

Jolanta MOLAS<sup>1</sup>

## COMPARISON OF THE CONTENT OF SOME NITROGEN COMPOUNDS IN WHITE HEAD CABBAGE CULTIVATED IN SOIL POLLUTED WITH HYDRATED NICKEL AND CHELATE NICKEL

### PORÓWNANIE ZAWARTOŚCI NIEKTÓRYCH ZWIĄZKÓW AZOTU W KAPUŚCIE GŁOWIASTEJ BIAŁEJ UPRAWIANEJ NA GLEBIE ZANIECZYSZCZONEJ HYDRATEM I CHELATEM NIKLU

**Abstract:** A pot experiment was study the effects of hydrate ( $\text{NiSO}_4 \cdot 7\text{H}_2\text{O}$ ) and chelate (Ni-EDTA) nickel on white head cabbage crop and on the content of N-total, free amino acids, protein and exogenous amino acids. Added to soil whose pH was 6.2 in the amount of  $75 \text{ mg} \cdot \text{kg}^{-1}$  nickel in both chemical forms decreased the crop of dry mass of cabbage, the content of total nitrogen and the analysed compounds of this element. It also reduced the nutritious value of protein, measured by the content of exogenous amino acids. The level of reduction of the crop of dry mass of cabbage and the content of the analysed nitrogen compounds was much bigger in the presence of hydrated nickel ( $\text{NiSO}_4 \cdot 7\text{H}_2\text{O}$ ) than in the presence of chelate form of this element, ie Ni-EDTA and corresponded to the content of nickel in the plants.

**Keywords:** amino acid, cabbage, nickel toxicity, nitrogen, soil pollution, protein

At the end of the last century nickel was included into the group of microelements, mainly because it is a component of urease (EC 3.5.1.5) and participates in nitrogen metabolism in plants [1-4]. Therefore, nickel is considered to be an important element mainly for plants applied with urea as a N source [3-5]. Nickel has a positive influence on N assimilation and its metabolism when it occurs in trace quantities [1, 3-5], but disturbs the course of these processes when it occurs in excessive amounts [6, 7]. As a result, the qualitative and quantitative composition of nitrogen compounds in plants changes [6, 7], which, in turn, determines the nutritional value of consumer plants [8].

Studies conducted so far have shown that phytoavailability and phytotoxicity of nickel depend mainly on its speciation in soil [9-12]. As a transition metal, nickel tends to form chelate compounds in soil solution and is accessible to plants in the form of complex ions [9-11]. Furthermore, it is introduced to the soil in chelate compounds together with organic fertilizers and waste deposit [11]. Taking this fact into account, the objective of this study was to compare the effect of hydrated ( $\text{NiSO}_4 \cdot 7\text{H}_2\text{O}$ ) and chelatic (Ni-EDTA) forms of nickel applied to the soil with excessive amount on the content of N-total and some nitrogen compounds in white head cabbage cv. Gloria di Enkhouizen 2.

#### Materials and methods

White head cabbage cv. Gloria di Enkhouizen 2 was grown in pot cultures (3 plants per pot containing 12 kg of soil), on average-quality soil, whose granulometric composition was that of silt. The soil had the following properties: float particles - 28%, organic

<sup>1</sup> Department of Plant Biology, Faculty of Agricultural Sciences in Zamosc, University of Life Sciences in Lublin, ul. Szczepbrzeska 102, 22-400 Zamość, phone 84 677 27 32, email: jolanta.molas@up.lublin.pl

C -  $0.8 \text{ mg} \cdot \text{kg}^{-1}$ , cation exchange capacity -  $11.8 \text{ cmol (+)} \text{ kg}^{-1}$ , pH 6.2, total content of Ni -  $11.8 \text{ mg} \cdot \text{kg}^{-1}$ , content of soluble Ni in  $1 \text{ mol} \cdot \text{dm}^{-3} \text{ HCl}$  -  $2.4 \text{ mg} \cdot \text{kg}^{-1}$ . Basic fertilization was applied in the following amount per pot: 1g of N in the forms of  $\text{KNO}_3$  and  $\text{Ca}(\text{NO}_2)_2 \cdot \text{H}_2\text{O}$  (nitrate form), 0.5 g of P -  $\text{Ca}(\text{H}_2\text{PO}_4)_2$ , 0.8 of K - KCl and 0.3 mg of Mg in the form of  $\text{MgSO}_4$ . The plants were grown in green house at  $20^\circ\text{C}$  and at relative humidity of 75% for a long (16-hour) photoperiod with light intensity of  $180 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ . Nickel was added to the soil at the stage of initiation of the leaf curling in the forms of  $\text{NiSO}_4 \cdot 7\text{H}_2\text{O}$  and Ni-EDTA (Me:L 1:1) in the amount which corresponds to the 2<sup>nd</sup> degree of pollution of average soil with this element, ie in the amount of  $75 \text{ mg Ni} \cdot \text{kg}^{-1}$  of soil.

The plants were harvested at the stage of loose head. Next, the crop of fresh and dry mass of head was measured. Ni content in dry mass of cabbage was determined using an atomic absorption spectrophotometer (Perkin Elmer, model 1100), according to the method described by Ostrowska et al [13]. Total-N content in dry mass of cabbage head was estimated by Kjeldahl method [13] on the Kjeltec Tecador automatic apparatus. Total protein content was calculated as a product of total-N concentration and protein coefficient (total-N x 6.25). The content of exogenous amino acids was determined on the automatic amino acid analyser (*Amino Acid Analyser T 339 M*) after a prior hydrolysis of the samples in  $6 \text{ mol} \cdot \text{dm}^{-3} \text{ HCl}$  for 24 h at  $110^\circ\text{C}$ . Sulphur amino acids were not determined. The content of free amino acids was determined according to the Weibull et al method [14], after deprotenization of the dried leaf tissues with 5% sulphosalicylic acid.

Statistical analysis: Variance analysis (ANOVA) of experimental plant parameters was carried out, followed by a test checking the significant difference with probability of 0.05 (LSD).

## Results and discussion

Added in excessive amounts to average soil, whose reaction was slightly acid (pH 6.2), nickel caused reduction of the crop of dry mass of head of white cabbage cv. Gloria di Enkhouizen 2; this element also affected the content of dry mass in fresh mass of head (Tab. 1). The effect depended on the chemical form of this element applied to the soil. When the soil was polluted with hydrated and chelate form of nickel, the crop of head dry mass was smaller by 36.9 and 16.9% respectively, than when the soil was not polluted with this metal. However the percentage of dry mass in the fresh mass of head was bigger than in control; by 9.8% and by 2% in the presence of hydrated nickel and chelate Ni-EDTA respectively (Tab. 1). These and earlier results imply that the observed increase in the content of dry mass in the fresh mass of cabbage head was connected with plant adaptation to growth in nickel stress conditions. As it has been shown earlier in excessive amount of nickel tissue density of the leaf increases and this organ acquires some xeromorphic properties [15].

Nickel used in the cultivation of cabbage in both chemical forms affected the content of N-total, free amino acids and protein (Tab. 1). It also affected the nutritional value of protein, defined by the content of endogenous amino acids (Tab. 2). In the conditions of excessive amount of inorganic nickel and Ni-EDTA chelate the content of N-total and total protein was reduced by 36.6 and 14.8%, respectively, as compared with the content of this element in control plants. The pool of free amino acids also decreased by as much as 65.5%

it the presence of  $\text{NiSO}_4 \cdot 7\text{H}_2\text{O}$  and by 12.2% in the presence of Ni-EDTA. Results of studies have shown that the observed differences in the effect of applied chemical forms of nickel on the level of N-total and its analysed organic compounds corresponded to the content of this metal in plants. When the soil was polluted with nickel in the form of  $\text{NiSO}_4 \cdot 7\text{H}_2\text{O}$ , the content of nickel in cabbage was bigger and the content of nitrogen and its compounds smaller than when the soil was polluted with Ni-EDTA chelate (Tab. 1). Inorganic nickel also reduced the nutritional value of protein to a greater extent than Ni-EDTA chelate (Tab. 2).

Table 1  
The crop of dry mass of head and the content of some nitrogen compounds in head of white cabbage

Parameter	Control object	$\text{NiSO}_4 \cdot 7\text{H}_2\text{O}$	Ni-EDTA	$\text{LSD}_{0.05}$
Crop of head dry mass [g per pot]	5.37	3.02	4.39	0.68
Dry mass of head [% of f.m.]	6.49	7.34	6.62	0.57
Ni content in head [ $\text{mg} \cdot \text{kg}^{-1}$ of d.m.]	19.64	237.36	163.19	17.05
Total-N [% of d.m.]	2.57	1.63	2.19	0.29
Total protein [% of d.m.]	16.06	10.19	13.69	1.78
Total exogenous amino acids [% of protein]	36.39	28.53	31.95	3.21
Total of free amino acids [ $\text{mg} \cdot \text{g}^{-1}$ of d.m.]	46.13	15.91	29.67	4.73

Table 2  
The content of exogenous amino acids in head of white cabbage

Amino acid [g per 100 g of protein]	Control object	$\text{NiSO}_4 \cdot 7\text{H}_2\text{O}$	Ni-EDTA	$\text{LSD}_{0.05}$
Lys	4.68	4.18	4.37	0.35
Phe	4.25	3.96	4.03	0.21
Ile	3.84	2.75	3.24	0.32
Leu	8.29	6.16	6.67	0.57
Thr	4.48	3.15	3.39	0.29
Val	4.44	3.63	4.07	0.30
His*	2.04	2.28	2.76	0.19
Arg*	4.37	2.42	3.42	0.64
EAAI**	0.75	0.58	0.65	-

\* exogenous amino acids for children aged below 12; \*\* EAAI - essential amino acid index (standard protein - chicken egg) [8].

Results of the studies conducted so far [3-5, 7, 16] have shown that the effect of nickel on the content of nitrogen compounds in plants depend on its content in the substrate (soil/water medium). Applied in high (excessive) concentrations, this metal depressed of nitrate assimilation and content of nitrogen compound in plants [7, 16]. Results of the conducted experiments have confirmed a negative effect of excess of nickel in the soil on the content of nitrogen and its organic compounds in cabbage plants. These results have also shown that both inorganic and chelatic nickel have this negative effect on the content

of nitrogen and its analysed organic compounds in cabbage. However, chemical form of nickel determines the content of this metal in plants, and the level of reduction of content of nitrogen and its organic compounds.

### Conclusion

Nickel added in excessive amounts ( $75 \text{ mg Ni} \cdot \text{kg}^{-1}$  of soil) to average soil whose pH was 6.2 both in its hydrate ( $\text{NiSO}_4 \cdot 7\text{H}_2\text{O}$ ) and chelate (Ni-EDTA) forms caused a reduction of the crop of dry mass of white head cabbage cv. Gloria di Enkhouizen 2 and a reduction of the content of N-total, free amino acids and protein. It also reduced the nutritional value of protein, determined on the basis of the content of exogenous amino acids. Hydrate nickel reduced cabbage crop, the content of nitrogen and of its analysed compounds to a much greater extent than Ni-EDTA chelate. These differences resulted from a bigger phytoassimilation of nickel from soil polluted with nitrogen sulphate than from soil polluted with Ni-EDTA.

### References

- [1] Brown P.H., Welch R.M. and Cary E.E.: *Nickel: a micronutrient essential for higher plants*. Plant Physiol., 1987, **76**(3), 801-803.
- [2] Dixon N.E., Gazzola C., Blakeley R.L. and Zerner B.: *Jack bean urease (EC 3.5.1.5). A metalloenzyme. A simple biological role for nickel?* J. Amer. Chem. Soc., 1975, **97**(14), 4131-4133.
- [3] Gerandás J. and Sattelmacher B.: *Significance of Ni supply for growth, urease activity and the concentration of urea, amino acids and mineral nutrients of urea-grown plants*. Plant and Soil, 1997, **190**, 153-162.
- [4] Gerandás J., Zhu Z. and Sattelmacher B.: *Influence of N and Ni supply on nitrogen metabolism and urea activity in rice (Oryza sativa L.)*. J. Exp. Bot., 1998, **49**(326), 1545-1554.
- [5] Tan X.W., Ikeda H. and Oda M.: *Effects of nickel concentration in the nutrient solution on nitrogen assimilation and growth of tomato seedlings in hydroponic culture supplied with urea or nitrate as the sole nitrogen source*. Sci. Hort., 2000, **84**, 265-273.
- [6] Mishra D. and Kar M.: *Nickel in plant growth and metabolism*. Bot. Rev., 1974, **40**(4), 395-452.
- [7] Kevrešan S., Petrović N., Popović M. and Kandrač J.: *Effect of heavy metals on nitrate and protein metabolism in sugar beet*. Biol. Plant., 1998, **41**(2), 235-240.
- [8] Jabłoński E.: *The factors determining and modifying the nutritional value of protein*. Ped. Wsp. Gastroenterol., Hepatol. Żyw. Dziecka, 2000, **2**(2), 83-87 (In Polish).
- [9] Kabata-Pendias A. and Pendias H.: *Biogeochemistry of Trace Elements*. Wyd. Nauk. PWN, Warszawa 1999 (in Polish).
- [10] Adriano D.C.: *Trace Element in Terrestrial Environment*. 2<sup>nd</sup> ed., Springer, New York 2001.
- [11] Nieminen T.M., Ukonmaanaho L., Rausch N. and Shotyk W.: *Biogeochemistry of nickel and its release into the environment*. Met. Ions Life Sci., 2007, **2**, 1-30.
- [12] Molas J. and Baran S.: *Relationship between the chemical forms of nickel applied to the soil and its uptake and toxicity to barley plants (Hordeum vulgare L.)*. Geoderma, 2004, **122**, 247-255.
- [13] Ostrowska A., Gawliński S. and Szczubińska Z.: *The Methods of Soils and Plants Analysis*. Wyd. IOŚ, Warszawa 1991 (in Polish).
- [14] Weibull J., Ronquist F. and Brishammar S.: *Free amino acid composition of leaf exudates and phloem sap*. Plant Physiol., 1990, **92**, 222-226.
- [15] Molas J.: *Changes in morphological and anatomical structure of cabbage (Brassica oleraceae L.) outer leaves in ultrastructure of their chloroplasts caused by an in vitro excess of nickel*. Photosynthetica, 1997, **34**(4), 513-522.
- [16] Athar R. and Ahmad M.: *Heavy metal toxicity: effect on plant growth and metal uptake by wheat, and on freeliving Azotobacter*. Water, Air and Soil Pollut., 2002, **138**, 165-180.

## **PORÓWNANIE ZAWARTOŚCI NIEKTÓRYCH ZWIĄZKÓW AZOTU W KAPUŚCIE GŁOWIASTEJ BIAŁEJ UPRAWIANEJ NA GLEBIE ZANIECZYSZCZONEJ HYDRATEM I CHELATEM NIKLU**

Katedra Biologii Roślin, Wydział Nauk Rolniczych w Zamościu, Uniwersytet Przyrodniczy w Lublinie

**Abstrakt:** W doświadczeniu wazonowym badano wpływ zanieczyszczenia gleby niklem w formie nieorganicznej ( $\text{NiSO}_4 \cdot 7\text{H}_2\text{O}$ ) i chelatowej (Ni-EDTA) na plon kapusty głowiastej białej oraz zawartość azotu, białka, egzogennych i wolnych aminokwasów. Nikiel dodany do gleby o pH 6,2 w ilości  $75 \text{ mg} \cdot \text{kg}^{-1}$  w obu formach chemicznych obniżał plon suchej masy kapusty oraz zawartość azotu i analizowanych jego związków. Obniżał także wartość odżywczą białka mierzoną zawartością egzogennych aminokwasów. Poziom redukcji plonu suchej masy kapusty i zawartości analizowanych związków azotu był znacznie większy w obecności niklu nieorganicznego  $\text{NiSO}_4 \cdot 7\text{H}_2\text{O}$  niż chelatu Ni-EDTA i dodatnio korelował z zawartością tego metalu w roślinach.

**Słowa kluczowe:** aminokwasy, azot, białko, kapusta, toksyczność niklu, zanieczyszczenie gleby