

POLISH JOURNAL OF ECOLOGY (Pol. J. Ecol.)	57	3	555–560	2009
--	----	---	---------	------

Short research contribution

Magdalena BŁASZAK, Dorota BIENKOWSKA

Department of Microbiology and Environmental Biotechnology,
West Pomeranian University of Technology, Słowackiego 17, 71-434 Szczecin, Poland
Magdalena.Blaszak@zut.edu.pl

EFFECT OF SOIL POLLUTION ON BACTERIAL RESISTANCE TO LEAD IONS: AN EXPERIMENTAL APPROACH

ABSTRACT: The objective of the laboratory experiment was to study the resistance to lead ions of bacteria isolated from soil with various level of contamination with this element. Lead (II) acetate trihydrate was applied to the soil (sandy loam) in two doses so that the total content of lead corresponded to weak (250 mg kg^{-1}) and strong (5000 mg kg^{-1}) contamination of soil with lead. From each of the soil (control, weakly and strongly contaminated soil) bacterial isolates were obtained and several dozen of them were randomly chosen for the experiment. The isolates were severally point wise cultured in three repetitions onto microbiological medium contaminated with lead (1000 mg dm^{-3}) and on the same control medium. The diameter of grown “twin” colonies in both cultures were compared. For each isolate separately the resistance index (*RI*) was described as a ratio of bacterial cultures diameter of the soil isolate cultured on the contaminated medium to the diameter of the same isolate cultured on the control medium. On the basis of the various *RI* results, the bacteria were divided into four resistance groups (RG) of approximate resistance index: I group, $RI = 0$ – entirely sensitive bacteria (no growth on the contaminated medium); II group $0 < RI \leq 0.5$ – very sensitive bacteria (colony diameter on contaminated medium is smaller or equal to half of the control bacteria colony diameter); III group, $0.5 < RI < 1$ – moderately sensitive bacteria (the colony diameter on contaminated medium

is bigger than a half of the diameter of the control colony); IV group, $RI \geq 1$ – resistant bacteria (the growth of bacteria on the contaminated medium is the same or bigger than on the control medium). The aim of this 150 days lasted experiment was the verification of the hypothesis of different lead resistance depending on bacteria origin. It was assumed that bacteria from lead contaminated soil should be more resistant to this metal when applied again to the microbiological medium contaminated with lead than bacteria from control medium. The results of the experiment partly prove this hypothesis. Three main conclusions appeared: 1) Control soil, with natural lead content (19 mg kg^{-1}) had variety of groups of bacteria regarding lead resistance. It was also noticed that the time of the experiment (since the beginning) in control soil had the least influence on the quantity in particular resistance groups. 2) Only in the strongly contaminated soil (5000 mg kg^{-1}) the sensitive bacteria (I RG) were entirely eliminated, contaminated soil with quantity corresponding to weak contamination (250 mg kg^{-1}) did not give such an effect. 3) The lack of strains of entirely sensitive bacteria (I RG) in strongly contaminated soil was observed only after 100 days of the experiment, the observations suggest the slow pace of succession. Replacing sensitive bacteria with more resistant forms lasted about 4 months.

KEY WORDS: bacteria, lead, resistance

Microorganisms' adaptation to heavy metals is a well-known phenomenon, especially with reference to particular bacterial strains (Vecchio *et al.* 1998, Roane 1999, Spain and Alm 2003, Joynt *et al.* 2006). However, the number of existing data concerning the influence of the heavy metals on soil microorganisms in relation to their whole community, taxonomical group or soil metabolism potential seems to be still insufficient. Bacterial defensive mechanisms against lead as heavy metal are various and they constitute the specific characteristic of given strain. There are the mechanisms connected with functioning of siderophores, passive adsorption of lead on bacterial cells components of walls and capsules, secretion of organic acids and hydrogen sulphide, which reacting with lead ions precipitates it in the form of insoluble sulphides and carbonates (Johnson 1998, Roane 1999). This knowledge, however, does not bring the information about the evolution of adaptive abilities of the whole community of the soil microorganisms. The current interest in the influence of lead ions on soil bacteria is rather due to its negative aspect. There are many works concerning the negative influence of lead on microorganisms and their activity (Johnson 1998, Przybulewska *et al.* 2003, Wyszowska and Kucharski 2003, Nguyen-Viet *et al.* 2007, Zeng *et al.* 2007). There are also experiments carried out on the lead influence on enzymatic activity of bacteria used in soil or water bioremediation from organic pollution (Joynt *et al.* 2006, Badani *et al.* 2007). Many researches are focused on the use of alive and dead bacterial biomass as an absorbent of heavy metals (including lead) from industrial and mine wastes (Nelson *et al.* 1995, Vecchio *et al.* 1998, Gong *et al.* 2005). In the present experiment a simple microbiological parameter was developed and used. It was called resistance index (*RI*), which shows susceptibility to lead of each analysed strain as well as the whole group of bacteria (median *RI*). The aim of the experiment was to determine the resistance of soil bacteria on lead contamination and indicate whether the bacteria from more contaminated soil are more resistant to lead added to the solid culture medium than the bacteria from uncontaminated soil.

The soil used for analyses was taken from humus level (0–10 cm) of a cultivable field. The soil had mechanical composition of sandy loam (58% loam fraction, 25% dust fraction and 17% fluming parts), $\text{pH}_{\text{H}_2\text{O}} = 6.5$ and of organic substance content = 1.5%. The natural lead content in the soil was $19 \text{ mg}\cdot\text{kg}^{-1}$. The soil was dried and in order to remove skeletal parts and mechanical impurities was passed through a sieve of 2 mm mesh diameter. The soil maximum water capacity was determined and it was kept at the state of 50% of the capacity through all the experiment. Lead (II) acetate trihydrate solution was applied to the soil in two doses, so that the total content of lead corresponded with weak (250 mg kg^{-1}) and strong (5000 mg kg^{-1}) soil contamination with lead (Kabata-Pendias *et al.* 1993). The research material was incubated at the temperature of 20°C in three repetitions. Using the method of surface inoculation of soil dilution several dozen of bacterial isolates were isolated (from the control, weakly and strongly contaminated soil) and from each soil forty were randomly chosen for the experiment (the cultures were cultured on medium: enriched agar – MPA, supplement by Biocorp). The five-day colonies (from MPA medium) were inoculated (transferred) into contaminated medium (MPA) with lead (1000 mg dm^{-3}) and on control medium (MPA without contamination) and cultured. Three repetitions were applied. After five days of incubation at the temperature of 25°C “twin” colonies were obtained (the first one cultured with the lead addition and the second one cultured on the control medium), both coming from the same source, that is, from the “mother” colony. This methodology of bacteria isolation was used in terms of 1st, 50th, 100th, 150th day, each time isolating new isolates from the soil. For each isolate separately the resistance index (*RI*) was determined as a ratio of bacterial cultures diameter of the soil isolate cultured on the medium contaminated with Pb^{+2} to the diameter of the same isolate cultured on the control medium. In case of irregular shape of bacteria colonies the diameter was measured in four dimensions and the result was given as a mean value of those four values. The mean resistance index (*RI*) is related to all the bacteria isolated from particular soil. On the

basis of various *RI* results the bacteria were divided into four resistance groups (RG):

I group, $RI = 0$ – entirely sensitive bacteria (no growth on the contaminated medium);

II group, $0 < RI \leq 0.5$ – very sensitive bacteria (colony diameter on contaminated medium is smaller or equal to half of the control bacteria colony diameter);

III group, $0.5 < RI < 1$ – moderately sensitive bacteria (the colony diameter on contaminated medium is bigger than a half of the diameter of the control colony);

IV group, $RI \geq 1$ – resistant bacteria (the growth of bacteria on the contaminated medium is the same or bigger than on the control medium).

The Duncan's multiple range test at the significance level of 0.05 was employed (STATSOFT, INC. 2007) to assess statistically significant differences in resistance of bacteria originated from soils with different level of contamination.

The resistance on heavy metals ions could be defined as the ability of organism to grow and function in presence of analysed metal (Hastrup *et al.* 2005). In present experiment

the resistance of several hundred bacterial isolates was examined on the basis of their growth abilities in conditions of lead contamination. Only in soil strongly contaminated with lead (5000 mg kg^{-1}) there was the succession of resistant microorganisms. At first, bacteria from not contaminated (control) soil and contaminated one showed similar lead resistance in microbiological medium, but in time in soil strongly contaminated with lead (5000 mg kg^{-1}) the resistant forms clearly started to dominate (Fig. 1). Only essential differences in bacterial resistance to lead ions occurred mainly at the final stage of the experiment (100th and 150th day). At that time among the bacteria isolated from strongly contaminated soil the completely resistant bacteria (IV RG) comprised on average 25% of all bacteria, whilst in control and weakly contaminated soil about 7.5% (Fig. 2). Similar conclusions refer to the experiments, which authors, examining the influence of lead on microorganisms, observed the total increase of microbial biomass or the amount of bacterial enzymes in soil (Przybulewska *et al.* 2003, Zeng *et al.* 2007). The rapid growth of

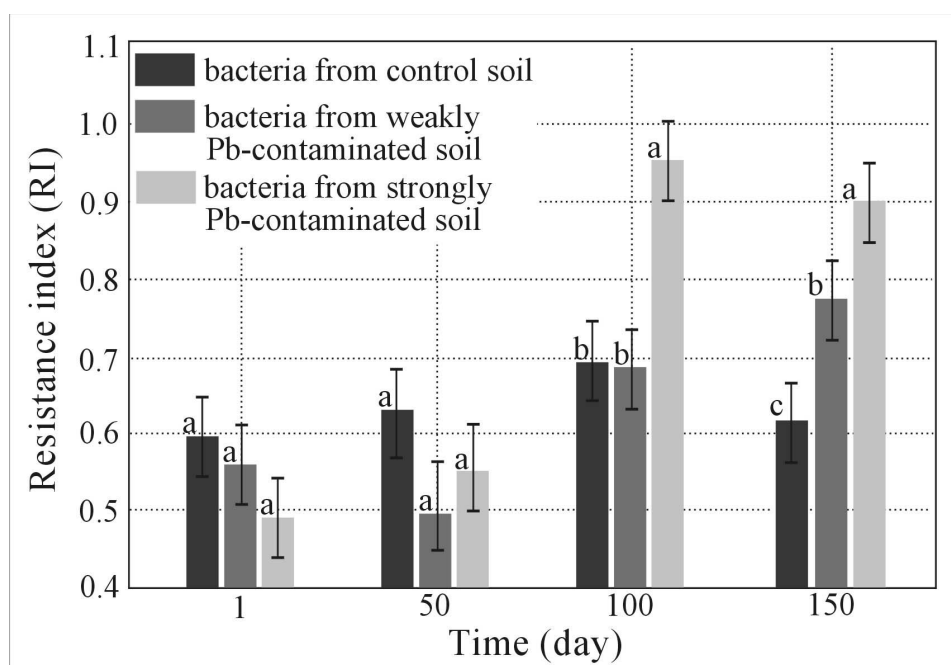


Fig. 1. Resistance index (*RI*) of whole bacteria community to lead: (the ratio of colony diameter of soil isolate cultured on lead-contaminated medium to colony diameter of the same isolates cultured on control medium). Bacteria were isolated from control, weakly (250 mg kg^{-1}) and strongly (5000 mg kg^{-1}) lead-contaminated soils. The same letters above columns mean the lack of essential statistical differences at $\alpha = 0.05$ according to Duncan's test.

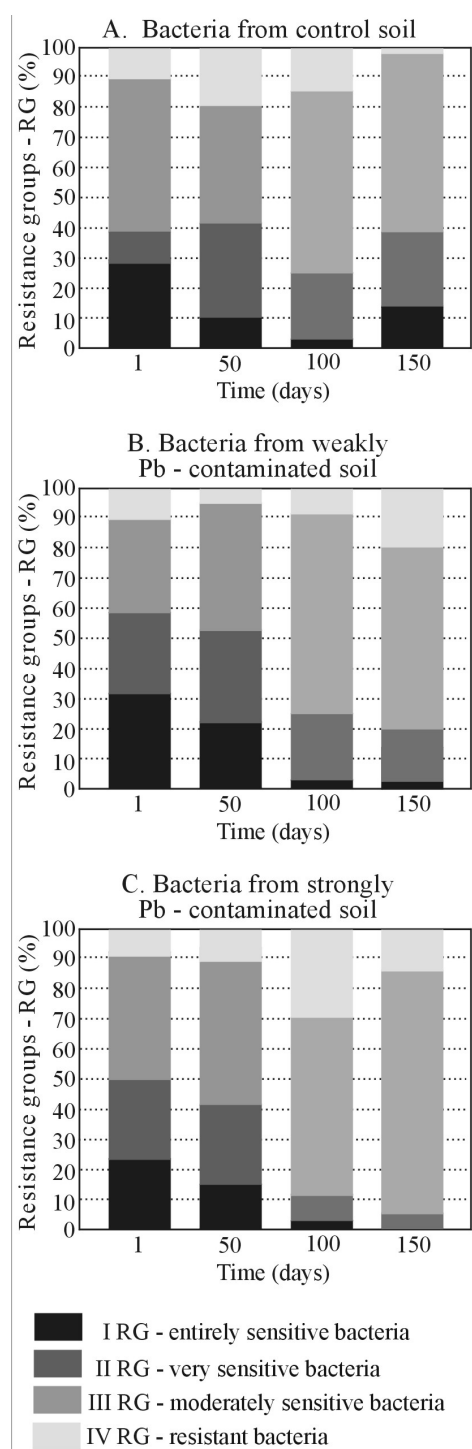


Fig. 2. Percentage participation of bacteria in particular resistance groups (RG). The value of resistance index (RI) decides upon the affinity of bacteria to one of the four resistance groups (RG): I Group, $RI = 0$ – entirely sensitive bacteria; II Group, $0 < RI \leq 0.5$ – very sensitive bacteria; III Group, $0.5 < RI < 1$ – moderately sensitive bacteria; IV Group, $RI \geq 1$ – resistant bacteria.

number of microorganisms and the increase of intensity of bacterial enzymes excretion could be caused by a sudden inflow of nutrient matter from dead susceptible cells, forming the nourishment for resistant bacteria, and in consequence, new, in qualitative and quantitative terms, microbial system emerges. If even low number of resistant bacteria are present in soil then the decrease of the total number of microorganisms in usually temporary. After some time susceptible bacteria are replaced by resistant ones (Feris *et al.* 2004, Joynt *et al.* 2006). However, through selection of susceptible bacteria the soil environment becomes less diverse. Resistant bacteria do not always have such versatile hydrolytic abilities and this, in consequence, has a negative influence on the whole environment (Nowak *et al.* 2004, Zeng *et al.* 2007). Unequivocal opinion of researchers on the influence of lead on bacteria concerns high doses of this metal. Many experiments proved that the increased concentration of lead in soil (from several hundred mg of lead in 1 kg soil) has an inhibiting effect on microorganisms growth and is the cause of qualitative and quantitative changes in contents of microbial biocenosis of soil. In such situation the functioning of even resistant microorganisms undergoes restrictions (Roane 1999, Nowak *et al.* 2004, Nguyen-Viet *et al.* 2007, Zeng *et al.* 2007).

Generally, it can be assumed that in all three soils during each period of experiment the bacteria from the group III, that is of moderate sensitivity on lead (30–80 % of all isolated strains) were dominating, and the number of bacterial isolates classified into other resistance groups differed in time between several and a dozen or so per cent (Fig. 2A, B, C). The entirely sensitive bacteria (I RG) and resistant ones (IV RG) are represented by the lowest number and in both cases the average participation of bacteria from those groups was less than several per cent (Fig. 2A, B, C). The soil contamination with lead ions did not seriously differentiate the resistance of bacteria population as moderately resistant isolates were always present in the biggest number and the smallest number of those extremely reacting on the lead presence in soil. Despite those general tendencies the results of Duncan's test showed statistically signifi-

cant differences, especially visible in two last analyses, when the average resistance index (*RI*) of bacteria isolated from strongly contaminated soil was the highest and amounted to 0.97 and 0.9, respectively. Moderately resistant and completely resistant bacteria comprised together almost 100% of total bacteria and at that time in control soil the average *RI* amounted to 0.7 and 0.6, respectively (Fig. 1). Bacterial isolates obtained from control soil were characterised by higher moderate resistance to lead on microbiological base. In first two terms these bacteria (from III RG) constituted 45%, and in two following terms almost 70% of the total bacteria. Besides the first term, when very sensitive bacteria comprised only several per cent, the number of bacterial isolates belonging to this group (II RG) was relatively constant, on average 25% of the total number of bacterial isolates. Similarly the number of bacterial isolates belonging to I and IV groups (completely sensitive and resistant) was at relatively constant level. Even though completely sensitive bacteria constituted almost 30% in the first term of experiment and 10% in next term, this value was constant thereafter. Beginning from 50th day of experiment the number of entirely resistant bacteria was decreasing and the number finally reduced to 3% (Fig. 2A). The majority of bacteria originating from soil weakly contaminated with lead were characterised by a moderate susceptibility to this metal in microbiological base. In the first term of the experiment these bacteria (III RG), similarly to completely sensitive bacteria (I RG) comprised approximately 30% of the total bacteria, however, with time the participation of the third group rapidly increased up to more than 70%. The reaction of entirely sensitive bacteria (I RG) was quite the opposite and their number decreased systematically reaching approximately 3%. Very susceptible strains (II RG) were represented by a not very numerous group with participation estimated for a dozen or so. The number of entirely resistant bacteria was low (several per cent), except the last term, when they constituted almost 30% of all isolated bacteria (Fig. 2B). As well as in other soils, in soil strongly contaminated with lead the domination of bacterial isolates moderately sensitive to lead (III RG) was observed. The number of bacterial

isolates of this group increased systematically, beginning with 40% and ending with 80% of the total number of bacteria. The strains with different resistance range did not exceed 30%. Completely and very sensitive bacteria (I and II RG) were diminishing in time, finally to complete absence of completely susceptible bacteria (I RG), when the number of bacterial isolates entirely resistant to lead (IV RG) was twice bigger in the second term of examination than in the first. This effect was linked to systematical elimination of bacteria entirely and very susceptible to lead (I and II RG) and growth of entirely resistant bacteria (IV RG) (Fig. 2C).

ACKNOWLEDGEMENTS: The author would like to thank Professor Andrzej Nowak for the essential advices and Miriam Lam for the translation.

REFERENCES

- Badani Z., Ait-Amara H., Si-Salah A. 2007 – The inhibitive effect of lead concentration on the biological treatment of wastewater of oil well drillings – *Desalination*, 206: 295–299.
- Feris K.P., Ramsey P.W., Rillig M., Moore J. N., Gannon J.E., Holben W.E. 2004 – Determining rates of change and evaluating group level resiliency differences in hyporheic microbial communities in response to fluvial heavy-metal deposition – *Appl. Environ. Microbiol.* 70: 4756–4765.
- Gong R., Ding Y., Liu H., Chen Q., Liu Z. 2005 – Lead biosorption and desorption by intact and pretreated *Spirulina maxima* biomass – *Chemosphere*, 58: 125–130.
- Hastrup A.S.C., Green III F., Clausen C.A., Jensen B. 2005 – Tolerance of *Serpula lacrymans* to copper-medium wood preservatives – *Int. Biodeterior. Biodegrad.* 56: 173–177.
- Johnson F.M. 1998 – The genetic effects of environmental lead – *Mutat. Res.* 410: 123–140.
- Joynt J., Bischoff M., Turco R., Konopka A., Nakatsu C.H. 2006 – Microbial Community Analysis of Soils Contaminated with Lead, Chromium and Petroleum Hydrocarbons – *Microb. Ecol.* 51: 209–219.
- Kabata-Pendias A., Motowicka-Terelak T., Piotrowska M., Terelak H., Witek T. 1993 – Ocena stopnia zanieczyszczenia gleb i roślin metalami ciężkimi i siarką. Ramowe wytyczne dla rolnictwa [The evaluation

- tion of contamination level of soils and plants with heavy metals and sulphur. Framework guidelines for agriculture] – Wydawnictwo IUNG – Polish Scientific Publishers, Puławy, 20 pp. (in Polish).
- Nelson Y.M., Lo W. Lion L.W., Shuler M.L., Ghiorse W.C. 1995 – Lead distribution in a simulated aquatic environment: effect of bacterial biofilms and iron oxide – *Wat. Res.* 29: 1934–1944.
- Nguyen-Viet H., Gilbert D., Mitchell E.A.D., Badot P.M., Bernard N. 2007 – Effects of Experimental Lead Pollution on the Microbial Communities Associated with *Sphagnum fallax* (Bryophyta) – *Microb. Ecol.* 54: 232–241.
- Nowak A., Szopa E., Błaszak M. 2004 – Wpływ metali ciężkich (Cd, Cu, Pb, Hg) na zawartość biomasy mikroorganizmów w glebie. [The influence of heavy metals (Cd, Cu, Pb, Hg) on amount of microbial biomass in soil] – *Acta Agr. Silv. ser. Agr.* 42: 335–339. (in Polish).
- Przybulewska K., Nowak A., Smolińska M. 2003 – Wpływ metali ciężkich na wybrane elementy cyklu przemian węgla [Influence of heavy metals on selected elements of carbon transformation cycle] – *Zesz. Probl. Post. Nauk Rol.* 492: 281–286. (in Polish).
- Roane T.M. 1999 – Lead Resistance in Two Bacterial Isolates from Heavy Metal-Contaminated Soils – *Microb. Ecol.* 37: 218–224.
- Spain A., Alm E. 2003 – Implications of microbial heavy metal tolerance in the environment – *Rev. Undergrad. Res.* 2: 1–6.
- Statsoft INC. 2007 – Statistica, version 7.1. – www.statsoft.com.
- Vecchio A., Finoli C., Di Simone D., Andreoni V. 1998 – Heavy metal biosorption by bacterial cells – *Fresenius J. Anal. Chem.* 361: 338–342.
- Wyszowska J., Kucharski J. 2003 – Liczebność mikroorganizmów w glebie zanieczyszczonej metalami ciężkimi [The number of microorganisms in soil contaminated with heavy metals] – *Zesz. Probl. Post. Nauk Rol.* 492: 435–442. (in Polish).
- Zeng L.S., Liao M., Chen C.L., Huang C.Y. 2007 – Effects of lead contamination on soil enzymatic activities, microbial biomass, and rice physiological indices in soil-lead-rice (*Oryza sativa* L.) system – *Ecotoxicol. Environ. Saf.* 67: 67–74.

Received after revision January 2009