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Growth and volatile compounds of *Martianthus leucocephalus* exposed to heavy metal stress

Crescimento e teor de compostos voláteis de *Martianthus leucocephalus* submetida ao estresse por metais

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ABSTRACT

Martianthus leucocephalus is found in the semi-arid Northeast of Brazil and has high pharmacological potential due to the production of volatile compounds. This study aimed to evaluate the Cd (15, 30, 45 and 60 $\mu\text{mol L}^{-1}$), Cu (30, 60, 90 and 120 $\mu\text{mol L}^{-1}$) and Zn (150, 300, 450 and 600 $\mu\text{mol L}^{-1}$) levels on the growth of this species and its volatile compounds production. Apical cuttings were placed in plastic trays containing 6.0L nutrient solution, and after rooting, they were submitted to Cd, Cu and Zn treatments for 15 days. All the metals evaluated reduced the succulence and growth of all parts of *M. leucocephalus*. This effect was accompanied by the increase of Cd, Cu and Zn concentrations in all plant tissues. Volatile compounds content produced by *M. leucocephalus* was increased only by Zn (86%).

Key words: *Lamiaceae*, metals, relative growth.

RESUMO

Martianthus leucocephalus é encontrada no semiárido nordestino e possui potencial farmacológico atribuído à produção de compostos voláteis. Este trabalho objetivou avaliar o efeito de doses de Cd (15, 30, 45 and 60 $\mu\text{mol L}^{-1}$), Cu (30, 60, 90 and 120 $\mu\text{mol L}^{-1}$) e Zn (150, 300, 450 and 600 $\mu\text{mol L}^{-1}$) no crescimento dessa espécie e na produção de compostos voláteis. Estacas apicais foram transferidas para bandejas plásticas contendo 6,0L de solução nutritiva e, após o enraizamento, foram submetidas aos tratamentos de Cd, Cu e Zn, durante 15 dias. Todos os metais avaliados reduziram a suculência e o crescimento de todas as partes de *M. leucocephalus*. Este efeito foi acompanhado pelo incremento das concentrações de Cd, Cu e Zn em todos os tecidos das plantas. Também foi observado que o teor de compostos voláteis, produzido por *M. leucocephalus*, foi aumentado apenas pelo Zn (86%).

Palavras-chave: *Lamiaceae*, metais, crescimento relativo.

INTRODUCTION

Family *Lamiaceae* is widely used in different parts of the world, both as a medicinal plant and in cooking. Several species, such as rosemary (*Rosmarinus* sp.), basil (*Ocimum* sp.) and mint (*Mentha* sp.) are well established in a number of regions (MARIUTTI & BRAGAGNOLO, 2007). In this family, the genus *Hyptis* is highly relevant due to its ability to produce a wide range of pharmacologically significant organic compounds (FALCÃO & MENEZES, 2003).

Formerly known as *Hyptis leucocephala* Mart. ex Benth, the *M. leucocephalus* species is restricted to semi-arid regions and exhibits high phytochemical and economic potential, attributed to the presence of volatile compounds, commonly known as essential oils (OLIVEIRA et al., 2011). According to Lucchese et al. (2006), the essential oil yield of the leaves is nearly 0.2%, which has antimicrobial activity against *Bacillus cereus*, *Staphylococcus aureus* and *Candida albicans*.

Despite its significant potential, few studies investigate the domestication and cultivation of *M. leucocephalus*, which would facilitate its sustainable economic use. Additional aspects should also be considered for the development of cultivation systems, including factors that can stimulate an

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increase in volatile compounds in leaves. In this respect, plant exposure to abiotic stress factors has been shown to modify secondary metabolic pathways (SELMAR & KLEINWÄCHTER, 2013), increasing the synthesis rate of some products.

One of the abiotic factors that act on secondary metabolism is the accumulation of metals in plants. According to MITHÖFER et al. (2004), a number of species synthesize and accumulate secondary metabolites after treatment with metals. *Rosmarinus officinalis* L. plants exposed to Cu exhibited an increase in essential oil content (DEEF, 2007). This trend was also observed in *Matricaria chamomilla* L. plants (GREJTOVSKY et al., 2006). Additionally, *Salvia miltiorrhiza* Bunge plants grown in soils containing high levels of Cd showed accumulation of lipid and water soluble bioactive compounds (LI et al., 2013). Although further research is needed to fully elucidate the effects of metal stress (NASIM & DHIR, 2010), some results indicated that these substances may be useful as elicitors in plants with a unique metabolism.

Conversely, exposure to high levels of metals also affects plant growth and development, both in the roots and aerial parts (NAGAJYOTI et al., 2010). The same authors also suggested that damage is associated with reduced photosynthetic capacity and excessive production of reactive oxygen species. Although excess metals are typically harmful to plants, the extent of damage may vary, depending on stress intensity, type of metal and plant species (NAGAJYOTI et al., 2010). This study aimed to determine the effect of Cd, Cu and Zn on the growth and volatile compound production of *M. leucocephalus*.

MATERIALS AND METHODS

Growth conditions and treatments

Apical cuttings of *M. leucocephalus* around 15cm long were obtained from the collection of medicinal and herbal plants in the tree nursery of Universidade Estadual de Feira de Santana, Bahia, Brazil. Then, the cuttings were immediately transferred to plastic trays containing six liters of HOAGLAND & ARNON's (1950) nutrient solution and placed in a greenhouse. The pH of the solution was adjusted to 6.0 ± 0.2 and monitored daily. After ten days, plants were placed in hydroponic system and treatments were applied as follows: control ($0.0 \mu\text{mol L}^{-1}$ metal), Cd (NO_3)₂ (15, 30, 45 and $60 \mu\text{mol L}^{-1}$), CuSO₄ (30, 60, 90 and $120 \mu\text{mol L}^{-1}$) and ZnSO₄ (150, 300, 450 and $600 \mu\text{mol L}^{-1}$). Metal concentrations were defined as a function of toxicity levels, established by preliminary

experiments and results reported in the literature for other species (ZHAO et al., 2010; VASSILEV et al., 2011; AMIRMORADI et al., 2012). Nutrient solutions were replaced once a week and the volume topped up daily. The system was maintained under intermittent aeration (15 minutes every three hours) using an air compressor coupled to a timer. The plants remained under these conditions for 15 days.

Growth analysis

Plants were collected one day before and 15 days after the application of treatments and weighed by using an analytical balance to determine the fresh weight of the leaves (LFW), stem (SFW) and roots (RFW). Next, the plant matter was placed in paper bags and transferred to a forced air oven (60°C) until constant weight in order to determine total dry weight (TDW) as well as that of leaves (LDW), stem (SDW), and roots (RDW). Based on these data, it was calculated the relative growth rate (RGR) and the succulence (Suc) in accordance with the methodology proposed by EL-LITHY et al. (2004), using the following equations:

$$\text{RGR (g g}^{-1} \text{ d}^{-1}) = (\text{nl TDW}_2 - \text{nl TDW}_1) / (t_2 - t_1)^{-1}$$

$$\text{SUC (g H}_2\text{O g}^{-1} \text{ DW)} = (\text{LFW}_2 - \text{LDW}_2) / \text{LDW}_2$$

where: LFW₂: final fresh weight of the leaves; LDW₂: final dry weight of the leaves; nl: Napierian logarithm; TDW₁: initial total dry weight; TDW₂: final total dry weight and (t₂-t₁) time interval (15d).

The experimental design was a completely randomized, with four replications per treatment. Results were submitted to variance analysis by F-test. When observed significance, the data were subjected to regression analysis using SISVAR statistical software (FERREIRA, 2003).

Volatile compounds analysis

Volatile compounds were extracted using a Clevenger distillation apparatus. For each sample, 1.5 to 3g LDW of *M. leucocephalus* was used. Distillation time was 3h. The volatile compounds and water were collected in a separatory funnel. We added 10mL dichloromethane three times in succession, followed by magnesium sulfate to separate residual water. Mixture was placed into a rotary evaporator to remove the dichloromethane. Volatile compound content was quantified by gravimetry on analytical balance and expressed in mg per 100g LDW, and mg per plant (leaves).

Metal concentration analysis

The extracts were obtained by acid digestion in concentrated HNO₃ and H₂O₂ (30%) using

a digestion block at 125°C for 60min, as described by JONES (2001). Digested material was adjusted to 50mL with deionized water. Cadmium, copper and zinc were measured by inductively coupled plasma optical emission spectroscopy (Simultaneous Varian VISTA-PRO ICP-OES).

RESULTS

Figures 1 and 2 illustrate that increased Cd, Cu and Zn concentrations in the nutrient solution reduced all the growth variable, whereas, Zn addition raised the volatile compound content (Figure 3).

Comparison of effects of metal treatments on each part of plants showed greater damage in the leaves, where Cd, Cu and Zn caused average reductions of 70, 81 and 85%, respectively (Figure 1). The part least effected by Cd was the root, with a 56% reduction at the highest tested dose of this metal compared to the 70 and 73% decreases recorded in LDW and SDW, respectively. However, in plants submitted to higher levels of Cu and Zn, the least effected region was the stem (63 and 75%). With respect to TDW, plants exposed to the highest doses of Cd and Cu exhibited around 75% decline in relation to controls (Figure 1). In plants submitted to the highest Zn level, the decline was greater (approximately 85%).

Results showed that increased levels of Cd, Cu and Zn in the nutrient solution increased the accumulation of these elements in all parts of *M. leucocephalus* (Figure 2). Regardless of the metal and dose applied, concentrations were always higher in roots than in other parts of plants (Figure 2). In plants submitted to Cu and Zn stress, levels of these metals were similar in stems and leaves. However, for plants exposed to Cd stress, concentrations of this metal were five times higher in stems than in the leaves.

Relative growth rate (RGR) values of plants exposed to the highest Cd, Cu and Zn levels displayed reductions of 59, 78 and 86%, respectively, in relation to controls (Figure 3). Succulence of leaves submitted to the largest doses of Cd, Cu and Zn fell by approximately half the amount observed in non-stressed plants (Figure 3). Among the metals tested, Zn induced an increased in volatile compound concentration of *M. leucocephalus*, at all doses. Treatment with 450µM of this metal produced an average of 0.31mg g⁻¹ leaf, equivalent to 86% increase in relation to the value reported in control plants (Figure 3). Treatment of 60µmol L⁻¹ of Cu also increased the volatile compounds yield in 42%. Conversely, all metals caused a decrease

in the production of volatile compounds of *M. leucocephalus*. Thus, in the higher doses of Cd, Cu and Zn, these reductions reached 68, 77 and 75% (Figure 3).

DISCUSSION

Assessing plant performance based on growth made possible to determine the toxicity of the elements applied in *M. leucocephalus*. Thus, Cu and Cd caused similar declines in TDW despite the fact that double the amount of Cu was applied, suggesting that Cd is more toxic in this species. Conversely, although effects of Zn stress on TDW were relatively more severe, concentration used was 10 and 5 times higher than that of Cd and Cu, respectively. Thus, results indicated increasing phytotoxicity of Cd>Cu>Zn on growth of *M. leucocephalus*.

Regardless of the metal applied, LDW was always more affected by stress than the other plant parts and levels of toxic metals in the leaves were always lower than those in the stem and roots. Thus, the data suggested that *M. leucocephalus* leaves are more sensitive to the accumulation of metals than other parts of the plant.

Roots accumulated more metals than leaves and stems, indicating low translocation from the root to the aerial part of the plant (ALI et al., 2012). In addition, the results confirmed that Cu and Zn are deemed to have low mobility in plants (MARSCHNER, 2012). Conversely, the internal transport rate of Cd varies considerably among species (CHOPRA & PATHAK, 2012). As such, results of this study demonstrated low mobility of this metal in *M. leucocephalus*. Additionally, the limited translocation of toxic elements from roots to aerial parts is also considered significant in plant tolerance to metal stress (YANG et al., 2003).

As with TDW, there was a decline in the relative growth rate (RGR) with the increased concentrations of all metals tested. This rate represents the periodic increase in plant DW in relation to the preexisting DW (EL-LITHY et al., 2004). Considering the more pronounced decrease in DW caused by the toxicity of these metals in leaves, data suggested that toxicity of metals tested can reduce photosynthetic capacity of *M. leucocephalus*. Photosynthetic disorders are often observed in plants exposed to high metal levels (NAGAJYOTI et al., 2010).

Metal stress has been reported as a factor that causes water stress in plants (PÁL et al., 2006), an effect also observed in *M. leucocephalus* and evident in its decreased succulence (Suc). When

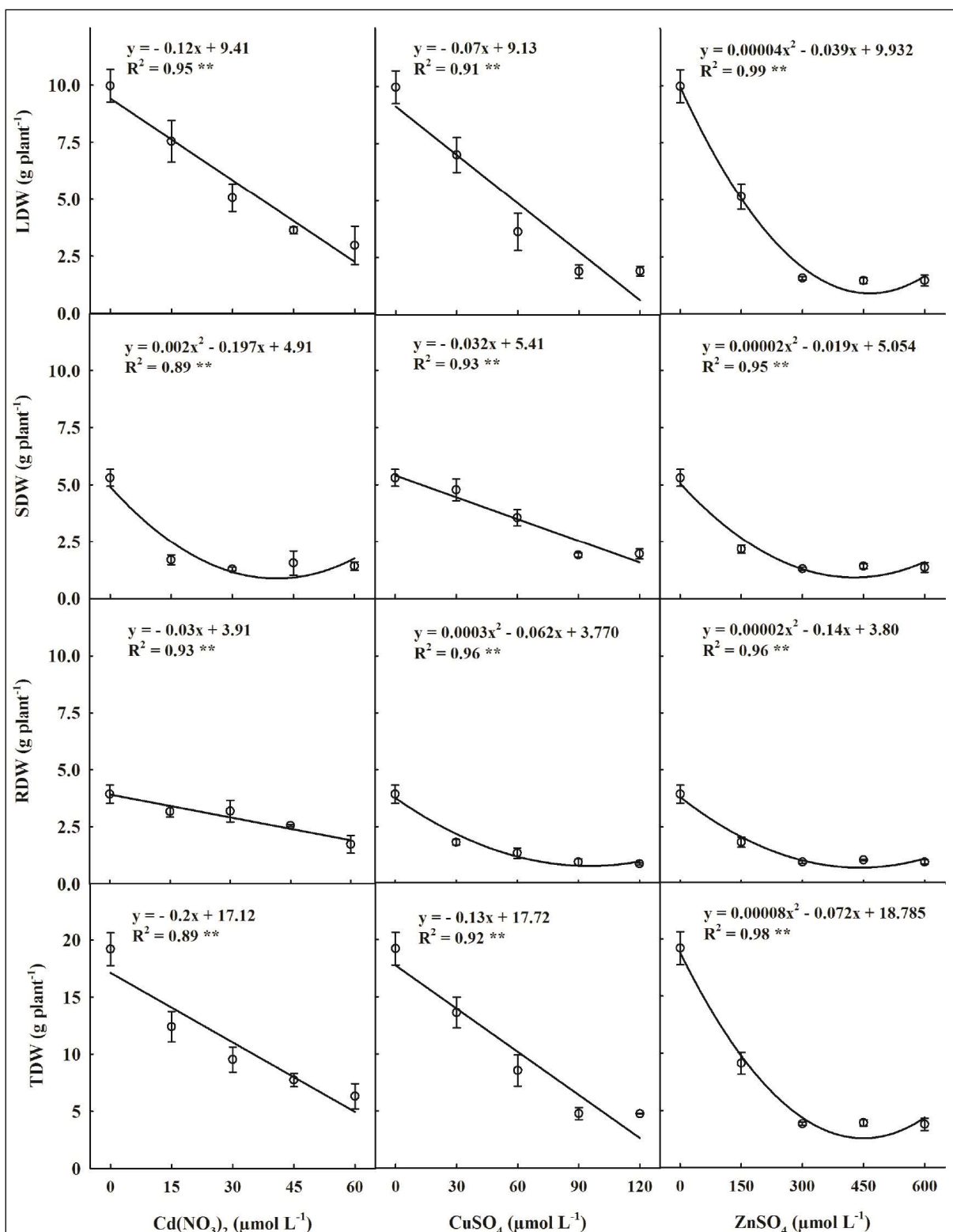


Figure 1 - Dry weight of leaves (LDW), stems (SDW), and roots (RDW) as well as total dry weight (TDW) of *Martianthus leucocephalus* (Mart. ex Benth.) J.F.B. Pastore plants cultivated using different levels of Cd, Cu, and Zn in a hydroponic system. Feira de Santana, 2014. **Significant at 1% probability.

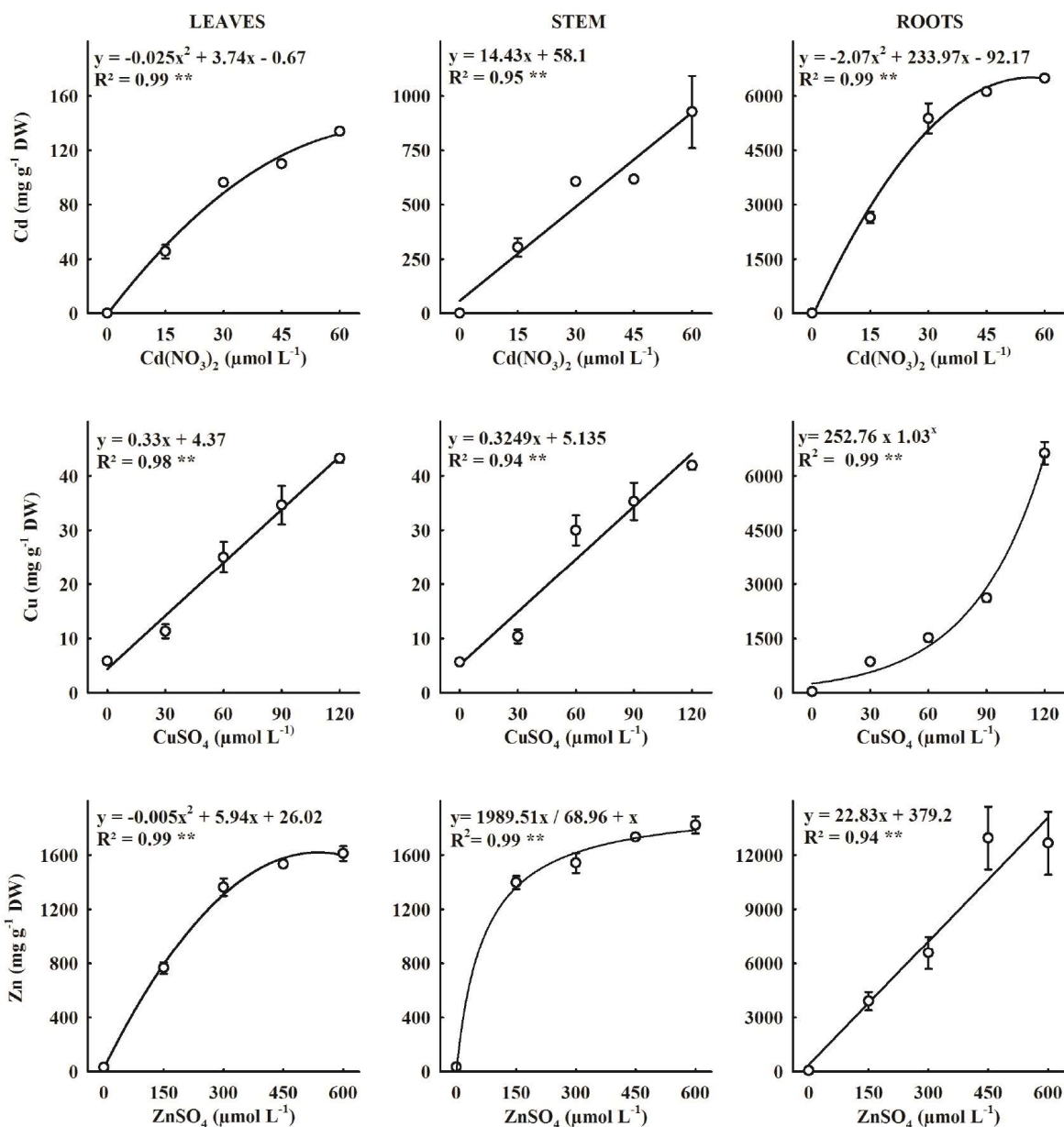
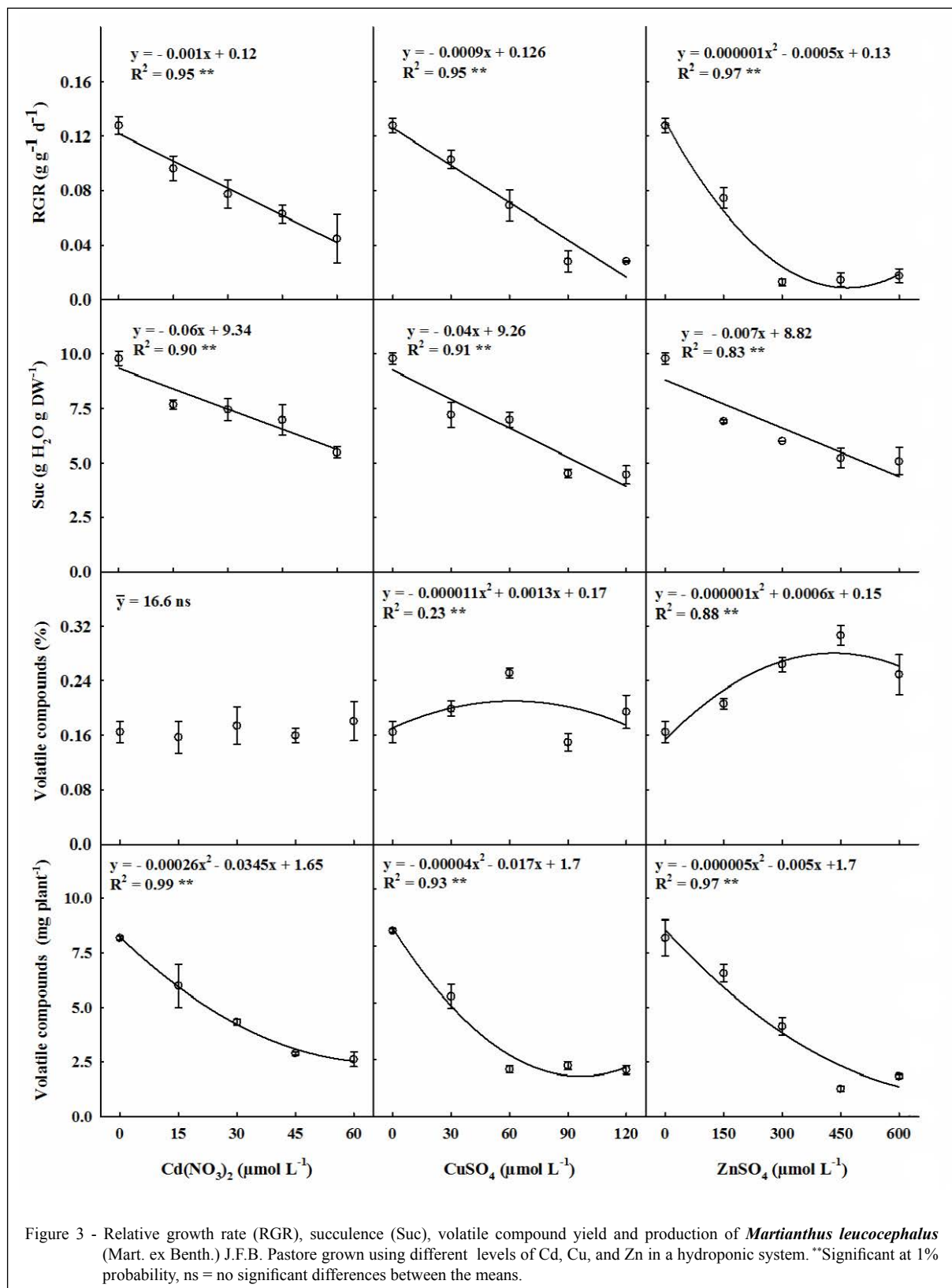


Figure 2 - Metal concentration in leaves, stems, and roots of *Martianthus leucocephalus* (Mart. ex Benth.) J.F.B. Pastore plants cultivated using different levels of Cd, Cu, and Zn in a hydroponic system. Feira de Santana, 2014. ^{**}Significant at 1% probability.

plants were exposed to higher levels of Cd, Cu and Zn, succulence fell by around half the values obtained in controls. Compromised water balance in plants exposed to heavy metals has been linked to reductions in membrane permeability and vascular bundle diameter, in addition to inhibiting formation of root hairs (PÁL et al., 2006). Water is an essential component in promoting growth and cell division (JALEEL et al., 2009). Thus, results obtained in this study suggested that reduced succulence in *M. leucocephalus* tissue submitted to treatment with Cd, Cu and Zn partially explains growth decline in this species.

Results illustrated that volatile compound content increased with Zn exposure (Figure 3). This increase in volatile compounds in plants exposed to Zn suggested a concentration effect, given the reduction in leaf growth. The observation that the volatile compounds production was lower in metals treated plants supports this hypothesis. Alternatively, Zn-induced stress may have increased activity of enzymes isopentenyl pyrophosphate: dimethylallyl pyrophosphate isomerase and phenylalanine ammonia lyase (LUCINI & BERNARDO, 2015). These are key enzymes in the terpenes and phenylpropanoids biosynthesis, the main components of plant essential



oils (MIGUEL, 2010). As the reduction in dry matter yield can be compensated by a higher plant density, *M. leucocephalus* cultivation with reduced spacing between plants may result in a real increase in volatile compound yield in Zn-contaminated soil.

Production of volatile compounds in plants may be an important mechanism in minimizing damage caused by abiotic stress (TURTOLA et al., 2003). A number of these substances can protect plant species from oxidative stress in adverse environmental conditions, such as high heavy metal levels (SELMAR & KLEINWÄCHTER, 2013). According to these authors, plants can maintain volatile compound production even under very negative conditions and with photosynthesis severely compromised. Accordingly, results of this study showed that growth was more affected by the presence of metals than volatile compound content, suggesting that these compounds play an important role in the metabolism of *M. leucocephalus*, even in adverse conditions.

CONCLUSION

The Cd, Cu and Zn stresses reduced growth of *M. leucocephalus*; however, volatile compounds concentration increase in the Zn presence. Considering that the growth reduction may be compensated by the increase in plant density, new researches should be performed in Zn contaminated soils, evaluating the effect of plant density in volatile compounds production of *M. leucocephalus*.

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