Letter to Editor

The Sensitivity of Bacteria to Heavy Metals in the Presence of Mineral Ship Motor Oil in Coastal Marine Sediments and Waters

K. Jankowska*, K.Olańczuk-Neyman, E.Kulbat

Gdańsk Technical University, Civil and Environmental Engineering Faculty,
G. Narutowicza Street 11/12 80-952 Gdańsk, Poland

Received: August 17, 2005
Accepted: June 1, 2006

Abstract

The sensitivity of bacteria, isolated from coastal marine sediments and waters of Sopot beach, Gdańsk Bay (Poland), to heavy metals in the presence of mineral ship motor oil were studied. All isolated heterotrophic bacteria were resistant to the 0.1 mM lead concentration occurring in the environment. Metal resistance of bacteria isolated from coastal water was strongly related to mineral ship motor oil concentration. At 0.1% mineral ship motor oil (stimulating bacteria growth) Pb and Cd resistance was lower compared to 1% oil concentration. The number of Pb-resistant bacteria, isolated from shoreline sediments, decreased with increasing metal and oil concentrations within the range occurring in the environment. The number of Cd-resistant bacteria at 0.1 mM Cd concentration was slightly stimulated by 1% concentration of mineral ship motor oil.

Keywords: coastal water, sandy sediment, heavy metals, mineral ship motor oil, heavy metal resistance

Introduction

The Baltic Sea is a semi-closed and low salinity sea. It is very sensitive to anthropogenic pollutant areas. [1, 2]. Anthropogenic hazards occur especially in bays and in coastal zones like the Gulf of Finland [3] or Gdańsk Bay [4]. In Gdańsk Bay toxic substances are introduced from atmospheric air, land effluents coming from wastewater treatment plants, and from harbours [5-7].

In coastal seawaters and shoreline sandy sediments the contamination of specific pollutants (such as mineral ship motor oils and heavy metals) may influence the composition of the microbial community and therefore inhibit the decomposition of organic pollutants [8].

The effect of heavy-metal pollution on bacterial communities has already been studied [9, 10]. Bacteria isolated from natural habitats may show metal tolerance and antibiotic resistance, which provide them with selective advantages [11]. Such mechanisms of resistance may be based on metal retention on the surface of a cell, on intracellular transformation into less toxic forms, on the release of metal from a cell with the help of polymers, and lowering the permeability of a cell membrane [12-15]. Many bacterial-resistant systems for toxic metals are encoded by plasmids [16]. The presence of the organisms that possess specific mechanisms of resistance to heavy metals increases destruction or transformation of toxic substances in the natural environment [17-20].

Oil derivatives that are found in the waters of the Baltic Sea are mainly of anthropogenic origin and are derived, first of all, from sea transport. Total mass of oil derivative substances which annually enters the Baltic Sea is twice the amount released into the waters of the North Sea and three times higher than that released into the North At-

*Corresponding author; e-mail: kjank@pg.gda.pl

lantic [21]. Oil derivative substances in the sea environment occur mainly as particles floating on the surface or are dissolved in water. In sediments they may also occur as agglomerates adsorbed on particle surfaces [22]. The presence of oil-derivative substances in water causes difficulties in gas exchange, limits transmission of solar radiation and may lead to deficiency of oxygen [23].

Contamination of the Gdańsk Bay region by oil derivatives is mainly connected with runoff. Concentrations recorded for open waters of Gdańsk Bay oscillate between 3 to 10 µg/l. In the streams flowing to Gdańsk Bay in Sopot (Swelina, Kolibianka, Kacza), clearly higher concentrations of oil derivatives (up to about 7.0 mg/l) were found just after rainfalls and spring thaws [24].

Oil derivatives present in the environment are gradually neutralized due to physico-chemical and biological processes. Biological degradation is based mainly on the utilization performed by chemoorganotrophic bacteria. In the regions contaminated with hydrocarbons an increased number of microorganisms adapted for degradation of such types of substances is observed [25, 26]. The occurrence of heavy metals together with oil derivatives in the environment may intensify their toxic activity. In this study the sensitivity of bacteria towards heavy metals, in the presence of mineral ship motor oil, in coastal marine sediments and waters of Sopot beach, Gdańsk Bay, Poland was studied.

Materials and Methods

Samples

Samples of water and sandy sediment were collected on the coast of Gdańsk Bay in Sopot (54°27'N.18°33'E) between the mouth of Grodowy Stream and Kamienny Stream (Fig.1).

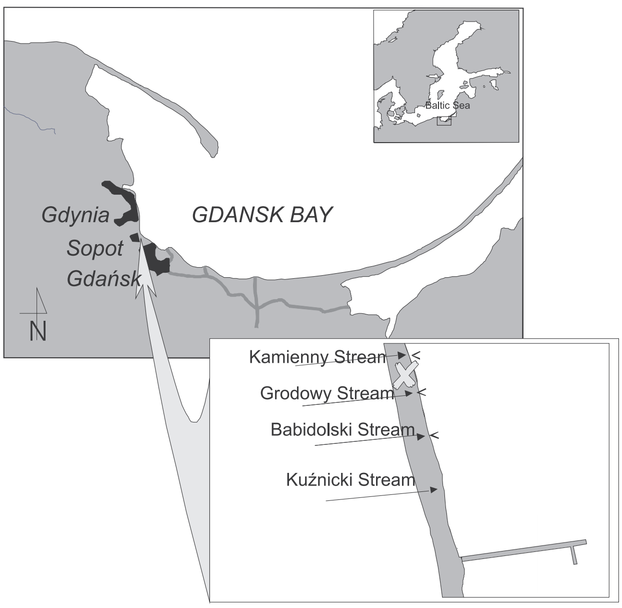

Fig.1. Study area.

Water samples were collected directly into sterile 100 ml glass bottles. A sterilized stainless steel sampler 10 cm in diameter was used for collecting sediments. Samples were collected at site I approx. 1-2 m from the waterline at the bottom about 30 cm deep.

Sand samples were collected at the three sites localized at different distances from the coastline (Fig.2): site I – app. 1-2 m from the waterline (water approx. 0.5 m deep), site II – at the water and the land boundary, and site III – in the sheltered region of the dune (approx. 60 m from the waterline, 2 m above the waterline). At site I, sandy sediment samples were taken on the surface layer (0-5 cm). At site II, sandy sediment samples were taken on the surface layer (0-5 cm) and 10-15 cm below. At site III sandy sediment samples were taken on the surface layer (0-5 cm) and 50-55 cm below. Three parallel samples were collected from each of three sites at a distance of about 50 m, and an average sample was obtained by mixing corresponding layers.

All samples were transported to the laboratory in a cool container (10°C±2°C). Microbiological analyses were performed within two hours of collection.

Physical and Chemical Determinations

A wet sand sample (2 kg) was sieved through a 63 µm plastic mesh for 60 min. Fine fraction (< 63 µm) was dried at 80°C for 24 h, homogenized in a porcelain mortar, and was wet mineralized with the mixture of $HClO_4$, HF and HNO_3 acids [27, 28, 29]. The determination of heavy metals (Pb, Cu, Cd) was performed in the Thermo Jarrel Ash atomic absorption spectrophotometer. The experiments were performed in triplicate.

Bacteriological Determinations

R2A Medium with 0.01 mM, 0.1 mM and 1.0 mM of Heavy Metals (Cd, Cu, Pb)

Appropriate volumes of stock 10 mM $CdSO_4$, 10 mM $CuSO_4$ and 10 mM $Pb(NO_3)_2$ solutions, sterilized via filtration, were added to a 100 ml liquid sterile R2A medium 15 minutes prior to inoculation.

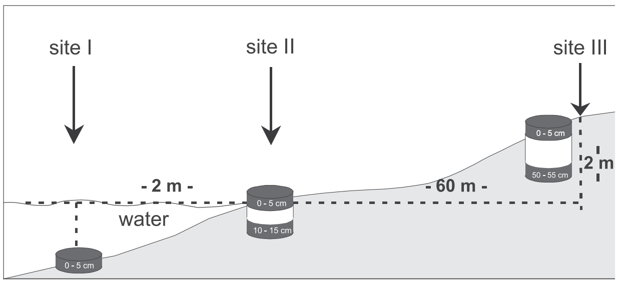

Fig. 2. Location of sampling point

R2A Medium with 0.1% and 1% of Mineral Ship Motor Oil

1g of mineral ship motor oil was dissolved in 30 ml of ethyl ether and mixed with 1 g silica gel. After ether evaporation appropriate amounts of mixture were added to R2A medium and then sterilized in an autoclave at 121°C for 20 min.

Sample Preparation

100 g sediment samples were mixed with 100 ml of Ringer solution (diluted 1:4) with 0.28% sodium pyrophosphate additive [30]. The solution was vigorously shaken for 20 minutes with a type 358S laboratory shaker and decanted for 2 minutes. Serial dilutions were prepared in Ringer solution (diluted 1:4) [31, 32].

Heterotrophic bacteria in water and sediment samples were enumerated by spread plate count. Plates were incubated at 20°C for 7 and 14 days.

Two cycles of bacteriological examination were conducted. In the first cycle (September 1997 – April 1998, five series), the influence of three heavy metals (Pb, Cu, Cd) as well as mineral ship motor oil towards bacteria growth was examined. The number of colony forming units of bacteria growing on a reference R2A medium [33] was compared to the number of bacteria growing on R2A medium supplemented with: 0.1 mM Pb and 1.0 mM Pb; 0.1 mM Cu and 1.0 mM Cu, 0.01 mM Cd and 0.1 mM Cd or mineral ship motor oil in concentrations of 0.1% and 1%. In the second cycle (September 1998 – April 1999; six series) heterotrophic bacterial growth was examined using R2A medium supplemented both with mineral ship motor oil in two concentrations (0.1% or 1%) and heavy metals: cadmium (0.01 or 0.1 mM) or lead (0.1 or 1 mM).

Calculation of Results

The results of bacteriological analyses are presented in semi–logarithmical scale denoting the colony-forming units (CFU) of heterotrophic bacteria per 1 g of dry sand mass or per 1 ml of water. All results were expressed in the form of the geometrical mean, maximal and minimal values, 50 percentiles, standard deviation and for the total time of investigation, testing site and layers. The results were analyzed using Statistica 5.0 software.

Results and Discussion

Concentrations of Cd, Cu, Pb mg/kg dry wt. of Sandy Coastal Sediment

Concentrations of heavy metals in fraction < 63 μm of sediment (Table 1) have been observed by other authors within the same range [34, 35]. Similar concentrations of heavy metals were supplied to R2A medium.

The Number of Heterotrophic Bacteria in the Presence of Heavy Metals

In many metal toxicity studies the importance of the time at which a metal is added to a medium is often overlooked [36, 37]. Hoffman has reported [38] that the degrees of inhibition did not change when cadmium was added 24 h before inoculation rather than when added within one minute after inoculation and variability in degrees of cadmium inhibition appeared to be somewhat dependent on cadmium bioavailability, which was lower after 12h in two of three media.

Taking into consideration the importance of the time at which a heavy metal is added to a medium in metal toxicity studies, metal salt additive was applied 15 minutes prior to inoculation in our experiment.

Bacteria isolated from water and sandy sediments were sensitive to metals supplied to the medium in the following concentrations: 0.1 mM Pb and 1.0 mM Pb; 0.1 mM Cu and 1.0 mM Cu, 0.01 mM Cd and 0.1 mM Cd.

In sand the largest inhibition of bacteria growth was observed in the case of copper in concentrations 1.0 mM ($\log_{10}$ 2.4 ± 0.6) and in the lower concentration (0.1 mM Cu) inhibition of bacteria growth is not so significant ($\log_{10}$ 0.3 ± 0.3). In the case of cadmium in concentrations 0.1mM and 0.01mM inhibition of bacteria growth was respectively $\log_{10}$ 2.3 ± 0.4 and $\log_{10}$ 0.9 ± 0.2 and in case of lead in concentrations 1.0mM and 0.1mM, respectively, $\log_{10}$0.7 ± 0.4 and $\log_{10}$0.3 ± 0.3.

However, in the presence of metals the number of bacteria isolated from water significantly decreased in cases of both concentrations of copper (in concentration 1.0 mM Cu about $\log_{10}$ 2.6 ±0.7 and 0.1 mM Cu $\log_{10}$ 0.8 ±0.6) and cadmium (respectively in concentration 0.1 mM Cd $\log_{10}$ 1.7 ± 0.6 and in 0.01 mM Cd $\log_{10}$ 0.9 ± 0.5). Lead did not so strongly influence the growth of bacteria isolated from water: in concentration 1.0 mM $\log_{10}$ 0.9 ± 0.5 and in 0. 1 mM $\log_{10}$ 0.2 ± 0.1 (Fig.3).

Also, other authors have published the evidence of metal-resistant bacteria in water and sandy sediments in different locations: along the Bay of Malaga, and the Guadalhorce River mouth in Malaga, Spain [39], as well as in the coastal environment at Gdańsk Deep [40, 41].

Table 1. Concentrations of Cd, Cu, Pb (mg/kg dry wt.) of sandy coastal sediment (fraction < 63 μm).

Site	deep (cm)	Cd	Cu	Pb
I	0 – 5	0.8 ± 0.1	9.4 ± 0.8	9.6 ± 0.3
II	0 – 5	1.1 ± 0.2	22.3 ± 4.1	12.6 ± 0.9
II	10 – 15	0.3 ± 0.1	5.9 ± 0.5	5.0 ± 0.5
III	0 – 5	1.3 ± 0.2	60.5 ± 18.2	45.8 ± 6.1
III	50 – 55	1.5 ± 0.1	26.3 ± 6.1	44.9 ± 4.3

Mineral Ship Motor Oil Influence on Bacterial Growth

Generally no harmful influence of oil concentrations (0.1% and 1%) was observed on the number of heterotrophic bacteria isolated from water. The geometric mean number of bacteria growing in the presence of 0.1% oil was 0.5 ± 0.2 log higher than in the reference and in the presence of 1% of oil was close to the reference. This indicates that bacteria occurring in the oil-contaminated water have the ability to utilize these substances as a source of carbon.

Bacteria isolated from sandy sediments were more sensitive towards petroleum-derivate substances present in their growing medium. The growth of bacteria in a lower concentration of oil (0.1%) was close to the reference. However, at ten times higher concentrations a reduction of bacteria number was recorded (Fig.4).

The toxic influence of oil derivatives on microorganisms in the sea water environment is stated by Atlas [42], LaMontagne [43] and Grant and Brigs [44].

Aggregate Influence of Mineral Ship Motor Oil and Heavy Metals on Bacteria Growth

Both metal and mineral ship motor oil contamination clearly have aggregated impact on growth inhibition of bacteria isolated from water. The primary factor was the mineral ship motor oil concentration. The bacteria growing in the presence of oil at a concentration of 0.1% were much more sensitive to the growing concentration of lead (from 0.1 to 1.0 mM) and cadmium (from 0.01 to 0.1 mM), compared to the bacteria cultivated in the presence of ten times higher oil concentrations (Fig. 5).

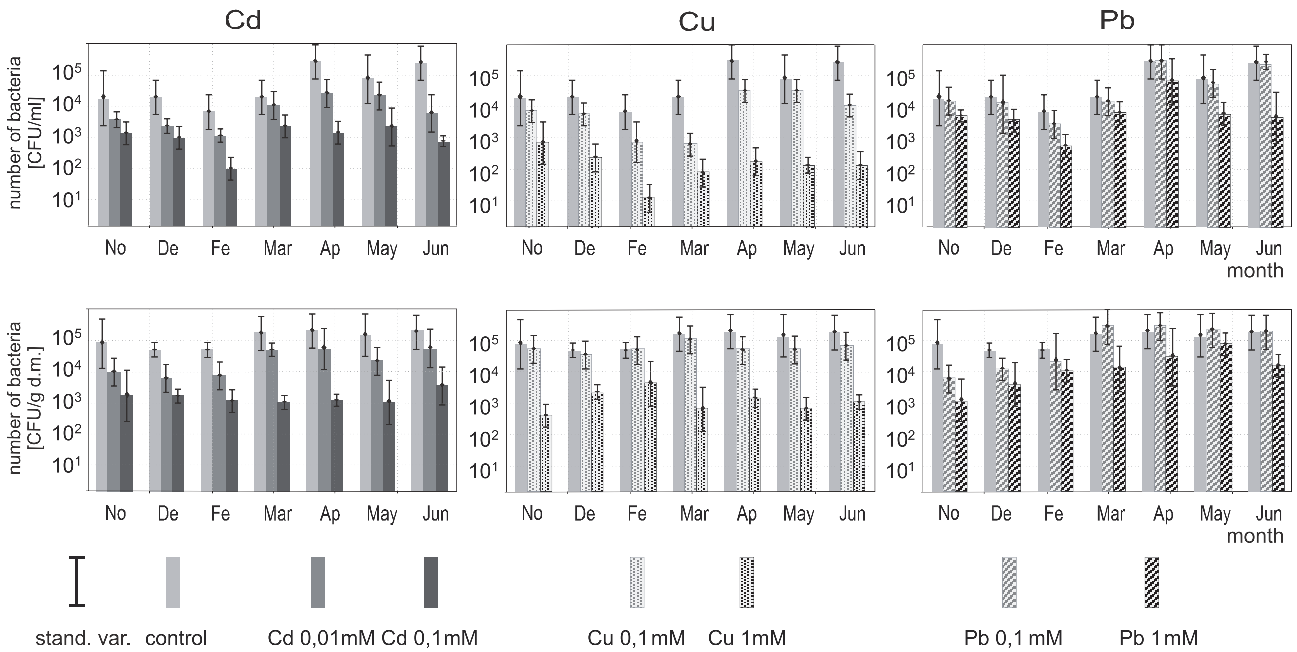

Fig.3. The number of heterotrophic bacteria in the presence of heavy metals (Pb, Cu, Cd) in 1; 0.1; 0.01 mM concentrations.

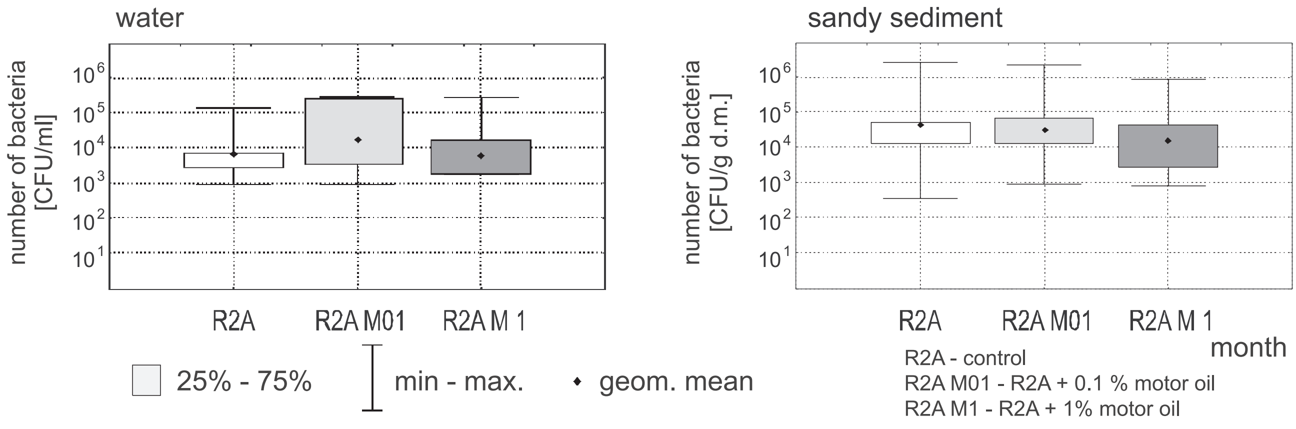

Fig.4. The number of heterotrophic bacteria in the presence of mineral oil in 1% and 0.1% concentrations.

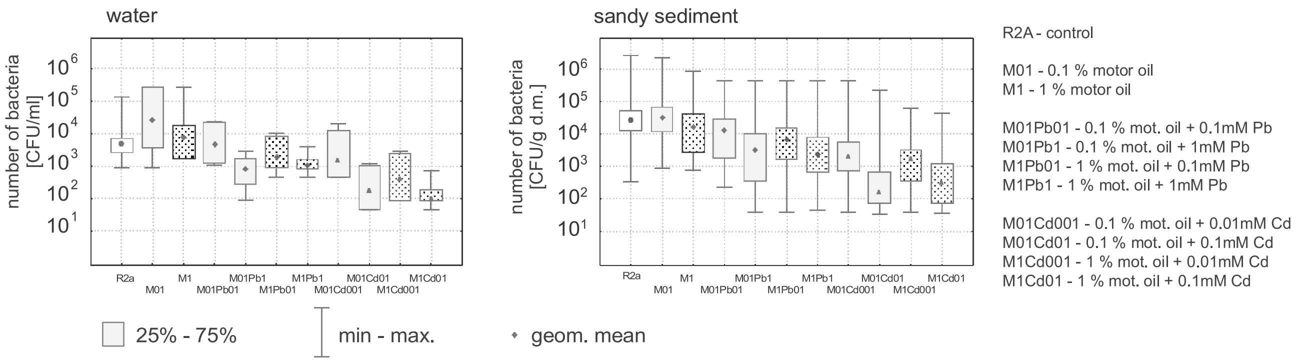

Fig.5. Number of bacteria resistant to heavy metals in the presence of mineral ship motor oil.

The number of bacteria isolated from sandy sediments grown in the presence of both oil concentrations (0.1% and 1.0%) decreased along with the growing concentration of lead in the medium. Conversely, a small stimulating impact of 1.0% oil concentration on number of bacteria resistant to 0.1mM cadmium concentration was observed.

Stronger inhibition of bacteria growth by heavy metals in simultaneous presence of mineral ship motor oil can be explained by:

a. The toxicity of oil which is a result of their sorption and solubilization through cell membrane, causing the disruption of membrane functioning as a physiological barrier [45].
b. The increased sensitivity of actively metabolizing cells in the presence of a stimulating growth concentration of a new available carbon source to the toxic substances.
c. The interaction between heavy metals and the oil itself affecting the availability of the metals to bacteria cells. However, the potential decrease of toxic metal influence in the presence of mineral ship motor oil, as a result of metal binding by reduced sulphur compounds in oil, cannot be omitted.

Summary

Different degrees of metal inhibition growth of heterotrophic bacteria isolated from the coastal environment in Gdańsk Bay in Sopot were observed.

The research has proved that heterotrophic bacteria isolated from the water and the sandy sediment are resistant to lead in 0.1mM, and only some of them are resistant to cadmium 0.01 mM. It has been proved that 0.1% and 1% oil concentration did not influence the growth of bacteria isolated from water. However, bacteria appearing in sediments are adopted to 0.1% oil, while 1% oil concentration insignificantly inhibits their growth (Fig.4). Our results have indicated that the bacteria in the presence of mineral ship motor oil showed increased sensitivity to lead and cadmium. The number of bacteria resistant to both metals depended on the mineral ship motor oil concentration of metal concentration.

The bacteria isolated from water, growing in the presence of oil in stimulating growth concentration (0.1%), were showing increased sensitivity to lead (0.1 and 1.0 mM) and cadmium (0.01 and 0.1 mM) compared to 1% oil concentration. Similarly inhibiting influence of increasing cadmium concentration on bacteria isolated from the sediments was stronger in the presence of 0.1% oil concentration (Fig.5).

The number of bacteria resistant to lead, which were isolated from sandy sediments, was decreasing along with the growing concentrations of metal and oil.

It can be expected that bacteria grown under optimal laboratory conditions can react differently to stress compared to bacteria grown under natural conditions.

Acknowledgements

The authors thank Anna Cieśla, Monika Szpakowska and Monika Skuza for their help in collection of materials and laboratory work. The authors also thank prof. Janusz Pempkowiak for enabling performance of analyses of heavy metals in the Marine Biogeochemistry Laboratory in the Marine Chemistry and Biochemistry Department at the Institute of Oceanology PAS, and Andrzej Staniszewski and Anna Malenga for help in performing these analyses. This work was financed by the State Committee for Scientific Research grant No. PO4 G06718.

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