

Copper phytotoxicity in agricultural crops cultivated in tropical soils

Fitotoxicidade do cobre em culturas agrícolas cultivadas em solos tropicais

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Highlights:

Cu is more toxic to plant species in Typic Hapludox.

Rice is the species most sensitive to Cu in soils.

It is expected that soils with 60 mg kg⁻¹ of Cu may keep the development of plants.

Abstract

Copper is a micronutrient for living organisms, but at high concentrations it may cause several deleterious effects. The objective of this study was to evaluate the behavior of agricultural crops to different concentrations of Cu in the soil. The species *Triticum aestivum* (wheat), *Zea mays* (maize), *Oryza sativa* (rice), *Phaseolus vulgaris* (dry bean), *Glycine max* (soybean), and *Raphanus sativus* (radish) were cultivated in the soils Typic Hapludox (TyHpx) and Rhodic Acrudox (RhAcx). The experiment was carried out in a greenhouse and the soils were set in pots in a completely randomized design, with four replicates per treatment adding the following Cu concentrations (as Cu-nitrate): 0, 75, 150, 300, 600, 800, 1000, 1200 mg kg⁻¹ dry soil. Measurements of biological variables, such as shoot dry weight (SDW), plant height, stem diameter, and number of leaves were carried out, in addition to determining Cu content in the SDW. At the TyHpx, a decreasing order of tolerance to Cu phytotoxicity was: dry bean, maize, soybean, wheat, radish, and rice. However, at the RhAcx, the decreasing order was: maize, soybean, radish, wheat, dry bean, and rice. The species that revealed the highest sensitivity to high Cu concentrations in soil was rice and that with the lowest sensitivity was maize.

Key words: Copper nitrate. Ecotoxicology. Micronutrient. Contamination.

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Resumo

O cobre é um micronutriente para organismos vivos, mas em altas concentrações pode causar vários efeitos deletérios. O objetivo deste estudo foi avaliar o comportamento de culturas agrícolas em diferentes concentrações de Cu no solo. As espécies *Triticum aestivum* (trigo), *Zea mays* (milho), *Oryza sativa* (arroz), *Phaseolus vulgaris* (feijão), *Glycine max* (soja) e *Raphanus sativus* (rabanete) foram cultivadas nos solos Latossolo Vermelho Amarelo distrófico típico (LVAd) e Latossolo Vermelho distroférico típico (LVdf). O experimento foi realizado em casa de vegetação e os solos foram colocados em vasos, em delineamento inteiramente casualizado, com quatro repetições por tratamento, adicionando às seguintes concentrações de Cu (como nitrato de Cu): 0, 75, 150, 300, 600, 800, 1000, 1200 mg kg⁻¹ de solo seco. Foram realizadas medidas das variáveis biológicas matéria seca da parte aérea (MSPA), altura da planta, diâmetro do caule e número de folhas, além disso foi determinado o teor de Cu na MSPA. No LVAd, a ordem decrescente de tolerância à fitotoxicidade do Cu foi: feijão, milho, soja, trigo, rabanete e arroz. No LVdf, a ordem decrescente foi: milho, soja, rabanete, trigo, feijão e arroz. A espécie que revelou maior sensibilidade às altas concentrações de Cu no solo foi o arroz e a que apresentou menor sensibilidade foi o milho.

Palavras-chave: Nitrato de cobre. Ecotoxicidade. Micronutriente. Contaminação.

Introduction

Soil protection, that is, to protect its diversity and ecological functions, has become an objective of environmental agencies around the world (Bone et al., 2010). Predict the possible consequences that a particular contaminant may cause in the environment is a need for decision making in contaminated sites management (Niva et al., 2016). The evaluation of ecotoxicological risks is based on a complex process of collecting, organizing, and analyzing environmental data to estimate adverse effects caused by contamination (Caetano, Marques, Gonçalves, Silva, & Pereira, 2016).

The relations between the contaminant source, exposure, and effects on organisms are complex and often specific to a particular location, since the environmental conditions and affected organisms are also specific. Therefore, ecotoxicological risk assessment should be site-specific to provide reliable information for decision-making (Jensen & Mesman, 2006). In such studies, the effects of varying concentrations of a substance on the biological variables of living organisms are evaluated. The results can be shown in calculated ecotoxicity parameters or indexes, such as the NOAEL (no observed adverse effect level),

LOAEL (lowest observed adverse effect level), EC_x (concentration that reduces *x* % compared to the control treatment), and HC_y (hazardous concentration to *y* % of the species) (Caetano et al., 2016; Verbruggen, Posthumus, & Van Wezel, 2001).

Successive agricultural management practices, such as soil fertilization and liming, and application of agricultural pesticides during the crop cycle, alongside the long period of cultivation in certain areas, may result in large amounts of metals added to soils, such as Cu. Copper is a micronutrient for plants acting on assorted metabolic processes, as member of several enzymes that catalyze the electron flow and oxidation reactions in plant cells (Yruela, 2005). However, at high concentrations, Cu in plants can cause disturbances in vegetative cells, such as inducing oxidative stress, which is determined by reactive oxygen species (ROS) formation and reduction in nutrient absorption, promoting considerable losses in productivity (Mantovani, 2009; Santos, Melo, Luz, & Tomasi, 2004; Toselli et al., 2008).

Some studies in Brazil have confirmed contamination with Cu in sites with intensive long-term agriculture and the contamination of soils with Cu-based pesticides has become a concern. Girotto

et al. (2016) observed that vineyards in the South of Brazil, had Cu contents above 400 mg kg⁻¹ in the soil after 30 years of cultivation, significantly affecting the physiology of black oat. Some studies were conducted in order to reduce soil Cu availability in Brazilian vineyards. Ferreira et al. (2018) studied the application of limestone (3 Mg ha⁻¹), Ca-silicate (3 Mg ha⁻¹), and vermicompost (30 g C kg⁻¹ of dry soil) to the soil to reduce Cu toxicity. They observed vermicompost was more efficient in reducing toxicity of Cu to the plants, since Cu is easily complexed with soil organic matter (SOM). High Cu concentrations in soils were also reported in areas with mango (*Mangifera indica*) trees, cultivated for more than 26 years in Pernambuco State (Silva, Nascimento, Biondi & Cunha, 2012), and with melon (*Cucumis melo*), cultivated for two years in Rio Grande do Norte State (Mendes, Duda, Nascimento, Lima, & Medeiros, 2010). In both cases, Cu contents were higher than the prevention value (60 mg kg⁻¹) allowed by the Brazilian legislation (Conselho Nacional do Meio Ambiente [CONAMA], 2009) for Cu in soils.

Soil characteristics, such as texture and SOM, directly affect Cu mobility in soils. Gharbi, Rejeb, Ghorbal and Morel (2005) observed greater transfer of Cu from soil to plant in a sandy loam than in a clay loam. These authors also found a difference in Cu toxicity between the species *Lactuca sativa* (lettuce) and *Spinacia oleracea* (spinach), with the latter being more affected by Cu. Copper accumulation also differed between plant organs, for instance, lettuce accumulated nearly 60 to 80% of the Cu in its roots (Gharbi et al., 2005). Cavani, Manici, Caputo, Peruzzi and Ciavatta (2016) reported alterations in the composition of bacterial communities, as well as decrease in biomass and microbial activities of the soil in Italian vineyards contaminated by the excessive use of cupric products. Lejon, Pascault and Ranjard (2010) also observed that Cu reduced bacterial diversity in soils with low organic matter content, but with the increase of SOM, there was a decrease in this deleterious effect. With the increase

of SOM there is the favoring of the formation of chelating agents in the soil, which complex the Cu, restricting its bioavailability (Ambrosini et al., 2015). Therefore, establishing relationships between soil characteristics and concentrations of Cu that can affect plants is extremely important for the most appropriate agricultural management.

This study aimed to verify the effect of Cu-nitrate in six crops: maize, rice, wheat, soybean, dry bean, and radish cultivated in two tropical soils. We also aimed to study how Cu-contaminated soils are able to influence growth variables for the studied species and the extension of Cu transfer to shoots.

Material and Methods

Ecotoxicology tests

The phytotoxicity tests were performed according to ISO 11.269-2 (International Organizations for Standardization [ISO], 2012). This standard protocol aims not to reproduce field situations, but rather to assess what happens at a worst-case contamination condition. Three monocotyledons were selected: *Triticum aestivum* cv. BRS 264 (wheat), *Zea mays* cv. RB 9004 PRO 2 (maize), and *Oryza sativa* cv. BRS Esmeralda (rice); and also three eudicotyledons: *Phaseolus vulgaris* L. cv. Madrepérola (dry bean), *Glycine max* cv. BRS 284 (soybean), and *Raphanus sativus* cv. Crimson Gigante (radish). These plants are widely cultivated throughout the world and the cultivars used in this study are commercial varieties found in Brazil.

Each experimental unit was made of a 500 cm³ pot filled with soil samples taken from the 0-20 cm depth of commonly found (in wet tropical zones of the Earth) soil types: a Typic Hapludox (TyHpx) (21°13'30" S and 44°57'38" W) and a Rhodic Acrudox (RhAcx) (21° 09' 18" S and 45° 05' 55" W) (Table 1) (Soil Survey Staff, 2014). Both soil samples were collected at the Lavras County, Minas Gerais State, Brazil. The soil samples were taken under natural vegetation (semideciduous tropical

rainforest), had low Cu content, and had not undergone visible major human impact.

The soil samples were ground, air dried, and successively sieved through 5 and 2 mm meshes. The samples were subsequently characterized and used in phytotoxicity tests. The particle size analysis was performed by the pipette method (Almeida et

al., 2012). The chemical characterization of soils was performed according to the methods proposed by Teixeira, Donagemma, Fontana and Teixeira (2017). Copper was determined according to United States Environmental Protection Agency [USEPA] (2007), method 3051A. The soil physical and chemical characteristics are presented in Table 1.

Table 1
Chemical and physical properties of the Typic Hapludox (TyHpx) and Rhodic Acrudox (RhAcx) soils (0-0.2 m) used in this study

Soils	Properties								
	pH ^a	Al	H + Al	CEC ^b at pH 7.0	O.M. ^c	Clay	Silt	Sand	Cu
		----- cmol _c dm ⁻³ -----				----- g kg ⁻¹ -----			mg kg ⁻¹
TyHpx	4.9	0.4	3.9	6.1	21.1	310	110	580	28.1
RhAcx	4.4	1.2	11.1	11.7	39.9	680	130	190	9.6

^a pH in water 1:2.5 soil:solution; ^b Cation exchange capacity; ^c Soil organic matter.

Fertilization was performed according to the critical level required for soils (Alvarez & Ribeiro, 1999) and minimum levels required for fertilization in pots (Malavolta, 1981). Watering was made throughout the experiment to keep water content at 60% of the total soil pore volume. Seventy-two hours after fertilization, different Cu concentrations were added to the soil (0, 75, 150, 300, 600, 800, 1000, and 1200 mg kg⁻¹) by different aliquots of a Cu(NO₃)₂ solution, following the guidelines of ISO 11.269-2 (ISO, 2012). Soils were then homogenized and incubated for 24 hours before sowing as suggested by ISO 11.269-2 (ISO, 2012). Ten seeds were sown per pot. After seedling emergence, the number of plants was reduced to six. The experiment lasted 21 days, after emergence of 50% of the control treatment.

Evaluation of biological variables

Seedling emergence was evaluated as suggested by Maguire (1962) and at the end of the experiment

the following vegetative growth variables were measured: shoot height (determined by graded rule), stem diameter (measured with digital caliper), number of leaves, and shoot dry weight (determined on a precision scale, after 70°C in a forced circulation oven until constant weight).

Copper concentration in shoots

The Cu concentration in plant material was determined according to USEPA (2007) method 3051A. Samples of approximately 0.5 g of dried plant material were milled in Wiley mill, then placed with 5.0 mL of concentrated HNO₃ into Teflon[®] vessels that were hermetically sealed and heated in a Mars-5 microwave digestion oven (CEM Corp, Matthews, NC) for 10 minutes. The still sealed vessels were removed from the oven and cooled to room temperature under a fume hood. After opening the vessels, the volume was added up to 10 mL with bidistilled water. The Cu concentration was determined in a graphite furnace atomic absorption

spectrophotometer. In each batch of material analyzed, one reference sample, with known and certified Cu content (NIST SRM 1573a - tomato leaves), was analyzed, as well as a blank sample, in order to ensure quality control and to calculate the method detection limit (MDL) (Penha et al., 2017).

Statistical analysis

For statistical purposes, each experiment consisted of one soil type and one plant species. The pots were in a completely randomized design, with four replicates per treatment. Biological variables were submitted to variance analysis ($p \leq 0.05$), followed by regression analysis ($p \leq 0.05$). Normality and variance homogeneity were checked by Kolmogorov-Smirnov and Bartlett tests, respectively. The NOAEL (no observed adverse effect level) and LOAEL (lowest observed adverse effect level) values were determined by the Dunnett test ($p \leq 0.05$).

EC_x values (concentration that reduces x % compared to the control treatment) and their 95% confidence limits were calculated for the following variables: emergence, plant height, stem diameter, and shoot dry weight using the non-linear regression models: Gompertz, Hormesis, and Logistic, inserted in the software Statistica, version 7.0. (Statsoft, 2004). The model that revealed the best determination coefficient (R^2) at 95% significance was used. These statistical models were used only for those data sets characterized by decreasing trends of values upon Cu addition (Environment Canada [EC], 2005; Rocheleau et al., 2010). The EC_x values of shoot dry weight (SDW) were used for calculating the HC_5 values (hazardous concentration to 5% of the species) through species sensitivity distribution (SSD) curves fit by ETX 2.0 software (Van Vlaardingen, Traas, Wintersen, & Aldenberg, 2004) at 95% significance.

Results and Discussion

All tested species were sensitive to Cu, since there was a reduction in the tested variables (emergence, height, stem diameter, number of leaves, and SDW) for both soils (Table 2), particularly for emergence and SDW (Figures 1 and 2). However, the sensitivity of each species was rather different due to morphophysiological characteristics of each species, such as cell wall thickness, root structure type and enzymatic defense mechanisms (Yruela, 2005).

In general, the emergence of monocotyledons showed a significant reduction after addition of 75 mg kg^{-1} of Cu (Table 2). For eudicotyledons, a significant reduction was observed after addition of 300 mg kg^{-1} of Cu (Table 2). According to Luchese, Gonçalves, Luchese and Braccini (2004), there is a decrease in the emergence of maize, a monocotyledon, as the concentration of Cu in soil increases. Singh, Nath and Sharma (2007) observed that Cu at high concentrations reduces the emergence and plant growth mainly due to inhibiting and reducing the activity of amylase, the enzyme responsible for the metabolization of starch present in the seed reserves used to generate energy for the germination process. In spite of the deleterious effects of Cu concentrations in soils, the eudicotyledons presented higher resistance to Cu at lower concentrations, $<300 \text{ mg kg}^{-1}$. Eudicotyledons are known for having seeds with the high starch reserves (Li, Li, Dunwell, & Zhang, 2012). On the other hand, according to Caetano et al. (2016), monocotyledons were less sensitive to concentrations of Cu in the soil (maximum concentration ranging from 497 to $951 \text{ mg Cu per kg of soil}$). The authors listed the species they studied in a decreasing order of Cu sensitivity: *Lactuca sativa* > *Lycopersicon esculentum* > *Zea mays* > *Avena sativa*. The lowest sensitivity of the monocotyledons presented by Caetano et al. (2016) does not match the results of the present study, possibly due to soil or varietal differences.

Table 2

NOAEL (no observed adverse effect level) and LOAEL (lowest observed adverse effect level) values according to phytotoxicological tests on maize, rice, wheat, soybean, radish, and dry bean in Typic Hapludox (TyHpx) and Rhodic Acrudox (RhAcx) with increasing concentrations of Cu. This table only shows data that followed normal distribution and were significant at 5% probability for Dunnett's test

Soils	Species	Parameter	Shoot dry weight	Plant height	Leaves	Seed emergence	Stem diameter
			----- mg kg ⁻¹ -----				
RhAcx	Maize	NOAEL	300	NV ^a	NV	150	NV
		LOAEL	600	NV	NV	300	NV
	Rice	NOAEL	75	75	75	0	150
		LOAEL	150	150	150	75	300
	Wheat	NOAEL	150	150	0	300	300
		LOAEL	300	300	75	600	600
	Soybean	NOAEL	300	150	150	600	NV
		LOAEL	600	300	300	800	NV
	Radish	NOAEL	150	600	300	300	600
		LOAEL	300	800	600	600	800
	Dry bean	NOAEL	300	800	NV	150	800
		LOAEL	600	1000	NV	300	1000
TyHpx	Maize	NOAEL	300	NV	NV	0	NV
		LOAEL	600	NV	NV	75	NV
	Rice	NOAEL	NV	0	75	150	75
		LOAEL	NV	75	150	300	150
	Wheat	NOAEL	NV	300	NV	150	300
		LOAEL	NV	600	NV	300	600
	Soybean	NOAEL	0	150	150	150	150
		LOAEL	75	300	300	300	300
	Radish	NOAEL	NV	NV	NV	150	NV
		LOAEL	NV	NV	NV	300	NV
	Dry bean	NOAEL	300	800	NV	800	800
		LOAEL	600	1000	NV	1000	1000

^a NV - Data not validated by ANOVA and Dunnett's test ($p < 0.05$).

The SDW was sensitive to increases of Cu concentrations in the two soil types and in all species evaluated. A significant reduction in the SDW of the monocotyledons grown in TyHpx is observed from the concentration of 300 mg kg⁻¹ (Figure 2), while the plants cultivated in RhAcx showed sensitivity

at concentrations higher than 400 mg kg⁻¹ (Figure 2). Regarding SDW, it was observed, just as in seed emergence, that eudicotyledons were more tolerant to Cu than the monocotyledons, particularly at higher concentrations.

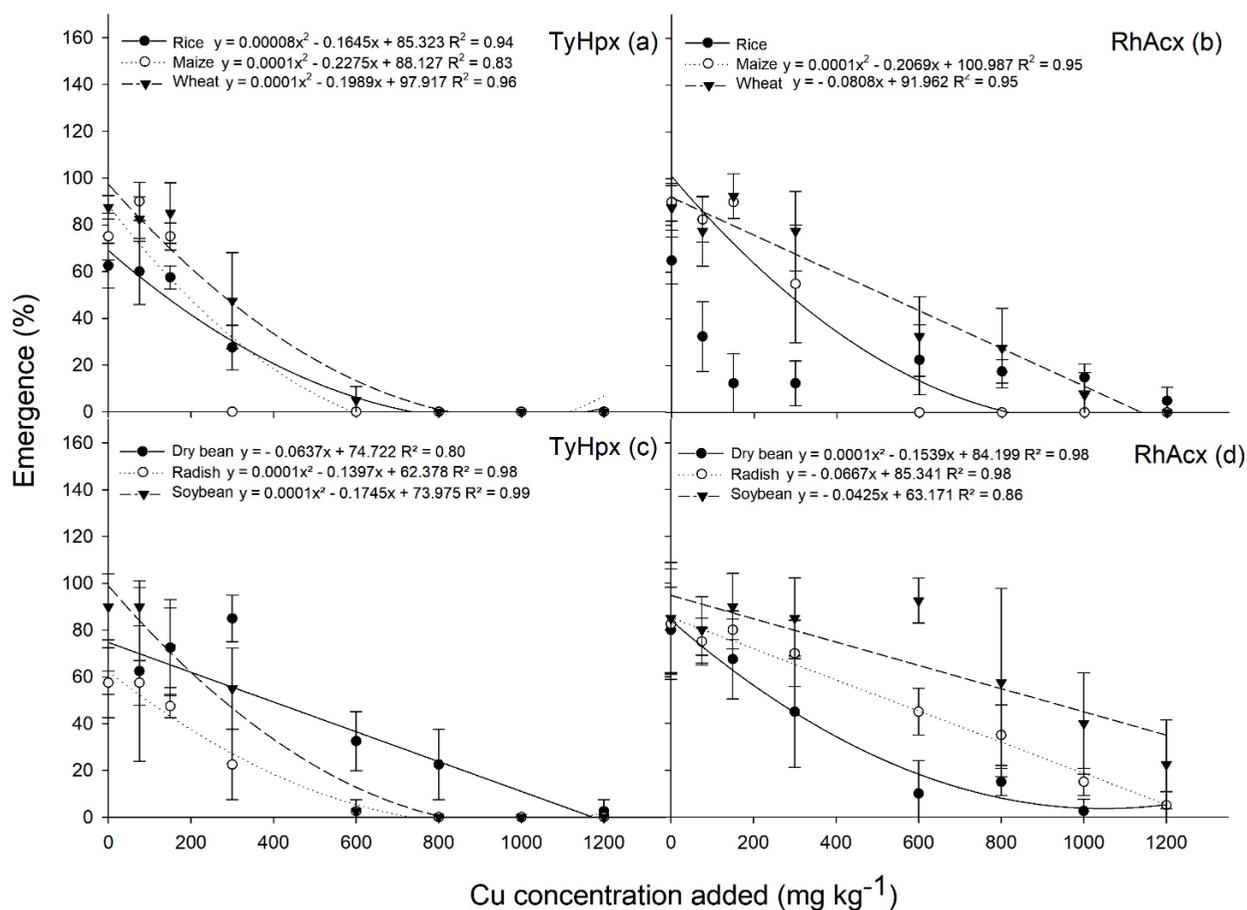


Figure 1. Seed emergence for rice, maize, wheat, dry bean, radish, and soybean after cultivation with increasing concentrations of Cu in Typic Hapludox (TyHpx) and Rhodic Acrudox (RhAcx). Vertical lines represent standard errors where they exceed the height of symbols. Points without error bars have standard errors smaller than the symbols.

Other vegetative growth variables, in addition to seed emergence and SDW, are recommended by ISO 11.269-2 (ISO, 2012) for ecotoxicological studies. Thus, evaluation of plant height and stem diameter was performed. Shoot height reduced with increasing Cu concentrations in both soil types. At lower concentrations of Cu, the species were not negatively affected (Table 2), because of the Cu being a plant nutrient with beneficial effects at lower concentrations. However, despite Cu being a plant nutrient, when in high concentrations, there are deleterious effects of the plants, as we observed in the monocotyledons (after addition of 75 mg kg⁻¹) and in the eudicotyledons (after addition of 300 mg kg⁻¹).

Higher concentrations of Cu (Figure 3) in plant tissues were observed from the concentration of 300 mg kg⁻¹ upwards. The species cultivated in TyHpx showed higher accumulation (Figure 4) even when growing less. The Cu content in SDW for some species almost doubled when comparing TyHpx with RhAcx. This effect is probably related to lower levels of clay and organic matter in the former. Clay and organic matter content are crucial for the soil buffering capacity in relation to metals (Nascimento & Fontes, 2004). Higher of organic matter, high CEC and high pH reduce the phytotoxic effect of Cu in soil (Li, Zhang, Ma, & McLaughlin, 2011; Cavani et al., 2016). But, beyond the soil's characteristics, plants can actively change their biochemical systems.

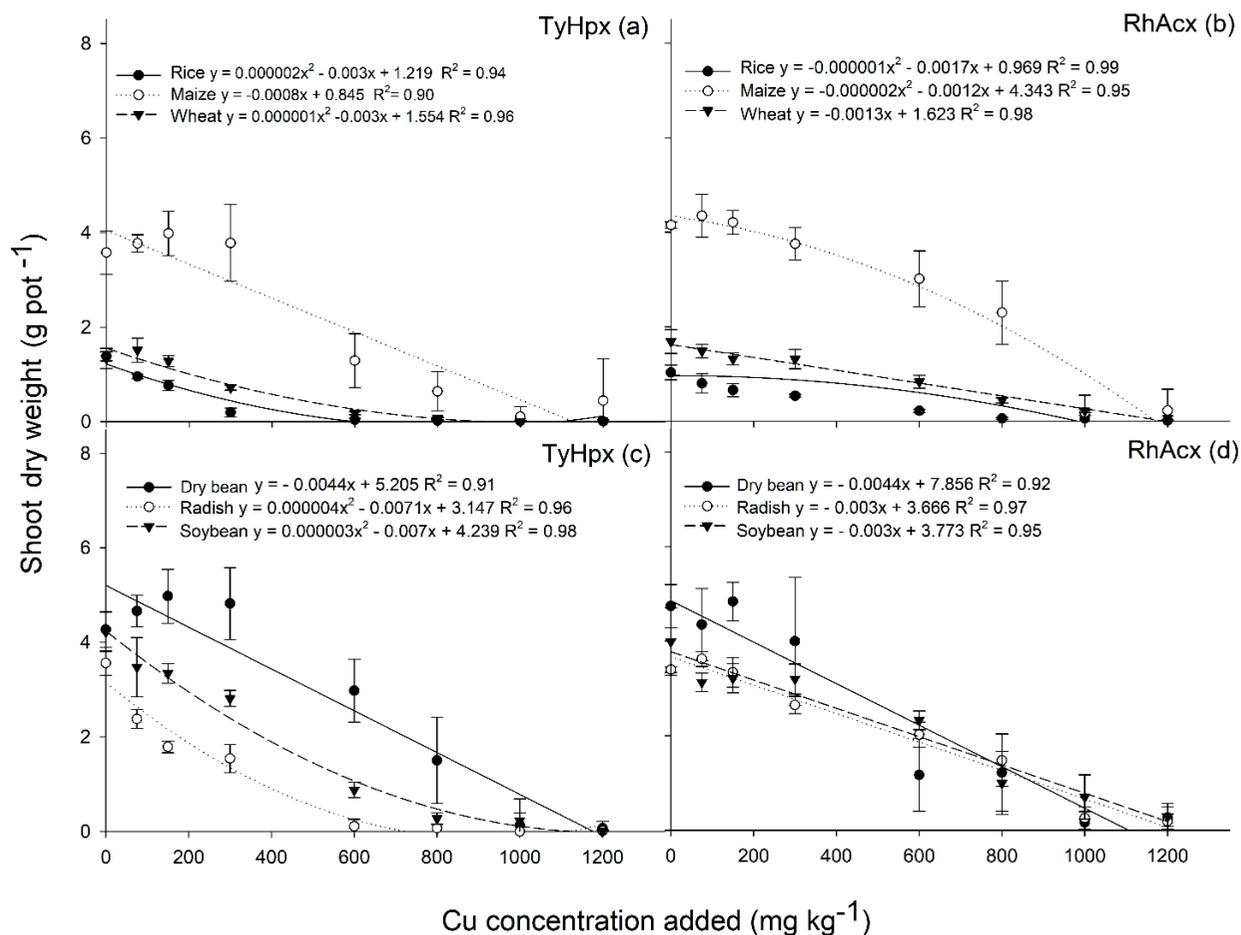


Figure 2. Shoot dry weight production for rice, maize, wheat, dry bean, radish, and soybean after cultivation with increasing concentrations of Cu in Typic Hapludox (TyHpx) and Rhodic Acrudox (RhAcx). Vertical lines represent standard errors where they exceed the height of symbols. Points without error bars have standard errors smaller than the symbols.

According to Guo, Zuo, Wang, Li and Ma (2010), Cu is a low mobile element in maize plants and Cu contents were found by them in the following order: leaf > stem > grains. The low mobility of Cu in plants led those authors to state that the presence of Cu in the soils they studied was not so worrying, because they demonstrated that, even under high application rates of Cu in soil (as CuCl₂ - 800 and 3,200 mg kg⁻¹). In our study, not all plant species could keep Cu concentrations in reduced levels in their tissues. According to Kabata-Pendias and Szeke (2015), doses of 20 and 100 mg kg⁻¹ in SDW are already

considered toxic for some species like citrus and legumes. Fatnassi, Chiboub, Saadani, Jebara and Jebara (2015) observed that *Vicia faba* plants grown in solution with 1 mM Cu responded metabolically with enzyme activation linked to the antioxidant activity of superoxide dismutase, catalase and ascorbate peroxidase, which increased Cu tolerance since the increase in the concentration of this metal may result in considerable losses in plant growth and production potential, with reduction in chlorophyll production and nitrate reduction (Luna, Casano, & Trippi, 1997).

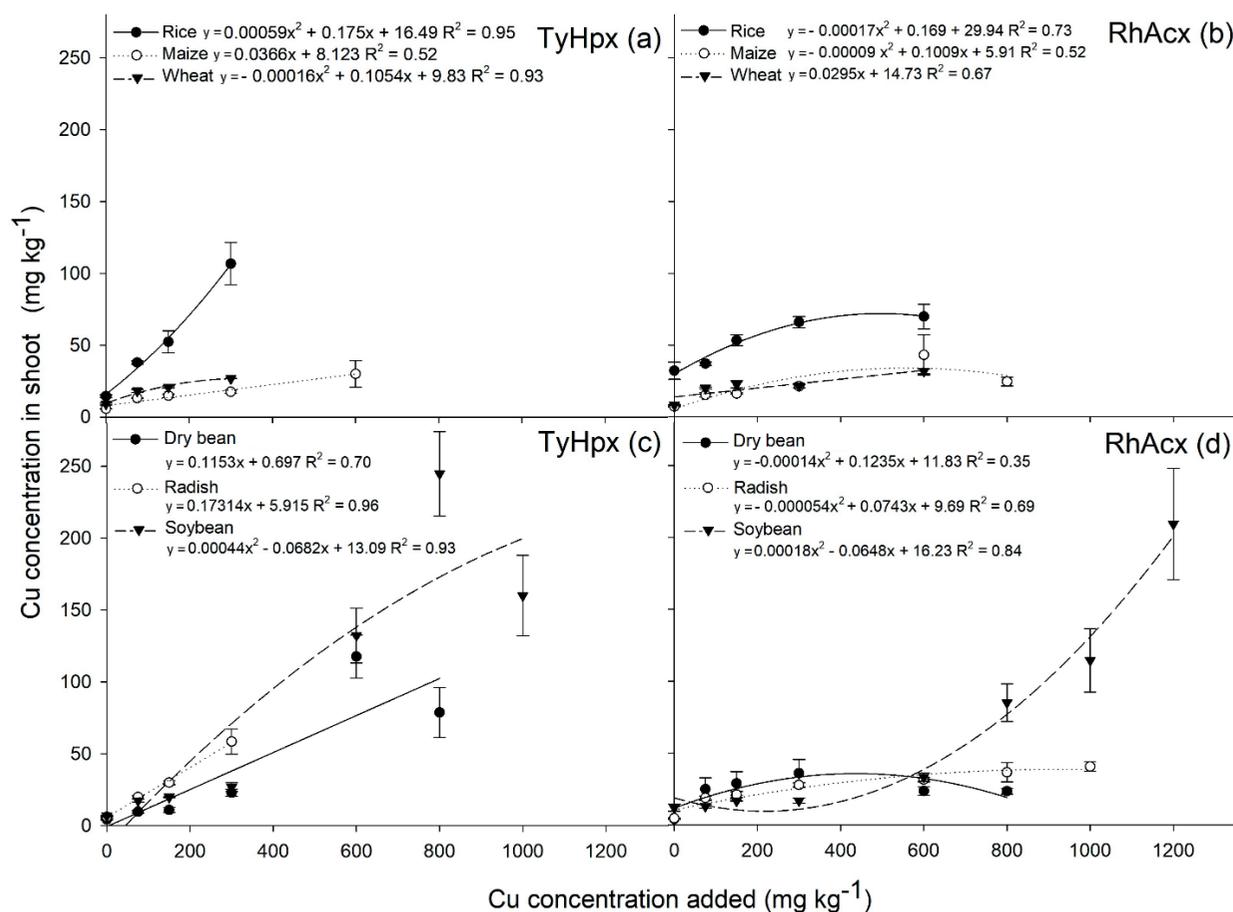


Figure 3 Copper concentration in plants (above ground tissue) of rice, maize, wheat, dry bean, radish, and soybean after cultivation with increasing concentrations of Cu in Typic Hapludox (TyHpx) and Rhodic Acrudox (RhAcx). Vertical lines represent standard errors where they exceed the height of symbols. Points without error bars have standard errors smaller than the symbols.

The values of NOAEL and LOAEL are shown in Table 2 and may be used in further ecotoxicological studies. However, some authors suggest that the evaluation of ecotoxicology based on NOAEL and LOAEL is not the best approach because these values are largely dependent on the concentrations used in each experiment, by the variability of data, by the level of significance, and by the sample size (Warne & Van Dam, 2008).

The values of EC₅₀, EC₂₀, and EC₁₀ varied widely between soils and plant species (Table 3), as they determine a specific concentration of effect and are not based on nominal treatment values. These fractions (50, 20 and 10% effect) were selected

because they are commonly used in ecotoxicology studies (Caetano et al., 2016; Hale et al., 2017). For the TyHpx, a decreasing sequence of tolerance to Cu phytotoxicity, evaluated by EC₅₀ for SDW, was: dry bean, maize, soybean, wheat, radish, and rice. However, at the RhAcx, the decreasing sequence was: maize, soybean, radish, wheat, dry bean, and rice (Figure 5). Based on our data, the most sensitive species to Cu was rice and the least were maize and soybean, with one exception observed for emergence in TyHpx. Xu et al. (2006) reported that rice is strongly affected by Cu in soils. According to them, yield was reduced about 10% at 100 mg kg⁻¹ of Cu; about 50% at 300-500 mg kg⁻¹;

and about 90% at a Cu concentration of 1000 mg kg⁻¹ applied to soil. Similar results were found in the present study, as the EC₅₀ (for SDW) was found to be 270 mg kg⁻¹ of Cu. It is noteworthy that different sensitivity rates for the same species can be related

to different cultivars, since Fageria (2007) found that rice was the most resistant species among soybean, wheat, common bean and corn, being able to show symptoms of toxicity only after 51 mg kg⁻¹ of Cu in soils, but a different cultivar was used.

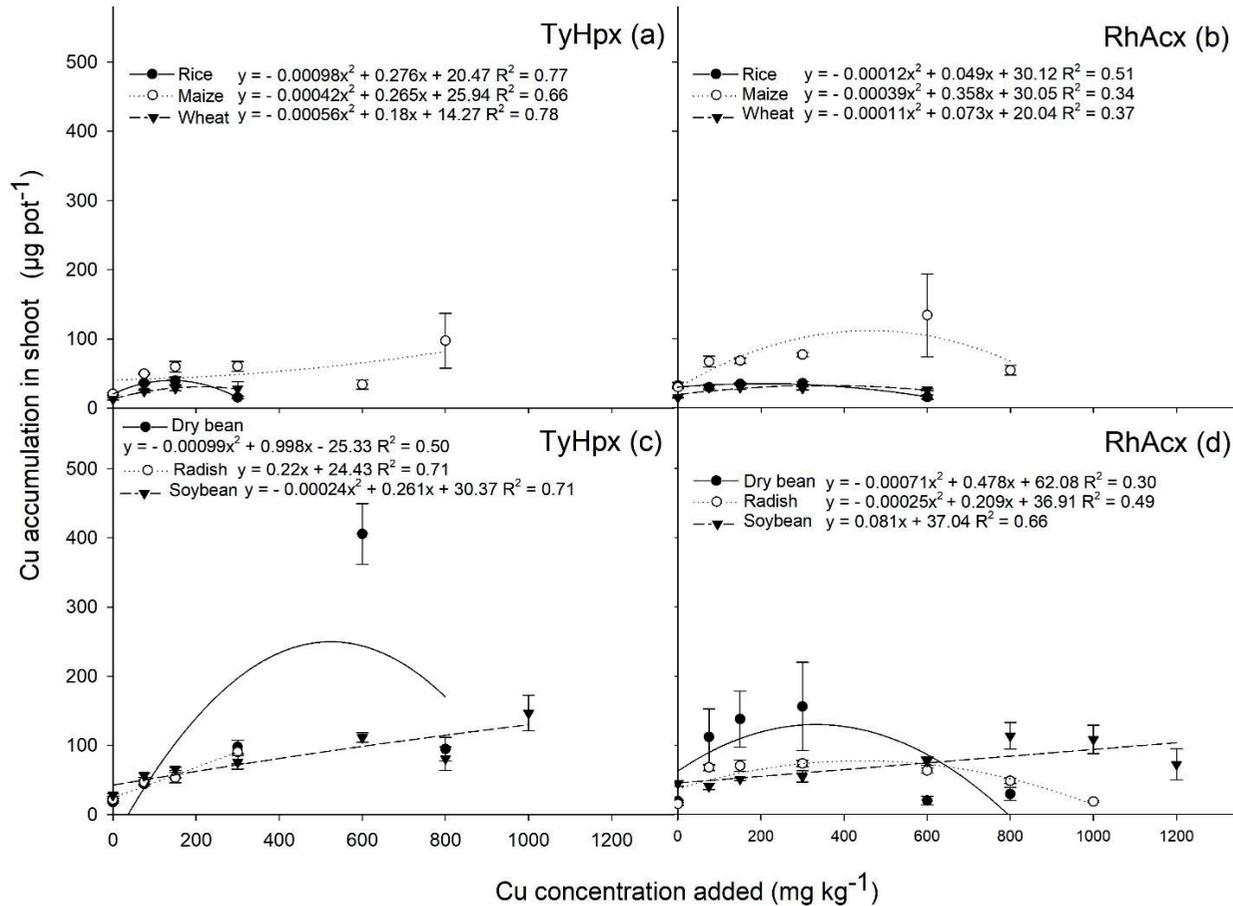


Figure 4 Copper accumulation in above ground plant material of rice, maize, wheat, dry bean, radish, and soybean after cultivation with increasing concentrations of Cu in Typic Hapludox (TyHpx) and Rhodic Acrudox (RhAcx). Vertical lines represent standard errors where they exceed the height of symbols. Points without error bars have standard errors smaller than the symbols.

Table 3

EC_{50} , EC_{20} , and EC_{10} (concentrations that reduces 50, 20, and 10% of the variable compared to the control treatment) values according to phytotoxicological tests on maize, rice, wheat, soybean, radish, and dry bean cultivated in Typic Hapludox (TyHpx) and Rhodic Acrudox (RhAcx) with increasing concentrations of Cu. This table shows data that followed normal distribution and were significant at 5% probability for non-linear regressions, \pm standard deviation of the means

Variable	Species	RhAcx				TyHpx			
		EC_{50}	EC_{20}	EC_{10}	R^2 (EC_{50})	EC_{50}	EC_{20}	EC_{10}	R^2 (EC_{50})
		----- mg kg ⁻¹ -----			%	----- mg kg ⁻¹ -----			%
Emergence	Maize	284 \pm 87	192 \pm 22	NV ^a	94	179 \pm 74	45 \pm 23	NV	90
	Rice	61 \pm 36	32 \pm 20	25 \pm 16	84	242 \pm 69	47 \pm 33	NV	95
	Wheat	569 \pm 57	227 \pm 23	113 \pm 12	92	314 \pm 30	236 \pm 48	199 \pm 60	98
	Soybean	896 \pm 87	720 \pm 124	634 \pm 149	87	331 \pm 52	204 \pm 59	148 \pm 62	97
	Radish	639 \pm 64	255 \pm 26	127 \pm 12	92	254 \pm 40	165 \pm 45	128 \pm 47	97
	Dry bean	335 \pm 109	150 \pm 94	88 \pm 75	92	651 \pm 134	478 \pm 215	390 \pm 259	84
Height	Maize	NV	NV	NV	-	878 \pm 46	773 \pm 68	710 \pm 85	95
	Rice	639 \pm 49	255 \pm 20	127 \pm 10	95	276 \pm 29	139 \pm 24	93 \pm 21	99
	Wheat	825 \pm 66	330 \pm 26	165 \pm 13	96	501 \pm 72	318 \pm 70	268 \pm 76	96
	Soybean	766 \pm 50	306 \pm 20	153 \pm 10	97	526 \pm 28	335 \pm 37	248 \pm 39	99
	Radish	868 \pm 58	651 \pm 86	562 \pm 131	95	455 \pm 58	297 \pm 73	224 \pm 75	97
	Dry bean	949 \pm 185	654 \pm 238	546 \pm 340	76	760 \pm 144	304 \pm 58	152 \pm 29	81
Stem diameter	Maize	NV	NV	NV	-	913 \pm 61	783 \pm 105	707 \pm 135	91
	Rice	855 \pm 110	342 \pm 44	171 \pm 22	90	350 \pm 62	123 \pm 39	67 \pm 28	97
	Wheat	1121 \pm 140	522 \pm 143	333 \pm 143	93	588 \pm 42	235 \pm 17	117 \pm 9	96
	Soybean	1106 \pm 60	860 \pm 102	728 \pm 133	90	714 \pm 28	565 \pm 46	484 \pm 127	98
	Radish	863 \pm 68	620 \pm 88	514 \pm 103	95	551 \pm 51	220 \pm 20	110 \pm 20	93
	Dry bean	701 \pm 115	280 \pm 46	140 \pm 23	84	914 \pm 85	787 \pm 121	721 \pm 149	83
Leaves	Maize	NV	NV	NV	-	NV	NV	NV	-
	Rice	469 \pm 92	118 \pm 53	47 \pm 30	97	299 \pm 44	106 \pm 28	58 \pm 20	98
	Wheat	NV	523 \pm 53	261 \pm 26	95	673 \pm 84	248 \pm 23	124 \pm 12	90
	Soybean	893 \pm 102	357 \pm 41	178 \pm 21	92	566 \pm 30	226 \pm 12	113 \pm 6	97
	Radish	NV	665 \pm 125	470 \pm 145	93	492 \pm 75	282 \pm 12	195 \pm 83	96
	Dry bean	NV	NV	NV	-	NV	NV	NV	-
Shoot dry weight	Maize	635 \pm 61	254 \pm 25	127 \pm 13	93	534 \pm 72	412 \pm 78	373 \pm 84	95
	Rice	270 \pm 70	92 \pm 46	45 \pm 31	96	148 \pm 18	61 \pm 15	34 \pm 12	98
	Wheat	597 \pm 37	239 \pm 15	119 \pm 7	97	303 \pm 34	129 \pm 47	112 \pm 38	98
	Soybean	630 \pm 59	252 \pm 24	126 \pm 12	94	516 \pm 41	206 \pm 16	103 \pm 8	94
	Radish	610 \pm 40	244 \pm 16	122 \pm 8	96	188 \pm 33	54 \pm 11	21 \pm 13	97
	Dry bean	335 \pm 109	150 \pm 94	88 \pm 75	92	651 \pm 134	478 \pm 215	390 \pm 250	84

^a NV - Data not validated by non-linear regression models ($p < 0.05$).

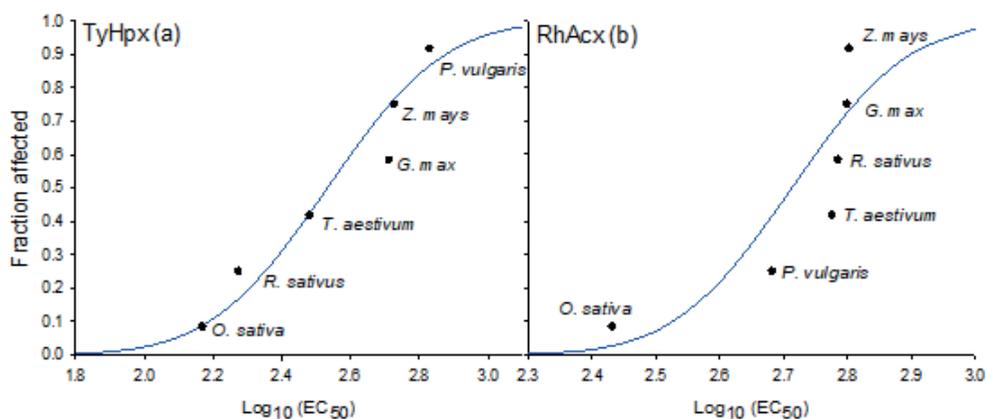


Figure 5 Species sensitivity distribution (SSD) curves built from EC_{50} of shoot dry weight for the soils Typic Hapludox (TyHpx) (a) and Rhodic Acrudox (RhAcx) (b).

Seed emergence was the variable with the highest variation among species and SDW the least variable. The variation in seed emergence between plant species may be related, if even in a small extent, to the nutritional reserve content of the seed (Bezerra, Momenté, & Medeiros, 2004; Marcos & Kikuti, 2006) and not the environmental conditions. Therefore, SDW is the best choice for building SSD curves. Besides, it was decided to present only SSDs built with EC_{50} values for avoidance graphic redundancy. Generally, it was observed a trend for higher EC_x values in RhAcx, compared to TyHpx. This may be due to higher values of CEC, clay, and SOM in the former. These variables can affect Cu availability because they may act as adsorbents (Kabata-Pendias & Szteke, 2015). According to Rooney, Zhao and McGrath (2006) the CEC is one

of the soil properties that has greatest relation with Cu toxicity. Thus, for RhAcx, probably a lower proportion of the applied Cu was available in the soil solution to cause toxicity.

The values of HC_5 (hazardous concentration to 5% of the species) were determined for the SDW (Table 4) based on the SSD curves. It was not possible to estimate HC_5 values for RhAcx because the data did not pass normality tests as the values for EC_x were very similar for the species tested (Figure 5b). On the other hand, the EC_x values obtained for the RhAcx were used to derive HC_5 for both soils (using the mean of EC_x for each species) and although the HC_5 values tended to be higher than those obtained TyHpx, it should be noted that confidence intervals overlap.

Table 4
 HC_5 values in Typic Hapludox (TyHpx), Rhodic Acrudox (RhAcx) and both soils (RhAcx + TyHpx) based on EC_x values for shoot dry weight

EC_x	HC_5		
	RhAcx ^a	TyHpx	RhAcx + TyHpx
	----- mg kg ⁻¹ -----		
EC_{50}	NV ^b	116.6 ± 81.4	220 ± 107
EC_{20}	NV	31.6 ± 39.7	74.3 ± 48.7
EC_{10}	NV	12.6 ± 23.4	33.5 ± 28.5

^a For each species, the EC_x from each soil was averaged and used to estimate the HC_5 .

^b NV - not validated in the normality test (the Anderson-Darling test, Kolmogorov-Smirnov test, Cramer von Mises test).

The experiments that evaluate the effects of Cu as nutrient use lower concentrations of Cu, because as shown by our results, greater concentration of Cu can be toxic. Moreira & Moraes (2019), for example, tested a maximum Cu concentration of 8 mg kg⁻¹ in soybeans, but the greatest grain yield was achieved at Cu concentrations of 4 mg kg⁻¹. Studies that focus in evaluating the toxic effects of Cu use greater Cu concentrations, such ours and Fageria (2007), where tested up to 96 mg kg⁻¹ of Cu in rice, common bean, soybean, wheat and corn crops.

The current maximum legal Cu concentration used by environmental agencies in Brazil, called “prevention value”, is 60 mg kg⁻¹. Up to this concentration, Brazilian soils are supposed to maintain their main ecological functions, like

the capability to grown plants (CONAMA, 2009; Conselho Estadual de Política Ambiental [COPAM], 2011). The results of the present study suggest that the current Brazilian prevention value for Cu is effective to ensure plant development. This claim is supported by HC₅ values calculated for TyHpx and for both soils (Table 4). Although 60 mg kg⁻¹ of Cu in the soil already leads to loss of plant growth, as shown in Table 5 using the prediction with the linear regression equations shown in Figure 2, the calculated HC₅ values for EC₅₀ are higher than the current Brazilian prevention value for Cu. Thus, in a Brazilian soil with 60 mg kg⁻¹ of Cu, a plant development of at least 50% may be expected for 95% of the species grown in such soil.

Table 5
Prediction of the loss of shoot dry weight using the the linear regression equations of rice, maize, wheat, dry bean, radish, and soybean cultivated in Typic Hapludox (TyHpx) and Rhodic Acrudox (RhAcx) with an addition of 60 mg kg⁻¹ of Cu

Cu added in soil (mg kg ⁻¹)	Rice	Maize	Wheat	Dry bean	Radish	Soybean
	shoot dry weight (g pot ⁻¹) - TyHpx					
0	1.219	0.845	1.554	5.205	3.147	4.239
60	1.046	0.797	1.378	4.941	2.735	3.830
	shoot dry weight losses (%)					
	-14.18	-5.68	-11.35	-5.07	-13.08	-9.65
	shoot dry weight (g pot ⁻¹) - RhAcx					
0	0.967	4.343	1.623	7.856	3.666	3.773
60	0.971	4.264	1.545	7.592	3.486	3.593
	shoot dry weight losses (%)					
	-0.37	-1.82	-4.81	-3.36	-4.91	-4.77

Conclusions

The species most sensitive to Cu was rice and the least were maize and soybean. The soil properties can intensify or decrease symptoms of toxicity, since Cu is more toxic to plant species in Typic Hapludox. This effect can be related to

the buffering effect of the clay and organic matter contents on metals toxicity. The current maximum legal Cu concentration for the prevention value used by environmental agencies in Brazil, 60 mg kg⁻¹, is protective enough for the tested species.

Funding information

Authors would like to thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) for the overall financial support during experiment and for the scholarship provided.

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