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**SOIL AND PLANT NUTRITION - Article**

# Nutritional evaluation of Guanandi seedlings fertilized with sewage sludge

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**ABSTRACT:** The generation of sewage sludge and the concern with their fate has greatly increased. The objective of this study was to assess the effects of using *in natura* (SS) or composted sewage sludge (SSC) in comparison with mineral fertilizer (MF) application and a control (CT) treatment on the nutritional status of Guanandi (*Calophyllum brasiliense* Cambess.) seedlings in a typical Red Oxisol in Brazil. The leaf-level nutritional responses of the Guanandi seedlings to these three different fertilizers and the control were evaluated at 90 and 180 days after planting. Nutritional data of leaf variables were compared between the treatments by means of one-way analysis of variance using the *F* test and a pair-wise comparison of means done by Tukey's test. The seedlings that received SS or SSC showed deficiencies in Ca, Mg, and Mn, which were confirmed by a visual diagnosis of their leaf symptoms. However, at 180 days after planting there were significant differences ($p < 0.05$) for K that was greater for MF, SS and SSC than CT, Mg, with SSC larger than SS, and for S that was greater with SS and SSC uses relative to MF. The results highlight the potential for using SS and SSC, after adding KCl and lime, although the Guanandi was highly demanding of Ca, Mg, and Mn. However, they were able to nutritionally supply seedlings, although the short-term cannot be conclusive as regards the exclusive use of them in place of mineral fertilizers.

**Key words:** *Calophyllum brasiliense*, organic fertilizer, mineral nutrition, oxisol, silviculture, Brazilian tree.

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# INTRODUCTION

As a result of rapid population growth, urbanization, and the improved coverage in wastewater treatment in the tropics, the amount of sewage sludge (SS) generated has increased greatly. This, coupled to the high cost of mineral fertilizers and escalating trends in their prices, has fostered a growing interest in using SS as a potential organic fertilizer (Usman et al. 2012) on agroforestry soils, which may offer a sustainable and economical alternative for augmenting nutrient supplies (Wang et al. 2004; Harrison et al. 2006), while also reducing the disposal costs of sewage treatment (FAO 2017).

In nutritional terms, SS typically contains approximately 40% of organic matter, 4% of N, 2% of P, and 0.1% of K, in addition to other micronutrients – Zn, Cu, Mn, Fe, Mo, and Ni – which, in high concentrations, may be toxic for plants. But heavy metals, such as Cd and Pb, can also be found in considerable quantities, especially if the SS comes from industrialized regions (Bettiol and Camargo 2006; Usman et al. 2012).

The rapid mineralization or lixiviation of N in the soil and the eutrophication caused by large available P content (Wang et al. 2004; Bettiol and Camargo 2006) could be minimized if SS were used in tropical forest soils. In general, forest soils have a high input of recalcitrant organic residues (Bachega et al. 2016), which can immobilize the soil N available (Strahm et al. 2005). Moreover, tropical soils are usually deep, nutritionally poor, and rich in iron oxides (Ker 1997); combined, these features favor the formation of an inner sphere complex with P, which would be characterized by the great energy of P-retention (Novais and Smith 1999). These conditions, when in association with a perennial root system, should contribute to minimizing the surface flow/outflow by greater infiltration rates (Harrison et al. 2003; Laclau et al. 2013).

Although SS can be used aiming to improve the chemical soil properties, there are some concerns associated with its use in the soil, such as the pathogens proliferation, emerging pollutants and contamination by heavy metals. In this sense, SS should be subjected to biological, chemical or thermal treatment, long-term storage or other appropriate process designed to reduce its fermentability and health hazards resulting from its use before being applied in soil (Water Research Center 1989; Eugenio et al. 2018).

The Guanandi tree is a shade-tolerant climax species (*Calophyllum brasiliense* Cambess.) that is found across much of Brazil. Ecologically, this specie has better answers in both clearing conditions (50% shading) and canopy (70% shading). The ideal conditions for seedling production and higher nutrient use efficiency (particularly for P) was obtained under 70% shading in the nursery conditions (Morandi et al. 2017). It has potential for commercial use owing to the quality of its wood and latex, and it is also useful in the degraded areas recovery (Lorenzi 2008). The economic importance of Guanandi has increased because its wood is similar to that of Big-leaf mahogany (*Swietenia macrophylla* King), but with the advantage of not being attacked by the well-known "shoot-borer", *Hypsipyla grandela* Zeller (Carvalho 1994). However, studies of Guanandi plantations nutritional requirements remain scarce (Furtini Neto et al. 2000), especially with respect to the use of organic residues as fertilizers (Artur et al. 2007). In general, the largest responses have been obtained with N, P, and Ca nutritional requirements. Organic fertilization usually has beneficial effects on the growth and development of forest species seedlings, such as those reported for Oiti (*Licania tomentosa* Benth.) fertilized with urban garbage compost (Alves and Passoni 1997) and Angelim (*Andira fraxinifolia* Benth.) fertilized with manure (Carvalho Filho et al. 2004).

We hypothesized that the use of sewage sludge (SS) and sewage sludge composted (SSC) will quickly (90 and 180 days after application) increase the nutrients foliar concentrations in the Guanandi seedlings in similar levels than mineral fertilizer (MF), not achieving high levels of heavy metals. In this context, the objective of this field study was to evaluate the effects of SS or SSC, in comparison with convention mineral fertilizer (MF), on N, P, K, Ca, Mg and S as macronutrients, B, Cu, Fe, Mo, Mn, Zn and Ni as micronutrients and Pb, Cd, and Cr as heavy metals concentrations in Guanandi leaves on a typical Red Oxisol in southeastern Brazil.

# MATERIAL AND METHODS

## Study area

The study was carried out in Sorocaba, state of São Paulo, Brazil (23°34'40" S and 47°31'17.8" W), in an area previously used as pasture (*Brachiaria decumbens*) for more than 30 years, which did not receive any input of fertilizer or liming in this period. The weather is characterized as Cfa (subtropical warm) according to the Köppen classification (Alvares et al. 2013), with average annual temperature of 21 °C, with a maximum of 30 °C in the summer and a minimum of 12 °C in the winter, and 1285 mm of annual precipitation (Ikematsu et al. 2007). The soil is classified as a typical Red Oxisol according to Soil Taxonomy (USDA 1999). Further details on the soil chemical attributes (sampled at 20 cm of deep) at the field are found in Table 1, which evidenced the soil pH equivalent to 5. The analyses were procedure according to Embrapa (1997).

The specie studied is best suited for shaded areas, therefore the experiment was initiated in the winter (dry period) to minimize plants stresses that are sensitive to high levels of solar radiation, especially in the initial stages of development.

## Treatments and experimental design

Leaves of Guanandi seedlings were evaluated nutritionally in response to three different sources: (1) sewage sludge (SS), (2) sewage sludge composted (SSC), and (3) mineral fertilizer (MF). A control treatment (CT) was included in the experiment (no SS, SSC and MF). The study plants had characteristics deemed suitable for experimentation in the field, according to the recommendations of Kalil et al. (2007). Guanandi seedlings were four months of age and were individually planted in cylindrical holes opened manually (0.3 m in depth and 0.2 m in diameter). Seedlings used in planting were acquired in the seedling nursery Tropical Flora Reflorestadora, in the municipality of Garça, São Paulo state.

The weeds were controlled in the beginning of the experiment by the application of Glyphosate herbicide.

The three fertilizers (SS, SSC, and Min Fert) were manually incorporated into the soil around the prepared holes. The spacing between plants was 3 × 2 m, with 16 seedlings per plot (12 × 8 m = 96 m$^2$) and 3 m single border to minimize any external interference; hence, there was a total of 4 seedlings replicates per plot (treatment) and 4 blocks, totalizing n = 16 replicates by treatment, in a reforestation area of 1200 m$^2$. The experiment was conducted from April 2011 to October 2011, in completely randomized in four blocks, and it analyzed the effects of four treatments, each with four seedlings in each one of the four replicates plots.

## Characterization of organic and inorganic fertilizers

The organic residues were characterized according to the local and national regulations for SS usage as a fertilizer (CETESB 1999; CONAMA 2006). The sludge was obtained from Jundiaí waste treatment plant (sanitation facility of Jundiaí company), in the municipality of Jundiaí, state of São Paulo, Brazil. The sewage treatment process was accomplished by thoroughly mixing the contents of aerated sewage lagoons, after which the material progressed to settling ponds. Then, the sludge was dehydrated by centrifugation, and polyelectrolytes were added. Sanitary conditions were promoted for the next 60 – 90 days by applying mechanical tillage on a covered surface (Galdos et al. 2004). The SS was processed using thermophilic composting after combining it with the chipped pruning residues of urban trees, crushed sugar cane and eucalyptus bark. This mixture was kept at a temperature above 55 °C for over 30 days and subjected to aeration by mechanical tumbling and by the oxidation activity of microorganisms, resulting in SSC. The residues were stabilized using a thermophilic composting process that contributes to a smaller fraction of biodegradability resistance and better material stability. The principal chemical composition of the residue is shown in Table 2 (Urzedo et al. 2013).

The SS, SSC, and MF applications were performed in the holes one single time over the entire experiment, in the first week of planting. The N dose for both mineral and organic sources was calculated considering the recommendation for Atlantic Forest trees (i.e., 60 kg·ha$^{-1}$) (van Raij et al. 1996); it was applied either as $NH_4NO_3$, according to recommendations of van Raij et al. (1996) or as an organic fertilizer. The mineralizable N rate in the organic fertilizers was set to 30% (Lambais and Carmo 2008), the default value for the SS treatment process as established by National Environmental Council (CONAMA 2006). The control treatment did not receive any fertilization, whereas SS, SSC, and MF were supplemented with KCl, and the MF was also supplemented with 80 kg·ha$^{-1}$ of $P_2O_5$ (van Raij et al. 1996).

**Table 1.** Soil chemical attributes from soil sample at 0 to 20 cm depth.

| OM (g·dm$^{-3}$) | pH in $CaCl_2$ | K | Ca | Mg | Al | H+Al | SB | CEC | V (%) | P | S | B | Cu | Fe | Zn | Mn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | (mmolc·dm$^{-3}$) | | | | | | | | | (mg·dm$^{-3}$) | | | | | | |
| 16 | 5 | 0.4 | 13 | 1 | 1 | 31 | 14 | 45 | 32 | 1 | 41 | 0.06 | 0.1 | 6 | < 0.1 | 0.6 |

OM = soil organic matter; CEC = cation exchange capacity; SB = sum of bases; V% = base saturation.

**Table 2.** Chemical characterization of the sewage sludge compost (SSC) and sewage sludge (SS) fertilizers used in the field experiment.

| Parameter | Unit[1] | SSC[2] | SS[2] | Maximum concentration allowed[3] | |
|---|---|---|---|---|---|
| | | | | CETESB | CONAMA |
| pH (in water 1:10) (V·V$^{-1}$) | - | 5,0 | 7.1 | - - | - |
| Umidity at 60 – 65 °C | % (m·m$^{-1}$) | 57.7 | 72.5 | - - | - |
| Total solids | % (m·m$^{-1}$) | 42.3 | 27.5 | - | - |
| Volatile solids | % (m·m$^{-1}$) | 45.8 | 58.4 | - | - |
| Organic carbon | g·kg$^{-1}$ | 248 | 333 | - | - |
| Nitrogen (Kjeldahl) | g·kg$^{-1}$ | 12 | 32.7 | - | - |
| Ammoniacal nitrogen | mg·kg$^{-1}$ | 461 | 1026 | - | - |
| Nitrogen nitrate-nitrite | mg·kg$^{-1}$ | 27.3 | 18.3 | - | - |
| Al | mg·kg$^{-1}$ | 13842 | 24390 | - | - |
| As | mg·kg$^{-1}$ | < 1.0[2] | < 1.0[2] | 75 | 41 |
| B | mg·kg$^{-1}$ | 17.3 | 11.2 | - | - |
| Cd | mg·kg$^{-1}$ | 2.5 | 6.1 | 85 | 39 |
| Ca | mg·kg$^{-1}$ | 55.1 | 10.5 | - | - |
| Pb | mg·kg$^{-1}$ | 49.7 | 123 | 840 | 300 |
| Cu | mg·kg$^{-1}$ | 109 | 219 | 4,300 | 1,500 |
| Total Cr | mg·kg$^{-1}$ | 115 | 170 | - | 1,000 |
| S | g·kg$^{-1}$ | 21.9 | 14.4 | - | - |
| Fe | mg·kg$^{-1}$ | 15,091 | 25,710 | - | - |
| P | g·kg$^{-1}$ | 4 | 9.5 | - | - |
| Mg | g·kg$^{-1}$ | 1 | 1.7 | - | - |
| Mn | mg·kg$^{-1}$ | 264 | 2.1 | - | - |
| Hg | mg·kg$^{-1}$ | < 1.0[2] | < 1.0[2] | 57 | 17 |
| Mo | mg·kg$^{-1}$ | 2 | 10 | 75 | 50 |
| Ni | mg·kg$^{-1}$ | 15.2 | 43.1 | 420 | 420 |
| Se | mg·kg$^{-1}$ | < 1.0[2] | < 1.0[2] | 100 | 100 |
| Zn | mg·kg$^{-1}$ | 590 | 1,900 | 7,500 | 2,800 |
| K | mg·kg$^{-1}$ | 1,126 | 1074 | - | - |
| Na | mg·kg$^{-1}$ | 708 | 1,240 | - | - |
| Ba | mg·kg$^{-1}$ | 540 | 460 | - | 1,300 |
| CEC[2] | mmol·kg$^{-1}$ | 333 | 534 | | |

[1]Results expressed in the sample on dry basis. [2]Sewage sludge compost (SSC), sewage sludge (SS), Cation exchange capacity (CEC). [3]Source: CETESB (1999) and CONAMA (2006). [4]Concentrations less than 1.0 mg·kg$^{-1}$ not detected.

The micronutrients concentrations of B, Cu, Fe, Mo, Mn, Zn and Ni, besides the heavy metals such as Cr, Pb and Cd, were determined according to Embrapa (1997).

## Leaf sampling and nutritional evaluation

Leaf sampling followed the methodology adopted by Santos et al. (2008) for native tree species, and it was carried out 90 and 180 days after planting. The third pair of leaves, from the growing tip (from pointer to the stem) of the 16 seedlings per treatment (4 per plot) at 90 and 180 days, were taken; they were washed to eliminate possible contaminants and atmospheric deposition, put into paper bags, and dried in an oven with forced air circulation at 65 to 70 °C, until constant weight. After drying, the leaves were ground with a Wiley-type mill.

The N concentration in the leaves was determined by using the micro Kjeldahl method, following Miyazawa et al. (2009). The other macronutrients (K, Ca, Mg and S via dry), micronutrients (Cu, B, Mo, Zn, Mn, Fe, Zn and Ni), and potentially toxic elements (Pb, Cd and Cr) were determined according to Miyazawa et al. (2009). Ground leaves were submitted to a nitric-perchloric digestion ($HNO_3$ + $HClO_4$, 3:1 v/v, respectively) in a digester block at 200 °C. Elemental determination was performed by Inductively coupled Plasma Optimal Emission Spectrometry (ICP OES, Santa Clara, USA).

## Initial growth of Guanandi

Monthly the Guanandi seedlings growth was measured by the height increment data (Carmo et al. 2014) with measuring tape, and the diameter at 5 cm apart from the base of the stem with a digital caliper.

## Statistical analysis

Data were subjected to Kolmogorov Smirnov and Levene's tests to check for homogeneity of variance. When detected normality and homoscedasticity, data were analyzed by analysis of variance (ANOVA) by the *F* test with P values for statistical significance of $p < 0.05$ and $p < 0.01$. In case of a significant treatment effect (i.e., four groups compared: SS, SSC, MF, and CT), pairwise differences between the means values of the treatment levels were evaluated using Tukey's test ($p < 0.05$). The statistical analyses were performed using ASSISTAT software beta v.7.6 (Silva 2012).

# RESULTS AND DISCUSSION

## Nutritional evaluation of Guanandi seedlings

Although the treatments did not present a significant difference regarding initial diameter at the base of stem (Fig. 1) and height (Carmo et al. 2014), some differences in the nutrient concentrations within the leaves were observed (Table 3). The increase in diameter consists of a secondary growth (Fig. 2), which is associated with more mature phases of the plants (Raven et al. 1996). In addition, the Guanandi is characterized as late secondary, with significant increases observed in the long term.

The organic fertilizers (SS and SSC) resulted in macronutrients leaf content increases, such as N, K and S, both at 90 and 180 days after planting (Fig. 3), though not statistically significant. Treatments that received K had similar nutrient contents at 180 days, suggesting there was no interaction between the organic fertilizers and the element availability over a longer timeframe of the experiment. Unexpectedly, there was no effect of the fertilizer treatments on the N leaf content after 180 days of planting. By contrast, many studies have reported an increased N leaf concentration in forestry tree species treated with SS, with effects seen from 2 months to 5 years after their application to the soil (Zabowski and Henry 1994; Wightman et al. 2001; Guedes and Poggiani 2003). In all treatment levels, and for both time periods, the N content was close to the lower limit of the suitable content. The highest S leaf concentrations were observed for both SSC and SS, but were lower in the CT

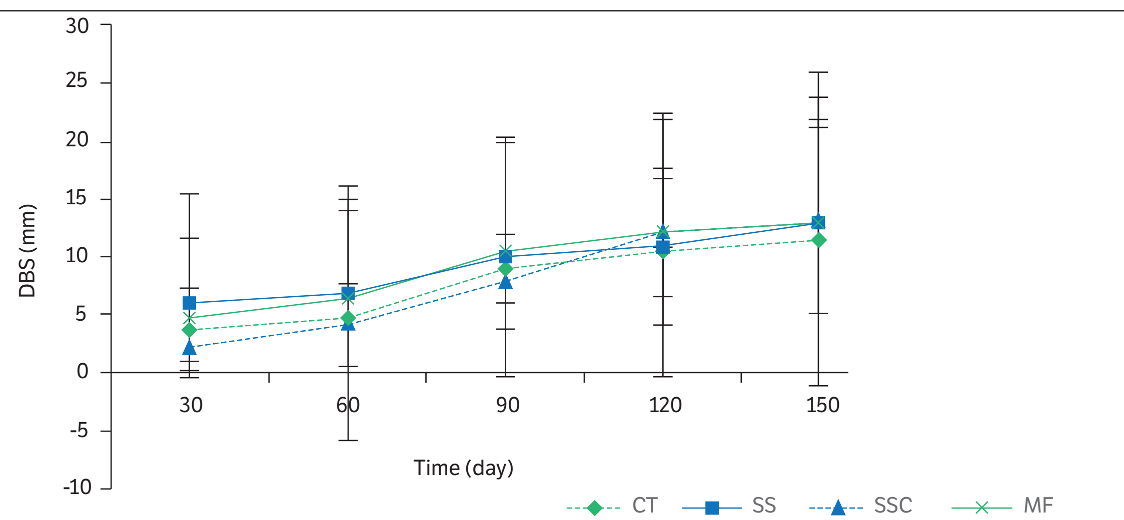


**Figure 1.** Average (n = 4) diameter at the base of stem (DBS) of Guanandi seedlings treated with no fertilization (CT; i.e., the control), sewage sludge (SS), sewage sludge compost (SSC) and mineral fertilizer (MF) for 150 days after planting.

**Table 3.** Average foliar concentrations of the macronutrients, micronutrients, and toxic elements in the Guanandi seedlings treated with sewage sludge (SS), sewage sludge compost (SSC), mineral fertilizer (Min Fert), and no fertilization (CT; i.e., the control), for each season collected. There are also shown the elemental concentrations considered suitable for forest species.

| Element | Initial | CT(1) | Min fert | SSC | SS | CV (%) | Test F(2) | Suitable content(3) |
|---|---|---|---|---|---|---|---|---|
| | | 3 months after application | | | | | | |
| N ($g{\cdot}kg^{-1}$) | 10.086 | 13.839 b | 16.483 ab | 14.345 ab | 17.583 a | 9.72 | 5.463* | 12 – 35 (Malavolta 1980) |
| P ($g{\cdot}kg^{-1}$) | 0.888 | 1.267 | 1.262 | 1.313 | 1.513 | 11.63 | 2.311ns | 1 – 2.3 (Malavolta 1980) |
| K ($g{\cdot}kg^{-1}$) | 7.381 | 6.752 b | 8.631 b | 11.233 a | 12.331 a | 9.58 | 29.294** | 10 – 15 (Malavolta et al. 1997) |
| Ca ($g{\cdot}kg^{-1}$) | 4.299 | 4.939 | 5.015 | 5.782 | 5.682 | 18.60 | 0.776ns | 3 – 12 (Malavolta et al. 1997) |
| Mg ($g{\cdot}kg^{-1}$) | 1.757 | 1.685 | 1.526 | 1.822 | 1.772 | 15.00 | 1.034ns | 1.5 – 5 (Malavolta et al. 1997) |
| S ($g{\cdot}kg^{-1}$) | 1.727 | 3.268 bc | 2.151 c | 5.042 a | 4.705 ab | 17.56 | 16.144** | 1.4 – 2 (Malavolta et al. 1997) |
| B ($mg{\cdot}kg^{-1}$) | 57.439 | 59.164 b | 69.318 ab | 74.808 ab | 84.974 a | 14.46 | 4.272* | 20 (Epsein 1975) |
| Cu ($mg{\cdot}kg^{-1}$) | 12.912 | 5.985 | 5.106 | 4.426 | 5.582 | 23.81 | 1.140ns | 6 (Malavolta 1980) |
| Fe ($mg{\cdot}kg^{-1}$) | 1,902.888 | 592.093 | 348.903 | 158.346 | 240.958 | 60.27 | 3.476ns | 100 (Epstein 1975) |
| Mo ($mg{\cdot}kg^{-1}$) | 23.969 | 32.337 | 26.972 | 46.851 | 35.991 | 28.19 | 2.814ns | 0.5 – 1 (Abreu et al. 2007) |
| Mn ($mg{\cdot}kg^{-1}$) | 39.148 | 20.347 | 23.397 | 21.214 | 27.060 | 18.56 | 1.965ns | 50 (Malavolta 1980) |
| Zn ($mg{\cdot}kg^{-1}$) | 69.439 | 52.708 b | 41.398 b | 59.273 ab | 84.040 a | 21.30 | 8.145** | 15 – 50 (Mills and Jones Junior 1996) |
| Ni ($mg{\cdot}kg^{-1}$) | 137.704 | 35.109 a | 13.112 ab | 6.074 b | 10.122 ab | 79.53 | 4.117* | 10 – 30 (Homer 1991) |
| Pb ($mg{\cdot}kg^{-1}$) | 1.868 | < 1.49(4) | < 1.49 | < 1.49 | < 1.49 | - | - | < 0.8 (Bovi et al. 2007) |
| Cd ($mg{\cdot}kg^{-1}$) | < 0.19(4) | < 0.19 | < 0.19 | < 0.19 | < 0.19 | - | - | < 0.1 (Bovi et al. 2007) |
| Cr ($mg{\cdot}kg^{-1}$) | 146.261 | 52.473 | 23.491 | 14.273 | 20.605 | 76.78 | 2.539ns | < 0.1 (Bovi et al. 2007) |
| **Element** | **Initial** | **CT(1)** | **Min fert** | **SSC** | **SS** | **CV (%)** | **Test F(2)** | **Suitable content(3)** |
| | | 6 months after application | | | | | | |
| N ($g{\cdot}kg^{-1}$) | 10.086 | 15.514 | 16.199 | 19.004 | 17.916 | 10.49 | 3.116ns | 12 – 35 (Malavolta 1980) |
| P ($g{\cdot}kg^{-1}$) | 0.888 | 1.314 | 1.711 | 1.828 | 1.433 | 18.08 | 2.700ns | 1 – 2.3 (Malavolta 1980) |
| K ($g{\cdot}kg^{-1}$) | 7.381 | 7.819 b | 11.144 a | 12.285 a | 12.063 a | 14.07 | 7.229** | 10 – 15 (Malavolta et al. 1997) |
| Ca ($g{\cdot}kg^{-1}$) | 4.299 | 4.415 | 4.290 | 4.420 | 3.761 | 16.08 | 0.677ns | 3 – 12 (Malavolta et al. 1997) |
| Mg ($g{\cdot}kg^{-1}$) | 1.757 | 1.192 ab | 1.184 ab | 1.377a | 0.889 b | 13.65 | 5.271* | 1.5 – 5 (Malavolta et al. 1997) |
| S ($g{\cdot}kg^{-1}$) | 1.727 | 1.829 b | 1.755 b | 3.263 a | 1.976 a | 22.81 | 7.800** | 1.4 – 2 (Malavolta et al. 1997) |
| B ($mg{\cdot}kg^{-1}$) | 57.439 | 91.806 | 93.588 | 89.563 | 87.072 | 7.15 | 0.656ns | 20 (Epsein 1975) |
| Cu ($mg{\cdot}kg^{-1}$) | 12.912 | 9.436 | 6.031 | 10.743 | 6.038 | 30.58 | 3.439ns | 6 (Malavolta 1980) |
| Fe ($mg{\cdot}kg^{-1}$) | 1,902.888 | 140.749 ab | 201.300 a | 113.656 b | 144.766 ab | 22.55 | 4.699* | 100 (Epstein 1975) |
| Mo ($mg{\cdot}kg^{-1}$) | 23.969 | 2.332 | 3.302 | 2.022 | 1.378 | 49.29 | 1.757ns | 0.5 – 1 (Abreu et al. 2007) |
| Mn ($mg{\cdot}kg^{-1}$) | 39.148 | 31.927 ab | 48.574 a | 26.082 b | 44.987 ab | 24.97 | 4.978* | 50 (Malavolta 1980) |
| Zn ($mg{\cdot}kg^{-1}$) | 69.439 | 44.633 | 82.684 | 81.727 | 69.505 | 80.28 | 0.402ns | 15 – 50 (Mills and Jones Junior 1996) |
| Ni ($mg{\cdot}kg^{-1}$) | 137.704 | 5.083 | 8.360 | 6.364 | 5.275 | 37.72 | 1.520ns | 10 – 30 (Homer 1991) |
| Pb ($mg{\cdot}kg^{-1}$) | 1.868 | < 1.49 | < 1.49 | < 1.49 | < 1.49 | - | - | < 0.8 (Bovi et al. 2007) |
| Cd ($mg{\cdot}kg^{-1}$) | < 0.19(4) | < 0.19 | < 0.19 | < 0.19 | < 0.19 | - | - | < 0.1 (Bovi et al. 2007) |
| Cr ($mg{\cdot}kg^{-1}$) | 146.261 | 9.446 b | 17.373 a | 8.581 b | 9.375 b | 24.90 | 8.482** | < 0.1 (Bovi et al. 2007) |

(1)Values with the same letter in the same line not showed significant difference at 5% by the Tukey's test for each sampling. (2) n.s., *, ** = non significant, significant at 5% and significant at 1% by Tukey's Test, respectively. (3)Suitable contents. (4) Contents less than the limit of quantification by the method.

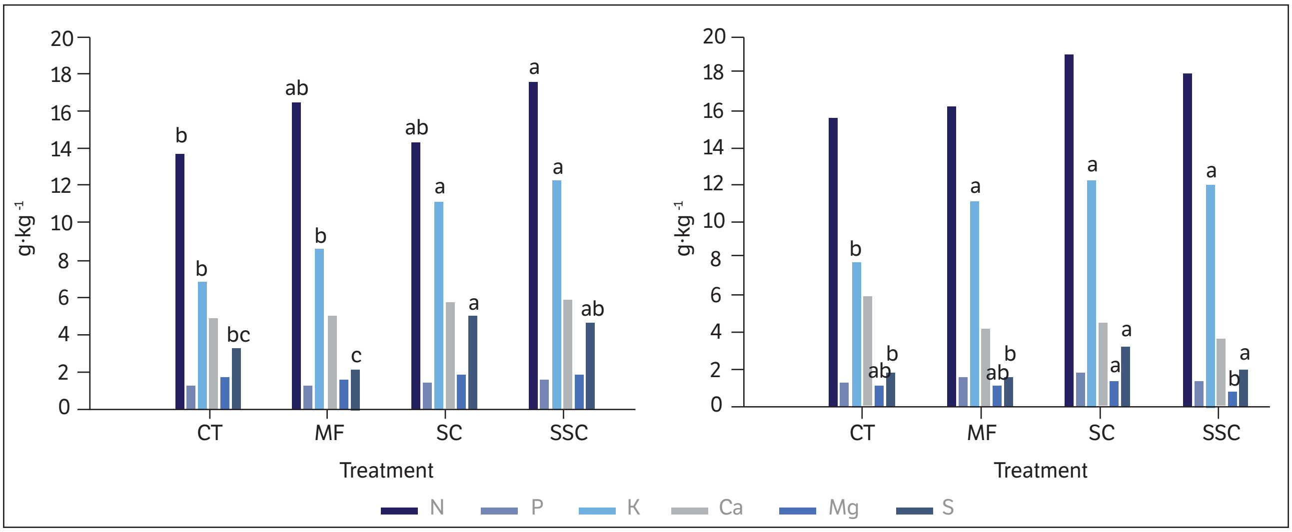


**Figure 2.** Macronutrients concentrations in Guanandi leaves at (A) 90 days and (B) 180 days after planting.

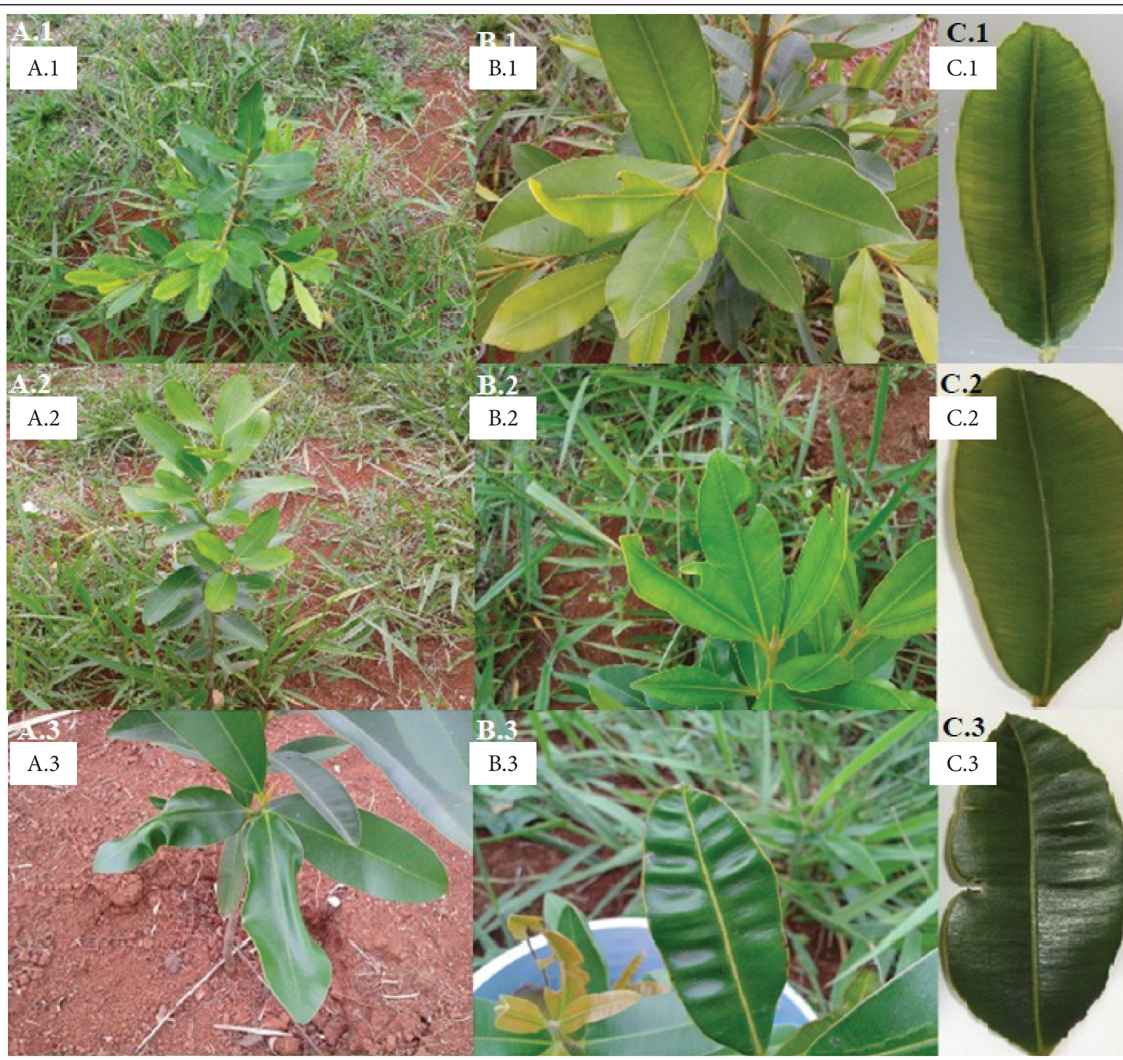


**Figure 3.** Magnesim, Mn, and Ca nutritional deficiencies observed through a visual diagnosis of the Guanandi seedlings. A, symptoms of Mg (A.1), Mn (A.2), and Ca (A.3) deficiency in the Guanandi seedlings; B, interneval chlorosis of old leaves (B.1), interneval chlorosis of young leaves (B.2), and the malformation and darkening of young leaves (B.3); C, leaf nutritional deficiency in Mg (C.1), Mn (C.2), and Ca (C.3)

and MF treated seedlings – since the recommendation for the inorganic fertilizer did not include S, and it is generally abundant in SS. The increased S leaf concentration in the Guanandi seedlings treated with SS agrees with Guedes and Poggiani (2003), who reported the lowest leaf S concentration in eucalypts seedlings fertilized with mineral fertilizers.

The concentration of Mg in the leaves was below the recommended range, both at 90 and 180 days after planting, in all treatments. There also was a decrease in this element content over the course of the experiment, suggesting that neither the organic fertilization nor the soil were efficient at supplying Mg. There were no significant differences in the concentration of P between the treatments over time, although it showed a slight increase. The highest P leaf concentration was observed for SS (0.200 $g \cdot kg^{-1}$ more than SSC) at 90 days, and for SSC (0.400 $g \cdot kg^{-1}$ more than SS) at 180 days after planting, although the treatments did not differ statistically. The low concentration of P observed in the leaves of *Calophyllum brasiliense* has been indicated as high efficiency in the internal use of this nutrient, though plants required it in large amounts (Gliessman 2001; Morandi et al. 2017). Santos et al. (2008) observed that Guanandi specie had also a higher presence of organic phosphorus in the leaves, although they had reduced absorption capacity of P, since the slow growth imply less development of the root system and soil exploration, which resulted in lower absorption of P.

Although the concentrations of Ca fell within the range of suitable values, symptoms of its deficiency were observed, as the plants showed poor formation of their young leaves and darkening (Fig. 3). Significantly lower Ca concentrations were observed in Guanandi seedlings in open-grown than in covered leaf tissues (Kellman 1985). Comparing the Ca results with the suitable foliar concentration suggests that Guanandi is highly demanding of this macronutrient, more than other forest species. The deficiency symptoms of Ca, Mg, and Mn exhibited by this species in the SS and SSC treatments indicate the need to supplement these nutrients, by enriching the organic residues via liming (Bellote and Neves 2001) to supply the initial nutritional requirements of newly planted Guanandi seedlings.

There was a significant treatments effect on the concentration of B, Zn, and Ni in the leaves at 90 days after planting (Table 3). The highest B and Zn leaf concentrations were observed in the SS treatment. By contrast, the highest Ni concentration was observed for CT and MF, though both treatments did not differed from SS. In this sense, it is important to highlight the higher pH value of SS relative to MF and SSC that evidenced pH value two units lower (Table 2). Specifically in relation to Ni, the increases in the pH values decrease Ni availability (Kellman 1985) due to dramatic increase in its retention in soil pH of pH 7.0 to 7.5.

No statistical difference about toxic elements was detected in the leaf content at 90 days after planting. However, the fertilizer treatment did have an effect on the concentration of Fe, Mn, and Cr in the leaves at 180 days after planting. It seems that SSC reduced the uptake of the Fe and Mn elements. Higher organic matter content in the SSC may have complexed metallic cations such as Fe and Mn, rendering them unavailable to plants, or can produce molecules that chelate micronutrients (van den Dreissche 1984).

According to several studies (e.g., Epstein 1975; Mills and Jones Junior 1996; Malavolta et al. 1997; Abreu et al. 2007; Bovi et al. 2007), most of the nutrients within the Guanandi leaves showed values close to those commonly found for the Atlantic Forest rainforest species. Nevertheless, the concentration of Cu and Mn in the leaves was lower than the suitable values for this tree species nutrition, whereas those of Zn and B exceeded them. According to Duboc and Guerrini (2009), the concentration of B in the leaves can be 3 to 40 times higher than the reference values, which highlights the lack of information regarding this micronutrient for possible comparisons between fertilizer treatments and plant species.

Leaf concentrations of Mg and Mn remained below their suitable values (Malavolta et al. 1997) in every treatment, which triggered symptoms of their deficiency (Fig. 1); interneval chlorosis of older leaves and interneval chlorosis with a thick reticulum of the younger leaves, respectively. The concentration of Fe in the leaves was high in both periods of evaluation when compared with their recommended contents (Epstein 1975); with the initial concentration (first days after planting and fertilizing) higher than that evaluated at 90 and 180 days after the application of treatments. Going from the 90 to 180 day-evaluation period, the concentration of Fe in the leaves decreased, yet the MF showed the highest concentration between the four treatments in all periods evaluated.

Although at 180 days after planting the concentration of Cr in the leaves was clearly reduced, all treatments exceeded the values established by Bovi et al. (2007), with the highest concentration of Cr found in the leaves of seedlings treated with MF. The presence of high concentration of Cr, irrespective of time and treatment, is of notable concern since matured

Guanandi trees produce fruits that are eaten by many animal species (Lorenzi 2008).

The concentration of Mo leaves decreased over time, with average values reduced 10-fold, but differences between treatments were not detected. At first glance this may suggest an analytical issue, but this is impossible, as all the samples were analyzed in a single batch. The concentration of Zn in the leaves surpassed that recommended by Abreu (2007). Specifically, the MF and SSC treatments had concentrations of Zn in the leaves increasing 41.3 and 22.4 mg·kg$^{-1}$, respectively, throughout the period evaluated. The concentration of B in the Guanandi seedling leaves increased greatly, from 90 to 180 days, though mainly in the MF treatment (+24.27 mg·kg$^{-1}$ vs. the control), leading to a far greater concentration than that recommended by Epstein (1975).

## CONCLUSION

The results highlight the potential for using SS and SSC, after adding KCl and lime, since Guanandi growing in Oxisol was highly demanding of Ca, Mg, and Mn, as evidenced by the leaf visual diagnosis of their deficiency symptoms. However, they were able to nutritionally supply Guanandi seedlings, although the short-term (180 days) cannot be conclusive as regards the exclusive use of them in place of mineral fertilizers. In addition, using sewage sludge composted or in natura imply in an alternative fate for their final disposal, which has been an issue of great concern.

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## AUTHORS' CONTRIBUTION

Conceptualization, C. B. Brandani, A. S. Kühl, V. E. Ilario and J. B. Carmo; Methodology, A. S. Kühl, and V. E. Ilario; Investigation, A. S. Kühl, V. E. Ilario, E. A. Pereira, L. C Oliveira, P. J. Ferreira Filho and J. B. Carmo; Writing – Original Draft, C. B. Brandani and J. B. Carmo; Writing – Review and Editing, C. B. Brandani and L. M. Pitombo; Supervision, L. M. Pitombo.

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