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Effect of liming on nickel availability for oilseed rape (*Brassica napus* L.) grown on a sod-podzolic soil from the region of Saint-Petersburg, Russia

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Abstract

Pollution of soils by heavy metals is one of the most serious problems in soil protection and for the production of safe and healthy food. In this context nickel (Ni) still remains one of the most harmful elements among heavy metals. The aim of this investigation was to quantify the effect of liming on the soil-plant transfer of Ni. In a pot experiment employing a sod-podzolic acid sandy-loam soil (pH_{KCl}4.1), oilseed rape was grown for up to 43 days with and without Ni contamination (20 mg kg^{-1} Ni) and increasing lime supply (0 - 2.1 g kg^{-1}). Lime application showed a distinct optimum in reducing Ni transfer into plants, which was most effective at a rate of 1.24 g kg^{-1}.

Key words: heavy metals, liming, nickel, oilseed rape, sod-podzolic soils, transfer factors

Zusammenfassung

Einfluss von Kalkung auf die Verfügbarkeit von Nickel für Körnerraps (*Brassica napus* L.) auf einem Podsol aus der Region Sankt Petersburg, Russland

Die Belastung von Böden mit Schwermetallen ist eines der ernsthaftesten Probleme des Bodenschutzes und für die Erzeugung sicherer und gesunder Nahrung. In diesem Kontext ist Nickel (Ni) eines der gefährlichsten unter den Schwermetallen. Ziel der hier vorgestellten Untersuchung war es, in einem Gefäßversuch den Einfluss von Kalkung auf den Transfer von Ni aus dem Boden in Rapspflanzen zu quantifizieren. Die Pflanzen wurden hierzu auf einem sauren lehmigen Sand aus Podsol (pH_{KCL}4,1) für 43 Tage mit und ohne Ni (20 mg kg^{-1} Ni) und bei steigenden Kalkgaben (0 - 2,1 g kg^{-1}) kultiviert. Der Einfluss der Kalkung auf die Reduzierung der Ni-Aufnahme der Pflanzen zeigte dabei ein deutliches Optimum bei 1,24 g kg^{-1}.

Schlüsselwörter: Kalkung, Nickel, Podsol, Raps, Schwermetalle, Transferfaktoren

1 Introduction

Pollution with heavy metals is one of the main problems in biosystems ecology. One of the most important subjects for research in this area is the development of methods for reducing the transfer of heavy metals into agricultural products and the food chain.

Nickel (Ni) is one of the most harmful elements (Iljin, 1991) but simultaneously Ni is essential for mammals as a micronutrient and occurs in many biological structures. At higher concentrations Ni turns out to become very toxic mainly inducing carcinogen processes (Saprikin, 1999). In plants Ni is found in the structure of urease (Walker et al., 1985), and is considered as an essential plant micronutrient.

The average total Ni concentration in soil on a global basis is estimated to 20 mg kg^{-1} Ni (Quipilg et al., 1984) with an overall range from 10 to 1000 mg kg^{-1} Ni (Jagodin et al., 1991). Nickel concentrations in anthropogenically-polluted soils can reach 200-26000 mg kg^{-1} Ni. The primary sources of Ni pollution are the burning of coal and oil, emissions of smelters and metal-works, municipal wastes, sewage, phosphate fertilisers and pesticides. In Russia the area most heavily polluted with Ni is the Kola Peninsula due to vast numbers of smelters and metal-works in this region. Only 5 km away from the smelting enterprise “Northnickel” close to the town Monchegorsk the Ni concentrations in the soil are 40 times higher than under natural conditions, 15 km away its 15-20 times and 25 km away, still 4-5 times (Evdokimova, 1990).

The risks of heavy metal transfer into the food chain are a function of the mobility of the heavy metal species and their availability in the soil (Richards, 1999). For the extraction of mobile forms of heavy metals different kinds of extractants are used: Large amounts of heavy metals are extracted by 1.0 M mineral acids and the species extracted are considered to represent the pool of the total concentration, which has potential to be mobilised. The concentrations extracted by an acetate-ammonium buffer solution characterises the more mobile pool of the element in question. Even more mobile is the exchangeable form of the element extracted by neutral salts, which is also considered as the most available fraction for plants (Gorbatov et al., 1987). Haq et al. (1980) evaluated the effectiveness of strong and weak acids, as well as chelates to extract Ni and revealed the following order of effectiveness: DTPA (Diaminetetraacetic acid) > EDTA (Ethylenediaminetetraacetic acid) > NTA (Nitrilotriacetic acid) > CH_3COOH (acetic acid) > H_2O. But obviously no extrac-

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tant can precisely measure the metal availability for plants. For this, it is necessary to take into account a wider range of soil properties such as clay, organic matter and hydrous oxides content, pH and CEC.

There are a lot of evidences that genotypical peculiarities of plants have considerable influence on heavy metal uptake (Kabata-Pendias and Pendias, 1991; Schnug and Strampe, 1988). Moreover heavy metal accumulation varies in different plant organs and depends on plant age (Ovcharenko M., 1997).

For a quantitative estimation of heavy metal availability for plants, commonly a coefficient is used, which takes into account both properties of plants and soil, the so called "Transfer Factor" [TF, ratio of element concentration in plants (mg kg^{-1}) to element concentration in soil (mg kg^{-1})]. Guo et al. (1995) reported from an experiment conducted on a Luvisol with a total Ni content of 29.7 mg kg^{-1} TF values of 0.40, 0.65 and 0.50 for beans, ryegrass and curly kale, respectively. In non contaminated controls the TF for the same plant species was 0.094, 0.100 and 0.094 respectively. In another experiment on a sandy soil with a Ni concentration of 50 mg Ni kg^{-1}, the TF for ryegrass was 2.98. The fact that the TF depends on the level of Ni contamination as well as the soil type has also been reported. In an experiment with ryegrass grown on an uncontaminated podzolic sandy soil, the TF was 0.53. In the same experiment, but on the chernozem soil, the TF was 4.5 times lower on contaminated soil and 1.4 times lower in the uncontaminated control (Vago et al., 1997).

Liming is considered to be an economically acceptable measure that generally helps to reduce transport of heavy metals into the food chain. Addition of $Ca(OH)_2$ decreased the concentration of the soluble + exchangeable Cd fraction but increased the concentration of inorganic-bound Cd fractions in soil (Bolan et al, 2003). Since there was no direct evidence for $CdCO_3$ or $Cd(OH)_2$ precipitation in the variable charge of the soil used for the plant growth experiment, Bolan et al. (2003) concluded that the alleviation of phytotoxicity can be attributed primarily to the immobilisation of Cd by an enhanced pH-induced increase in negative charge. This is confirmed by findings of Nebolsin (2000) who reported from an experiment with vetch and barley plants a general decrease of Ni uptake with increasing lime doses. In an experiment conducted by Cho et. al. (1996) with radish the Ni content in shoots and roots was negatively correlated with an increasing pH.

The objective of the research presented in this paper was to quantify the effects of liming on the soil/plant transfer of Ni and its translocation in *Brassica napus* plants during the vegation period.

2 Materials and methods

The pot experiment was conducted at the Pushkin branch of the Agricultural Physical Research Institute in Saint-Petersburg, Russia. An acid sod-podzolic sandy-loam soil (according to the Russian soil classification system) or a dystric cambisol (according to the FAO-Unesco soil classification) was used for this study. It is a typical arable soil in the territories of ancient glacier transition. This soil is characterised by a low organic matter content (1.7 %), acid reaction (pH_{KCl} 4.1, hydrolytic acidity 3.9), and low contents of plant available (1M HCl) phosphorus and potassium (42 mg P kg^{-1} and 83 mg K kg^{-1} soil dry weight basis). The moist soil was passed through a 5-mm sieve before mixing thoroughly with fertiliser, lime and $Ni(NO_3)_2 \cdot 6H_2O$. The plastic pots employed in the experiment contained 5 kg of soil (dry weight basis).

The investigated crop was a spring variety of oilseed rape (*Brassica napus* L.) which was sown in May at a density of 15 plants per pots. The experiment was conducted outdoors under a plastic shelter. Nutrients and water were applied sufficiently for optimum growth.

The experimental design included 4 treatments plus a control:

1. Control
2. 20 mg Ni kg^{-1} + lime 0.83 g kg^{-1}
3. 20 mg Ni kg^{-1} + lime 1.25 g kg^{-1}
4. 20 mg Ni kg^{-1} + lime 1.66 g kg^{-1}
5. 20 mg Ni kg^{-1} + lime 2.10 g kg^{-1}

All treatments were carried out in three replicates.

Table 1:
Changes of soil pH (KCl) as influenced by lime application and plant growth (numbers in brackets are standard deviations)

Lime dose, g kg^{-1}	Sampling time, days after planting				
	14	21	29	36	43
	pH				
0	4.11 ± 0.02	4.04 ± 0.01	3.97 ± 0.02	3.83 ± 0.07	3.80 ± 0.03
0.83	4.84 ± 0.07	4.69 ± 0.05	4.52 ± 0.04	4.23 ± 0.01	4.22 ± 0.02
1.25	5.16 ± 0.07	5.14 ± 0.05	4.61 ± 0.04	4.41 ± 0.07	4.40 ± 0.01
1.66	5.54 ± 0.04	5.60 ± 0.05	4.97 ± 0.08	5.09 ± 0.08	5.13 ± 0.04
2.10	5.73 ± 0.01	5.42 ± 0.07	5.17 ± 0.05	5.17 ± 0.01	5.25 ± 0.01

Table 2:
Parameters for the sigmoidal logistic function, type 3 description of dry matter development of *Brassica napus* grown on a sod podzolic soil from Saint Petersburg area as affected by lime application.

Lime dose, g kg^{-1}	R^2, %	M_{max}, g per pot	M_0, g per pot	μ, days^{-1}	T, days
Control	95	3.2 ±0.5	0.011±0.026	0.21±0.10	3.3
0.83	99	10.3 ± 2.1	0.048 ± 0.034	0.15 ± 0.03	4.6
1.25	99	14.0 ± 0.3	0.008 ± 0.003	0.23 ± 0.01	3.0
1.66	99	18.3 ± 0.3	0.004 ± 0.002	0.27 ± 0.02	2.6
2.10	99	19.9 ± 0.8	0.004 ± 0.005	0.29 ± 0.04	2.4

Remarks: R^2 - % of variability in dry matter yield explained by a logistic function, numbers apply to Equ. 1 in the text; T – period of time in which dry matter is doubled; T = 0.693μ^{-1}

Soil and plants were sampled at 14, 21, 29, 36 and 43 days after planting. The last sampling time coincided with the flowering phase. All yields are given on a dry matter basis.

2.1 Chemical analysis

a) Soil analysis:
- pH in 1M KCl suspension (soil:solution = 1:2.5);
- plant available phosphorus (P) and potassium (K) extracted by 1.0M HCl (soil:acid = 1:5);
- Ca extracted by 1M KCl (soil:solution = 1:2.5);
- Ni extracted by ammonium-acetate buffer pH 4.8 (soil:buffer = 1:10) and determined by atomic absorption spectrometry (Anonym, 1993).

b) Plant analysis:
- determination of Ca and Ni by atomic absorption spectroscopy following wet digestion with $HClO_4/HNO_3$.

2.2 Statistical analysis

Statistical analysis was conducted by means of the software package ORIGIN 6.0 using the routine for analysis of variance (ANOVA). Differences in the concentrations and yields were tested for significance with the t-test for normal distribution.

3 Results and discussion

3.1 Development of dry matter growth during the vegetation period

Addition of 20 mg Ni kg^{-1} to soil had no negative effect on growth and development of the plants. Also no visual symptoms of Ni toxicity on any plant part were detected. Lime addition considerably influenced the dry matter production beginning from the lowest dose 0.83 g kg^{-1} (table 2). This implicates a growth promoting effect of the lime application. However, it has to be mentioned here, that the control variant did receive no mineral fertilisers. Therefore the differences in absolute dry matter yield between the lime treatments are well comparable, but may be distorted when lime treatments are compared with control.

The dry matter development is described with a S-shaped curve, which is well approximated by a logistic function (Walter and Lampereht, 1976; Kletschenko, 1986; Warpholomeev and Kalyuzhny, 1990):

$$M_{(t)} = \frac{M_{\max}}{1 + \frac{M_{\max}}{M_0} e^{-\mu t}} \qquad \text{(Equ. 1)}$$

where:
M_{max} - maximum yield, g per pot;
M_0 - mass of seeds sown in the pot in g;
μ - speed constant for dry matter growth, days^{-1};
t - time in days.

M_0 - is a calculated value, the average is 0.015 ± 0.018 g per pot. Taking into consideration a determination error of calculated M_0 this value coincides virtually with the mass of seeds sown in each pot (M_0 = 0.04). The coincidence of these values allows to use the logistic equation in cited form and shows a high reliability of its parameters.

The maximum yield in the treatments with lime application exceeded the maximum yield in the control treatment without liming by 3.0, 4.4, 5.7 and 6.0 times, respectively. The most intensive dry matter production was observed in all treatments 23 to 35 days after sowing. The dry matter production decreased during the flowering of the plants (figure 1; days 35-43), which is common for most of plants (Batygin, 1986).

In the treatments not only the amount of yield was increased but also the specific speed of growth (μ, days^{-1}) was enhanced with each lime level. Unfortunately in scientific literature there are no data available describing lime influence on this important parameter, so it is impossible to compare the obtained values.

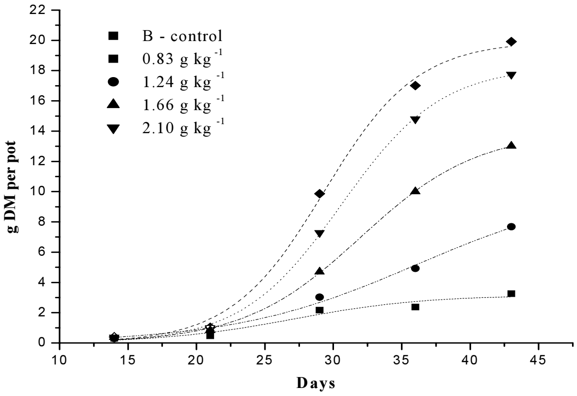

Fig.1:
Dry matter production of *Brassica napus* grown on a sod-podzolic soil from the Saint Petersburg as affected by lime application.

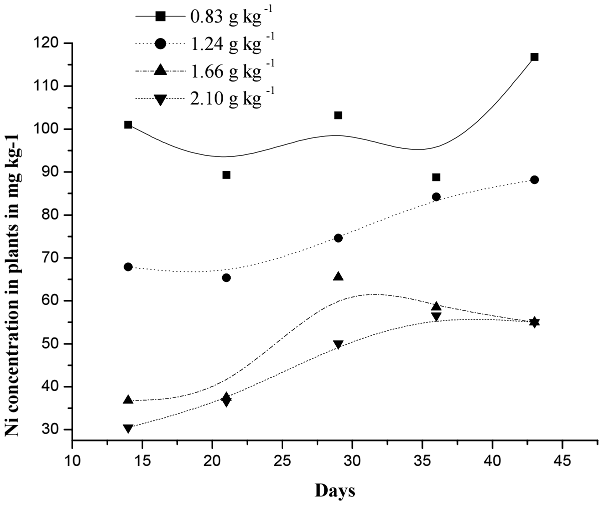

Fig. 2:
Influence of lime application on Ni concentrations during the vegetation period in *Brassica napus* grown on a sod-podzolic soil from the Saint Petersburg area.

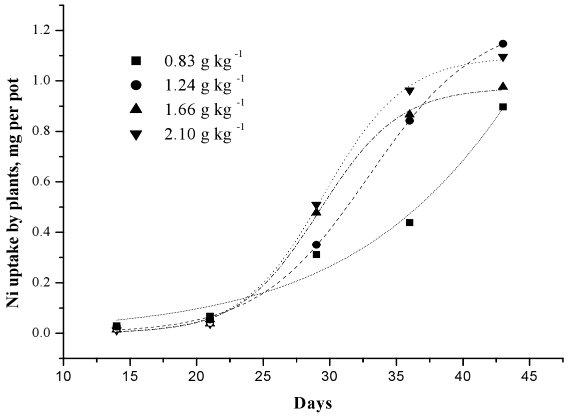

Fig. 3:
Influence of lime application on Ni uptake during vegetation period by *Brassica napus* grown on a sod-podzolic soil from the Saint Petersburg area.

3.2 Development of Ni concentrations in plants and Ni uptake during the vegetation period

In general Ni concentrations increased in the plants dry matter over time. Figure 2 shows clearly that increasing lime applications decreased Ni concentrations in the dry matter of the plants during vegetation period. The replicates of the 0.83 g kg^{-1} treatment showed an exceptional high variability, which caused also a larger variability of this particular treatment in Ni uptakes (figure 3).

But finally the differences in Ni concentrations between lime treatments did not show up when Ni uptake of the plants is to be compared. From figure 3 it is obvious that the increase in dry matter production by liming fully compensated for the decrease of Ni concentrations in the dry matter.

For the mathematical description of the change of Ni uptake by plants the following equation was employed:

$$A(t)=\frac{A_{\max}}{1+\frac{A_{\max}}{A_0}e^{-\varepsilon t}} \qquad \text{(Equ. 2)}$$

where:
$A_{\max}$: maximum value of Ni uptake by plants, mg per pot,
A_0: Ni uptake by seedlings, mg per pot,
ε: speed constant of Ni uptake, days^{-1},
t : time in days.

With lime dose increasing, the constant of speed (ε) of Ni uptake by plants increased (table 3). Thus on the last two treatments, the periods of time which is needed to double the Ni uptake (T, table 3) are smaller than those describing the speed of plant growth (T, table 2). The difference in constants of speeds should have an effect on the dynamics of an element concentration in plants during vegetation. If the speed of an element uptake surpasses the speed of plant growth, the concentration should increase in time (Drichko et al., 1994). This hypothesis is proved by the results given in figure 2. The expected pattern became most clearly apparent in the treatments with lime doses of 1.25-1.66 g kg^{-1}.

3.3 Development of transfer factors for Ni (TF Ni) during the vegetation period

Transfer Factors (TF) were calculated as the ratio of Ni concentration in plants to Ni concentration in soil, extracted with acetate-ammonium buffer pH 4.8. The tendency to decrease of TF factors with increasing lime doses (table 4) was observed.

The lime application showed a distinct optimum in reducing Ni transfer into the plants, which was most effec-

Table 3:
Parameters for the sigmoidal logistic function, type 3 describing Ni uptake by *Brassica napus* grown on a sod-podzolic soil from the Saint Petersburg area as affected by lime applications (see figure 3)

Lime dose, g kg^{-1}	R^2	A_{max}, mg/pot	A_0, mg/pot	ε, days^{-1}	T, days	C_0
0.83	98	4.90 ± 21	0.012 ± 0.036	0.10 ± 0.05	6.9	1.100
1.25	99	1.25 ± 0.03	0.0004 ± 0.00014	0.24 ± 0.01	2.9	0.008
1.66	99	0.97 ± 0.02	0.0005 ± 0.00005	0.34 ± 0.04	2.0	0.006
2.10	99	1.10 ± 0.03	0.00006 ± 0.00006	0.33 ± 0.04	2.1	0.015

Remarks: R^2 - % of variability in Ni uptake explained by a logistic function, numbers apply to Equ. 2 in the text; T – period of time in which Ni uptake is doubled; T = 0.693ε^{-1}; C_0 – Ni concentration in seedlings.

tive in interval of doses 1.25-1.66 g kg^{-1}. The minimum TF was observed on first phases of plants development, with a tendency of TF increasing simultaneously with increasing plants age (maturation), what is in line with investigations of Andreeva (2003), who showed the same tendency in an experiment with *Avena sativa* grown on Ni contaminated (25 mg kg^{-1}) sod-podzolic soil. In that experiment Ni Transfer Factor of oat plants was 1.24 in the phase of layering and increased up to 2.7 in the panicle phase.

Table 4:
Factors for Ni transfer into *Brassica napus* depending on lime application on a sod-podzolic soil from the Saint Petersburg area.

Lime dose, g kg^{-1}	Sampling time, day after planting				
	14	21	29	36	43
0.83	14.8	14.7	17.8	15.0	19.2
1.25	12.0	12.9	13.4	14.2	13.5
1.66	7.7	7.0	13.3	10.8	12.1
2.10	6.2	7.0	10.4	10.9	10.8

3.4 Dynamics of Ni concentration in plants depending on lime doses

For the description of the dynamics of changes in Ni concentrations in plants depending on lime doses the following equation for a sigmoidal Boltzman function was employed:

$$C_{(D)} = \frac{C_{\max} - C_{\min}}{1 + e^{(D - D_0)/\Delta D}} + C_{\min} \qquad \text{(Equ. 3)}$$

where:

C_{min} - Ni concentration in plants grown on soil with high lime dose,
C_{max} - Ni concentration in plants grown on soil with low lime dose,
D_0 - a lime dose under which the change of Ni concentration in plants on the unit of change of Ca concentration in soil is maximum,
$_{\Delta}D$ - constant, describing the velocity of changes in Ni concentrations from maximum towards minimum.

During the first phase of plants development the Ni concentration smoothly decreased with increasing lime dose. This tendency is kept on more mature phases of plants development, but in the flowering phase, which is critical for all plants, there was an extremely sharp change of Ni concentration (figure 4). It is characterised by the parameter $D_{1/2}$. The lower this parameter, the greater the changes of Ni concentrations in plants (table 5).

Due to negative D_0 the data calculated for the 29-th day of grwoth are unreliable. Applying a linear function for the description of Ni behaviour at this plant phase development might be more reasonable. In all periods of observation the parameter D_0 was almost constant and equal to 1.24 g kg^{-1}, which implies that D_0 is a characteristic parameter for Ca-Ni interactions.

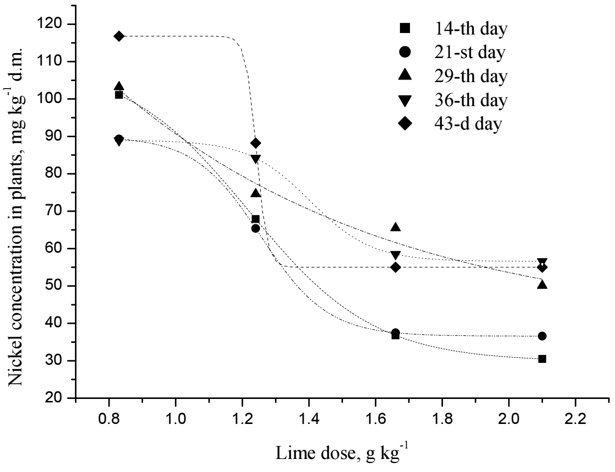

Fig. 4:
Influence of liming on Ni concentrations in *Brassica napus* grown on a sod-podzolic soil from the Saint Petersburg area.

Table 5:
Parameters of the sigmoidal Boltzman function for the description of changes in Ni concentrations in *Brassica napus* grown in a pot trial depending on lime applications on a sod-podzolic soil from the Saint-Petersburg area.

Sampling time, day after planting	R^2	C_{min}	C_{max}	D_0	$_{\Delta}D$
14	1.00	29.7	109.8	1.22	0.19
21	1.00	36.6	90.0	1.26	0.09
29	0.98	38.8	802.0	-1.0	0.38
36	1.00	56.6	88.0	1.24	0.09
43	1.00	55.0	116.8	1.24	0.01

Remarks: R^2 - % variability in Ni concentration in plants over time explained by a sigmoidal Boltzman function, numbers apply to Equ. 3 in the text.

4 Conclusions

The development of dry matter growth during vegetation period on all experiment treatments corresponded with the classical S-shaped curve, which is well approximated by the logistic equation. Specific speed of plant growth (μ) was increased by 43 % with three times increasing of Ca dose (figure 1, table 2). The same effect in the increase of the specific growth speed was shown in an experiment with timothy conducted by Drichko et al. (1994). In the first year of their experiment, doubling K doses did not influence the parameter μ at all, but in the second year of experimentation a 40 % increase was observed.

Ni uptake by plants during the vegetation period followed a S-shaped curve which is well described by logistic equation. It was shown that lime doses influenced the specific speed of Ni uptake to a greater extent than the specific speed of plants growth. Results have shown an increase of the specific speed of Ni uptake by 3.3 times with increasing lime dose (figure 3, tables 2 and 3).

Plants grown on limed soils usually contained less heavy metals than those grown on soils without lime additions (Alekseev, 1987). In the experiment presented here, lime doses influenced Ni concentration in plants the same way as it did on most of heavy metals. The dynamics of Ni concentration in plants depending on lime dose was described by a sigmoidal logistic function type 3 (figure 4, table 5). In conditions of this experiment the lime dose 1.24 g kg^{-1} was the distinct optimum for reducing Ni concentrations in plants (parameter D_0, table 5). Under this conditions the application of lower or higher doses seems to be non-effective. If the experiment conditions are changed (another kind of soil, plant, etc.), it is very likely that the parameter D_0 will be different as well. But anyway within the existing variability of acidity of agricultural soils the decrease in Ni concentration in plants due to interaction with Ca is possible not more than twice. This conclusion is in line with that of Drichko and Tsvetkova (1990) who analysed other pairs of elements, such as K-Cs, Sr-Ca, Sb-P, S-Se.

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