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## Organic compounds with high Ni content: Effects on soil and strawberry production

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phytotoxicity  
biosolids  
residues**ABSTRACT**

The use of organic residues can improve soil chemical and physical conditions and plant growth. However, these residues often contain heavy metals that can interfere with growth. Here, a pot experiment was performed to compare the effects of organic compound (OC) containing a high nickel (Ni) load with those of a mineral Ni source (NiCl<sub>2</sub>, a soluble salt) on the production, quality and nutritional status of strawberry and on soil chemistry. Six dosages of Ni (0, 9, 18, 36, 72 and 144 mg kg<sup>-1</sup>) were added to the soil using both OC and NiCl<sub>2</sub>. The use of high-Ni OC resulted in increased productivity and fruit weight, whereas the soluble NiCl<sub>2</sub> salt caused reduction in fruit weight. Application of NiCl<sub>2</sub> at concentrations >18 mg kg<sup>-1</sup> also caused internervial chlorosis on leaves, suggesting that strawberry is highly sensitive to Ni. The mineral Ni source was almost 15-fold more effective than the organic source at increasing leaf Ni content. Application of OC caused an increase of organic matter, Ca, sum of bases, cation exchange capacity, base saturation, Zn, Mn, and Cu, but reduced the levels of Mg in soil.

**Palavras-chave:**metais pesados  
fitotoxidez  
biossólido  
resíduos

## Composto orgânico com altos níveis de Ni: Efeitos no solo e na cultura de morango

**RESUMO**

O uso de resíduos orgânicos pode promover não apenas melhorias nas condições químicas e físicas do solo e da planta mas também a entrada de metais pesados no ciclo biológico. Objetivando avaliar o efeito de um composto orgânico (CO) com altos teores de níquel (Ni) e do cloreto de Ni na produção, qualidade, nutrição do morango e nos aspectos químicos do solo, conduziu-se um experimento em vasos. Aplicaram-se seis doses de Ni 0, 9, 18, 36, 72 e 144 mg Ni kg<sup>-1</sup> de solo por meio de um CO e um sal solúvel. Avaliaram-se os efeitos na produtividade, qualidade de frutos e composição química (macro e micronutrientes) em tecido da planta tal como efeitos no solo. A aplicação de CO com altos teores de Ni proporcionou aumentos na produção e massa de frutos, porém o uso isolado exerceu decréscimos na massa dos frutos; já na fonte mineral e nas maiores doses provocou clorose internervial nas folhas. A cultura do morango mostrou alta sensibilidade nas maiores concentrações de Ni; enfim, o CO promoveu incrementos de MO, Ca, soma de bases, CTC, saturação de base, Zn, Mn e Fe mas reduziu os teores de Mg.



## INTRODUCTION

The use of sewage sludge, alone or in combination with other organic composts (OC), increases soil organic matter and inorganic nutrients and corrects soil pH (Boechat et al., 2013). As a result, sewage sludge has been shown to improve plant productivity (Khan et al., 2013). However, sewage sludge can also increase the levels of heavy metals such as nickel (Ni) in the soil-plant system (Pathak et al., 2009). Nickel bioavailability to plants is a complex process that depends on the concentration and chemical form of the metal and on its interaction with other elements (Fuentes et al., 2004). Organic compounds have a high affinity to Ni and decrease its availability by specific adsorption (Yassen et al., 2007). However, changes in soil pH and interaction with other chemicals can cause Ni to precipitate in soils, thus changing its potential assimilation by plants (Misra & Pande, 1974). The simple release of Ni into the soil does not ensure its absorption by plants (Richards et al., 1997), and it is less damaging to plants when combined with OC. Plants also have mechanisms for mitigating Ni phytotoxicity by limiting its absorption and redistribution, for example, free radicals that can immobilize metals in the cell wall (Richards et al., 1997). Ni is considered an essential plant nutrient at low concentrations (Wood et al., 2006), but at high concentrations, Ni can interfere with the absorption of other compounds, thus leading to physiological damage or plant death (Sengar et al., 2008).

Strawberry (*Fragaria x ananassa* Duch) crops have high nutrient demand and strong potential for the use of OC in its cultivation. There are no published data about the effects of Ni on strawberry. In this context, the aim of this study was to evaluate the use of an OC with high Ni content on the quality, production and nutritional status of strawberry plants and on the chemical characteristics of the soil.

## MATERIAL AND METHODS

This experiment was performed in April 2009 at Campo Largo, Paraná, Brazil (25° 27' 34" S; 49° 31' 40" W; 95 m a.s.l.). The area is in a mesothermal humid subtropical climate zone (Köppen's classification). The experiment was conducted in open air using 7.5-L pots (30 cm diameter) filled with Inceptisols (Ustepts) (USDA, 2013). The soil was extracted from 0-50 cm depth in a fallow area. Following homogenization, a soil sample was taken for analysis of macro and micronutrients according to Camargo et al. (2009), with the resulting values: pH (soil-water), 4.4; P, 0.1 mg L<sup>-1</sup>; organic matter (OM), 40 g kg<sup>-1</sup>; base saturation (V%), 20%. Calcium (Ca, mmol c L<sup>-1</sup>), 13; it was determined as the sum of bases: K, 2; Mg, 6; H + Al, 105; and cation exchange capacity (CEC), 126 mmol c L<sup>-1</sup>. Boron (B) content was 0.06 mg kg<sup>-1</sup>; copper (Cu) was 0.95 mg kg<sup>-1</sup>; and iron (Fe), manganese (Mn), zinc (Zn) and Ni were 52, 1.25, 0.35, and 0.37 mg kg<sup>-1</sup>, respectively. The soil was corrected for pH (10 Mg ha<sup>-1</sup> of dolomitic limestone) and enriched with N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O (40, 260, and 80 kg ha<sup>-1</sup>, respectively). Soil correction was performed before filling the pots and applying the treatments.

The OC was obtained from a petrochemical company close to the experimental area and consisted of residues produced

in the industrial area combined with sewage from the facility. The industrial wastewater sludge was treated by alkalization with sodium hydroxide and was partially dried to produce a doughy material (sewage sludge). This sludge was then mixed with pine sawdust (80:20 sawdust:sludge), and 0.08% urea was added to accelerate the composting process. Composting was used to reduce the initially high Ni concentration by dilution. However, the Ni concentration (720 mg kg<sup>-1</sup>) remained above the legal limit of 420 mg kg<sup>-1</sup> (CONAMA, 2013). The composting process was conducted in open air for 6 months (180 d).

Eight subsamples of the composted material were collected and mixed to form a composite sample, which was sent to the laboratory for analysis and had the following composition: C/N, 17:8; pH (in water) 7.6; density 0.5 g cm<sup>-3</sup>; humidity 3.7%; OC, 87 g kg<sup>-1</sup>. Macronutrient contents (g kg<sup>-1</sup>) were N, 4.9; P, 0.7; Ca, 68.7; Mg, 1.5; K, 1.9; and S, 1.0. Micronutrient contents (mg kg<sup>-1</sup>) were as follows: B, 34.8; Cu, 20; Fe, 10; Cd, 0.5; Ni, 720 (which corresponds to Ni: OM 0.0072); Mn, 278; Zn, 234; Pb, 12; and Cr, 23. The analysis included digestion of the samples with nitric and perchloric acids followed by chemical determination using inductively coupled plasma-atomic emission spectrometry (ICP-AES). Organic matter was estimated by dry combustion through the evolution of carbonic dioxide. Nitrogen content was determined by titration following digestion of the sample with sulfuric acid and distillation of the resulting solution (Kjeldahl method). Phosphorus was quantified by spectroscopy (Camargo et al., 2009).

To isolate the effect of Ni on the plants, six experimental dosages were applied using OC (the organic source) and a soluble Ni salt (NiCl<sub>2</sub>, the mineral source). The dosages were 0, 9, 18, 36, 72, and 144 mg Ni kg soil<sup>-1</sup>, corresponding to OC application rates of 0, 50, 100, 200, 400 and 800 m<sup>3</sup> ha<sup>-1</sup>, respectively. This followed the recommendation of Brazilian environmental agencies (maximum concentration of 420 mg kg<sup>-1</sup> or a total of 72 kg Ni ha<sup>-1</sup>), so that the first dosage levels were within the allowable concentration. The Ni dosages (from organic and mineral sources) were mixed into the soil to obtain a volume of 7.5 L, and the mixture was then added to the pots for the planting of strawberry seedlings.

The experimental design was completely randomized, including the two sources of Ni (organic and mineral), six dosage rates and four replicates, with five plants each (one plant per pot), for a total of 240 experimental units. Strawberry (*Fragaria x ananassa* Duch 'Camarosa') was planted in each pot and maintained during the full experimental cycle (180 days). Fertilization of surface soil was accomplished by adding urea at a rate of 40 kg ha<sup>-1</sup> in three applications, calculated on a per-plant basis.

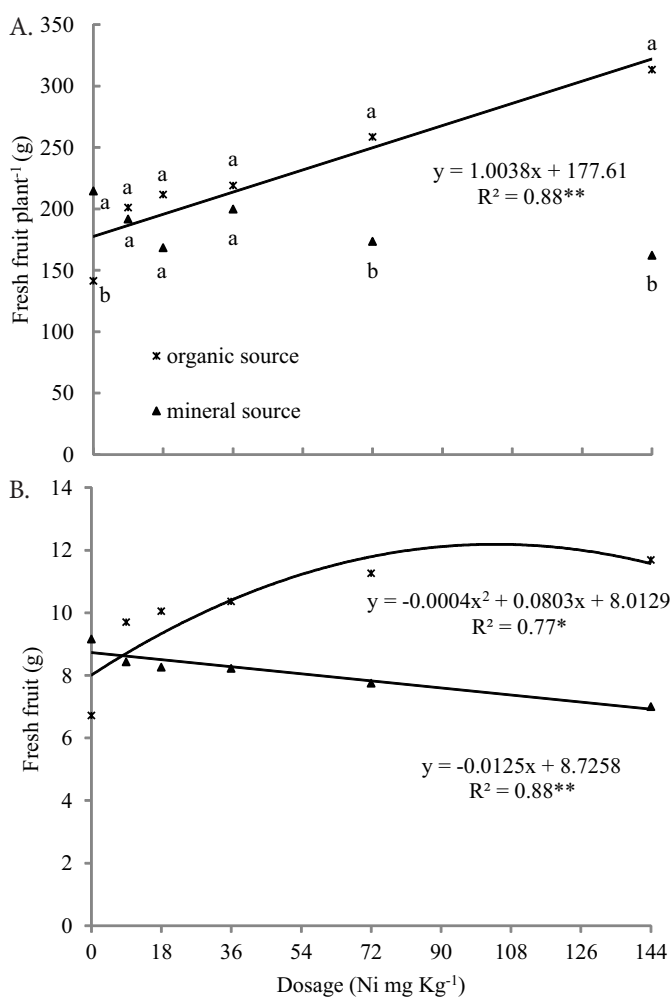
Productivity was estimated by counting the total number of fruits and estimating fruit weight per plant. Harvesting was performed between July and December, and fruits at commercial maturity stage (75% red epidermis) were collected two to three times per week. In addition, fruit quality was evaluated at the beginning, middle and end of the experimental cycle by randomly selecting fruits, which were sliced and centrifuged for their juice. The acidity of the extracted juice

was evaluated by titration, and soluble solids were determined using a refractometer (AOAC, 2005).

The third and fourth newly developed leaves (without petioles) were collected and subjected to chemical analysis. Leaf levels of Ca, Mg, K, P, Fe, Mn, Zn, B, Cu and Ni were obtained following calcination of the samples at 550 °C and solubilization in HCl (3 mol L<sup>-1</sup>). To analyze nickel content, dry matter (2.5 g) was dry-digested and the extract was filtered using one-quarter of the original volume, thus increasing the concentration of the resulting solution eight-fold. Phosphorous was determined by colorimetry and total C and N were estimated by dry combustion. The remaining chemicals were determined with conventional absorption spectroscopy using air/acetylene flame.

At the end of the experiment, the soil was homogenized to obtain samples for analysis of macro- and micronutrients, pH, aluminum, and organic matter according to the methods described above. Plants were separated, and their roots, crowns and aerial parts were washed, dried and weighed to quantify dry matter.

The data were analyzed using analysis of variance. For significant results, differences in mean values between treatments were assessed with Tukey tests (0.05 significance level) using regression models.



Different letters for the same dosage indicate significant differences ( $p < 0.05$ ); \* and \*\* Significant at 0.05 and 0.01 levels

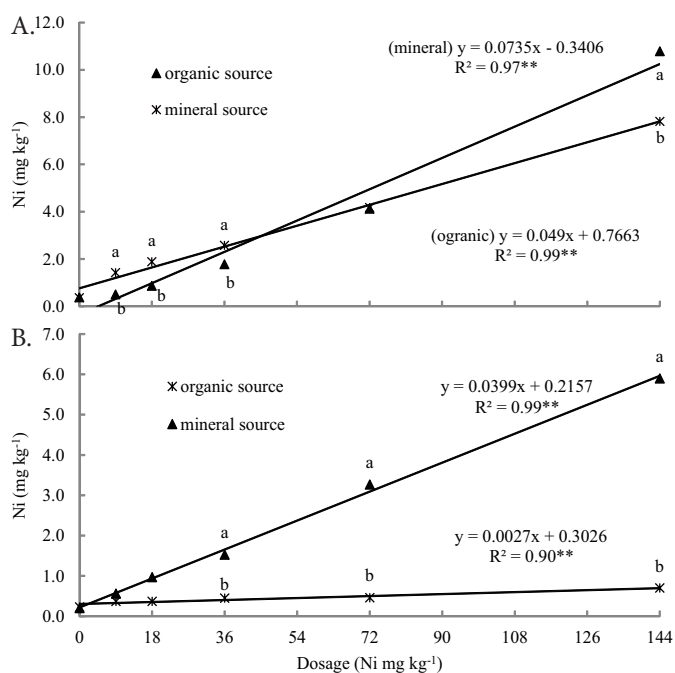
Figure 1. Mean productivity per plant (A); average weight of fresh fruits in relation to Ni dosages and sources (B)

## RESULTS AND DISCUSSION

A positive increase in fruit production and weight was observed for plants treated with OC (Figure 1). In contrast, in plants treated with the mineral Ni source, average fruit weight decreased linearly, but there was no effect on productivity. The number of fruits was not affected by the dosage or source of Ni, and the same was true for dry matter, acidity, and soluble solids. The increased productivity was likely related to the positive effects of the use of organic residues, as demonstrated for other plants (Khan et al., 2013), and may have been related to improvements in soil chemistry and biophysical characteristics. Thus, the high Ni load of the OC used in this study apparently had no negative effects on strawberry production. The same result was found by Melo et al. (2007), who attributed the absence of phytotoxic effects to complexation of this heavy metal in their OC.

The addition of both organic and mineral Ni sources to the soil resulted in a linear increase in its availability (Figure 2). However, the organic source had a lower increase compared with the mineral source. Levels of Ni in the leaves changed in relation to source and dosage, with a clear linear increase with the application of mineral Ni, which suggested much higher availability of Ni from this source (Figure 2).

Differences in Ni availability between the sources may be related to the higher specific adsorption of organic molecules, which can limit heavy metal absorption by plants (Vandenhove et al., 2009), thus reducing the toxic effects of Ni in soil, even at high concentrations. According to Misra & Pande (1974), the addition of organic matter to the soil resulted in solubilization of autochthonous Ni during the first few days, due to the production of organic acids that led to complexation of Ni with the organic matter, thus increasing its availability to the plants.



Different letters for the same dosage indicate significant differences ( $p < 0.05$ ); \*\* Significant at the 0.01 level

Figure 2. Nickel availability in soil (extracted with DTPA) (A); Ni levels in strawberry leaves as a function of dosages and source of Ni added to the soil (B)

In contrast, the addition of Ni via mineral source caused a steady decline in average fruit weight with increasing dosage (Figure 1), leading to smaller fruits with lower market value. The variability in productivity was associated with fruit weight. Molasa & Baran (2004) observed a phytotoxic effect of Ni delivered in a soluble source, suggesting higher bioavailability in this form. The lack of changes in fruit acidity and soluble solids content suggests that fruit quality was not affected by the use of OC and that those parameters are less susceptible than productivity. Similar effects were observed by Hargreaves et al. (2008), who found no differences in fruit quality in terms of soluble solids between organic (i.e. organic residues) and traditional systems.

No phytotoxic effects were observed when Ni was delivered through the organic source. The complexing effect of Ni was exerted mainly by organic matter acting as a natural multilinker to metals, being held by the functional groups participating in the interaction of organic material x metal, which are carboxylic, phenolic, alcoholic, phenolic and also some of the carbonyl group (Fuentes et al., 2004), also reported by Melo et al. (2007). However, clear symptoms of toxicity, in the form of an internerval chlorosis in older leaves, were detected for the two highest Ni dosages from the mineral source (Figure 3). These symptoms persisted until the leaves were shed.

Internerval chlorosis of strawberry leaves (Figure 3) was also observed by other researchers (Pandey & Gopal, 2010) and may be linked to the toxicity of Ni. This chlorosis is due to depressed synthesis and rapid oxidative degradation of chloroplast pigments caused by the Ni (Pandey & Gopal, 2010). The average Ni concentration in strawberry leaf tissue reported by Mikiciuk & Mikiciuk (2010) was 2.8 mg kg<sup>-1</sup>. Wood et al. (2006) found that Ni was toxic to radish (*Raphanus sativus* L.) at concentrations >120 mg kg<sup>-1</sup> and that symptoms of Ni deficiency occurred in pecan (*Carya illinoensis* Koch) at concentrations of 0.5 mg kg<sup>-1</sup>. Thus, strawberry was shown to be very sensitive to Ni, displaying symptoms of toxicity at average Ni concentrations (5.9 mg kg<sup>-1</sup>) lower than those found in other studies. On the other hand, strawberry did not show symptoms of Ni deficiency at concentrations <0.5 mg kg<sup>-1</sup>, as found in pecan (Wood et al., 2006).

The use of the organic source caused increases in most of the soil chemical characteristics, including OM, Ca, SB, CEC, V%, Zn, Cu, and Mn. The opposite was found for H + Al and Mg (Figure 4), and there was no effect on pH, P, or K (data not shown). There was no clear influence of OC application on Fe and B availability. In contrast, the mineral source had no effect on any of the chemical characteristics.

In tomatoes, Palacios et al. (1998) found that Ni restricted the absorption of P, increased the absorption of K and reduced the absorption of Mg, Fe, Mn, Cu, and Zn, with Mn displaying the highest reduction. Ni-induced changes in the absorption of other nutrients are probably only expressed at higher levels of toxicity, when Ni can severely interfere with the absorption metabolism. Those effects would not occur if Ni concentrations are close to the tolerance limit of the species, which may partially explain these results.

The enhanced levels of organic matter in the soil (Figure 4) were directly related to the use of OC, as observed in other

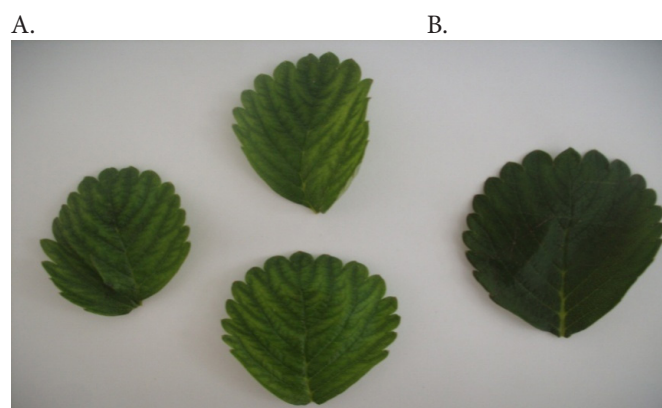


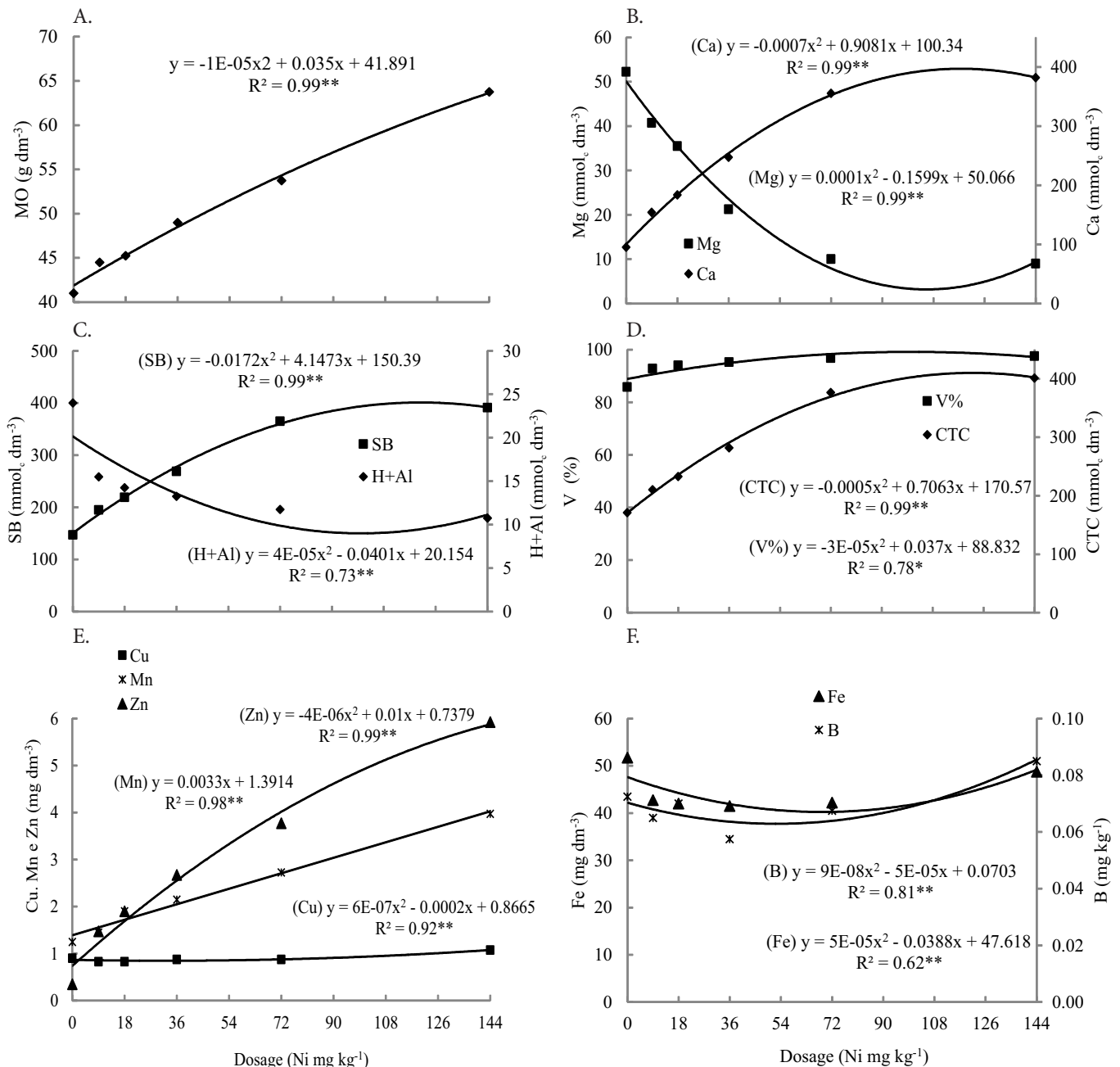
Figure 3. Leaflets of strawberry with symptoms of Ni toxicity (A); Healthy leaflets (B)

studies (Khan et al., 2013). In addition, increases in organic matter affect colloidal complexes, creating stable molecules with high surface charge, which normally leads to a significant increase in CEC in the soil (Pan et al., 2003) as observed here (Figure 4). Increases in Ca, Mn, Cu, and Zn (Figure 4) in soil were also related to the use of the OC. In addition, increased Ca was reflected in the sum of bases and base saturation. Kidd et al. (2007) found increased Ca levels in soil and attributed this to the high concentration of Ca used in the chemical stabilization of the industrial wastewater sludge used in their study. Other authors also reported increased concentrations of Zn, Mn, Fe, and Cu in soil following the application of sewage sludge (Warman & Termeer, 2005).

The low values for potential acidity (H + Al, Figure 4) were related to the reaction of organic molecules with the soil, but were not related to soil pH (data not shown). The lack of changes in pH was probably a result of the high pH (average = 6.7) of the experimental soil, because the amendments were added before the seedlings were planted. In addition, acids produced during decomposition of the organic matter and from nitrification can limit the increase in pH (Franchini et al., 2001).

Decreased soil Mg levels in treatments that received the organic source (Figure 4) were related to the high concentrations of that element in the soil. Less soil was required to bring pots containing Ni from the organic source up to the experimental volume (7.4 L), and this might have caused the fall in Mg levels. In contrast, Hashemimajd & Somarinti (2011) found increased soil Mg content when using OC. On the other hand, the lack of effects on soil K concentrations was related to the naturally lower levels of this element in sewage sludge (Warman & Termeer, 2005). In addition, it is well established that K has high solubility in organic composts, which may have reduced its concentration in soil by the end of the experiments.

The OC application also caused small changes in nutrient concentrations in the leaves and fruits, concentrations were within the normal standard for the crop. There were increases in the concentrations of Ca, Mn, and Zn in leaves, and increases of Mg and Zn and decreases of K, Fe and Cu in fruit (data not shown). However, the change was small and probably would have little impact on plant nutrition.



\*\* significant at the 0.01 level; \* significant at the 0.05 level

Figure 4. Mean soil values of (A) OM, (B) Ca, Mg, (C) Sb, H + Al, (D) V%, CEC, (E) Cu, Mn, Zn and (F) B and Fe as a function of increasing nickel dosages from the organic source

## CONCLUSIONS

1. The addition of Ni via OC applied at concentrations equal to and 1.7- and 3.8-fold higher than the allowable limit lead to increases in strawberry fruit production and weight, but its use yielded decreases in fruit weight.

2. Ni delivered in a mineral source resulted in tissue Ni concentrations up to 10-fold higher than that of Ni delivered in OC, and the highest dosages caused internervous chlorosis.

3. Addition of OC to the soil caused increases in OM, Ca, SB, CEC, V%, Zn, Mn, and Cu, and a decrease in Mg.

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