# Effect of interactions between nickel and other heavy metals on the soil microbiological properties

**J. Wyszkowska, E. Boros, J. Kucharski**

*University of Warmia and Mazury in Olsztyn, Olsztyn, Poland*

**ABSTRACT**

A pot greenhouse experiment was performed to determine the effect of contamination with nickel interacting with other heavy metals on the microbiological properties of soil. The study was conducted on samples of soils classified under natural conditions as typical Eutric Cambisol developed from heavy loamy sand and typical Eutric Cambisol developed from light silty loam. Soil material was contaminated with nickel in the amount of 50 and 200 mg $Ni^{2+}$/kg. The treatments with 200 mg $Ni^{2+}$/kg were additionally contaminated with other heavy metals ($Zn^{2+}$, $Cu^{2+}$, $Pb^{2+}$, $Cd^{2+}$, $Cr^{6+}$), in the amount of 50 mg/kg soil. The following treatments, in which the soil was contaminated with heavy metals applied alone or in combinations, were compared in the study: Ni, Zn, Cu, Pb, Cd, Cr, NiZn, NiCu, NiPb, NiCd, NiCr, NiZnCu, NiZnPb, NiZnCd, NiZnCr, NiZnCuPb, NiZnCuCd, NiZnCuCr, NiZnCuPbCd, NiZnCuPbCr, NiZnCuPbCdCr. The experiment was carried out in four replications. A microbiological analysis was performed on days 28 and 56. The tested crop was oat. It was found that the impact of particular heavy metals on microbiological properties of soils depended on their type, interactions between nickel and zinc, copper, lead, cadmium and chromium (VI), date of analysis and soil species. Soil contamination with heavy metals reduced the population size of *Azotobacter* spp. The counts of other microbial groups, i.e. copiotrophic bacteria, spore-forming copiotrophic bacteria, oligotrophic bacteria, spore-forming oligotrophic bacteria, ammonifying bacteria, nitrogen immobilizing bacteria, cellulose-decomposing bacteria, *Arthrobacter* spp., *Pseudomonas* spp., actinomyces and fungi, showed varied susceptibility to heavy metals.

**Keywords:** soil microbes; heavy metals; soil

Heavy metals are permanently bound to the sorption complex, which may result in their accumulation in soil. Heavy metals exert a significant effect on soil microbes and soil processes, thus disturbing the biological equilibrium of soil, followed by soil degradation (Huang and Shindo 2000). Babich and Stotzky (1997) demonstrated that heavy metals are highly toxic to soil microbes. The impact of heavy metals on microorganisms and on enzymatic activity depends, among others, on soil pH, content of organic and mineral colloids, as well as on the type of heavy metals and their chemical properties (Kucharski and Wyszkowska 2004).

Nickel is one of the most toxic heavy metals. Its geochemical properties are similar to cobalt and iron. Geochemical properties of nickel result in similar distribution and influence of this element in environment. Nickel in soils appears most often on the second and the third degree of oxidation. Fertilization of sewage sludge as well as composts from different wastes, and also wide utilization of this metal in paper, food and chemical industry are the causes of local contamination of environment with this element. Nickel is used for production of laboratory apparatus, medical instruments, steel melting, artificial materials, electrodes, and batteries, and to cover metal objects (Kabata-Pendias and Pendias 2001). The wide use of nickel in different branches of industry exposes environment to its uncontrolled emission into atmosphere, water and soil. Its influence on microbiological properties of the soil is less recognized than that of other heavy metals.

Literature (Pandey and Sharma 2002) provides abundant information on the influence of single heavy metals on soil metabolism as well as on the growth and development of different plant species. However, data on combined effects of several heavy metals on the microbiological and biochemical properties of soil as well as on plants are scarce (Giridhara and Siddaramappa 2002); still in a natural environment heavy metal pollution is in most cases caused by some heavy metals.

Hence, the aim of the present study was to determine the impact of nickel interacting with other heavy metals (zinc, copper, lead, cadmium, chromium) on the microbiological activity of soil. In this investigation all heavy metals were applied alone in the dose of 50 mg/kg, whereas nickel as a less recognized element was used in two doses: 50 and 200 mg/kg. 200 mg $Ni^{2+}$ was a background of soil contamination for application of other heavy metals.

## MATERIAL AND METHODS

The experiment was performed in a greenhouse. Plastic pots were filled with 3 kg of typical Eutric Cambisol developed from heavy loamy sand and 3 kg of typical Eutric Cambisol developed from light silty loam. The detailed characteristics of soils are given in Table 1. The experiment was carried out in four replications. All treatments were regularly fertilized with macro- and micronutrients, as follows (pure component in mg/kg soil): N – 100 [$CO(NH_2)_2$], P – 44 [$KH_2PO_4$], K – 83 [$KH_2PO_4$ + KCl], Mg – 20 [$MgSO_4.7\ H_2O$], Cu – 5 [$CuSO_4.5\ H_2O$], Zn – 5 [$ZnCl_2$], Mn – 5 [$MnCl_2.4\ H_2O$], Mo – 5 [$Na_2MoO_4.2\ H_2O$], B – 0.33 [$H_3BO_4$].

Different heavy metals contamination was the main variable experimental factor: $Ni^{2+}$ ($NiCl_2.6\ H_2O$) – 50 or 200 mg/kg soil and 50 mg/kg soil of $Zn^{2+}$ ($ZnCl_2$), $Cu^{2+}$ ($CuCl_2$), $Pb^{2+}$ [$Pb(CH_3COO).3\ H_2O$], $Cd^{2+}$ ($CdCl_2.2.5\ H_2O$), $Cr^{6+}$($K_2Cr_2O_7$). The treatments contaminated with 200 mg $Ni^{2+}$/kg were additionally contaminated with the following heavy metals: Zn, Cu, Pb, Cd, Cr, ZnCu, ZnPb, ZnCd, ZnCr, ZnCuPb, ZnCuCd, ZnCuCr, ZnCuPbCd, ZnCuPbCr, ZnCuPbCdCr. In this investigation the soil uncontaminated with heavy metals was studied as well.

Prior to the establishment of the experiment, soil samples were weighed on an individual basis, fertilizer components were added and the soil was contaminated with heavy metals. The samples were mixed thoroughly and put into pots. Oat (cv. Bajka) was sown when a moisture content level of 60% capillary water capacity of soil was reached. After emergence oat plants were thinned to 12 per pot. The plants were collected at the panicle differentiation stage (day 56 of the experiment) and their mass was determined. Simultaneously, soil samples were taken for a microbiological analysis. A microbiological analysis of soil was also performed on day 28, and the obtained results are presented as means of two measurements. For microbiological analyses 10 g of soil samples was taken. All analyses were done in 6 replications for each sample.

The analysis included the determination of the counts of oligotrophic bacteria, copiotrophic bacteria, spore-forming oligotrophic bacteria and spore-forming copiotrophic bacteria – on the Hattori's medium (Hattori and Hattori 1980); ammonifying bacteria, nitrogen immobilizing bacteria and cellulose-decomposing bacteria – on the Zaborowska's medium (Zaborowska et al. 2006); *Azotobacter* spp. – by the method of Fenglerowa (Fenglerowa 1965); *Arthrobacter* spp. – on the Mulder and Antheumisse's medium (Mulder and Antheumisse 1963); *Pseudomonas* spp. – on the Mulder and Antheumisse's medium containing nystatin (Mulder and Antheumisse 1963); actinomyces – on the Kuster and William's medium containing nystatin and actidion (Parkinson et al. 1971); and fungi – on the Martin's medium (Martin 1950).

The results were verified statistically by the Duncan's multiple range test and a two-factorial analysis of variance, using the Statistica software (StatSoft, Inc. 2003).

Table 1. Some physicochemical properties of soils used in the experiment

| Soil species | Granulometric composition (mm) | | | $pH_{KCl}$ | Hh | S | $C_{org}$ (g/kg) |
|---|---|---|---|---|---|---|---|
| | 1–0.1 | 0.1–0.02 | < 0.02 | | mmol(+)/kg soil | | |
| | % | | | | | | |
| hls | 66 | 17 | 17 | 6.90 | 11.25 | 89.30 | 7.50 |
| lsl | 42 | 32 | 26 | 7.00 | 8.77 | 159.00 | 11.15 |

hls – heavy loamy sand; lsl – light silty loam; Hh – hydrolytic acidity; S – sum of exchangeable basic cations; $C_{org}$ – organic carbon content

## RESULTS AND DISCUSSION

The elements used in the experiments and the interactions between nickel and these elements changed the population size of all tested microbial groups (Tables 2–4). This impact was different and depended on the type of heavy metal, soil species and date of analysis, as confirmed by the coefficients of correlation between the doses of nickel and other heavy metals ($Zn^{2+}$, $Cu^{2+}$, $Pb^{2+}$, $Cd^{2+}$, $Cr^{6+}$), mean oat yield and the counts of soil microbes (Table 5).

Table 2. Effect of soil contamination with nickel and other heavy metals on the counts of oligotrophic and copiotrophic bacteria (cfu/kg dm)

| Metals* | Olig × $10^8$ | | $Olig_p$ × $10^7$ | | Cop × $10^8$ | | $Cop_p$ × $10^8$ | |
|---|---|---|---|---|---|---|---|---|
| | soil species | | | | | | | |
| | hls | lsl | hls | lsl | hls | lsl | hls | lsl |
| 0 | 83 ± 3 | 79 ± 1 | 32 ± 1 | 42 ± 1 | 49 ± 3 | 68 ± 10 | 49 ± 3 | 53 ± 4 |
| $Ni_{50}$ | 99 ± 10 | 74 ± 6 | 24 ± 3 | 36 ± 3 | 55 ± 5 | 68 ± 12 | 24 ± 4 | 75 ± 7 |
| $Ni_{200}$ | 91 ± 7 | 100 ± 4 | 29 ± 3 | 136 ± 18 | 195 ± 11 | 81 ± 8 | 64 ± 3 | 56 ± 2 |
| Zn | 49 ± 7 | 61 ± 2 | 19 ± 1 | 44 ± 2 | 188 ± 14 | 79 ± 10 | 72 ± 12 | 80 ± 5 |
| Cu | 66 ± 8 | 131 ± 2 | 12 ± 2 | 132 ± 8 | 138 ± 20 | 91 ± 4 | 68 ± 7 | 90 ± 6 |
| Pb | 65 ± 11 | 105 ± 5 | 16 ± 3 | 120 ± 14 | 206 ± 11 | 92 ± 2 | 50 ± 5 | 50 ± 7 |
| Cd | 35 ± 4 | 57 ± 3 | 16 ± 2 | 112 ± 9 | 204 ± 21 | 65 ± 6 | 47 ± 4 | 76 ± 12 |
| Cr | 90 ± 6 | 90 ± 5 | 40 ± 2 | 86 ± 9 | 303 ± 37 | 70 ± 1 | 42 ± 5 | 51 ± 3 |
| NiZn | 64 ± 13 | 59 ± 1 | 29 ± 4 | 93 ± 5 | 121 ± 4 | 71 ± 5 | 36 ± 4 | 59 ± 2 |
| NiCu | 89 ± 5 | 154 ± 4 | 26 ± 4 | 122 ± 9 | 130 ± 12 | 57 ± 5 | 51 ± 5 | 87 ± 13 |
| NiPb | 70 ± 8 | 114 ± 7 | 13 ± 2 | 118 ± 11 | 171 ± 11 | 89 ± 25 | 45 ± 1 | 91 ± 10 |
| NiCd | 123 ± 11 | 136 ± 1 | 18 ± 4 | 99 ± 5 | 166 ± 25 | 81 ± 2 | 59 ± 6 | 81 ± 9 |
| NiCr | 199 ± 14 | 189 ± 6 | 33 ± 3 | 61 ± 6 | 175 ± 13 | 108 ± 8 | 46 ± 4 | 76 ± 10 |
| NiZnCu | 79 ± 12 | 142 ± 8 | 31 ± 4 | 57 ± 11 | 102 ± 18 | 47 ± 8 | 52 ± 1 | 54 ± 4 |
| NiZnPb | 81 ± 4 | 81 ± 10 | 25 ± 6 | 138 ± 16 | 103 ± 22 | 93 ± 3 | 160 ± 11 | 109 ± 6 |
| NiZnCd | 101 ± 2 | 148 ± 10 | 25 ± 7 | 99 ± 4 | 208 ± 5 | 150 ± 16 | 63 ± 4 | 77 ± 2 |
| NiZnCr | 197 ± 19 | 264 ± 19 | 27 ± 3 | 104 ± 8 | 152 ± 7 | 71 ± 9 | 31 ± 8 | 105 ± 8 |
| NiZnCuPb | 143 ± 21 | 107 ± 3 | 32 ± 4 | 59 ± 6 | 162 ± 9 | 104 ± 3 | 49 ± 8 | 84 ± 7 |
| NiZnCuCd | 96 ± 13 | 118 ± 7 | 30 ± 2 | 60 ± 6 | 78 ± 18 | 64 ± 7 | 50 ± 5 | 92 ± 6 |
| NiZnCuCr | 119 ± 12 | 195 ± 4 | 25 ± 5 | 36 ± 3 | 124 ± 16 | 105 ± 8 | 51 ± 8 | 83 ± 1 |
| NiZnCuPbCd | 55 ± 5 | 81 ± 3 | 26 ± 3 | 114 ± 3 | 119 ± 31 | 106 ± 9 | 31 ± 1 | 88 ± 5 |
| NiZnCuPbCr | 166 ± 20 | 312 ± 2 | 19 ± 0 | 97 ± 9 | 373 ± 68 | 67 ± 4 | 35 ± 5 | 55 ± 1 |
| NiZnCuPbCdCr | 219 ± 20 | 203 ± 6 | 77 ± 2 | 61 ± 4 | 222 ± 48 | 170 ± 6 | 78 ± 2 | 44 ± 3 |
| $LSD_{0.05}$ | | | | | | | | |
| a | 11 | | 7 | | 21 | | 71 | |
| b | 3 | | 2 | | 61 | | 21 | |
| a, b | 15 | | 10 | | 291 | | 101 | |

*all heavy metals except for nickel were applied in the amount of 50 mg/kg dm soil; nickel was applied with other heavy metals in the amount of 200 mg/kg dm soil

$Ni_{50}$ – 50 mg/kg dm soil; $Ni_{200}$ – 200 mg/kg dm soil

hls – heavy loamy sand; lsl – light silty loam; Olig – oligotrophic bacteria; $Olig_p$ – spore-forming oligotrophic bacteria; Cop – copiotrophic bacteria, $Cop_p$ – spore-forming copiotrophic bacteria

LSD for: a – types of soil contamination, b – soil species

Table 3. Effect of soil contamination with nickel and other heavy metals on the counts of ammonifying bacteria, nitrogen immobilizing bacteria, *Azotobacter* spp. and *Arthrobacter* spp. (cfu/kg dm)

| Metals* | Am × $10^8$ | | Im × $10^8$ | | Az × $10^3$ | | Art × $10^9$ | |
|---|---|---|---|---|---|---|---|---|
| | soil species | | | | | | | |
| | hls | lsl | hls | lsl | hls | lsl | hls | lsl |
| 0 | 110 ± 15 | 112 ± 15 | 165 ± 10 | 123 ± 15 | 28 ± 4 | 51 ± 4 | 9 ± 1 | 14 ± 2 |
| $Ni_{50}$ | 158 ± 20 | 167 ± 20 | 277 ± 38 | 154 ± 7 | 27 ± 4 | 39 ± 3 | 7 ± 1 | 16 ± 4 |
| $Ni_{200}$ | 167 ± 13 | 111 ± 13 | 179 ± 2 | 105 ± 14 | 0 | 36 ± 6 | 8 ± 1 | 17 ± 3 |
| Zn | 180 ± 18 | 152 ± 18 | 175 ± 19 | 176 ± 13 | 27 ± 2 | 47 ± 7 | 8 ± 1 | 11 ± 3 |
| Cu | 180 ± 18 | 177 ± 18 | 146 ± 12 | 142 ± 5 | 17 ± 1 | 35 ± 2 | 8 ± 1 | 11 ± 4 |
| Pb | 98 ± 2 | 101 ± 2 | 170 ± 27 | 195 ± 18 | 14 ± 2 | 41 ± 2 | 15 ± 4 | 19 ± 4 |
| Cd | 67 ± 10 | 101 ± 10 | 124 ± 13 | 98 ± 18 | 15 ± 2 | 37 ± 6 | 4 ± 1 | 12 ± 3 |
| Cr | 189 ± 27 | 109 ± 27 | 104 ± 8 | 66 ± 4 | 0 | 2 ± 1 | 8 ± 1 | 11 ± 2 |
| NiZn | 123 ± 3 | 96 ± 3 | 132 ± 1 | 77 ± 8 | 0 | 19 ± 2 | 4 ± 1 | 15 ± 3 |
| NiCu | 63 ± 9 | 119 ± 9 | 140 ± 15 | 140 ± 22 | 0 | 17 ± 3 | 8 ± 1 | 12 ± 2 |
| NiPb | 88 ± 15 | 151 ± 15 | 90 ± 3 | 112 ± 13 | 0 | 25 ± 2 | 4 ± 1 | 8 ± 1 |
| NiCd | 139 ± 13 | 100 ± 13 | 120 ± 4 | 79 ± 7 | 0 | 17 ± 1 | 8 ± 1 | 15 ± 4 |
| NiCr | 357 ± 33 | 199 ± 33 | 167 ± 15 | 121 ± 7 | 0 | 0 | 12 ± 3 | 15 ± 4 |
| NiZnCu | 81 ± 14 | 220 ± 14 | 103 ± 3 | 151 ± 16 | 0 | 17 ± 1 | 15 ± 5 | 10 ± 1 |
| NiZnPb | 225 ± 27 | 115 ± 27 | 63 ± 6 | 89 ± 8 | 0 | 15 ± 4 | 14 ± 3 | 7 ± 1 |
| NiZnCd | 197 ± 8 | 69 ± 8 | 143 ± 11 | 124 ± 15 | 0 | 21 ± 4 | 14 ± 1 | 20 ± 3 |
| NiZnCr | 382 ± 12 | 134 ± 12 | 84 ± 11 | 103 ± 5 | 0 | 2 ± 2 | 10 ± 2 | 3 ± 1 |
| NiZnCuPb | 243 ± 24 | 30 ± 24 | 125 ± 13 | 76 ± 15 | 0 | 14 ± 3 | 20 ± 3 | 6 ± 1 |
| NiZnCuCd | 233 ± 19 | 85 ± 19 | 117 ± 9 | 110 ± 9 | 0 | 8 ± 2 | 9 ± 1 | 9 ± 1 |
| NiZnCuCr | 121 ± 10 | 112 ± 10 | 107 ± 12 | 116 ± 14 | 0 | 1 ± 1 | 22 ± 2 | 17 ± 4 |
| NiZnCuPbCd | 115 ± 12 | 90 ± 12 | 125 ± 6 | 155 ± 10 | 0 | 4 ± 1 | 13 ± 5 | 8 ± 1 |
| NiZnCuPbCr | 144 ± 21 | 130 ± 21 | 113 ± 13 | 166 ± 14 | 0 | 1 ± 1 | 9 ± 1 | 16 ± 3 |
| NiZnCuPbCdCr | 215 ± 9 | 155 ± 9 | 185 ± 10 | 131 ± 24 | 0 | 0 | 19 ± 3 | 14 ± 4 |
| $LSD_{0.05}$ | | | | | | | | |
| a | 15 | | 14 | | 2 | | 1 | |
| b | 5 | | 4 | | n.s. | | <1 | |
| a, b | 22 | | 19 | | 3 | | 2 | |

Am – ammonifying bacteria; Im – nitrogen immobilizing bacteria; Az – *Azotobacter* spp.; Art – *Arthrobacter* spp.
*explanations as in Table 2

The population size of microorganisms in light silty loam and heavy loamy sand was considerably affected by nickel and other heavy metals examined in the experiment (Tables 2–4). More significant changes were usually caused by nickel applied at a dose of 200 mg/kg, compared to 50 mg/kg. The higher dose of nickel contributed to the proliferation of total oligotrophic bacteria, spore-forming oligotrophic bacteria, total copiotrophic bacteria, spore-forming copiotrophic bacteria (Table 2), ammonifying bacteria, *Arthrobacter* spp. (only in more compact soil) (Table 3) and cellulose-decomposing bacteria, as well as fungi and actinomyces in more compact soil (Table 4). Nickel applied at the higher dose inhibited the growth of bacteria of the genus *Azotobacter* in both types of soil, and the growth of actinomyces in heavy loamy sand. A comparison of the impact of nickel and other heavy metals, applied at an identical dose (50 mg/kg), shows that the most distinct changes

Table 4. Effect of soil contamination with nickel and other heavy metals on the counts of cellulose-decomposing bacteria, *Pseudomonas* spp., actinomyces and fungi (cfu/kg dm)

| Metals* | Cel × $10^6$ | | Ps × $10^9$ | | Act × $10^8$ | | Fun × $10^6$ | |
|---|---|---|---|---|---|---|---|---|
| | soil species | | | | | | | |
| | hls | lsl | hls | lsl | hls | lsl | hls | lsl |
| 0 | 46 ± 6 | 45 ± 2 | 14 ± 3 | 17 ± 4 | 172 ± 17 | 107 ± 3 | 37 ± 2 | 34 ± 2 |
| $Ni_{50}$ | 54 ± 1 | 69 ± 9 | 22 ± 4 | 20 ± 4 | 151 ± 17 | 84 ± 2 | 32 ± 1 | 35 ± 4 |
| $Ni_{200}$ | 81 ± 5 | 66 ± 4 | 13 ± 1 | 19 ± 3 | 109 ± 10 | 186 ± 2 | 35 ± 5 | 76 ± 8 |
| Zn | 55 ± 2 | 54 ± 1 | 16 ± 3 | 19 ± 2 | 148 ± 7 | 108 ± 6 | 30 ± 3 | 29 ± 4 |
| Cu | 53 ± 3 | 71 ± 2 | 13 ± 2 | 18 ± 2 | 208 ± 5 | 130 ± 12 | 35 ± 6 | 23 ± 3 |
| Pb | 55 ± 5 | 48 ± 1 | 18 ± 5 | 23 ± 1 | 172 ± 7 | 138 ± 12 | 57 ± 7 | 50 ± 5 |
| Cd | 26 ± 2 | 39 ± 2 | 13 ± 5 | 13 ± 3 | 76 ± 5 | 74 ± 4 | 17 ± 2 | 33 ± 1 |
| Cr | 71 ± 5 | 49 ± 5 | 23 ± 3 | 11 ± 3 | 167 ± 19 | 87 ± 4 | 27 ± 2 | 26 ± 3 |
| NiZn | 49 ± 4 | 74 ± 3 | 13 ± 3 | 17 ± 3 | 193 ± 17 | 88 ± 6 | 60 ± 7 | 69 ± 1 |
| NiCu | 72 ± 5 | 44 ± 3 | 20 ± 5 | 18 ± 2 | 165 ± 13 | 105 ± 3 | 88 ± 3 | 103 ± 3 |
| NiPb | 60 ± 4 | 75 ± 5 | 15 ± 5 | 27 ± 2 | 90 ± 11 | 217 ± 25 | 68 ± 16 | 33 ± 2 |
| NiCd | 55 ± 5 | 51 ± 3 | 9 ± 2 | 10 ± 2 | 181 ± 21 | 87 ± 12 | 38 ± 5 | 64 ± 6 |
| NiCr | 77 ± 3 | 37 ± 5 | 16 ± 4 | 22 ± 3 | 186 ± 20 | 100 ± 12 | 76 ± 10 | 46 ± 3 |
| NiZnCu | 86 ± 7 | 35 ± 4 | 22 ± 4 | 14 ± 3 | 128 ± 12 | 98 ± 3 | 105 ± 22 | 47 ± 2 |
| NiZnPb | 61 ± 1 | 73 ± 1 | 13 ± 2 | 12 ± 4 | 92 ± 6 | 109 ± 3 | 98 ± 7 | 133 ± 1 |
| NiZnCd | 64 ± 3 | 39 ± 1 | 18 ± 3 | 30 ± 2 | 121 ± 21 | 117 ± 20 | 62 ± 7 | 85 ± 3 |
| NiZnCr | 39 ± 3 | 29 ± 4 | 26 ± 3 | 15 ± 3 | 172 ± 3 | 91 ± 10 | 100 ± 6 | 43 ± 1 |
| NiZnCuPb | 68 ± 3 | 62 ± 8 | 16 ± 1 | 13 ± 3 | 215 ± 23 | 81 ± 11 | 97 ± 7 | 55 ± 3 |
| NiZnCuCd | 84 ± 5 | 73 ± 7 | 14 ± 3 | 17 ± 3 | 144 ± 4 | 108 ± 11 | 76 ± 8 | 76 ± 3 |
| NiZnCuCr | 88 ± 7 | 31 ± 3 | 7 ± 1 | 21 ± 1 | 247 ± 13 | 127 ± 1 | 99 ± 4 | 71 ± 3 |
| NiZnCuPbCd | 68 ± 4 | 93 ± 3 | 12 ± 2 | 21 ± 4 | 93 ± 5 | 99 ± 9 | 88 ± 11 | 73 ± 4 |
| NiZnCuPbCr | 53 ± 5 | 68 ± 3 | 33 ± 4 | 17 ± 3 | 280 ± 31 | 185 ± 6 | 117 ± 1 | 70 ± 3 |
| NiZnCuPbCdCr | 46 ± 4 | 49 ± 6 | 43 ± 2 | 25 ± 1 | 260 ± 7 | 108 ± 2 | 122 ± 21 | 60 ± 4 |
| $LSD_{0.05}$ | | | | | | | | |
| a | 5 | | 3 | | 14 | | 8 | |
| b | 2 | | 1 | | 4 | | 2 | |
| a, b | 7 | | 4 | | 20 | | 11 | |

Cel – cellulose-decomposing bacteria; Ps – *Pseudomonas* spp.; Act – actinomyces; Fun – fungi
*explanations as in Table 2

in microbial counts (including an increase and a decrease) were caused by cadmium. Cadmium exerted a significant influence on the population size of 10 microbial groups, copper on 9, lead on 8, zinc and chromium on 7, and nickel only on 6.

Among all heavy metals tested in the study chromium was found to be the most toxic to bacteria of the genus *Azotobacter*. These bacteria completely disappeared in chromium-contaminated heavy loamy sand, whereas in light silty loam their count decreased 27-fold. Cadmium was the second most toxic heavy metal to *Azotobacter* spp. (Table 3), followed by copper, lead, nickel and zinc.

In the treatments in which nickel was applied together with other heavy metals *Azotobacter* spp. disappeared completely in heavy loamy sand, irrespective of the number of soil-contaminating heavy metals, while in light silty loam the count of these bacteria decreased significantly, but they were not totally destroyed even in soil samples

Table 5. Coefficients of correlation among the doses of nickel and other heavy metals, mean oat yield and the counts of soil microbes

| Variable | Dose | Yield | Olig | $Olig_p$ | Cop | $Cop_p$ | Am | Im | Cel | Ps | Art | Az | Act | Fun |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Heavy loamy sand** | | | | | | | | | | | | | | |
| Dose | 1.00 | | | | | | | | | | | | | |
| Yield | −0.59** | 1.00 | | | | | | | | | | | | |
| Olig | 0.54** | −0.33** | 1.00 | | | | | | | | | | | |
| $Olig_p$ | 0.25* | −0.32** | 0.45** | 1.00 | | | | | | | | | | |
| Cop | 0.21 | −0.14 | 0.26* | −0.02 | 1.00 | | | | | | | | | |
| $Cop_p$ | −0.04 | 0.01 | −0.01 | −0.03 | 0.08 | 1.00 | | | | | | | | |
| Am | 0.20 | −0.11 | 0.62** | 0.28* | 0.07 | −0.05 | 1.00 | | | | | | | |
| Im | −0.46** | 0.39** | 0.04 | 0.10 | −0.14 | 0.05 | −0.05 | 1.00 | | | | | | |
| Cel | 0.27* | −0.40** | 0.10 | 0.31* | −0.15 | 0.03 | 0.11 | −0.16 | 1.00 | | | | | |
| Ps | 0.33** | −0.08 | 0.58** | 0.37** | 0.43** | 0.03 | 0.15 | 0.17 | −0.21 | 1.00 | | | | |
| Art | 0.59** | −0.17 | 0.43** | 0.32** | 0.03 | 0.16 | 0.14 | −0.02 | 0.38** | 0.24* | 1.00 | | | |
| Az | −0.71** | 0.81** | −0.38** | −0.28 | −0.27 | 0.03 | −0.18 | 0.58** | −0.47** | −0.10 | −0.26 | 1.00 | | |
| Act | 0.32** | −0.06 | 0.59** | 0.20 | 0.29* | 0.05 | 0.16 | 0.14 | −0.01 | 0.45** | 0.42** | −0.07 | 1.00 | |
| Fun | 0.82** | −0.45** | 0.57** | 0.30* | 0.05 | −0.05 | 0.17 | −0.37** | 0.31** | 0.44** | 0.61** | −0.59** | 0.37** | 1.00 |
| **Light silty loam** | | | | | | | | | | | | | | |
| Dose | 1.00 | | | | | | | | | | | | | |
| Yield | −0.71** | 1.00 | | | | | | | | | | | | |
| Olig | 0.65** | −0.53** | 1.00 | | | | | | | | | | | |
| $Olig_p$ | −0.07 | −0.02 | 0.01 | 1.00 | | | | | | | | | | |
| Cop | 0.42** | −0.21 | 0.30* | −0.03 | 1.00 | | | | | | | | | |
| $Cop_p$ | 0.19 | −0.14 | 0.06 | 0.24* | −0.03 | 1.00 | | | | | | | | |
| Am | −0.17 | 0.13 | 0.14 | −0.19 | −0.13 | −0.11 | 1.00 | | | | | | | |
| Im | −0.05 | 0.40** | 0.08 | −0.18 | 0.01 | −0.19 | 0.37** | 1.00 | | | | | | |
| Cel | 0.00 | 0.14 | −0.36** | 0.29* | −0.03 | 0.13 | −0.19 | −0.10 | 1.00 | | | | | |
| Ps | 0.10 | 0.09 | 0.23 | −0.04 | 0.59** | −0.10 | 0.09 | 0.43** | 0.01 | 1.00 | | | | |
| Art | −0.19 | 0.23 | 0.01 | −0.11 | 0.20 | −0.55** | −0.06 | 0.35** | −0.18 | 0.40** | 1.00 | | | |
| Az | −0.84** | 0.84** | −0.62** | 0.04 | −0.26* | −0.12 | 0.02 | 0.25* | 0.03 | 0.05 | 0.21 | 1.00 | | |
| Act | −0.08 | 0.12 | 0.19 | 0.44** | 0.07 | −0.10 | 0.10 | 0.22 | 0.34** | 0.38** | 0.12 | 0.16 | 1.00 | |
| Fun | 0.43** | −0.39** | 0.13 | 0.34** | 0.14 | 0.27* | −0.36** | −0.12 | 0.14 | −0.01 | 0.07 | −0.33** | 0.03 | 1.00 |

Olig – oligotrophic bacteria; $Olig_p$ – spore-forming oligotrophic bacteria; Cop – copiotrophic bacteria; $Cop_p$ – spore-forming copiotrophic bacteria; Am – ammonifying bacteria; Im – nitrogen immobilizing bacteria; Az – *Azotobacter* spp.; Art – *Arthrobacter* spp.; Cel – cellulose-decomposing bacteria; Ps – *Pseudomonas* spp.; Act – actinomyces; Fun – fungi

*r* – coefficient of correlation significant at: $^{**}P < 0.01$, $^{*}P < 0.05$, $n = 138$

contaminated with NiZnCuPbCdCr. Regardless of the number of heavy metals used for soil contamination, chromium and cadmium inhibited the growth of bacteria of the genus *Azotobacter* to the highest extent. Therefore, these elements can be considered the most dangerous to soil microbes, irrespective of whether they occur alone or in combination with other heavy metals. Cadmium applied alone was found to be a stronger inhibitor of the growth of *Azotobacter* spp. than chromium. Nickel applied at a dose of 50 mg $Ni^{2+}$/kg had no significant effect on these bacteria.

As regards soil contamination with multiple heavy metals, the highest number of microbial groups (8–9) was significantly modified by NiCd, NiPb and NiCr. Substantial changes in bacterial counts were also caused by a combination of the following heavy metals: NiZnCuPbCdCr (Tables 2–4). Despite the fact that the total level of soil contamination in this treatment was 450 mg of heavy metals per kg dm of soil, the counts of particular microbial groups increased as a result of their combined effect as follows: total oligotrophic bacteria – 2.6-fold, spore-forming oligotrophic bacteria – 1.9-fold, total copiotrophic bacteria – 3.3-fold, spore-forming copiotrophic bacteria – 1.2-fold, ammonifying bacteria – 1.8-fold, nitrogen immobilizing bacteria – 1.1-fold, *Arthrobacter* spp. – 1.4-fold, *Pseudomonas* spp. – 2.1-fold, actinomyces – 1.3-fold, and fungi – 2.6-fold. This increase was statistically significant. The population size of cellulose-decomposing bacteria remained unchanged while that of *Azotobacter* spp. decreased markedly.

The yield of oat grown on uncontaminated light silty loam and heavy loamy sand was comparable: 23.74 g dm per pot and 22.12 g dm per pot, respectively. However, differences in the growth and development of oat plants were observed in the treatments where soil was artificially contaminated with heavy metals (Table 6). Nickel applied in the amount of 200 mg $Ni^{2+}$/kg was more toxic to oat grown on heavy loamy sand than on light silty loam, although oat yield was significantly reduced in both cases. Nickel applied at the lower dose (50 mg $Ni^{2+}$/kg) caused no statistically significant inhibition of the growth and development of oat plants, nor did copper, zinc and lead applied at identical dose. Only cadmium and chromium decreased the yield of oat grown on both types of soil. Chromium, compared to cadmium, had a greater adverse effect on oat growth. In the treatments in which soil was contaminated with nickel and additionally with one of the tested heavy metals (Zn, Cu, Pb, Cd or Cr) the percentage yield of oat considerably declined. The toxic effect of contamination with two heavy metals was significantly higher in the case of oat plants grown on lighter soil, which was reflected in yield. The yield patterns of oat grown on soil contaminated with three heavy metals seem interesting. In the case of heavy loamy sand no further yield decrease was observed, and in some treatments (NiZnCu, NiZnPb) the yield was even higher, compared to soil samples contaminated with NiZn, NiCu, NiPb and NiCd. A further increase in the number of soil-contaminating heavy metals did not enhance their toxicity towards oat plants. In the combination NiZnCuPbCdCr a significant decrease in oat yield was noted when all heavy metals (NiZnCuPbCdCr) were applied simultaneously to the soil. It follows that the greatest changes in oat yield resulted from soil contamination with chromium applied alone, and with nickel applied in combination with two other heavy metals, especially when oat was grown on lighter soil.

Literature data (Šimon 1999, Šmejkalová et al. 2003, Vasundhara et al. 2004) indicate that heavy metals are considered inhibitors of the microbiological and biochemical activity of soil. However, the results of this study suggest that heavy metals may either inhibit or stimulate the growth of soil

Table 6. Percentage decrease in the yield of oat resulting from soil contamination with nickel and other heavy metals

| Treatments | Soil species | |
|---|---|---|
| | heavy loamy sand | light silty loam |
| $Ni_{50}$ | 9.27 ± 0.74 | 5.74 ± 1.20 |
| $Ni_{200}$ | 64.36 ± 1.55 | 39.51 ± 0.93 |
| Zn | 7.96 ± 1.05 | 7.28 ± 1.67 |
| Cu | 0.08 ± 1.37 | 6.37 ± 0.85 |
| Pb | 6.23 ± 1.84 | 11.12 ± 0.46 |
| Cd | 14.91 ± 1.17 | 17.45 ± 0.98 |
| Cr | 53.96 ± 0.88 | 60.08 ± 1.21 |
| NiZn | 69.84 ± 1.57 | 38.74 ± 0.07 |
| NiCu | 70.60 ± 0.98 | 48.19 ± 0.20 |
| NiPb | 64.03 ± 1.36 | 36.62 ± 1.87 |
| NiCd | 63.31 ± 0.72 | 51.94 ± 0.35 |
| NiCr | 51.35 ± 0.59 | 60.22 ± 1.89 |
| NiZnCu | 44.86 ± 1.69 | 54.84 ± 0.33 |
| NiZnPb | 54.00 ± 0.48 | 42.13 ± 1.88 |
| NiZnCd | 66.76 ± 1.68 | 49.19 ± 0.87 |
| NiZnCr | 55.81 ± 0.44 | 63.97 ± 1.25 |
| NiZnCuPb | 57.16 ± 0.85 | 61.89 ± 1.11 |
| NiZnCuCd | 66.47 ± 0.56 | 55.38 ± 0.45 |
| NiZnCuCr | 62.47 ± 1.23 | 51.85 ± 1.55 |
| NiZnCuPbCd | 61.75 ± 1.44 | 53.98 ± 0.36 |
| NiZnCuPbCr | 50.84 ± 0.89 | 45.71 ± 0.21 |
| NiZnCuPbCdCr | 68.20 ± 1.18 | 54.02 ± 1.12 |
| $LSD_{0.05}$ | a – 1.94, b – 0.57, a × b – 2.74 | |

$Ni_{50}$ – 50 mg/kg dm soil; $Ni_{200}$ – 200 mg/kg dm soil microbes, depending on the group (type) of these microbes. For instance, the heavy metals tested in the present experiment decreased the population size of *Azotobacter*, but increased the counts of oligotrophic and copiotrophic bacteria as well as actinomyces and fungi, and even ammonifying bacteria. However, conclusions on the negative or positive impact of heavy metals should not be drawn based on an increase in bacterial counts. Heavy metals may increase the total population size of soil microbes, but some beneficial species, improving soil fertility, may disappear, which was the case in this study. Our results are consistent with the findings of Zaborowska et al. (2006). These authors demonstrated that the growth of some microbial groups may be stimulated by heavy metals in soils having neutral reaction (as in the present experiment), but inhibited in acid soils. This is related to the influence of soil pH on the mobility of heavy metals.

The number of heavy metals contaminating the soil is also important. It would be difficult to consider an increase in the counts of some microbial groups caused by the combined effect of multiple elements (NiZnCuPbCdCr) as desirable, since it results in disturbances in the natural biological equilibrium, often stronger than those caused by heavy metals applied alone. It should be also remembered that the treatment with the largest number of soil-contaminating heavy metals was characterized by the highest levels of both qualitative and quantitative contamination, because each of these heavy metals (except for nickel) was applied in the amount of 50 mg/kg dm soil. As a result, the total contamination level in this treatment was as high as 450 mg/kg. Despite this fact the population size of some microbial groups increased, and bacteria of the genus *Azotobacter* were not completely destroyed in more compact soil (light silty loam). According to Giller et al. (1998), soil microbes have developed numerous defense mechanisms, so an increased concentration of heavy metal ions or metabolites in the environment leads to their bioaccumulation in the cells of these microorganisms.

Heavy metal contamination has an adverse effect not only on the biological activity of soil, but also on the growth and development of crops (Tibazarwa et al. 2001, Pandey and Sharma 2002). Jasiewicz and Antonkiewicz (2000) conducted experiments on plants and found that heavy metals applied at low doses (Cd – 5, Cu – 20, Ni – 15, Pb – 30, Zn – 50 mg/kg) had no negative impact on the growth of development of the plants. However, when they were applied at higher doses (Cd – 80, Cu – 320, Ni – 240, Pb – 480, Zn – 800 mg/kg) these heavy metals caused a substantial yield decrease. No increasing doses of heavy metals were applied in this study, but their number was increased, which contributed to a higher total level of soil contamination and finally resulted in a decrease in oat yield.

## REFERENCES


Babich H., Stotzky G. (1997): Sensitivity of various bacteria including actinomyces and fungi to cadmium and influence of pH on sensitivity. Appl. Environ. Microbiol., *33*: 681–695.

Fenglerowa W. (1965): Simple method for counting *Azotobacter* in soil samples. Acta Microbiol. Pol., *14*: 203–206.

Giller K.E., Witter E., McGrath S.P. (1998): Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils: a review. Soil Biol. Biochem., *30*: 1389–1414.

Giridhara M., Siddaramappa R. (2002): Effect of heavy metals on urease activity in soil. Curr. Res. Univ. Argric. Sci. Bangalore, *31*: 4–5.

Hattori R., Hattori T. (1980): Sensitivity to salts and organic compounds of soil bacteria isolated on diluted media. J. Gen. Appl. Microbiol., *26*: 1–14.

Huang Q., Shindo H. (2000): Effect of copper on the activity and kinetics of free and immobilized acid phosphatase. Soil Biol. Biochem., *32*: 1885–1892.

Jasiewicz Cz., Antonkiewicz J. (2000): Effect of heavy metal soil contamination on the physicochemical properties of soil, yield and chemical composition of Jerusalem artichoke (*Helianthus tuberosus* L.). Agricultura, *84*: 141–146. (In Polish)

Kabata-Pendias A., Pendias H. (2001): Trace Elements in Soils and Plants. CRC Press, Boca Raton.

Kucharski J., Wyszkowska J. (2004): Inter-relationship between number of microorganisms and spring barley yield and degree of soil contamination with copper. Plant Soil Environ., *50*: 243–249.

Martin J. (1950): Use of acid, rose bengal and streptomycin in the plate method for estimating soil fungi. Soil Sci., *69*: 215–233.

Mulder E.G., Antheumisse J. (1963): Morphologie, physiologie et ecologie des *Arthrobacter*. Ann. Inst. Pasteur, *105*: 46–74.

Pandey N., Sharma C.P. (2002): Effect of heavy metals $Co^{2+}$, $Ni^{2+}$ and $Cd^{2+}$ on growth and metabolism of cabbage. Plant Sci., *163*: 753–758.

Parkinson D., Gray F.R.G., Williams S.T. (1971): Methods for Studying the Ecology of Soil Microorganism. Blackweel Sci. Publ. Oxford and Edinburgh, IBP Handbook: 19.

Šimon T. (1999): The effect of increasing rates of nickel and arsenic on the growth of radish and soil microflora. Rostl. Výr., *45*: 421–430. (In Czech)

Šmejkalová M., Mikanová O., Borůvka L. (2003): Effects of heavy metal concentrations on biological activity of soil microorganisms. Plant Soil Environ., *49*: 321–326.

StatSoft, Inc. (2003): Statistica (data analysis software system). Version 6.0. Available at: www.statsoft.com

Tibazarwa C., Corbisier P., Mench M., Bossus A., Solda P., Mergeay M., Wyns L., van der Lelie D. (2001): A microbial biosensor to predict bioavailable nickel in soil and its transfer to plants. Environ. Pollut., *113*: 19–26.

Vasundhara G., Jayashree G., Muraleedhara-Kurup G. (2004): Sequestration of nickel and copper by *Azotobacter chroococcum* SB1. Bull. Environ. Contam. Toxicol., *72*: 1122–1127.

Zaborowska M., Wyszkowska J., Kucharski J. (2006): Microbial activity in zinc contaminated soil of different pH. Pol. J. Environ. Stud., *15*: 569–574.

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*Corresponding author:*

Prof. dr. hab. Jadwiga Wyszkowska, University of Warmia and Mazury in Olsztyn, Department of Microbiology, Plac Łódzki 3, 10-727 Olsztyn, Poland
e-mail: jadwiga.wyszkowska@uwm.edu.pl