https://doi.org/10.1590/1678-4499.20180242

SOIL AND PLANT NUTRITION - Article

Effects of nickel fertilization on soybean growth in tropical soils

Camila de Carvalho Braga Levy[1,*], Estêvão Vicari Mellis[3], Marina Krey Murrer[3], Carlos Repiso Inglés[1], Cathal N. Daynes[2], Edilson Cavalli[2], Marcio Koiti Chiba[3]

1.Trade Corporation International - R&D Agro - Madrid - Spain.
2.Trade Corporation International - Technical Department - Madrid - Spain.
3.Instituto Agronômico - Centro de Solos e Recursos Ambientais - Campinas (SP), Brazil.

ABSTRACT: Although nickel (Ni) is recognized as plant nutrient since the turn of the 21st century, uncertainty remains about its optimum application rates and forms. This paper focuses on Ni application in soils, relating to its effect as a plant micronutrient. Specifically, the effect of Ni on the activity of urease in soybean plants is examined. The effects of applying four Ni rates in two different soil types were tested. A full factorial 2 × 4 blocked design experiment was established under controlled conditions. Ni rates (0, 0.25, 0.5 and 1.0 mg of Ni·kg^{-1} of soil) were applied in two soils with contrasting clay contents. The addition of Ni increased the urease activity in soybean plants but was affected by soil textural differences. The highest urease activity was achieved by the application of 1.0 mg·kg^{-1} Ni in the sandy soil. The absorption of Ni by the plants and its availability was found to be soil texture dependent. The rate of 0.25 mg·kg^{-1} Ni increased the soybean dry matter production by 25% in the sandy soil. In conclusion, Ni was effective in promoting plant growth and biomass accumulation although depending on soil clay proportion. For soybean, there was no correlation between urease activity and biomass accumulation. The results of this study indicate a clear Ni effect in different type of soils in São Paulo state, serving as a solid initial doses indicator for soybean fertilization programs and future studies on nickel in Soybean.

Key words: urease, clay, sand, Oxisol, *Glycine max*.

*Corresponding author: camilalevy@yahoo.com.br

Received: Jun. 30, 2018 – Accepted: Dec. 9, 2018

INTRODUCTION

Soybean is one of the most important crops in the world, as it constitutes a major source of protein for both animal and human food, in addition to its recent use as a feedstock for the manufacture of biofuels (Alovisi et al. 2011). although soybean is one of the most produced grain crops in the world, the average yield in many countries, such as Brazil, can still be considered low with increased production possible through a better understanding of its nutritional requirements. Proper management of micronutrients is one of the major factors that can increase soybean productivity (Masuda 2009; Alovisi et al. 2011).

Among the essential micronutrients for soybeans, Ni has received increasing study in recent years (Seregin and Kozhevnikova 2006; Moraes et al. 2010; Alovisi et al. 2011; Kutman et al. 2013; 2014; Syam et al. 2016; Tezotto et al. 2016). An obvious reason is that Ni was the latest element to have its nutritional essentiality recognized and confirmed for plant species (Brown et al. 1987), therefore the literature was scant.

Although Ni concentration in plant tissue is critical for plant development, it is relatively low in comparison to other micronutrients (0.1 mg·kg^{-1}, dry mass basis) (Epstein and Bloom 2005; Chen et al. 2014). However, Ni has an important function in nitrogen metabolism (Brown and Sunderman Jr. 1980) due to its structural and functional role on urease, the enzyme that breaks down urea to CO_2 and NH_4^+ (Dixon et al. 1975; Bai et al. 2006). Deficiency of Ni inhibits urease activity leading to accumulation of urea related N-compounds, which results in toxicity and decreased plant growth (Eskew et al. 1983; Brown et al. 1990; Dechen et al. 2007; Witte 2011). Some authors emphasize the importance of a suitable supply of Ni to increase hydrogenase activity in Bacteroides, increasing nodulation and biological nitrogen fixation (BNF) in soybean (Sellstedt and Smith 1990; Klucas et al. 1983; Ureta et al. 2005). According to McClure and Israel (1979), the fixed nitrogen transport is done by ureides that catalyze the end of the transport of urea molecule, which is further metabolized by the enzyme urease. As regards to the Ni fraction within urease, Fabiano et al. (2015) reported that besides urease, Ni can also activate an isoform of glyoxalase I, which performs an important role in the degradation of a potent cytotoxic compound – methylglyoxal. This fact suggests that Ni may play an important role in the metabolism of antioxidants in plants, especially under stress.

Despite its recognized importance, fertilization with Ni in soybean growth on tropical soils is still not recommended (Lavres et al. 2016), due to the lack of studies on the effect of Ni application in the crop, absorption both foliar and from the soils and the later assimilation of Ni (Alovisi et al. 2011).

According to a literature review by Adriano (1986), the total content of Ni in soils is usually between 20 and 40 ppm worldwide and can range from 100 to 7000 ppm in serpentine soils. In São Paulo state, the highest Ni content found in soils was 127 ppm of Ni in clayey soils (Rovers et al. 1983). In Brazil, Ni has been used in soybean fertilization to improve seed quality, at a rate of 0.25 kg·ha^{-1}, applied during flowering period (Mazzafera et al. 2013). The application of 0.5 mg·kg^{-1} of Ni in soil resulted in within health safety tolerance levels of the micronutrient in grains for human consumption, and yield gains of up to 1,502 kg·ha^{-1} in field conditions (Freitas et al. 2018).

To consider the adsorptive capacity of Ni by soil colloids, it is imperative to consider the application of Ni in some specific types of soil. Some factors such as pH, organic matter and iron oxides favor Ni adsorption, especially in tropical soils (Pombo et al. 1989; Mellis et al. 2004). Thus, it would seem reasonable to conclude that differences in soil clay content will affect the adsorption of Ni and thus its availability. This information is relevant to improve the application rate recommendations as well as formulation types of Ni in the fertilizer industry.

Therefore this study aims to evaluate the effect of Ni at different rates of application when applied in soils with different clay contents, using the performance of soybean plants and associated urease activity to determine optimum rates.

MATERIALS AND METHODS

Soil characterization and fertilization

Two different types of soils (USDA classification) were used in the experiment: Dystrophic red-yellow Oxisol sandy texture (sandy soil) and Red Eutrudox Oxisol clayey texture (clayey soil). Soil samples were collected in the state of São Paulo, from the 0–0.2 m top layer. Soil preparation included air-drying, 2 mm sieving and analysis for chemical and physical characterization (Table 1). Soil organic matter (acid dichromate oxidation), pH (0.01M $CaCl_2$ solution), available P, exchangeable K, Ca, and Mg (extraction by ion

→

exchange resin), H and Al (by SMP-buffer solution), cation exchange capacity (CEC), soil base saturation (V%), sulfate (S in $Ca(H_2PO_4)_2$ $0.01 M \cdot L^{-1}$), B (hot water), and Cu, Fe, Mn and Zn (extraction with DTPA TEA at pH 7.0) were measured according to the methods proposed by Raij et al. (2001). Particle size was measured using the pipette method (Camargo et al. 1986).

Table 1. Two tropical soils (sandy and clayey) chemical characteristics and particle size characterization prior to the experiment.

Characteristic	Sandy	Clayey
pH, $CaCl_2$	4.2	4.8
MOS ($g \cdot dm^{-3}$)	18	39
Phosphorus (P) $_{(resin)}$ ($g \cdot dm^{-3}$)	3.0	14.0
Potassium (K) ($mmol_c \cdot dm^{-3}$)	0.4	3.4
Al^{3+} ($mmol_c \cdot dm^{-3}$)	11.00	ND
H+ Al^{3+} ($mmol_c \cdot dm^{-3}$)	38.0	47.0
V (%)	12	53
Cu ($mg \cdot dm^{-3}$)	0.3	7.0
Fe ($mg \cdot dm^{-3}$)	53.0	21.0
Mn ($mg \cdot dm^{-3}$)	1.8	40.2
Zn ($mg \cdot dm^{-3}$)	0.8	3.9
Ni ($mg \cdot dm^{-3}$)	0.01	0.27
Sand ($g \cdot kg^{-1}$)	792	548
Silt ($g \cdot kg^{-1}$)	58	5.8
Clay ($g \cdot kg^{-1}$)	151	394

ND = not detected.

Before treatments with Ni, soil base saturation was increased to 70% by using dolomitic limestone (Effective Calcium Carbonate Equivalent = 71%). After liming, the soil was incubated for 30 days maintaining soil moisture at field capacity. Each experimental unit received 60 $mg \cdot dm^{-3}$ of N, 200 $mg \cdot dm^{-3}$ of P_2O_5 and 60 $mg \cdot dm^{-3}$ of K_2O. In the sandy soil 0.5 $mg \cdot dm^{-3}$ of B, 1 $mg.dm^{-3}$ of Cu, 2.5 $mg \cdot dm^{-3}$ of Mn and 5 $mg \cdot dm^{-3}$ of Zn were applied, according to Abreu et al. (1994). In addition, it should be noted that the soybean seeds were inoculated with *rhizobium*, but nodulation was not observed in the roots. As a consequence, there was no biological nitrogen fixation, requiring the application of nitrogen at 40 days after planting (DAP).

Once the soybean plants were cut, the soil from each pot was sampled, air-dried and passed through a 2 mm sieve. Soil Ni availability were measured by extraction with DTPA TEA at pH 7.0 according to the method proposed by Raij et al. (2001).

For a better understanding of the results and in the attempt to determine the optimal dose of Ni in the studied conditions, further studies of adsorption were carried out.

Experimental design and treatments

The experiment was conducted in pots of 5 dm^{-3} capacity in a greenhouse at Campinas, São Paulo state, Brazil (Coordinates 22°53′31.18′ S, 47°3′51.96′ W), during spring/summer growing season. The experimental design was based on a randomized block with a factorial combination of two types of soils and four rates of Ni using $NiCl_2$ PA (240 $g \cdot kg^{-1}$ Ni) as the source, with four replications, resulting in 64 pots. Ni rates applied directly in the soil by using a solution were equivalent to 0, 0.25, 0.5 and 1 $mg \cdot kg^{-1}$ Ni. As suggested by Malavolta (2006), for experimental purposes it was decided to use a dose similar to that recommended for Cobalt (Co).

Plant material and experimental conduction

The soybean variety used was Anta 82, a transgenic variety with very early ripening and tolerant to lodging with a full cycle of approximately 110 days. Before sowing, the soybean seeds were treated with *rhizobia* inoculant (0.1 $g \cdot kg^{-1}$ seed), Co (0.015 $mg \cdot g^{-1}$ seed) and Molybdenum (Mo) (0.3 $mg \cdot g^{-1}$ seed). A total of 10 seeds were sown per pot. Twenty days later thinning was performed to maintain six plants per pot. The soil moisture was kept at field capacity by using deionized water to avoid contamination of the metal quantities.

Each pot had two individuals collected at 40 (flowering stage) and 70 (grain filling stage) days after planting (DAP).

Urease activity

To determinate urease activity, one individual of each experimental unit was analyzed for fresh matter. The urease enzyme activity was determined using a methodology adapted from Hogan et al. (1983), based on the quantification of the ammonium produced by the enzymatic hydrolysis of urea. About 100 mg of fresh leaf discs were cut and incubated in glass tubes with a buffer solution of NaH_2PO_4 (0.20 $mol \cdot L^{-1}$) with Na_2HPO_4 (0.50 $mol \cdot L^{-1}$) + n-propanol (0.66 $mol \cdot L^{-1}$) and urea (0.21 $mol \cdot L^{-1}$) at 30 °C for 3 h in a water-bath. After the incubation, 0.5 mL of the buffer solution was taken and mixed with 2.5 mL of a solution made of 0.1 $mol \cdot L^{-1}$ phenol and 170 $mmol \cdot L^{-1}$ of sodium nitroprusside, plus 2.5 mL of a second solution of 0.125 mol·L^{-1} of NaOH and 0.15 mol·L^{-1} of $Na_2HPO_4 \cdot 12H_2O$ + NaOCl (3% of Cl_2). N-NH_4 production was estimated by colorimetrical spectrophotometry at 625 nm.

Mineral nutrition analysis

Another individual of each pot was collected at 40 and 70 DAP followed by oven drying at 65 °C for 72 h. Dry matter (DM) was recorded and the Ni and N concentration determined. The Ni concentration was determined by nitric-perchloric acid digestion and the N concentration by sulfuric digestion. The methods were performed according to Bataglia et al. (1988).

Growth and yield assessment

At the end of the cycle (110 DAP) the two remaining plants in each pot were harvested and the number of pods and seeds per pod was determined. In addition, the cumulative Ni content in the soybean grains was determined according to the method described by Bataglia et al. (1988).

Statistical analysis

The statistical analysis was performed with R software (R Development Core Team 2008). Data were analyzed by preliminarily descriptive parameters: mean, standard deviation, coefficient of variation, analysis of variance, F-test. When the effects of Ni doses were significant, regression analysis was performed. For all analyses described above, the level of significance was 5% ($p < 0.05$).

RESULTS AND DISCUSSION

Ni availability in soils

The content of Ni available in the soil was increased linearly with the application of micronutrient doses, independently of soil type, by 571.4% (Fig. 1). It is important to note that the sandy soil contained almost no Ni and the control was not supplemented to reach the same content of clayey soil (Table 2). This point alone is of agronomic significance as it can be hypothesized that in this area of Brazil soybean grown on sandy soil becomes deficient in Ni if and when the seed reserves of Ni are depleted. As a result, on the sandy soils the application of supplemental Ni by growers should be seriously considered as a result of the first principles hypothesis above, which is supported by the following data.

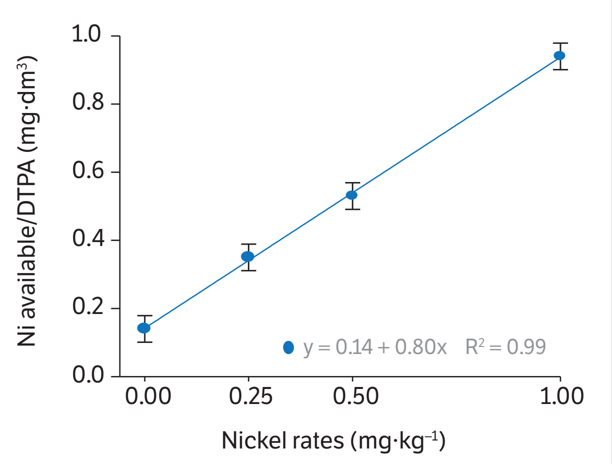

Figure 1. Ni available content (Ni-DTPA – mg·dm^{-3}) in soil after cultivation of soybean (*Glycine max*) in pots and correspondent applied doses of Ni (mg·kg^{-1}) in tropical soil.

There would also be a particular risk of Ni deficiency in second-generation seed grown in the sandy soils. This is because the Ni will be diluted and depleted in the first generation so that when the crop sets seed it is likely to be deficient in Ni. This seed, if used as seed source for plants to be grown in this same sandy soil, would present an enhanced probability of Ni deficiency in the second generation. As a result, on sandy soils the application of supplemental Ni by growers should be seriously considered because of both the hypotheses.

There was no interaction between micronutrient dose and soil type. However the clayey soil had an average higher availability of Ni content than the sandy soil, at all doses, because the initial higher Ni content remained in the clay soil (Table 2).

Table 2. Ni content (mg·dm^{-3}) in two tropical soils (sandy and clayey) and correspondent applied doses of Ni.

Ni rates	Sandy	Clayey
(mg·kg^{-1})	(mg·dm^{-3})	
0	0	0.28
0.25	0.15	0.55
0.50	0.45	0.60
1.00	0.75	1.13
Mean	0.34 b	0.64 a
Texture	Significant effect at the 5% level ($p < 0.05$)	
Dose	Significant effect at the 5% level ($p < 0.05$)	
T × D	Not significant	

Results followed by different lowercase letters indicate a significant effect at the level of 5% by Tukey test.

Plant dry-matter and leaf foliar area

The application of the Ni treatments in soils influenced both the dry matter (DM) and leaf foliar area (LFA) of soybean. At 40 DAP the DM and LFA fitted a quadratic regression model regardless of soil type (Figs. 2 and 3). The maximum production of dry matter (5.5 g·plant^{-1}) and larger leaf foliar area (761.9 mm^{-2}) was achieved at Ni dose of 0.63 mg·kg^{-1}. These increments corresponded to increases of 26.7% and 38.6% dry matter and leaf foliar area, respectively, in comparison to the control with no supplemental Ni. At 70 DAP, there was a significant interaction between rates of Ni and type of soil to DM (Fig. 2). The DM fitted a linear model in the two studied soils. However, for the LFA the effect was linear only for rates of Ni applied (Fig. 3). In sandy soils, dry matter was increased by 25% under Ni application, from 9.6 g·plant^{-1} without treatment to 12.0 g·plant^{-1} on plots with a supply of 1 mg·kg^{-1} of Ni in soil. In clayey soil, an increment of 12.56% was achieved, from 13.85 g·plant^{-1} at the dose of 0 to 15.59 g·plant^{-1} at maximum rate. Leaf foliar area varied from 885.8 to 931.5 mm^{-2} independent of soil type, or rather, Ni application increased LFA up to 5.16%.

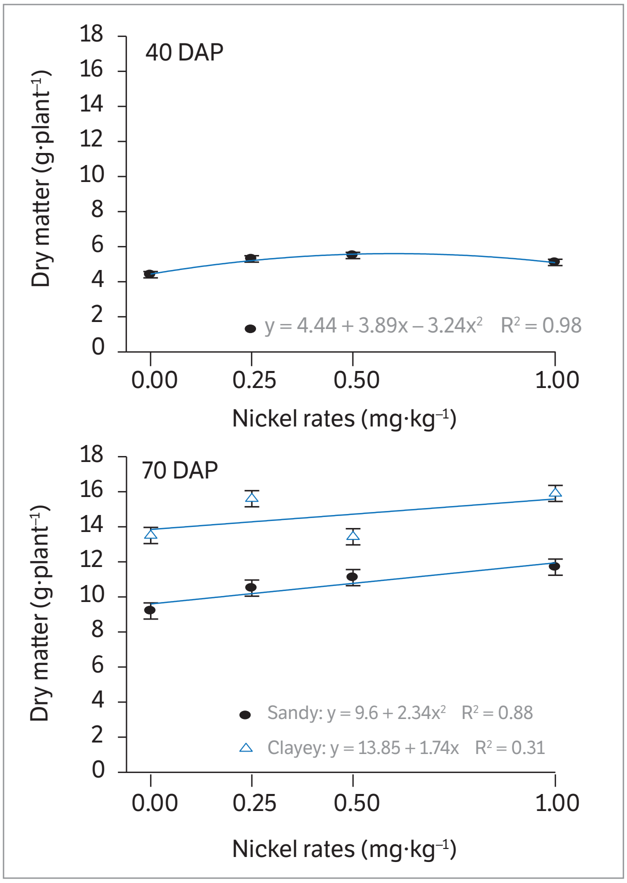

Figure 2. Soybean (*Glycine max*) dry matter production (g·plant^{-1}) at 40 and 70 DAP and correspondent applied doses of Ni (mg·kg^{-1}) in two tropical soils (sandy and clayey).

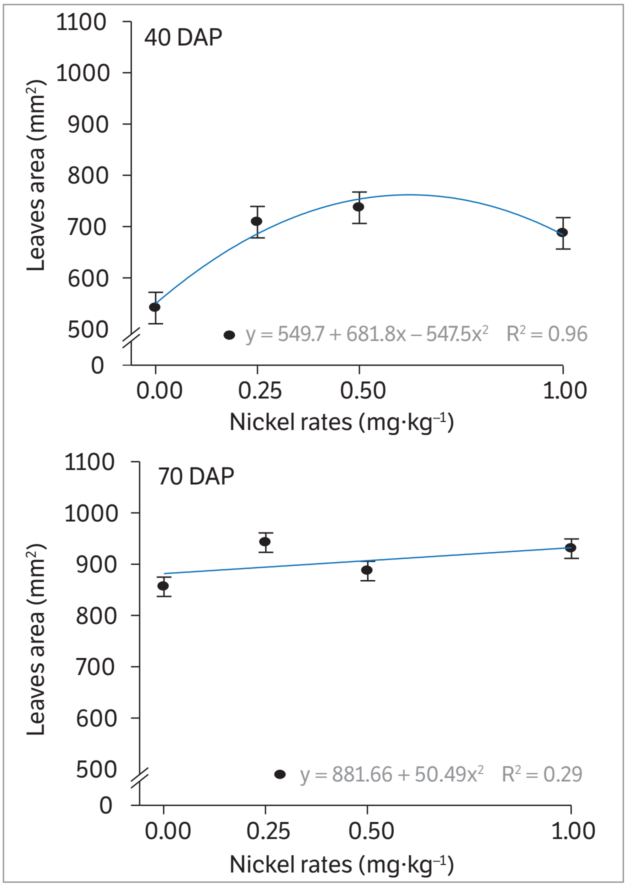

Figure 3. Soybean (*Glycine max*) leaf foliar area production (mm^{2}) at 40 and 70 DAP and correspondent applied doses of Ni (mg·kg^{-1}) in tropical soil.

Plant height

Forty DAP plant height was significantly affected by texture and also by Ni dose with no interaction effect between texture and dose (Table 3). In sandy soils plant height had the largest increase (+14.5%) observed between the control and the lowest dose of Nickel. Thereafter plant height continued to increase with increasing applications of Ni but to a lesser extent as the plant moved from probable Ni deficiency in the control to sufficiency at doses of 0.5 mg·kg^{-1} and above. This growth pattern indicating diminishing returns from increased applications is typical of what can be expected in plant nutritional studies where a plant moves from a nutrient deficiency to a nutrient sufficiency situation, as confirmed in other studies for soybean

→

Rodak (2014)[4]. Plant growth was quadratically adjusted independent of the soil sampled. The highest growth was obtained at the dose of 0.75 mg·kg^{-1} (Fig. 4). No significant results were observed at 70 DAP.

Recent studies in Ni handling in soybean crops also showed significant effects of this micronutrient on soybean growth and dry mass production (Queiroz 2012[5]; Rodak 2014; Franco 2015[6]). Kutman et al. (2013) studied the effects of soybean seeds enrichment with Ni and verified that seeds with low levels of Ni presented limited growth when submitted to urea foliar applications. Lavres et al. (2016) analyzed the effects of soybean seeds submitted to different Ni doses. The authors obtained a 64% increment in dry matter related to an increasing rate of Ni from 0 to 180 mg·kg^{-1}. From this dose onwards, dry matter production started to decrease, indicating that Ni doses higher than 180 mg·kg^{-1} in seeds treatment can be toxic. In the present work, linear growth in dry matter production was observed in both soils, indicating that the application of Ni via soil reduces the risk of phytotoxicity.

Table 3. Soybean (*Glycine max*) cultivated in pots plant growth (height in cm) at 40 and 70 DAP and correspondent applied doses of Ni in two tropical soils (sandy and clayey).

Dose (mg·kg^{-1})	Plants height			
	Sandy (cm)	Clayey (cm)	Sandy (cm)	Clayey (cm)
	40 DAP		70 DAP	
0.00	26.0	32.5	61.3	66.6
0.25	29.7	36.6	60.4	61.5
0.50	30.5	33.8	62.3	62.8
1.00	31.0	35.6	63.5	61.5
Mean	20.9B	34.6A	61.9A	63.1A
Variance analysis summary				
CV%	6.8		6.3	
Texture	*		not significant	
Dose	*		not significant	
Texture × Dose	not significant		not significant	

*Significant effect at the 5% level ($p < 0.05$). Results followed by different capital letters indicate a significant effect at the level of 5% by Tukey test.

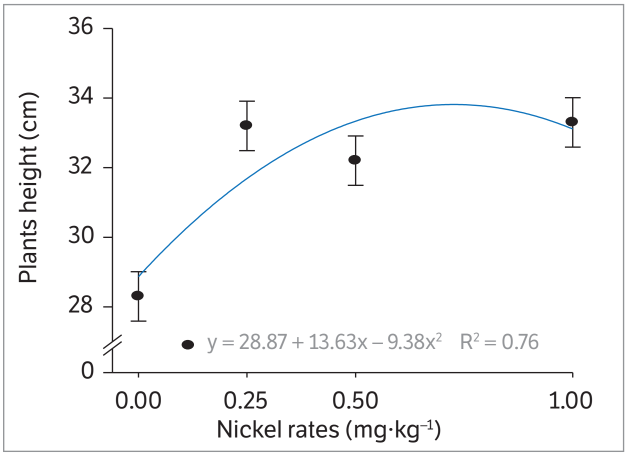

Figure 4. Soybean (*Glycine* max) plants height (cm) at 40 DAP and correspondent applied doses of Ni (mg·kg^{-1}) in tropical soil.

Ni and N concentration in shoots

The application of different Ni rates in the soil had a positive influence on the accumulation of this micronutrient in the shoots of the soybean plants at 40 and 70 DAP (Fig. 5). There was significant interaction between Ni doses and soil type on both samplings. The Ni content in plants grown in sandy soil increased quadratically as doses increased. At 40 DAP the maximum Ni concentration (3.75 mg·kg^{-1}) in vegetative organs was obtained by applying 1.0 mg·kg^{-1} on soil. On the other hand, at 70 DAP the maximum accumulation of Ni in aerial tissues (2.58 mg·kg^{-1}) was achieved at 1.2 mg·kg^{-1} dose. Although Ni content in grown plants increased with higher doses in clayey soil, this raise does not fit the models studied at 40 DAP. At 70 DAP the Ni accumulation followed a linear correlation from 0.36 mg·kg^{-1} at dose 0 to 0.74 mg·kg^{-1} at maximum dosage. Rodak et al. (2015) studied methods for Ni quantification in soil and in plants grown in different tropical soils from Brazil. Their research determined that Ni absorption by plants increases along with raised supplied doses. According to these authors, the availability and absorption of Ni by soybean is higher in sandy soils. This is due to the adsorption capacity of this element to iron and aluminum oxides. Mellis et al. (2004) studied the adsorption of Ni in Brazilian soils and verified high affinity of this micronutrient for soil colloids, which makes the availability of Ni lower in clayey textures.

---

[4] Rodak, B. W. (2014). Níquel em solos e na cultura de soja. (Master's Dissertation). Curitiba: Universidade Federal do Paraná.

[5] Queiroz, C. S. (2012). Níquel, outros micronutrientes e silício e a ferrugem asiática (Phakopsora pachyrhizi) na cultura da soja (Glycine Max). (Master's Dissertation). Goiânia: Universidade Federal de Goiás.

[6] Franco, G. C. (2015). Tratamento de sementes de soja com níquel para o aumento da fixação biológica e atividade da urease. (Master's Dissertation). Piracicaba: Universidade de São Paulo.

Ni doses affected negatively the nitrogen (N) concentration in shoots from the samples collected at 40 DAP. The N content was not affected in samples collected at 70 DAP. This is probably due to an additional N based fertilizer applied at 40 DAP (Table 4). Although N leaf content was indirectly correlated to Ni doses at 40 DAP, there was no difference between the treatments concerning accumulated N content. This implies that N decrease relates to a higher growth rate of these plants (Table 3), which suggests a dilution effect by higher dry mass. Kutman et al. (2013), studying the effect of seed enrichment and Ni supplementation via nutrient solution aiming to reduce foliar damage by urea application, also did not verify nodulation in potted soybean plants. Lavres et al. (2016) evaluated the effect of increasing doses of Ni in the treatment of potted soybean seeds. They observed a linear increase of N content in the shoots of the soybean plants corresponding to increased doses of the micronutrient. The authors attributed linear N increase to the positive effect of Ni application on soybean nodulation, which did not occur in the present experiment. Another factor that may have affected N accumulation was the applied limestone to raise soil saturation until 70%. Macedo et al. (2016) analyzed liming effect on Ni availability and N-metabolism on soybean in Alfisol. For this purpose, different doses of Ni were applied – 0, 0.1, 0.5, 1.0 and 10 mg·dm^{-3} – and saturation was increased by base addition to 50% and 70%. It was verified that at 70%, Ni alone increased the N accumulation in the shoots with 10 mg·dm^{-3} of Ni, 10 times the highest dose used in this study.

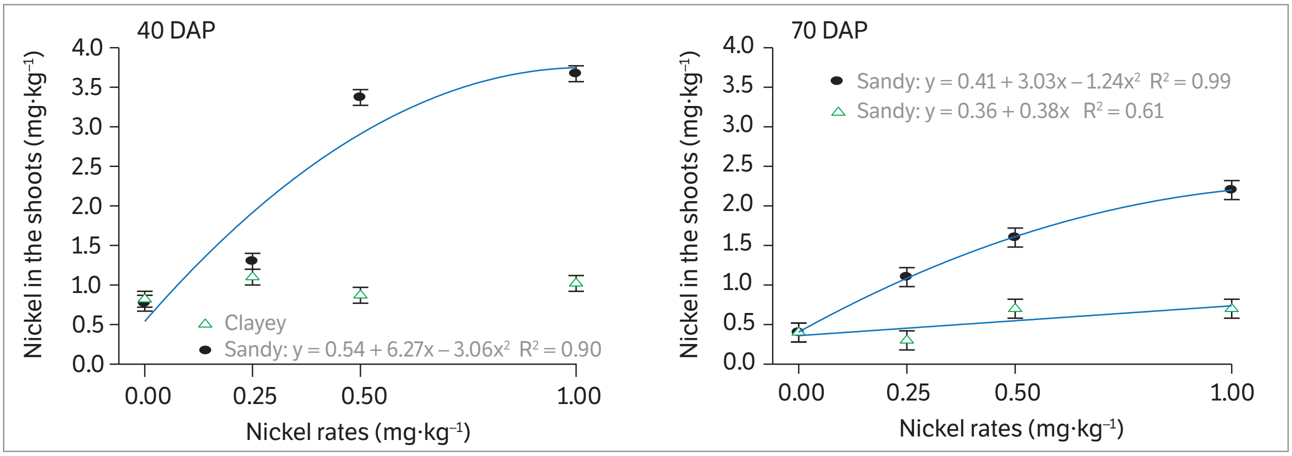

Figure 5. Ni content (mg·kg^{-1}) in soybean (*Glycine max*) shoots at 40 and 70 DAP and correspondent applied doses of Ni (mg·kg^{-1}) in two tropical soils (sandy and clayey).

Table 4. N content in soybean (*Glycine max*) shoots at 40 and 70 DAP and corresponding correspondent applied doses of Ni.

Dose (mg·kg^{-1})	40 DAP				70 DAP			
	N content in leaves		Stored N		N content in leaves		Stored N	
	Sandy	Clayey	Sandy	Clayey	Sandy	Clayey	Sandy	Clayey
	(g·kg^{-1})		(mg·plant^{-1})		(g·kg^{-1})		(mg·plant^{-1})	
0.00	42.4	36.2	72.4	116.3	27.1	23.7	136.8	167.3
0.25	41.7	37.3	67.3	125.7	26.7	26.0	139.1	348.3
0.50	34.9	31.7	61.8	110.4	27.5	25.7	152.3	161.1
1.00	37.2	31.2	58.0	103.7	27.2	25.9	158.2	206.0
Mean	39.0A	34.1B	64.9B	114.0A	27.1A	25.3A	146.6B	220.7A
CV%	7.70		22.2		11.24		45.9	
Texture	*		*		ns		*	
Dose	*		ns		ns		ns	
Texture × Dose	ns		ns		ns		ns	

*Significant effect at the 5% level ($p < 0.05$). Results followed by different capital letters indicate a significant effect at the level of 5% by Tukey test; ns = not significant.

Urease activity

The urease activity in soybean was positively influenced by the correlation of several rates of Ni and soil types (Fig. 6). This result corroborates with Freitas et al. (2018) and Barcelos et al. (2017), who showed leaf urease activity was very responsive to Ni fertilization. In samples collected at 40 DAP increased urease activity by Ni fertilization fitted a quadratic model in the sandy soil and a linear model in clayey soil. In the clayey soil, an increase of urease activity of 33.31 mmol·g^{-1}·h^{-1} was obtained with the application of the highest dose of Ni. In the sandy soil, an increase of 194.8 mmol·g^{-1}·h^{-1} was observed with the same dose (0.70 mg·kg^{-1} of Ni in the soil). In the samples collected at 70 DAP the correlation was quadratic in both soils. In the clayey soil, the highest Ni dose was 0.86 mg·kg^{-1}, leading to an increase of 145.2 mmol·g^{-1}·h^{-1} compared to dose 0. In the sandy soil, the highest Ni dose was 0.76 mg·kg^{-1}, leading to an increase of 301.9 mmol·g^{-1}·h^{-1} in comparison to dose 0.

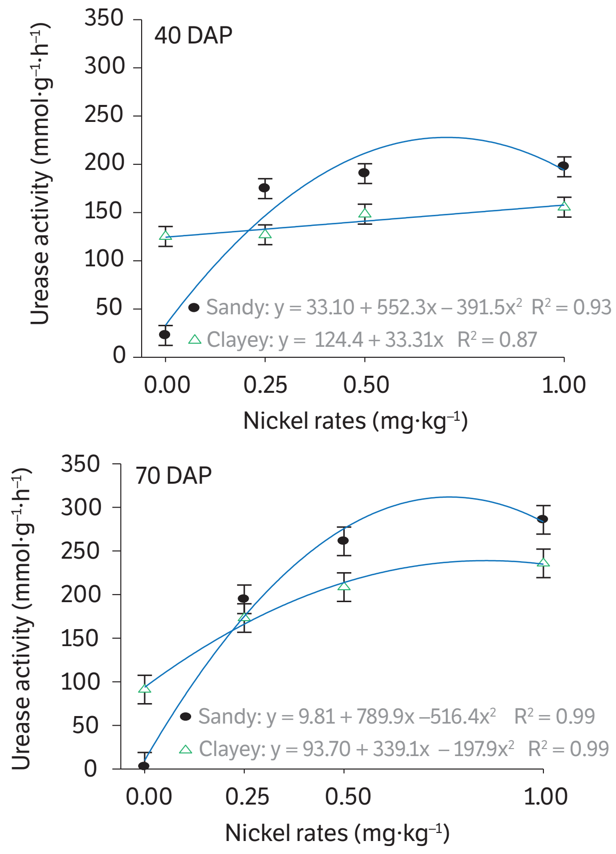

Figure 6. Urease activity (mmol·g^{-1}·h^{-1}) and correspondent applied doses of Ni (mg·kg^{-1}) in two tropical soils (sandy and clayey).

Rodak (2014) also studied the effect of several Ni doses in soybean, varying from 0 to 5 mg·dm^{-3} on two tropical soils with contrasting textures in the state of Paraná, Brazil. The author obtained a maximum urease activity when using 0.50 mg·dm^{-3} of Ni in sandy soil and 0.60 mg·dm^{-3} of Ni, which corroborates the results found in this work. According to him, the application of 5 mg·dm^{-3} of Ni in the soil decreased the urease activity in soybean due to phytotoxicity. Lavres et al. (2016) and Barcelos et al. (2017) studied Ni application effects both through seed and leaf treatments in tropical soils and described a similar urease activity to the one found in the present work. It evidences the importance of supplying this micronutrient in appropriate amount to the N metabolism in soybean plants.

Number of pods and grain production

Although productivity was not increased by Ni supply (Table 5), a quadratic effect was observed in the number of pods in soybeans treated plants, regardless of soil type. At 0.5 mg·kg^{-1} the maximum number of pods was 24, two more than the treatment without Ni (Fig. 7). In a similar work, Rodak (2014) observed a reduction in the number of soybean pods with the application of Ni doses higher than 0.7 mg·dm^{-3} in a clayey soil in Brazil that presented signs of phytotoxicity.

Table 5. Grain mass (g) and number of pods of soybean (*Glycine max*) cultivated in pots and correspondent applied doses of Ni.

Dose (mg·kg^{-1})	Grains		Pods	
	Sandy	Clayey	Sandy	Clayey
	(g)		(number per plant)	
0.00	10	13	21	22
0.25	10	13	25	24
0.50	9	13	23	23
1.00	9	13	21	22
Mean	9.5B	13A	22.5	22.7
Variance analysis summary				
CV%	5.62		5.35	
Texture	*		ns	
Dose	ns		*	
Texture × Dose	ns		ns	

*Significant effect at the 5% level ($p < 0.05$). Results followed by different capital letters indicate a significant effect at the level of 5% by Tukey test; ns = not significant.

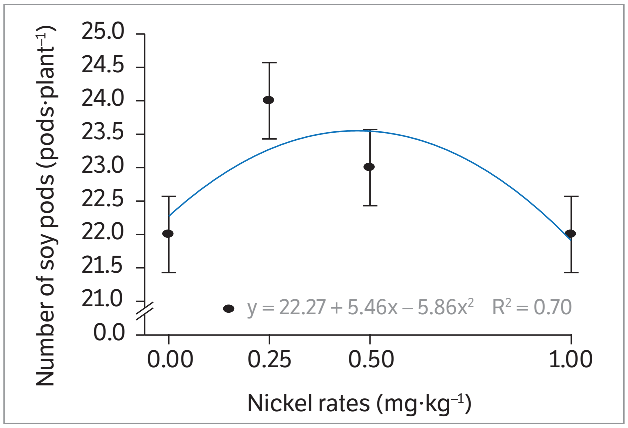

Figure 7. Number of pods per plant of soybean (*Glycine max*) and correspondent applied doses of Ni (mg·kg^{-1}) in tropical soil.

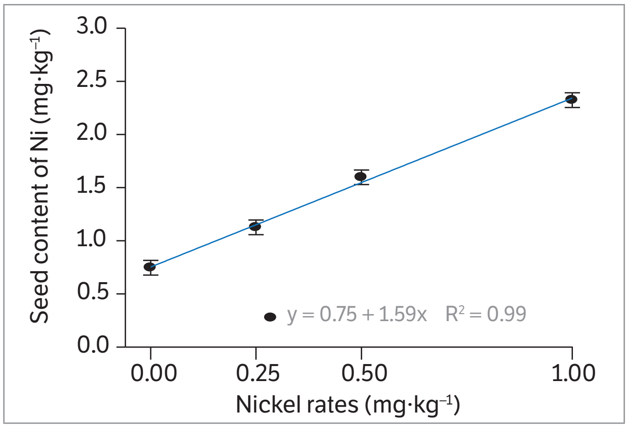

Figure 8. Ni content (mg·kg^{-1}) in seeds of soybean (*Glycine max*) and correspondent applied doses of Ni (mg·kg^{-1}) in tropical soil

Ni content in soybean grains

The Ni content in soybean grains was linearly increased regardless of the type of soil (Fig. 8). The Ni content in grains also increased from 0.74 mg·kg^{-1} between no Ni applied to 2.32 mg·kg^{-1} of Ni in grains at a dose of 1 mg·kg^{-1}. Rodak et al. (2015) observed an average accumulation in soybean grains in the order of 3.22 mg·kg^{-1} by the application of Ni in the soil. Lavres et al. (2016), when studying the application of Ni in the treatment of soybean seeds in a clayey soil, obtained the maximum content of 4.0 mg·kg^{-1} with the application of 360 mg·kg^{-1}. Barcelos et al. (2017), studying the effect of Ni application via foliar, found that the Ni content in soybean grains was caused to be linearly increased. In that study, the maximum Ni content in the grains was 14.0 mg·kg^{-1} with the application of 100 g Ni·ha^{-1}. Flyvholm et al. (1984), determined Ni levels in soybeans consumed in the human diet, finding an average of 5.2 mg·kg^{-1}. The mean values found in soybeans in this study are below the maximum tolerance range established by the Brazilian National Health Surveillance Agency (ANVISA 1965), which is 5.0 mg·kg^{-1} of Ni. Thus, when comparing the results obtained in the present study with the others aforementioned, the application of low doses of Ni via soil is apparently safer than other methods. As mentioned by Freitas et al. (2018) and after the results obtained by this study, it is clear the need of more studies to set an accurate Ni rate for higher crops. The grain mass was not affected by Ni doses in any of the studied soils. The results obtained in this study regarding productivity corroborate Alovisi et al. (2011), Kutman et al. (2013), Lavres et al (2016) and Rodak (2014), who studied the application of Ni, via foliar, seed and soil, obtaining no increase in the yield of soybean plants.

CONCLUSION

This was the first study to review Ni application rates in different types of soils of São Paulo state. Soil texture influences the availability of Ni for soybean plants and consequently its response to the addition of this micronutrient. Ni addition via soil increases the activity of urease in soybean plants, although there was no evidence of yield gain due to this effect. There was a positive effect of increasing doses of Ni applied via soil in sandy soil on the number of pods per plant. The Ni doses used in this experiment had a negative effect on the dry mass of the final shoot in both sandy and clayey texture, except for the 0.25 mg.kg^{-1} dose in the clayey soil. The effect of Ni was negative for the dry mass of grains in sandy soil, except for 0.25 mg.kg^{-1}. In relation to dry matter production of shoot, grain and number of pods per plant, the dose of Ni with the best performance to both sandy and clayey soil was 0.25 mg.kg^{-1}. For future studies, this dose should be further experimented in different types of soil to increase the scientific and practical knowledge about Ni.

AUTHOR'S CONTRIBUTION

Conceptualization, Levy C. C. B., Mellis E. V., Chiba, M. K.; Methodology, Levy C. C. B., Mellis E. V., Chiba M. K.; Investigation, Levy C. C. B., Murrer M. K., Cavalli E.;

→

Writing – Original draft, Levy C. C. B., Mellis E. V., Cavalli E., Chiba M. K.; Writing – Review and Editing, Levy C. C. B., Mellis E. V., Murrer M. K., Inglés C. R., Daynes C. N.; Funding Acquisition, Mellis E. V., Chiba M. K.; Resources, Levy C. C. B., Murrer M. K., Cavalli E.; Supervision, Mellis E. V., Chiba M. K.

ORCID IDs

C. C. B. Levy
https://orcid.org/0000-0003-1265-7598
E. V. Mellis
https://orcid.org/0000-0002-7492-3823
M. K. Murrer
https://orcid.org/0000-0002-1210-4961
C. R. Inglés
https://orcid.org/0000-0002-2539-4320
C. N. Daynes
https://orcid.org/0000-0002-1292-4572
E. Cavalli
https://orcid.org/0000-0003-0824-0861
M. K. Chiba
https://orcid.org/0000-0002-7468-2660

REFERENCES

Abreu, C. A., Novais, R. F., Raij, B. and Ribeiro, A. C. (1994). Comparação de métodos para avaliar a disponibilidade do manganês em solos. Revista Brasileira de Ciência do Solo, 18, 81-90.

Adriano, D. C. (1986). Trace elements in the terrestrial environment. New York: Springer-Verlag.

Alovisi, A. M. T., Magri, J., Dutra, J. E., Magri, E., Santos, M. J. G. and Alovisi, A. A. (2011). Foliar fertilization with nickel sulphate in soybean. Tests and science: biological sciences. Agriculture and Health, 15, 25-32.

Bai, C., Reilly, C. C. and Wood, B. W. (2006). Nickel deficiency disrupts metabolism of ureides, amino acids, and organic acids of young pecan foliage. Plant Physiology, 140, 433-443. https://doi.org/10.1104/pp.105.072983

Barcelos, J. P. Q., Osorio, C. R. W. S., Leal, A. J. F., Alves, C. Z., Santos, E. F., Reis, H. P. G. and Reis, A. R. (2017). Effects of foliar nickel (Ni) application on mineral nutrition status, urease activity and physiological quality of soybean seeds. Australian Journal of Crop Science, 11, 184-192.

Bataglia, O. C., Cardoso, M. and Carretero, M. V. (1988). Situação nutricional de seringais produtivos no Estado de São Paulo. Bragantia, 47, 109-123.

[ANVISA] Agência Nacional de Vigilância Sanitária (1965). Decreto no 55.871, de 26 de março de 1965. Modifica o Decreto no 50.040, de 24 de janeiro de 1961, referente a normas reguladoras do emprego de aditivos para alimentos, alterado pelo Decreto no 691, de 13 de março de 1962. Brasília: DOU.

Brown, S. S. and Sunderman Jr., F. W. (1980). Nickel toxicology. London: Academic Press.

Brown, P. H., Welch, R. M. and Cary, E. E. (1987). Nickel: a micronutrient essential for all higher plants. Plant Physiology, 85, 801-803. https://doi.org/10.1104/pp.85.3.801

Brown, P. H., Welch, R. M. and Madison, J. T. (1990). Effect of nickel deficiency on soluble anion, amino acid, and nitrogen levels in barley. Plant and Soil, 125, 19-27. https://doi.org/10.1007/BF00010740

Camargo, O. A., Moniz, A. C. Jorge, J. A. and Valadares, J. M. A. S. (1986). Métodos de análise química, mineralógica e física de solos. Campinas: IAC.

Dechen, A. R. and Nactigall, G. R. (2007). Ill elements required for plant nutrition. In: R. F. Novais, V. V. H. Alvarez, N. F. Barros, R. L. F. Sources, R. B. Cantarutti and J. C. L. Neves (Eds.), Soil Fertility (p. 91-132). Viçosa: Brazilian Society of Soil Science.

Dixon, N. E., Gazzola, C., Blakeley, R. L. and Zerner, R. (1975). Jack bean urease (EC 3.5.1.5) Metalloenzyme. A simple biological role for nickel. Journal of the American Chemical Society, 97, 4131-4133. https://doi.org/10.1021/ja00847a045

Epstein, E. and Bloom, A. J. (2005). Mineral nutrition of plants: principles and perspectives. Sunderland: Sinauer Associates.

Eskew, D. L., Welch, R. M. and Cary, E. E. (1983). Nickel: an essential micronutrient for legumes and possibly all higher-plants. Science, 222, 621-623. https://doi.org/10.1126/science.222.4624.621

Fabiano C. C., Tezotto T., Favarin J. L., Polacco J. C. and Mazzafera P. (2015). Essentiality of nickel in plants: a role in plant stresses. Front Plant Science, 6, i.754. https://doi.org/10.3389/fpls.2015.00754

Flyvholm, M. A., Nielsen, G. D. and Andersen, A. (1984). Nickel content of food and estimation of dietary intake. Z. Lebensm. Unters. Forsch., 179, 427-431. https://doi.org/10.1007/BF01043419

Freitas, D. S., Rodak, B. W., Reis, A. R., Reis, F. B., Carvalho, T. S., Schulze, J., Carneiro, M. A. C. and Guilherme, L. R. G. (2018). Hidden nickel deficiency? nickel fertilization via soil improves nitrogen metabolism and grain yield in soybean genotypes. Frontiers in Plant Science, 9, 614. https://doi.org/10.3389/fpls.2018.00614

Hogan, M. E., Swift, I. E. and Done, J. (1983). Urease assay and ammonia release from leaf tissues. Phytochemistry, 22, 663-700. https://doi.org/10.1016/S0031-9422(00)86958-7

Klucas, R. V., Hanus, F. J., Sterling, A. R. and Evans, H. J. (1983). Nickel: a micronutrient element for hydrogen-dependent growth of *Rhizobium japonicum* and for expression of urease Activity In Soybean Leaves. Proceedings of the National Academy of Sciences, 80, 2253-2257. https://doi.org/10.1073/pnas.80.8.2253

Kutman, B. Y., Kutman, U. B. and Cakmak I. (2013). Nickel-enriched seed and externally supplied nickel improve growth and alleviate foliar urea damage in soybean. Plant and Soil, 363, 61-75. https://doi.org/10.1007/s11104-012-1284-6

Kutman, B. Y., Kutman, U. B. and Cakmak, I. (2014). Effects of seed nickel reserves or externally supplied nickel on the growth, nitrogen metabolites and nitrogen use efficiency of urea-or nitrate-fed soybean. Plant and Soil, 376, 261-276. https://doi.org/10.1007/s11104-013-1983-7

Lavres, J., Franco G. C. and Câmara G. M. S. (2016). Soybean seed treatment with nickel improves biological nitrogen fixation and urease activity. Front. Environ. Science, 4, 37. https://doi.org/10.3389/fenvs.2016.00037

Macedo, F. G., Bresolin, J. D., Santos, E. F., Furlan, F. L., Silva, W. T., Polacco, J. C. and Lavres, J. (2016). Nickel availability in soil as influenced by liming and its role in soybean nitrogen metabolism. Front. Plant Science, 7, 1358. https://doi.org/10.3389/fpls.2016.01358

Malavolta, E. (2006). Manual de nutrição mineral de plantas. São Paulo: Ceres.

Masuda, T. (2009). Production world. Soybean Research Bulletin, 13. Rondonópolis: Mt Foundation.

Mazzafera, P., Tezotto, T. and Polacco, J. C. (2013). Nickel in plants. In R. H. Kretsinger, V. N. Uversky and E. A. Permyakov (Eds.), Encyclopedia of Metalloproteins. New York: Springer.

Mcclure, P. R. and Israel, D. W. (1979). Transport of nitrogen in the xylem of soybean plants. Plant Physiology, 64, 411-416. https://doi.org/10.1104/pp.64.3.411

Mellis, E. V., Cruz, M. C. P., Casagrande, J. C. (2004). Nickel adsorption by soils in relation to pH, organic matter and iron oxides. Scientia Agricola, 61, 190-195. https://doi.org/10.1590/S0103-90162004000200011

Moraes, M. F., Abreu Junior, C. H., Lavres Junior, J. (2010). Micronutrientes. In L. I. Prochnow, V. Casarn and S. R. Stipp (Eds.), Boas práticas para uso eficiente de fertilizantes (p. 205-278). Piracicaba: International Plant Nutrition Institute.

Pombo, L., Salgado, V., Volkweiss, S. and Klamt, E. (1989). Adsorção de níquel por dois solos: terra bruna estruturada similar e podzólico vermelho-amarelo. Pesquisa Agropecuária Brasileira, 24, 593-598.

Raij, B., Quaggio, J. A., Cantarella, H. and Abreu, C. A. (2001). Os métodos de análise química do sistema IAC de análise de solo no contexto nacional. In B. van Raij, L. J. C. Andrade, H. Cantarella and J. A. Quaggio (Eds.), Análise química para avaliação da fertilidade em solos tropicais (p. 251-256). Campinas: Instituto Agronômico.

R Development Core Team (2008). R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing.

Rodak, B. W., Moraes, M. F., Pascoalino, J. A. L., Oliveira Junior, A., Castro, C. and Pauletti, V. (2015). Methods to quantify nickel in soils and plant tissues. Revista Brasileira de Ciência do Solo, 39, 788-793. https://doi.org/10.1590/01000683rbcs20140542

Rovers, H., Camargo, O. A. and Valadares, J. M. A. S. (1983). Níquel total e solúvel em DTPA em solos do Estado de São Paulo. Revista Brasileira de Ciência do Solo, 7, 217-220.

Sellstedt, A. and Smith, G. D. (1990). Nickel is essential for active hydrogenase in free-living *Frankia* isolated from *Casuarina*. FEMS Microbiology Letters, 70, 137-140. https://doi.org/10.1111/j.1574-6968.1990.tb13966.x

Seregin, I. V. and Kozhevnikova, A. D. (2006). Physiological role of nickel and its toxic effects on higher plants. Russian Journal of Plant Physiology, 53, 285-308. https://doi.org/10.1134/S1021443706020178

Syam, N., Wardiyati, T., Maghfoer, M. D., Handayanto, E., Ibrahim, B. and Muchdar, A. (2016). Effect of Accumulator Plants on Growth and Nickel Accumulation of Soybean on Metal-contaminated Soil. Agriculture and Agricultural Science Procedia, 9, 13-19. https://doi.org/10.1016/j.aaspro.2016.02.109

Tezotto, T., Souza, S. C. R., Mihail, J., Favarin, J. I., Mazzafera, P., Bilyeu, K. and Polacco, J. C. (2016). Deletion of the single UreG urease activation gene in soybean NIL lines: characterization and pleiotropic effects. Theor Exp Plant Physiology, 28, 307-320. https://doi.org/10.1007/s40626-016-0052-z

Ureta, A. C., Imperial, J., Ruiz-Argüeso, T. and Palacios, J. M. (2005). *Rhizobium leguminosarum* biovar *viciae* symbiotic hydrogenase activity and processing are limited by the level of nickel in agricultural soils. Applied and Environmental Microbiology, 71, 7603-7606. https://doi.org/10.1128/AEM.71.11.7603-7606.2005

Witte, C. P. (2011). Urea metabolism in plants. Plant Science, 180, 431-438. https://doi.org/10.1016/j.plantsci.2010.11.010