# Inter-relationship between number of microorganisms and spring barley yield and degree of soil contamination with copper

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**ABSTRACT**

The purpose of the study has been to determine the effect of soil contamination with copper on the modification of microbial number and spring barley yield and to assess whether spores of actinomycete *Streptomyces odorifer* and *Streptomyces viridis* were used for detoxification of copper polluted soils. The tests were performed in a pot experiment, which was conducted on two types of soil: Eutric Cambisol soil derived from light loamy sand and Eutric Cambisol soil derived from light loam. The results showed that number of all analysed microorganisms was significantly negatively correlated with a degree of soil contamination by copper, but positively correlated with the yield of spring barley. In general, the adverse impact of copper on the development of oligotrophic bacteria and their spores, eutrophic bacteria and their spores as well as actinomycetes and fungi was much weaker in more compact (light loam) than lighter (light loamy sand) soil. Copper had a strong toxic effect on spring barley and significantly inhibits the growth and development of the plants. An inoculum containing spores of *Streptomyces viridis* and *Streptomyces odorifer* did not alleviate the negative response of spring barley to copper contamination of soil, although it had positive influence on the growth of some microorganisms.

**Keywords:** soil contamination with copper; spring barley yield; number of microorganisms

Microorganisms are characterised by varied sensitivity to the presence of heavy metals in soil environment. The uptake and accumulation of heavy metals in cells depend on the kind of heavy metal, degree of utilisation, type of compound in which the heavy metal occurs and environmental factors. According to Chmielewski and Kłapcińska (1984), the uptake of heavy metals by microorganisms is associated with specific transport of metal ions that involves binding proteins and carriers localised in the membrane, with synthesis and release of chelating compounds, which bind and transport ions dissolved in the environment, and with non-specific accumulation of metals, which consists in binding by the wall complex biopolymers.

In this connection, the capability of some bacteria to accumulate heavy metals may find a future application in detoxification of copper polluted environments (Travieso et al. 1999, Wakatsuki 1995). A certain role in the cleaning up of copper contaminated areas can also be attributed to actinomycetes, which are quite readily adaptable to increasing levels of heavy metals in soil, mainly because of biosorption and bioaccumulation (Badura et al. 1986, Golab et al. 1995). The present study has been undertaken to determine the interdependence between the degree of soil contamination with copper and number of microorganisms and the yield of spring barley. Another objective was to assess whether it would be possible to use spores of two actinomycetes species, *Streptomyces odorifer* and *Streptomyces viridis*, to detoxicate copper contaminated soils.

## MATERIAL AND METHODS

The tests were conducted in a greenhouse, in plastic pots (with four replications) filled with 3.2 kg soil each. The experiment was carried out on two soils simultaneously: Eutric Cambisol soil derived from light loamy sand of pH 5.6 in 1 mol KCl/dm$^3$, hydrolytic acidity 18.0 mmol($H^+$)/kg, total exchange bases 65.6 mmol(+)/kg, organic carbon content 6.0 g/kg, and Eutric Cambisol soil derived from light loam of pH 5.9 in 1 mol KCl dm$^3$, hydrolytic acidity 14.3 mmol($H^+$)/kg, total exchange bases 81.0 mmol(+)/kg and organic carbon content 8.9 g/kg. Prior to putting into the pots, soil was fertilised with the following amounts (expressed as pure substances in mg/kg of soil dry matter), of macro- and microelements: N = 120 [$CO(NH_2)_2$]; P = 75 [$K_2HPO_4$]; K = 120 [$K_2HPO_4$ + KCl]; Mg = 40 [$MgSO_4.7\ H_2O$]; Zn=5 [$ZnCl_2$]; Cu=5 [$CuSO_4.5\ H_2O$]; Mn = 5 [$MnCl_2.4\ H_2O$]; Mo = 5 [$Na_2MoO_4.2\ H_2O$]; B = 0.33 [$H_3BO_3$]. Mineral fertilisers were applied to soil in a single treatment before sowing, by mixing them with a whole batch of soil per pot. At the same time, the soil was contaminated with

copper, using $CuSO_4.7\ H_2O$ in the following rates expressed as a pure substance: 0, 400, 800 and 1200 mg Cu/kg soil.

The trials were accomplished in two series with four replications. No microorganisms were added to the soil in the first series, whereas in the second one the effect of increasing rates of copper was examined after conidia of *Streptomyces odorifer* in the amount of $100.10^6$/kg of soil and *Streptomyces viridis* in the amount of $42.10^6$/kg of soil were added. The conidia number was determined with the plate method.

*Streptomyces odorifer* and *Streptomyces viridis* were obtained from the Department of Microbiology's own collection. For seven days conidia were cultured on slants at 28°C. After that, each culture was washed off with 3 $cm^3$ of 0.85% (final concentration) NaCl aqueous sterile solution. The suspension from 60 slants were poured into a conical flask of 1 $dm^3$ volume and mixed. A 5 $cm^3$ portion of the suspension was measured out per pot (3.2 kg soil) and mixed with the soil on the day of establishing the experiment. Cultures of *Streptomyces odorifer* and *Streptomyces viridis* were grown on a medium composed of 10.0 g soluble starch, 0.3 g casein, 2.0 g $KNO_3$, 2.0 g NaCl, 2.0 g $K_2HPO_4$, 0.05 g $MgSO_4.7\ H_2O$, 0.02 g $CaCO_3$, 0.01 g $FeSO_4$, 20.0 g agar and up to 1 $dm^3$ distilled water, pH 7.0.

The experiment was carried out for 62 days. During the first twenty days the soil was not sown. On day 20 soil samples were taken for microbiological analyses and cv. Rabel spring barley (15 plants per pot) was sown. Spring barley was harvested in the phase of flowering. The vegetation period was 42 days. During that time, soil samples were collected for microbiological analyses. During the whole period of the experiment (62 days) soil moisture was maintained at the level of 60% of capillary water holding capacity.

The determination of colony forming units (cfu) number carried out on the day of soil sampling with the plate method. The following microorganisms were examined: oligotrophic bacteria (Olig) and their spores ($Olig_p$) and eutrophic bacteria (Cop) and their spores ($Cop_p$) on a medium with peptone and meat broth according to Onta and Hattori (1983), *Azotobacter* spp. with Fenglerowa's method (1965), cellulolytic bacteria (Cel) on Winogradsky medium (1953), actinomycetes (Act) with the method by Küster and Williams (1964) using nystatin and actidion according to the procedure presented by Parkinson et al. (1971) and fungi (Fun) on peptone-glucose agar according to Martin (1950). Spores of oligotrophic and eutrophic bacteria were determined in the material that had been pasteurised at 85°C for 15 minutes.

The results were elaborated statistically using Duncan's test and three-factor analysis of variance. Statistical analysis was completed using Statistica software (StatSoft Inc. 2001). The results of microbiological determinations were given as means for the two dates of soil sampling.

## RESULTS AND DISCUSSION

Assessment of the impact of excessive copper levels in soil should not be limited to the observation of the growth and development of plants exclusively. It also needs to take into consideration the effect of copper on the biota of soil. Giller et al. (1998, 1999) reported that copper could be highly toxic to the biota of soil, with microorganisms being much more sensitive than plants to high concentrations of heavy metals in environment. This finding, however, is different from the results of our study on spring barley. Although copper present in soil modified the number of all physiological and taxonomic groups of microorganisms (Tables 1 and 2), its toxic effect on spring barley was much stronger. The extent of copper affected on microorganisms depended on soil granulometric composition, its pH and inoculation with actinomycetes. In both types of soil, formed from light loamy sand and from light loam, the average numbers of oligotrophic bacteria and their spores, eutrophic bacteria and their spores, cellulolytic bacteria, *Azotobacter* spp. bacteria, actinomyces and fungi decreased (Tables 1 and 2) under the effect of copper introduced to soil in the form of $CuSO_4.5\ H_2O$, regardless of the inoculation of soil with *Streptomyces*. Thus, the development of soil microorganisms was largely determined by copper contamination. The number of oligotrophic bacteria and their spores in both of the analysed soils was significantly negatively correlated with the rate of copper. In light loamy sand contaminated with 1200 mg Cu/kg of soil, the growth of oligotrophic bacteria was inhibited by an average 55% than that of oligotrophic sporulating bacteria by 36%. In this soil was the largest decreased number of eutrophic bacteria (by 39%), eutrophic sporulating bacteria (by 61%), actinomycetes (by 34%) and fungi (by 60%).

The adverse impact of copper on the development of oligotrophic bacteria and their spores, eutrophic bacteria and their spores, actinomycetes and fungi was usually weaker in more compact soil (light loam) than in lighter soil (light loamy sand) (Tables 1 and 2). Under the effect of the highest concentration of copper (1200 mg Cu/kg) in the former type of soil, the number of nearly all bacteria decreased by 12–24%. The results indicate that copper was most probably adsorbed more readily by light loam, therefore its concentration as a biologically active substance was lower.

Table 1. Effect of soil contamination with copper on number of microorganisms in light loamy sand (cfu/kg soil dry matter)

| Cu dose (mg/kg d.m.) | Olig × $10^8$ | $Olig_p$ × $10^7$ | Cop × $10^8$ | $Cop_p$ × $10^7$ | Az × $10^3$ | Cel × $10^6$ | Act × $10^8$ | Fun × $10^6$ |
|---|---|---|---|---|---|---|---|---|
| **Control – without *Streptomyces* inoculation** | | | | | | | | |
| 0 | 143.75 | 26.66 | 76.11 | 44.96 | 1.26 | 19.94 | 106.31 | 38.65 |
| 400 | 103.48 | 21.62 | 69.01 | 35.74 | 0.00 | 18.16 | 91.86 | 31.91 |
| 800 | 48.02 | 16.36 | 57.75 | 32.46 | 0.00 | 14.39 | 86.18 | 23.40 |
| 1200 | 40.53 | 15.90 | 31.59 | 15.51 | 0.00 | 13.17 | 60.36 | 14.01 |
| Average | 83.94 | 20.13 | 58.61 | 32.16 | 0.32 | 16.41 | 86.18 | 26.99 |
| *r* | –0.97 | –0.96 | –0.96 | –0.96 | –0.77 | –0.98 | –0.97 | –1.00 |
| ***Streptomyces viridis*** | | | | | | | | |
| 0 | 97.48 | 31.42 | 48.85 | 43.10 | 1.08 | 28.64 | 60.56 | 29.50 |
| 400 | 81.87 | 31.65 | 50.76 | 35.53 | 0.00 | 25.31 | 64.37 | 23.53 |
| 800 | 76.37 | 30.63 | 43.66 | 23.07 | 0.00 | 22.48 | 53.91 | 20.82 |
| 1200 | 61.55 | 33.41 | 33.08 | 15.37 | 0.00 | 20.12 | 46.85 | 11.98 |
| Average | 79.32 | 31.78 | 44.09 | 29.27 | 0.27 | 24.14 | 56.42 | 21.46 |
| *r* | –0.99 | 0.54 | –0.89 | –0.99 | –0.77 | –1.00 | –0.86 | –0.98 |
| ***Streptomyces odorifer*** | | | | | | | | |
| 0 | 77.70 | 38.91 | 61.55 | 44.17 | 1.44 | 22.85 | 78.64 | 22.69 |
| 400 | 58.40 | 19.04 | 58.31 | 28.35 | 0.00 | 16.45 | 78.22 | 17.36 |
| 800 | 58.50 | 14.98 | 56.44 | 28.27 | 0.00 | 15.95 | 66.60 | 14.85 |
| 1200 | 42.01 | 13.05 | 49.35 | 20.28 | 0.00 | 16.38 | 54.91 | 10.70 |
| Average | 59.15 | 21.49 | 56.41 | 30.26 | 0.36 | 17.90 | 69.59 | 16.40 |
| *r* | –0.95 | –0.89 | –0.96 | –0.93 | –0.77 | –0.78 | –0.95 | –0.99 |
| ***LSD**** | | | | | | | | |
| a | 10.42 | 1.16 | 2.28 | 2.15 | 0.18 | 1.68 | 8.12 | 1.35 |
| b | 9.06 | 1.01 | 1.98 | 1.87 | n.s. | 1.46 | 7.06 | 1.18 |
| a × b | 18.03 | 2.01 | 3.94 | 3.72 | n.s. | 2.90 | 14.05 | 2.35 |

Olig – oligotrophic bacteria, $Olig_p$ – oligotrophic endosporous bacteria, Cop – eutrophic bacteria,
$Cop_p$ – eutrophic endosporous bacteria, Az – *Azotobacter* spp., Cel – cellulolytic bacteria, Act – actinomyces, Fun – fungi
**LSD* for: a – copper dose, b – *Streptomyces* inoculation, n.s. – non-significant
*r* – correlation coefficients

Khan and Scullion (2000), who tested the effect of copper contamination of five different types of soil, also found out that the toxic effect of this element on microorganisms was greater in light soil and weaker in heavier soil, which contained more mineral and organic colloids.

*Azotobacter* spp. bacteria were unquestionably most sensitive to soil contaminated with copper. Both in leached brown soil formed from light loamy sand and that formed from light loam, even the lowest concentration of copper (400 mg Cu/kg) caused complete disappearance of *Azotobacter* spp. cells.

In both types of soil inoculation with actinomycetes conidia significantly influenced on the amounts of all the microbial groups (Tables 1 and 2). As well as in light loamy sand and in light loam *Streptomyces viridis* and *Streptomyces odorifer* were favourable for the development of cellulolytic and oligotrophic sporulating bacteria, but in light loam they inhibited the growth of eutrophic bacteria and fungi. The way in which the inoculation with actinomycetes affected soil microorganisms was altered when copper sulfate was added to soil which inhibited the influence of

Table 2. Effect of soil contamination with copper on number of microorganisms in light loam (cfu/kg soil dry matter)

| Cu dose (mg/kg d.m.) | Olig × $10^8$ | $Olig_p$ × $10^7$ | Cop × $10^8$ | $Cop_p$ × $10^7$ | Az × $10^3$ | Cel × $10^6$ | Act × $10^8$ | Fun × $10^6$ |
|---|---|---|---|---|---|---|---|---|
| **Control – without *Streptomyces* inoculation** | | | | | | | | |
| 0 | 115.62 | 19.67 | 70.25 | 40.13 | 0.90 | 12.25 | 95.05 | 33.44 |
| 400 | 87.29 | 18.92 | 52.79 | 40.78 | 0.00 | 12.37 | 77.26 | 35.98 |
| 800 | 79.59 | 16.89 | 38.64 | 43.15 | 0.00 | 11.44 | 51.98 | 31.71 |
| 1200 | 70.49 | 15.03 | 33.41 | 32.09 | 0.00 | 8.36 | 44.22 | 25.09 |
| Average | 88.25 | 17.63 | 48.77 | 39.04 | 0.23 | 11.10 | 67.13 | 31.56 |
| *r* | –0.95 | –0.98 | –0.98 | –0.58 | –0.77 | –0.87 | –0.98 | –0.81 |
| ***Streptomyces viridis*** | | | | | | | | |
| 0 | 87.23 | 20.34 | 59.65 | 37.85 | 1.13 | 17.88 | 81.62 | 32.47 |
| 400 | 89.24 | 19.69 | 59.26 | 34.27 | 0.00 | 15.87 | 70.40 | 32.65 |
| 800 | 79.12 | 17.34 | 51.61 | 29.68 | 0.00 | 14.74 | 63.36 | 31.41 |
| 1200 | 39.00 | 14.71 | 60.31 | 25.51 | 0.00 | 13.00 | 60.58 | 29.74 |
| Average | 73.65 | 18.02 | 57.71 | 31.83 | 0.28 | 15.37 | 68.99 | 31.57 |
| *r* | –0.85 | –0.97 | –0.18 | –1.00 | –0.77 | –0.99 | –0.97 | –0.91 |
| ***Streptomyces odorifer*** | | | | | | | | |
| 0 | 115.91 | 22.47 | 54.74 | 44.87 | 0.76 | 24.32 | 83.10 | 44.47 |
| 400 | 103.73 | 22.98 | 67.61 | 48.45 | 0.00 | 15.96 | 81.57 | 41.62 |
| 800 | 112.12 | 15.59 | 69.73 | 36.28 | 0.00 | 15.41 | 76.83 | 37.03 |
| 1200 | 87.82 | 11.41 | 48.82 | 19.87 | 0.00 | 13.38 | 69.23 | 37.31 |
| Average | 104.89 | 18.11 | 60.22 | 37.37 | 0.19 | 17.26 | 77.68 | 40.11 |
| *r* | –0.79 | –0.94 | –0.20 | –0.88 | –0.77 | –0.89 | –0.96 | –0.94 |
| ***LSD**** | | | | | | | | |
| a | 9.23 | 1.40 | 2.88 | 1.90 | 0.34 | 2.02 | 7.08 | 1.87 |
| b | 8.03 | n.s. | 2.50 | 1.65 | n.s. | 1.76 | 6.16 | 1.63 |
| a × b | 15.97 | 2.45 | 5.03 | 3.28 | n.s. | 3.50 | 12.26 | 3.24 |

Olig – oligotrophic bacteria, $Olig_p$ – oligotrophic endosporous bacteria, Cop – eutrophic bacteria,
$Cop_p$ – eutrophic endosporous bacteria, Az – *Azotobacter* spp., Cel – cellulolytic bacteria, Act – actinomyces, Fun – fungi
**LSD* for: a – copper dose, b – *Streptomyces* inoculation, n.s. – non-significant
*r* – correlation coefficients

the inocula. The addition of actinomycetes to soil only partly alleviated the negative effect of copper on the soil biota. Although *Streptomyces viridis* and *Streptomyces odorifer* conidia did not stimulate the growth of eutrophic bacteria, actinomycetes or fungi in the uncontaminated objects, they significantly reduced the unfavourable impact of copper on the growth of these microorganisms. In the series of the experiment without streptomycete inoculation (mean for the two soils) following the application of the highest dose of copper (1200 Cu/kg soil), the number of eutrophic bacteria was reduced by 55%, actinomycetes by 48% and fungi by 44%. In contrast, in the series with *Streptomyces viridis*, the respective reduction was 16, 24 and 32%. When soil was inoculated with *Streptomyces odorifer*, the number of the three microbial groups declined by 15, 23 and 38%, respectively.

Modification in the microbial number may have been caused not only by soil contamination with copper but also by mutual interactions between groups of microorganisms, as the growth of one group of microorganisms can stimulate or retard that of others. This assumption has been based

on the results of Pearson's simple regression coefficients calculated for the rate of copper versus the biological activity of soil, which were mostly highly significant or significant (Table 3).

The negative response of all the analysed groups of microorganisms to excessive amounts of copper in soil agrees with the highly significantly adverse effect of this metal on yield of spring barley (Table 4). The tests performed suggest that by analysing modifications in the number of particular groups of microorganisms in soil it may be possible to obtain some information on the direction and scale of changes in the arable layer of soil.

The study presented herein has proved that soil contamination with copper has a considerable effect on the growth and development of spring barley. This has been made evident by highly significant negative correlation between the rate of this metal and yield of aboveground parts of the crops, grown on Eutric Cambisol soil derived from light loamy sand ($r$ = –0.97) as well as on Eutric Cambisol soil derived from light loam ($r$ = –0.95). Symptoms of the toxic effect of copper, consisting in disturbed water management (wilting), chlorosis of new leaves and damage of the growth tip and roots, were observed even in the spring barley grown in soil contaminated with the smallest rate of copper (400 mg/kg soil) and intensified at higher concentrations of the metal. Consequently, in all the objects polluted with 1200 mg/kg soil, also those inoculated with actinomycetes spores, the yield of aboveground parts decreased by 95% on Eutric Cambisol soil derived from light loamy sand and by 97% on Eutric Cambisol soil derived from light loam.

The inoculation of soils with *Streptomyces viridis* and *Streptomyces odorifer* conidia did not affect sig-

Table 3. Coefficients of Pearson's simple correlation between spring barley yield and number of soil microorganisms

| Variable | Yield | Olig | Cop | $Olig_p$ | $Cop_p$ | Az | Cel | Act | Fun |
|---|---|---|---|---|---|---|---|---|---|
| **Light loam** | | | | | | | | | |
| Yield | 1.00 | 0.66* | 0.52** | 0.77** | 0.59** | 0.87** | 0.61** | 0.83** | 0.46** |
| Olig | 0.66** | 1.00 | 0.45** | 0.52** | 0.57** | 0.42** | 0.49** | 0.73** | 0.69** |
| Cop | 0.52** | 0.45** | 1.00 | 0.41** | 0.23 | 0.32* | 0.40* | 0.82** | 0.46** |
| $Olig_p$ | 0.77** | 0.52** | 0.41** | 1.00 | 0.85** | 0.49** | 0.59** | 0.58** | 0.49** |
| $Cop_p$ | 0.59** | 0.57** | 0.23 | 0.85** | 1.00 | 0.32* | 0.34* | 0.42** | 0.46** |
| Az | 0.87** | 0.42** | 0.32* | 0.49** | 0.32* | 1.00 | 0.48** | 0.62** | 0.19 |
| Cel | 0.61** | 0.49** | 0.40* | 0.59** | 0.34* | 0.48** | 1.00 | 0.55** | 0.74** |
| Act | 0.83** | 0.73** | 0.82** | 0.58** | 0.42** | 0.62** | 0.55** | 1.00 | 0.66** |
| Fun | 0.46** | 0.69** | 0.46** | 0.49** | 0.46** | 0.19 | 0.74** | 0.66** | 1.00 |
| **Light loamy sand** | | | | | | | | | |
| Yield | 1.00 | 0.77** | 0.60** | 0.55** | 0.92** | 0.90** | 0.64** | 0.53** | 0.76** |
| Olig | 0.77** | 1.00 | 0.65** | 0.47** | 0.74** | 0.62** | 0.53** | 0.60** | 0.91** |
| Cop | 0.60** | 0.65** | 1.00 | –0.02 | 0.78** | 0.44** | 0.03 | 0.90** | 0.73** |
| $Olig_p$ | 0.55** | 0.47** | –0.02 | 1.00 | 0.41** | 0.57** | 0.82** | –0.09 | 0.32* |
| $Cop_p$ | 0.92** | 0.74** | 0.78** | 0.41** | 1.00 | 0.77** | 0.53** | 0.68** | 0.83** |
| Az | 0.90** | 0.62** | 0.44** | 0.57** | 0.77** | 1.00 | 0.52** | 0.40* | 0.58** |
| Cel | 0.64** | 0.53** | 0.03 | 0.82** | 0.53** | 0.52** | 1.00 | –0.17 | 0.42** |
| Act | 0.53** | 0.60** | 0.90** | –0.09 | 0.68** | 0.40* | –0.17 | 1.00 | 0.76** |
| Fun | 0.76** | 0.91** | 0.73** | 0.32* | 0.83** | 0.58** | 0.42** | 0.76*** | 1.00 |

*significance for $p < 0.05$, ** for $p < 0.01$, $n = 36$

Olig – oligotrophic bacteria, $Olig_p$ – oligotrophic endosporous bacteria, Cop – eutrophic bacteria, $Cop_p$ – eutrophic endosporous bacteria, Az – *Azotobacter* spp., Cel – cellulolytic bacteria, Act – actinomyces, Fun – fungi

Table 4. Effect of soil contamination with copper on spring barley yield (g dry matter per pot)

| | Cu dose (mg/kg d.m.) | Control – without *Streptomyces* inoculation | *Streptomyces viridis* | *Streptomyces odorifer* | Average |
|---|---|---|---|---|---|
| Light loam | 0 | 14.80 | 13.84 | 14.47 | 14.37 |
| | 400 | 7.06 | 6.93 | 7.14 | 7.04 |
| | 800 | 0.84 | 1.91 | 4.19 | 2.31 |
| | 1200 | 0.68 | 0.29 | 0.43 | 0.46 |
| | average | 5.84 | 5.74 | 6.56 | 6.05 |
| | *r* | –0.94 | –0.97 | –0.98 | –0.97 |
| Light loamy sand | 0 | 15.12 | 15.00 | 14.31 | 14.81 |
| | 400 | 6.58 | 7.76 | 5.65 | 6.66 |
| | 800 | 2.19 | 1.54 | 3.08 | 2.27 |
| | 1200 | 0.73 | 0.53 | 0.76 | 0.67 |
| | average | 6.15 | 6.21 | 5.95 | 6.10 |
| | *r* | –0.95 | –0.96 | –0.94 | –0.95 |
| *LSD** | a = 0.34; b = n.s.; c = n.s.; a × b = 0.48; a × c = 0.58; b × c = 0.41; a × b × c = 0.82 | | | | |

**LSD* for: a = copper dose, b = *Streptomyces* inoculation, c = soil type, n.s. = non-significant
*r* – correlation coefficients

nificantly the volume of spring barley yields in the control and copper contaminated objects. In other words, it did not alleviate the toxic effect of copper on the growth and development of plants, which may be an indirect proof that *Streptomyces* have a very small contribution to the bio-acummulation of this heavy metal. In some earlier research (Wyszkowska et al. 2001), an inoculum composed of *Streptomyces* conidia turned out to be ineffective as a detoxifying agent in soils contaminated with chromium. Nonetheless, Dahm et al. (1986) suggested that actinomycetes were capable of allievating the noxious effect of heavy metals through their active participation in degradation of organic matter.

Toxic influence of excessive amounts of copper in soil on plants has been confirmed by the studies conducted by Kucharski and Wyszkowska (1998) or Ruszkowska and Wojcieska-Wykupajtys (1996). The studies Dumestre et al. (1999) seemed to prove that copper could be incorporated into a trophic chain via plants, which were able to uptake it actively or passively. Under the conditions of increased copper concentrations in soil, after the metal is taken up by plants, it is bound in their roots by phytochelates, with only small amounts accumulated in aboveground parts (Ebbs and Kochian 1997, Gigliotti et al. 1996). This, however, does not protect the plants from the toxic effect of copper, which has also been revealed by the present experiment on spring barley.

Recapitulating, it can be assumed that all microorganisms are vulnerable to excessive levels of heavy metals, including copper, in soil. Both from our own research (Kucharski and Wyszkowska 1998, Kucharski et al. 2001) and the reports of other researchers (Dumestre et al. 1999, Giller et al. 1998, 1999, Khan and Scullion 2000) provide sufficient evidence. Modification in the soil biological equilibrium caused by excessive quantities of copper, which occurred in the present tests, may be attributed to upset physiological functions, protein denaturation, and destruction of cell membranes of soil microorganisms. Long-term presence of heavy metals in soil produces some influence on the metabolism and growth of microorganisms, which leads to their decreased biomass (Khan and Scullion 1999).

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**ABSTRAKT**

**Vztahy mezi množstvím mikroorganismů a výnosem jarního ječmene a stupněm kontaminace půdy mědí**

Cílem studie bylo stanovit vliv kontaminace půdy mědí na změny množství mikroorganismů a na výnos jarního ječmene a prokázat, zda spory aktinomycet *Streptomyces odorifer* a *Streptomyces viridis* mohou být použity pro detoxikaci půd kontaminovaných mědí. Byla zvolena forma nádobového pokusu s použitím dvou typů půd: Eutric Cambisol odvozená od lehké hlinitopísčité půdy a Eutric Cambisol odvozená od lehké půdy. Výsledky ukazují, že množství všech zjišťovaných mikroorganismů byla ve významné negativní korelaci se stupněm kontaminace mědí, avšak v pozitivní korelaci s výnosem jarního ječmene. Nepříznivý vliv mědi na vývoj oligotrofních a eutrofních bakterií a jejich spor, stejně jako aktinomycet a hub byl značně slabší na kompaktnějších (lehkých půdách) než na lehčích (hlinitopísčitých) půdách. Měď měla silný toxický vliv na jarní ječmen a významně inhibovala růst a vývoj rostlin. Dodáním inokula spor *Streptomyces odorifer* a *Streptomyces viridis* nedošlo ke zmírnění negativního účinku mědi na jarní ječmen, ačkoli byl pozitivně ovlivněn růst některých mikroorganismů.

**Klíčová slova:** kontaminace půdy mědí; výnos jarního ječmene; množství mikroorganismů

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