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EFFECTS OF NICKEL AND NITROGEN SOIL FERTILIZATION ON LETTUCE GROWTH AND UREASE ACTIVITY[1]

Thomas Carlos Oliveira[2], Renildes Lúcio Ferreira Fontes[3], Sebastião Tavares de Rezende[4] & Víctor Hugo Alvarez V.[3]

SUMMARY

Nickel is a micronutrient involved in nitrogen metabolism and a constituent of the urease molecule. Plant growth and urease activity were evaluated in lettuce (*Lactuca sativa* L.) grown in soil-filled pots in a 2 x 8 factorial design with two nitrogen (N) sources and eight Ni rates, with five replications. Nitrogen was applied at 200 mg dm^{-3} (half the dose incorporated into the soil at seedling transplanting and half top-dressed later) using the sources NH_4NO_3 (AN) and $CO(NH_2)_2$ (Ur). The Ni treatments (0, 2, 4, 8, 12, 16, 24 and 32 mg dm^{-3}) were applied as $NiCl_2$. The shoot dry-matter yield, leaf urease activity, Ni levels in the lettuce leaves and Ni levels extracted from soil with Mehlich-3 (M-3) and DTPA were determined. In the plants supplied with AN, the shoot dry-matter yield was higher than in those supplied with Ur. There was no difference in shoot dry matter in response to soil-applied Ni. The leaf urease activity increased with Ni application, regardless of the N source. The extractions with M-3 and DTPA were efficient to evaluate Ni availability for lettuce in the Red-Yellow Latosol.

Index terms: ammonium nitrate, urea, plant nutrition, *Lactuca sativa* L.

RESUMO: *EFEITOS DA ADUBAÇÃO COM NÍQUEL E NITROGÊNIO NO CRESCIMENTO E NA ATIVIDADE DA UREASE EM ALFACE*

Níquel é um micronutriente envolvido no metabolismo de nitrogênio e é componente da molécula da urease. Foram avaliados o crescimento de plantas e a atividade da urease em alface (Lactuca sativa L.) *cultivada em solo em vasos, em experimento fatorial 2 x 8, com duas fontes de nitrogênio (N) e oito doses de Ni, com cinco repetições. O N foi aplicado ao solo (200*

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mg dm^{-3}), incorporando-se metade no transplantio das mudas e a outra metade, mais tarde, na superfície do solo, utilizando-se nitrato de amônio (NH_4NO_3) e ureia [$CO(NH_2)_2$]. Os tratamentos com Ni (0, 2, 4, 8, 12, 16, 24 e 32 mg dm^{-3}) foram aplicados, usando-se cloreto de níquel ($NiCl_2$). Produção de matéria seca, atividade da urease e do Ni nas folhas de alface e Ni no solo (Mehlich-3 e DTPA) foram determinados. A produção de matéria seca nas plantas supridas com NH_4NO_3 foi maior do que nas supridas com $CO(NH_2)_2$. Não houve diferença na produção de matéria seca das folhas em resposta à aplicação de Ni ao solo. A atividade da urease aumentou com o incremento das doses de Ni, mas não se alterou em razão da fonte de N utilizada. As extrações com Mehlich-3 e DTPA foram eficientes para avaliar a disponibilidade de Ni para alface no Latossolo Vermelho-Amarelo.

Termos de indexação: nitrato de amônio, ureia, nutrição de plantas, Lactuca sativa *L.*

INTRODUCTION

Nearly seven decades ago, crop responses to the application of nickel (Ni) fertilizer in common bean (*Phaseolus vulgaris* L.), potato (*Solanum tuberosum* L.) and wheat (*Triticum aestivum* L.) were described by Roach & Barclay (1946). In 1975, Dixon et al. (1975) found that Ni is a component of urease, a ubiquitous metalloenzyme present in plants, and later on, based on works by Eskew et al. (1983, 1984) and Brown et al. (1987), Ni was classified as a micronutrient (Salisbury & Ross, 1992; Marschner, 2008). However, research with Ni has mainly addressed its toxic effects on plants and on how Ni-hyperaccumulator plants respond to high Ni concentrations. Apparently, Ni^{2+} reaches toxicity levels in the shoots first, but higher Ni^{2+} activities can also be toxic to the roots, decreasing growth and inhibiting lateral root formation (Kopittke et al., 2007). Although Ni is an important environmental contaminant, its action mechanism in plants is not yet clear (Sengar et al., 2008)

It is known that Ni is directly related to the nitrogen (N) metabolism of plants because Ni deficiency impairs the urease activity, resulting in accumulation of urea in the plant. Further evidence of the Ni requirement for N metabolism comes from the observation that the accumulation of urea in Ni-free urea-based media caused growth suppression and a deficiency of physiological N (Gerendás et al., 1998). The urea absorbed by a plant has to be broken down to CO_2 and NH_3 for N assimilation and to prevent urea accumulation in the plant (Marschner, 2008). The Ni-metalloenzyme urease catalyzes the initial step of the urea hydrolysis to CO_2 and NH_3 (Mulrooney & Hausinger, 2003). Therefore, higher urease activity mediated by Ni (Eskew et al., 1984; Brown et al., 1987; Marschner, 2008) helps to prevent urea accumulation in the plant. Gerendás & Sattelmacher (1997a,b) verified the need for Ni for urease activation, for plant growth on urea-based media and for recycling endogenous urea. In rice grown in nutrient solution, Gerendás et al. (1998) found that in plants supplied with urea and Ni, the growth was impaired at the lower levels of Ni, whereas in plants supplied with NH_4NO_3 and Ni, growth was not affected by the micronutrient.

In nodules of N-fixing plants, the hydrogenase catalysis of the reversible oxidation of molecular hydrogen increases in response to Ni (Klucas et al., 1983), suggesting that there is a role of Ni in the symbiotic fixation of N. Recently, Bai et al. (2006) reported that both C respiration and N metabolism are sensitive to Ni nutrition and that circumstantial evidence indicates the possibility of undiscovered roles of Ni in plant nutritional physiology. Malavolta et al. (2006) showed that high levels of Ni in citrus flowers increased the urease activity with production of NH_3. Ni applied in the flowering stage would increase the flowering and the percentage of fruit set; hence, the foliar application of Ni associated to $(NH_2)_2CO$ would increase the crop yield. According to Lovatt et al. (1988), NH_3 improves the induction of citrus flowering. Although Ni is essential for plant growth, its physiological role is poorly understood (Bai et al., 2008).

Methods of soil analysis to estimate Ni availability to plants are not routinely used in soil-fertility laboratories, in contrast to studies of other micronutrients. These methods focus on soil-fertility aspects and search for the correction of nutrient deficiencies as related to plant requirements for adequate growth. Universal extracting solutions may be used to simultaneously determine the soil availability of several nutrients in the same extract (Raij, 1994; Abreu et al., 1995; Eckert & Watson, 1997). The availability of P, K, Ca, Mg, Mn, Cu, Zn, Mo, and B has been determined in multi-element extraction using Mehlich-3 solution (Mehlich, 1984; Sims, 1989; Jones Junior, 1990).

This work evaluated the urease activity in the leaves and the growth of lettuce plants in soil enriched with Ni and fertilized with urea and ammonium nitrate. Additionally, the Ni availability in the soil was evaluated using Mehlich-3 and DTPA extractors.

MATERIAL AND METHODS

Plant growth

Lettuce (*Lactuca sativa* L.) plants were grown in a Red-Yellow Brazilian Latosol in Viçosa, State of

Minas Gerais. The experiment was conducted in a greenhouse of the Soil Science Department, Federal University of Viçosa. Soil samples collected in the 0-20 cm layer were air-dried, passed through a 2 mm sieve and analyzed for chemical and physical characterization (Table 1). Nitrogen (200 mg dm^{-3}) was supplied by two sources (NH_4NO_3 and $CO(NH_2)_2$) and Ni (doses 0, 2, 4, 8, 12, 16, 24, and 32 mg dm^{-3} Ni) was supplied as $NiCl_2.6H_2O$. The experiment was arranged in a 2 x 8 factorial design with five replications in randomized blocks, and the experimental unit consisted of a 3 dm^3 plastic pot containing soil with the treatments and two lettuce plants. After liming to reach a 70 % base saturation index (CFSEMG, 1999), the soil was incubated for 15 days maintaining soil moisture at field capacity. Each experimental unit received 300 mg dm^{-3} P (KH_2PO4), 150 mg dm^{-3} K (K_2SO_4), 2.5 mg dm^{-3} Fe (Fe-EDTA), 3 mg dm^{-3} Mn ($MnCl_2.4H_2O$), 4 mg dm^{-3} Zn ($ZnSO_4.7H_2O$), 1.0 mg dm^{-3} Cu ($CuSO_4.5H_2O$), 1.0 mg dm^{-3} B (H_3BO_3), and 0.15 mg dm^{-3} Mo ($Na_2MoO_4.2H_2O$). The soil moisture was maintained at field capacity during plant growth. Nitrogen fertilization was split into two parts: the first (100 mg dm^{-3}) was applied prior to seedling transplanting, incorporated into the soil, and the second (100 mg dm^{-3}) was divided into four 25 mg dm^{-3} portions that were broadcast on the soil surface, sequentially, every 10 days. Ur was applied in a mixture with a commercial product containing 2 % urease inhibitor N-(n-butyl) thiophosphoric triamide (NBPT). The plants were harvested after 63 days of growth. From each experimental unit, the fresh matter of one plant was analyzed for determination of urease activity. The other plant was oven-dried at 65 °C for 72 h, and the dry mass was recorded and the mineral composition determined.

Table 1. Red Yellow Latosol physical and chemical properties

Property	Value
pH (H_2O) (1:2.5)	4.80
Organic matter (dag kg^{-1})(1)	3.00
P (mg dm^{-3})(2)	0.80
K (mg dm^{-3})(2)	24.0
Al^{3+} ($cmol_c$ dm^{-3})(3)	0.20
Ca^{2+} ($cmol_c$ dm^{-3})(3)	0.08
Mg^{2+} ($cmol_c$ dm^{-3})(3)	bdl
H+Al ($cmol_c$ dm^{-3})(3)	4.80
$CEC_{pH\ 7.0}$ ($cmol_c$ dm^{-3})(4)	7.04
Base saturation index (%)	2.00
Al saturation index (%)	58.80
Ni (mg dm^{-3})(2)	bdl
Fe (mg dm^{-3})(2)	44.10
Zn (mg dm^{-3})(2)	0.26
Cu (mg dm^{-3})(2)	0.24
Mn (mg dm^{-3})(2)	3.60
Sand (%)(5)	22.00
Silt (%)(5)	4.00
Clay (%)(5)	74.00

(1)Walkley-Black method; (2)Mehlich-1; (3)KCl 1 mol L^{-1}; (4)$Ca(OAc)_2$ 0.5 mol L^{-1}, pH 7.0 (Defelipo & Ribeiro, 1981); (5)Pipette (Embrapa, 1997); bdl: below detection limit.

The N concentration in the leaves was determined using the Kjeldhal method after sulfuric digestion, and the Ni leaf concentration by atomic emission spectroscopy (ICP/AES) after nitric-perchloric acid digestion (Zazoski & Burau, 1977).

After the lettuce plants were cut, the soil from each pot was sampled, air-dried and passed through a 2 mm sieve. Soil Ni was extracted with Mehlich-3 (Mehlich, 1984) or DTPA (Raij et al., 2001), and the extracts were analyzed to determine Ni levels using atomic emission spectroscopy (ICP/AES).

Urease activity on leaves

The phenol hypochlorite assay of Witte & Medina-Escobar (2001), with modifications of the reaction time and temperature to 2 h and 40 °C, respectively, was used to analyze the urease activity in the lettuce-leaf extracts. The reagents used for the assay were a solution of 50 mmol L^{-1} phosphate buffer (PBS), Reagent A and Reagent B. The 50 mmol L^{-1} phosphate buffer solution (PBS) was prepared with 3.403 g KH_2PO_4 and 4.355 g K_2HPO_4 dissolved in 400 mL distilled water, with additional water added for a final volume of 1 L, and the pH was adjusted to 7.5. Reagent A was prepared with 34 mg of $Na_2[Fe(CN)_5NO]\cdot 2H_2O$ (disodium pentacyanonitrosylferrate) and 7 g of C_6H_5OH (phenol) dissolved in 80 mL distilled water, completing the volume to 100 mL (stored in a dark flask at 4 °C). Reagent B was prepared with 2.96 g of NaOH dissolved in 140 mL distilled water plus 22.26 g of Na_2HPO_4 and 40 mL NaOCl (6 %), completing the volume to 200 mL, with the pH adjusted to 12 (stored in a dark flask at room temperature).

For protein extraction, discs collected from six fresh lettuce leaves (two from the lower portion of the plant, two from the top, and two from the middle) were ground and homogenized in a Petri plate. Samples of 1.0 g were ground in a previously cooled mortar with 5 mL of PBS, plus 120 µL of 825 mmol L^{-1} dithiothreitol (DDT), plus 20 µL of 26 mmol L^{-1} phenylmethylsulfonyl fluoride (PMSF) (in ethylic alcohol) plus 30 mg of polyvinylpolypyrrolidone (PVPP). After extraction, the samples were placed in Eppendorf tubes and centrifuged at 14.000*g* for 10 min at 4 °C. The supernatant was collected, transferred into new tubes and centrifuged again at 14.000*g* for 20 min at 4 °C. After the final centrifugation, the extract, a clear protein solution, was removed and kept on ice to determine the urease activity.

To identify the adequate time of reaction in the phenol hypochlorite assay (Witte & Medina-Escobar, 2001), preliminary tests were performed with the

lettuce-leaf extracts after 1.0, 1.5, 2.0, 2.5, and 3.0 h of reaction. The absorbance was measured at 636 nm, and the absorbance readings were plotted as a function of the reaction times.

For urease-activity analysis, 20 µL of 5 mol L^{-1} urea was added to a test tube containing 180 µL of leaf extract, and the tube was shaken and placed to heat in a water bath for 2 h at 40 °C (heated extract). To 20 µL of heated extract, 980 µL distilled water, 100 µL of Reagent A and 200 µL of Reagent B were added. The tubes were sealed and placed in a water bath for 20 min at 50 °C to reach the endpoint of color development. The absorbance was read at 636 nm, and the urease activities in the leaf extracts were calculated. The results were expressed in katal (kat), a unit of the International System of Units for enzyme activity. One katal (1 kat) is the amount of enzyme required for transformation of 1 mol s^{-1} of substrate at defined conditions (Dybkaer, 2001). Standards of NH_4Cl were prepared at 0, 4, 10, 20, 40, and 80 µmol L^{-1} concentrations and assayed as described for the leaf extracts.

Statistics

Analyses of variance were conducted for the lettuce plants dry-matter yield, leaf urease activity, shoot Ni and N concentrations and Ni levels extracted from soil using Mehlich-3 (M-3) and DTPA.

Regression equations were adjusted for the dry-matter yield of lettuce shoots and the concentrations and contents of Ni in the shoots as a function of the doses of Ni applied to soil, within each N source (AN and Ur). The regression equations were adjusted for urease activity in the fresh matter of the lettuce leaves as a function of the Ni doses applied to soil, within each N source (AN and Ur).

The software SAEG (Statistical Analysis System) (Ribeiro Jr., 2001) was used for the statistical analysis.

RESULTS AND DISCUSSION

Plant growth

The lettuce dry-matter yield was greater in the plants supplied with NH_4NO_3 (AN) (5.07 g/plant) than in the plants supplied with $(NH_2)_2CO$ (Ur) (4.20 g/plant) (Figure 1, Tables 2 and 3). Vitti et al. (2002) found similar results for sugarcane in the field, with a yield of 66 t ha^{-1} for plants supplied with N-AN and of 57 t ha^{-1} when supplied with N-Ur. Gerendás & Sattelmacher (1997b) observed improved growth of zucchini plants in a nutrient solution containing AN, compared to plants supplied with Ur as N source.

There was no response in lettuce growth to the addition of Ni to soil, regardless of the N source, be it AN or Ur (Figure 1, Table 2). Similarly, in a nutrient solution, rye did not respond to increased doses of Ni;

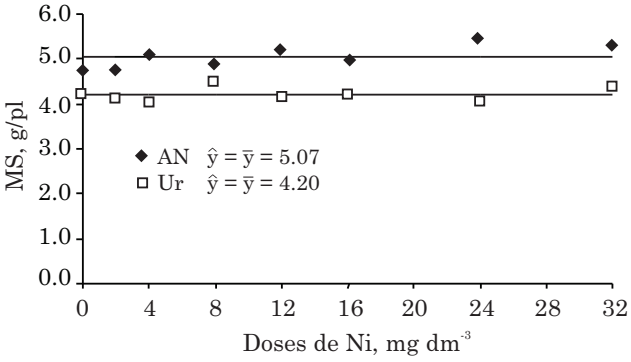

Figure 1. Lettuce dry-matter (DM) shoot production as a function of Ni doses applied to the soil and under plant N supply by additions of ammonium nitrate (AN) and urea (Ur) to the soil.

however, the dry-matter yield of soybean, cucumber and sunflower plants increased (Gerendás & Sattelmacher, 1997a). The shoots of plants grown in soil without Ni (0.0 mg dm^{-3} treatment) contained 0.75 mg kg^{-1} Ni when treated with AN and 0.19 mg kg^{-1} Ni when Ur was the N source (Table 2). The plant growth without Ni supply was similar to the plant growth in all treatments with Ni soil application (Figure 1). This pattern shows that the Ni levels added to the soil had no effect on lettuce growth, and although no available Ni (based on Mehlich-1 extraction) in the soil was detected (Table 1), no Ni deficiency was observed in the plants. Because it is unlikely that Ni in the lettuce seeds (0.45 ng/seeds) provided adequate Ni for growth, the inputs used in the soil treatment may have contributed to supply the plants with Ni. Gerendás & Sattelmacher (1997a, b) reported that experiments investigating Ni deficiency in nutrient solutions require special care to remove Ni from the growth medium, with the mandatory use of high-purity chemicals and a rigorous cleaning of all equipment. Whereas Ni deficiency is not commonly reported in plants, toxicity is the focus of many studies, mainly in sludge-treated soils.

Urease activity

The 2 h reaction time used for the urease-activity determination in the leaf extracts was based on results of preliminary tests, which showed a linear relationship between the absorbance readings (at 636 nm) and reaction times tested at 40 °C (Figure 2). This linear behavior for the relationship between NH_3 production and time spent for the occurrence of the enzymatic reaction is a criterion to ensure that no agents are interfering with the reaction medium (Witte & Medina-Escobar, 2001).

The leaf urease activity increased as a function of the increment of Ni applied to soil, independently of whether AN or Ur was used (Figure 3, Table 2). This activity increase was strong up to the concentration of 2 mg dm^{-3} Ni, less evident at the higher Ni levels and remained practically unchanged at concentrations

Table 2. Nickel (Ni) and nitrogen (N) concentrations in the dry matter of lettuce plants grown in soil amended with Ni doses and fertilized with two N sources. The regression equations for the dry-matter Ni and N are functions of the Ni doses applied to the soil

N source	Ni doses (mg dm^{-3})							
	0	2	4	8	12	16	24	32
				Ni (mg kg^{-1})				
NH_4NO_3	0.75	1.28	2.02	3.93	5.5	7.16	11.08	13.67
				$\hat{y}$ = 0.5432 + 0.4188***x R^2 = 0.99				
$(NH_2)_2CO$	0.19	1.20	2.05	3.54	5.17	6.24	9.59	11.75
				$\hat{y}$ = 0.5275 + 0.3625***x R^2 = 0.99				
				N total (dag kg^{-1})				
NH_4NO_3	3.68	3.61	3.70	3.76	3.49	3.76	3.49	3.48
				$\hat{y} = \bar{y}$ = 3.60				
$(NH_2)_2CO$	3.99	4.04	3.90	4.00	4.06	3.95	4.00	3.91
				$\hat{y} = \bar{y}$ = 4.00				

Table 3. Analysis of variance for plant dry-matter yield (DMY), leaf urease activity (LUrA), Ni and N concentrations in lettuce, and Ni extracted from the soil with Mehlich-3 (M-3) and DTPA

Source of variation	DF	MS					
		DMY	LUrA	Ni	N	M-3	DTPA
		plant				Ni (soil)	
Blocks	4	1.89^{ns}	$1{,}743.50^{***}$	3.88^{**}	0.14^{ns}	0.81^{ns}	1.37^{ns}
N sources	1	15.31^{***}	$1{,}115.50^{ns}$	9.93^{**}	2.98^{***}	5.72^{**}	6.18^{*}
Ni within AN	7	0.28^{ns}	$1{,}782.30^{***}$	110.99^{***}	0.06^{ns}	83.94^{***}	30.74^{***}
Ni within Ur	7	0.11^{ns}	$2{,}175.30^{***}$	83.26^{***}	0.02^{ns}	76.53^{***}	20.52^{***}
Residue	60	0.95	197.30	0.92	0.07	0.79	1.28
CV (%)		20.10	21.80	18.03	7.21	20.58	46.49

^{ns}, ** and *** : not significant and significant at 1 and 0.1 %, respectively.

of over 8 mg dm^{-3} Ni. There was no difference in the leaf urease-activity responses to AN and Ur (Figure 3, Table 2). In the plants supplied with AN, the maximum urease activity (1.35 µkat kg^{-1} in the fresh matter) was reached with 18.40 mg dm^{-3} soil Ni, whereas with Ur, the maximum was 1.42 µkat kg^{-1} for 20.40 mg dm^{-3} soil Ni (Figure 3). The 18.40 mg dm^{-3} and 20.40 mg dm^{-3} Ni doses corresponded to plant Ni concentrations equal to 8.25 mg kg^{-1} for the AN treatment and 7.92 mg kg^{-1} for Ur, respectively. These Ni concentrations in leaves are in the 1 to 10 mg kg^{-1} range (Marschner, 2008) found for Ni in the vegetative tissues of most plants. Krogmeier et al. (1991) found increased soybean-leaf urease activity in response to Ni, which controlled the absorption of the foliar-applied urea, preventing urea accumulation in the leaves.

Plant Ni and N

For both N sources (AN and Ur), the Ni level in the shoots increased linearly in response to the Ni applied to the soil, with higher values found in the plants on soil supplied with AN (Tables 2 and 3). For most of the plants, the leaf Ni concentrations (Table 2) were below 10 mg kg^{-1}, which is the phytotoxic limit for sensitive species (Marschner, 2008). In the plants supplied with 24 and 32 mg dm^{-3} Ni (AN) and 32 mg dm^{-3} Ni (Ur), the Ni concentrations in leaves were 11.08, 13.67 and 11.75 mg kg^{-1}, respectively (Table 2). These values are above the 10 mg kg^{-1} toxicity limit but below 50 mg kg^{-1} Ni, which is the limit for moderately tolerant species (Marschner, 2008). Fontes et al. (2008) observed lettuce leaves with 4.7 mg kg^{-1} Ni in a clayey Latosol (73 % clay) amended with 32 mg dm^{-3} Ni, whereas in a sandy Latosol (70 % sand), the leaf Ni concentration was 40.0 mg kg^{-1}. Here, the soil clay content was 74 % (Table 1), and the average leaf Ni concentrations in the AN and Ur treatments were 12.7 mg kg^{-1} (Table 2), which is closer to the Ni leaf concentrations found by Fontes et al. (2008) in a clayey soil. According to Marschner (2008), the Ni concentration range in the vegetative tissues of most plants is between 1 and 10 mg kg^{-1} and

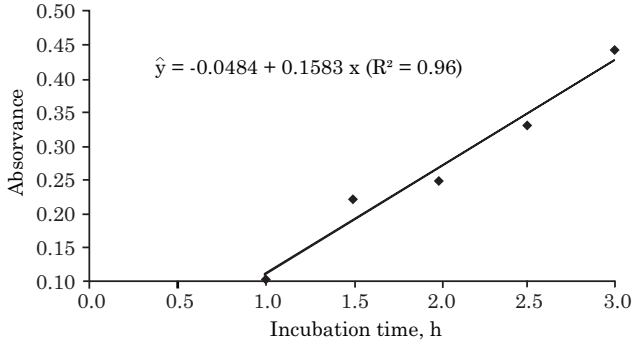

Figure 2. Absorbance (at 636 nm) due to NH_3 production (mediated by urease activity) in lettuce-leaf extracts as a function of the reaction periods (incubation time, IT) for NH_3 production at 40 °C.

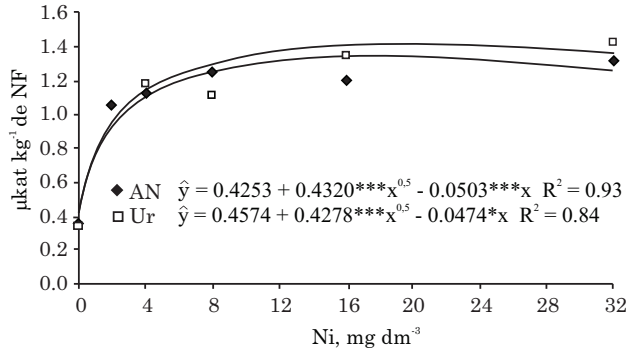

Figure 3. Urease activity in the fresh matter (FM) of lettuce leaves as a function of Ni doses added to soil fertilized with N supplied as ammonium nitrate (AN) and urea (Ur).

expresses the plant characteristics related to the efficiency of absorption and transport from roots to shoots. The Ni lettuce leaf concentration of 12.7 mg kg^{-1} (average of plants treated with 32 mg dm^{-3} soil-applied Ni in either AN or Ur as N source) in a soil with 74 % clay (high cation-retention capacity) indicates the efficiency of lettuce for Ni uptake and translocation. It is important to note that no Ni-toxicity symptoms were observed in the plants.

The N concentrations in the leaves of the plants from the Ur treatments were higher than those in the treatments with AN (Tables 2 and 3). The urease-inhibitor application assured the persistence of N in the soil, making N available for root absorption. Nitrogen loss from urea occurs through NH_3 volatilization due to urea enzymatic hydrolysis (Da Ros et al., 2005), a reaction mediated by urease and intensified when urea is spread on the soil surface. Because the dry-matter yield was greater in the plants supplied with AN (Figure 1), it seems that some adverse effect occurred in the treatments with Ur application. Similarly, in zucchini plants grown in a nutrient solution supplied with different N sources, plant dry weight in AN treatments was greater than under Ur supply (Gerendás & Sattelmacher, 1997b).

Regarding the plant response to Ni soil application, the shoot N concentrations varied little in the treatments with AN and Ur, varying from 3.5 to 3.8 dag kg^{-1} for AN and from 3.9 to 4.1 dag kg^{-1} for Ur (Table 2). These values are in agreement with the optimum range for N concentration (2.0-5.0 dag kg^{-1}) in lettuce leaves in the pre-harvest growth period (Hochmuth et al., 1991; Jones Junior et al., 1991; Ludwick, 2002; Hartz & Jonhstone, 2007).

Ni in soil

Lindsay & Norvell (1978) and Whitney (1988) used Mehlich-3 and DTPA extractors for the extraction of cationic micronutrients (Fe^{2+}, Mn^{2+}, Zn^{2+} and Cu^{2+}) from the soil. Here, the Ni concentrations extracted with Mehlich-3 and DTPA increased linearly as a function of the Ni applied to soil (Table 3). The Mehlich-3 approach recovered more Ni than DTPA (Table 3) because DTPA (calcium chloride and triethanolamine) removes Ni only by forming complexes, whereas Mehlich-3 (acetic acid, ammonium nitrate, nitric acid, ammonium fluoride and EDTA) additionally removes Ni by exchange reactions. The efficiency of Ni-extraction methods for the analysis of soil Ni availability to plants on a routine basis is still a matter of study. The efficiency of Mehlich-3 for the determination of soil Ni availability for tobacco (Mulchi et al., 1991), common bean (Abreu et al., 1995), sorghum (Revoredo & Mello, 2006), and lettuce and common bean (Fontes et al., 2008) has been reported. For DTPA soil extraction, the Ni recovered from soil correlated with Ni in corn leaves (Oliveira, 1995) and with Ni in the leaves and grains of common bean (Berton et al., 2006). Here, the Ni concentrations recovered by DTPA and M-3 were correlated with the Ni concentrations and contents in lettuce shoots (Table 5), indicating the efficiency of these extractors for Ni analysis in the Red-Yellow Latosol studied.

In the soil treated with AN, the Ni levels recovered with M-3 and DTPA were higher than the levels in the Ur-treated soil (Tables 3 and 4). Urea hydrolysis generates NH_3 (Mulrooney & Hausinger, 2003), which may be lost by volatilization. It seems that NH_3 loss contributed to decrease the NH_4^+ concentration in the Ur-treated soil, lowering the competition with Ni^{2+} for soil adsorption sites and allowing greater Ni^{2+} adsorption. In contrast, a higher NH_4^+concentration in the AN-treated soil increased the competition with Ni^{2+}, forcing its release from the soil exchange sites. A high soil clay content (74 %) (Table 1) magnified the Ni^{2+}-adsorption effect. In addition to the absence of NH_4^+competition in Ur-treated soil, Ni utilization by microorganisms

Table 4. Nickel concentrations in soil amended with Ni and fertilized with two N sources. Regression equations for the Ni recovered from the soil (Mehlich-3 and DTPA extractions) are a function of the Ni doses applied to the soil

N source	Ni doses (mg dm^{-3})							
	0	2	4	8	12	16	24	32
	mg dm^{-3}							
	Ni recovered (Mehlich-3)							
NH_4NO_3	0.00	0.78	1.17	3.17	5.19	6.01	8.73	11.57
	$\hat{y}$ = 0.1233 + 0.3635***x R^2 = 0.99							
$(NH_{2)2}CO$	0.00	0.33	1.36	2.28	4.17	5.04	8.10	11.06
	$\hat{y}$ = - 0.2173 + 0.3477***x R^2 = 0.99)							
	Ni recovered (DTPA)							
NH_4NO_3	0.00	0.49	0.76	1.83	2.73	3.51	5.46	6.95
	$\hat{y}$ = 0.00138 + 0.2205***x R^2 = 0.99							
$(NH_2)_2CO$	0.00	0.34	0.79	1.30	2.19	2.55	4.26	5.84
	$\hat{y}$ = - 0.0443 + 0.1799***x R^2 = 0.99							

Table 5. Linear coefficients for the correlations between Ni contents and concentrations in the lettuce shoots and the concentrations recovered from the soil with DTPA and Mehlich-3

Extractor	Ni shoot	Ni contents
	Ammonium Nitrate	
Mehlich-3	0,91*	0,83*
	0,79*	0,72*
	Urea	
DTPA	0,97*	0,95*
	0,96*	0,94*

Significant at 5 % based on a *t*-test.

in the synthesis of urease required for Ur breakdown may have contributed to the lower Ni extraction from the Ur-treated soil.

CONCLUSIONS

1. Nickel additions to the soil in the range of 0.0 to 32 mg dm^{-3} had no effect on lettuce growth in soil fertilized with AN or Ur, and no toxicity symptoms were observed.

2. The urease activity in lettuce leaves had a quadratic response to the addition of Ni to soil.

3. There was no difference between the urease activity in the leaves of lettuce grown in soil fertilized with AN or Ur.

4. With AN and Ni additions to soil (from 0.0 to 32 mg dm^{-3}), the maximum lettuce-leaf urease activity was reached with 18.40 mg dm^{-3} Ni. With Ur fertilization, this maximum activity was reached with 20.40 mg dm^{-3} Ni.

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