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RESPONSE OF MICROORGANISMS TO SOIL CONTAMINATION WITH CADMIUM, NICKEL AND LEAD

REAKCJA DROBNOUSTROJÓW NA ZANIECZYSZCZENIE GLEBY KADMEM, MIEDZIĄ, CYNKIEM I OŁOWIEM

Abstract: In pot experiment the effect of loamy sand contaminated with cadmium, copper, zinc and lead on number of oligotrophic bacteria, actinomycetes and fungi was studied. Heavy metals were applied to soil as a single pollution and in mix each other. Two level of heavy metals pollution were examined: 1st level (mg · kg^{-1} of soil): Cd – 4, Cu – 150, Pb – 100 and Zn – 300; 2nd level (mg · kg^{-1} of soil): Cd – 12, Cu – 450, Pb – 300 and Zn – 900. It was found that oligotrophic bacteria were the most sensitive on contamination of soil with cadmium, copper, zinc and lead, slightly less – actinomycetes and the least susceptible were fungi. The negative effect of heavy metals on microorganisms was not the sum of single heavy metals action.

Keywords: heavy metals, bacteria, fungi, actinomycetes

In most cases, heavy metals have negative influence on microorganisms. They reduce microbial biomass, counts, structure and activity [1–4]. They cause disorders in enzymatic activities by forming complexes with substrates or blocking reactive functional groups of enzymes [3]. They also impair the resistance of microorganisms to other stresses [1, 5]. Heavy metals disturb proper functioning of ecosystems by producing adverse effect on the course of many processes, such as those involved in transformations of carbon and nitrogen compounds [6, 7]. The actual effect of heavy metals on microbiological properties of soil depends on the degree of soil contamination, properties of chemical compounds which occur in the soil and on any other types of pollution present in the environment.

Unlike model studies, which often focus on the effect of individual heavy metals on the microbiological activity of soil, this experiment dealt with the influence of mixtures on microorganisms. Thus, our objective has been to analyze the effect of cadmium, copper, zinc and lead on oligotrophic bacteria, actinomycetes and fungi. The effect produced by individual heavy metals was compared with that exerted by their mixtures.

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Material and methods

The experiments (in four replications) were conducted in polyethylene pots placed in a greenhouse. The test soil was loamy sand soil with pH in 1 mol KCl · dm^{-3} – 5.60; hydrolytic acidity (Hh) – 1.31 cmol(H^+) · kg^{-1} soil; C_{org} – 5.00 g · kg^{-1}; total exchangeable bases (S) – 5.71 cmol(+) · kg^{-1}; exchangeable capacity of the sorptive complex (T) – 7.02 cmol(+) · kg^{-1}; base cation saturation ratio (V) – 81.34 %. Prior to the trials, soil samples (3 kg each) were mixed with mineral fertilizers and heavy metals. All the treatments received identical macro- and microelement fertilization consisting of N –100 [$CO(NH_2)_2$], P – 44 [KH_2PO_4]; K – 83 [KH_2PO_4 + KCl], Mg – 20 [$MgSO_4 \cdot 7H_2O$], Zn – 5 [$ZnCl_2$], Cu – 5 [$CuSO_4 \cdot 5H_2O$], Mn – 5 [$MnCl_2 \cdot 4H_2O$], Mo – 5 [$Na_2MoO_4 \cdot 2H_2O$], B – 0.33 [H_3BO_3] expressed as pure element in mg per kg soil. The soil samples prepared as described above were contaminated with cadmium ($CdCl_2 \cdot 2^1/_2H_2O$), copper ($CuSO_4 \cdot 5H_2O$), lead ($PbCl_2$) and zinc ($ZnCl_2$). Two levels of contamination were applied. The first one corresponded to the maximum permissible concentrations of heavy metals contained in the Decree of the Minister for Environment of 9th September 2002 [8] and comprised, [mg · kg^{-1}], 4 Cd, 150 Cu, 100 Pb and 300 Zn. The other level was 3-fold higher than that and equalled, [mg · kg^{-1} soil], 12 Cd, 450 Cu, 300 Pb and 900 Zn. In addition to this, cadmium polluted objects received other heavy metals (Cu, Pb and Zn). The following objects contaminated with heavy metals were compared: Cd, Cu, Pb, Zn, CdCu, CdPb, CdZn, CdCuPb, CdCuZn, CdPbZn and CdCuPbZn. The effect of the heavy metals was verified according to the control (non-polluted) objects. The trials were conducted in two series: soil cropped with oats cv. Borowik (12 plants per pot) and uncropped soil.

Having been mixed with the fertilizers and heavy metals, the soil was brought to a moisture content of 60 % water capillary capacity and maintained at this level for two weeks. On day 14 samples for microbiological analyses were collected and oats was sown in the cropped soil series. The pots in the other series were maintained unsown for 61 days. In this period constant moisture (60 % of capillary water capacity) of soil was maintained. After that time, oats plants (in the inflorescence stage) were harvested, the yields were measured and the soil samples obtained from both series were assayed for counts of microorganisms. Thus, counts of microorganisms were assayed twice, on day 14 and day 61 of the experiment. The microbiological assays consisted of determinations of counts of oligotrophic bacteria on Onta and Hattori medium [9], actinomycetes – on Kuster and William medium supplemented with nystatin and actidione [10] and fungi – on Martin medium [11].

The results underwent statistical elaboration using Duncan's multiple range test and two-factor analysis of variance. All statistical calculations were performed with an aid of Statistica software [12].

Results and discussion

Counts of oligotrophic bacteria in soil sown with oats were 1.34-fold higher than in uncropped soil (Table 1). Independent of the soil use, heavy metals depressed rather substantially the populations of oligotrophic bacteria. Higher rates of pollution produced particularly strong negative effects although these were not proportional to the increase in the pollution degree.

Oligotrophic bacteria proved to be very sensitive to copper. Their population decreased by 36 % in the soil polluted with the higher rate of copper and by 30 % when the lower dose of the contaminant was applied. A similar effect was produced by cadmium, which depressed populations of oligotrophic bacteria by 34 % and 20 %, respectively. Lead and zinc had a weaker influence on this group of microorganisms.

Soil contamination with cadmium in conjunction with copper, lead or zinc tended to be more toxic than the treatments involving single elements. However, less notable effects occurred after adding more pollutants, such as mixtures of three heavy metals. Larger modifications in the populations of oligotrophic bacteria were observed under the total contamination with all the four test elements, cadmium, copper, lead and zinc, especially in uncropped soil. The aggregated effect of these four heavy metals caused a 46 % decrease in the count of oligotrophic bacteria. This can be compared with a 36 % decline as a result of the contamination with three metals, 34 % – with two metals and 26 % – with a single metal.

Table 1

Number of oligotrophic bacteria [cfu $10^8 \cdot kg^{-1}$ d.m. of soil]

Object	Contamination level			
	1st		2nd	
	Manner of soil management			
	unsown	sown	unsown	sown
Control	137 ± 7	183 ± 5	137 ± 9	183 ± 6
Cd	115 ± 9	140 ± 6	90 ± 9	122 ± 7
Cu	106 ± 7	115 ± 5	90 ± 4	115 ± 5
Pb	112 ± 6	135 ± 5	115 ± 8	124 ± 7
Zn	104 ± 9	133 ± 7	119 ± 9	138 ± 6
CdCu	101 ± 6	121 ± 6	97 ± 6	104 ± 6
CdPb	88 ± 6	122 ± 6	96 ± 10	114 ± 9
CdZn	99 ± 9	111 ± 6	99 ± 10	113 ± 7
CdCuPb	88 ± 6	93 ± 6	104 ± 7	119 ± 7
CdCuZn	95 ± 9	117 ± 6	97 ± 6	106 ± 6
CdPbZn	104 ± 6	119 ± 6	85 ± 9	101 ± 6
CdCuPbZn	86 ± 6	104 ± 6	65 ± 5	94 ± 6
Average	103 ± 2	124 ± 2	99 ± 1	119 ± 2
LSD	a – 2.9; b – 1.2; c – 1.2; a · b – 4.0; a · c – 4.0; b · c – 1.6; a · b · c – 5.7			

LSD for: a – kind of contamination; b – contamination level; c – manner of soil management: 1st contamination level [mg · kg^{-1} d.m. of soil]: Cd – 4; Cu – 150; Pb – 100; Zn – 300; 2nd contamination level [mg · kg^{-1} d.m. of soil]: Cd – 12; Cu – 450; Pb – 300; Zn – 900.

Counts of actinomycetes (Table 2) was 2.15-fold higher in cropped than uncropped soil, which meant that the differences between the two types of soil use were much larger here than in the case of oligotrophic bacteria. On the other hand, toxic effect of particular heavy metals on actinomycetes was much weaker than that produced on oligotrophic bacteria. Actually, zinc was the only metal which depressed the count of actinomycetes (by 17–23 % in uncropped soil and 9 % in soil under oats), but this result was observed only when the lower rate of this metal had been introduced to soil. In the soil polluted with the triple doses of the metals relative their permissible levels, cadmium produced a negative effect on actinomycetes although its influence was much weaker than that of zinc. In the treatment consisting of unropped soil and the lower cadmium rate, the metal did not inhibit the growth of actinomycetes. Moreover, in the soil sown with oats, the same rate of cadmium stimulated the multiplication of these microorganisms. Cadmium added to soil together with copper had a significant negative effect on actinomycetes. The aggregated toxic effect produced by these two elements was much stronger when the lower doses of the metals were added to soil.

Table 2

Number of actinomycetes [cfu $10^8 \cdot kg^{-1}$ d.m. of soil]

Object	Contamination level*			
	1st		2nd	
	Manner of soil management			
	unsown	sown	unsown	sown
Control	48 ± 1	103 ± 7	48 ± 1	103 ± 7
Cd	48 ± 3	160 ± 14	46 ± 3	93 ± 8
Cu	63 ± 4	108 ± 8	44 ± 4	119 ± 11
Pb	47 ± 4	155 ± 12	59 ± 3	101 ± 5
Zn	40 ± 2	94 ± 7	37 ± 4	103 ± 5
CdCu	35 ± 2	89 ± 4	46 ± 4	101 ± 9
CdPb	36 ± 4	91 ± 6	58 ± 5	95 ± 9
CdZn	51 ± 4	88 ± 5	36 ± 3	62 ± 4
CdCuPb	49 ± 5	68 ± 6	55 ± 3	94 ± 7
CdCuZn	48 ± 3	105 ± 6	37 ± 2	80 ± 3
CdPbZn	49 ± 3	94 ± 6	40 ± 3	85 ± 8
CdCuPbZn	46 ± 3	126 ± 10	26 ± 2	76 ± 4
Average	47 ± 1	107 ± 2	44 ± 1	93 ± 1
LSD*	a – 3.8; b – 1.5; c – 1.5; a · b – 5.3; a · c – 5.3; b · c – 2.17; a · b · c – 7.5			

* Explantions are under Table 1.

Cadmium applied in conjunction with lead produced similar effects, except in uncropped soil with the triple doses of the contaminants. The strongest toxic effects on actinomycetes produced by a combination of these two elements occurred in uncropped soil treated with the stronger doses of cadmium and lead. Actinomycetes were the most sensitive to the joint effect produced by cadmium and zinc. When introduced at the triple doses, these two heavy metals depressed the population of actinomycetes by 40 % in soil under oats. When lead was added to an analogous object, it significantly reduced the negative effect of soil contamination on actinomycetes, even though the total amount of contaminates had increased. Lead nearly completely alleviated the negative impact of soil contamination in combination with cadmium and copper, but this effect was observed only in uncropped soil at either of the levels of pollution. In turn, zinc applied together with cadmium and copper – at the lower dose – completely neutralised the toxic effect of copper and cadmium. In contrast, when applied at the higher dose it significantly rose the toxicity of a cadmium and copper mixture. The total contamination with all the four heavy metals, although the highest in terms of the quantity and quality of the pollution, did not cause any larger modifications in the population of actinomycetes that those generated by a mixture of cadmium and zinc.

Table 3

Number of fungi [cfu $10^6 \cdot kg^{-1}$ d.m. of soil]

Object	Contamination level*			
	1st		2nd	
	Manner of soil management			
	unsown	sown	unsown	sown
Control	30 ± 2	31 ± 3	30 ± 2	31 ± 3
Cd	34 ± 2	39 ± 2	32 ± 2	37 ± 3
Cu	42 ± 3	35 ± 1	33 ± 2	44 ± 3
Pb	33 ± 2	31 ± 2	34 ± 3	39 ± 4
Zn	30 ± 2	49 ± 3	36 ± 2	41 ± 2
CdCu	25 ± 2	32 ± 1	20 ± 2	25 ± 2
CdPb	33 ± 2	28 ± 2	30 ± 2	22 ± 2
CdZn	31 ± 2	48 ± 3	31 ± 2	30 ± 3
CdCuPb	37 ± 3	35 ± 3	34 ± 3	35 ± 3
CdCuZn	25 ± 2	34 ± 4	28 ± 1	30 ± 2
CdPbZn	29 ± 2	37 ± 2	35 ± 3	37 ± 3
CdCuPbZn	34 ± 2	37 ± 3	28 ± 3	36 ± 3
Average	32 ± 1	36 ± 1	31 ± 1	34 ±1
LSD*	a – 1.9; b – 0.8; c – 0.8; a · b – 2.7; a · c – 2.7; b · c – ns; a · b · c – 3.8			

* Explantions are under Table 1.

In contrast to oligotrophic bacteria and actinomycetes, counts of fungi were independent from the type of soil use and proved to be comparable in uncropped soil and soil under oats (Table 3). Fungi clearly differed from actinomycetes and, even more so, from oligotrophic bacteria, in their response to soil contamination with heavy metals. Each of the tested heavy metals, when applied individually, made fungal populations to grow. The highest increase in fungi was caused by zinc (on average, a 28 % increase), followed by copper (26 %), cadmium (16 %) and lead (12 %). In the soil under oats treated with the higher doses of the metals, a significant drop in the count of fungi was noticed under the influence of a combination of cadmium and copper, cadmium and lead or cadmium and zinc. The number of fungi fell most substantially when cadmium was added together with lead; the smallest decline in fungal counts occurred under the joint effect of cadmium and zinc. The addition of lead to an object treated with cadmium and copper not only alleviated the negative influence of the two latter metals but contributed to a significant increment in the population of fungi. The count of fungi was also depressed when soil had received a mixture of cadmium, copper and zinc but an addition of a fourth element, lead, significantly reduced the adverse effect of the three former elements.

The present investigations have demonstrated that the mechanism of heavy metals affecting soil microorganisms is far from being completely recognised. Differences in the response to soil pollution observed between oligotrophic bacteria, actinomycetes and fungi could be attributed to some specific characteristics of these three groups of microogranisms, which in turn originate from the specific structure of their cells. The most sensitive cells die under the effect of heavy metals while other cells can adjust themselves to new conditions through certain physiological or genetic modifications. Some other cells, also tolerant to the presence of heavy metals, can be removed from microbial populations as a result of competition between microorganisms which can survive pollution with heavy metals [1]. Therefore, the growth of fungal populations observed under the effect of the four tested metals, when applied singly, cannot be considered as a positive outcome as long as we lack detailed knowledge of the structure of fungal cells. Some research [2] suggests that soil pollution with heavy metals can cause such large modifications in the species that the whole metabolism of soil can be disrupted [6].

The results on the response of microorganisms to heavy metals contained in this paper are further confirmed by several other reports [3, 4, 13, 14]. All these findings prove that the exact effect produced by heavy metal contamination of soil is conditioned not only by the amounts of heavy metals in soil environment but also by the presence of other metals, which can diminish toxicity of single metals [15–17].

Conclusions

1. Oligotrophic bacteria were most sensitive to soil pollution with heavy metals (cadmium, copper, lead and zinc). Acinomycetes were slightly less sensitive to such conditions and fungi proved to be the least sensitive microogranisms.

2. The adverse effect of heavy metals on microogrniams is not additive in character, ie it is not a total of the effects produced by single metals.

3. Lead, applied as $PbCl_2$, proved to mask most efficiently the effect produced by the other three heavy metals tested.

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REAKCJA DROBNOUSTROJÓW NA ZANIECZYSZCZENIE GLEBY KADMEM, MIEDZIĄ, CYNKIEM I OŁOWIEM

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Abstrakt: W doświadczeniu wazonowym badano wpływ zanieczyszczenia piasku gliniastego kadmem, miedzią, cynkiem i ołowiem oraz ich mieszaninami w różnych proporcjach na liczebność bakterii oligotroficznych, promieniowców i grzybów. Zastosowano 2 poziomy zanieczyszczenia, które wynosiły [mg · kg^{-1} gleby]: I poziom: Cd – 4; Cu – 150; Pb – 100; Zn – 300; II poziom: Cd – 12; Cu – 450; Pb – 300; Zn – 900. W wyniku badań stwierdzono, że bakterie oligotroficzne były najbardziej wrażliwe na zanieczyszczenie gleby metalami ciężkimi (kadmem, miedzią, ołowiem i cynkiem), nieco mniej – promieniowce, a najmniej – grzyby. Negatywne oddziaływanie metali ciężkich na drobnoustroje nie miało charakteru addytywnego, tzn. nie było sumą skutków działania pojedynczych metali.

Słowa kluczowe: metale ciężkie, bakterie, grzyby, promieniowce