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PRESSURE EXERTED BY ZINC ON THE NITRIFICATION PROCESS*

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Abstract

The objective of this study was to evaluate the nitrification rate in soil polluted with zinc. The experimental protocol was as follows: soil (sandy loam) collected from the 0-20 cm layer of a cropped field was passed through a 2 mm mesh sieve, placed in 150 cm^3 glass beakers, 100 g of soil in each, and polluted with the following doses of Zn^{2+} per 1 kg d.m. of soil: 0, 300, 600, 1 200 and 2 400. Zinc was applied in the form of $ZnCl_2$ aqueous solution. Afterwards, ammonia nitrogen as $(NH_4)_2SO_4$ was added to the soil material in two doses: 0 and 240 mg N kg^{-1} d.m. Once zinc chloride and ammonium sulphate had been thoroughly mixed with the soil, water was added until the soil moisture content reached 50% of capillary water holding capacity and then the beakers were placed in a laboratory incubator at 25°C. After 10, 20, 30 and 40 days, the incubated soil was tested to determine the content of N-NH_4 and N-NH_3. Additionally, after 10 and 40 days of incubation, the most probable counts of nitrifying bacteria involved in the first and second step of the nitrification process were determined. The experiment was run with three replicates for each day. Two determinations of each parameter were performed in the soil samples placed in beakers. In total, 6 results were obtained for each experimental variant. Based on the determinations, the amounts of nitrified and immobilized nitrogen were calculated and the resistance (RS) and resilience (RL) of the nitrification process and nitrifying bacteria to the contamination of soil with zinc were expressed.

It has been experimentally demonstrated that excess zinc in soil significantly disturbs the nitrification rate. As little as 300 mg Zn^{2+} kg^{-1} d.m. of soil significantly inhibits nitrification. Zinc contamination interferes with nitrification and other metabolic process which affect soil nitrogen, which is confirmed by depressed nitrogen immobilization at higher rates of soil contamination with this element.

The adverse effect of zinc on nitrification is primarily due to the negative impact of this element in the soil environment on nitrifying bacteria. Zinc more strongly inhibits the first than the second step nitrification bacteria, but ammonia-oxidizing bacteria recover more quickly than nitrate forms. The RS parameters for the nitrification process towards zinc pollution were on a low level and tended to decrease as the degree of zinc contamination rose. The resistance of nitrifying bacteria to zinc decreased parallel to the increasing amounts of zinc in soil.

Keywords: nitrification, zinc contamination, resistance of nitrification of contamination, nitrifying bacteria.

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PRESJA CYNKU NA PROCES NITRYFIKACJI

Abstrakt

Celem badań było określenie przebiegu procesu nitryfikacji w glebie zanieczyszczonej cynkiem. Procedura doświadczenia była następująca. Glebę (glinę piaszczystą) pobraną z użytku rolnego z warstwy od 0 do 20 cm przesiano przez sito o oczkach o średnicy 2 mm, następnie umieszczono po 100 g w szklanych zlewkach o pojemności 150 cm^3 i zanieczyszczono następującymi dawkami Zn^{2+} w przeliczeniu na 1 kg s.m.: 0, 300, 600, 1200 i 2400. Cynk stosowano w postaci wodnego roztworu $ZnCl_2$. Do tak przygotowanego materiału glebowego wprowadzono azot amonowy w postaci $(NH_4)_2SO_4$ w ilości 0 i 240 mg N kg^{-1} s.m. Po dokładnym wymieszaniu chlorku cynku oraz siarczanu amonu z glebą, glebę uwilgotniono do poziomu 50% kapilarnej pojemności wodnej, a następnie zlewki wstawiono do cieplarki o temp. 25°C. Po upływie 10, 20, 30 i 40 dni, w inkubowanej glebie oznaczono zawartość N-NH_4 i N-NO_3, i dodatkowo po 10 i 40 dniach najbardziej prawdopodobną liczbę bakterii nitryfikacyjnych (NPL) I i II fazy nitryfikacji. Doświadczenie wykonano w 3 powtórzeniach dla każdego terminu badań. W próbkach umieszczonych w zlewkach wykonano po 2 oznaczenia każdego parametru. Łącznie dla każdego obiektu uzyskano po 6 wyników. Na podstawie oznaczeń wyliczono ilość azotu znitryfikowanego oraz zimmobilizowanego, a także oporność (RS) procesu nitryfikacji i bakterii nitryfikacyjnych na zanieczyszczenie gleby cynkiem oraz zdolność powrotu tych cech do stanu równowagi (RL).

Stwierdzono, że nadmiar cynku w glebie powoduje istotne zaburzenia procesu nitryfikacji. Nawet 300 mg Zn^{2+} kg^{-1} s.m. gleby istotnie hamuje ten proces. Zanieczyszczenie cynkiem zakłóca nie tylko proces nitryfikacji, ale także inne procesy metabolizmu azotu glebowego, o czym świadczy zmniejszająca się immobilizacja azotu wraz ze zwiększeniem stopnia zanieczyszczenia gleby tym pierwiastkiem.

Niekorzystne oddziaływanie cynku na proces nitryfikacji wynika głównie z negatywnego oddziaływania nadmiaru tego pierwiastka w środowisku glebowym na bakterie nitryfikacyjne. Cynk w większym stopniu hamuje rozwój bakterii I fazy nitryfikacji niż II fazy, ale bakterie I fazy nitryfikacji szybciej powracają do stanu równowagi niż II fazy. Współczynniki oporności (RS) procesu nitryfikacji na zanieczyszczenie cynkiem kształtowały się na niskim poziomie i malały wraz z pogłębiającym się stopniem zanieczyszczenia. Oporność bakterii nitryfikacyjnych na działanie cynku była tym mniejsza, im większa jego ilość znajdowała się w glebie.

Słowa kluczowe: nitryfikacja, zanieczyszczenie cynkiem, oporność nitryfikacji na zanieczyszczenie, bakterie nitryfikacyjne.

INTRODUCTION

Nitrification is a key process in nitrogen cycling in the environment (Ros et al. 2011, Gómez-Rey et al. 2012, He et al. 2012). Without nitrification it would be impossible to remove excess amounts of nitrogen compounds from water and wastewater (Munz et al. 2012, Zeng et al. 2012) as it precedes the process of denitrification, which leads to reduction of nitrates to volatile nitrogen oxides and molecular nitrogen. On the other hand, nitrification in the soil environment creates a risk of nitrogen loss due to the leaching of NO_3^- (Abaas et al. 2012) outside the rhizosphere of plants and *via* nitrogen escape because nitrates are acceptors of electrons from the organic matter undergoing oxidation by denitrifying bacteria.

Both ammonium and nitrate nitrogen are easily absorbed by plants (Dzida et al. 2012) , although the ammonium cation is more stable in soil than the nitrate anion (Abaas et al. 2012). Beside, whenever nitrification becomes too intensive, for example due to over-fertilization, plants and especially nitrophilous species tend to accumulate excessive amounts of nitrates (Kucharski 1985, Smoleń et al. 2012). Nonetheless, the factors which favour an intensive course of nitrification in soil are also the ones which create optimal conditions for the growth and development of most crops. Soils in which nitrification proceeds undisturbed are well tilled for plant cultivation, their C:N ratio is close to 12-16, the soil reaction is neutral and the physicochemical properties are suitable for growing crops (Szukics et al. 2012). For this reason, the nitrification process in soil is often erroneously claimed to play a positive part in agricultural practice (Kucharski 1985). But the same characteristics make nitrification a good indicator of soil contamination, for example with herbicides (Kucharski, Wyszkowska 2008, Baćmaga et al. 2012), aromatic hydrocarbons (Kucharski et al. 2009, 2010, Wyszkowski, Sivitskaya 2012) and heavy metals (Donner et al. 2010, Ruyters et al. 2010, Kucharski et al. 2011, Trevisan et al. 2012). All the factors which retard the activity of soil enzymes also modify the enzymes responsible for oxidation of ammonia nitrogen (Trevisan et al. 2012). The same effect is produced by heavy metals (Mertens et al. 2010), although in this case their toxic effect on nitrifying bacteria plays a role as well (He et al. 2012).

Zinc is one of the heavy metals which can interfere with the metabolism of soil (Donner et al. 2010, Ryuters et al. 2010, Kucharski et al. 2011, Trevisan et al. 2012). Excessive amounts of zinc in soils are possible in industrial regions. Zinc may also occur as a point pollutant in soils lying in agricultural regions. The above considerations have encouraged us to undertake the present study with an aim of analyzing the course of nitrification in soil polluted with zinc.

MATERIAL AND METHODS

The study was conducted in laboratory conditions. Sandy loam, whose characteristics are specified in Table 1, was used as the test soil. The protocol of the experiment was as follows: soil sampled from the 0-20 cm layer of a cropped field was passed through a 2 mm mesh sieve and then placed in 150 cm^3 glass beakers, 100 g of soil in each, where it was polluted with the following doses of zinc per 1 kg d.m. of soil: 0, 300, 600, 1 200 and 2 400. Zinc was added in the form of $ZnCl_2$ aqueous solution. Afterwards, ammonia nitrogen as $(NH_4)_2SO_4$ was added to the soil material in the amounts of 0 and 240 mg N kg^{-1} d.m. Once zinc chloride and ammonia sulphide had been carefully mixed with the soil, the soil moisture content was raised to 50% of capillary water holding capacity and then the beakers were transfer-

Table 1

Some physicochemical properties of the soil

Soil texture (grain diameter in mm)			C_{org} (g kg^{-1})	Zn^{2+} (mg kg^{-1})	pH_{KCl}	Hh	S	T	V (%)
2 – 0.05	0.05–0.002	< 0.002							
Content (%)						(mmol$^{(+)}$ kg^{-1} d.m. soil)			
72	21	7	7.05	16.60	7.0	8.00	111.00	119.00	93.28

C_{org} – organic carbon content, Zn^{2+} – total zinc content, pH_{KCl} – pH in 1 M KCl, Hh – hydrolytic acidity, S – sum of exchangeable cations, T – total soil adsorption capacity, V – base saturation

red to a laboratory incubator set at a temperature of 25°C. After 10, 20, 30 and 40 days, the incubated soil was analyzed to determine the concentrations of N-NH_4 and N-NO_3. Additionally, after 10 and 40 days of incubation, the most probable number (MPN) of bacteria of the first and second step of nitrification were determined. The experiment was run with three replications for each of the four determination days. The samples kept in the beakers were submitted to determinations in two replicates. In total, 6 results were obtained for each experimental variant. A separate experimental series had been prepared for each determination day and – once the content of the nitrogen forms and counts of the bacteria were determined – it was disposed of.

Mineral nitrogen was extracted from soil with 1% aqueous solution of K_2SO_4. The soil to potassium sulphate ratio was 1 : 5. The extracts underwent determinations of the content of N-NH_4 with Nessler's reagent and N-NO_3 with phenoldisulphic acid (Kucharski et al. 2009). The most probable number of the first and second stage nitrification bacteria was determined according to Kucharski et al (2009).

The results of mineral nitrogen determinations were used to calculate amounts of nitrified nitrogen (N_{nit}) and immobilized nitrogen (N_{im}) from the following formulas:

$$N_{nit} = \frac{N_0}{N_d} \cdot 100,$$

where:

N_{nit} – nitrified nitrogen in %,

N_0 – content of N-NO_3 in the fertilized treatment minus the content N-NO_3 in the unfertilized treatment,

N_d – content of N-NH_4 and N-NO_3 in the fertilized treatment minus the content of N-NH_4 and N-NO_3 in the unfertilized treatment,

100 – conversion factor to %,

$$N_{im} = \frac{D - N_d}{D} \cdot 100,$$

N_{im} – immobilized nitrogen in %,

D – dose of N-NH_4,

N_d – content of N-NH_4 and N-NO_3 in the fertilized treatment minus the content of N-NO_3 in the unfertilized treatment,

100 – conversion factor to %.

Additionally, the resistance (RS) of the nitrification process and nitrifying bacteria to soil contamination with zinc and the resilience of nitrification and nitrifying bacteria in soils contaminated with zinc were calculated from the formulas proposed by ORWIN and WARDLE (2004):

$$RS = 1 - \frac{2\left|D_0\right|}{C_0 + \left|D_0\right|},$$

$$RL = \frac{2\left|D_0\right|}{\left(\left|D_0\right| + \left|D_x\right|\right)} - 1,$$

D_0 – difference between the control (C_0) and contaminated soil (P_0) in time t_0,

D_x – difference between the control (C_x) and contaminated soil (P_x) in time t_x.

The results were submitted to three-factor analysis of variance (Anova), using the Duncan's test. All the calculations were aided with the Statistica version 10 software (StatSoft, Inc. 2011).

RESULTS AND DISCUSSION

The content of ammonia nitrogen (Table 2) and nitrate nitrogen (Table 3) in soil was a function of the soil contamination with zinc, ammonia sulphate fertilization and incubation time. In the unpolluted treatments, the amount of nitrogen increased significantly until day 20 of incubation, but began to decline on later days. In the nitrogen fertilized treatments, it decreased steadily as the incubation continued, so that on day 40 it was nearly 11-fold lower than on day 10. Zinc contamination of soil resulted in maintaining a high pool of ammonia nitrogen (Table 1) and a low amount of nitrate

Table 2

Content $N\text{-}NH_4$ in 1 kg d.m. soil (mg N)

Dose of Zn^{2+} (mg kg^{-1} d.m. soil)	Dose N (mg kg^{-1})							
	0				240			
	days of soil incubation							
	10	20	30	40	10	20	30	40
0	19.094	58.862	40.618	4.950	142.062	66.199	48.950	13.341
300	16.871	57.857	39.504	2.552	164.528	165.849	154.744	119.540
600	23.997	56.789	41.039	25.453	171.899	216.431	250.632	203.198
1200	13.362	55.443	58.406	21.891	171.305	237.602	272.062	207.142
2400	9.503	46.032	74.558	18.903	184.466	241.782	284.254	208.343
r	-0.776	-0.971	0.972	0.543	0.843	0.755	0.783	0.710
LSD	*a* – 1.925; *b* – 1.722; *c* – 1.218; *ab* – 3.851; *ac* – 2.723; *bc* – 2.436. *abc* – 5.446							

LSD for *a* – zinc dose, *b* – incubation time, *c* – N dose, *r* – coefficient correlation

Table 3

Content of $N\text{-}NO_3$ in 1 kg d.m. soil (mg N)

Dose of Zn^{2+} (mg kg^{-1} d.m. soil)	Dose N (mg kg^{-1})							
	0				240			
	days of soil incubation							
	10	20	30	40	10	20	30	40
0	27.281	49.549	40.443	40.482	60.674	208.722	229.711	221.440
300	7.948	27.095	32.999	27.065	21.983	85.624	109.346	104.225
600	6.612	8.893	10.162	10.812	18.123	27.566	25.795	29.384
1200	3.151	7.314	7.895	7.858	14.344	20.348	19.260	24.403
2400	1.228	6.116	7.722	7.723	11.364	18.312	18.612	19.259
r	-0.720	-0.735	-0.762	-0.761	-0.678	-0.694	-0.708	-0.714
LSD	*a* – 0.725; *b* – 0.648; *c* – 0.458; *ab* – 1.449; *ac* – 1.025; *bc* – 0.917. *abc* – 2.050							

Explanations see Table 1

nitrogen (Table 3) in soil. The content of nitrate nitrogen in both fertilized and unfertilized soil decreased as the degree of zinc pollution increased. The coefficients of the correlation between the content of $N\text{-}NO_3$ and dose of zinc were significantly negative and ranged from -0.720 to -0.762 in soil not fertilized with nitrogen and from -0.678 to -0.714 in nitrogen amended soil.

The intensity of nitrification in the analyzed soil was relatively high, as nearly 96% of ammonia nitrogen in the control treatment, with no zinc contamination, had been oxidized to nitrate nitrogen before day 20 of incubation (Table 4). Zinc, however, significantly inhibited the process. On day 40 of incubation, the dose of 300 mg Zn^{2+} kg^{-1} depressed the amount of nitrified nitrogen by 2.4-fold, whereas the highest dose, i.e. 2 400 mg Zn^{2+} kg^{-1}, reduced it by almost 17-fold. The coefficients of the correlation between the dose of zinc and the amount of nitrified nitrogen were significantly negative on each day of the determinations.

The values of the resistance index (RS) of the nitrification process to zinc pollution fluctuated on a low level (Table 5) and tended to decline under higher degrees of pollution. In the treatments polluted with 300 mg Zn^{2+} kg^{-1}, they varied from 0.171 to 0.689, and in the ones contaminated with 2 400 mg Zn^{2+} kg^{-1} they ranged from 0.023 and 0.106. The adverse effect of excess zinc in soil was persistent, which is confirmed by the negative values of the soil resilience index (Table 6). They varied from -0.181 to -0.179 in unfertilized soil and from -0.504 to -0.637 in soil fertilized with nitrogen.

The surplus zinc in soil also contributed to lower counts of nitrifying bacteria in soil (Tables 7, 8). The most probable count of the first step nitrification bacteria under the effect of 300 mg Zn^{2+} kg^{-1} declined by 3.5-fold, under 600 mg Zn^{2+} kg^{-1} it was 8.8-fold lower, the dose of 1 200 mg Zn^{2+} kg^{-1}

Table 4

Amounts of nitrified nitrogen (%)

Dose of Zn^{2+} (mg kg^{-1} d.m. soil)	Days of soil incubation				Average
	10	20	30	40	
0	21.356	95.594	95.784	95.568	77.075
300	8.680	35.148	39.850	39.743	30.855
600	7.221	10.472	6.941	9.460	8.524
1200	6.618	6.677	5.050	8.199	6.636
2400	5.476	5.865	4.937	5.740	5.504
r	-0.662	-0.686	-0.691	-0.699	-0.692

Table 5

Resistance index (RS) values of nitrification to zinc contamination of soil

Dose of Zn^{2+} (mg kg^{-1} d.m. soil)	Dose N (mg kg^{-1})							
	0				240			
	days of soil incubation							
	10	20	30	40	10	20	30	40
300	0.171	0.376	0.689	0.502	0.221	0.258	0.312	0.308
600	0.138	0.099	0.144	0.154	0.176	0.071	0.059	0.071
1200	0.061	0.080	0.108	0.107	0.134	0.051	0.044	0.058
2400	0.023	0.066	0.106	0.105	0.103	0.046	0.042	0.045
r	-0.946	-0.661	-0.630	-0.662	-0.933	-0.663	-0.629	-0.657

Table 6

Resilience index (RL) values of nitrification

Dose of Zn^{2+} (mg kg^{-1} d.m. soil)	Dose N (mg kg^{-1})	
	0	240
300	0.181	-0.504
600	-0.179	-0.637
1200	-0.150	-0.619
2400	-0.114	-0.608
r	-0.457	-0.428

Table 7

First step nitrification bacteria (MPN 10^6 kg^{-1} d.m. soil)

Dose of Zn^{2+} ($mg\ kg^{-1}$ d.m. soil)	Dose N ($mg\ kg^{-1}$)					
	0			240		
	days of soil incubation					
	10	40	average	10	40	average
0	10.933	1.093	6.013	10.933	0.787	5.860
300	3.666	0.787	2.227	2.533	0.787	1.660
600	1.093	0.480	0.787	1.093	0.243	0.668
1200	1.093	0.243	0.668	0.361	0.098	0.230
2400	0.046	0.011	0.029	0.072	0.011	0.041
r	-0.711	-0.927	-0.736	-0.666	-0.846	-0.692
LSD	*a* – 0.184; *b* – 0.116; *c* – 0.116; *ab* – 0.260; *ac* – 0.260; *bc* – 0.164; *abc* – 0.367					

Table 8

Second step nitrification bacteria (MPN 10^6 kg^{-1} d.m. soil)

Dose of Zn^{2+} ($mg\ kg^{-1}$ d.m. soil)	Dose N ($mg\ kg^{-1}$)					
	0			240		
	days of soil incubation					
	10	40	average	10	40	average
0	36.667	25.333	31.000	48.000	22.267	35.133
300	25.333	3.667	14.500	25.333	2.533	13.933
600	25.333	2.533	13.933	25.333	2.147	13.740
1200	18.067	0.787	9.427	2.533	1.093	1.813
2400	14.000	0.243	7.121	1.093	0.787	0.940
r	-0.895	-0.625	-0.768	-0.865	-0.589	-0.805
LSD	*a* – 1.552; *b* – 0.982; *c* – 0.982; *ab* – 2.195; *ac* – 2.195; *bc* – 1.388; *abc* – 3.104					

depressed the count by more than 25-fold and the highest dose, 2 400 mg Zn^{2+} kg^{-1}, resulted in a nearly 143-fold lower count of bacteria. The same doses of zinc caused smaller changes in counts of nitrite-oxidizing bacteria than ammonia-oxidizing ones (Table 8). Thus, the lowest dose of zinc, i.e. 300 mg Zn^{2+} kg^{-1}, decreased the most probable count of the second step nitrification bacteria by 2.5-fold, the second dose – by 2.6-fold, the third dose – by 19.4-fold and the fourth dose – by 37.4-fold.

The resistance of nitrifying bacteria to zinc tended to weaken as the quantity of the pollutant in soil rose (Table 9). However, the resistance of ammonia-oxidizing bacteria was weaker on day 10 of incubation than on day 40, whereas that of nitrite-oxidizing bacteria was stronger on day 10 than on day 40. The first step nitrification bacteria recovered faster than the second step nitrification bacteria from the disorders caused by zinc (Table 10). The mean RL index, irrespective of the fertilization or zinc dose, was 0.888 for the first step nitrification bacteria and 0.004 for the second step nitrification bacteria. Such a low RL index for nitrate forming bacteria was due to the negative values of this parameter in the treatments not fertilized with nitrogen.

The intensity of nitrification is manifested by the correlations between the concentrations of ammonia nitrogen and nitrate nitrogen. In soil with optimal conditions for the development of nitrifying bacteria, $N\text{-}NH_4$ is relatively quickly oxidized to $N\text{-}NO_3$, meaning that as the amount of nitrate

Table 9

Resistance index (RS) values of nitrifying bacteria to zinc contamination of soil

Dose of Zn^{2+} (mg kg^{-1} d.m. soil)	Nitrification bacteria							
	first step				second step			
	dose N (mg kg^{-1})							
	0		240		0		240	
	days of soil incubation							
	10	40	10	40	10	40	10	40
300	0.201	0.563	0.131	1.000	0.528	0.078	0.358	0.060
600	0.053	0.281	0.053	0.182	0.528	0.053	0.358	0.051
1200	0.053	0.125	0.017	0.066	0.327	0.016	0.027	0.025
2400	0.002	0.005	0.003	0.007	0.236	0.005	0.012	0.018
r	-0.782	-0.888	-0.819	-0.702	-0.945	-0.902	-0.855	-0.915

Table 10

Resilience index (RL) values of nitrifying bacteria

Dose of Zn^{2+} (mg kg^{-1} d.m. soil)	Nitrification bacteria			
	first step		second step	
	dose N (mg kg^{-1})			
	0	240	0	240
300	0.919	1.000	-0.313	0.069
600	0.883	0.895	-0.336	0.060
1200	0.841	0.878	-0.138	0.365
2400	0.819	0.867	-0.051	0.372
r	-0.924	-0.726	0.936	0.845

nitrogen increases, the content of ammonia nitrogen falls down (SZUKICS et al. 2012). This dependence could also be observed in treatments not polluted with zinc (Tables 2, 3), but excessive amounts of zinc introduced to soil interfered with its homeostasis, which was evidenced by the negative response of the first (Table 7) and second (Table 8) step nitrification bacteria to this contamination, as a result of which the rate of ammonia nitrogen oxidation was reduced (Table 4) or even almost completely halted when higher doses of zinc had been added to soil. The adverse effect of zinc on nitrification is most probably caused by both the direct toxic effect of excess zinc on nitrifying bacteria (RUYTERS et al. 2010) and its influence on the enzymes responsible for this process (TREVISAN et al. 2012). The negative influence of zinc on autochthonous soil microorganisms is well-documented in literature (KUCHARSKI et al. 2000, ZABOROWSKA et al. 2006, WYSZKOWSKA et al. 2007, 2008, RUYTERS et al. 2010) and proven by a rapidly depleting pool of immobilized

Table 11

Amounts of immobilized nitrogen (%)

Dose of Zn^{2+} (mg kg^{-1} d.m. soil)	Days of soil incubation				Average
	10	20	30	40	
0	34.850	30.620	17.667	21.105	26.060
300	32.628	30.617	20.172	19.105	25.630
600	33.578	25.702	6.156	18.201	20.909
1200	29.527	18.670	6.242	15.918	17.589
2400	22.875	13.356	8.089	16.260	15.145
r	-0.981	-0.969	-0.621	-0.834	-0.935

nitrogen under its effect (Table 11). The present study shows that the average immobilization of nitrogen in the control treatment was 26%, but in the one contaminated with 2 400 mg Zn^{2+} kg^{-1} it fell down to 15%. Irrespective of the dose of zinc, nitrogen immobilization oscillated from 35% to 8% on particular days of determinations.

CONCLUSIONS

1. Excess zinc in soil causes considerable disorders in the process of nitrification. Even the lowest tested dose, 300 mg Zn^{2+} kg^{-1} d.m. of soil, significantly inhibited nitrification.

2. Apart from interfering with nitrification, zinc contamination also disrupts other soil nitrogen metabolic processes, which is demonstrated by the decreased nitrogen immobilization under the effect of a higher degree of soil pollution with zinc.

3. The adverse influence of zinc on nitrification is mainly due to the negative effect of excess zinc in the soil environment on nitrifying bacteria.

4. Zinc more strongly inhibits the development of the first than the second step nitrification bacteria, but the former bacteria recover faster.

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