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SOIL SCIENCE

Ecotoxicity test as an aid in the determination of copper guideline values in soils

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ABSTRACT: *The process of identifying a contaminated area involves a preliminary assessment consisting of chemical analysis and comparison with guideline values. In Brazil, these values are included in the CONAMA Resolution no. 420/2009, based on chemical analysis and phytotoxicity. The objective of this research was to evaluate the ecotoxicity of copper in two natural soils in the state of Rio de Janeiro, a Haplic Planosol (sandy) and a Red-Yellow Argisol (medium texture), to verify if the values established by the legislation offer protection to the soil fauna. Lethality and reproduction tests were performed with Eisenia andrei worms and Folsomia candida springtails. Results indicated copper values in Planosol as LC50 435 mg kg 1 and EC50 29 mg kg 1 for E. Andrei and LC10 137 mg kg 1 and EC50 117 mg kg 1 for F. candida. In Argisol, these results were LC50 690 mg kg 1 and EC50 61 mg kg 1 for E. andrei, and LC10 42 mg kg 1 and EC50 138 mg kg 1 for F. candida. Values reported are lower than the research value (200 mg kg-1) established by the CONAMA resolution 420/09, indicating that concentrations lower than the limit values may affect these organisms, depending on the type of soil. Worms were more sensitive than springtails to copper contamination, and sandy soil was more susceptible to ecotoxicity due to copper contamination, probably due to the greater bioavailability of the metal. Results of such ecotoxicity tests should be considered in the development of soil guideline values.*
Key words: *contaminated areas, risk assessment, terrestrial ecotoxicology, soil invertebrates, metals, natural soils.*

Ensaios de ecotoxicidade como auxílio na determinação de valores orientadores de cobre em solos

RESUMO: *O processo de identificação de uma área contaminada envolve uma avaliação preliminar que consiste em análise química e comparação com valores orientadores. No Brasil, esses valores constam na Resolução CONAMA nº 420/2009, baseados em análises químicas e fitotoxicidade. O objetivo deste trabalho foi avaliar a ecotoxicidade do cobre em dois solos naturais do Estado do Rio de Janeiro, um Planossolo Háplico (arenoso) e um Argissolo Vermelho-Amarelo (textura média), para verificar se os valores estabelecidos pela legislação oferecem proteção à fauna do solo. Foram realizados ensaios de letalidade e de reprodução com minhocas da espécie Eisenia andrei e com colêmbolos da espécie Folsomia candida. Os resultados indicaram no Planossolo valores de cobre para E. andrei de CL_{50} 435 mg kg^{-1} e CE_{50} 29 mg kg^{-1} e para F. candida CL_{10} 137 mg kg^{-1} e EC_{50} 117 mg kg^{-1}. No Argissolo esse resultado foi para E. andrei CL_{50} 690 mg kg^{-1} e CE_{50} 61 mg kg^{-1}, e para F. candida CL_{10} 42 mg kg^{-1} e CE_{50} 138 mg kg^{-1}. Os valores encontrados são menores que o valor de investigação (200 mg kg^{-1}) estabelecidos pela resolução 420/09 do CONAMA, indicando que concentrações menores do que os valores-limite podem afetar esses organismos, a depender do tipo de solo. As minhocas mostraram-se mais sensíveis que os colêmbolos à contaminação por cobre, e o solo arenoso mostrou-se mais suscetível a apresentar ecotoxicidade diante de uma contaminação por cobre, devida, provavelmente, à maior biodisponibilidade do metal. Os ensaios de ecotoxicidade devem ser considerados no desenvolvimento de valores orientadores para solos.*
Palavras-chave: *áreas contaminadas, avaliação de risco, ecotoxicologia terrestre, invertebrados do solo, metais, solos naturais.*

INTRODUCTION

The process of identifying a contaminated area involves preliminary assessment, which includes, among other things, a comparison of chemical analysis results with guiding values. In Brazil, the establishment of guiding values began in the State of São Paulo, by CETESB, in 1997, while the federal level was specified by Resolution CONAMA 420 published in 2009 (BRASIL, 2009). For the determination of the guiding values, CETESB relied on the values established by the Dutch List, with data

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related to human toxicology, and on the natural values of metals in some soils in the State of São Paulo, using C-Soil risk assessment (RIVM) and phytotoxicity tests to estimate toxicity (CETESB, 2001). Subsequently, CONAMA used the values presented by CETESB in Resolution No. 420/2009, establishing the values for the prevention and investigation of substances in soils, and determined that each state should establish its quality reference values (BRASIL, 2009). However, the values specified in this legislation were not based on ecotoxicity tests involving soil invertebrates, and soil types were also not considered.

Chemical analyses are essential for the assessment of soil contamination, but provide limited information on its ecotoxicity. Ecotoxicity test is a necessary complement to chemical analysis to assess the impact of metals on invertebrates (XU et al., 2009; SANTORUFO et al., 2012; DUAN, et al., 2016). In these tests, organism-tests are used because of the close relationship of such organisms with the environment in which they live, have measurable behavioral, reproductive, or metabolic reactions that can indicate some change in that environment, when do not lead them to death (ANDRÉA, 2008).

The bioavailability of metals in soils and their toxicity to soil organisms are determined by several factors, such as the organic matter, clay, and iron oxides contents, along with the pH (LOCK et al., 2000; LUO et al., 2014). Due to these variations, LUO et al. (2014) and DUAN et al. (2016) argue that a variety of soils should be used in the construction of toxicity prediction models as the behavior of metals in soils can be different, especially in terms of their bioavailability.

Considering the complexity involved in the definition of guideline values, the different types of natural soils that exist in Brazil, and the protection of their edaphic organisms, this research proposed to apply ecotoxicity tests in evaluating the effects of copper on edaphic macro and mesofauna in two types of soil, verifying if the effective concentrations are covered by the values proposed by Brazilian legislation.

MATERIALS AND METHODS

Test soil

The soil samples were collected in non-anthropized areas located in the state of Rio de Janeiro. A Haplic Planosol sample collected inside the campus of Universidade Federal Rural do Rio de Janeiro (UFRRJ), and a Yellow-Red Argisol sample collected from the experimental field of EMBRAPA Agrobiologia in Seropédica, was used. The samples were collected with the aid of a shovel and hoe in the 0-20 cm soil layer. The pH value and physical and chemical analyses of soils were determined according to the method proposed by EMBRAPA (TEIXEIRA et al., 2017). The electrical conductivity (C.E.) was determined using a conductivity meter (ION brand). For the determination of the metal content, the samples were digested in a closed system according to method 3051A described by USEPA (2007) using the Mars Xpress digester. Results of the analyses of the sample are shown in table 1. The pseudo-total contents of Planosol were 1 mg.kg^{-1} of Cu, 0.35 mg kg^{-1} of Cd, 2.4 mg kg^{-1} of Pb, and 7.5 mg kg-^{1} of Zn; in Argisol the contents were 8.0 mg kg-^{1} of Cu, lower than the detection limit of Cd, 26.21 mg kg-^{1} of Pb, and 46.7 mg kg-^{1} of Zn.

Soil contamination

The copper concentrations used in the tests were based on the guideline values of Resolution No. 420 of CONAMA (BRASIL, 2009), where the prevention value corresponds to 60 mg kg^{-1} and the investigation value corresponds to 200 mg kg^{-1}. The copper concentrations applied in the tests were: 0,

Table 1 - Physical and chemical attributes of soil.

	Cam(1)	AR(2)	AN(3)	Sil(4)	GA(5)	AF(6)	GF(7)	Sil/Ar(8)	pH	CTC(9)	V(10)	Corg (11)	C.E(12)
	cm	----------g kg^{-1}----------					%			cmolc/dm^3	%	g kgl	uS
Argisol	-0.20	37	24	7	39	18	35	0.19	5.48	6.9	57.9	4.2	25.0
Planoso l	-0.20	5	2	6	76	13	60	0.75	5.51	3.09	38.5	3.6	63.7

(1) Layer; (2) Clay; (3) Clay naturally dispersed in water; (4) Silt; (5) Coarse sand; (6) Fine sand; (7) Degree of flocculation; (8) Silte/clay; (9) CTC; (10) Base saturation; (11) Organic carbon; (12) Electrical conductivity.

30, 60, 120, 200, 350, 700 mg kg^{-1}. A concentrated solution of copper nitrate ($Cu(NO3)_2.3H2O$, 331,2 mg L^{-1}) was used to contaminate the samples. For each treatment, 4.5 kg of soil was weighed and the diluted solution was added each in its specific concentration, until reaching 60% of the water retention capacity. The contaminated soils remained in incubation for 30 days until the beginning of the tests in order so that the metal reactions in the soil were stabilized. After this period, soil digestion was performed using the USEPA 3051A method (USEPA, 2007), to confirm the pseudo-total contents and the geochemical fractionation to verify the bioavailability of this metal in the soil (Table 3).

Biogeochemical fractionation

Sequential extraction was performed with the BCR method of the European Community Bureau of Reference (URE et al., 1993). The method consisted of adding solutions to the sample to extract metals at different degrees of adsorption. Samples remained in falcon tubes shaking for 20 hours, after this period, the sample was centrifuged, solute removed, dilution completed, and taken for measure. The first extraction step determined the water-soluble and weak acid metals, that is, the exchangeable and weakly retained metals on the sediment surface, extracted using 0.11 mol L^{-1} acetic acid (F1 fraction) corresponding to the bioavailable fraction. The second step determined the metals bound to the iron and manganese oxides; these are unstable under reducing conditions due to changes in the redox potential of the sediment and extracted using 0.1 mol L^{-1} hydroxylamine hydrochloride at pH 2.0 (F2 fraction). The third stage involved the degradation of organic matter under oxidizing conditions, with the release of metals bound to sulfides and complexes in organic matter, extracted using 8.8 mol L^{-1} hydrogen peroxide at pH 2.0 - 3.0 and 1 mol L^{-1} ammonium acetate at pH 2.0 (Fraction F3); the fourth and last step of the BCR procedure is the total digestion of the residue from the previous steps (Fraction F4) and the residual fraction, which was obtained by subtracting the sum of fractions F1, F2, and F4 from the pseudo-total contents.

Ecotoxicity test

In all tests, the Tropical Artificial Soil (TAS), proposed by GARCIA et al. (2004), was used as environmental control. The worms of the *Eisenia andrei* species Bouché, 1972 (Annelida: Lumbricidae) were cultivated using cow manure and coconut shell powder, according to the recommendations of ISO 11268-2 (ISO, 2012). The *Folsomia candida* springtails Willem, 1902 (Collembola: Isotomidae) were cultivated in a mixture of gypsum and activated charcoal, with synchronized age, according to ISO 11267 (ISO, 2014). The experiments were arranged to be entirely randomized, in which each dose had four replicates.

Chronic worm breeding test and acute lethality test were performed simultaneously. The reproduction test was based on OECD 222 (OECD, 2004) and ISO 11268-2 (ISO, 2012), and the lethality assay was based on OECD 207 (OECD, 1984) with the exposure period modified from 15 to 28 days. Ten adult organisms were placed in a container with 500g of soil, where they remained for 28 days, fed on oatmeal every two weeks. On the 28th day, adult individuals were removed from the container and the quantity of surviving organisms was checked and weighed. The soil remained in the container for another 28 days for the development of the cocoons and birth of juveniles. After that period, the number of juveniles was counted in the test. The total test period was 56 days.

With the *F. candida* springtails, the reproduction test was based on the ISO 11267 methodology (ISO, 2014). Ten individuals aged 10–12 days were inserted into each test container with 30 g of soil, where they remained for 28 days. During this period, the organisms were fed with biological yeast (*Saccharomyces cerevisiae*) on the 1st and 14th day. At the end of the test, the number of surviving, adults juveniles were counted. For this, water and stamp ink were added inside each replica, which brings the organisms to the surface of the water, allowing the registration per image for later counting in the *Image Tool for Windows 3.0* program.

Data analysis

The data from the reproduction tests were analyzed with the *Statistica 7.0* program to apply the normality and homogeneity tests (Cochran and Barllet; $p<0.05$), and subsequent analysis of variance (ANOVA), followed by the Dunnet test to determine the No Observed Effect Concentration (NOEC). The regression curve used to determine the Effective Concentration of 50% of the population (EC) was estimated using a non-linear equation. The effect of copper on the Argisol springtails was evaluated using the logistic model and on Planosol using the exponential model. The effect on earthworms was evaluated using the logistic model, in both soils. To define the Lethal Concentration of 50% of the population (LC), the lethality data were evaluated using the *Priprobit* program ($P<0.05$).

RESULTS AND DISCUSSION

The results of the reproduction and lethality test with *E. andrei* are shown in figure 1 and table 2. The development of juveniles of this organism in Planosol showed significant reduction for the dose of 30 mg kg^{-1}, varying from 32 individuals in the control to 16 individuals for this dose, and this variation generated a value of EC_{50} = 28.8 mg kg^{-1} (Table 2). This value was lower than the prevention value (60 mg kg^{-1}) determined by the CONAMA legislation (BRASIL, 2009). From the dose of 120 mg kg^{-1}, the juveniles of *E. andrei* were not reported in the evaluated treatments. The lethality of adult organisms occurred in the first weeks of the test, from the dose of 350 mg kg^{-1}. At the dose of 700 mg kg^{-1}, more than 50% lethality of adult organisms was observed on Planosol where the LC_{50} was 435.25 mg kg^{-1}.

In Argisol, significant reduction in the reproduction of *E. andrei* occurred at the dose of 60 mg kg^{-1}. While the average number of individuals in the control was 34, it was 18 at the dose of 60 mg kg^{-1} (Figure 1). There was no presence of juvenile earthworms at the dose of 350 mg kg^{-1}. This result generated a value of EC_{50} = 61 mg kg^{-1}, which is higher than that obtained in Planosol, indicating lower ecotoxicity of copper when applied in Argisol. This value is very close to the prevention value and lower than the investigation value (200 mg kg^{-1}) of CONAMA (BRASIL, 2009), showing divergence with the value indicated in the legislation. The investigation value indicated that investigative actions should be carried out; however, in areas with this level of contamination, would no longer find young *E. andrei* in the soil.

There was 50% adult mortality at the highest dose tested (700 mg kg^{-1}), resulting in a LC_{50} value of 690.27 mg kg^{-1} (Figure 2 and Table 2). Low food consumption by surviving adults was observed in both soils at the end of the first phase of the test and; consequently, there was a reduction in weight compared to the start of the test.

The reproduction results of *F. candida* show a significant reduction in the reproduction of this organism in Planosol from the dose of 120 mg kg^{-1}, ceasing its reproduction at the dose of 700 mg kg^{-1} (Figure 2). These results generated a value of EC_{50} = 117 mg kg^{-1} (Table 2), and this value was

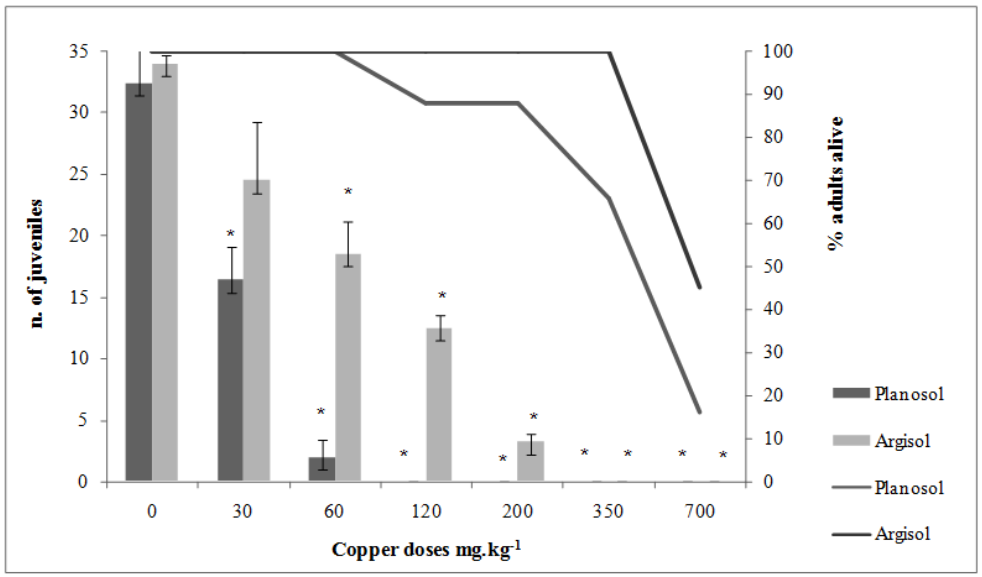

Figure 1 - Development of *E. andrei* in Copper-contaminated Planosol and Argisol.

The bars indicate the number of juveniles (average ± standard deviation) and the lines indicate the survival of adults (%). Asterisks indicate statistically significant differences (P<0.05) in relation to the control in the Dunnet test.

Table 2 - No observed effect concentration values (NOEC), effective concentration for 50% of the population (EC_{50}), lethal concentration for 50 and 10% of the population (LC_{50} and LC_{10}, respectively) of copper in mg kg^{-1}, obtained in reproduction and lethality tests with *Eisenia andrei* (earthworms) and *Folsomia candida* (springtails).

	Eisenia andrei			*Folsomia candida*		
	NOEC	EC50	LC50	NOEC	EC50	LC10
	mg kg-1					
Planosol	<30	29 (26.35-31.24)	435	<120	117 (83-151)	137
Argisol	<30	61 (42.95-79.07)	690	<30	138 (73-203)	42

Estimated values by statistical programs, Statistics for EC and NOEC and Priprobit for LC.

lower than the investigation value. Adult lethality was less than 50%, with 60% survival, so that it was not possible to determine the LC_{50}, but the LC_{10} of 137 mg kg^{-1} was estimated for lethality value of 10% of adult individuals (Table 2). In Argisol, differences in reproduction were observed from the first dose of 30 mg kg^{-1} itself, decreasing gradually with each dose until the EC_{50} of 138 mg kg^{-1} (Table 2 and Figure 2). As in Planosol, it was not possible to calculate LC_{50}, but LC_{10} = 42 mg kg^{-1} was determined. In both soils, at the highest doses, adult organisms did not develop well and remained the same size as at the beginning of the test. Low food consumption was also observed, which favored the development of fungi in the soil.

The *E. andrei* species was more sensitive than *F. candida* in the tests. SANTURUFO et al. (2012) also observed lower sensitivity of *F. candida* than the *Enchytreids albidus* and *Eisenia fetida* oligochaetas, verifying that *F. candida* accumulated less metal in its organism than the other organisms studied. Due to the difference in body structure and habits among the species, the sensitivity of the organisms also should be considered in the determination of the guideline values, because the organisms are affected differently, producing a variation in the results of the effective and lethal concentrations, which may cause divergence during the establishment of the values specified in the legislation.

The variation in EC and LC values between the soils studied is an important point to pay attention, as they showed that the ecotoxicological effects of the metal on edaphic fauna depend on the type of soil. Using *E. andrei*, SPURGEON et al. (1994), analyzed metal content in artificial soil and reported that, when adding copper nitrate solutions, values of EC_{50}=53 mg kg^{-1} and LC_{50}=555 mg kg^{-1} are obtained. DUAN et al. (2016), analyzing 15 natural soils from different parts of China, obtained EC_{50} values ranging from 27.7 mg kg^{-1} to 383.7 mg kg^{-1}, demonstrating a variation in ecotoxicity between the different soils. For springtails, NATAL-DA-LUZ- et al. (2011), on studying *F. candida,* found that EC_{50} = 42.4 mg kg^{-1}. BRUUS PEDERSEN et al. (2000) obtained the value of EC_{50} = 657 mg kg^{-1} using copper sulphate. The authors also observed that the organisms did not feed appropriately or have grown when compared to the control, as we reported in our as well. It is observed that the use of soils with different physical and chemical properties leads to different EC and LC results, which can be quite discrepant. Therefore, when establishing guideline values, considering different soil types can present to a safer result to protect the edaphic organisms.

The variability in the results is due to variation in the bioavailability of the metal in the soil, depending on the number of adsorption sites, which varies according to CTC, pH, clay and organic matter content, and these properties vary according to the type of soil (LOCK et al., 2000). Studying the correlation of soil properties with metal bioavailability, SANTORUFO et al. (2012) reported that these elements had accumulated considerably in organisms in soils with lower pH value and lower organic matter content. DUAN et al. (2016) analyzed 15 natural soils in China and investigated the bioavailability of metals and their relationship with the behavior and accumulation in earthworms. It was pointed out from correlation analysis that CTC, exchangeable Mg, organic carbon (CO) content, clay content, and silt content showed significant correlations with the bioavailability of metals in the soil, and CTC and CO directly influenced the

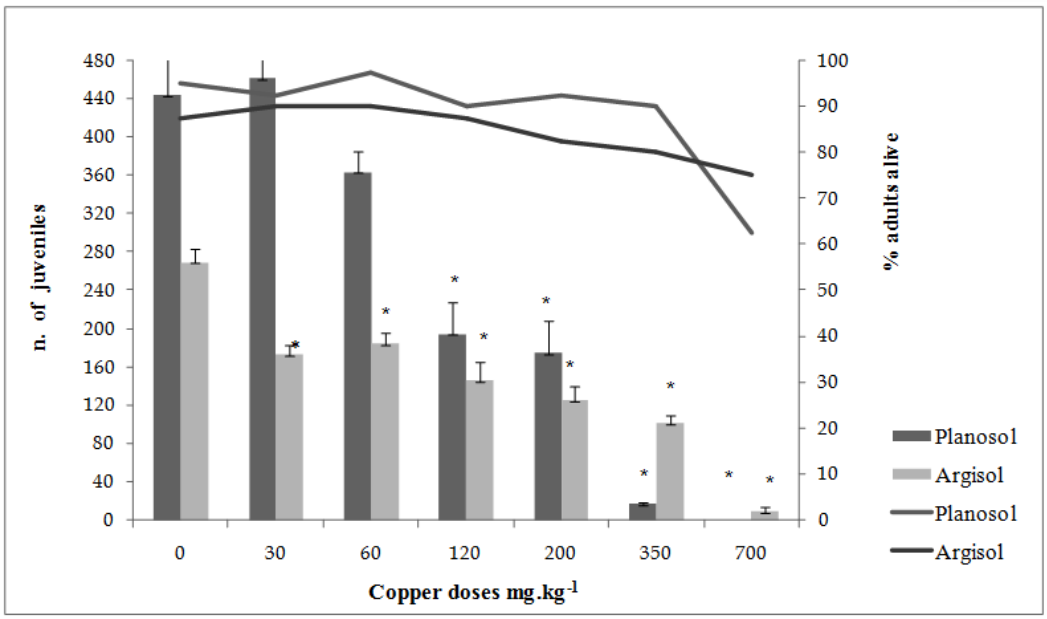

Figure 2 - Development of *F. candida* in copper-contaminated Planosol and Argisol.

The bars indicate the number of juveniles (average ± standard deviation) and the lines indicate the survival of adults (%). Asterisks indicate statistically significant differences (P<0.05) in relation to the control in the Dunnet test.

effective concentrations determined. DUAN et al. (2016) affirmed that, theoretically, the bioavailability of the metal; and consequently, the toxicity, is closely related to the behavior of particles and speciation of the metal in the soil, whose adsorption capacity is determined by the number and type of sites available.

The sensitivity of the two organisms to copper was higher in the Planosol in most of the tests. Planosol is the sandiest soil with lower levels of clay and organic matter; and consequently, lower CTC values, factors correlated with the increased bioavailability of copper. Thus, this soil shows less adsorption and copper becomes more available (Table 3). CORINGA et al. (2016) and SOARES et al. (2017) analyzed the geochemical fractioning in soils with distinct characteristics and found different percentages of copper adsorption in the soil. They also analyzed the influence of the factors cited above and affirm that copper has little mobility in the soil. The physical and chemical differences between the soils in this study probably promoted different levels of metal adsorption on the soil surface, leaving them less available in medium-textured soil than in sandy soil, which reflected the results of LC and EC.

Differences in metal bioavailability and toxicity between different soil types have been considered for the development of soil quality criteria in several countries (DEFRA, 2002, USEPA, 2003, CCME, 2006). For example, in Germany, the precautionary values of Cd, Pb, Cr, Cu, Ni, Hg, and Zn consider clay, silty and sandy soils separately. In China, the protection values of Ni, Cu, Zn, Cd, Cr, Hg, Pb, and As, were different in soils with pH <6.5, 6.5 and 7.5 and > 7.5, and the effects of soil CTC were also considered in the development and application of protection values for Cr and As (DUAN et al. , 2016).

The EC values obtained in all trials were lower than the intervention value suggested by CONAMA (BRASIL, 2009), whose guidelines are used to assess contaminated areas. Such values were not based on ecotoxicity tests with edaphic fauna, which can be an uncertainty factor in the application of these values. The use of these tests would contribute to the determination of values that can prevent damage to soil fauna; and consequently, to the ecosystem services in which these organisms are involved. The use of ecotoxicological tests with different test organisms and different types of soils would make the

Table 3 - Geochemical fractionation of copper contaminated soils in the different fractions in relation to the concentration of copper in the soil (mg kg^{-1}).

----------Planosol----------							
	0	30	60	120	200	350	700
F1	N.D	6.0	10.2	22.4	42.6	72.3	117
F2	N.D	7.0	3.0	N.D	13.0	30.2	40.3
F3	N.D	N.D	2.1	5.2	9.1	15.0	9.2
F4	0.35	29.5	58.7	115.3	188.4	347.0	676.6
res	0.35	17.5	43.4	87.7	123.7	229.5	510.1
----------Argisol----------							
	0	30	60	120	200	350	700
F1	0.3	5.1	7.0	20.05	37.3	44.5	78.05
F2	N.D	3.0	N.D	2.03	6.0	12.7	20.3
F3	N.D	N.D	N.D	1.3	10.7	28.3	40.4
F4	8.0	34.3	66.0	128.2	205.4	360.3	687.3
res	7.7	26.2	59.0	104.82	151.4	274.8	548.55

F1 = bioavailable fraction; F2 = oxide binding; F3 = organic matter binding; F4 = pseudo-total; res = residual (pseudo-total - (F1 + F2+ F3).

application of the guideline values safer in cases of soil contamination in Brazil, which should be the next step in improving Brazilian legislation.

This research indicated the need for the use of ecotoxicity tests for the determination of guideline values, and also demonstrates the importance of tests in the ecological risk assessment of contaminated areas, since chemical analyses itself may not reflect the effects of bioavailability on different soil types and groups of organisms. The development of regional protection values that consider different types of soils is one of the challenges for the improvement of legal instruments in Brazil regarding soil protection (NIVA et al., 2016). Although, ecotoxicity tests are used worldwide and standardized by ISO and OECD, and in Brazil by ABNT, their application in the Brazilian scenario still difficult, especially as few studies have been conducted. It is important to develop such tests for natural soils in order to better understand the effects of waste contamination and the changes it may cause in soil fauna.

CONCLUSION

Soils with different physical and chemical characteristics present different ecotoxicity results because of the differences in the bioavailability of metals in the soil, due to adsorption factors. Soils with sandy textures increase the bioavailability of copper compared to those with medium textures.

The EC values obtained indicated that the concentrations of copper lower than the limit values established by CONAMA Resolution 420/09 affecting the development of *E. andrei* and *F. candida*, depending on the type of soil. As a bioindicator organisms, *E. andrei* have been reported to be more sensitive than *F. candida* to copper contamination. Therefore, the application of ecotoxicity tests can contribute to the establishment of soil quality values in legislation and help to reduce the uncertainties in decision making on the management of contaminated and waste areas.

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DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed to statistical analysis, discussion of results and development of the article. The author

Bruna Faria Simões not only developed the actions mentioned but also carried out the laboratory analyses.

REFERENCES

ANDRÉA, M. M. **Biodindicadores ecotoxicológicos de Agrotóxicos**. São Paulo, Instituto Biológico. Comunicado Técnico, v83, 2008

BRASIL, **Resolução CONAMA Nº 420 de dezembro de 2009**. Available from: <http://www.mma.gov.br/port/conama/legiabre.cfm?codlegi=620>. Accessed: Aug. 31, 2018.

BRUUS PEDERSEN, M.; et al. Effects of Copper on reproduction of two collembolan species exposed through soil, food, and water. **Environmental Toxicology and Chemistry**, vol.19, n.10, p.2579-2588, 2009. Available from: <https://setac.onlinelibrary.wiley.com/doi/epdf/10.1002/etc.5620191026>. Accessed: Mar. 29, 2020. doi: 10.1002/etc.5620191026.

CANADIAN COUNCIL OF MINISTERS OF THE ENVIRONMENT (CCME). **A Protocol for the Derivation of Environmental and Human Health Soil Quality Guidelines**. PN 1332. Canada, Winnipeg, MB. 2006.

COMPANHIA AMBIENTAL DO ESTADO DE SÃO PAULO (CETESB), **Relatório de estabelecimento de Valores Orientadores para Solos e Águas Subterrâneas no Estado de São Paulo**. / CASARINI, DCP.; et al. São Paulo, Biblioteca, 2001.

CORINGA, J. E. S.; et al. Distribuição geoquímica e biodisponibilidade de metais traço em sedimentos no Rio Bento Gomes, Poconé - MT, Brasil, **Acta Amazônica**, 46(2), p.161-174, 2016. Available from: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0044-59672016000200161&lng=pt&tlng=pt>. Accessed: Mar. 29, 2020. doi: 10.1590/1809-4392201502215.

DEPARTMENT FOR ENVIRONMENT, FOOD AND RURAL AFFAIRS, AND THE ENVIRONMENT AGENCY (DEFRA). **The Contaminated Land Exposure Assessment (CLEA) Model**: Technical Basis and Algorithms. Contaminated Land Research Report 10.The Environment Agency, Bristol, United Kingdom. 2002.

DUAN, X.; et al. Effects of soil properties on copper toxicity to earthworm Eisenia fetida in 15 Chinese soils. **Chemosphere**, v.145, p.185-192, 2016. Available from: <https://www.sciencedirect.com/science/article/pii/S0045653515304306?via%3Dihub>. Accessed: Mar. 29, 2020. doi: 10.1016/j.chemosphere.2015.11.099.

GARCIA, M. V. B.; et al. Proposal for an artificial soil substrate for toxicity tests in tropical regions. In: 25th **Annual Meeting of Society of Environmental Toxicology and Chemistry (SETAC)**, Portland. 25th Annual Meeting of SETAC, 2004. Available from: <http://abstracts.co.allenpress.com/pweb/setac2004/document/?id=41943>. Accessed: Mar. 29, 2020.

INTERNATIONAL ORGANIZATION FOR STANDARDIZATION (ISO). **Soil quality – Effects of pollutants on earthworms (Eisenia andrei).Part 2. Determination of Effects on Reproduction**. ISO 11268-2, International Standard Organization, Geneva. 2012.

INTERNATIONAL ORGANIZATION FOR STANDARDIZATION (ISO). **Soil quality – Inhibition of reproduction of Collembola (Folsomia candida) by soil pollutants**. ISO 11267, International Standard Organization, Geneva. 2014.

LOCK, K.; et al. Multivariate test designs to assess the influence of zinc and cadmium bioavailability in soils on the toxicity to Enchytraeus albidus. **Environmental Toxicology and Chemistry**, v.19, n.11, p.2666–2671, 2000. Available from: <https://setac.onlinelibrary.wiley.com/doi/abs/10.1002/etc.5620191108>. Accessed: Mar. 29, 2020. doi: 10.1002/etc.5620191108.

LUO, W.; et al. Contribution of soil properties of shooting fields to lead bioavailability and toxicity to Enchytraeus crypticus. **Soil Biologyand Biochemistry**, v.76, p.235-241, 2014. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0038071714001850>. Accessed: Mar. 29, 2020. doi: 10.1016/j.soilbio.2014.05.023.

NATAL-DA-LUZ, T.; et al. Toxicity to Eisenia andrei and Folsomia candida of a metal mixture applied to soil directly or via an organic matrix. **Ecotoxicology and Environmental Safety**, v.74, p.1715–1720, 2011. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S014765131100162X?via%3Dihub>. Accessed: Mar. 29, 2020. doi: 10.1016/j.ecoenv.2011.05.017.

NIVA, C.; et al. Soil ecotoxicology in Brazil is taking its course. **Environmental Science and Pollution Research International**, v. online, 2016, p.1-16. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/27072030>. Accessed: Mar. 29, 2020. doi: 10.1007/s11356-016-6597-1.

ORGANIZATION FOR ECONOMIC COOPERATION AND DEVELOPMENT (OECD) **Guideline for Testing of Chemicals. Earthworm**, Acute Toxicity Tests. 207, 1984.

ORGANIZATION FOR ECONOMIC COOPERATION AND DEVELOPMENT (OECD) **Guideline for Testing of Chemicals**. Earthworm reproduction test, 222, 2004.

SANTORUFO, L.; et al. Ecotoxicological assessment of metal-polluted urban soils using bioassays with three soil invertebrates. **Chemosphere**, v.88, p.418-425, 2012. Available from: <https://www.sciencedirect.com/science/article/pii/S0045653512002585?via%3Dihub>. Accessed: Mar. 29, 2020. doi: 10.1016/j.chemosphere.2012.02.057.

SOARES, R.; et al. Avaliação do Risco Ambiental e Comportamento Geoquímico de Metais em Área Impactada por Resíduos Industriais em Queimados (RJ). **Revista Virtual Quimica**, v.9, n.5, 2017. Available from: <http://rvq.sbq.org.br/detalhe_artigo.asp?id=841>. Accessed: Mar. 29, 2020.

SPURGEON, D. J.; et al. Effects of Cadmium, Copper, Lead and Zinc on Growth, Reproduction, and Survival of the Earthworm Eisenia fetida (savigny): Assessing the Environmental Impact of point-source metal contamination in terrestrial ecosystems. **Environmental Pollution**, v.84, p.123-130, 1994. Available from: <https://www.sciencedirect.com/science/article/pii/0269749194900949>. Accessed: Mar. 29, 2020. doi: 10.1016/0269-7491(94)90094-9.

TEIXEIRA, P. C.; et al. **Editores técnicos, Manual de métodos de análise de solo**. Brasília, DF, EMBRAPA, 3 ed., 2017, e-book.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY (USEPA). **Guidance for Developing Ecological Soil Screening Levels**. OSWER Directive 92857-55.Office of Solid Waste and Emergency and Remedial Response, Washington DC. 2003.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY (USEPA). **Method 3051A**, 2007. Available from: <https://www.epa.gov/sites/production/files/2015-12/documents/3051a.pdf>. Accessed: Aug. 12, 2018.

URE, A. M.; et al. Speciation of heavy metals in soils and sediments. An account of the improvement and harmonization of extraction techniques undertaken under the auspices of the BCR of the Commission of the European Communities. **International Journal of Environmental Chemistry**, 51, p.135-151, 1993. Available from: <https://www.tandfonline.com/doi/abs/10.1080/03067319308027619>. Accessed: Mar. 29, 2020. doi: 10.1080/03067319308027619.

XU, J.; et al. Evaluation of growth and reproduction as indicators of soil metal toxicity to the Collembolan, Sinella curviseta. **Insect Science**. V.16, p.57-63, 2009. Available from: <https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1744-7917.2009.00254.x>. Accessed: Mar. 29, 2020. doi: 10.1111/j.1744-7917.2009.00254.x.