

TOLERANCE OF ENVIRONMENTAL BACTERIA TO HEAVY METALS

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ABSTRACT

The aim of this study was to isolate microorganisms with bioremediation potential from soil contaminated with heavy metal compounds, and to analyze the tolerance of these microorganisms to various heavy metal concentrations. In total, 7 bacterial strains were isolated and cultivated. The sensitivity of microorganisms to heavy metals proved to be dependent on the type of medium. The use of liquid media during the 8-hour and 24-hour toxicity tests resulted in reduced tolerance of isolates to the concentrations of selective factors used, relative to culture on solid substrates. In addition, optical density increases in the bacterial cultures have been observed at the highest concentrations of some heavy metals. Briefly, during the 48-hours test 3 strains showed increased optical density values in the presence of 1 mM copper sulfate and 2 strains displayed this ability also in the presence of 1 mM cadmium sulfate and lead nitrate.

Key words: autochthonous bacteria, bioremediation, soil, heavy metals, toxicity

INTRODUCTION

Soil is a storage of water, nutrients, minerals, inorganic and organic compounds [Kabała 2015]. Therefore, toxic and harmful compounds can also be accumulated in soil, including that used in plant cultivation and animal breeding. These toxic compounds are mainly polycyclic aromatic hydrocarbons and heavy metals. Heavy metals are described as metals (and metalloids) with relatively high density (above $5 \text{ g} \cdot \text{cm}^{-3}$) that are toxic to living organisms in low concentrations [de Lima e Silva et al. 2012]. The elements belonging to this group are e.g. lead (Pb), mercury (Hg), cadmium (Cd), copper (Cu), nickel (Ni), manganese (Mn), arsenic (As), cobalt (Co), chromium (Cr) and zinc (Zn). They are ubiquitous in many environments due to both natural (e.g. volcano eruptions) and industrial processes [Wuana and Okieimen 2011, Dixit et al. 2015]. Some of these metals, like Zn, Cu, Co and Ni are considered as microelements and in small doses are crucial to the biochemical processes of living organisms. However, anthropogenic activities lead to the rise of their concentrations in soil and different environments to alarming levels [Marzan et al. 2017]. These compounds can affect the work of enzymes involved in repair pro-

cesses, cell metabolism, cause DNA damage and lead to cancer development [Tchounwou et al. 2012, Baltazar et al. 2019].

Soil is an environment remarkably rich in microorganisms. The number of bacteria in one gram of dry soil ranges from $4 \cdot 10^6$ to $2 \cdot 10^9$ [Whitman et al. 1998]. Some microorganisms under the influence of the environmental pressure adapt to these unfavorable living conditions. Thus, these microorganisms may have a bioremediation potential, supporting soil purification from substances posing a threat to human and animal health [Kang et al. 2016]. At the same time, it is possible that metals, specifically metal nanoparticles, can stimulate the metabolic activity of bacteria [Augustyniak et al. 2016], which could enhance their bioremediation potential. Interestingly, bacteria tolerance to heavy metals may be connected with antibiotic resistance. That phenomenon is explained by the presence of efflux pumps which discard all harmful substances and molecules from bacterial cells including both antibiotics and heavy metals [Tomova et al. 2015].

The aim of this study was to isolate microorganisms with bioremediation potential from soil exposed on con-

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tamination with heavy metal compounds, such as copper, cadmium, lead and mercury, and to analyze the tolerance of these microorganisms to various heavy metal concentrations. The impact of incubation time of supernatants obtained from soil suspension on the number of isolated microorganisms was also tested, as well as comparison of the sensitivity of microorganisms to heavy metals in solid and liquid medium.

MATERIAL AND METHODS

Source of isolation. The source of bacteria isolation were soil samples taken from the former, now closed, airport in the West Pomeranian Voivodeship, Poland. The soil was exposed on contamination with petroleum and heavy metal compounds: copper, cadmium, lead and mercury. 20 g of soil was taken from 31 places, from a depth of 20 cm. All samples were mixed thoroughly and then 10 g of soil mixture was weighed and divided into two homogeneous samples. Afterwards, the samples were suspended in 100 mL of 0.85% NaCl solution and shaken at 110 RPM for 24 h at room temperature ($22 \pm 2^\circ\text{C}$) (Biosan, PSU-10i, Latvia). Subsequently, soil suspensions sedimented for 4 hours. The supernatant obtained from one sample was immediately used for further research, while the supernatant from the second sample was further incubated for 7 days at room temperature.

Mixtures of heavy metals solutions. Three mixtures of different heavy metal concentrations in sterile distilled water were prepared. Initial concentrations of metal salts: CuSO_4 , CdSO_4 , $\text{Pb}(\text{NO}_3)_2$ and HgCl_2 were 100 mM, 100 mM, 30 mM and 30 mM respectively (Table 1). To completely dissolve the precipitated salt crystals, solutions were sonicated (VEVOR Ultrasonic Cleaner, PS-30A, Germany) at 50°C . Each metal solution was passed through a PES syringe filter with a diameter of 26 mm and a pore diameter of $0.22 \mu\text{m}$ (Sartorius Stedim BiotechTM MinisartTM PES Venting Filter, Thermo Fisher Scientific, USA). Solutions were stored at 4°C .

The following mixtures of heavy metal salt solutions were prepared: I, II and III. Concentrations of heavy metal salts in each mixture are shown in Table 1.

To control the purity of metal mixtures, 100 mL of each mixture was plated on TSA and incubated at room temperature for 120 hours.

TSA medium with selective factors

Prepared heavy metal mixtures I, II and III were added to the Trypticase Soy Agar (TSA, BioMaxima Poland). The final concentrations of individual heavy metals in TSA are given in Table 2. The final concentrations of copper, cadmium, lead and mercury used in this work were taken from Richards et al. [2001], Teitzel and Parsek [2003] and Raja et al. [2009].

Bacterial isolation

100 μL of each supernatant was spread by the surface method onto Trypticase Soy Agar (TSA) (BioMaxima, Poland) without selective factors and onto TSA with heavy metal mixtures I, II or III (Table 2). Inoculation of the TSA without selective factors served as control of the presence and estimated number of microorganisms in the supernatants. Cultures were incubated for 96 hours at room temperature. Morphologically distinct colonies growing on TSA with heavy metals were selected for further study.

Characterization of the bacteria

Gram stained preparation was made from each isolated colony. The ability of isolates to produce catalase was assessed by the use of 3% H_2O_2 and oxidase activity by the use of strip tests was determined (Erba Mannheim, OXItest, Germany).

Strains were stored in Trypticase Soy Broth (TSB) (BioMaxima, Poland) with the addition of 10% glycerol at -20°C .

Growth curves of the bacteria in liquid cultures without the addition of heavy metals

The ability of bacteria to grow in liquid media and the growth rate were evaluated in TSB (BioMaxima, Poland) cultures run in 96-well microtiter plates. Each of the bacterial strains was inoculated in TSB medium and incubated with shaking (110 RPM) at room temperature for 48 hours and diluted in TSB medium until an optical density of 0.5 McFarland standard for each strain was reached. The wells were loaded with 200 μL of each strain suspension and incubated at 110 RPM for 24 hours at room temperature. Sterile TSB medium was used as a control of the purity of the medium. All cultures were analysed in triplicate. Optical density was measured using a spectrophotometer (TECAN, Nanoquant Infinite m200pro, Switzerland) at $\lambda = 600 \text{ nm}$ for 8 hours at room temperature with measurements taken every 30 min without shaking. Growth curves were made according to Mytilinaios et al. [2011]. Microsoft Excel and GraphPad Prism 8 were used to analyze the results and determine the growth curves.

To exclude contamination, after the absorbance measurements were completed, 10 μL of each suspension was plated by the surface method on TSA medium and incubated at room temperature for 48 hours.

8-hour and 48-hour heavy metal toxicity test

Heavy metal solutions were prepared up to 24 hours prior to testing by suspending them in sterile distilled water and sonicated in an ultrasonic bath (VEVOR Ultrasonic

Cleaner, PS-30A, Germany) for 30 min at 50°C. The mixtures were then passed through PES syringe filters with a diameter of 26 mm and a pore diameter of 0.22 µm (Sartorius Stedim Biotech™ Minisart™ PES Venting Filter, Thermo Fisher Scientific, USA).

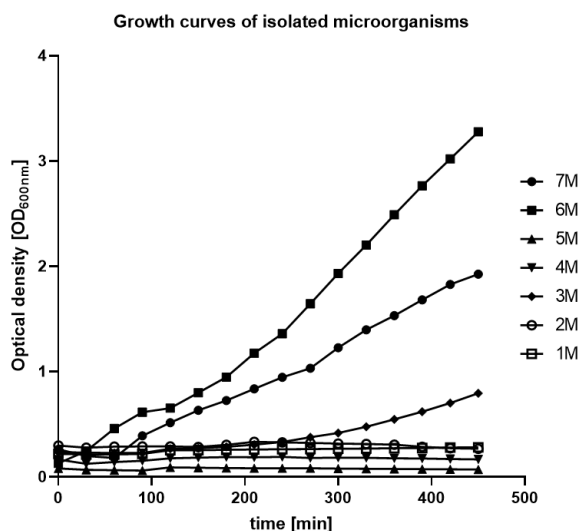


Fig. 1. Strains growth curves during 8h incubation in TSB medium

Rys. 1. Krzywe wzrostu szczepów w ciągu 8h inkubacji w podłożu TSB

Each of the bacterial strains was inoculated in TSB medium and incubated with shaking (110 RPM) at room temperature for 48 hours until an optical density of 0.5 McFarland standard was reached. Bacterial suspensions were used for further testing within 30 minutes after their preparation. Strain tolerance to 5 concentrations of heavy metal salts (Table 3) was tested using 96-well polystyrene microtiter plates according to the methodology proposed by Raja et al. [2009], with modifications described as follows. To selected wells of the microtiter plate, 50 µL of heavy metal solutions at various concentrations (Table 3) and 50 µL of bacterial suspension were added, so that the final density of the suspension in each well was 5×10^5 CFU mL⁻¹. The purity checks of each of the test solutions were also performed as well as the sterility check of TSB medium. TSB medium with addition of the heavy metals solutions served as a negative control. The growth of each strain in TSB, without the addition of heavy metals, has also been applied. All samples were analysed in duplicate. Plates were incubated at room temperature without shaking. Each measurement took place every 30 min for 8 hours for fast-growing strains (6M and 7M). In contrast, strains with a slower growth rate (1M, 2M, 3M, 4M and 5M) were tested for 48 hours, with measurements performed at 6 measuring points. Readings were taken at room temperature using a spectrophotometer (TECAN,

Nanoquant Infinite m200pro, Switzerland) at $\lambda = 600$ nm. Microsoft Excel and GraphPad Prism 8 were used to analyze the results and determine the growth curves.

RESULTS

Bacterial isolation

The growth of numerous microorganisms was noted on the TSA medium without the addition of a selective factors. However, less bacteria were recorded from the supernatant tested immediately after 4 hours of soil sedimentation than from the supernatant subjected to an additional 7 days incubation. On the TSA medium with the addition of the metal mixture I, no microorganisms have grown. Whereas different bacterial colonies were noted on the TSA medium with the addition of metal mixture II and III. In total, 7 isolates (1M – 7M) with different morphology and characteristics were selected (Table 3). The 7M isolate grew both on the medium with the addition of mixture II and III, however, the colony from the TSA with mixture II (higher metal concentration) was selected for further studies. The growth rate of isolates ranged from 24 hours (6M isolate) to 72 hours.

To confirm the tolerance or intolerance of bacteria to heavy metal concentrations contained in the mixtures, each strain was once again plated on TSA with mixtures I, II and III. Once again, no growth of any of the analysed bacteria was noted on TSA with mixture I. Bacteria originating from TSA with mixture III (strain 1M – 6M) did not grow on TSA with mixture II. In turn, the strain isolated from TSA with mixture II (strain 7M) again grew on TSA with mixture II and III.

Characterization of the bacteria

Among the 7 bacteria analysed, 4 Gram-negative rods, 2 Gram-variable rods and 1 Gram-variable bacilli were identified. All bacteria were catalase positive and oxidase activity was noted in 4 isolates (Table 4).

Growth curves of the isolated bacteria in liquid cultures without the addition of heavy metals

All strains grew in TSB medium within 48 h. A growth curve was made for each of the isolated strain (Fig. 1). The fastest growth rate was observed for the 6M and 7M strains, with a visible proliferation starting after 60 and 90 minutes respectively. The 3M strain showed the increase in optical density after 4 hours. In contrast, strains 1M, 2M, 4M and 5M did not show any optical density increase within 8 hours.

Table 1. Heavy metal salt concentrations in each mixture

Tabela 1. Stężenia soli metali w każdej mieszaninie

Heavy metal Metal ciężki	Output metal concentration Wynikowe stężenie metalu	Mixture I Mieszanina I	Mixture II Mieszanina II	Mixture III Mieszanina III
		Heavy metal concentrations in mixtures Stężenia metali ciężkich w mieszaninach		
CuSO ₄	100 mM	4 mM	1 mM	0.1 mM
CdSO ₄	100 mM	8 mM	1 mM	0.02 mM
Pb(NO ₃) ₂	30 mM	3.6 mM	1.2 mM	0.6 mM
HgCl ₂	30 mM	3 mM	1.8 mM	0.6 mM

Table 2. Heavy metal concentrations in TSA medium

Tabela 2. Stężenia metali ciężkich w podłożu TSA

Heavy metal Metal ciężki	TSA + mixture I TSA + mieszanina I	TSA + mixture II TSA + mieszanina II	TSA + mixture III TSA + mieszanina III
	Heavy metal concentrations in TSA		
CuSO ₄	2 mM	0.5 mM	0.05 mM
CdSO ₄	4 mM	0.5 mM	0.01 mM
Pb(NO ₃) ₂	1.8 mM	0.6 mM	0.3 mM
HgCl ₂	1.5 mM	0.9 mM	0.3 mM

Table 3. Concentrations of heavy metal salts solutions used in the toxicity test

Tabela 3. Stężenia soli metali ciężkich użytych w teście toksyczności

Heavy metal Metal ciężki	Concentrations of heavy metal salts in liquid cultures Stężenia soli metali ciężkich w kulturach płynnych				
	1)	2)	3)	4)	5)
CuSO ₄	1 mM	0.5 mM	0.25 mM	0.1 mM	0.05 mM
CdSO ₄	1 mM	0.5 mM	0.1 mM	0.05 mM	0.01 mM
Pb(NO ₃) ₂	0.9 mM	0.75 mM	0.6 mM	0.45 mM	0.3 mM
HgCl ₂	1.2 mM	0.9 mM	0.75 mM	0.6 mM	0.45 mM

8-hour and 48-hour heavy metal toxicity test

Toxicity tests of heavy metals: copper, cadmium, lead and mercury were performed for all tested strains. Strains 1M, 2M, 3M, 4M, 5M did not show any optical density increase during the 8-hour test, therefore the test duration was extended to 48 hours. The optical density of 6M strain culture increased at all tested concentrations of copper, cadmium and lead salts during 8h tests, while in the presence of mercury salts it did not change and stayed the same as the negative control (Fig. 2). Copper sulphate incorporation at every concentration used, did not influence the optical density of 6M strain suspension in comparison to the control. There was also no effect of 0.01 mM cadmium sulphate concentration on the tested strain. The use of higher concentrations of this heavy

metal resulted in a gradual decrease of optical density of 6M strain as the cadmium concentration increased. Lead nitrate given in high concentrations (0.9 mM and 0.75 mM) showed an increase of optical density measurement of 6M strain. In turn, the addition of mercury chloride at each concentration tested, inhibited the growth of the strain to the level of pure TSB medium (Fig. 2). The 7M strain showed a higher value of optical density in the medium with the addition of copper sulphate at all concentrations tested, at a level not deviating from the control. Increase in the optical density of the analysed strain was also observed in the medium with the addition of cadmium sulphate, with the decrease in visible proliferation level only at its highest concentration of 1 mM (Fig. 2). In case of lead nitrate and mercury chloride, no growth was

Table 4. Identification of isolated bacteria

Tabela 4. Identyfikacja wyizolowanych bakterii

Isolate Izolat	Source of isolation Źródło izolacji	Morphology Morfologia	Gram staining Barwienie metodą Grama	Catalase Katalaza	Oxidase Oksydaza
1M	TSA + mixture III TSA + mieszanina III	Rods – Pałeczki	Gram-variable Gram-zmienne	+	–
2M	TSA + mixture III TSA + mieszanina III	Bacilli	Gram-variable Gram-zmienne	+	–
3M	TSA + mixture III TSA + mieszanina III	Rods – Pałeczki	Gram-variable Gram-zmienne	+	+
4M	TSA + mixture III TSA + mieszanina III	Rods – Pałeczki	Gram-negative Gram-ujemne	+	+
5M	TSA + mixture III TSA + mieszanina III	Rods – Pałeczki	Gram-negative Gram-ujemne	+	–
6M	TSA + mixture III TSA + mieszanina III	Rods – Pałeczki	Gram-negative Gram-ujemne	+	+
7M	TSA + mixture II TSA + mieszanina II	Rods – Pałeczki	Gram-negative Gram-ujemne	+	+

observed in any of the metal concentrations used (data not shown).

In addition, during the 8-hour test, no visible proliferation was observed for any of the strains analysed, at any of the mercury concentrations tested. In the 48-hour test, 1M, 2M, 4M and 5M strains did not show any OD increase at any concentrations of any of the heavy metals tested, which may indicate high toxicity of the salts for the above-mentioned strains. The optical density of 3M strain rose in all tested copper sulphate concentrations, although the increase was slightly limited, which indicates a moderate tolerance of this strain to this metal. In the sample with the lowest concentrations of cadmium and lead, a slight rise of the OD was observed after 30 hours, while higher concentrations inhibited it. In the mercury-added medium, the 3M strain OD was at the level of the negative control regardless of the concentration used (Fig. 3). The 6M strain was able to show optical density increase in the TSA medium with the addition of all concentrations of copper salt. Except the lowest copper concentration (0.05 mM), each remaining concentration showed an increase of optical density of 6M strain samples in relation to the control, proportionally to the increase in the concentration of copper sulphate in the medium (Fig. 4). The addition of cadmium in any of the tested concentrations caused a gradual inhibition of the OD of 6M strain. Growth inhibition was greater, the higher the metal concentration (Fig. 4). In turn, in the presence of lead nitrate, the inhibition of OD of the tested strain was relatively low in comparison to control. 6M strain did not proliferate in the medium with mercury, regardless of the concentration used (Fig. 4). The 7M strain optical density increased in TSA media with the addition

of all tested concentrations of copper and cadmium salts, however a decrease in bacterial tolerance to heavy metals was noted as concentrations increased. High concentrations of lead nitrate (0.9 mM, 0.75 mM and 0.6 mM) lead to the increase of the optical density of 7M strain (Fig. 5). Also, strain 7M did not show any OD increase in the medium with mercury at any concentration. During the 48-hour toxicity test, no strains proliferated at any of the tested mercury chloride concentrations.

DISCUSSION

The elements chosen for this research are Cd, Hg, Pb and Cu. First three of them are considered not to have any significant biological role and are toxic even in very low concentrations [Rathnayake, et al. 2010, Jarosławska and Piotrowska-Seget 2014, Khan et al. 2016]. The occurrence of cadmium in the human organism has been linked to osteoporosis, chronic pulmonary problems and oncogene activation [Khan et al. 2016]. Exposure to lead have been connected to impairment of development, plumbism and weakness of the joints and nausea [Wuana and Okieimen 2011]. Mercury is highly toxic in volatile, liquid, and solid form and is related to fatigue, hair loss, kidney and lung failure or brain damage [Dixit et al. 2015]. On the contrary, copper is an essential metal as it serves as a cofactor of enzymatic reactions related to oxidative stress and is involved in hemoglobin formation. However, ability of copper to change from oxidized to reduced form leads to production of radicals. In addition, overexposure to copper may lead to Wilson's disease [Tchounwou et al. 2012].

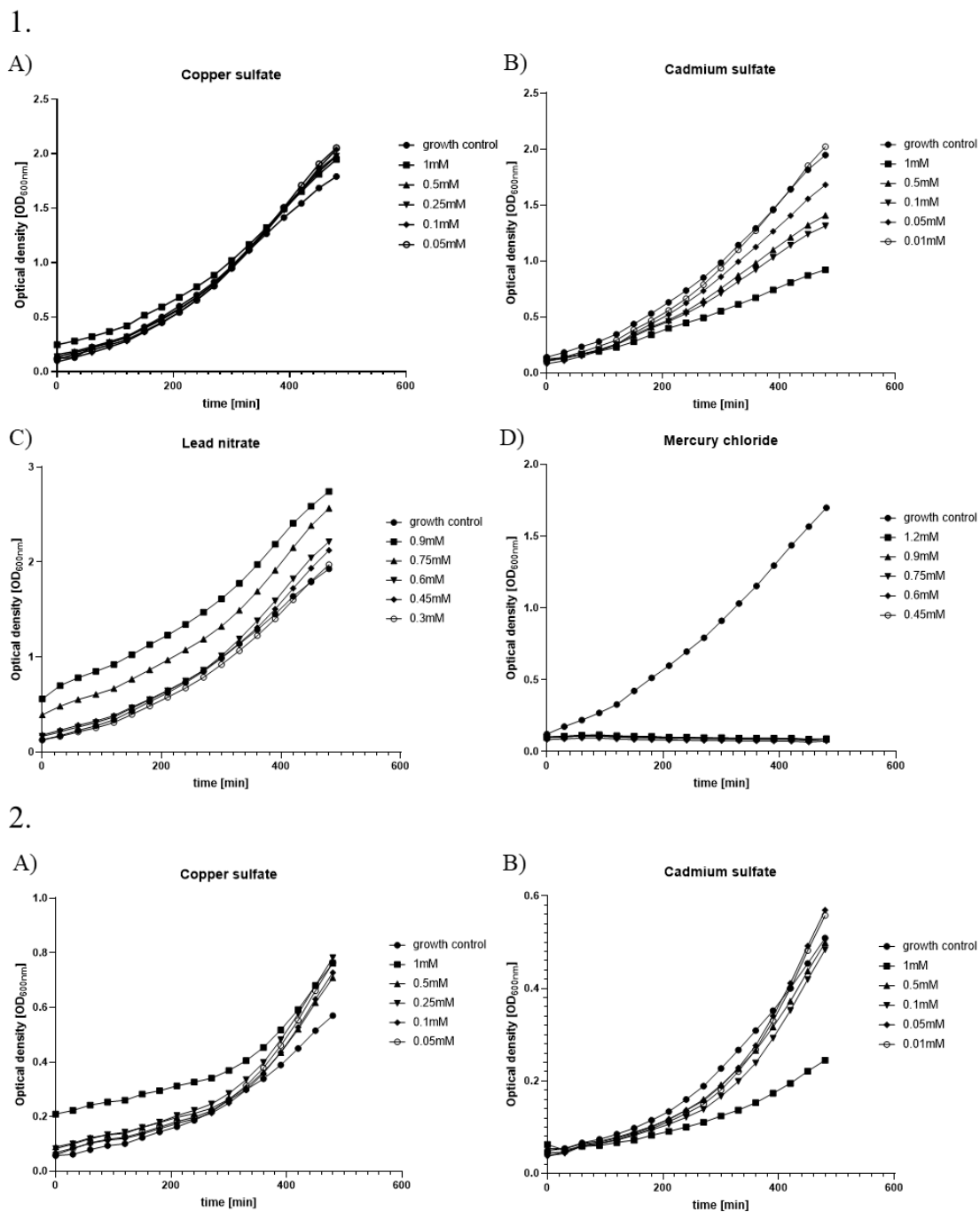


Fig. 2. Heavy metal toxicity test against strains (1.) 6M and (2.) 7M conducted for 8 hours, using: A) CuSO_4 ; B) CdSO_4 ; C) $\text{Pb}(\text{NO}_3)_2$; D) HgCl_2

Rys. 2. Test toksyczności metali ciężkich względem szczepów (1.) 6M i (2.) 7M w trakcie 8 godzin, z użyciem: A) CuSO_4 ; B) CdSO_4 ; C) $\text{Pb}(\text{NO}_3)_2$; D) HgCl_2

Many approaches have been developed in order to remove heavy metal pollution from the environment, such as ion-exchange, chemical precipitation, membrane technologies. oxidation and reduction reactions or reverse

osmosis [Dixit et al. 2015]. However, these methods can be expensive and inefficient if the concentration of heavy metal did not reach a certain level. For this reason, many researches search for more environment-friendly

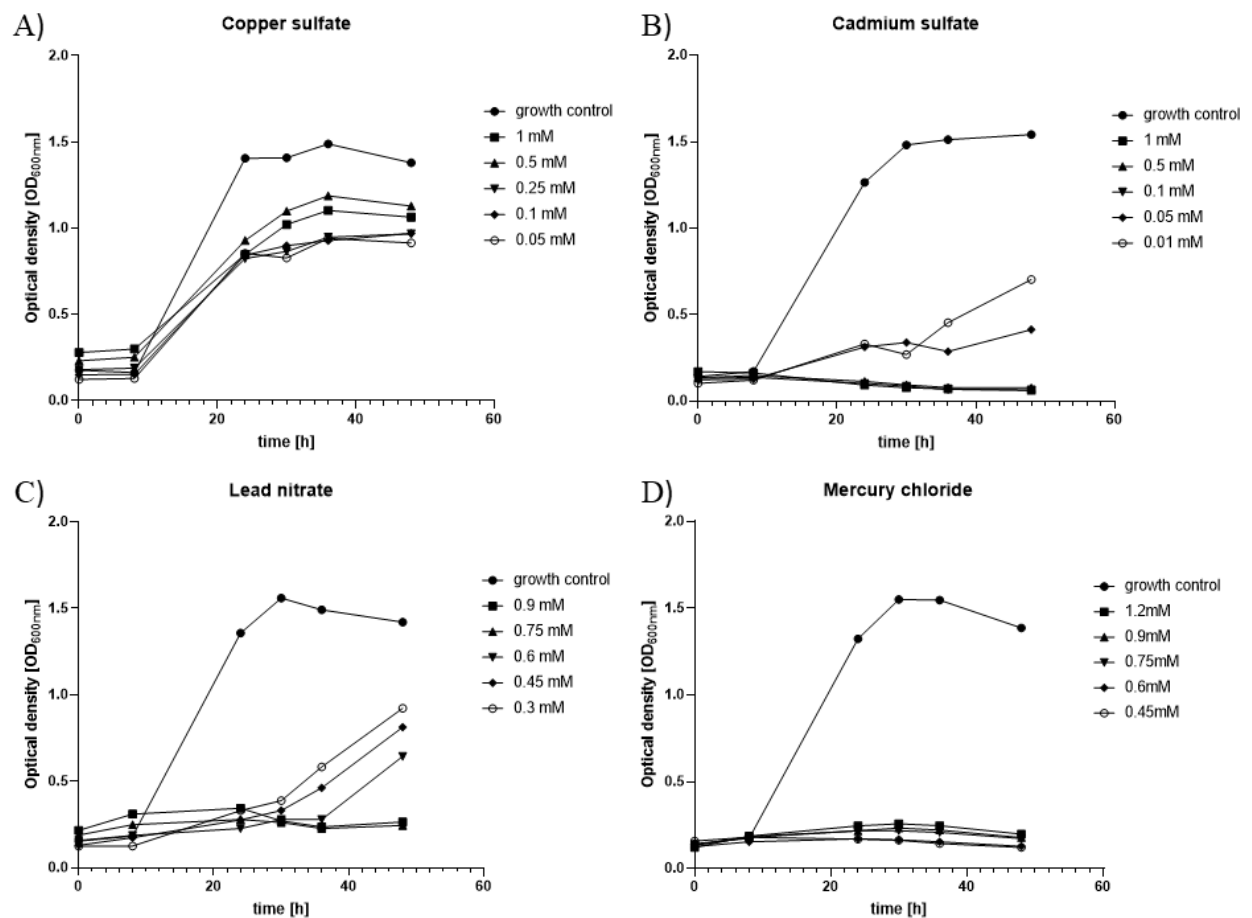


Fig. 3. Heavy metal toxicity test against strain 3M conducted for 48 hours, using: A) CuSO₄; B) CdSO₄; C) Pb(NO₃)₂; D) HgCl₂

Rys. 3. Test toksyczności metali ciężkich względem szczepu 3M w trakcie 48 godzin, z użyciem: A) CuSO₄; B) CdSO₄; C) Pb(NO₃)₂; D) HgCl₂

and cost-effective techniques. One of them is bioremediation – a technology using microorganisms in order to remove or reduce the concentration of pollutants in soil and water.

Some microorganisms are able to produce siderophores or polymers that trap a metal ion in the extracellular space or on the surface of the cell. Other mechanisms are storing metals in bacterial cytoplasm or periplasmic space, the use of efflux pumps or chemical modification of a metal to less toxic form [Lemire et al. 2013]. These mechanisms are called biosorption, bioaccumulation, biotransformation, bioleaching, biomineralization and redox reactions [Dixit et al. 2015].

Seven bacteria strains were isolated from the soil contaminated with heavy metals from the former airport in the West Pomeranian Voivodeship. Four isolates were Gram-negative and three Gram-variable. Six of them were rod-shaped and one bacilli-shaped. Four strains were oxidase positive and all were catalase pos-

itive. Gram-negative bacteria usually produce dense capsules which may be crucial in metals adsorption and this feature increases bacteria tolerance to heavy metals. Moreover, two layers of cell membrane help them to tolerate and grow at higher metal concentration than Gram-positive bacteria. Furthermore, the catalase enzyme acts as a defense mechanism against the reactive oxygen species which eases the metabolic efficiency [Bhojiya and Joshi 2015]. The source of isolation was chosen due to its historical exploitation which led to likely soil contamination. Bacteria living in such harsh conditions had to create mechanisms which would allow them to survive [Bhojiya and Joshi 2015]. Another, alternative spots rich in microorganisms resistant to heavy metals may be tailing sites, sludges or waste waters [Hassen et al. 1998, Bhojiya and Joshi 2015]. During isolation procedure the length of samples incubation time had crucial impact on the number of isolated microorganisms. When duration of sedimentation was 4 hours, only 1 strain was isolated

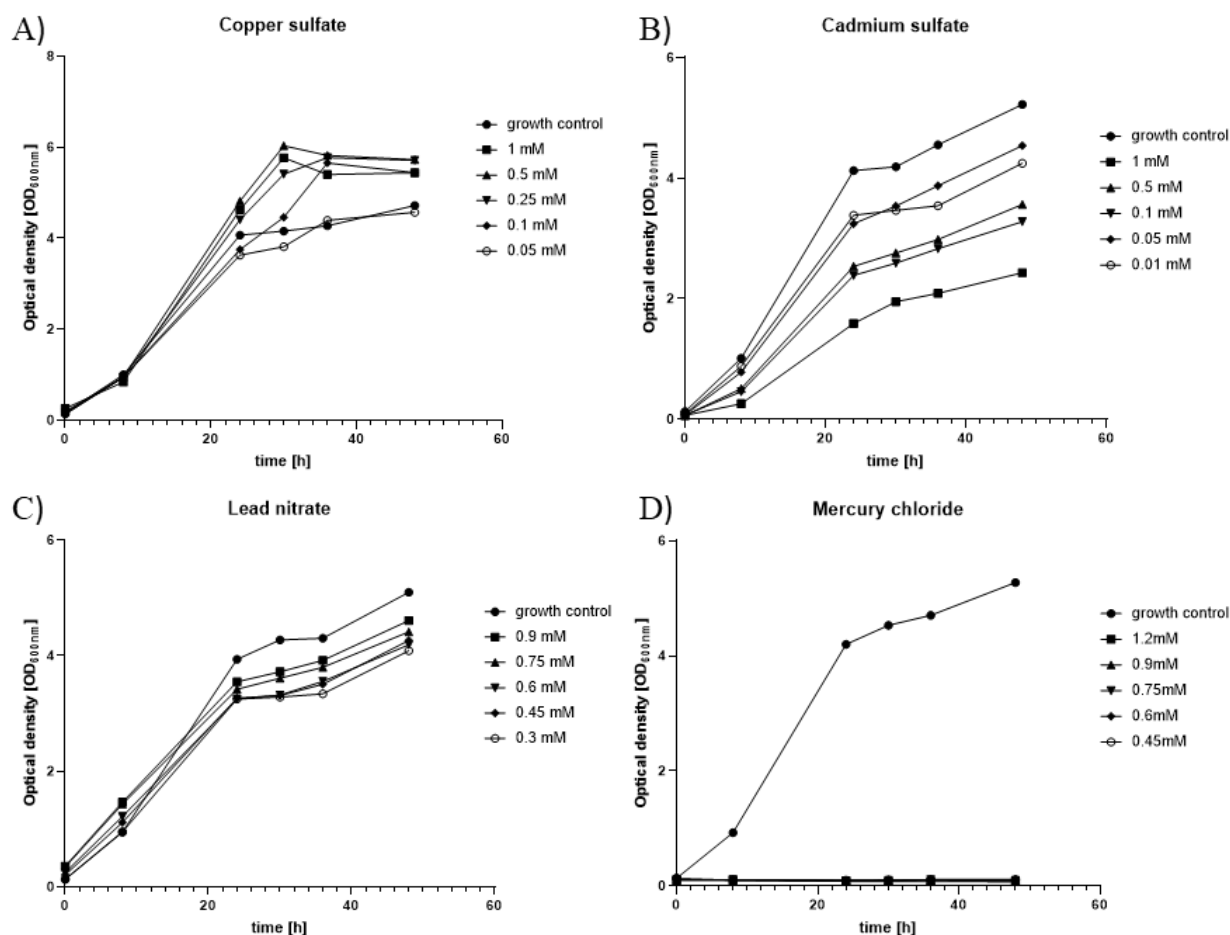


Fig. 4. Heavy metal toxicity test against strain 6M conducted for 48 hours, using: A) CuSO_4 ; B) CdSO_4 ; C) $\text{Pb}(\text{NO}_3)_2$; D) HgCl_2

Rys. 4. Test toksyczności metali ciężkich względem szczepu 6M w trakcie 48 godzin, z użyciem: A) CuSO_4 ; B) CdSO_4 ; C) $\text{Pb}(\text{NO}_3)_2$; D) HgCl_2

versus 6 strains isolated after additional 7 days. That may relate to better regeneration of microorganisms and release from soil clumps. Similar conclusion was showed by Limmathurotsakul et al. [2012], where different methodologies were tested for *Burkholderia pseudomallei* isolation from soil. Scientists obtained 27.7% and 41.5% of isolates after 1 hour and overnight sedimentation time, respectively.

In this study tolerance of isolated strains to four heavy metals was evaluated. Strains which initially were able to survive on solid medium enriched with all tested heavy metals, then were subjects for further analysis. The main aim of this research was to establish isolated bacteria tolerance of heavy metals in liquid medium, due to better diffusion and complexation proprieties of metals. Many scientists noticed differences between bacteria tol-

erance of heavy metals in liquid and solid media. There is a tendency that microorganisms are more sensitive to stress factors if analysis is conducted in liquid medium [Hussein and Joo 2013, Bhojiya and Joshi 2015]. Chosen concentrations of heavy metals were comparable to those used in work presented by other authors: CuSO_4 (0.05mM – 1mM), CdSO_4 (0.01mM – 1mM), $\text{Pb}(\text{NO}_3)_2$ (0.3mM – 0.9mM), HgCl_2 (0.45mM – 1.2mM) [Teitzel and Parsek 2003, Raja et al. 2009]. Three from seven strains were growing when copper, cadmium or lead salts were present in the liquid medium. Strains 1M, 2M, 4M and 5M despite growing on solid medium with mixture of heavy metals were not able to proliferate in liquid cultures enriched with tested stress factors. None of the analysed isolates survived mercury chloride stress. It may implicate that this metal is the most toxic among tested.

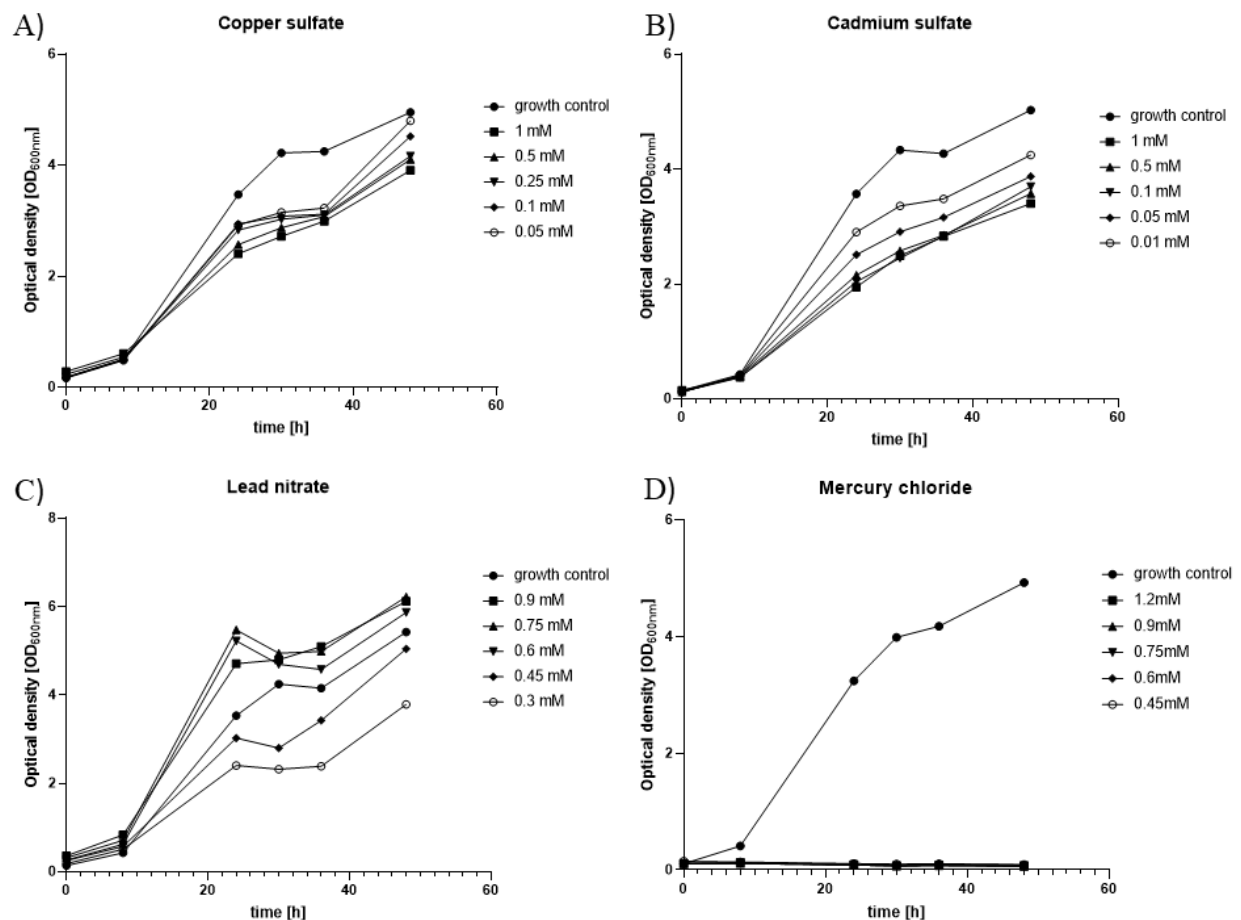


Fig. 5. Heavy metal toxicity test against strain 7M conducted for 48 hours, using: A) CuSO₄; B) CdSO₄; C) Pb(NO₃)₂; D) HgCl₂

Rys. 5. Test toksyczności metali ciężkich względem szczepu 7M w trakcie 48 godzin, z użyciem: A) CuSO₄; B) CdSO₄; C) Pb(NO₃)₂; D) HgCl₂

Similar results were confirmed by [Hassen et al. \[1998\]](#). The least toxic metal was copper which exhibited neutral effects on strains 3M, 6M and 7M. Copper in some concentrations is essential for bacteria for optimal functioning of the metabolic pathways, however it is toxic in doses higher than optimal ones. Three tested strains grew on medium supplemented with 1mM CuSO₄, higher doses of this metal were not analysed and we did not estimate the minimal inhibitory concentration (MIC) for these strains. In turn, [Rathnayake et al. \[2010\]](#) obtained MIC for soil bacteria with much greater copper concentration, as much as 3.5 mM.

In our study, in some cases, the increase of optical density of cultures with high concentrations of lead and copper was observed (Figures 2, 4, 5). These observations may reflect an enhanced proliferation, however different explanations of this fact are probable, e.g. capsule production or binding metals with structures released by bacterium, metal chelation or formation of complexes

that can lead to reduction of metal activity [[Hassen et al. 1998](#)]. This phenomenon was not further investigated in this work. [Hassen et al. \[1998\]](#) also underlined that some ingredients of the medium may interact with metal ions, although in our research, control samples that consisted of medium and metal solution (in chosen concentrations) excluded the possibility of such interaction, since the measurements of the optical density in comparison to the control of the medium were on the same levels.

The tolerance of soil bacteria to heavy metals is considered as an indicator of their potential toxicity to other form of biota [[Tomova et al. 2015](#)]. Moreover, the ability to survive in such strict conditions may be associated with an ability to bond heavy metals by bacteria and neutralize them. Bacteria able to survive in environments contaminated with heavy metals may create mechanisms described above which lead to elimination of harmful substances. Those bacteria may have serious biotechnological poten-

tial in bioremediation process and may be useful in recultivation of contaminated areas around the world.

CONCLUSIONS

In conclusion, response of isolated microorganisms to heavy metals was diversified. Toxicity tests conducted with the use of liquid medium resulted in reduced tolerance of isolates, compared to the results obtained on solid medium. Nevertheless, optical density increases in the bacterial cultures have been observed at the highest concentrations of some heavy metals. Results obtained in this study proves that microbes isolated from soil exposed on contamination with heavy metals may have the bioremediation potential.

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TOLERANCJA BAKTERII ŚRODOWISKOWYCH NA METALE CIĘŻKIE

STRESZCZENIE

Celem niniejszej pracy była izolacja mikroorganizmów o potencjale bioremediacyjnym z gleby zanieczyszczonej związkami metali ciężkich oraz analiza tolerancji tych mikroorganizmów na różne stężenia metali ciężkich. W sumie wyizolowano 7 szczepów bakteryjnych. Wrażliwość mikroorganizmów na metale ciężkie okazała się zależna od rodzaju użytego podłoża. Zastosowanie płynnych pożywek podczas 8-godzinnego i 24-godzinnego testu toksyczności spowodowało obniżoną tolerancję izolatów na stężenia zastosowanych czynników selektywnych w stosunku do hodowli na podłożach stałych. Ponadto zaobserwowano wzrost gęstości optycznej w kulturach bakteryjnych przy najwyższych stężeniach niektórych metali ciężkich.

Słowa kluczowe: bakterie autochtoniczne, bioremediacja, gleba, metale ciężkie, toksyczność

