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# RESPONSE OF BACTERIA TO SOIL CONTAMINATION WITH HEAVY METALS

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Abstract

The effect of contamination of loamy sand with single heavy metals ($Cd^{2+}$, $Cu^{2+}$, $Zn^{2+}$, $Pb^{2+}$) and with their mixtures on the number of copiotrophic, ammonifying, nitrogen immobilising, cellulolytic bacteria and bacteria of the *Arthrobacter* and *Pseudomonas* genera was examined in a pot experiment. The research was performed in two series: with soil sown with oat and unsown soil.

It was found that the sensitivity of bacteria to $Cd^{2+}$, $Cu^{2+}$, $Zn^{2+}$ and $Pb^{2+}$ is a specific characteristic related to the content of these metals in soil and to the method of soil use. The development of the bacteria of *Arthrobacter* and *Pseudomonas* was most strongly inhibited in the soil sown with oat, while ammonifying, nitrogen immobilising, and cellulolytic bacteria were most inhibited in the unsown soil. Copiotrophic, cellulolytic, nitrogen immobilising and ammonifying bacteria proved to be more resistant to this contamination than bacteria of *Arthrobacter* and *Pseudomonas* genera. Increasing the number of heavy metals simultaneously contaminating the soil to two ($Cd^{2+}$ and $Cu^{2+}$; $Cd^{2+}$ and $Zn^{2+}$; $Cd^{2+}$ and $Pb^{2+}$) and to three ($Cd^{2+}$, $Cu^{2+}$ and $Zn^{2+}$; $Cd^{2+}$, $Cu^{2+}$, and $Pb^{2+}$; $Cd^{2+}$, $Pb^{2+}$ and $Zn^{2+}$) generally did not increase the intensity of their effect on the examined bacteria. Changes brought about by these mixtures were usually similar to changes caused by individual heavy metals.

Key words: heavy metals in soil, bacteria count, copper, zinc, cadmium, lead.

## REAKCJA BAKTERII NA ZANIECZYSZCZENIE GLEBY METALAMI CIĘŻKIMI

Abstrakt

W doświadczeniu wazonowym badano wpływ zanieczyszczenia piasku gliniastego pojedynczymi metalami ciężkimi ($Cd^{2+}$, $Cu^{2+}$, $Zn^{2+}$, $Pb^{2+}$) i ich mieszaninami na liczebność bakterii kopiotroficznych, amonifikacyjnych, immobilizujących azot, celulolitycznych oraz bak-

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terii z rodzaju *Arthrobacter* i *Pseudomonas*. Badania wykonano w dwóch seriach: z glebą obsianą owsem i nieobsianą.

Stwierdzono, że wrażliwość bakterii na $Cd^{2+}$, $Cu^{2+}$, $Zn^{2+}$ i $Pb^{2+}$ jest cechą specyficzną związaną z zawartością tych metali w glebie oraz sposobem jej użytkowania. Rozwój bakterii z rodzaju *Arthrobacter* oraz *Pseudomonas* był intensywniej hamowany w glebie obsianej owsem, natomiast bakterii amonifikacyjnych, immobilizujących azot oraz celulolitycznych – w glebie nieobsianej. Bardziej odporne na te zanieczyszczenia okazały się bakterie kopiotroficzne, celulolityczne, immobilizujace azot i amonifikacyjne niż bakterie z rodzaju *Arthrobacter* i *Pseudomonas*. Zwiększenie liczby metali ciężkich jednocześnie zanieczyszczających glebę do dwóch ($Cd^{2+}$ i $Cu^{2+}$; $Cd^{2+}$ i $Zn^{2+}$; $Cd^{2+}$ i $Pb^{2+}$) i trzech ($Cd^{2+}$, $Cu^{2+}$ i $Zn^{2+}$; $Cd^{2+}$, $Cu^{2+}$ i $Pb^{2+}$, $Cd^{2+}$, $Pb^{2+}$ i $Zn^{2+}$) z reguły nie zwiększało intensywności ich oddziaływania na badane bakterie. Zmiany wywołane przez te mieszaniny były zazwyczaj zbliżone do zmian powodowanych przez pojedyncze metale ciężkie.

Słowa kluczowe: metale ciężkie w glebie, liczebność bakterii, miedź, cynk, kadm, ołów.

# INTRODUCTION

Heavy metals present in soil are a serious threat to human and animal health. Neither are they neutral to plants (BELYAEVA et al. 2005) or microorganisms (WYSZKOWSKA et al. 2007). They can have an inhibitory effect on the development of bacteria, fungi and actinomycetes (BOROS et al. 2007, LUGAUSKAS 2005). Heavy metals reduce biomass of microorganisms and lower their soil activity (WYSZKOWSKA et al. 2008a, MIN et al. 2005), and even if they do not reduce their number, they depress their biodiversity (MOFFETT 2003).

The mechanism of heavy metals affecting the environment in the soil-plant relation has not been completely clarified. On the one hand, soil bacteria immobilize heavy metals. On the other hand, they contribute to higher mobility of heavy metals, which is mainly due to metabolites they produce (KUFFNER et al. 2008). It is particularly difficult to establish mutual relations when soil is simultaneously contaminated with a number of various heavy metals. Under such conditions, the task of establishing which heavy metal predominantly destroys soil microbiological properties becomes complicated, since the joint influence of several metals may not be the sum of individual effects.

The aim of the research was to establish the effect of soil contamination with single heavy metals: cadmium, zinc, copper and lead, and with their mixtures on the count of bacteria of *Arthrobacter* and *Pseudomonas* genera, as well as on copiotrophic, ammonifying, nitrogen immobilising and cellulolytic bacteria.

## MATERIALS AND METHODS

The research was carried out in a pot experiment, in four replications. The subject of the research was the soil of the granulometric composition of loamy sand ($pH_{KCl}$ – 5.60, $C_{org.}$ : N ratio – 11.8), uncontaminated and contaminated with individual heavy metals ($Cd^{2+}$, $Cu^{2+}$, $Zn^{2+}$, $Pb^{2+}$) and with their mixtures containing two metals ($Cd^{2+}$ and $Cu^{2+}$; $Cd^{2+}$and $Zn^{2+}$; $Cd^{2+}$and $Pb^{2+}$), three metals ($Cd^{2+}$, $Cu^{2+}$ and $Zn^{2+}$; $Cd^{2+}$, $Cu^{2+}$, and $Pb^{2+}$; $Cd^{2+}$, $Pb^{2+}$ and $Zn^{2+}$) and all the heavy metals ($Cd^{2+}$, $Cu^{2+}$, $Pb^{2+}$ and $Zn^{2+}$). The soil was contaminated with the following doses of heavy metals in mg $kg^{-1}$ of soil: $Cd^{2+}$ – 4 and 12; $Cu^{2+}$ – 150 and 450; $Pb^{2+}$ – 100 and 300; $Zn^{2+}$ – 300 and 900. These elements were introduced to the soil in the form of the following compounds: cadmium – $CdCl_2 \cdot 2''H_2O$, copper – $CuSO_4 \cdot 5H_2O$, lead – $PbCl_2$ and zinc – $ZnCl_2$. After mixing the soil with compounds containing heavy metals and macroelements satisfying alimentary requirements of the experimental plant (oat), soil portions of 3 kg each were placed in plastic pots. The same mineral fertilization as in the previous research (Wyszkowska et al. 2006) was applied. The soil in pots was brought to a moisture content corresponding to 60% capillary water capacity using distilled water and left for 14 days unsown. On day fourteen, soil samples were collected, microbiological analyses were performed, and cv. Borowik oat was sown in a series with four replications (after plant germination, 12 plants were left in each pot), while the other series, which was also carried out with four replications, the soil was left unsown. On the harvest day (in the inflorescence phase), soil samples were collected from both series of experiments and microbiological analysis were conducted. Their scope included determination of the number of the following bacteria: *Arthrobacter, Pseudomonas*, ammonifying, nitrogen immobilising, cellulolytic and copiotrophic, using the colony-count method. A detailed procedure of determining these microorganisms has been provided in Wyszkowska at al. (2008b).

The results of microbiological analysis were statistically analysed using Duncan's multiple range test, applying a three factor variance analysis. The statistical analysis was accomplished with Statistica software (StatSoft, Inc....2006).

## RESULTS AND DISCUSSION

The number of copiotrophic bacteria in soil sown with oat was 1.25-fold higher than in the unsown soil (Table 1). It was also influenced by the level and type of heavy metal contamination. In the soil contaminated with smaller doses of metals, no negative effects of cadmium, copper, lead or zinc on these bacteria were found. Neither were they affected by cadmium with an

Table 1

Number of copiotrophic bacteria (cfu $10^8$ $kg^{-1}$ of d.m. soil)

| Object | Contamination level | | | |
|---|---|---|---|---|
| | I | | II | |
| | soil use | | | |
| | unsown | sown | unsown | sown |
| 0 | 88 ± 3 | 110 ± 6 | 88 ± 3 | 110 ± 6 |
| Cd | 87 ± 7 | 129 ± 5 | 68 ± 3 | 97 ± 4 |
| Cu | 87 ± 5 | 129 ± 6 | 64 ± 4 | 90 ± 4 |
| Pb | 85 ± 5 | 126 ± 5 | 69 ± 6 | 93 ± 8 |
| Zn | 85 ± 6 | 126 ± 7 | 86 ± 4 | 111 ± 7 |
| CdCu | 88 ± 7 | 104 ± 4 | 85 ± 7 | 109 ± 8 |
| CdPb | 88 ± 5 | 104 ± 6 | 60 ± 6 | 92 ± 9 |
| CdZn | 85 ± 5 | 104 ± 4 | 82 ± 3 | 117 ± 5 |
| CdCuPb | 85 ± 6 | 104 ± 7 | 57 ± 3 | 78 ± 4 |
| CdCuZn | 98 ± 5 | 140 ± 8 | 72 ± 6 | 100 ± 8 |
| CdPbZn | 98 ± 5 | 140 ± 7 | 71 ± 3 | 99 ± 5 |
| CdCuPbZn | 97 ± 4 | 111 ± 5 | 33 ± 2 | 74 ± 5 |
| Mean | 89 ±1 | 119 ± 2 | 70 ± 1 | 97 ± 2 |
| LSD | a – 3.3, b – 1.4, c – 1.4, a · b – 4.7, a · c – 4.7, b · c – 1.9; a · b · c – 6.7 | | | |

LSD for: a – kind of contamination; b – contamination level; c – soil utilization, d – analysis term
I contamination level in mg per kg d.m. of soil: Cd – 4; Cu – 150; Pb – 100; Zn – 300
II contamination level in mg per kg d.m. of soil: Cd – 12; Cu – 450; Pb – 300; Zn – 900

addition of copper, lead or zinc, or by cadmium with an addition of copper and lead. Mixtures of cadmium and copper, zinc and lead as well as of copper, zinc and lead caused a significant increase in the number of copiotrophic bacteria. In the soil under oat, at this level of contamination, single metals stimulated bacterial growth. Cadmium with one additional metal or with the three other metals had no significant influence on microorganisms, but when applied with copper and zinc or with zinc and lead, it produced a stimulating effect,

In the soil contaminated by higher doses of heavy metals, regardless of the soil use, cadmium, copper and lead significantly reduced the count of copiotrophic bacteria. However, zinc had no such effect. Cadmium reduced total contamination with copper or zinc, but it increased the additional contamination with copper and lead. The bacterial count was most severely reduced as a result of the joint effect of all the four metals, in the unsown soil – by 63%, and in the sown soil – by 33%.

The number of ammonifying bacteria depended both on the method of soil use and on the type and extent of contamination with heavy metals (Table 2). In the soil sown with oat but uncontaminated with metals, it was 13% higher than in the unsown soil, and in the contaminated soil it was higher by up to 47% on average. A stronger, unfavourable effect on these bacteria was produced by metals in the unsown soil. Lead and zinc reduced their count by 22% - 23% on average, while cadmium and copper – by 13% and 16%, respectively. Cadmium applied with zinc lowered their number by as much as 43%, but applied with copper – by 18%, and with lead – by 22%, the same as lead alone. The joint effect of cadmium and zinc was mitigated by lead. The introduction of lead into the soil contaminated with cadmium and zinc reduced the results of the negative effect of those two elements from 43% to 29%, although it depended the negative effect of contaminating soil simultaneously with cadmium and copper. The highest inhibition of ammonifying bacteria in the unsown soil was found in the sample of soil con-

Table 2

Number of ammonifying bacteria (cfu $10^8$ $kg^{-1}$ of d.m. soil)

| Object | Contamination level* | | | |
|---|---|---|---|---|
| | I | | II | |
| | soil use | | | |
| | unsown | sown | unsown | sown |
| 0 | 137 ± 8 | 162 ± 9 | 137 ± 12 | 162 ± 12 |
| Cd | 123 ± 5 | 155 ± 10 | 115 ± 9 | 150 ± 10 |
| Cu | 123 ± 8 | 155 ± 7 | 106 ± 5 | 153 ± 9 |
| Pb | 106 ± 2 | 134 ± 5 | 109 ± 8 | 138 ± 9 |
| Zn | 106 ± 7 | 134 ± 10 | 105 ± 9 | 146 ±14 |
| CdCu | 111 ± 5 | 169 ± 10 | 114 ± 5 | 158 ± 7 |
| CdPb | 111 ± 7 | 169 ± 8 | 103 ± 5 | 159 ± 5 |
| CdZn | 90 ± 7 | 122 ± 7 | 65 ± 4 | 125 ± 5 |
| CdCuPb | 90 ± 6 | 122 ± 6 | 98 ± 4 | 158 ± 5 |
| CdCuZn | 114 ± 6 | 180 ± 7 | 88 ± 6 | 149 ±6 |
| CdPbZn | 114 ± 7 | 180 ±11 | 80 ± 4 | 143 ± 9 |
| CdCuPbZn | 102 ± 5 | 162 ±9 | 50 ± 2 | 115 ± 5 |
| Mean | 111 ± 1 | 153 ±2 | 97 ± 2 | 146 ± 2 |
| LSD | a – 4.5, b – 1.8, c – 1.8, a · b – 6.3, a · c – 6.3, b · c – 2.6, a · b · c · 8.9 | | | |

* designations under Table 1

taminated by the four heavy metals at the same time. The count was reduced under the influence of the metal mixture by 45%, on average. It was observed for the soil of the second degree of contamination to a higher extent (bz 64% than for the first degre (by 26%). In the soil sown with oat, the highest reduction of ammonifying bacteria was found as a result of lead contamination (by 16%) and zinc contamination (by 14%), as well as resulting from joint contamination with cadmium and zinc (by 24%). An increasing number of metals in the soil contaminating pool produced a more intense effect on the number of bacteria in unsown soil in comparison with soil under oat.

The count of nitrogen immobilising bacteria in the soil sown with oat and free from heavy metals was significantly higher than in the unsown soil, although the difference was much smaller than in the case of other groups of bacteria (Table 3). In contaminated soil, these differences grew in favour of sown soil, as in the case of ammonifying bacteria. It proves that heavy metals have a more negative effect on ammonifying bacteria deprived of plant cover. However, in the soil contaminated with the lowest rates of

Table 3

Number of nitrogen immobilizing bacteria (cfu $10^8$ $kg^{-1}$ of d.m. soil)

| Object | Contamination level* | | | |
|---|---|---|---|---|
| | I | | II | |
| | soil use | | | |
| | unsown | sown | unsown | sown |
| 0 | 58 ± 3 | 66 ± 4 | 58 ± 4 | 66 ± 5 |
| Cd | 56 ± 2 | 100 ± 2 | 57 ± 4 | 71 ± 4 |
| Cu | 56 ± 4 | 100 ± 7 | 51 ± 3 | 60 ± 3 |
| Pb | 58 ± 5 | 111 ± 5 | 41 ± 2 | 69 ± 6 |
| Zn | 58 ± 4 | 111 ± 6 | 33 ± 2 | 56 ± 3 |
| CdCu | 37 ± 1 | 45 ± 3 | 53 ± 2 | 43 ± 4 |
| CdPb | 37 ± 2 | 45 ± 4 | 63 ± 2 | 68 ± 3 |
| CdZn | 46 ± 3 | 63 ± 3 | 43 ± 1 | 47 ± 3 |
| CdCuPb | 46 ± 5 | 63 ± 3 | 53 ± 4 | 64 ± 5 |
| CdCuZn | 52 ± 4 | 72 ± 4 | 26 ± 3 | 40 ± 3 |
| CdPbZn | 52 ± 2 | 72 ± 2 | 34 ± 3 | 47 ± 3 |
| CdCuPbZn | 37 ± 3 | 57 ± 4 | 20 ± 2 | 47 ± 3 |
| Mean | 49 ± 1 | 76 ± 2 | 44 ± 1 | 57 ± 1 |
| LSD | a – 2.2, b – 0.9, c – 0.9, a · b – 3.1, a · c – 3.1, b · c – 1.3, a · b · c – 4.5 | | | |

* designations under Table 1

single heavy metals, there was no reduction in the count of ammonifying bacteria, while a higher concentration of lead and zinc significantly reduced their number, particularly as regards unsown soil. Zinc proved to be most toxic to this group of bacteria. It reduced the number of ammonia forming bacteria by as much as 43%, while cadmium had no toxic effect on these microbes. On the other hand, excessive quantities of heavy metals in soil, but only if present in twos, had an explicit, negative effect on ammonia forming bacteria. $Cd^{2+}$ with $Cu^{2+}$ inhibited their development by 28%, and $Cd^{2+}$ with $Zn^{2+}$ by 20% and $Cd^{2+}$ with $Pb^{2+}$ by 14%, on average. Having more metals in the contaminating pool did result in any further reduction in bacterial counts. It was only a joint contamination with $Cu^{2+}$, $Cd^{2+}$, $Pb^{2+}$ and $Zn^{2+}$ that caused an average reduction by 36% as regards the population of nitrogen immobilising bacteria, which in the unsown soil was reduced by up to 51%.

The count of cellulolytic bacteria, in contrast to the other groups and types, was significantly higher in the unsown soil than in the sown soil (Table 4). The effect of heavy metals on these bacteria was the weakest.

Table 4

Number of cellulolytic bacteria (cfu $10^6$ $kg^{-1}$ of d.m. soil)

| Object | Contamination level* | | | |
|---|---|---|---|---|
| | I | | II | |
| | soil use | | | |
| | unsown | sown | unsown | sown |
| 0 | 44 ± 2 | 24 ± 1 | 44 ± 3 | 24 ± 2 |
| Cd | 35 ± 2 | 22 ± 1 | 42 ± 3 | 24 ± 2 |
| Cu | 35 ± 4 | 22 ± 2 | 38 ± 2 | 22 ± 2 |
| Pb | 41 ± 3 | 18 ± 1 | 37 ± 3 | 24 ± 1 |
| Zn | 41 ± 3 | 18 ± 2 | 39 ± 2 | 22 ± 2 |
| CdCu | 44 ± 2 | 23 ± 2 | 41 ± 3 | 22 ± 2 |
| CdPb | 44 ± 2 | 23 ± 1 | 43 ± 1 | 20 ± 1 |
| CdZn | 32 ± 3 | 21 ± 3 | 36 ± 3 | 22 ± 2 |
| CdCuPb | 32 ± 3 | 21 ± 2 | 35 ± 1 | 24 ± 2 |
| CdCuZn | 37 ± 2 | 23 ± 2 | 36 ± 2 | 23 ± 2 |
| CdPbZn | 37 ± 3 | 23 ± 2 | 38 ± 3 | 26 ± 2 |
| CdCuPbZn | 38 ± 2 | 24 ± 1 | 33 ± 3 | 19 ± 2 |
| Mean | 39 ± 1 | 22 ± 1 | 38 ± 1 | 23 ± 1 |
| LSD | a – 1.4, b – n.s.; c – 0.6, a · b – 2.0, a · c – 2.0, b · c – n.s.; a · b · c – 2.8 | | | |

* designations under Table 1

The reduction in the number of these bacteria ranged on average from 5% in the sample jointly contaminated with $Cd^{2+}$ and $Cu^{2+}$ to 18% in the soil contaminated with four heavy metals. In addition, heavy metals in the soil deprived of plant cover revealed a stronger effect on these bacteria.

The number of bacteria of the genus *Arthrobacter* was over three-fold higher in the soil sown with oat than in the unsown soil (Table 5). All the heavy metals had a stronger inhibitory effect on the development of these bacteria in the unsown soil. An average reduction in their count in the sown soil resulting from individual metals ranged from 30% ($Pb^{2+}$) to 51% ($Zn^{2+}$), while in the unsown soil, cadmium and copper had no effect on these bacteria, and zinc and lead reduced their number by 5% – 18%.

While examining the effects of heavy metals, disregarding the method of soil usage, it is clear that their effect on the bacteria of *Arthrobacter* genus was not directly proportional to the number of metals used in the contamination pool. The highest changes were caused by cadmium, lead, copper and zinc when they individually contaminated the soil. As a result of their activity, the count was reduced by 21%-28%. In those samples, in cas-

Table 5

Number of *Arthrobacter* spp. (cfu $10^6$ $kg^{-1}$ of d.m. soil)

| Object | Contamination level* | | | |
|---|---|---|---|---|
| | I | | II | |
| | soil use | | | |
| | unsown | sown | unsown | sown |
| 0 | 39 ± 3 | 122 ± 8 | 39 ± 2 | 122 ± 7 |
| Cd | 34 ± 3 | 73 ± 6 | 45 ± 3 | 64 ± 3 |
| Cu | 39 ± 4 | 61 ± 4 | 43 ± 3 | 62 ± 3 |
| Pb | 29 ± 3 | 104 ± 8 | 35 ± 2 | 67 ± 4 |
| Zn | 37 ± 2 | 62 ± 4 | 37 ± 2 | 58 ± 3 |
| CdCu | 31 ± 3 | 75 ± 5 | 44 ± 3 | 53 ± 4 |
| CdPb | 35 ± 4 | 71 ± 6 | 43 ± 3 | 65 ± 4 |
| CdZn | 42 ± 4 | 79 ± 8 | 28 ± 2 | 58 ± 3 |
| CdCuPb | 34 ± 2 | 66 ± 3 | 36 ± 2 | 63 ± 5 |
| CdCuZn | 36 ± 4 | 56 ± 5 | 32 ± 2 | 42 ± 5 |
| CdPbZn | 44 ± 3 | 63 ± 5 | 42 ± 3 | 66 ± 4 |
| CdCuPbZn | 27 ± 3 | 59 ± 4 | 26 ± 3 | 44 ± 3 |
| Mean | 36 ± 1 | 74 ± 1 | 38 ± 1 | 64 ± 1 |
| LSD | a – 2.5, b – 1.0, c – 1.0, a · b – 3.6, a · c – 3.6, b · c – 1.45, a · b · c – 5.0 | | | |

* designations under Table 1

es when cadmium was applied together with copper, lead or zinc, the reduction of the count was similar and amounted to 22% - 26%. It was by about 10% higher when cadmium was applied together with copper and zinc, but it did not increase in the result of joint activity of cadmium with copper and lead or cadmium with lead and zinc. It was only under the influence of the contamination of soil with all four heavy metals ($Cd^{2+}$, $Cu^{2+}$, $Pb^{2+}$, $Zn^{2+}$) that the reduction by 45% was observed as regards the number of these bacteria.

The count of bacteria of the *Pseudomonas* genus was 1.5-fold higher in the soil sown with oat than in the unsown soil, while heavy metals in soil under oat reduced their number to a higher extent (Table 6). Therefore, they had a similar effect on these bacteria as on the bacteria of the *Arthrobacter* genus. Irrespective of the level of contamination, as regards the soil covered with oat, the highest negative effects on *Pseudomonas* was generated by cadmium and zinc. These metals reduced the number of *Pseudomonas* by 49% and 43%, respectively. A weaker effect was found for copper and lead, which reduced the number of the bacteria by 35% and 29%. In the

Table 6

Number of *Pseudomonas* spp. (cfu $10^6$ $kg^{-1}$ of d.m. soil)

| Object | Contamination level* | | | |
|---|---|---|---|---|
| | I | | II | |
| | soil use | | | |
| | unsown | sown | unsown | sown |
| 0 | 107 ± 5 | 162 ± 3 | 107 ± 4 | 162 ± 4 |
| Cd | 72 ± 3 | 85 ± 4 | 77 ± 5 | 79 ± 3 |
| Cu | 120 ± 8 | 93 ± 5 | 126 ± 6 | 117 ± 6 |
| Pb | 127 ± 10 | 101 ± 5 | 113 ± 9 | 129 ± 7 |
| Zn | 92 ± 6 | 91 ± 5 | 106 ± 8 | 95 ± 6 |
| CdCu | 82 ± 7 | 59 ± 4 | 85 ± 6 | 78 ± 6 |
| CdPb | 88 ± 8 | 98 ± 5 | 125 ± 6 | 120 ± 5 |
| CdZn | 104 ± 9 | 77 ± 6 | 63 ± 7 | 55 ± 4 |
| CdCuPb | 104 ± 8 | 82 ± 6 | 92 ± 8 | 73 ± 5 |
| CdCuZn | 91 ± 6 | 113 ± 7 | 85 ± 6 | 89 ± 4 |
| CdPbZn | 128 ± 6 | 129 ± 7 | 73 ± 3 | 84 ± 5 |
| CdCuPbZn | 89 ± 5 | 103 ± 3 | 48 ± 3 | 55 ± 4 |
| Mean | 100 ± 2 | 99 ± 2 | 92 ± 2 | 95 ± 1 |
| LSD | a – 3.6, b – 1.5, c – n.s., a · b – 5.2, a · c – 5.2, b · c – 2.1, a · b · c – 7.3 | | | |

* designations under Table 1

unsown soil, cadmium reduced the count of *Pseudomonas*, by 30% reduction and zinc only by 7%; in contrast, copper and lead increased the amount of these bacteria by 5% - 12%. In the soil sown with oat, a negative effect of cadmium was intensified by a simultaneous contamination with copper and zinc, while lead contamination lowered this effect. Copper resulted in a further 9% and zinc to a 10% reduction in the number of *Pseudomonas* as compared to cadmium alone, while lead mitigated the effect of cadmium by 16%. On the other hand, copper and zinc, and lead and zinc applied together with cadmium revealed a less toxic effect on bacteria of the genus *Pseudomonas*, as compared to cadmium alone, while joint contamination with the four heavy metals ($Cd^{2+}$, $Cu^{2+}$, $Pb^{2+}$, $Zn^{2+}$) had a similar effect as the contamination with cadmium alone.

The heavy metals demonstrated a clearly negative effect on soil bacteria, which agrees with other research (Boros et al. 2007, Lugauskas 2005, Oliveira, Pampulha 2006). They had a stronger effect on bacteria of the *Arthrobacter* and *Pseudomonas* genera than on individual physiological groups. This is primarily related to a higher tolerance to heavy metals possessed by qualitatively richer groups of microbes in comparison to individual genera and types (Loc, Janssen 2005, Mertens et al. 2007). An interesting and unexplained phenomenon is the reciprocal neutralization of the effects of metals on bacteria when they are applied in a mixture. Tolerance of bacteria to individual metals was often higher than in the case of their mixtures. One exception was the mixture of all the four metals, which resulted in the most unfavourable changes in the bacterial count, which was caused by the joint effect of the highest number of metals in the soil-contaminating pool as well as the higher contamination rate, being the sum of contamination with individual metals.

## CONCLUSIONS

1. The sensitivity of bacteria to $Cd^{2+}$, $Cu^{2+}$, $Zn^{2+}$ and $Pb^{2+}$ is a specific characteristic, related to the content of those metals in soil and the method of its use. The development of the bacteria of *Arthrobacter* and *Pseudomonas* spp. was most intensively inhibited in the soil sown with oat, while for ammonifying, nitrogen immobilising, cellulolytic bacteria it was in the unsown soil. Copiotrophic, cellulolytic, nitrogen immobilising and ammonifying bacteria proved to be more resistant to these contaminations than bacteria of the *Arthrobacter* and *Pseudomonas* genera.

2. Copper had the strongest effect on copiotrophic bacteria; lead and zinc – on ammonifying bacteria; zinc – on nitrogen immobilising bacteria; while *Arthrobacter* was most strongly affected by zinc and *Pseudomonas* – by cadmium and zinc.

3. Increasing the number of heavy metals simultaneously contaminating the soil to two ($Cd^{2+}$ and $Cu^{2+}$; $Cd^{2+}$ and $Zn^{2+}$; $Cd^{2+}$ and $Pb^{2+}$) and to three ($Cd^{2+}$, $Cu^{2+}$ and $Zn^{2+}$; $Cd^{2+}$, $Cu^{2+}$, and $Pb^{2+}$; $Cd^{2+}$, $Pb^{2+}$ and $Zn^{2+}$) generally did not increase the intensity of their effect on the examined bacteria. Changes caused by these mixtures were usually similar than changes caused by individual heavy metals.

4. The quality and quantity threshold of soil contamination in relation to bacteria was evidently exceeded by a joint application of the four heavy metals ($Cd^{2+}$, $Cu^{2+}$, $Zn^{2+}$ and $Pb^{2+}$).

5. Further studies are required in order to determine the mechanism of mutual neutralization of the effect of heavy metals on bacteria.

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