synthesis and enzymes action (Foster 1983, Cursino *et al.* 1999). However, many bacteria have developed several different and efficient mechanisms for tolerating the mercury compounds. These mechanisms include five ways by which the bacteria defend themselves from action of mercury: (1) the lowering of the cell wall

permeability to mercury, (2) the formation of insoluble mercury sulphides, (3) the conversion of mercurial compounds into metal mercury, (4) the conversion of non-volatile mercurial compounds to volatile ones by means of methylation, and (5) the rapid excretion of mercury from cells into the medium (Osborn 1999). These mechanisms allow the bacteria to carry out reactions that transform mercury between its three major forms; Hg^0 , Hg^{2+} and org-Hg (Barkay *et al.* 1992).

The reduction of Hg^{2+} and degradation of org-Hg are detoxification processes allowing bacterial growth in the presence of mercury. The enzyme that catalyses the reduction of Hg^{2+} is the intracellular cytoplasmic, FAD-containing mercuric reductase, which is a soluble cytoplasmic protein about 63.000 molecular weight. Strains, which detoxify organomercurials specify a second cytoplasmic enzyme, organomercurial lyase. This cleaves C-Hg bonds to release Hg^{2+} , which is in turn volatilised by the reductase (Foster 1983, Barkay *et al.* 1992).

Because microorganisms in aquatic ecosystem are involved in many basic ecological processes such as organic matter degradation, biogeochemical cycling and detoxification of toxic contaminants (Martinez *et al.* 1991) the object of the present study was to determine the influence of mercury ions on the numbers and respiratory activity of neustonic and planktonic bacteria inhabiting the estuarine lake.

2. MATERIALS AND METHODS

2.1. Study area

The studies were carried out in an estuarine Lake Gardno situated in the World Biosphere Reserve – Słowiński National Park (Baltic Coast, Poland). The lake is very shallow (1.3 m average depth) but covers a large area (2.500 ha). The shallow depth and large area as well as the lack of shielding winds make possible a full mixing of water in both vertical and horizontal profiles. As a result, the lake can be regarded as a polymictic basin in which no thermal or oxygen stratification is observed. The emergent macroflora covers 4% of the Lake Gardno surface forming a 20–100 m wide offshore belt, which constitutes home to many bird species. The main species of macrophytes

are: *Typha angustifolia*, *Phragmites australis*, *Scirpus lacustris* and *Schoenoplectus lacustris*.

Lake Gardno is characterised by intermediate conditions between marine and inland environment. On one hand it is supplied by the water of the River Łupawa, on the other hand via a 1.3 km channel it is connected with the Baltic Sea (Fig.1) whose large volumes of sea water abundantly penetrate into the lake. Therefore, the water of the lake, or its part acquires seawater properties, resulting in 2–5‰ salinity. Consistently with the Venetian system, Lake Gardno can be classified as mixo-oligohaline type (0.5–5.0‰) (Dethier 1992).

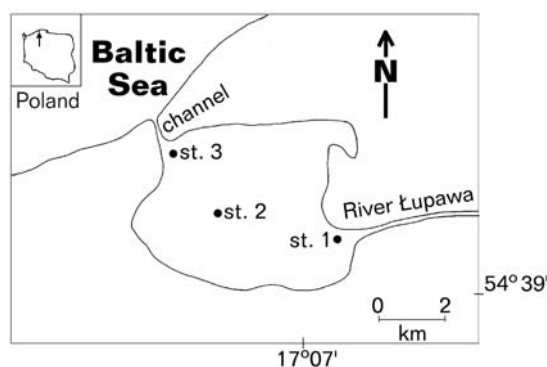


Fig. 1. Sampling stations location in Lake Gardno (Baltic Coast, Poland).

2.2. Sampling

Water samples were taken during June 2000 from three stations (Fig.1): one near the River Łupawa inflow (freshwater zone) (station 1), one in near-sea part (seawater zone) (station 3) and at one station in mid-lake (mixed zone) (station 2). Water samples for bacteriological analyses were taken from three layers. Film layer (FL) samples (thickness of $90 \pm 17 \mu\text{m}$) were taken with a 30×30 glass plate (Harvey and Burzell 1972), surface layer (SL) samples (thickness of $242 \pm 40 \mu\text{m}$) were collected with a 40×50 cm Garrett net (24 mesh net of 2.54 cm length) (Garrett 1965). Glass plate and polyethylene net were rinsed with ethyl alcohol and distilled with sterile water prior to sampling. The water from subsurface layer (SUB) was sampled from the depth of about 10–15 cm. The all water samples were collected into sterile glass bottles and stored in an ice-box, where the

temperature did not exceed 7°C until they were taken for analysis. The time between sample collection and performance of the analyses usually did not exceed 6–8 h.

2.3. Determination of the influence of mercury on the numbers of neustonic and planktonic bacteria

In order to study the effect of mercury ions (HgCl_2) on the numbers of neustonic and planktonic heterotrophic bacteria an iron-peptone agar (IPA) medium was used (Ferrer *et al.* 1963). The final concentrations – of mercury chloride ions (Merck AG) in IPA medium were as follows: 0.0, 10.0, 15.0, 20.0, 50.0, 100.0, 200.0 mg dm^{-3} (Hermansson *et al.* 1987). The collected surface (FL and SL layer) and subsurface (SUB) water samples were diluted with sterile buffered water (Daubner 1967) and inoculated by the spread method in three parallel replicates on IPA medium with various concentrations of mercury chloride. IPA medium containing no HgCl_2 was used as controls. After a 7 day-cultivation at 20°C, the colonies of neustonic (FL, SL) and planktonic (SUB) bacteria were counted and the results were calculated as a number of bacteria per cm^3 water. Subsequently, nine bacterial colonies from each water layer and station growing on media without heavy metal were picked from the plates and were transferred to semiliquid IPA medium (5.0 g of agar dm^{-3} of medium). The cultures maintained on this medium, after purity control were kept at 4°C and used for further investigation in order to determine the influence of mercury on respiratory activity of these bacteria.

2.4. Estimation of the influence of mercury chloride on bacteria respiratory activity

The influence of mercury chloride on respiratory activity of neustonic and planktonic bacteria was determined as oxygen uptake by these organisms using Clark's electrode (Rank Brothers Ltd. Model 10) (Konopka and Zakharova 1999). The respiratory activity of nine bacterial strains from each water layer and station was determined. Pure cultures of bacteria were multiplied on IPA agar slants for 48–72 h at 20°C. Subsequently, they were washed off

from the slants with phosphate buffer (0.01 M, pH 7.0), centrifuged at 15,000 rpm for 15 min and washed twice with the buffer. The washed bacteria were resuspended in the same buffer and adjusted to the turbidity of 4 Mac Farland standards. Typically, 1 cm^3 of such a suspension contained 10^9 bacteria. Casein hydrolyzate (Casamino acids vitamin-free – Difco) was used as a respiratory substrate in this study. This substrate (0.5 mg cm^3) was dissolved in phosphate buffer containing different concentrations (0.0, 1.0, 2.0, 4.0, 8.0, 20.0, 30.0, 50.0 $\mu\text{g cm}^{-3}$) of mercury chloride.

Before measurements, the respiratory chamber of Clark's electrode was calibrated with sodium dithionate at the polarizing voltage of 6.0 V. After calibration, 1.5 cm^3 of the bacterial suspension and 30 μl of respiratory substrate were put into the respiratory chamber. Changes in voltage on the electrode were recorded by an analogue XY Line Record TZ 5000 recorder and stored in a computer program BS81x – BS51x Data Recording System Ver. 3.3.05. The number of measurements was set at 30, taken every 6 seconds. During the measurements, the Clark's electrode was connected to a flow stabilizer of temperature, which ensured thermal stability in the respiratory chamber. Respiratory activity of each strain of bacteria was measured in triplicates. Data were corrected for endogenous respiration and the oxygen uptake was converted into $\mu\text{l O}_2 \text{ h}^{-1}$ per 10^9 cells.

3. RESULTS

Figure 2A presents the results on the impact of mercury chloride on the number of bacteria inhabiting film layer (FL), surface layer (SL) and subsurface (SUB) water layers in Lake Gardno. The given data show that a low (10–15 mg dm^{-3}) mercury chloride concentrations in the medium caused the increase in the numbers of bacteria inhabiting the film layer. However, the same HgCl_2 concentration in a culture medium caused the decrease in bacteria numbers in the surface layer. At concentration 20 mg dm^{-3} of mercury chloride, the bacteria inhabiting both (FL, SL) surface water layers decreased in number. A dramatic drop in the number of neustonic bacteria (FL, SL) was recorded in the presence of 50–200 mg dm^{-3} mercury chlo-

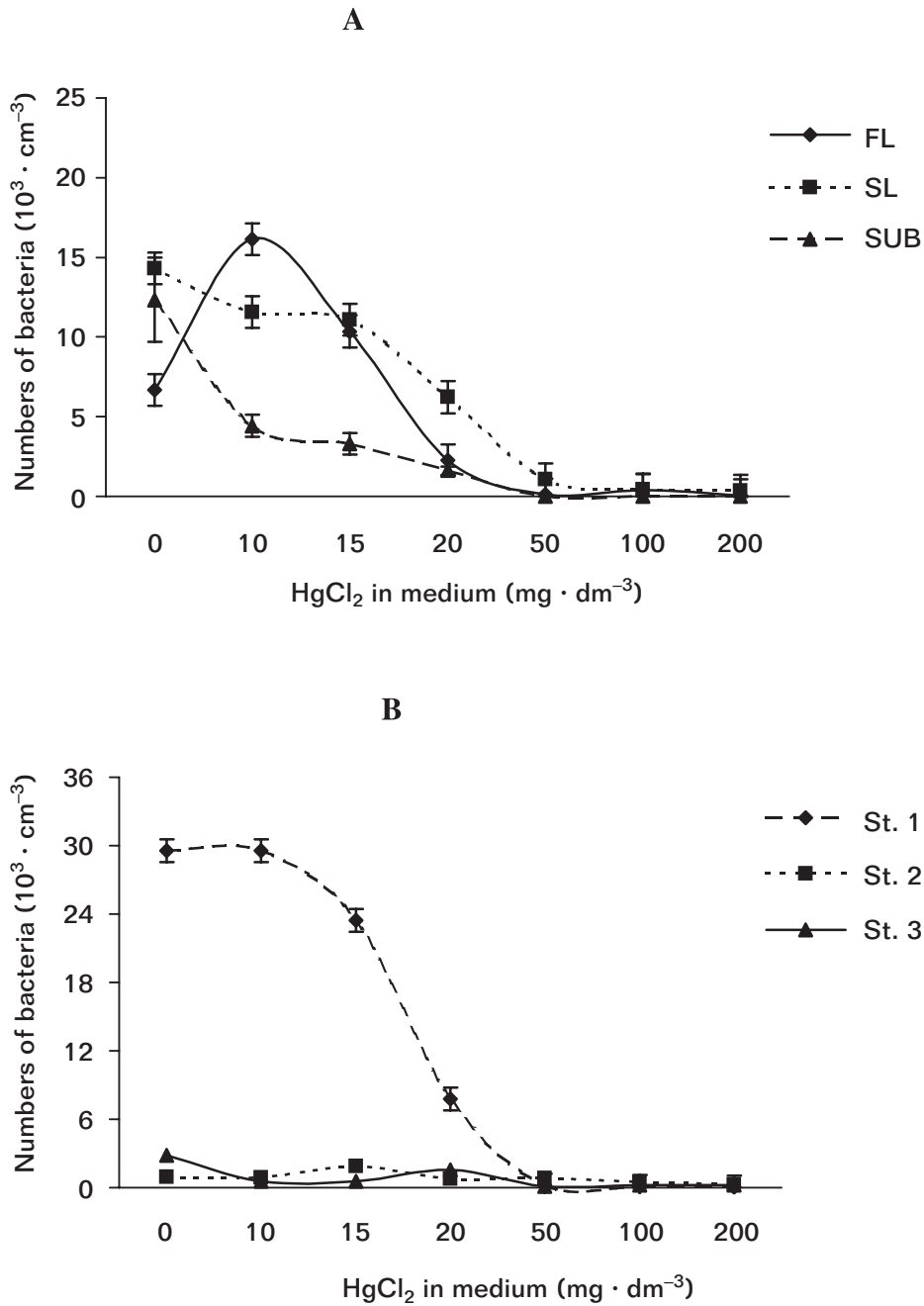


Fig. 2. The effect of different mercury chloride concentrations in media on numbers of bacteria isolated from different water layers (A) (average from the pooled data of all stations) and stations (B) (average from the pooled data of all layers). Vertical bars represented standard deviation of the mean, $n = 9$. Points without visible bars indicate that standard deviation was less than the size of the point. FL – film layer, SL – surface layer, SUB – subsurface layer, St. 1-3 (see Fig.1).

ride. However, at such great HgCl_2 concentration in the culture medium, low number of neustonic bacteria were able to survive under the same conditions. In the subsurface water layer (SUB), the plankton bacte-

ria numbers dropped with the increasing mercury ions concentration in the culture medium. Even the lowest (10 mg dm^{-3}) tested concentration of mercury ions caused a two-fold reduction in the number of

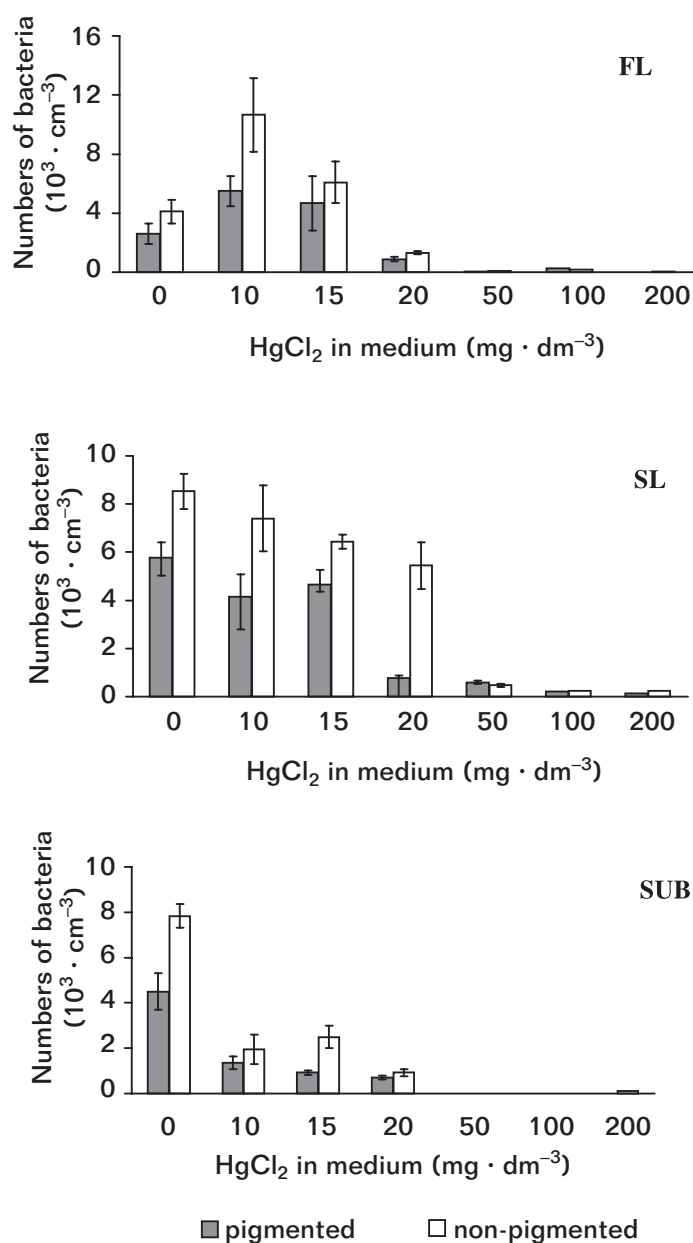


Fig. 3A. Influence of HgCl_2 concentrations on numbers of the pigmented and non-pigmented bacteria from different water layers. For explanations see Figs 1 and 2. Vertical bars represented standard deviation division of the mean, $n = 9$. Points without visible bars indicate that standard deviation was less than the size of the point.

those microorganisms. No growth of planktonic bacteria occurred in media with concentration above 20 mg dm^{-3} .

The data presented in figure 2B shows that the bacteria isolated from particular parts of Lake Gardno exhibited various levels of tolerance to the presence of mercury in the culture medium. It was found out that the bacteria isolated at station

1 close to the River Łupawa outflow into lake Gardno were most tolerant to the increasing concentration of mercury ions.

Figure 3 presents the results concerning mercury effect on the growth of pigmented and non-pigmented bacteria inhabiting Lake Gardno. The data obtained indicate that in all studied water layers (Fig. 3A) and stations (Fig. 3B) non-pigmented bac-

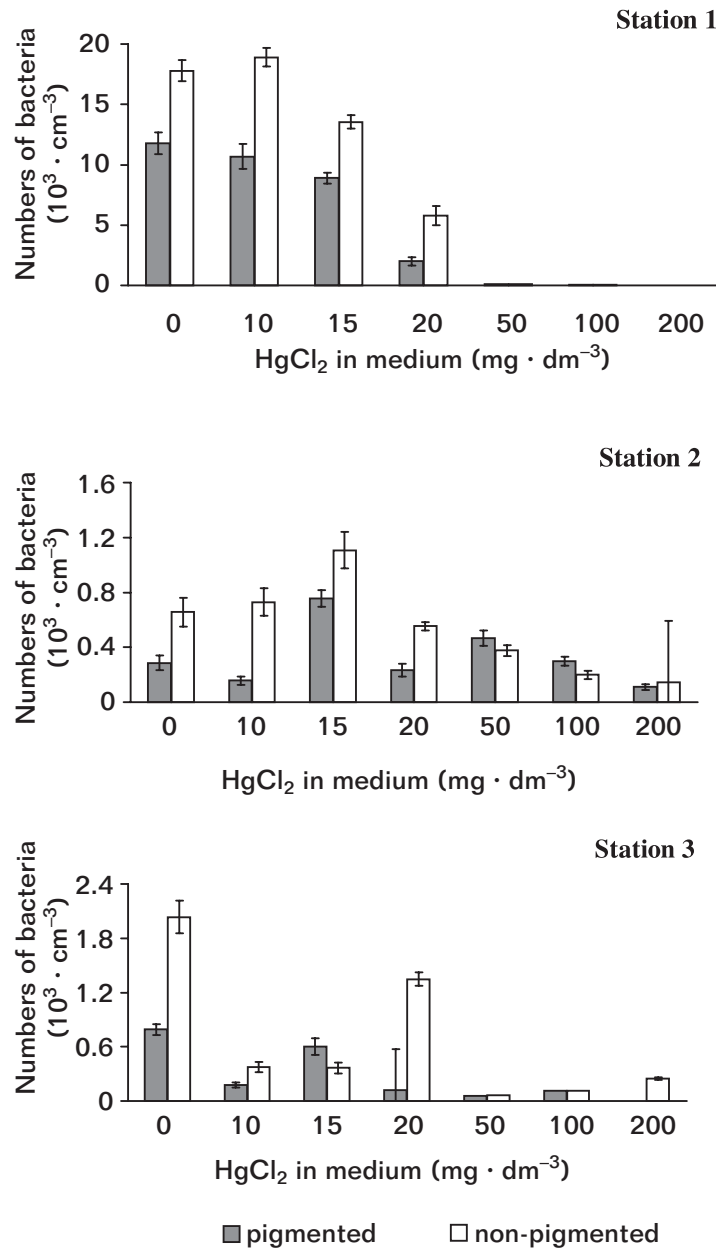


Fig. 3B. Influence of HgCl₂ concentrations on numbers of the pigmented and non-pigmented bacteria from different stations. For explanations see Figs 1 and 2. Vertical bars represented standard deviation division of the mean, $n = 9$. Points without visible bars indicate that standard deviation was less than the size of the point.

teria were most likely to tolerate the higher mercury ions concentrations in culture medium than the pigmented ones.

Figure 4A presents data on the influence of different concentrations of mercury chloride on the respiratory activity of neustonic and planktonic bacteria. It was revealed that even a low ($1-4 \mu\text{g cm}^{-3}$) con-

centration of mercury chloride in the respiratory substrate affected the amount of oxygen uptake by bacteria, whereas a concentration of $8 \mu\text{g cm}^{-3}$ HgCl₂ dramatically hindered the process. Further increase in this heavy metal concentration in the respiratory substrate blocked completely the respiratory process in neustonic and plank-

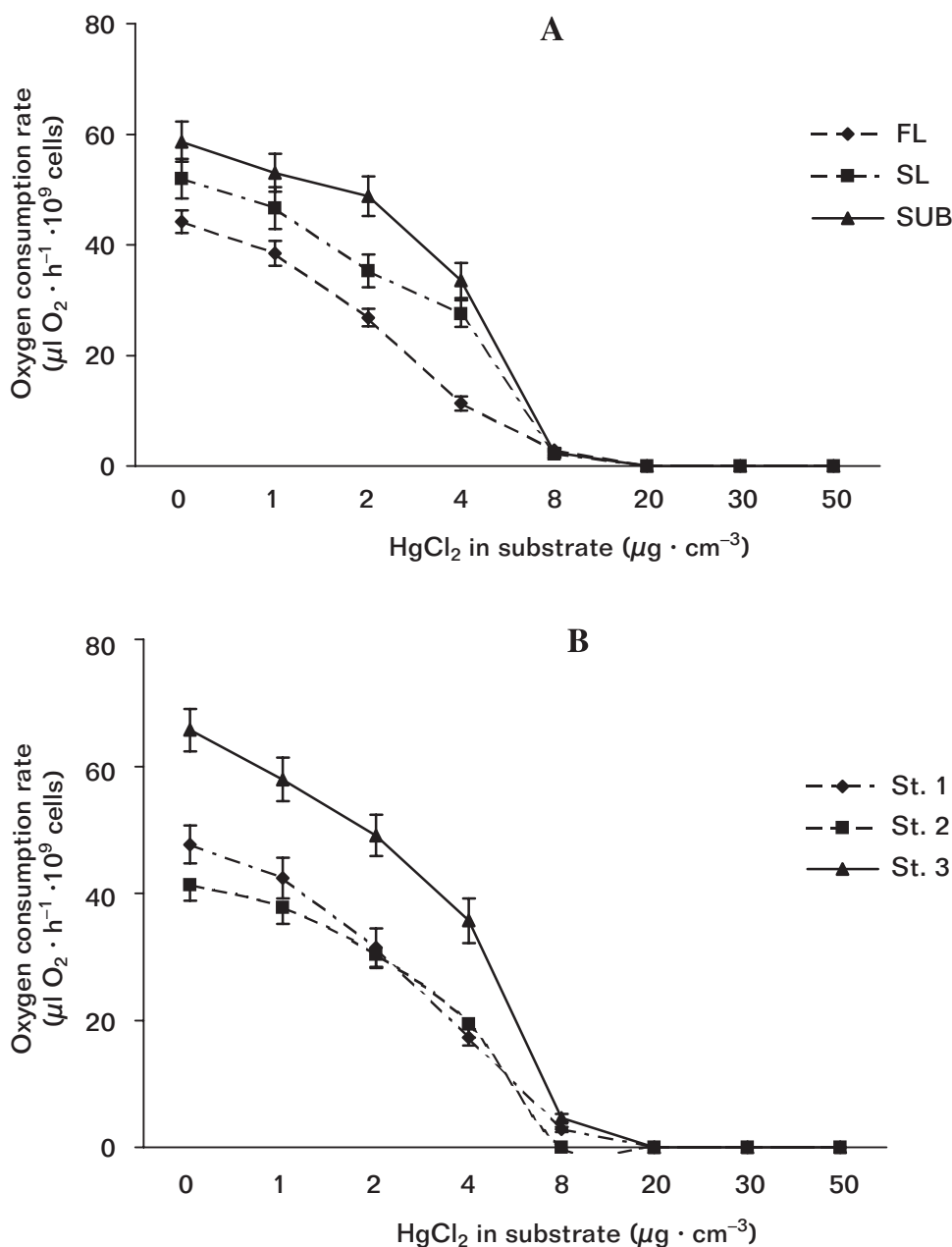


Fig. 4. Oxygen consumption rate by bacteria from different water layers (A) and stations (B) at different mercury chloride concentrations in the respiratory substrate. For explanations see Figs 1 and 2. FL, SL, SUB (see Fig. 2) St. 1-3 (see Fig. 1) Vertical bars represented standard deviation of the mean, $n = 9$ Points without visible bars indicate that standard deviation was less than the size of the point.

tonic bacteria. The bacteria isolated from the subsurface water proved to be most tolerant to the increasing mercury concentrations in the respiratory substrate, while those occurring in the film layer appeared to be the least tolerant.

Following the data shown in Figure 4B, one can note that the $8 \mu\text{g cm}^{-3}$ HgCl₂ con-

centration in the respiratory substrate dramatically limited the process of oxygen uptake by the bacteria isolated from the different parts of Lake Gardno. At the same time, the concentration of $20\text{--}50 \mu\text{g cm}^{-3}$ of mercury chloride hindered completely the bacteria respiration. It was found out that the bacteria isolated from the sea zone (sta-

tion 3) were the least vulnerable to mercury present in the respiratory substrate.

4. DISCUSSION

Many studies (Hermansson *et al.* 1987, Niewolak *et al.* 1996, Donderski *et al.* 1997, Lopez-Amoros *et al.* 1997) show the toxic effects of mercury ions on the number of aquatic bacteria. It has been reported (Martinez *et al.* 1991) that Hg is more toxic to bacteria than other frequently occurring heavy metals in aquatic ecosystems. Results of these studies, along with data published in the present paper, reveal a diminishing bacterial growth occurring along with increasing concentration of mercury chloride in culture medium. This results from the fact that activity of many bacterial enzymes, such as: proteases, phosphatases, reductases, liases, ureases, dehydrogenases, arylsulphatases are inhibited by mercury (Foster 1983, Bogdanova *et al.* 1992, Diaz-Ravina *et al.* 1994).

Among the neustonic and planktonic bacteria that inhabit Lake Gardno many pigmented organisms were found (Mudryk 1987). Therefore, it is interesting to assess and compare Hg influence on the number of pigmented and non-pigmented bacteria. In Lake Gardno, non-pigmented bacteria usually displayed greater levels of tolerance to different mercury ions concentrations in culture medium than the non-pigmented ones. These data do not correspond in any way to the data obtained by Nair *et al.* (1992), who showed that the pigmented bacteria isolated from the Arabian Sea displayed greater tolerance to heavy metals than non-pigmented ones.

Donderski *et al.* (1997), who investigated the effect of heavy metals on the numbers of bacterioneuston and bacterioplankton inhabiting the eutrophic Lake Jeziorak Mały (north-eastern Poland) observed that neustonic bacteria showed higher level of tolerance to various concentrations of heavy metals than planktonic ones. Also in marine environments, mercury resistance occurs at higher frequencies among air-water interface bacteria than among the bulk community (Kim 1985, Hermansson and Lindberg 1994, Lopez-Amoros *et al.* 1997). The results presented in the paper correspond to these data. Neustonic bacteria inhabiting Lake Gardno showed greater toleran-

ce to Hg ions than the planktonic ones. There are different possible explanations of this observation. Large amounts of organic substance accumulated in surface water (Williams *et al.* 1986) could limit the toxicity of heavy metals to heterotrophic bacteria living in this special biotope. Thus, in the surface microlayer the toxic effects of heavy metals are reduced or masked through the formation of complexes of metals with organic compounds. The cations of these chemical elements bind easily with small organic molecules (amino acids, simple sugar) and large molecules (proteins, nucleic acids) creating complex molecules (Piotrowicz *et al.* 1972, Albright and Wilson 1974, Lion *et al.* 1982). Also heavy metals are probably continuously present in the surface microlayer making the bacteria adapted to these substances (Kim 1985).

Some previous papers (Farrell *et al.* 1993, Fabiano *et al.* 1994, Perez-Garcia *et al.* 1993, Konopka and Zakharova 1999) based on laboratory experiments, showed that the short response to toxic metals is connected with a large reduction in metabolic-respiratory activity of aquatic bacteria. According to Ranjard *et al.* (1997) mercury exercises a particularly great inhibitory influence on bacteria respiration. Mills and Colwell (1977) and Mudryk *et al.* (2000) found that Hg inhibited intensively glucose and casein hydrolyzate oxidation by marine bacteria. Results presented in this paper also confirm the inhibitory influence of such heavy metal as mercury on respiration processes of estuarine neustonic and planktonic bacteria inhabiting Lake Gardno.

Barkay *et al.* (1992) and Cursino *et al.* (1999) report that bacteria probably play a key role in the recycling and detoxification of Hg in aquatic ecosystems. This suggests that in the future the impact of mercury contamination in these environments may be reduced by human intervention in order to accelerate natural detoxification processes by bioremediation based on the introduction of genetically engineered micro-organisms into ecosystems.

5. SUMMARY

The studies were carried out in an estuarine Lake Gardno situated in the Słowiński National Park (Baltic Coast, Poland). Lake Gardno is characterised by intermediate conditions between

marine and inland environment. On one hand it is supplied by the water of the River Łupawa, on the other hand via – a 1.3 km channel – it is connected with the Baltic Sea (Fig. 1) whose large volumes of sea water abundantly penetrate into the lake. Water samples were taken from three zones (Fig. 1): freshwater zone (station 1), seawater zone (station 3) and at one station in mid-lake mixed zone (station 2). Water samples for bacteriological analyses were taken from film layer (FL) (thickness of $90 \pm 17 \mu\text{m}$), surface layer (SL) (thickness of $242 \pm 40 \mu\text{m}$) and sub-surface layer (SUB) (10–15 cm). Object of the present study was to determine the influence of mercury ions on the number and respiratory activity of neustonic and planktonic bacteria inhabiting this lake. In order to study the effect of mercury ions (HgCl_2) on the numbers of neustonic and planktonic heterotrophic bacteria an iron-peptone agar (IPA) medium with different concentrations of mercury chloride ions was used. The influence of mercury chloride on respiratory activity of neustonic and planktonic bacteria was determined as oxygen uptake by these organisms using Clark's electrode. The neustonic bacteria showed higher levels of tolerance to various concentrations of Hg than the planktonic ones (Fig. 2A). Bacteria isolated from particular parts of Lake Gardno exhibited various levels of tolerance to the presence of mercury in the culture medium (Fig. 2B). Non-pigmented bacteria from all studied water layers (Fig. 3A) and stations (Fig. 3B) were most likely to tolerate the higher mercury ions concentrations in culture medium than the pigmented ones. High concentrations (above $8 \mu\text{g cm}^{-3}$) of mercury chloride in the respiratory substrate blocked completely oxygen uptake by neustonic and planktonic bacteria (Fig. 4A). It was found out that the bacteria isolated from the sea zone were least vulnerable to mercury present in the respiratory substrate (Fig. 4B).

6. REFERENCES

- Albright J. L., Wilson M. L. 1974 – Sublethal effects of several metallic salts-organic compounds combinations upon the heterotrophic microflora of natural water – *Wat. Res.* 8: 181–195.
- Barkay T., Gilman M., Liebert C. 1990 – Genes encoding mercuric reductases from selected gram-negative aquatic bacteria have a low degree of homology with mer A of transposon Tn501 – *Appl. Environ. Microbiol.* 56: 1695–1701.
- Barkay T., Turner R. M., Saouter E., Horn J. 1992 – Mercury biotransformations and their potential for remediation of mercury contamination – *Biodegradation*, 3: 147–159.
- Bogdanova E. S., Mindlin S. Z., Pakrova E., Kocur M., Rouch D. A. 1992 – Mercuric reductase in environmental Gram-positive bacteria sensitive to mercury – *FEMS Microbiol. Lett.* 97: 95–100.
- Cursino L., Oberda S. M., Cecilio R. V., Moreira R. M., Chartone-Souza E., Nasicimento A. M. A. 1999 – Mercury concentration in the sediment at different gold prospecting sites along the Carmo stream, Minas Gerais, Brazil, and frequency of resistant bacteria in the respective aquatic communities – *Hydrobiologia*, 394: 5–12.
- Daubner I. 1967 – *Mikrobiologia vody* – Slov. Akad. Vied. Press, Bratislava.
- Dethier M. N. 1992 – Classifying marine and estuarine natural communities: An alternative to the coward in system – *J. Nat. Areas*, 12: 90–100.
- Diaz-Ravina M., Bååth E., Frostegard A. 1994 – Multiple heavy metal tolerance of soil bacterial communities and its measurement by a thymidine incorporation technique – *Appl. Environ. Microbiol.* 60: 2238–2247.
- Donderski W., Głuchowska M., Wódkowska A. 1997 – Effect of heavy metal ions on neustonic and planktonic bacteria isolated from lake Jeziorak Mały – *Pol. J. Environ. Stud.* 6: 29–34.
- Fabiano M., Danovaro R., Magi E., Maz-zucotelli A. 1994 – Effects of heavy metals on benthic bacteria in coastal marine sediments: a field result – *Mar. Poll. Bull.* 28: 18–23.
- Farrell R. E., Germida J. J., Huang P. M. 1993 – Effects of chemical speciation in growth media on the toxicity of mercury (II) – *Appl. Environ. Microbiol.* 59: 1507–1514.
- Ferrer E. B., Stapert E. M., Sokolski W. T. 1963 – A medium for improved recovery of bacteria from water – *Can. J. Microbiol.* 9: 420–422.
- Foster T. J. 1983 – Plasmid-determined resistance to antimicrobial drugs and toxic metal ions in bacteria – *Microbiol. Rev.* 47: 361–409.
- Garrett W. D. 1965 – Collection of slick-forming materials from the sea surface – *Limnol. Oceanogr.* 10: 602–605.
- Harvey G., Burzell L. A. 1972 – A simple microlayer method for small samples – *Limnol. Oceanogr.* 17: 156–157.
- Hermansson M., Lindberg C. 1994 – Gene transfer in the marine environment – *FEMS Microbiol. Ecol.* 15: 47–54.
- Hermansson M., Jones G. W., Kjelleberg S. 1987 – Frequency of antibiotic and heavy metal resistance, pigmentation and plasmids in bacteria of the marine air-water interface – *Appl. Environ. Microbiol.* 53: 2338–2342.
- Kim S-J. 1985 – Effect of heavy metals on natural populations of bacteria from surface

- microlayers and subsurface water – *Mar. Ecol. Prog. Ser.* 26: 203–206.
- Konopka A., Zakharova T. 1999 – Quantification of bacterial lead resistance via activity assays – *J. Microbiol. Meth.* 37: 17–22.
- Lion L. W., Harvey R. W., Leckie J. O. 1982 – Mechanisms for trace metal enrichment at the surface microlayer in an estuarine salt marsh – *Mar. Chem.* 11: 235–244.
- Lopez-Amoros R., Vives-Rego J., Garcia-Lara J. 1997 – Exogenous isolation of Hg^r plasmids from coastal Mediterranean waters and their effect on growth and survival of *Escherichia coli* in sea water – *Microbios*, 92: 109–112.
- Martinez J., Soto Y., Vives-Rego J., Bianchi M. 1991 – Toxicity of Cu, Ni and alkylbenzene sulfonate on the naturally occurring bacteria in the Rhone river plume – *Environ. Toxicol. Chem.* 10: 641–647.
- Mills A. L., Colwell R. R. 1977 – Microbial effects of metal ions in Chesapeake Bay water and sediment – *Bull. Environ. Contam. Toxicol.* 18: 99–103.
- Mudryk Z. 1987 – Some physiological properties of water bacteria isolated from the estuarine lake Gardno – *Stud. Mat. Oceanol.* 51: 269–282.
- Mudryk Z., Donderski W., Skórczewski P., Walczak M. 2000 – Effect of some heavy metals on neustonic and planktonic bacteria isolated from the Deep of Gdańsk – *Oceanol. Stud.* 29: 89–99.
- Nair S., Chandramohan D., Bharathi P. A. 1992 – Differential sensitivity of pigmented and non-pigmented marine bacteria to metals and antibiotics – *Wat. Res.* 26: 431–434.
- Niewolak S., Kopij H., Chomutowska H. 1996 – Influence of some heavy metals on the survival of heterotrophic bacteria in bottom sediments of eutrophic lake – *Pol. J. Environ. Stud.* 5: 21–27.
- Osborn A. M., Bruce K. D., Strike P., Ritchie D. A. 1997 – Distribution, diversity and evolution on the bacterial mercury resistance *mer* operon – *FEMS Microbiol. Rev.* 9: 239–262.
- Ostrovskii D. N., Lysak E. I., Demina G. P., Binyukov V. I. 2000 – Interaction of bacteria with mercuric compounds – *Microbiology*, 69: 516–523.
- Perez-Garcia A., Codina J. C., Cazorola F. M., de Vicente A. 1993 – Rapid respirometric toxicity test: sensitivity to metals – *Bull. Environ. Contam. Toxicol.* 50: 703–708.
- Piotrowicz S. R., Ray B. J., Hoffman G. L., Duce R. A. 1972 – Trace metal enrichment in the sea-surface microlayer – *J. Geophys. Res.* 77: 5243–5254.
- Ranjard L., Richaume A., Jocteur-Monrozier L., Nazaret S. 1997 – Response of soil bacteria to Hg (II) in relation to soil characteristics and cell location – *FEMS Microbiol. Ecol.* 24: 321–331.
- Reyes N. S., Frischer M. E., Sobecky P. A. 1999 – Characterization of mercury resistance mechanisms in marine sediment microbial communities – *FEMS Microbiol. Ecol.* 30: 273–284.
- Williams P. M., Carlucci A. F., Henrichs S. M., van Vleet E. S., Horrigan S. G., Redi F. M., Robertson K. J. 1986 – Chemical and microbiological studies of sea-surface film in the southern Gulf of California and the west coast of Baja California – *Mar. Chem.* 19: 7–98.

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