Nickel: The last of the essential micronutrients

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ABSTRACT

The knowledge about the role of Ni (Ni) in the nutrition, physiology and metabolism of the majority of crops is limited, whereas is considered to be an essential element for the higher plants starting from the 80’s of the twentieth century. The primary function of Ni in plants is defined in terms of its importance for the hydrolysis of urea; however, Ni may have an importance in other physiological processes, such as nitrogen fixation. Although the deficiencies of Ni in plants are relatively rare events, the positive response of yield and nitrogen use efficiency to applications of Ni are shown for different species. The present work summarizes the data about the essentiality of Ni and its function in plant metabolism as well as its agronomic importance for the crops.

Key words: mineral nutrition, essential element, mineral deficiencies, nitrogen cycle.

Introduction

Knowledge about the role of Ni in nutrition, physiology and metabolism of most crops is currently limited (Bai et al., 2006). However, the evidence of essentiality of this element for higher plants is not a new issue, but still goes back to the 70’s of the twentieth century, when a group of researchers suggested the possible role of Ni in the metabolism of nitrogen through its participation in the structure of the enzyme urease (Dixon et al., 1975). Already in the 80’s Eskew et al. (1984a), through studies of soybeans, demonstrated the essential role of Ni in nitrogen metabolism of leguminous plants, a role that was independent of the form of available nitrogen (NO3− or NH4+). The evidence generated by this research suggested the essentiality of Ni for higher plants (Eskew et al., 1984b).

The lack of evidence for the role of Ni in non-leguminous plants was caused by the fact that at that time the studies about the essentiality were incomplete and, therefore, its essentiality was not accepted. This gap of knowledge was supplied by Brown et al. (1987) who established the essential role of Ni in non-leguminous plants, specifically in barley. These results together with those obtained previously by Eskew et al. (1984a) led Brown et al. (1987) to propose a more support towards the addition of Ni to the group of micronutrients. Although these studies were, possibly, the most significant ones in determining the essentiality of Ni, there also stood out the studies forwarded by Roach and Barclay (1946) in plants of potato (Solanum tuberosum), wheat (Triticum aestivum) and bean (Phaseolus vulgaris) in England that indicated an increase in plant production as a result of foliar application of Ni. Additionally, Cataldo et al. (1978) studied the dynamics and transport of Ni in soybean plants, while Eskew et al. (1983) founded toxic levels of urea in the tips of soybean leaves poor in Ni, a behavior similar to that reported by Walker et al. (1985) in plant Vignia unguiculata.

The previous studies allowed including Ni within the group of essential mineral nutrients (Marschner, 2002; Taiz and Zeiger, 2004; Epstein and Bloom, 2005; Azcon-
Bieto and Talón, 2008) and, therefore, it is understood that plants may not complete the life cycle in the absence of this nutrient (Arnon and Stout, 1939). Recently, the Department of Agriculture of the USA and the Association of American Plant Food Control Officials included Ni as an essential element for plants, making it possible in the USA the manufacture and sale of fertilizers containing Ni (Bai et al., 2006).

Due to the fact that this mineral element is a new one in the list of essential micronutrients, the objectives of the present review were to illustrate the current state of research on functions of Ni in plants, in particular, describe the dynamics of Ni in non-accumulator and accumulator species, clarify the physiological functions of Ni in plants as well as symptoms of deficiency and toxicity caused by Ni in plants and identify plant responses to the applications.

**Dynamics of nickel in soils and plants**

At level of dynamics in soil, Ni is abundant metal in the earth crust with about 3% of the composition of the earth. In agricultural soils, typical contents of this element vary from 3 to 1,000 mg kg⁻¹, however, the soils derived from basic igneous rocks can contain from 2,000 to 6,000 mg kg⁻¹ of Ni. Soil pH plays an important role in the availability of Ni, and at pH > 6.7 Ni exists in form of poorly soluble hydroxides, while at pH < 6.5 increases the presence of relatively soluble compounds (Brown, 2006).

It is considered that the system of Ni²⁺ uptake by roots is similar to that of Cu²⁺ and Zn²⁺, a conclusion obtained after confirmation of competitive inhibition in absorption of these three nutrients. When the available concentration of Ni²⁺ in the substrate is low (0.5 - 30 mM), the process of its absorption by roots is dependent on the expenditure of ATP, a characteristic that indicates the presence of an active transport of high affinity (Brown, 2006).

Once Ni is absorbed by the root, its movement to the aboveground parts of plants is closely linked to the formation of organic complexes (Cataldo et al., 1978, 1988; Bhatia et al., 2005). In general, potential ligands of metals in plants could be grouped into three classes: oxygen donor ligands (carboxylates: malate, citrate, malonate, succinate, and oxalate), sulfur donor ligands (metallothioneins and phytochelatins), and nitrogen donor ligands (amino acids) (Baker et al., 2000). In the case of Ni (Ni⁺), there was reported complex formation for its transport in the xylem with amino acids histidine (Krämer et al., 1996; Brown, 2006) and nicotinamime (Mari et al., 2006) and organic acids citrate, malate, and malonate (Cataldo et al., 1988; Robinson et al., 2003; Bhatia et al., 2005), although in the case of complex with nicotinamine, this one was reported for the species tolerant or accumulators of Ni (Mari et al., 2006). At the same time, Homer et al. (1995) suggested that formation of complexes of Ni in the xylem with molecules of high molecular weight, such as metallothioneins and phytochelatins, is an unlikely process.

Complex formation is dependent on pH, such as at low pH the organic acids are better chelating agents for Ni than amino acids, whereas at high pH amino acids increase their capacity to act as ligands (Bhatia et al., 2005). Brown (2006) indicates that at pH below 6.5 histidine is the most significant ligand for Ni, while at pH < 5 citrate is the most important chelating agent. In oak Quercus ilex, Araujo et al. (2009) evaluated the effect of four different ligands (histidine, oxalic acid, aspartic and citric acids) present in the xylem sap on the movement of Ni²⁺ in the xylem. The order of affinity of ligands towards Ni²⁺ reported in this research was: oxalic acid > citric acid > histidine > aspartic acid. In contrast, the amount of Ni bound to the walls of the xylem was higher when Ni was present as free cation, followed by Ni-aspartic acid, Ni-histidine, Ni-citric acid, and Ni-oxalic acid (Araujo et al., 2009).

Addition of chelating agents to soils with high contents of Ni may be an effective practice to increase the metal concentration in soil solution, but have a low effect on increasing of Ni absorption by plants, as showed the study of Molas and Baran (2004) in barley. This research evaluated several Ni containing compounds: Ni-citrate, Ni-glutamate, Ni-EDTA and NiSO₄·7H₂O and found that the rate of absorption of Ni by plants arranged from highest to lowest as NiSO₄·7H₂O > Ni-citrate > Ni-glutamate > Ni-EDTA. In Ni non-accumulating species, after being absorbed and transported, is used to ensure the functioning of urease, and, thus, to ensure the hydrolysis of urea to produce ammonia and carbon dioxide (Marschner, 2002; Taiz and Zeiger, 2004). Ni in the phloem may be retraslocated rapidly from the leaves to young tissues, especially during reproductive growth (Tiffin, 1971); this movement is associated with the formation of complexes with organic acids and amino acids (Brown, 2006). Thus, Ni is considered an element mobile in the phloem (Cataldo et al., 1978; Page and Feller, 2005), whose mobility is higher than that of cobalt (Zeller and Feller, 1999). In soybeans, over 70% Ni present in the leaves could be retranslocated to the seeds and accumulated mainly in the cotyledons (Tiffin, 1971; Cataldo et al., 1978).
Nickel hyperaccumulator plants

Ni hyperaccumulator species (metallophytes), such as *Stackhousia tryonii*, *Hybanthus floribundus*, *Thlaspi caerulescens*, *Halimione portulacoidae*, *Berkheya coddii*, *Brassica juncea*, and *Typha latifolia* are known to accumulate high concentrations of Ni, among 0.1 and 3.0%, in shoots and leaves (Ye et al., 1997; Robinson et al., 2003; Bidwell et al., 2004; Bhatia et al., 2005; Duarte et al., 2006; Mari et al., 2006; Hsiao et al., 2007). The latex of *Sebertia acuminate* (Sapotaceae), a tree native to New Zealand, contains 25.74% dry weight Ni (Sagner et al., 1998) as well as other cases of exceptionally high accumulation of Ni in the aboveground parts of plants are reported; the explanations for Ni hyperaccumulation are related to the defense role played by high concentrations of Ni in plant tissues against herbivores and pathogens (Baker et al., 2000).

It is known that the members of ZIP protein families (Zinc Regulated Transporters / Iron Regulated Transporters), NRAMP (Natural Resistance Associated Macrophage Protein), and YSL (Yellow Stripe Like) are involved in the transport of Ni in different organisms. The transformation of yeasts with ZNT1 or ZNT2 partially conferred Ni tolerance correlated with the input of Zn, which inhibits the absorption of Ni. In contrast, transformation with NRAMP4 conferred sensitivity to Ni in yeasts explained by a release of Ni from the vacuole (Tejada-Jiménez et al., 2009). In transgenic plants of *Arabidopsis* sp., the overexpression of gene AtIREG2 causes increased tolerance to high concentrations of Ni. Thus, it appears that the physiological function of AtIREG2 may be accumulation of excess of Ni accompanied by a counter ion (nitrate or sulphate) in the vacuole to maintain the ionic balance of cells (Schaaf et al., 2006).

Pianelli et al. (2005) suggested that, in response to elevated contents of Ni, nicotinamine is translocated from the leaves of hyperaccumulators to the roots, where it forms complexes with Ni and facilitates its transport to the shoot. In *Arabidopsis* sp., overexpression of nicotinamine synthase confers tolerance to Ni. In addition, the tolerance of plants to Ni also results from the chelating of Ni in the root with histidine or organic acids, such as citrate (Tejada-Jiménez et al., 2009).

Ni hyperaccumulator plants differ from non-accumulators with the route of transport of this element in the root cortex. The absorption of Ni via the apoplast of the roots of corn, a non-accumulating plant, ranged from 81.3 to 88.0%, while that of *Leptoplax emarginata*, a hyperaccumulator of Ni, was from 90.6 to 95.5% (Redjala et al., 2010). The root cell wall in both species had similar affinity for the Ni but, in hyperaccumulator plants, more Ni was absorbed via the apoplast. This suggests, according to the authors, that symplastic absorption is not the main factor associated with hyperaccumulation, and the transport system of Ni can not be similar in these two species (Redjala et al., 2010).

In hyperaccumulator species, after absorption and transport via xylem, Ni can be accumulated in vacuoles of leaf epidermal cells (Krämer et al., 1996; Küpper et al., 2001; Bidwell et al., 2004; Schaaf et al., 2006), in the cuticle of the upper epidermis (Robinson et al., 2003) or remain in the apoplast occupying certain sites in the cell wall (Krämer et al., 1996; Bidwell et al., 2004). The accumulation of Ni in the vacuole of epidermal cells is related to the decrease in the concentrations of K+ and Na+ (Bidwell et al., 2004) as a likely consequence of a competitive effect between these cations.

Montargès-Pelletier et al. (2008) reported the carboxylic acids (citric and malic) as the main responsible agents for the transfer of Ni in hyperaccumulator plants *Alyssum murale* and *Leptoplax emarginata*. In their research, citrate was the main ligand of Ni found in stems, whereas in leaves this function corresponded to malate. Histidine was not detected in leaves, stems, and roots of plants under study. In contrast, McNear et al. (2010) founded that, in *Alyssum murale*, Ni was in the sap of xylem in a greater proportion together with histidine, followed by malate and other low molecular weight molecules. The authors based on their results adapt a model, in which Ni is transported from roots to leaves in complexes with histidine and then stored in the epidermis of leaves and stem in complexes with malate, other organic acids of low molecular weight and counter-ions, such as sulfate SO4−2 (McNear et al., 2010).

Nickel functions in plants: hydrolysis of urea

Ni is chemically related to iron (Fe) and cobalt (Co). Oxidation state of Ni in biological systems is Ni2+, but it could also exist as Ni3+ and Ni+ (Marschner, 2002). Ni is a functional constituent of seven enzymes, six of which are present in bacteria and animals, while only one, urease (urea amidohydrolase, EC 3.5.1.5), occurs in plants (Brown, 2006). Constituent participation of Ni in the structure of urease was first documented by Dixon et al. (1975) after its isolation and description from *Canavalia ensiformis*. Of the seven Ni-dependent enzymes two have non-redox functions (urease and glyoxylase) and the remaining five are involved in oxidation-reduction reactions (Ni-superoxide...
dismutase, methyl coenzyme M reductase, carbon monoxide dehydrogenase, acetyl coenzyme A synthase and hydrogenase) (Brown, 2006).

Metalloenzyme urease is a ubiquitous (everywhere present) (Malavolta and Moraes, 2007) enzyme that consists of six identical spherical subunits, each with two atoms of Ni (Dixon et al., 1980; Hirai et al., 1993) whose molecular mass is reported in the range of 473–590 kDa (Fishbein et al., 1973; Dixon et al., 1980). Within the subunits, the union of Ni is coordinated by ligands containing N- and O- (Marschner, 2002).

Although it is considered that Ni is not required for the synthesis of urease, this element is an essential metal component in the structure and catalytic function of the enzyme (Hirai et al., 1993; Marschner, 2002). In soybean urease, its synthesis is directed by a long chain of RNA consisting of 3,000 to 3,500 nucleotides and their participation on the total weight of extractable seed protein is of the order of 0.2% (Polacco and Sparks, 1982).

The role of urease is to catalyze the hydrolysis of urea CO(NH$_2$)$_2$, to ammonia (NH$_3$) and carbon dioxide (CO$_2$), a reaction that occurs mainly in leaves (Marschner, 2002; Taiz and Zeiger, 2004; Malavolta and Moraes, 2007; Azcon-Bieto and Talón, 2008). The above statement may indicate that the functionality of Ni is restricted to those crops, where nitrogen inputs are derived from urea, however, this assumption is not correct; the essentiality of Ni is due to the formation of urea interior of plants as a result of metabolic pathways common to all plants that include the catabolism of purines (adenine and guanine), ureides and protein catabolism of arginine via ornithine cycle and conversion of canavanine to canaline in certain plants (Walker et al., 1985).

**Other functions of nickel in plants**

Ni is also involved in symbiotic nitrogen fixation through its role as an active center of hydrogenase, a process documented in strains of nitrogen-fixing bacteria Bradyrhizobium japonicum, Bradyrhizobium sp. (Lupinus sp.), Rhizobium tropici, Rhizobium leguminosarum, and Azo-rhizobium caulino-dans (Palacios, 1995). Hydrogenase is an enzyme responsible for oxidizing the hydrogen produced by nitrogenase during symbiotic nitrogen fixation resulting in the production of ATP and, therefore, this enzyme increases the efficiency of symbiotic process, and decreases the inhibitory activity of hydrogen in the bacteroids (Palacios, 1995; Ruiz-Argueso et al., 2000). Thus, the low level of Ni in agricultural soils may limit the activity of hydrogenase from R. leguminosarum and, therefore, the efficiency of symbiotic nitrogen fixation in legumes (Ruiz-Argueso et al., 2000; Malavolta and Moraes, 2007).

Zobiole et al. (2010) in Brazil showed that application of glyphosate can negatively influence symbiotic nitrogen fixation in soybeans grown in soils with low native concentrations of Ni in response to a decrease in the foliar concentration of this element. In Matricaria chamomilla, accumulation of chlorogenic acid, an important antioxidant compound, was increased almost fourfold in response to the application of 120 mM Ni to the substrate (sand). It is, therefore, proposed that Ni may have antioxidant properties of phenolic metabolites (Kovacik et al., 2009).

Being similar to cation of iron, cation of Ni may have beneficial functions for the formation of anthocyanins that contain iron or aluminum as structural elements. According to Aziz et al. (2007), applications of Ni to soil contributed to accumulation of anthocyanins and flavones in plants of Hibiscus sabdariffa when applying 20-25 mg kg$^{-1}$ Ni.

**Deficiencies and toxicities of nickel in plants**

Ni deficiency in legumes and other dicots causes a decrease in the activity of enzyme urease, a condition that causes accumulation of toxic levels of urea and is manifested as necrosis at the tip of the leaves (Eskew et al., 1983; Walker et al., 1985; Malavolta and Moraes, 2007). In soybean, low levels of Ni in soil reduced nodulation (Zobiole et al., 2010) and seed yield, a phenomenon that is explained by the involvement of Ni in hydrogenase activity of bacteroids (Brown, 2006). At the same time, due to the relatively low requirements of plants in Ni, the events of Ni deficiencies in the field are few, while the toxicities caused by Ni are more common (Mengel and Kirkby, 2001).

Decreased urease activity in non-timber species can induce the deficiency of nitrogen and affect the contents of amino acid amides (asparagine and glutamine) and intermediates of urea cycle (arginine, ornithine, and citrulline) (Bai et al., 2006). In grasses, on the other hand, deficiency symptoms include interenal chlorosis and necrotic spots on young leaves. In general, urea accumulation in the tip of the leaves (necrosis) of both monocotyledonous and dicotyledonous plants is diagnostically symptom of Ni deficiency (Brown, 2006).

One of the best documented cases of the deficiency of Ni is the perennial timber pecan Carya illinoinensis. In this
species, Ni deficiency is known as “mouse ear” or “little leaf disorder” (Malavolta and Moraes, 2005; Bai et al., 2006) and was first proved as a deficiency of Ni in 2004 by Wood and colleagues (Malavolta and Moraes, 2007). However, the symptom is reported in the United States since 1918 and is characterized by the presence of round dark spots on the tips of new leaves and curving of leaf blade to make the appearance of the ear of a mouse (Malavolta and Moraes, 2005).

In pecan, Ni deficiency affects nitrogen metabolism via ureide catabolism, amino acid metabolism and ornithine cycle intermediates and metabolism of carbon through the accumulation of lactic acid and oxalic acids that accumulate on the edges of leaf blade and would also be linked to necrosis of the tips of the leaves (Bai et al., 2006).

Ni deficiency in pecan could be corrected by foliar application of Ni; however, the dose of Ni reported in the literature is variable. Thus, Brown (2006) indicates that a dose of Ni equal to 100 mg L\(^{-1}\) is sufficient to correct the deficiency, while Malavolta and Moraes (2005) recommended spraying a solution of 0.8 g L\(^{-1}\) Ni mixed with a dose of 4.8 g L\(^{-1}\) urea.

Malavolta and Moraes (2005) and Brown (2006) indicated that the main factors that favor the development of Ni deficiency are: a) excess of Cu and Zn that competitively inhibits the absorption of Ni by roots, b) soil pH > 6.5 (formation of low soluble hydroxides and Ni oxides), c) soils with high contents of Fe, Mn, Ca, or Mg, d) excessive doses of nitrogen or excessive liming, e) high levels of soil phosphorus that favor the formation of phosphates of Ni and decrease the absorption of Ni by plants; f) inhibition of urease activity by accumulation of Cu in plants.

In soils developed over ultrabasic rocks, high levels of Ni, such as exceeding 250 mg kg\(^{-1}\) soil, may lead to Ni toxicity in non-accumulator plants (Mengel and Kirkby, 2001). The symptoms of Ni toxicity may resemble the symptoms of iron deficiency due to a reduced absorption of iron in soils high in Ni (Mengel and Kirkby, 2001). The critical level of Ni in leaves varies according to species, but generally a suitable range is considered between 1 and 10 mg kg\(^{-1}\) dry matter basis (Marschner, 2002), higher than 25 mg kg\(^{-1}\) lead to Ni toxicity in non-accumulator species (Malavolta and Moraes, 2007) through distortions in the growth of root system and leaf buds (Brown, 2006). In wheat, the addition of 50 and 100 mM Ni to the growth substrate resulted in decrease of fresh weight of shoot, the nitrate content, a reduction in the activity of nitrate and nitrite reductase, 40 and 80% less, respectively (Gajewska and Sklodowska, 2009). In contrast, an increase in ammonium content, proline concentration, and the activity of NADH-glutamate synthase in plants treated with toxic levels of Ni was reported (Gajewska and Sklodowska, 2009). The toxicity of Ni in plants may be alleviated by liming or application of phosphate fertilizers that reduce availability of Ni to the plants (Mengel and Kirkby, 2001).

**Plant yield response to applications of nickel**

The response of plants to applications of Ni is wide and includes effects on nitrogen fixation, seed germination and disease suppression. However, a much higher effect could be seen when nitrogen is provided in the form of urea or symbiotically fixed (Brown, 2006).

The first evidence of the yield response to Ni was documented by Roach and Barclay (1946), who reported a significant increase in crop yields of potato (Solanum tuberosum), wheat (Triticum aestivum) and bean (Phaseolus vulgaris) as a result of foliar application of Ni from dilute solutions.

In soybean, it was found that the addition of 40 g ha\(^{-1}\) Ni increases nodulation and crop yield (Malavolta and Moraes, 2007), an effect attributed to the proper functioning of the symbiosis between soybean and Rhizobium sp. (Brown, 2006). In parsley (Petroselinum crispum) growing in plastic containers with clay, the addition of 50 mg kg\(^{-1}\) soil Ni from NiSO\(_4\) source increases the yield and quality of leaves, reduces the accumulation of NO\(_3^-\) and NH\(_4^+\) and increases the accumulation of essential oil aroma constituents (Atta-Aly, 1999). On the other hand, in Brazil the application of 0.03 mg L\(^{-1}\) Ni in nutrient solution of umbu seedlings (Spondias tuberosa) increased dry mass production by 81.52% compared to untreated control (Caires et al., 2007).

In rose of Jamaica (Hibiscus sabdariffa), Aziz et al. (2007) found that a joint application of cobalt and Ni in doses of 20 and 25 mg kg\(^{-1}\) soil, respectively, increases the total mass of the plants, branch number and dry weight and fresh weight of flowers. In addition, these applications promoted an increase in the concentration of N, P, K, Co, Ni, Mn, Zn, and Cu, both in leaves and flowers of the plants (Aziz et al., 2007).

Gad et al. (2007) in tomato (Lycopersicon esculentum) grown in sand found that the addition of 30 mg kg\(^{-1}\) sand Ni significantly increased the total mass of the plant,
number of branches, leaf area, root length, contents of auxins and gibberellins. Similarly, the addition of Ni in the aforementioned doses improved fruit quality variables such as size, fresh weight, diameter, dry weight, contents of vitamin C, total soluble solids, and soluble sugars. In addition, the application of Ni caused the decrease in the contents of NO₃⁻ and NH₄⁺ as well as acidity, favorable characteristics for consumer health (Gad et al., 2007).

Finally, it has been shown a beneficial effect of Ni in the management of agents causing fungal diseases, such as rust of cereal crops (Brown, 2006; Malavolta and Moraes, 2007). The beneficial effect is attributed to the alleged role of this element in reactions involving enzymes, such as superoxide dismutase, changes in nitrogen metabolism due to the contribution of Ni (Brown, 2006) and the possible toxicity of Ni to the pathogen (Malavolta and Moraes, 2007).

Changes in nitrogen metabolism may involve the decrease in amount of free amino acids, a substrate used by most pathogens for growth and proliferation (Strengbom et al., 2002). The accumulation of free amino acids, such as valine, leucine, isoleucine, tyrosine, tryptophan, and arginine, in response to Ni deficiency was reported by Bai et al. (2006). In practical terms, the efficiency of foliar sprays of urea in different crops can be improved by their joint application with Ni (NiSO₄) at levels not exceeding 40 g ha⁻¹ of Ni for crop cycle.

The application of Ni may have positive effects on nitrogen use efficiency in crops that extract high content of this mineral nutrient from soil and where nitrogen fertilizers are applied using urea as the main source, such as in case of rice. However, such effects could only be verified by conducting a research involving Ni as a case study.

Conclusions

The current state of research dedicated to physiology of Ni in plants illustrates the essentiality of this micronutrient for plants, in particular, its importance for the processes related to the metabolism of nitrogen. The primary function of Ni is defined in terms of its importance for the hydrolysis of urea; however Ni may have an importance in other physiological processes, such as nitrogen fixation and synthesis of anthocyanins. Although the deficiencies of Ni in plants are relatively rare events, the positive response of crop yield and nitrogen use efficiency to applications of Ni are shown for different species.

Literature cited


