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Environment

Cultivation of *Tagetes patula* L. in copper contaminated soils

Cultivo de *Tagetes patula* L. em solos contaminados por cobre

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ABSTRACT

Leaf quality is one of the most valued commercial parameters in the cultivation of flowers and ornamental plants in vases, with soil or substrate conditions playing a fundamental role in its successful practice. Thus, the objective of this study was to evaluate the vegetative development of marigold (*Tagetes patula* L.) cultivated in soils contaminated by Cu, seeking to verify the soil´s phytoremediation potential for an agriculture with low environmental impact. The experiment took place in a greenhouse, using a completely randomized, 5x2 factorial design (added levels of Cu in the soil: 0 (no addition: control), 250, 500, 750, and 1,000 mg kg^{-1} of copper sulfate, and soil pH corrections: 5.5 and 6.0, according to the soil analysis report), with five replications. This work evaluated phytotechnical parameters and translocation factors, bioaccumulation, bioconcentration, and Cu extraction rate in plants. It was observed that the development of marigold plants was similar on both soil pH corrections according to the addition of Cu, affecting the aesthetic quality of the plants. In this context, it is concluded that the marigold species is tolerant to cultivation in soils with excess Cu and is characterized as a Cu extracting plant with low soil phytoremediation potential.

Keywords: Phytoremediation; Marigolds; Crop tolerance factors

RESUMO

No cultivo de flores e plantas ornamentais em vaso, a qualidade foliar é um dos parâmetros comerciais mais valorizados, sendo as condições de solo ou substrato fundamentais para o sucesso deste cultivo. Assim, o objetivo foi avaliar o desenvolvimento vegetativo de tagetes (*Tagetes patula* L.) cultivado em solos contaminado por Cu, buscando verificar seu potencial fitorremediativo do solo e uma agricultura de baixo impacto ambiental. O experimento ocorreu na casa de vegetação, utilizando delineamento inteiramente casualizado, com fatorial 5x2 (teores adicionados de Cu no solo: 0 (sem adição: testemunha), 250, 500, 750 e 1.000 mg kg^{-1} de sulfato de cobre e correções de pH do solo: 5,5 e 6,0 de acordo com o laudo da análise do solo), com cinco repetições. Avaliaram-se parâmetros fitotécnicos e fatores de translocação, bioacumulação, bioconcentração e taxa de extração de Cu na planta. Observou-se que o desenvolvimento das plantas de tagetes foi similar para ambas as correções de pH do solo conforme a adição de Cu, afetando a qualidade estética das plantas. Neste contexto, conclui-se que a espécie de tagetes apresenta tolerância de cultivo em solo com excesso de Cu e caracteriza-se como planta extratora de Cu, com baixos potenciais de fitorremediação do solo.

Palavras-chave: Fitorremediação; Tagetes; Fatores de tolerância de cultivo

1 INTRODUCTION

The emergence of new technologies for agricultural production constantly faces the challenge of generating sustainable development and an increasingly production demand. Phytosanitary control through pesticides plays an important role in different production systems. However, the indiscriminate use of these chemical compounds has been generating environmental impacts that affect the soil-water-plant-atmosphere system, as it occurs in wine production (Menegaes et al., 2017; 2019).

Among the most frequent diseases in grapevines (*Vitis* spp.), the incidence of mildew (*Plasmopara viticola*) stands out, for which one of the forms of sanitary management is the use of the Bordeaux mixture [$(CuSO_4.5H_2O)$ + Ca $(OH)^2$)], which has been used as a fungicide and a bactericide. However, its constant use in vineyards has promoted the accumulation of copper (Cu) on the soil surface, causing a negative impact on the development and productivity of the vines (Mackie et al., 2012; Menegaes et al., 2020).

Plants may have their development impaired when the soil has nutritional deficiencies, and it is important to properly manage the soil-plant system. Agricultural management include practices such as green manuring, mineral and organic fertilizers, among others (Gonçalves Junior et al., 2013). Xia and Shen (2007) state that Cu is present in the physiological processes of plants as one of the essential nutrients for plant development. However, it may become a toxic element depending on the dosage. Thus, plants have developed several strategies, encompassing a complex network of metal transport pathways, with the aim of regulating plant homeostasis as a function of environmental changes in Cu concentration. Such strategies evolve in order to prevent the accumulation of the metal in its free and reactive form.

Cu has a high affinity with organic matter, when in contact it generates soluble and insoluble complexes. In the surface horizon of the soil, through complexation reactions with humic and fulvic acids, this element is retained. When in low concentrations in the solution, it will be immobilized mainly by humic acids and as the stronger binding sites are saturated, a greater amount of Cu will be solubilized by fulvic acids or by simpler organic compounds. In acidic soils with low organic matter content, copper mobility will be affected (Oorts, 2013).

Research studies regarding the effects of Cu when accumulated in winegrowing soils have been carried out around the world. These show that some species of plants, called bioaccumulators, can reduce the concentration of Cu in the soil or make it unavailable, thus avoiding its toxic effects. The species that stand out for having a good phytoremediation potential are mostly plants intended for green manure and/or soil cover, such as oats (*Avena sp.*), alshotlzia (*Elshotlzia splendens* N. F. M.) (Mackie et al., 2012), and ornamental species such as calla lily (*Zantedeschia* spp.) and chinese pink (*Dianthus chinensis* L.) (Menegaes et al., 2020).

In this context, the objective of this study is to evaluate the vegetative development of marigold (*Tagetes patula* L.) cultivated in soils contaminated by Cu, verifying its phytoremediation potential on the soil, seeking agriculture practices with a lower environmental impact.

2 MATERIALS AND METHODS

The experiment was carried out in a greenhouse, with glass cover and zenith windows, in the L-W Direction, at the Floriculture Sector of the Federal University of Santa Maria Department of Phytotechnics in Santa Maria, RS (29º43’ S; 53º43’ W, altitude of 95 m), in 2021. The climate in the region is humid subtropical (Cfa), according to the Köppen-Geiger classification, with an average annual rainfall of 1,769 mm, average annual temperature close to 19.2º C and air humidity around 78.4% (ALVARES et al., 2013).

The experimental design was completely randomized, in a 5x2 factorial scheme (added levels of Cu in the soil and soil pH corrections), with five replications, where each experimental unit was composed of one vase. The Cu contents were composed by added concentrations of Cu per kilogram of soil, in the amounts of zero (no addition: control), 250, 500, 750 and 1,000 mg kg^{-1} of copper sulfate ($CuSO_4$). Soil pH corrections were pH 5.5, without the addition of lime, according to the soil analysis report (Table 1), and pH 6.0, with addition of PRNT 76% lime. The soil used was collected from a winegrowing area in the municipality of Santa Maria, RS, at a depth of 0-20 cm. The soil in the region is classified as Alisol.

Table 1 – Soil analysis report

Ca	**Mg**	**Al**	**H+Al**	**CTC efet.**	**CTC pH7**	**Saturation (%)**		**Index**
			$cmol_c$ dm^{-3}			**Al**	**Bases**	**SMP**
3,3	0.9	0.0	2.0	4.6	6.6	0.0	69.3	6.7
pH water	S	K	Cu	Zn	B	% MO	% Argila	Texture
1:1			mg dm^{-3}			m v^{-2}		
5.5	10.1	136.0	14.01	14	0.37	1.2	8.0	4.0

Source: UFSM Soil Laboratory (2021)

Marigold seeds came from an experimental cultivation area in the Floriculture Sector, from the 2019/2020 harvest. Sowing took place directly in the pots, with three seeds per pot. Thinning was performed 15 days after sowing (DAS), with the total emergence of seedlings, leaving only one plant per pot. The vases used were number 15, with 1.3 L in volume, 14.5 cm of upper diameter and 11 cm of lower diameter, 12 cm in height, made of black plastic material, with a distribution of 10 vases m^{-2}.

Evaluations on the evolution of plant height development, length and width of leaves (L), the diameters of plant canopy in relation to the vase (with a millimeter ruler) and the number of leaves and buds (by manual counting method) were carried out in a four-day interval between 20 and 72 days after transplantation (DAT). Leaf area was calculated by Equation 1:

$$LA: (L \times W) \times k \tag{1}$$

where k assumes the value of 0.44, due to the leaf clippings (Mainardi et al., 2004).

At 72 DAT, the dry phytomass of the inflorescences, aerial part and roots were evaluated by drying the plants in a forced ventilation oven at 65° C until reaching constant mass. Subsequently, subsamples of dry plant material were ground in a Willey-type mill, submitted to nitric-perchloric digestion and the quantification of Cu concentrations in plant tissues was performed using an optical emission spectrometer with inductively coupled plasma (ICP-OES, Avio 500 - Perkin Elmer). This equipment had the possibility of using plasma in axial and radial configuration. The procedure for sampling preparation and digestion of samples was validated according to Salazar et al. (2011) and performed by the methods described by Gomes et al. (2016). Soil Cu contents after cultivation were sampled by chemical analysis.

The calculations of the effective number of total plants (PENt) and the effective number of shoots of plants (PENpa) were carried out according to the methodology described by Garcia et al. (2004) and Sun et al. (2008). Phytoremediative evaluations followed the methodologies described by Caille et al. (2005) and Yoon et al. (2006) for the translocation factor (FCA) (Equation 2), the shoot bioaccumulation factor (FCA) (Equation 3), and the root bioconcentration factor (FCO) (Equation 4) and, the rate of metal extraction (MER) (Equation 5) as described by Mertens et al. (2005),

$$FT = Cu_{AP} / Cu_{R} \tag{2}$$

$$FCA = FCA = Cu_{AP} / Cu_{S} \tag{3}$$

$$FCO = Cu_{R} / Cu_{S} \tag{4}$$

$$MER = \{(Cu_T \times DM_T) / (Cu_S \times M_R)\} \times 100 \quad (5)$$

where: Cu_{AP} = Cu concentration in aerial part dry mass; Cu_R = Cu concentration in root dry mass; Cu_T = Cu concentration in the total dry mass; Cu_S = Cu content available in the soil after cultivation; DM_T = total dry mass; e M_R = mass of soil volume rooted by species.

The results were detected by analysis of variance (ANOVA) and the averages were detected by regression ($p<0.05$) with the aid of the SISVAR statistical program (Ferreira, 2014).

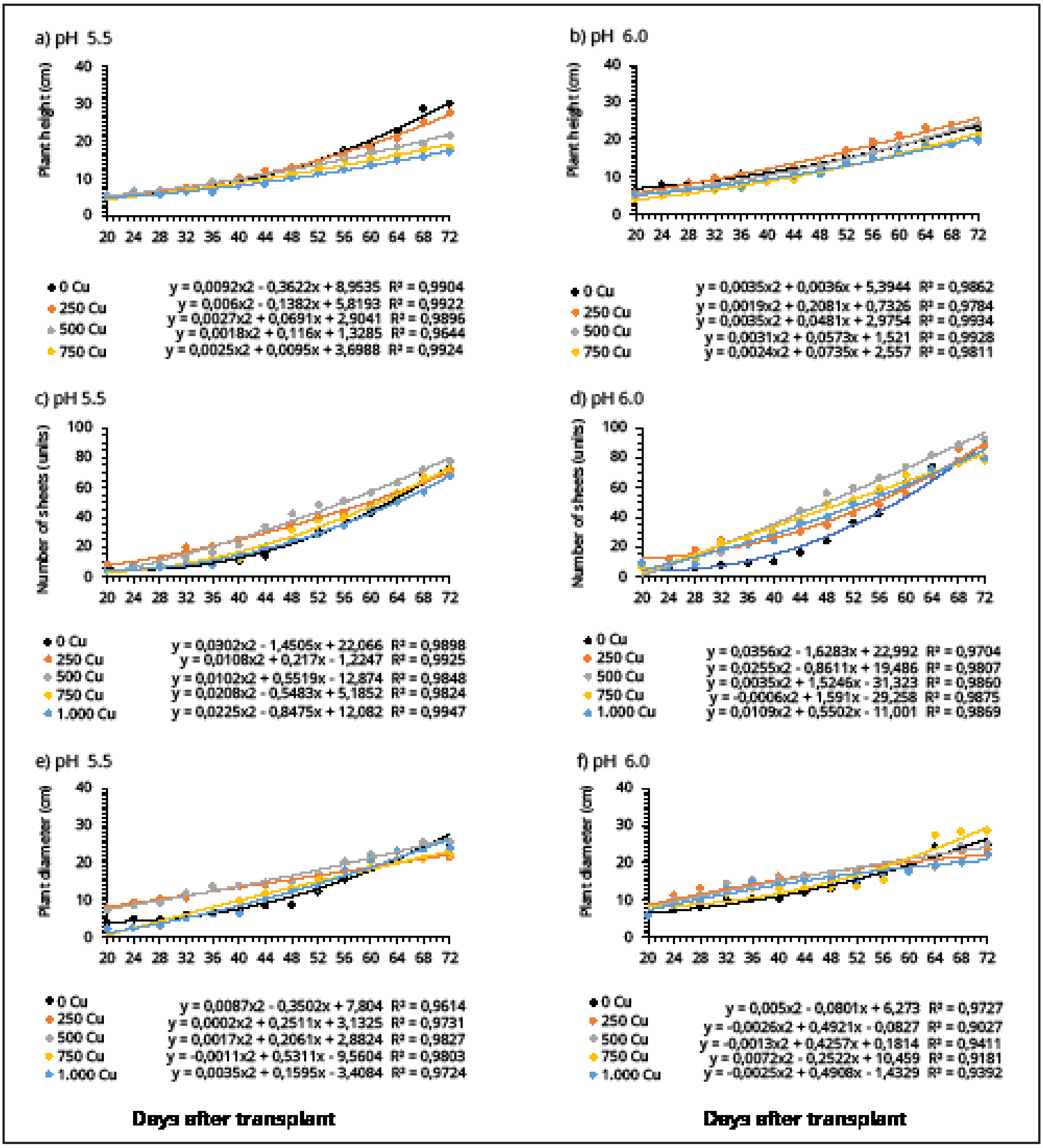
3 RESULTS AND DISCUSSION

It was verified that the evolution of the vegetative development of marigold plants cultivated in soil with different pH and added levels of Cu follow the same trend for the biometric parameters of plant height, number of leaves and plant diameter (Fig. 1). This indicates that the *tagetes patula* L. species presents cultivation tolerance to soils containing high levels of Cu (Figure 2).

Similar results were presented by Menegaes et al. (2020) for the species garden rosewood, cala-lily and chrysanthemum (*Dendranthema grandiflora* Tzevelev). However, the authors report different levels of tolerance to different levels of Cu in the soil. It was observed that the height of marigold plants was similar at the pH 6.0 correction throughout the cultivation cycle (Figures 1a and 1b). However, this similarity at the pH 5.5 correction was up to 48 DAT, when treatments without (control) and with 250 mg kg^{-1} of Cu resulted in taller plants in comparison to the other levels of Cu added to the soil.

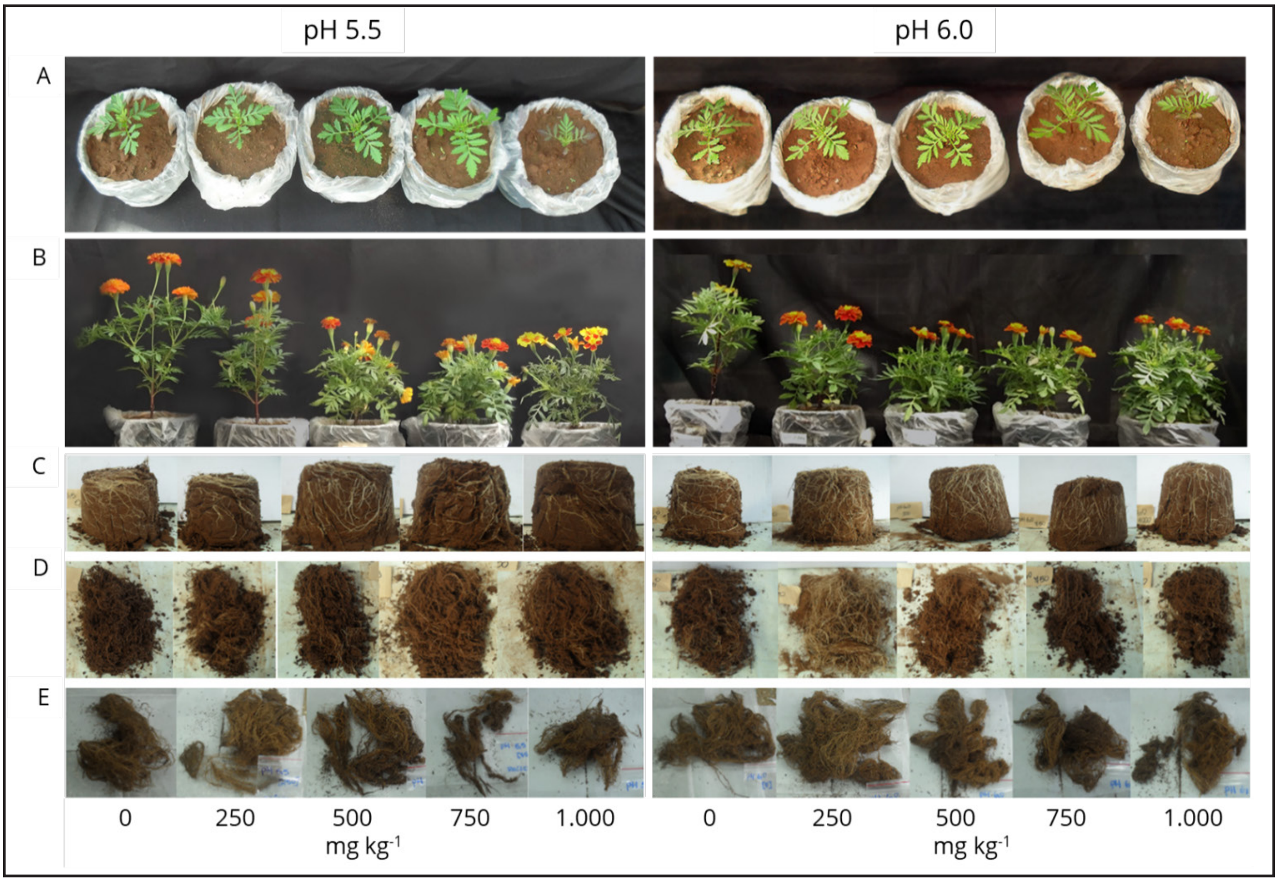
Bellé (2000) recommends the cultivation of marigold plants (*Tagetes* spp.) in soil or substrate pH above 6.3 for their full development, with an average plant height of 30 cm. Nachtigall et al. (2007) observed that the availability of Cu in the soil occurs at a pH close to 6.0. This may explain the variation in the height of marigold plants between pH 5.5 and 6.0, without (control) and with addition of Cu in the soil, in the same way that there is a compaction of these plants, ideal for ornamentation.

Figure 1 – Evolution of the development of marigold plants (*Tagetes patula* L.) cultivated in soil at pH 5.5 and 6.0 and Cu levels of 0; 250; 500; 750 and 1,000 mg kg^{-1}. Plant height (a; b), number of leaves (c; d) and plant diameter (e; f)



Source: elaborated by the authors

Figure 2 – *Tagetes patula* L. cultivated in soil at pH 5.5 and 6.0 and with added Cu content of 0; 250; 500; 750 and 1,000 mg kg^{-1}. Leaf expansion (A) at 20 days after transplanting (DAT) and flowering plants (B), whole clod (C), mass of roots without clod (D) and roots without soil (E) at 72 DAT



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In the experiment carried out by Sonmez *et al*. (2006), tomato plants (*Solanum lycopersicum* L.) were exposed to high concentrations of Cu 0; 1,000 and 2,000 mg kg^{-1}, in a soil with pH 6.5, resulting in a decrease in pl Eduarda Victória Bisognin ant height. On average, there was a reduction of 39% and 50% in plant height, for the respective treatments, 1,000 and 2,000 mg kg^{-1}, when compared to the treatment without additional Cu.

Regarding the number of leaves (Figures 1c and 1d), there was similarity between Cu concentrations for both pH corrections. From 44 DAT approximately, the amount of leaves increased, mainly in the intermediate treatments with contents of 250; 500

and 750 mg kg^{-1} of Cu. It can be assumed that this effect is a possible adaptation of the plant, since at the content of 1,000 mg kg^{-1} of Cu added to the soil there was an increase in the number of leaves from 60 DAT onwards.

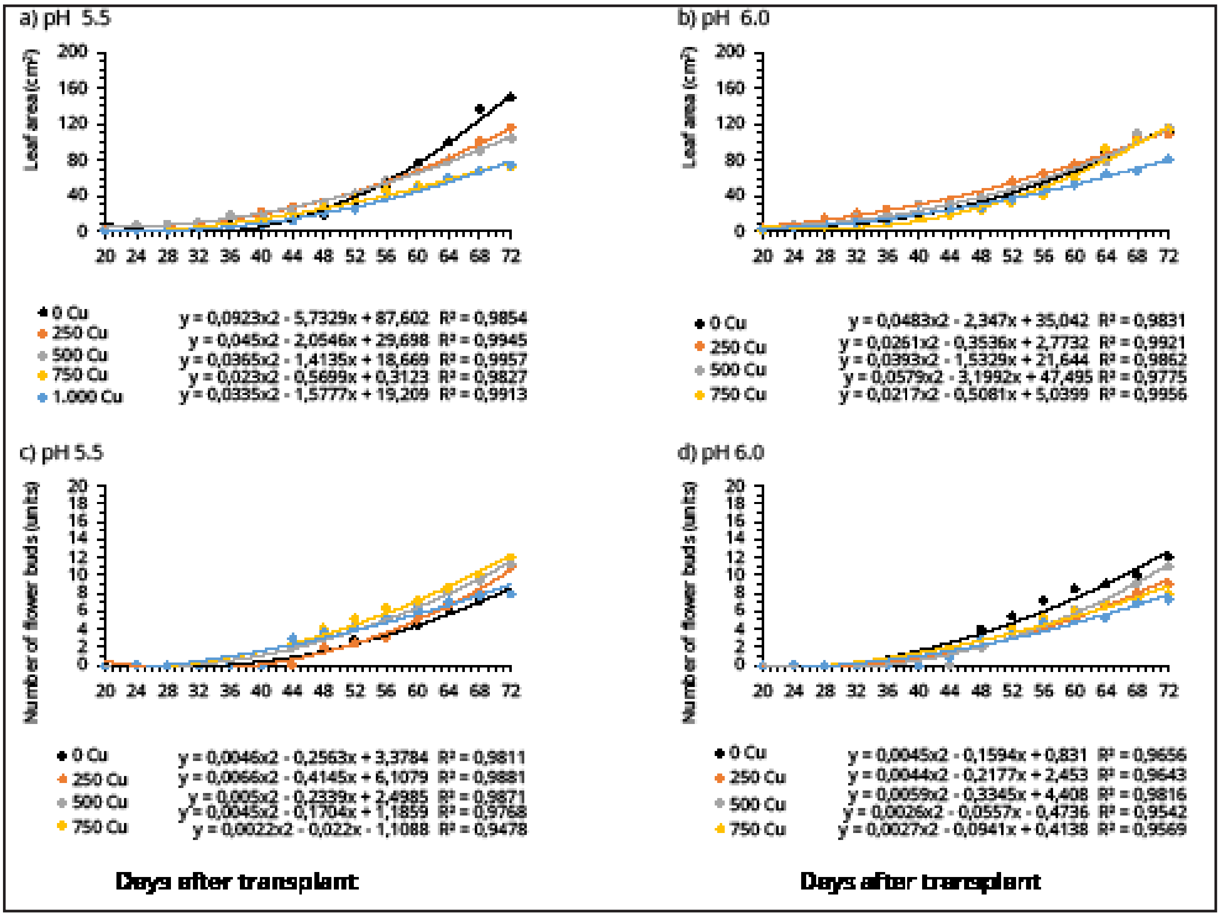
For the diameters of the plants in both pH corrections (Figures 1e and 1f), it was observed that the vegetative development did not present atypical forms, maintaining a tendency from the highest levels of Cu added in the soils. Regarding the leaf areas of *tagetes patula* L. plants (Figures 3a and 3b), it was verified that in the pH 5.5 correction, the treatment without addition of Cu (control) showed greater leaf area from 56 DAT.

The development of flower buds (Figures 3c and 3d) occurred from 44 DAT onwards in soil correction at pH 5.5. It was observed that plants with treatments of 500 and 750 mg kg^{-1} of Cu developed a greater number of floral buds when compared to the treatment without addition of Cu. For soil correction at pH 6.0, the highest number of flower buds occurred in the treatment without adding Cu, which may be explained by the low availability of Cu and pH close to the ideal standards for the cultivation of this crop.

The results of the biometric parameters of marigold plants corroborate the results of Tavares et al. (2013) on sunflower plants (*Helianthus annuus* L.), and Menegaes et al. (2020) on garden carnation, cala-lily and chrysanthemum plants, where the authors attribute the variation in vegetative development to the individual adaptation of each species to the different levels of contaminants present in the soil, in evidence of phytotoxicity, thus contributing to the tolerance of marigold plants being cultivated in soils containing high amounts of Cu, regardless of soil pH.

It was found that the vegetative and floristic development of marigold plants was satisfactory in all treatments containing different Cu contents and soil pH, without typical changes in plant plasticity (Figures 2; 3 and 4). Menegaes et al. (2019) verified the same conditions of vegetative and floristic development for wild carnation plants grown in Cu-contaminated soils.

Figure 3 – Evolution of the development of marigold plants (*Tagetes patula* L.) cultivated in soil at pH 5.5 and 6.0 and Cu levels of 0; 250; 500; 750 and 1,000 mg kg^{-1}. Leaf area (a; b) and number of flower buds (c; d)



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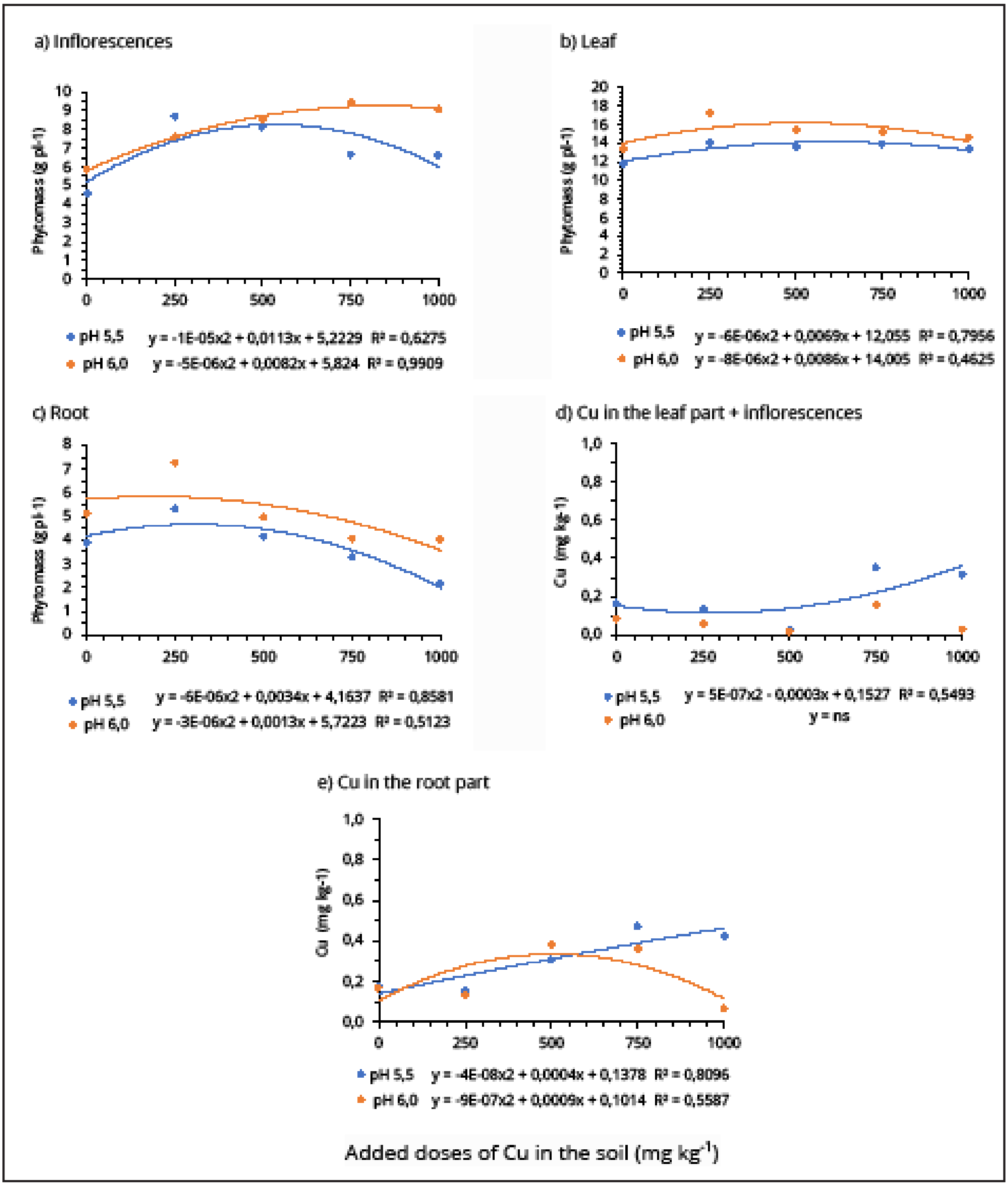
At 72 DAT, the parameters of dry mass and Cu contents contained in marigold plants were evaluated (Figure 4). It was verified that the partition of the dry phytomass was in the aerial part (inflorescences and leaves) in relation to the root part.

It was verified that the phytomass of the inflorescences was higher in the added content of 250 mg kg^{-1} of Cu at pH 5.5 and in the contents of 750 and 1,000 kg^{-1} of Cu at pH 6.0. In relation to the leaf phytomass, they remained approximate, however, root phytomass showed a decrease according to the increase of Cu contents in the soil for both soil pH corrections.

Figure 4 – Dry phytomass of inflorescences (a), aerial part (b) and root part (c); Cu content in the leaf part + inflorescences (c) and in the root (d) of marigold plants (*Tagetes patula* L.) grown in soil at pH 5.5 and 6.0 and added Cu content of 0; 250; 500; 750 and 1,000 mg kg^{-1}. ns: not significant



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In the work by Menegaes et al. (2017) on chrysanthemum cv. Dark Fiji cultivated in different levels of Cu in the soil, it was verified that the partitioning of Cu in the dry phytomass relates the concentrations of this element in the aerial part (leaves and inflorescences) and, in the roots, it was increasing according to the increase of the added Cu content, therefore, without large accumulations.

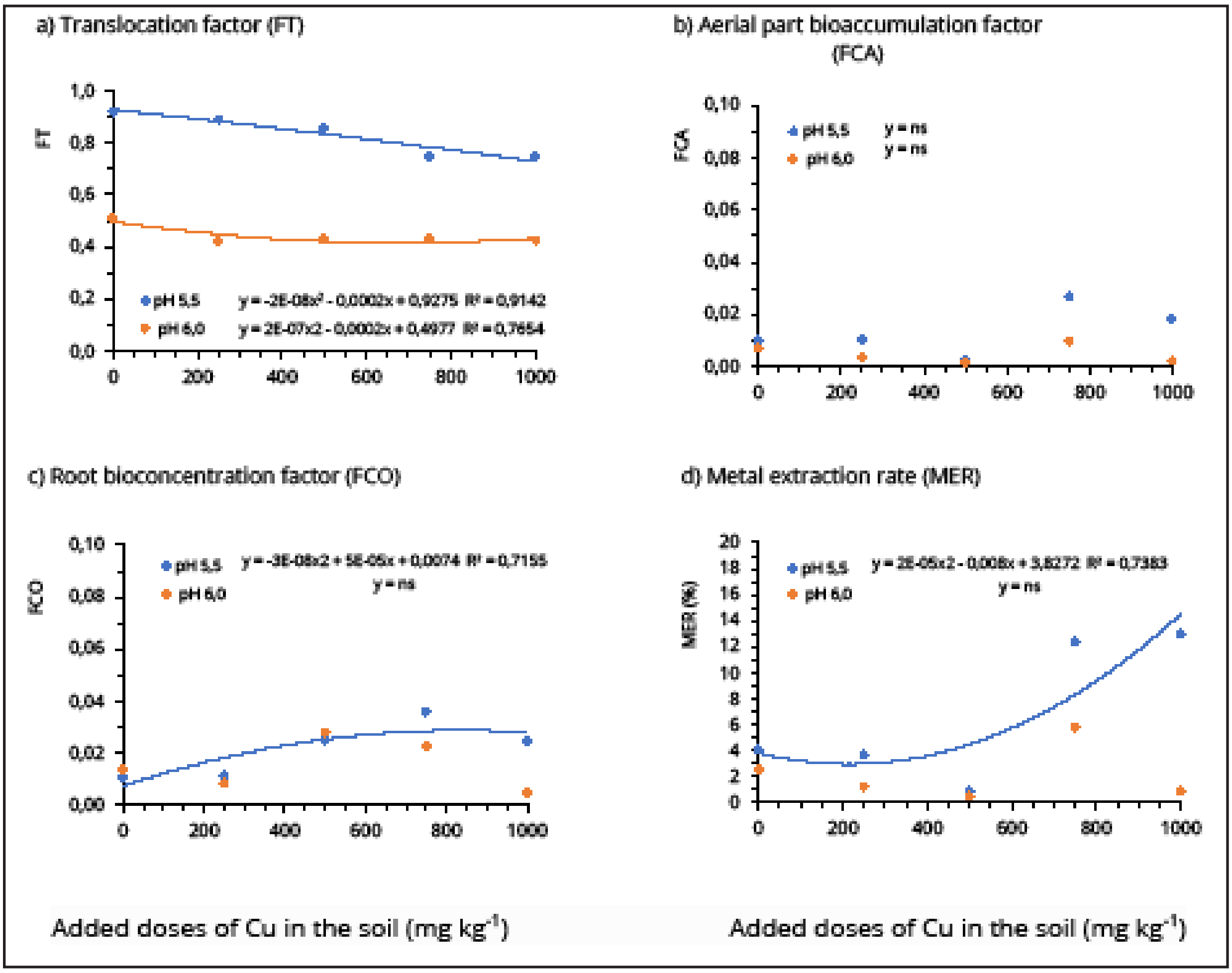
Andreazza et al. (2015) report that roots, due to direct contact with metals present in the soil, tend to accumulate them, especially Cu, resulting in reduced root length. Some plants are capable of reducing the diffusion of the cation through the interior of the tissue in order to protect it from intoxication, remaining tolerant to the excess of this element in the root-soil system. According to Marsola et al. (2005), bean plants (*Phaseolus vulgaris* L.), when exposed to high doses of Cu (0 to 5 mmol kg^{-1}), showed differences in Cu concentrations between shoots and roots, using this mechanism as a defense.

Considering the ratio between the Cu contents in the shoot and in roots, it cannot be considered that the marigold species is efficient with regard to translocation (Figure 5a). The possibility that was verified is that, as the Cu concentration increases, the translocation factor (TF) decreases.

The shoot bioaccumulation factors (FCA) were not significant between the soil pH and the Cu contents, and for the root bioconcentration factors (FCC), Figure 5c, it was observed that there was a low correlation of the data for the pH 5.5 and a non-significant relationship for pH 6.0, indicating that marigold plants are tolerant to the cultivation in soils containing excess Cu.

The tolerance can also be confirmed by the metal extraction rate (MER), which is directly related to the soil pH, in Figure 5d, it was observed that at pH 5.5 there was a higher MER compared to pH 6.0. The cultivation of *tagetes patula* L., according to Bellé (2000), is best in soil pH above 6.3, however, Oorts (2013) reports that the Cu available in the soil can be absorbed by the plant in average of 33% at pH 6.0 and 100% at pH 4.0. Menegaes et al. (2019) found a higher MER of Cu by the rosewood plants in a soil pH of 5.8 compared to the other crops. However, in the work by Menegaes *et al*. (2017) it was found that chrysanthemum plants cv. Dark Fiji were characterized as Cu-extracting plants, with low soil phytoremediation potentials.

Figure 5 – Translocation factor (FT; a), shoot bioaccumulation factor (FCA; b), root bioconcentration factor (FCO; c) and metal extraction rate (MER; d) from marigold plants (*Tagetes patula* L.) grown in soil at pH 5.5 and 6.0 and Cu content of 0; 250; 500; 750 and 1,000 mg kg^{-1}. ns: not significant



Source: elaborated by the authors

The authors Marques et al. (2011), Mackie et al. (2012) and Menegaes et al. (2019; 2020), highlight the importance of studying plants that are tolerant to excess Cu in the soil, especially in areas where vines are cultivated, with cover crops intended to help control erosion and improve soil fertility. Thus, the use of ornamental cover crops becomes an alternative for growing areas contaminated with Cu, providing low cost and low environmental impact, while beautifying it.

CONCLUSION

The Cu contents added to the soil affected the aesthetic quality of the plants. The higher the Cu dosage, the lower the plant development of marigold plants was in both soil pH corrections. The *Tagetes patula* L. species is tolerant to cultivation in soil with excess Cu and is characterized as a Cu extracting plant, with low soil phytoremediation potential.

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