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Availability of nutrients and toxic heavy metals in marigold plants

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ABSTRACT. Availability of nutrients and toxic heavy metals in marigold plants. This study was performed aiming to assess the availability of nutrients and toxic heavy metals present in marigold plants (*Calendula officinalis*) treated with different fertilizers. The treatments were arranged in factorial scheme (2 x 2 x 3) in a completely randomized experimental design (CRD), with two textures of soil (sandy and clayey), two forms of fertilization (organic and chemical) and three fertilization levels (without fertilization, recommended dose, and twice the recommended dose) totaling 12 treatments, with four replications. The results showed that the clayey soil promoted the availability of N P, K, Mg, Cu, Zn and Fe; on the other hand, the sandy soil favored the availability of Ca, Mn, Pb and Cr. The organic fertilization provided higher levels of P and Fe, while the leaf tissue of marigold plants chemically fertilized presented higher concentrations of K and Mn.

Keywords: medicinal plants, chemical fertilization, organic fertilization.

Disponibilidade de nutrientes e metais pesados tóxicos em plantas de calêndula

RESUMO. Realizou-se este trabalho com o objetivo de avaliar a disponibilidade de nutrientes e de metais pesados tóxicos presentes em plantas de calêndula (*Calendula officinalis*) após diferentes tipos de adubação. Os tratamentos foram arranjados em esquema fatorial (2 x 2 x 3) dispostos em delineamento experimental inteiramente casualizado (DIC), sendo duas texturas de solo, (argilosa e arenosa), duas formas de adubação (química e orgânica) e três doses de adubação (sem adubação, dose recomendada e o dobro da dose recomendada), totalizando 12 tratamentos com quatro repetições. Os resultados demonstraram que os solos argilosos favoreceram a disponibilidade de N P, K, Mg, Cu, Zn e Fe; os solos de textura arenosa favoreceram a disponibilidade de Ca, Mn, Pb e Cr. A adubação orgânica disponibilizou maiores teores de P e Fe, enquanto o tecido foliar de plantas de calêndula adubadas com adubação química apresentaram concentrações maiores de K e Mn.

Palavras-chave: plantas medicinais, adubação química, adubação orgânica.

Introduction

The synthesis of principle actives in medicinal plants is result from the secondary metabolism (COSTA et al., 2008). Gobbo-Neto and Lopes (2007) asserted that the sources of nutrients and their availability can influence the production of different secondary metabolites.

Animal wastes had been widely used as a fertilizer prior the advent of chemical fertilizers. However, the concern about soil degradation has grown the interest on using manure in agriculture (GONÇALVES JUNIOR et al., 2007). Santos et al. (2003) stated that mineral fertilizers and pesticides frequently contain impurities, among them, the heavy metals.

Heavy metals are chemical elements (metals and some semimetals) that have density higher than

5 g cm⁻³, and atomic number higher than 20 (GONÇALVES JUNIOR; PESSOA, 2002). Gonçalves Junior et al. (2009) say that metals such as copper (Cu), zinc (Zn), nickel (Ni) and chromium (Cr) are used in biological metabolism and considered essential, whereas lead (Pb) and cadmium (Cd) are not essential, thus considered toxic, even at trace levels. The essential metals can also produce toxic effects at high concentrations. Therefore, not all heavy metal is essentially toxic.

Plants can accumulate these metals in all tissues and transfer them to the trophic chain, and nowadays, this accumulation is a subject of environmental interest, not only due to the phytotoxicity of many of these elements, but also due to the potential adverse effects on animal and human health (SHWANTZ et al., 2008).

In this way, this study was conducted to evaluate the content of toxic heavy metals Cd, Pb and Cr, and of essential elements N, P, K, Ca, Mg, Zn, Fe and Mn present in marigold plants (*Calendula officinalis*) subjected to different treatments with organic fertilization and conventional fertilization on soils with different textures.

Material and methods

The trial was conducted in an experimental area of protected cultivation of the State University of West of Paraná (Unioeste), at the municipality of Marechal Cândido Rondon, Paraná State. The treatments were arranged in factorial scheme ($2 \times 2 \times 3$), in a completely randomized design (CRD), with two textures of soil (sandy and clayey), two forms of fertilization (organic and chemical) and three fertilization levels (without fertilization, recommended dose, and twice the recommended dose), totaling 12 treatments with four replications, arranged in 48 plastic pots with capacity of 5 kg of soil.

Characterization of soils used in the experiment

The soils used in the experiment were collected at the layer 0-20 cm deep. The clayey soil was collected in the municipality of Marechal Cândido Rondon, Paraná State, and classified as Oxisoil (LVe), and the sandy soil was collected in the municipality of Palotina, Paraná State, and classified as Ultisol (PVd) (EMBRAPA, 1999). In order to determine the distribution of primary soil particles (sand, silt and clay), it was performed a granulometric analysis according to the methodology of Coelho et al. (2009). Table 1 lists the results from the chemical analysis of the soils accomplished according to Pavan et al. (1992).

Chemical characterization was carried out from samples taken from the layers 0-10 and 10-20 cm deep. After collection, samples were dried using a forced air circulation oven at 65°C for

48h, characterized as oven-dried fine soil and crushed in a hammer-type mill, and sieved (2 mm diameter) to remove clods and impurities (SEIDEL et al., 2010).

Chemical fertilization

For the recommendation of chemical fertilization, we used the chemical analysis of the soils (Table 1). The dose values used for chemical fertilization, i.e., the recommended dose and twice the recommended dose were, respectively, for the clayey soil: 30 and 60 kg ha⁻¹ for N, 50 and 100 kg ha⁻¹ for P, 20 and 40 kg ha⁻¹ for P; and for the sandy soil: 30 and 60 kg ha⁻¹ for N, 120 and 240 kg ha⁻¹ for P, 80 kg ha⁻¹ and 180 ton. ha⁻¹ for P (RAIJ et al., 1997). The sources of N, P and K used were ammonium sulfate ((NH₄)₂SO₄), monoammonium phosphate (MAP) and potassium chloride (KCl), respectively.

Organic fertilization

For the recommendation of organic fertilization, we used the Bulletin 100 from the Agronomic Institute of Campinas (IAC) that suggests from 20 to 50 ton. ha⁻¹ of fertilizer for medicinal plants (RAIJ et al., 1997). In this way, based on the result of chemical analysis of the soil (Table 1), the recommended dose for clayey soil was 30 ton. ha⁻¹ and twice was 60 ton. ha⁻¹, while for the sandy soil the recommended dose was 40 ton. ha⁻¹ and twice was 80 ton. ha⁻¹ of organic compost.

The organic compost used in the experiment was prepared with swine manure removed from a settling pond of waste and sun-dried, and with fragments of grass cutting according to the methodology described by CENTEC (2004). The organic compost was chemically characterized by nitroperchloric digestion (AOAC, 2005) and the determination of the elements was made through atomic absorption spectrometry, at flame mode (EAA/flame) (WELZ; SPERLING, 1999). The results are presented in Table 1.

Table 1. Chemical analysis of Oxisoil (LVe), Ultisol (PVd) and of organic compost.

	P	MO	pH	H+Al	Al ³⁺	K ⁺	Ca ²⁺	Mg ²⁺	SB	CTC	V
	mg dm ⁻³	g dm ⁻³	CaCl ₂ 0.01 mol L ⁻¹								%
LVe	8.40	21.20	5.12	3.49	0.10	0.60	4.17	0.86	5.60	9.10	61.80
PVd	2.19	6.84	4.46	2.81	0.15	0.04	0.60	0.16	0.80	3.61	22.16
			Cu	Zn	Fe	Mn	Cd	Pb	Cr		
			g kg ⁻¹								
LVe			14.90	173.00	45.20	3.40	3.00	48.00	5.00		
PVd			2.80	72.00	8.61	0.60	1.00	20.00	ND		
			Organic compost								
N	P	K	Ca	Mg	Cu	Zn	Fe	Mn	Cd	Pb	Cr
			g kg ⁻¹								
13.13	10.83	2.00	41.30	5.20	314.00	250.00	416.00	536.00	2.00	62.00	17.00

Installation and conduction of the experiment

The soils were sieved (5 mm) to be used in the experiment. For the sandy soil, a liming process was needed to raise the base saturation to 50%, using limestone as a corrective (GATIBONI et al., 2003).

About ten marigold seed were sown per pot and, at 15th day after sowing, we performed the thinning to let only four plants per pot. The removal of invasive plants was made daily, with no need to control pests or diseases in the culture. The pots were watered daily aiming to keep the field capacity of the soil.

Completion of the experiment

At 90 days of cultivation, all the plants were cut close to the ground and washed with tap water and then with distilled deionized water. After washing, the plant material was weighed and packed separately in identified paper bags and dried in a forced air circulation oven at 65°C (LACERDA et al., 2009).

After drying, the material was crushed in Wiley-type mill with sieve of 2 mm. Crushed samples were packed in polyethylene bags, individually separated to be chemically analyzed, in order to determine the contents of the toxic heavy metals Cd, Pb and Cr and of the elements K, Ca, Mg, Cu, Zn, Fe and Mn through nitroperchloric digestion (AOAC, 2005), followed by determination in EAA/flame, with a device GBC 932AA (WELZ; SPERLING, 1999). As a detection limit of the analyzed elements, it was used the manufacturer's manual (GBC, 1998). To quantify N and P, the sulfuric acid digestion was employed (AOAC, 2005) followed by ultraviolet visible spectrometry (UV-VIS) to determine total P, and distillation in micro-distiller Kjeldhal to determine total N (MANTOVANI et al., 2005).

The results were subjected to an analysis of variance and Tukey's test, using the software Sisvar, considering 5% probability, in order to test the effect

of the treatments on the availability of elements evaluated in the marigold cultivation.

Results and discussion

Plant tissue

The soil texture influenced significantly ($p < 0.05$) on the absorption of most elements (Table 2). For the fertilization sources used in this experiment, significant differences were detected for the elements P, K, Fe and Mn in marigold plants (Table 2).

In the interaction between soil and fertilization, there was a significant difference for the contents of N, P, K, Zn, Fe and Mn. In the triple interaction between soil, fertilization, and doses, a significant difference was detected among all elements evaluated, except for Cu and Cd (Table 2). For all the interactions, the Cd values were below the detection limit of this metal for the method used, 0.009 mg L⁻¹ (GBC, 1998).

The Table 2 shows the concentrations of N, P, K, Ca, Mg, Cu, Zn, Fe, Mn, Pb and Cr found in marigold plants as a function of the two textures of the soil used in the experiment.

Marigold plants cultivated in clayey soil had higher concentration of N, P, K, Mg, Cu, Zn and Fe in the leaf tissue (Table 2). The higher concentrations of N in clayey soil is explained by Sangoi et al. (2003) that state that this texture has higher holding capacity of N, mainly as NH₄⁺, in comparison to sandy soils. The higher water holding capacity of clayey soils reduces the percolation of soluble nitrates to lower soil layers, making this element available to the plants. The same is true in relation to P in clayey soils, since according to Falcão and Silva (2004), in studies performed with soils from Manaus (Amazonas State), it was verified that the maximum absorption capacity of P presented positive correlation with the clay content in the soil.

Table 2. Mean values of the concentrations of nutrients and heavy metals in marigold plants in the interactions soil, fertilization and soil x fertilization.

Mean values of the concentrations of N, P, K, Ca, Mg, Zn, Fe, Mn and of toxic heavy metals Pb and Cr in the soil after harvest of marigold plants											
	N	P	K	Ca	Mg	Cu	Zn	Fe	Mn	Pb	Cr
	g kg ⁻¹					mg kg ⁻¹					
LVe	33.51a	1.67a	22.30a	24.48b	10.17a	21.75a	45.50a	1076.67a	294.50b	13.46b	3.50b
PVd	10.90b	1.44b	8.55b	29.76a	7.56b	12.75b	31.83b	781.29b	379.67a	19.67a	6.42a
Mean values of the concentrations of P, K, Fe and Mn in the fertilization interaction											
	g kg ⁻¹					mg kg ⁻¹					
	P		K		Fe		Mn				
Chemical	1.49b		16.78a		782.90b		449.46a				
Organic	1.62 ^a		14.07b		1075.33a		224.71b				
Mean values of the concentrations of N, P, K, Zn, Fe, Mn and Cr in the interaction between soil and fertilization											
	N	P	K	Zn	Fe	Mn	Cr				
	g kg ⁻¹							mg kg ⁻¹			
LVe	Chemical	30.70a	1.55b	21.73a	51.42a	975.22a	384.67a	4.08a			
	Organic	36.31b	1.80a	22.87a	39.58b	1178.67a	204.33b	2.92a			
PVd	Chemical	11.66a	1.44a	11.82a	26.92b	590.58b	514.25a	5.08b			
	Organic	10.14a	1.45a	5.27b	36.75a	972.00a	245.08b	7.75a			

For each soil, mean values followed by a same letter, in the columns, are not different by the Tukey's test, at 5% probability.

The fact that marigold plants have content of P higher than its content in sandy soil is due to the greater cation exchange capacity (CET) of clayey soils, which allows higher retention of this element in the soil, preventing losses by leaching and, consequently higher availability to plants (WERLE et al., 2008). The availability of the cations Zn^{2+} , Cu^{2+} , Fe^{2+} are also influenced by the CET of clayey soil (VITTI et al., 2008).

Also, the marigold plants cultivated in sandy soil presented higher availability of Ca, Mn, Pb and Cr in the leaf tissue (Table 2). Prado and Natale (2004), analyzing the root system of guava trees, argued that liming increases the availability and absorption of Ca by the plant. In the experiment, the liming was performed only in the sandy soil, with greater availability of Ca in the leaf tissue of plants cultivated in this type of soil.

The Mn is found in the soil solution as Mn^{2+} , Mn^{3+} and Mn^{4+} and is absorbed by the plants in the divalent form, and the availability of this element decreases with the pH raise, through the liming process, in organic soils where it remains complexed (MOTTA et al., 2007). In this way, it is justified the the Mn content found in the leaf tissue of marigold plant cultivated in sandy soil, according to the results listed in Table 2.

The toxic heavy metals Pb and Cr had higher concentration in the leaf tissue of marigold plants cultivated in sandy soil, different from the results found by Korf et al. (2008) that, examining the retention of toxic heavy metals in soils of areas for disposal of urban solid waste, have observed the significant retention potential of these elements in clayey soils (Table 2).

In the Table 2 are presented the concentrations of P, K, Fe and Mn found in marigold plants as a function of the two types of fertilization used in the experiment. The fertilization sources had no statistical difference for the availability of the other elements evaluated in the leaf tissue. The concentrations of P and Fe were higher in the leaf tissue of plants cultivated with organic compost. Dortzbach (2009) verified increase in the contents of P and K in organic cultivation using swine manure as source of compost. Nevertheless, the marigold plants had higher concentration of K when fertilized with chemical fertilizer (Table 2). The elements Mn and K presented higher contents in the plant tissue of plants chemically fertilized.

Motta et al. (2007) asserted that besides presenting the same valence, in the absorbed form, and similar size, Fe and Mn are very susceptible to the oxiredution process, which contributes to the occurrence of interaction between these two

elements, the rising concentration of one causes the deficiency of the other. In this experiment, the concentration of Fe was higher in marigold plants fertilized with organic compost, unlike the Mn (Table 2).

The concentrations of N, P, K, Zn, Fe, Mn and Cr presented statistical difference in the interaction between the two soil textures (clayey and sandy) and two fertilization sources (chemical and organic) (Table 2). The elements N and P had significant differences only for the clayey soil, and N presented higher concentrations in the tissue of plants chemically fertilized, and P had higher contents in plants fertilized with organic compost. This is due to the higher CET of the clayey soil that enables it to retain these elements and make them available to the plants (WERLE et al., 2008).

A significant statistical difference ($p < 0.05$) was found for the elements K, Fe and Cr, only considering the sandy soil; the K was found at higher contents in the tissue of plants chemically fertilized, and the elements Fe and Cr are found at higher concentrations in plants fertilized with organic compost. As the sandy soils have less organic matter, the most available forms of these elements are found in chemical fertilizers (LUCENA et al., 2004).

For the elements Zn and Mn, there was significant difference between the fertilization sources for the two soil textures. In the clayey soil, the Zn and Mn presented higher contents in plants under chemical fertilization. In the sandy soil, the Mn content was higher in plants chemically fertilized and the Zn had higher contents in plants fertilized with organic compost.

Conclusion

In conclusion, the soil textures (clayey and sandy) and the sources of fertilization (chemical and organic) have influenced significantly the availability of elements and toxic heavy metals evaluated in cultivated marigold plants.

The results evidenced that the clayey soils promoted the availability of N P, K, Mg, Cu, Zn and Fe; and the sandy soils favored the availability of Ca, Mn, Pb and Cr. The organic fertilization provided higher levels of P and Fe, whereas the leaf tissue of marigold plants under chemical fertilization presented higher concentrations of K and Mn. The marigold plants cultivated in clayey soils presented greater height and higher values of dry and fresh masses.

For the toxic heavy metals, only the sandy soil presented significant difference for the concentration of Cr and Pb in the tissue of marigold plants.

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