Impact of Heavy Metals on Development of Metal Resistance in Soil Microbiota

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Abstract

Heavy metal pollution caused by anthropogenic activity is a great concern of the present days. Widespread use of substances containing metals inevitably lead to their deposition in soil affecting soil microbiota, which plays important role in maintaining soil functions. The aim of our study was to determine number of heavy metal resistant isolates acquired from the soil from heavy metal polluted area of dump near Hnúšťa. Soil samples were obtained from two collection sites (48° 36´ 4,47502´´ N, 19° 57´32,654´´ E and 48° 36´ 4,4634´´ N, 19° 57´ 32,67´´ E) and mixed together. The microorganisms used in this study were routinely cultivated and screened for resistance to different concentrations of four heavy metals – Zn (2–500 mg/l), Cu, Ni, Pb (all three metals tested at concentrations from 0,5 to 125 mg/l). Very high resistance against Cu, Ni and Pb even at the highest tested concentrations was found at majority of tested bacterial strains. Almost all 89 from 89 isolates show resistance against these metals at tested concentrations. Only in case of Zn we determined the MIC (minimal inhibitory concentration) – 125 mg/l. The results point out very high resistance pattern in soil bacteria.

Keywords: heavy metals, soil microbiota, metal resistance

Introduction

Environmental stress could be defined as a physiological response of individuals which can affect their wellbeing and performance. It is a force shaping evolution and adaptation in continuously changing environment and it is a characteristic of both – the stressed and the stressor (Bijlsma, 2005). Environmental stress is considered as a biological response to the physical parameters of the environment. Extrinsic stress that stems from changes in abiotic factors such as climate factors, temperature or chemical components, either man-made or naturally occurring, is regarded as the most important stress agent (Lindgren-Laurila, 2005; Sørensen et al., 2005). Rapidly growing human population causes major changes in the abiotic and biotic environment at a fast rate (Frankham, 2005).

Heavy metal pollution mainly caused by anthropogenic activity is widespread in the environment. Generally, heavy metals play a basic role in physiological and metabolic processes in humans, plants and microorganisms. Metals like Cu, Ni, Zn, Co and Cr act as micronutrients and are necessary in redox processes. The non-essential heavy metals like Cd, Pb, As, Hg or Ag have no biological importance to living organisms and are very toxic when present in the ecosystem (D´Amore et al., 2005). The occurrence of higher concentrations of heavy metals in the environment is a serious health problem due to their non-degradative property which enables them to be persistent in the nature for a long time with a long-term harmful effects on the ecosystem (Singh et al., 2011).

The natural population of soil microbiota is severely affected by the heavy metal pollution which can lead to the loss of bacterial species responsible for nutrient cycling what results in a negative effect on the functioning of the whole ecosystem. There are several soil physicochemical conditions which can strongly influence soil bacterial response to metal pollution. Soil texture, pH, organic matter content or clay content are main factors which affect metal toxicity and bioavailability (Azarbad et al., 2013).

Only a long-lasting exposure to the heavy-metal pollution results in the development of adapted microbial communities that can persist and survive in such habitat (Giller et al., 1998). It is believed that some microorganisms have evolved metal resistance shortly after the life begun because of the exposure to the toxic metals. And some microorganisms are believed to evolve metal resistance as a result of metal pollution exposure over the recent 50 years (Gomathy-Sabarinathan, 2010). To be able to survive under these toxic conditions of the environment, some bacteria have evolved several ways to withstand the potentially lethal conditions (Piotrowska-Seget et al., 2005). The resistance mechanisms developed by microorganisms include efflux pumps, permeability barriers, enzymatic detoxification, intra- and extra- cellular sequestration and reduction (Nies, 1999). Genetic determinants responsible for coding of these mechanisms are found in several bacterial strains. Most of them are plasmid encoded and show tendency to be specific for a particular metal, whereas others are more general and confer resistance to a variegation of metals (Gomathy-Sabarinathan, 2010). However, metal resistance genes and antibiotic resistance genes can be linked together if located on the same mobile genetic element in the cell what means that metal pollution may also sustain antibiotic resistance in microorganisms (Ghosh et al., 2000). The fact, that metal contaminated soil, in comparison to the non-metal contaminated soil, contain higher percentage of antibiotic resistant isolates have led to the conclusion, that metal pollution may play a part in increased bacterial antibiotic resistance through an indirect selection. Agricultural soils amended with metal containing fertilizers are considered a significant pool of antibiotic resistance genes in

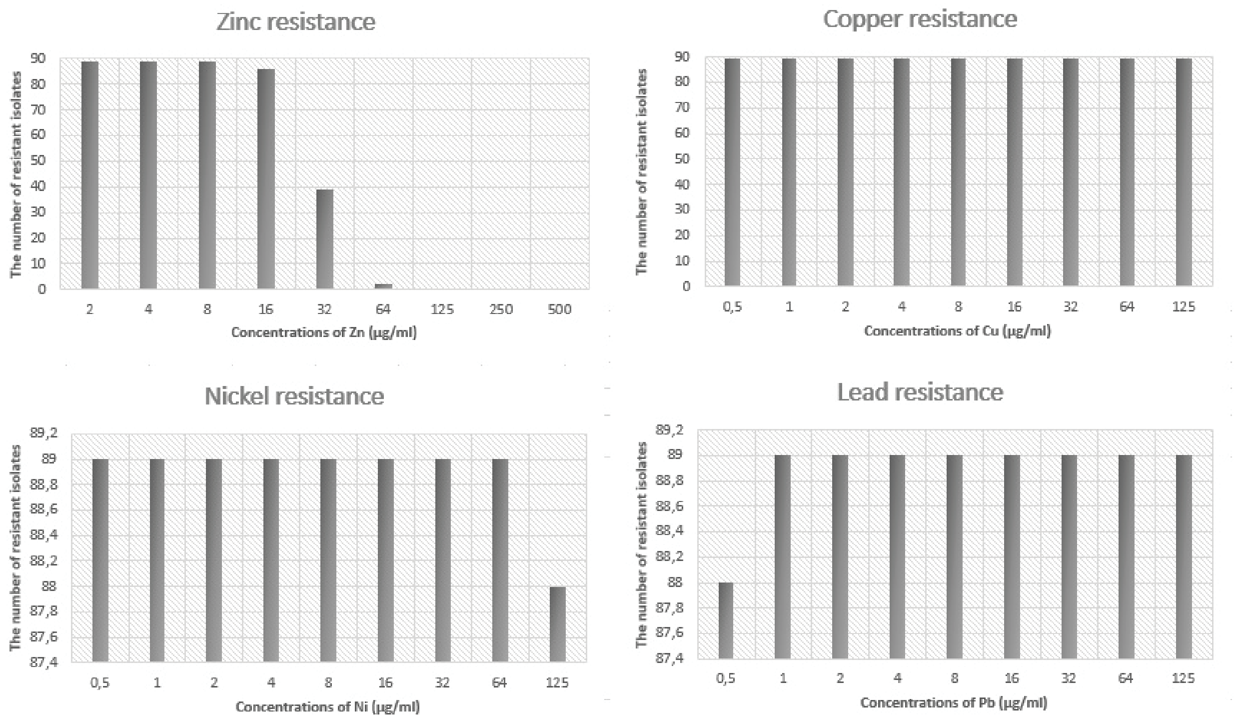

Fig. 1. The number of resistant isolates against zinc; copper; nickel; lead

Rys. 1. Liczba odpornych izolatów przeciwko cynkowi; miedzi; niklu; ołowiowi

the environment and a potential source of antibiotic resistance in bacterial pathogens infecting humans and animals (Alonso et al., 2001; Berg et al., 2005). Accordingly, accumulation of antibiotic resistance genes in soils and their assumed linkage to the metal resistance genes in soil microbiota are of concern. Hence, the main objective of this study was to determine the number of metal resistant bacterial isolates isolated from the metal contaminated soil of dump.

Materials and methods

Two soil samples (approx. 100g) were collected from the top layer (0–10 cm) at two collection sites located near the area of Hnúšťa (SK) (48° 36´ 4,47502´´ N, 19° 57´32,654´´ E and 48° 36´ 4,4634´´ N, 19° 57´ 32,67´´ E). In these sites, high concentrations of heavy metals have been accumulated for decades due to the mining activity. Both soil samples were mixed together thoroughly. 1g of the soil was suspended in 10ml PBS-T and intensively shaken for 30 minutes. Then, 50µl of serial 10-fold dilutions of soil suspensions were plated onto the TSA (Tryptic Soy Agar) agar plates which were cultivated at 25°C for 48 hours to determine CFU (colony forming units). Colonies of bacteria used for later metal resistance testing were selected based on their variable phenotype on TSA agar. Metal resistance testing of bacterial isolates obtained from the primary isolation was performed on MH (Mueller-Hinton) agar using the dilution method with the addition of metals- Zn, Cu, Ni, Pb. The metals were added to the medium in the form of water stock solution of their salts in an appropriate amount to the achievement of final concentrations of metal ions in the medium. The metal concentrations were as follows: Zn (2–500 mg/l), Cu, Ni, Pb (all three metals tested at concentrations from 0,5 to 125 mg/l). The plates were incubated at 25°C for 48 hours and then the growth of bacterial isolates was evaluated.

Results and discussion

In our experiment, we determined the number of bacterial CFU in metal contaminated soil after the 48 hours incubation of isolates of the primary isolation – 3.02x107. The count is approximately in accordance with the findings of Berg et al. (2005), who set CFU value of contaminated soil on 1.5x106 CFU. Based on the different phenotype of our colonies, we selected 89 bacterial colonies from these isolates to examine their metal resistance to heavy metals at variable concentrations.

All bacterial isolates exhibited very high resistance pattern (Figure 1) even at the highest concentrations of Ni, Cu and Pb- nearly all of them were resistant to all concentrations of these three metals, what is in accordance with the findings of Joshi (2013) who performed an experiment with the metal contaminated soil of industrial waste. Bacterial isolates obtained from this soil showed maximum tolerance to metals tested (Pb, Zn, Ni, Cu and others).

In our experiment, only the isolates growing on the medium amended with elevated concentration of Zn (concentration 125 mg/l and higher) showed sensitivity to this heavy metal. It could be caused by the fact, that some elements, like Zn, provide micronutrition for biochemical reactions as enzyme co-factors thus are essential for the cell (Bernier- Surette, 2013). In addition to this, Bernier and Surette (2013) also claimed, that bacterial responses to metals are concentration dependent- at higher concentrations they become toxic what can lead to cellular stress responses or even cell death. This statement was confirmed by our experiment in the case of Zn. In the case of other three metals tested, concentrations which were used in our research, was not high enough to demonstrate this tendency.

According to Spain and Alm (2003) zinc displays comparatively less toxic effect on bacterial cells in comparison with other heavy metals what is not in accordance with our results, because in our experiment zinc showed toxicity to bacteria at concentration 125 mg/l, whereas others heavy metals tested were not toxic to microorganisms even at higher concentrations.

However, there is one interesting fact, that all our isolates were of multi-resistant phenotype- to some extent they exhibited resistance against all four metals tested. All of the isolates were resistant to copper at all of its concentrations tested; 88/89 isolates showed resistance to nickel and lead at all of their concentrations tested, but resistance to zinc at different concentrations was variable- it ranged from 2 to 125µg Zn/ml.

Conclusion

The results proved very high resistance pattern in microorganisms isolated from contaminated soil of dump. Bacteria exposed to high concentrations of heavy metals in their natural habitat were forced to develop resistance mechanisms maintaining their fitness even under the stressful conditions of the environment. The widespread presence of metal resistance mechanisms is of concern because of the linkage between metal resistance genes and antibiotic resistance genes, what makes this issue interesting also from the medical point of view. Linkage and subsequent interspecific co-transfer these two groups of genes via the horizontal transport could become a serious health problem which can cause difficulties with the therapy of bacterial diseases in human.

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Literatura – References

1. ALONSO, Ana et al. Environmental selection of antibiotic resistance genes. Environmental Microbiology [online]. 2001, vol. 3, no. 1, January [cit. 2018-04-10]. Dostupný z WWW: https://onlinelibrary.wiley.com/doi/abs/10.1046/j.1462-2920.2001.00161.x. ISSN 1462-2920.
2. AZARBAD, Hamed et al. Microbial community structure and functioning along metal pollution gradients. Environmental Toxicology and Chemistry [online]. 2013, vol. 32, no. 9, September September [cit. 2018-04-10]. Dostupný z WWW: https://setac.onlinelibrary.wiley.com/doi/full/10.1002/etc.2269. ISSN 1552-8618.
3. BERG, Jeanette et al. Copper amendament of agricultural soil selects for bacterial antibiotic resistance in the field. Letters in Applied Microbiology [online]. 2005, vol. 40, no. 2, February [cit. 2018-04-10]. Dostupný z WWW: https://onlinelibrary.wiley.com/doi/full/10.1111/j.1472-765X.2004.01650.x. ISSN 1472-765X.
4. BERNIER, Steve P.- SURETTE, Michael G. Concentration-dependent activity of antibiotics in natural environments. Frontiers in Microbiology [online]. 2013, vol. 4, no. 20, February [cit. 2018-04-10]. Dostupný z WWW: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3574975/. ISSN 1664-302X.
5. BIJLSMA, Rita- LOESCHCKE, Volker. Environmental stress, adaptation and evolution: an overview. Journal of Evolutionary Biology [online]. 2005, vol. 18, no. 4, July [cit. 2018-04-09]. Dostupný z WWW: https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1420-9101.2005.00962.x. ISSN 1420-9101.

6. D'AMORE, J et al. Methods for speciation of metals in soils. Journal of Environmental Quality [online]. 2005, vol. 34, no. 5, January [cit. 2018-04-09]. Dostupný z WWW https://dl.sciencesocieties.org/publications/jeq/abstracts/34/5/1707. ISSN 0047-2425.

7. FRANKHAM, Richard. Stress and adaptation in conservation genetics. Journal of Evolutionary Biology [online]. 2005, vol. 18, no. 4, July [cit. 2018-04-09]. Dostupný z WWW: https://www.ncbi.nlm.nih.gov/pubmed/16033545. ISSN 1420-9101.

8. GHOSH, Arpan et al. Characterization of large plasmids encoding resistance to toxic heavy metals in Salmonella abortus equi. Biochemical and Biophysical Research Communications [online]. 2000, vol. 272, no. 1, May [cit. 2018-04-10]. Dostupný z WWW: https://www.sciencedirect.com/science/article/pii/S0006291X0092727X?via%3Dihub. ISSN 0006-291X.

9. GILLERA, Ken. E. et al. Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils: A review. Soil Biology and Biochemistry [online]. 1998, vol. 30, no. 10-11, September [cit. 2018-04-10]. Dostupný z WWW: https://www.sciencedirect.com/science/article/pii/S0038071797002708?via%3Dihub. ISSN 0038-0717.

10. GOMATHY, M.- SABARINATHAN, K. G. Microbial mechanisms of heavy metal tolerance- review. Agricultural Reviews . [online]. 2010, vol. 31, no. 2, [cit. 2018-04-10]. Dostupný z WWW: http://www.arccjournals.com/uploads/articles/ar312007.pdf. ISSN 0976-0539.

11. JOSHI, B. H.- MODI, K. G. Screening and characterization of heavy metal resistant bacteria for its prospects in bioremediation of contaminated soil. Journal of Environmental Research And Development [online]. 2013, vol. 7, no. 4A, April-June [cit. 2018-04-11]. Dostupný z WWW: http://webcache.googleusercontent.com/search?q=-cache:XDjGaUSKta0J:www.jerad.org/ppapers/dnload.php%3Fvl%3D7%26is%3D4A%26st%3D1531+&cd=3&hl=sk&ct=clnk&gl=sk&client=firefox-b-ab. ISSN 2319-5983.

12. LINDGREN, Beatrice- LAURILA, Anssi. Proximate causes of adaptive growth rates: growth efficiency variation among latitudinal populations of Rana temporaria.. Journal of Evolutionary Biology [online]. 2005, vol. 18, no. 4, July [cit. 2018-04-09]. Dostupný z WWW: https://www.ncbi.nlm.nih.gov/pubmed/16033553. ISSN 1420-9101.

13. NIES, Dietrich H. Microbial heavy-metal resistance. Applied Microbiology and Biotechnology [online]. 1999, vol. 51, no. 6, June [cit. 2018-04-10]. Dostupný z WWW: https://link.springer.com/content/pdf/10.1007/s002530051457.pdf. ISSN 1432-0614.

14. PIOTROWSKA-SEGET, Zofia et al. Metal-tolerant bacteria occurring in heavily polluted soil and mine spoil. Applications in Soil Ecology [online]. 2005, vol. 28, no. 3, March [cit. 2018-04-10]. Dostupný z WWW: https://www.sciencedirect.com/science/article/pii/S0929139304001064?via%3Dihub. ISSN 0929-1393.

15. SINGH, Reena et al. Heavy metals and living systems: An overview. Indian Journal of Pharmacology [online]. 2011, vol. 43, no. 3, May-June [cit. 2018-04-09]. Dostupný z WWW: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3113373/. ISSN 1998-3751.

16. SØRENSEN, Jesper Givskov et al. Altitudinal variation for stress resistance traits and thermal adaptation in adult Drosophila buzzatii from the New World. Journal of Evolutionary Biology [online]. 2005, vol. 18, no. 4, July [cit. 2018-04-09]. Dostupný z WWW: https://www.ncbi.nlm.nih.gov/pubmed/16033554. ISSN 1420-9101.

17. SPAIN, Anne- ALM, Elizabeth. Implications of Microbial Heavy Metal Tolerance in the Environment. Rev Undergraduate Res [online]. 2003, vol. 2, no. 1–6, [cit. 2018-04-11]. Dostupný z WWW: http://www.ruf.rice.edu/~rur/issue2_files/PDF_Final/spain.pdf.

Wpływ metali ciężkich na rozwój odporności na zawartość metali w mikroflorze w glebie

Zanieczyszczenie metalami ciężkimi wywołane działalnością antropogeniczną jest wielkim problemem współczesności. Powszechne stosowanie substancji zawierających metale nieuchronnie prowadzi do ich odkładania się w glebie co wpływa na mikroflorę glebową, która odgrywa ważną rolę w utrzymywaniu funkcji gleby. Celem badań było określenie liczby izolatów odpornych na metale ciężkie pozyskanych z gleby z zanieczyszczonego obszaru składowiska metali ciężkich w pobliżu Hnúšťa. Próbki gleby pobrano z dwóch miejsc (48° 36' 4,47502'' N, 19° 57'32,654'' E i 48° 36' 4,4634''bN, 19° 57'32,67'' E) i wymieszano razem. Mikroorganizmy wykorzystane w tym badaniu były rutynowo hodowane i badane pod kątem odporności na różne stężenia czterech metali ciężkich: Zn (2–500 mg/l), Cu, Ni, Pb (wszystkie trzy badane metale w stężeniach od 0,5 do 125 mg/l). Bardzo wysoką odporność na Cu, Ni i Pb nawet przy najwyższych testowanych stężeniach stwierdzono w większości badanych szczepów bakteryjnych. Prawie wszystkie z 89 izolatów wykazują odporność na te metale w testowanych stężeniach. Tylko w przypadku Zn określiliśmy MIC (minimalne stężenie hamujące) = 125 mg/l. Wyniki wskazują na bardzo wysoki wzór oporności w bakteriach glebowych.

Słowa kluczowe: metale ciężkie, mikroflora gleby, odporność na zawartość metali