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Effect of Cadmium and Magnesium on Microbiological Activity in Soil

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Abstract

The aim of these experiments was to apply magnesium (50 and 100 mg Mg kg^{-1} soil) to neutralise the potentially negative effects of soil contamination by cadmium (10, 20, 30 and 40 mg Cd kg^{-1} soil) on the number of some groups of microorganisms. Another objective was to determine the relationship between the number of these microorganisms relative to yield of yellow lupine and some physicochemical properties of soil.

Soil contamination with high rates of cadmium (30 and 40 mg Cd kg^{-1} of soil) caused a significant decrease in the number of oligotrophic bacteria, oligotrophic sporulating bacteria, and copiotrophic and copiotrophic sporulating bacteria in soil, especially at the yellow lupine shoots elongation phase. Low (10 mg Cd kg^{-1} soil) and medium (20 mg Cd kg^{-1} soil) concentrations of cadmium in soil stimulated a proliferation of organotrophic bacteria. Magnesium fertilisation stimulated the proliferation of soil microorganisms at the phase of shoot elongation but did not inhibit the negative effect of high cadmium rates. The number of organotrophic, copiotrophic, and oligotrophic sporulating bacteria in soil during the yellow lupine harvest was lower than during the phase of shoot elongation. On the other hand, the number of oligotrophic and copiotrophic sporulating bacteria was higher. The number of soil microorganisms was positively correlated with the yield of aboveground parts and weight of yellow lupine roots, especially in the objects fertilised with magnesium.

Keywords: cadmium contamination, magnesium fertilisation, number of soil microorganisms, yellow lupine yield.

Introduction

By decomposing organic matter and improving the soil structure, soil microorganisms play an important role in metabolism of various organic compounds, nitrogen, phosphorus, sulphur and other elements [1]. According to Brokes [2], such microbiological parameters as the number, weight and activity of microorganisms can be good indicators of soil contamination with heavy metals, including cadmium. As a rule, cadmium has a negative effect on the growth of soil microorganisms as it can greatly depress their numbers [3, 4, 5, 6]. On one hand, the number of microorganisms in soil depends on the total content and concentrations of particular forms of heavy metals. On the other hand, it is conditioned by several other factors, such as the granulometric composition of soil, quantity and quality of organic matter, especially carbohydrate rich organic matter, pH, total exchange capacity, nutrient availability, moisture, temperature and oxygen availability [3, 7, 8]. The influence of cadmium and other heavy metals on the proliferation of soil microorganisms is stronger in light sandy soils than in

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clay or organic soils [3]. Although liming or organic matter supplementation are the two most popular methods to reduce or eliminate the negative effect of heavy metals on the properties of soil and development of plants [9], new substances are being sought that could accelerate reclamation of contaminated soils.

The aim of this research was to use magnesium to neutralise the potentially negative influence of soil contamination by cadmium on the number of some groups of microorganisms. Another objective was to determine the relationships between the number of microorganisms and the yield of yellow lupine or some physicochemical soil properties.

Methods

The experiment, carried out in a greenhouse at the University of Warmia and Mazury in Olsztyn, comprised simulated soil contamination with cadmium at 0, 10, 20, 30 and 40 mg Cd kg^{-1} of soil and constant fertilisation with other microelements: nitrogen (25 mg N), phosphorus (33 mg P) and potassium (75 mg K kg^{-1} soil). Cv. Juno yellow lupine was grown in polyethylene pots filled with 10 kg soil (granulometric composition of heavy loamy sand). The soil was acid and had the following characteristics: pH_{KCl} - 5.0, hydrolytic acidity (Hh) - 22.5 mmol H^+ kg^{-1}, total exchange capacity (T) - 83.5 mmol (+)kg^{-1}, base saturation (V) - 73.1%, P content - 99.2 mg, K content - 110.3 mg and Mg content - 9.1 mg kg^{-1} soil. Cadmium was applied in the form of $CdCl_2$; nitrogen was used as $CO(NH_2)_2$; phosphorus as KH_2PO_4; potassium as KH_2PO_4+KCl, and magnesium as $MgSO_4{:}7H_2O$. Cadmium, nitrogen, phosphorus, potassium and magnesium compounds were introduced once during the establishment of the experiment by dissolving the chemicals in water and carefully mixing them with soil.

Soil samples were taken on two occasions during the vegetative phase of lupine: at the shoots elongation phase (1st date) and at the harvest of yellow lupine plants (2nd date). Soil samples were analysed by the plate number to determine the number of: organotrophic bacteria by Bunt Rovir's method [10], total oligotrophic bacteria, oligotrophic sporulating bacteria on 100-fold diluted nutrient bullion, and total copiotrophic and copiotrophic sporulating bacteria on concentrated nutrient bullion using the method of Onta and Hattori [11]. Spores of oligotrophic and copiotrophic bacteria were determined in material that had been pasteurised for 15 min at 358 K.

The results were interpreted statistically using the ANOVA three-factor analysis of variance. Correlations of the number of soil microorganisms with soil contamination by cadmium, yield of aboveground parts of yellow lupine plants, yield of roots, number and weight of root nodules were determined with the help of polynominal regression equations and correlation coefficients, whereas the correlations of cadmium contamination and magnesium fertilisation versus chemical and microbiological properties of soil were derived from Pearson's simple correlation coeffcients. Statistical calculations were performed using Statistica software package [12].

Results and Discussion

Soil contamination with cadmium upset the biological balance of soil, regardless of magnesium fertilisation or the date of soil sampling. Fluctuations in the proliferation of all the analysed groups of microorganisms were observed. In the objects without magnesium fertilisation, small rates of cadmium were observed to stimulate the number of organotrophic bacteria in soil (Tab. 1). At the shoots elongation phase, the number of these bacteria grew over two-fold (r=0.46) in response to 20 mg Cd kg^{-1} of soil. On the other hand, during the harvest the number of organotrophic bacteria increased by a 9% (r=0.57) in the objects treated with 10 mg Cd·kg^{-1} of soil. Although magnesium fertilisation increased the number of those bacteria in the control objects and in the pots containing small rates of cadmium (10 mg Cd kg^{-1} of soil), it did not neutralise the negative effect of high cadmium rates on

Table 1. Number of organotrophic bacteria in 1 kg d.m. soil (cfu 10^8).

Cd dose in mg kg^{-1} of soil	1st date	2nd date	Mean
Without Mg			
0	73.43	56.01	64.72
10	116.32	61.17	88.75
20	158.05	59.42	108.73
30	119.87	61.40	90.64
40	114.98	59.81	87.39
Mean	116.53	59.56	88.05
r	0.46	0.57	0.48
50 mg Mg kg^{-1} of soil			
0	100.43	64.91	82.67
10	156.86	56.31	106.58
20	159.34	53.05	106.20
30	105.81	45.82	75.81
40	88.61	49.75	69.18
Mean	122.21	53.97	88.09
r	-0.35	-0.89	-0.53
100 mg Mg kg^{-1} of soil			
0	149.20	55.49	102.35
10	135.08	64.84	99.96
20	121.64	62.66	92.15
30	119.41	48.74	84.07
40	113.58	37.55	75.57
Mean	127.78	53.86	90.82
r	-0.96	-0.74	-0.99
LSD	a – 19.42**; b – n.s.; c – 12.29**; axb – n.s.; axc – n.s.; bxc – n.s.; axbxc – n.s.		

LSD for: a - Cd dose, b - Mg dose, c - date,
n.s. - non-significant, * significant at p=0.05, ** significant at p=0.01

the growth of organotrophic bacteria. The number of those bacteria in soil during the yellow lupine harvest was from 49% (the series without magnesium) to 58% (100 mg Mg kg^{-1} of soil) lower than during the vegetation of the crop.

Simulated contamination of soil with cadmium had a greater effect on the number of organotrophic than oligotrophic bacteria (Tab. 2). During the vegetatation of yellow lupine, the growth of oligotrophic bacteria was significantly depressed by cadmium at the shoots elongation phase, causing the number of the bacteria decrease by 43% (r=-0.96). During the harvest no such effect was observed.

Table 2. Number of oligotrophic bacteria in 1 kg d.m. soil (cfu 10^8).

Cd dose in mg kg^{-1} of soil	1st date	2nd date	Mean
Without Mg			
0	64.07	66.82	65.45
10	58.89	67.40	63.14
20	57.47	68.39	62.93
30	44.77	73.23	59.00
40	36.27	68.19	52.23
Mean	52.29	68.80	60.55
r	-0.96	0.53	-0.93
50 mg Mg kg^{-1} of soil			
0	68.39	81.04	74.72
10	57.40	65.32	61.36
20	56.09	64.71	60.40
30	61.39	62.05	61.72
40	59.77	49.75	54.76
Mean	60.61	64.57	62.59
r	-0.44	-0.93	-0.85
100 mg Mg kg^{-1} of soil			
0	84.06	71.77	77.92
10	82.06	61.78	71.92
20	68.17	59.70	63.93
30	71.06	56.92	63.99
40	64.00	49.00	56.50
Mean	73.87	59.83	66.85
r	-0.92	-0.97	-0.97
LSD	a – 11.24**; b – n.s; c – n.s.; axb – n.s.; axc – n.s.; bxc – 12.31**; axbxc – n.s.		

Explanations under Table 1.

Magnesium fertilisation had a positive influence on the proliferation of oligotrophic bacteria only at the lupine shoots elongation phase and elevated the negative effect of cadmium on their number. The difference in the number of oligotrophic bacteria between the series without magnesium and the series with 100 mg Mg kg^{-1} of soil was on average 41%. The growth of oligotrophic bacteria at the shoots elongation phase was higher in the series without magnesium and with 50 mg Mg and lower in the series with 100 Mg kg^{-1} of soil than during yellow lupine harvest.

In response to cadmium contamination, the number of copiotrophic bacteria in magnesium untreated soil increased considerably on the first date but did not show any significant changes on the second date of analyses (Tab. 3). Although the growth of copiotrophic bacteria was higher in magnesium fertilised soil (50 and 100 mg Mg kg^{-1} of soil) uncontaminated or contaminated with the lowest Cd rate compared to the control objects, the toxic effect of cadmium on the number of these bacteria was becoming evident as the cadmium concentration grew. The number of copiotrophic bacteria in the series with the higher magnesium rate (100 mg Mg kg^{-1} of soil) was on average 40% higher at the shoots elongation phase and 9% higher during the lupine harvest than in the objects without magnesium. The mean number of

Table 3. Number of copiotrophic bacteria in the 1 kg d.m. soil (cfu 10^8).

Cd dose in mg kg^{-1} of soil	1st date	2nd date	Mean
Without Mg			
0	32.76	44.80	38.78
10	41.80	42.12	41.96
20	44.90	44.47	44.68
30	52.16	45.39	48.77
40	51.14	45.71	48.43
Mean	44.55	44.50	44.53
r	0.95	0.57	0.97
50 mg Mg kg^{-1} of soil			
0	52.20	46.94	49.57
10	41.35	43.17	42.26
20	42.16	44.39	43.28
30	45.86	45.09	45.48
40	45.71	39.04	42.37
Mean	45.46	43.73	44.59
r	-0.31	-0.74	-0.58
100 mg Mg kg^{-1} of soil			
0	88.06	50.68	69.37
10	59.55	58.35	58.95
20	54.90	49.68	52.29
30	57.14	43.90	50.52
40	51.42	40.41	45.92
Mean	62.22	48.61	55.41
r	-0.81	-0.80	-0.96
LSD	a – 9.89*; b – 7.66**; c – n.s.; axb – n.s.; axc – n.s.; bxc – n.s.; axbxc – n.s.		

Explanations under Table 1.

Table 4. Number of oligotrophic sporulating bacteria in 1 kg d.m. soil (cfu 10^7).

Cd dose in mg kg^{-1} of soil	1st date	2nd date	Mean
Without Mg			
0	32.40	26.26	29.33
10	39.62	33.33	36.48
20	28.74	33.26	31.00
30	32.19	31.27	31.73
40	27.93	28.57	28.25
Mean	32.17	30.54	31.36
r	-0.56	0.13	-0.34
50 mg Mg kg^{-1} of soil			
0	43.20	30.07	36.63
10	36.72	37.16	36.94
20	35.01	32.36	33.68
30	36.48	31.75	34.11
40	27.07	23.73	25.40
Mean	35.70	31.01	33.35
r	-0.89	-0.59	-0.85
100 mg Mg kg^{-1} of soil			
0	48.76	33.30	41.03
10	38.49	28.99	33.74
20	30.86	24.47	27.66
30	31.14	20.09	25.61
40	25.90	14.66	20.28
Mean	35.03	24.30	29.66
r	-0.94	-1.00	-0.98
LSD	a – 6.70**; b – n.s.; c – 4.24**; axb – n.s.; axc – n.s.; bxc – n.s.; axbxc – n.s.		

Explanations under Table 1.

Table 5. Number of copiotrophic sporulating bacteria in 1 kg d.m. soil (cfu 10^7).

Cd dose in mg kg^{-1} of soil	1st date	2nd date	Mean
Without Mg			
0	20.52	70.30	45.41
10	19.27	98.90	59.08
20	17.60	81.84	49.72
30	13.69	78.18	45.94
40	13.42	65.52	39.47
Mean	16.90	78.95	47.92
r	-0.97	-0.37	-0.55
50 mg Mg kg^{-1} of soil			
0	44.64	69.31	56.97
10	39.57	62.31	50.94
20	38.94	56.81	47.88
30	33.59	50.14	41.87
40	30.24	40.95	35.59
Mean	37.40	55.90	46.65
r	-0.98	-1.00	-0.99
100 mg Mg kg^{-1} of soil			
0	50.22	62.52	56.37
10	33.41	62.17	47.79
20	27.63	58.21	42.92
30	26.37	43.53	34.95
40	24.05	41.85	32.95
Mean	32.33	53.65	42.99
r	-0.89	-0.93	-0.98
LSD	a – 10.63**; b – n.s.; c – 6.73**; axb – n.s.; axc – n.s.; bxc – 11.65**; axbxc – n.s.		

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these bacteria in soil not fertilised with magnesium was similar on both dates of analyses, and in the series with 100 mg Mg kg^{-1} of soil it declined during the vegetative period of yellow lupine.

Soil contamination with 10 mg Cd kg^{-1} of soil significantly stimulated the growth of oligotrophic sporulating bacteria in soil on either assay date but of copiotrophic sporulating bacteria - only during the yellow lupine harvest (Tabs. 4 and 5). Higher rates of cadmium caused decrease in the number of these bacteria in all series of experiments, and especially in the series with 100 mg Mg kg^{-1} of soil. However, the mean number of both groups of bacteria in the objects fertilised with magnesium at the phase of shoots elongation phase was higher than in the objects without magnesium. The proliferation of oligotrophic sporulating bacteria during vegetative growth decreased, whereas that of copiotrophic sporulating bacteria increased, reaching the maximum during the yellow lupine harvest. The highest (a nearly 5-fold increase in the number of copiotrophic sporulating bacteria) was recorded in the series without magnesium.

The results of polynominal regression equations (Fig. 1) and Pearson's simple correlation coefficients (Tab. 6) seem indicative of a significant, negative correlation between the number of soil microorganisms and cadmium contamination, which reaffirms the research of Hiroki [4]. The number of soil microorganisms were positively correlated with the yield of yellow lupine aboveground parts and weight of roots, in the objects with magnesium and, partly, in the series without magnesium (Fig. 2, Tab. 6).

Proliferation of soil microorganisms was also positively correlated with the weight of the roots nodules of yellow lupine and, positively or negatively (depending on the type of microorganisms) with some physicochemical properties of soil, especially with soil hydrolytic acidity, total exchange capacity, degree of base saturation, phosphorus and potassium content (Tab. 6). A very high correlation between the number of each group of microorganisms and yellow lupine yield was confirmed by calculating polynominals of theoretical yields, which were

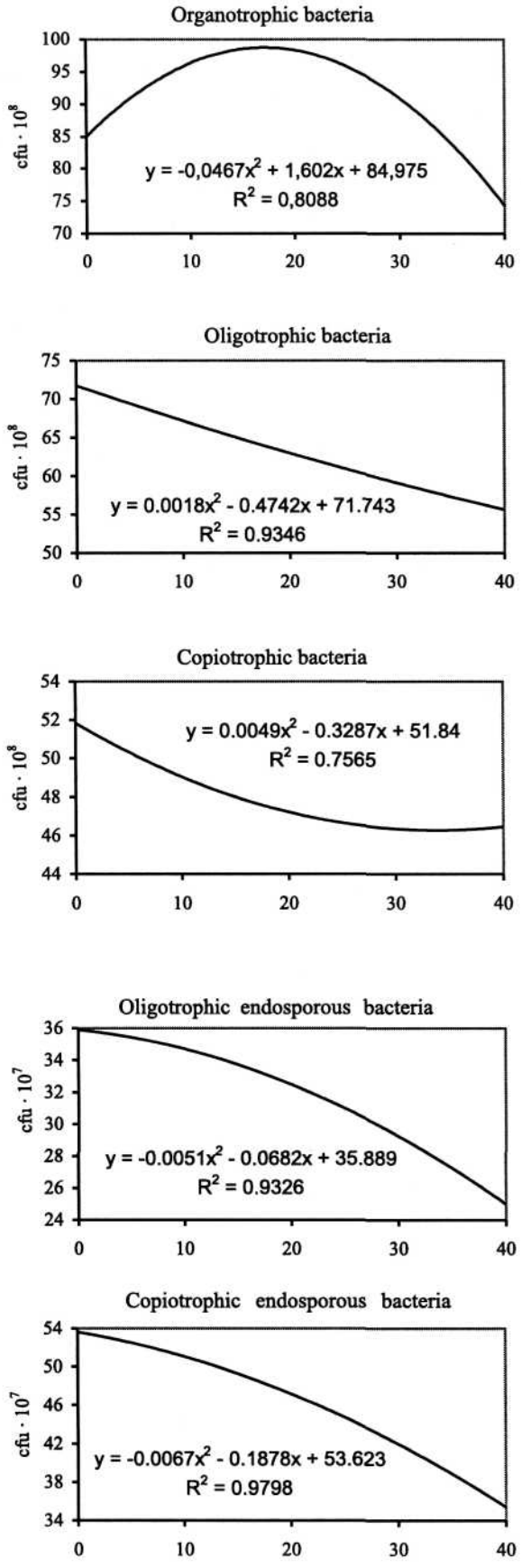

Fig. 1. Correlation between Cd rate versus number of microorganisms in 1 kg d.m. of soil, independent of magnesium fertilisation and date of soil analysis.

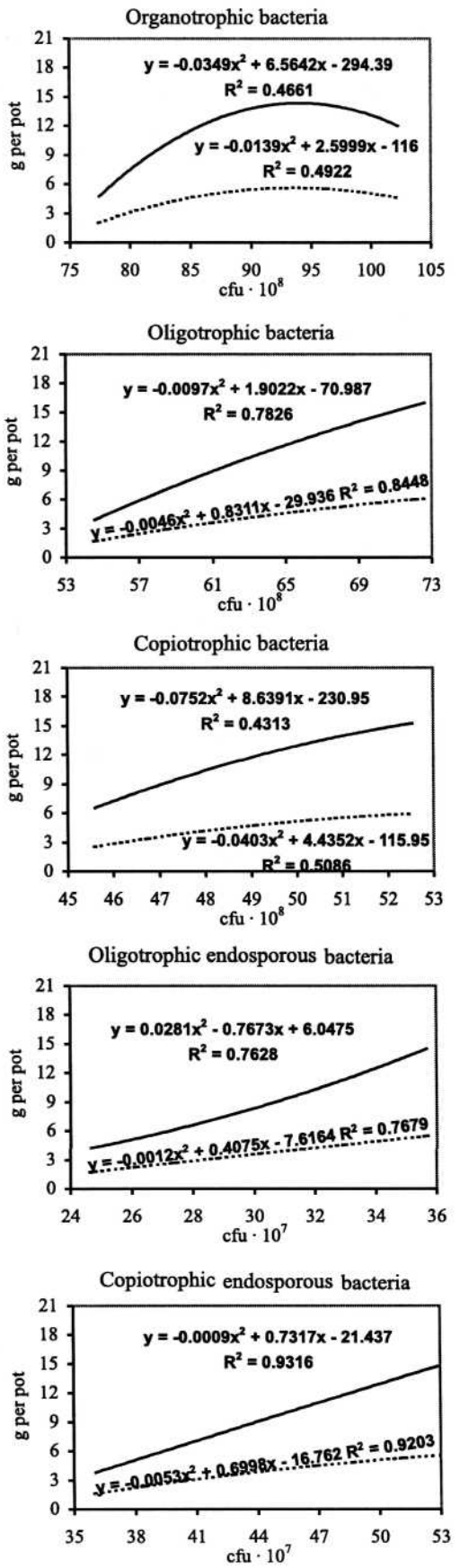

Fig. 2. Correlation between the number of microorganisms in 1 kg d.m. of soil versus the yield of yellow lupine.
——— above ground parts; ---------- roots

Table 6. Pearson's simple correlation coeffcients between Cd rates, Mg rates, yellow lupine yield, some chemical and biochemical soil properties, independent of the date of assay (mean for all series with magnesium).

Variable	Org	Olig	$Olig_p$	Cop	Cop_p
Cd dose	-0.08	-0.33**	-0.37**	-0.12	-0.26*
Mg dose	0.03	0.15	– 0.07	0.29**	– 0.08
Aboveground parts yield	0.07	0.27**	0.30**	0.01	0.24*
Roots yield	0.10	0.32**	0.30**	0.10	0.22*
Number of nodules	0.12	0.17	0.33**	– 0.01	0.23*
Nodules weight	0.07	0.30**	0.32**	0.05	0.23*
pH_{KCl}	– 0.66**	0.09	– 0.16	– 0.12	0.65**
H	0.73**	– 0.06	0.31**	0.26*	– 0.65**
S	– 0.09	0.18	0.36**	0.18	0.42**
T	0.60**	0.06	0.52**	0.36**	– 0.32**
V	– 0.68**	0.10	– 0.19	– 0.19	0.70**
C	0.34**	0.12	0.11	0.09	– 0.26*
P	0.48**	– 0.08	0.32**	0.01	– 0.24*
K	0.51**	– 0.03	0.10	0.24*	– 0.52**
Mg	0.15	0.19	0.03	0.36**	– 0.13
Org	1.00	– 0.13	0.35**	0.16	– 0.41**
Olig	– 0.13	1.00	0.22*	0.15	0.23
$Olig_p$	0.35**	0.22*	1.00	0.32**	– 0.03
Cop	0.16	0.15	0.32**	1.00	0.07
Cop_p	– 0.41**	0.23*	– 0.03	0.07	1.00

H - hydrolytic acidity; S - total exchange bases; T - sorptive capacity; V - degree of base saturation; C - C content in soil; P - P content in soil; K - K content in soil; Mg - Mg content in soil;
number of: Org - organotrophic bacteria; Olig - oligotrophic bacteria; $Olig_p$ - oligotrophic endosporous bacteria; Cop - copiotrophic bacteria; Cop_p - copiotrophic endosporous bacteria;
Correlation coefficient: * significant at p=0.05 (n=90) and ** significant at p=0.01 (n = 90).

approximately the same as the actual yields (Tab. 7). The number of soil microorganisms could be a complementary indicator in forecasting crop yields, next to the activity of soil enzymes.

Table 7. Theoretical yield of yellow lupine calculated from polynominals obtained from the actual yield and the number of soil microorganisms, in g·pot^{-1}.

	Aboveground parts	Roots
Actual yield	10.20	4.04
Theoretical yield		
Org	10.16	3.99
Olig	10.61	4.09
Cop	10.29	3.96
$Olig_p$	10.19	4.00
Cop_p	10.29	3.95

* Theoretical yield was calculated on the basis of polynomials shown in Fig. 2

In our studies, small amounts of cadmium (10 mg Cd kg^{-1} of soil) caused an increase in the number of organotrophic bacteria, which confirms the results obtained by Dusek [13] and Hiroki [14]. The increase in the numbers of some microorganisms in response to low rates of cadmium, observed also in our experiments, is explained by Dusek [13] by the supply of easily available substrate from degradation of dead cells of bacteria and fungi intolerant to cadmium.

Cadmium introduced to soil at a higher dose (30 and 40 mg Cd kg^{-1}) depressed the number of soil microorganisms, which is consistent with the results reported by Hiroki [4], Jiang-Xianjun *et al.* [5] and Milosevic *et al* [6]. Also in the research done by Jiang-Xianjun *et al.* [5], the number of bacteria in soil contaminated with cadmium applied with or without other heavy metals was significantly smaller than in the control objects (without Cd).

Some authors [4, 15, 16] tried to arrive at a systematic description of the effect of cadmium on every group of soil microorganisms. Hiroki [4] pointed to a large decrease in the amount *of Actinomyces*, a slightly lower decrease in bacteria and the smallest decrease in fungi. According to Doelman *et al.* [15] and Dias-Junior *et al.* [16]

the tolerance of soil microorganisms to cadmium decreases in the order: fungi>*Actinomyces*>bacteria.

The research on the effect of magnesium on number of microorganisms is scarce and usually deals with groups of microorganisms other than those assayed in our study [17, 18, 19]. Desirable effects of magnesium fertilisation consisting in increasing bacterial populations, as observed during our study, was also noted by Park-Hyun *et al.[l]*.

Conclusions

1. Contamination of soil with high rates of cadmium (30 and 40 mg $Cd \cdot kg^{-1}$ of soil) caused a significant decrease in the number of oligotrophic bacteria, oligotrophic sporulating bacteria, and copiotrophic and copiotrophic sporulating bacteria in soil, especially at the yellow lupine shoots elongation phase.

2. Magnesium fertilisation stimulated the proliferation of soil microorganisms at the phase of shoot elongation but did not inhibiting the negative effect of high cadmium rates.

3. The numbers of organotrophic, copiotrophic, and oligotrophic sporulating bacteria in soil during the yellow lupine harvest was lower than during the phase of shoot elongation. On the other hand, the number of oligotrophic and copiotrophic sporulating bacteria was higher.

4. The number of soil microorganisms was positively correlated with the yield of aboveground parts and weight of yellow lupine roots, especially in the objects fertilised with magnesium.

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