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Bioavailability of soil Cu, Fe, Mn and Zn from soil fractions

Biodisponibilidade de Cu, Fe, Mn, e Zn em frações do solo

Renildes Lúcio Ferreira Fontes^{1*}; Gilvan Barbosa Ferreira²;
Victor Hugo Alvarez V.¹; Júlio Cesar Lima Neves¹;
Arlindo Ferreira de Faria³; Maurício Paulo Ferreira Fontes¹

Highlights

Sequential extraction may help soil fertility evaluation for soil fertilization.

Cu, Fe, Mn, and Zn in soil fractions are a reservoir of bioavailable forms to plants.

Plant Cu, Fe, Mn, and Zn correlate to soil availability and soil fractions forms.

Abstract

Cationic micronutrients bioavailability depends on the chemical characteristics of soil fractions. Fourteen soils received individual doses of five micronutrients (Cu, Fe, Mn, Zn, B) arranged in seven treatments set according a Baconian Matrix. The soils incubated with treatments during 15 days had corn cultivated in greenhouse for 30 days, in three consecutive growth cycles. The cationic micronutrients were determined in the corn shoots after each growth cycle. Soil samples collected before the first and after each growth cycle had the available concentrations of Cu, Fe, Mn and Zn determined by single extractions (Mehlich-1 and DTPA-pH 7.3) and by sequential extraction. Correlation analysis was performed for the Cu, Fe, Mn and Zn concentrations determined in the corn shoots, the available concentrations of Cu, Fe, Mn and Zn in the soils (Mehlich-1 and DTPA) and the concentrations of Cu, Fe, Mn and Zn in the soil fractions (sequential extraction). The distribution of available metals forms in fractions reflected their affinity with soil components. Soil available Cu correlated with Cu bound to organic matter. The exchangeable fraction was the main source of soil available Mn and Zn. The Fe availability related mainly to the Mn oxides, Fe oxides, and exchangeable fractions. The plants absorbed Cu mainly from the Mn-oxides and organic matter fractions. Manganese absorbed by plants originated from the exchangeable and Mn-oxides fractions. The Zn absorbed by plants originated mainly from the exchangeable fraction. Correlations of single metal extractions (Mehlich-1 and

¹ Profs., Universidade Federal de Viçosa, UFV, Viçosa, MG, Brasil. E-mail: renildes@ufv.br; vhav@ufv.br; julio_n2003@yahoo.com.br; mpfontes@ufv.br

² Pesquisador, Empresa Brasileira de Pesquisa Agropecuária, EMBRAPA, Campina Grande, PB, Brasil. E-mail: gilvan.ferreira@embrapa.br

³ Discente do Curso de Doutorado do Programa de Pós-Graduação em Solos e Nutrição de Plantas, PPGSNP, UFV, Viçosa, MG, Brasil. E-mail arlindo.faria@ufv.br

* Author for correspondence

DTPA) with Cu, Mn and Zn contents in plants were positive.

Key words: Availability. Cationic micronutrients. Sequential extraction. Soil. Zea mays.

Resumo

A biodisponibilidade dos micronutrientes catiônicos depende das características químicas das frações do solo. Quatorze solos receberam doses individuais de cinco micronutrientes (Cu, Fe, Mn, Zn, B) em sete tratamentos, estabelecidos conforme uma Matriz Baconiana. Os solos, com os tratamentos, foram incubados em vasos plásticos durante 15 dias, cultivando-se milho durante 30 dias, em três ciclos consecutivos. Amostras de solo e planta foram coletadas após cada ciclo de crescimento. Os metais foram determinados na parte aérea das plantas e correlacionados com sua disponibilidade no solo (DTPA/Mehlich-1) e concentrações nas frações do solo (extração sequencial). A distribuição dos metais disponíveis nas frações refletiu sua afinidade com os componentes do solo. O Cu disponível correlacionou-se com o ligado à matéria orgânica. Enquanto a fração trocável foi a principal fonte de Mn e Zn disponíveis, a disponibilidade de Fe esteve ligada às frações óxidos de Mn, óxidos de Fe, e fração trocável. As plantas absorveram o Cu, principalmente, das frações óxidos de Mn e matéria orgânica; o Mn das frações trocável e óxidos de Mn; e o Zn da fração trocável. As extrações com DTPA e Mehlich-1 se correlacionaram positivamente com Cu, Mn e Zn absorvidos pelas plantas.

Palavras-chave: Extração sequencial. Disponibilidade. Micronutrientes catiônicos. Solo. Zea mays.

Introduction

The dynamics of labile forms of cationic micronutrients in soil fractions as related to soil fertility status have been reported over the years in several studies. Chemical fractionated Cu and Zn in soil was correlated to concentrations in tissues of soybean, alfalfa, corn grain, orchardgrass and sudangrass (Shober, Stehowertehouwer, & MacNeal, 2007). The distribution of Cu and Zn was studied in soil fractions after application in soluble forms (Orroño & Lavado, 2009). Fractionation by sequential extraction evaluated Fe and Mn in a nutrient-poor forest soil in area exposed to acid rain for years (Walna, Spsychalski, & Ibragimow, 2010). Mescouto et al. (2011) evaluated the distribution and availability of Cu, Fe, Mn, and Zn in different fractions in soil from the Amazon Region and Wali, Colinet and Ksibi (2014) used soil sequential extraction

to evaluate the speciation of Cu, Fe, Mn, and Zn in contaminated soils from Tunisia. Shahid et al. (2016) studied the balance of Cu, Fe, Mn, and Zn in soil and changes in uptake by rice and Dalpisol et al. (2017) evaluated Cu, Mn, and Zn availability in soils that received alkaline sewage sludge. Many of the studies deal with soils poor in micronutrients and no evaluation is made on the relationship between extractable micronutrient availability and the concentrations in soil chemical fractions related to the soil potential to supply the micronutrient to crops. According to Oviasogie, Aghimien and Ndiokwere (2011), information about Zn and Cu dynamics in soil chemical fractions, associated to data about Zn and Cu bioaccumulation in wetland soils palms plantations may enhance the fertility management options. Leite, Muraoka and Colzato (2019) extracted Zn with DTPA and Mehlich-1 and observed positive correlation

with Zn from soil labile fractions, which indicates that Zn is bioavailable. Sequential extraction of micronutrients in soils generates data that may improve the knowledge about extractor specificities for determining micronutrient availability levels for crop fertilization. It may help to assess the relation between the extracted form and the form in soil fractions, and the fraction that contributes with the micronutrient bioavailable to plants. Knowing the plant-available cationic micronutrients in the soil and the potential of the soil fractions to supply them for plants may contribute for their sustainable management in crop fertilization programs. The work aims the evaluation of the relationship between the contents of Cu, Fe, Mn, and Zn in corn plants and their concentrations in soil fractions (sequential extraction) and bioavailable concentrations

determined in Mehlich-1 and DTPA-pH 7.3 soil extracts.

Material and Methods

Soils

Samples of 14 soils, classified according Santos et al. (2003), were collected from the 0-20 cm depth layer, air dried, sieved to 2 mm, and analyzed for determination of organic matter by Walkley-Black (Empresa Brasileira de Pesquisa Agropecuária [EMBRAPA], 2007), Cu, Fe, Mn, and Zn (Mehlich-1 and DTPA-pH 7.3 extractions), Al^{3+} (KCl 1 mol/L extraction); H+Al ($C_4H_6O_4$ 0.5 mol/L-pH 7.0 extraction), texture (EMBRAPA, 1997), and clay mineralogy (Resende, Bahia, & Braga, 1987) (Table 1).

Table 1
Classification, geographic location, and chemical and physical properties of soils

| Classification | GPS Coordinates | Identification |
|------------------------|--------------------|----------------|
| Red-Yellow Oxisol | -19°49'S, -43°21'W | RYO-1 |
| Inceptisol | -19°57'S, -43°24'W | INC-1 |
| Red-Yellow Oxisol | -19°42'S, -43°28'W | RYO-2 |
| Oxisolic Inceptisol | -20°06'S, -43°02'W | oINC |
| Inceptisol | -19°57'S, -43°24'W | INC-2 |
| Red-Yellow Oxisol | -19°57'S, -43°24'W | RYO-3 |
| Fluvic Entisol | -19°49'S, -42°18'W | FENT |
| Red-Yellow Oxisol | -19°21'S, -42°34'W | RYO-4 |
| Red Oxisol | -18°48'S, -42°36'W | RO-1 |
| Red Oxisol | -18°31'S, -42°44'W | RO-2 |
| Red Oxisol | -18°39'S, -43°05'W | RO-3 |
| Red-Yellow Oxisol | -19°13'S, -42°19'W | RYO-5 |
| Red Oxisol | -19°27'S, -44°14'W | RO-4 |
| Dystroferic Red Oxisol | -20°55'S, -46°59'W | dyRO |

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| Properties | Soils | | | | | | |
|--|-------|-------|-------|-------|-------|-------|-------|
| | RYO-1 | INC-1 | RYO-2 | oINC | INC-2 | RYO-3 | FENT |
| pH (H ₂ O) 1:2.5 | 4.35 | 4.56 | 4.69 | 4.51 | 4.70 | 4.65 | 6.24 |
| Org. Matter, dag/kg ⁽¹⁾ | 3.32 | 2.06 | 2.94 | 4.69 | 1.98 | 1.95 | 1.03 |
| Cu mg/dm ³⁽²⁾ | 0.56 | 0.33 | 0.13 | 0.16 | 0.93 | 0.67 | 2.50 |
| Cu, mg/dm ³⁽³⁾ | 0.63 | 0.29 | 0.20 | 0.29 | 0.46 | 0.47 | 1.68 |
| Fe, mg/dm ³⁽²⁾ | 56.70 | 66.96 | 67.51 | 71.96 | 76.49 | 27.41 | 11.56 |
| Fe, mg/dm ³⁽³⁾ | 66.03 | 49.76 | 50.36 | 52.59 | 33.89 | 20.46 | 9.03 |
| Mn mg/dm ³⁽²⁾ | 1.09 | 1.37 | 2.27 | 1.82 | 2.12 | 4.41 | 61.09 |
| Mn, mg/dm ³⁽³⁾ | 0.46 | 0.72 | 0.97 | 0.85 | 0.82 | 2.32 | 43.58 |
| Zn mg/dm ³⁽²⁾ | 4.69 | 1.64 | 0.80 | 1.14 | 2.75 | 1.52 | 2.90 |
| Zn, mg/dm ³⁽³⁾ | 3.17 | 0.83 | 0.22 | 0.28 | 1.25 | 0.76 | 1.07 |
| Al ³⁺ , cmol _c /dm ³⁽³⁾ | 1.60 | 2.06 | 2.58 | 1.70 | 1.54 | 1.52 | 0.00 |
| H+Al, cmol _c /dm ³⁽³⁾ | 9.51 | 7.16 | 8.85 | 11.06 | 6.17 | 6.70 | 2.05 |
| Sand, % ⁽³⁾ | 57 | 46 | 39 | 33 | 43 | 40 | 67 |
| Silt, % ⁽³⁾ | 10 | 12 | 20 | 17 | 17 | 13 | 12 |
| Clay, % ⁽³⁾ | 33 | 42 | 41 | 50 | 40 | 47 | 21 |
| Kaolinite, dag/kg ⁽³⁾ | 16.9 | 29.5 | 33.4 | 32.5 | 35.3 | 31.0 | 24.1 |
| Gibbsite, dag/kg ⁽³⁾ | 18.3 | 19.6 | 20.1 | 23.9 | 15.6 | 17.1 | 8.3 |
| Hematite, dag/kg ⁽³⁾ | 3.74 | 0.0 | 0.0 | 6.1 | 0.0 | 0.0 | 0.0 |
| Goethite, dag/ kg ⁽³⁾ | 0.2 | 1.2 | 2.9 | 0.3 | 1.8 | 4.8 | 4.4 |
| | RYO-4 | RO-1 | RO-2 | RO-3 | RYO-5 | RO-4 | dyRO |
| pH (H ₂ O) 1:2.5 | 4.96 | 4.75 | 4.43 | 4.72 | 4.39 | 5.63 | 5.40 |
| Org. Matter, dag/kg ⁽¹⁾ | 4.44 | 4.65 | 4.96 | 4.53 | 3.73 | 7.79 | 2.51 |
| Cu mg/dm ³⁽²⁾ | 0.09 | 0.76 | 1.10 | 0.19 | 0.51 | 0.71 | 6.27 |
| Cu, mg/dm ³⁽³⁾ | 0.54 | 0.81 | 1.36 | 0.26 | 0.48 | 0.76 | 4.51 |
| Fe, mg/dm ³⁽²⁾ | 40.49 | 46.79 | 29.92 | 23.13 | 58.11 | 26.70 | 34.72 |
| Fe, mg/dm ³⁽³⁾ | 37.93 | 56.67 | 70.94 | 33.33 | 56.54 | 37.00 | 15.73 |
| Mn mg/dm ³⁽²⁾ | 1.52 | 3.02 | 1.75 | 0.94 | 3.37 | 25.75 | 35.31 |
| Mn, mg/dm ³⁽³⁾ | 0.56 | 1.76 | 1.11 | 0.54 | 1.75 | 14.06 | 25.53 |
| Zn mg/dm ³⁽²⁾ | 1.19 | 0.85 | 1.40 | 0.95 | 0.90 | 1.56 | 0.98 |
| Zn, mg/dm ³⁽³⁾ | 0.32 | 0.26 | 0.46 | 0.32 | 0.21 | 0.46 | 0.35 |
| Al ³⁺ , cmol _c /dm ³⁽⁴⁾ | 0.72 | 1.02 | 1.40 | 1.04 | 1.54 | 0.36 | 0.12 |
| H+Al, cmol _c /dm ³⁽⁵⁾ | 10.80 | 11.29 | 12.87 | 10.27 | 10.03 | 11.88 | 6.93 |
| Sand, % ⁽⁶⁾ | 58 | 30 | 23 | 6 | 22 | 19 | 31 |
| Silt, % ⁽⁶⁾ | 7 | 11 | 9 | 12 | 7 | 16 | 22 |
| Clay, % ⁽⁶⁾ | 35 | 59 | 68 | 82 | 71 | 65 | 47 |
| Kaolinite, dag/kg ⁽⁷⁾ | 12.8 | 26.7 | 37.6 | 34.3 | 42.3 | 41.2 | 10.0 |
| Gibbsite, dag/kg ⁽⁷⁾ | 25.1 | 33.3 | 26.9 | 41.3 | 24.7 | 28.08 | 40.9 |
| Hematite, dag/kg ⁽⁷⁾ | 0.9 | 12.6 | 12.2 | 11.5 | 11.0 | 10.9 | 21.5 |
| Goethite, dag/ kg ⁽⁷⁾ | 6.0 | 0.6 | 0.6 | 0.6 | 0.5 | 0.5 | 1.1 |

Walkley-Black, ⁽²⁾ Mehlich-1 Defilippo & Ribeiro (1997), ⁽³⁾ DTPA pH 7.3 Lindsay & Norvell (1978), ⁽⁴⁾ KCl 1 mol/L, ⁽⁵⁾ CaOAc 0.5 mol/L at pH 7,0 (Defilippo & Ribeiro, 1997). ⁽⁶⁾ EMBRAPA (1997). ⁽⁷⁾ ⁽⁹⁾ Resende et al. (1987).

Experimental design and plant cultivation

A randomized block design arranged in a factorial 14 x 7 (soil types x micronutrient treatments), with three replications was set. Cu, Fe, Mn, Zn, and B ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, Fe-EDTA, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, and H_3BO_3) at the doses 2, 10, 5, 5, and 0.5 mg/dm (respectively) were added to the soils for corn cultivation under greenhouse conditions. Treatments were set according to a Baconian Matrix which is characterized as an exploratory matrix used

to define the studied factors priorities and choose the factors doses to give structure to the treatments in a process for analysis of an agricultural system (Turrent, 1979). Treatments were set as: Treatment 1= no micronutrient addition; Treatments 2 to 6 = Zn, Cu, Fe, Mn, B, each one, individually added, at the dose used for corn, with no addition of the others; Treatment 7 = Zn, Cu, Fe, Mn, B, all added together, each one at the used dose for corn (Table 2).

Table 2
Micronutrient treatments according to a Baconian Matrix

| Treatment | Micronutrient | | | | |
|-----------|----------------------------------|------|-----|-----|-----|
| | Cu | Fe | Mn | Zn | B |
| | ------(mg/dm ³)----- | | | | |
| 1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 2 | 2.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 3 | 0.0 | 10.0 | 0.0 | 0.0 | 0.0 |
| 4 | 0.0 | 0.0 | 5.0 | 0.0 | 0.0 |
| 5 | 0.0 | 0.0 | 0.0 | 5.0 | 0.0 |
| 6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.5 |
| 7* | 2.0 | 10.0 | 5.0 | 5.0 | 0.5 |

*Doses used for corn fertilization.

The experimental unit was a 2.0 dm³ plastic pot with soil, one plant per pot. Pots distributed at random inside each block and rotated weekly in the greenhouse benches. Each pot received 300 mg/dm³ N (150, just before planting, 75, 10 days after emergence, and 75, 20 days after emergence); 186 to 312 mg/dm³ P; 48 to 84 mg/dm³ S; K to reach 200 mg/dm³; 2.0 cmolc/dm³ Ca; 0.5 cmolc/dm³ Mg; and 0.3 mg/dm³ Mo. After 15 days incubation,

10 seeds of Al³⁺ tolerant corn cultivar Vencedor were sowed per pot and plants cultivated in three consecutive cycles or cultivation periods (30 days growth for the 10 seeds per pot, per cycle). Thinning was done 10 days after planting, leaving 5 plants per pot. Soil moisture was maintained between 70-100% of the field capacity, by weighing and daily visual verification, while the plants were grown for 30 days after emergence.

Given the appearance of symptoms of Mg and P deficiency in the first growth period, 0.8 cmolc dm⁻³ of Ca and 0.2 cmolc dm⁻³ of Mg, and 40 cmolc dm⁻³ of P were applied to the soil before the second growth period. During the 2nd cultivation, due to the visual symptoms of P, K and S deficiency in some plants, 150 cmolc dm⁻³ of P, 75 cmolc dm⁻³ of K and 31 cmolc dm⁻³ of S were applied together with the N in cover. In the 3rd cycle, it was applied 120, 95, 63 and 22 cmolc dm⁻³ P, K, S and Mg, respectively, with the N application.

Plant analysis

At the end of each cultivation period, corn aerial part was cut near soil surface, the roots were removed and the soil was sieved to 2 mm. The soil sieved after each cultivation period (2 mm mesh) was saved for the next growth cycle. Shoot samples were oven dried at 70°C for 72 hours and dry matter weight determined. Plant analysis is presented for the cationic micronutrients (Cu, Fe, Mn, and Zn), without approaching the anionic micronutrient B. The analysis of Cu, Fe, Mn, and Zn in the dry matter digested by nitric-perchloric solution (Zazoski & Burau, 1977) was performed by atomic absorption spectrophotometry (Varian SpectrAA 200) (Jordão et al., 2006). All reagents were analytical grade and all glassware and materials thoroughly cleaned.

Soil analysis

Each experimental unit had a soil sample (100 cm³) withdrawn before the first cycle and after each cultivation cycle, and divided in two sub-samples of 50 cm³ (1 and 2) for the soil analysis. The application of the

micronutrients occurred only before the first corn cultivation, with a potential exhaustion of the cationic micronutrients in the experimental units along the growth cycles. Soil analysis is presented for the cationic micronutrients (Cu, Fe, Mn, and Zn), without approaching the anionic micronutrient B.

Sub-sample 1 - Analysis by single extractions - micronutrient available forms

Concentrations of Cu, Fe, Mn and Zn were determined by extractions with DTPA (DTPA-pH 7.3) (Lindsay & Norvell, 1978) and Mehlich-1 (Defelipo & Ribeiro, 1997). The soil cationic micronutrient availability was determined by atomic absorption spectrophotometry (Varian SpectrAA 200) (Jordão et al., 2006), using analytical grade reagents. All glassware and materials were thoroughly cleaned.

Sub-sample 2 - Analysis by sequential extraction - micronutrient linked to soil fractions

Sequential extraction took place according to Shuman (1985), with modifications (2.5 g of soil instead of 10 g for the Exchangeable and Organic Matter fractions; combination of the two extracts from successive extractions with NaClO for organic matter fraction; 0.50 g of soil instead of 1.00 g for extraction of Mn-Oxides, AFeOxides and CFeOxides; Residual Fraction calculated as the difference between the metal in Total Attack, and the sum of metals from previous fractions) as presented in Table 3. The six soil fractions to which the metals were bound were named Exchangeable (Exch), Organic Matter (OM), Mn

Oxides (MnOx), Amorphous Fe Oxides (AFeOx), Crystalline Fe Oxides (CFeOx), and Residual (Res) fractions). The cationic micronutrients in soil fraction extracts were determined by

atomic absorption spectrophotometry (Varian SpectrAA 200) (Jordão et al., 2006), using analytical grade reagents, with all glassware and materials being thoroughly cleaned.

Table 3
Sequential extraction of Fe, Mn, Zn, and Cu, adapted from Shuman (1985)

| Phases ⁽¹⁾ | Fraction | Extracting Solution (ExtSol) | Soil (g) | ExtSol (mL) | Conditions ⁽²⁾ |
|-----------------------|-------------------------------|--|---------------------|-------------|--|
| 1 | Exchangeable (Exch) | Mg(NO ₃) ₂ 1 mol/L, pH 7.0 | 2.50 | 10 | 2 h shaking |
| 2 | Organic Matter (OM) | NaClO 0.7 mol/L, pH 8.5 | 2.50 ⁽³⁾ | 5 | Water-bath (boiling by 30 min, stirring occasionally). Repeat and combine the extracts |
| 3 | Mn Oxides (MnOx) | NH ₂ OH.HCl 0.1 mol/L in HNO ₃ 0.01 mol/L, pH 2.0 | 0.50 ⁽⁴⁾ | 10 | 30 min shaking |
| 4 | Amorphous Fe Oxides (AFeOx) | (NH ₄) ₂ C ₂ O ₄ .H ₂ O 0.2 mol/L in H ₂ C ₂ O ₄ 0.2 mol/L, pH 3.0 | 0.50 | 25 | 4 h without shaking, in the dark |
| 5 | Crystalline Fe Oxides (CFeOx) | (NH ₄) ₂ C ₂ O ₄ .H ₂ O 0.2 mol/L + H ₂ C ₂ O ₄ 0.2 mol/L, in ascorbic acid 0.1 mol/L, pH 3.0 | 0.50 | 25 | Water-bath (boiling by 30 min, stirring occasionally) |
| 6 | Residual (Res) | - | - | - | (Calculated by difference) |
| 7 | Total (Total) | HF/HNO ₃ /HCl | 1.00 | 20 | + 30 min in microwave centrifugation: 50 min at 0.83 MPa (4000 rpm/5 min) |

⁽¹⁾NaCl 0.5 mol/L washing the samples among phases, with shaking by 3 min (10 mL after phases 1 and 2; and 2.5 mL after phases 3 and 4); ⁽²⁾Separation of liquid and solid phases by centrifugation at 4000 rpm by 5 min. ⁽³⁾2.5g from phase 2 dry until constant weight, passed through a 35 mesh sieve and sieved; ⁽⁴⁾0.5 g weighed to continue the extraction.

Statistical analysis

Analysis of variance (ANOVA) and regression analysis provided the statistics for the Cu, Fe, Mn, and Zn determined in soils and corn plants, under a randomized block design with treatments arranged in a factorial 14 x 7 (soils x micronutrients), with three replications.

Since the values of standard deviations of the variables (metals in DTPA/Mehlich-1 extracts, metals in soil fractions extracts, and metals in plant extracts) were proportional to their means, the data was transformed using the $y = \ln(x+1)$ formula. Multiple linear regression analysis process (Stepwise) modeled the relationship between the extracted concentrations of Cu,

Fe, Mn, and Zn by Mehlich-1 and DTPA and the metal concentrations in the soil fractions. The same procedure modeled the contents of Cu, Fe, Mn and Zn in plant shoots ($\mu\text{g}/\text{dm}^3$), the soil concentrations extracted by DTPA/Mehlich-1 (mg/dm^3) and the concentrations in the soil fractions (mg/kg). The soil parameters were the independent variables and the plant shoot micronutrient contents the dependent variables in the stepwise linear regression analysis models, similar to the used for cationic micronutrient contents in wheat grains and soil parameters (Ayoubi, Mmenatkesh, Jalalian, Sahrawat, & Gheysari, 2014). Due to discrepant characteristics in the FENT, RO-4 and dyRO soils as compared to the others (Table 1), the correlation and regression analysis excluded the data from these soils, since they did not fit well the model (outliers) and the linear correlations above 0.8 between their variables could cause inter correlations or inter associations among the independent variables (multicollinearity).

Results and Discussion

Availability of Cu, Fe, Mn, and Zn in soils, as related to their forms linked to the soil fractions

DTPA-pH 7.3 extracted 72 to 79 % of soil Cu, and Mehlich-1 36 to 55 % of soil Cu, which is the Cu promptly available for plants. The correspondent sequential extraction showed that these available Cu forms originated mainly from those linked to the organic matter (Tables 4 and 5). The next fraction responsible for supplying available Cu was the Mn oxides fraction, where 10-11 % (DTPA) and 15-23 % (Mehlich-1) of the soil

Cu was retained by occlusion. In the sequence, the crystalline Fe oxides fraction contributed with the Cu availability (1-3 with DTPA and to 2-5% with Mehlich-1). The amorphous Fe oxides fraction had a smaller contribution (2 % or less), which was similar to the contribution of the residual fraction (2 %) (Tables 4 and 5). This emphasizes the importance of organic matter as the main supplier of Cu for plant uptake, storing Cu in available forms, which is crucial mainly in weathered soils. This pattern for Cu availability as related to Cu fractions is consistent with the data reported by Shuman (1985) and Nascimento, Fontes and Melicio (2003). Furthermore, Nascimento and Fontes (2004) reported high correlation between Cu and soil organic carbon, based on the Langmuir and Freundlich constants for Cu adsorption. More recently, in acidic soils, Joshi, Srivastava, Dwivedi, Pachauri and Shukla (2015) found that the Cu availability to plants depends, mainly, on the organically bound, with soil Cu components more soluble to water having also contribution. Higher amount of Cu extracted with DTPA from organic fraction (Table 5) was due to the extractor's capability to compete with soil organic matter for the ionic metallic chelation in the soil (Shuman, 1985; Sims, 1986; Nascimento, Fontes, & Melicio, 2003). The metals in soils occur predominantly in insoluble forms, however some of them are linked to soil particles in a reversibly equilibrium, with the soil solution ions bound to inorganic and organic ligands, for Cu, very low amount is free Cu^{2+} in soil solution. The organic matter is one of the components of the equilibrium established by the various soil pools of Cu (Minnich & McBride, 1987; Minnich, McBride, & Chaney, 1987), and very important to maintain the pool of available forms.

Table 4

Regression equations for Cu, Fe, Mn and Zn extracted with DTPA and M-1 as related to them in soil fractions (Exch; OM; MnOx; AFeOx; CFeOx; Res)⁽¹⁾ and to soil pH (in H₂O), before and after each corn growth cycle (metals in soil fractions are in mg/kg and in DTPA-Mehlich-1 extracts in mg /dm³)

| Metal ⁽²⁾ - Cycle ⁽³⁾ | Regression Equation ⁽⁴⁾ | R ² |
|---|---|----------------|
| ----- DTPA extraction as a function of the chemical fractions and pH ----- | | |
| Cu - 0 | $\hat{Y} = 0.32 + 1.24^{***}OM - 0.09^{***}CFeOx - 0.005^{***}Res$ | 0.83 |
| Cu - 1 | $\hat{Y} = 0.24 + 0.877^{***}OM - 0.004^{*}Res$ | 0.75 |
| Cu - 2 | $\hat{Y} = -0.05 + 0.49^{***}OM + 0.11^{***}MnOx - 0.04^{**}CFeOx$ | 0.84 |
| Cu - 3 | $\hat{Y} = -0.07 + 0.65^{***}OM + 0.11^{***}MnOx - 0.02^{\circ}AFeOx - 0.03^{**}CFeOx$ | 0.88 |
| Fe - 0 | $\hat{Y} = 120 + 1.19^{***}Exch + 0.88^{***}OM + 0.35^{***}MnOx - 0.01^{***}AFeOx + 0.0004^{***}Res - 24^{***}pH$ | 0.90 |
| Fe - 1 | $\hat{Y} = 171 + 0.92^{**}Exch + 1.48^{***}OM + 0.44^{***}MnOx - 0.01^{***}AFeOx - 34^{***}pH$ | 0.87 |
| Fe - 2 | $\hat{Y} = 113 + 0.82^{***}Exch + 0.92^{***}OM + 0.38^{***}MnOx - 0.01^{***}AFeOx - 23^{***}pH$ | 0.82 |
| Fe - 3 | $\hat{Y} = 150 + 1.12^{***}Exch + 0.89^{***}OM + 0.52^{***}MnOx - 0.01^{***}AFeOx + 0.0001^{***}Res - 30^{***}pH$ | 0.91 |
| Mn - 0 | $\hat{Y} = -6.19 + 0.40^{***}Exch + 0.84^{**}OM - 0.003^{**}Res + 1.10^{***}pH$ | 0.92 |
| Mn - 1 | $\hat{Y} = -6.12 + 0.55^{***}Exch - 0.69^{*}OM + 0.34^{***}MnOx + 0.03^{\circ}CFeOx - 0.004^{**}Res + 0.92^{**}pH$ | 0.97 |
| Mn - 2 | $\hat{Y} = -4.66 + 0.40^{***}Exch + 0.28^{***}MnOx - 0.002^{**}Res + 0.73^{***}pH$ | 0.97 |
| Mn - 3 | $\hat{Y} = -6.71 + 0.40^{***}Exch + 0.10^{***}AFeOx + 1.17^{***}pH$ | 0.96 |
| Zn - 0 | $\hat{Y} = -4.11 + 1.37^{***}Exch + 0.30^{***}MnOx - 0.16^{*}AFeOx + 0.09^{**}CFeOx - 0.75^{***}pH$ | 0.97 |
| Zn - 1 | $\hat{Y} = -2.12 + 1.42^{***}Exch + 0.26^{***}MnOx + 0.11^{***}CFeOx + 0.32^{\circ}pH$ | 0.96 |
| Zn - 2 | $\hat{Y} = -0.12 + 1.18^{***}Exch + 0.08^{*}MnOx + 0.08^{*}AFeOx$ | 0.97 |
| Zn - 3 | $\hat{Y} = 0.18 + 1.19^{***}Exch + 0.38^{***}MnOx - 0.01^{**}Res$ | 0.87 |
| ----- Mehlich ⁻¹ extraction as a function of the chemical fractions and pH ----- | | |
| Cu - 0 | $\hat{Y} = -0.02 + 1.24^{***}OM + 0.28^{***}MnOx - 0.17^{***}CFeOx - 0.01^{*}Res$ | 0.68 |
| Cu - 1 | $\hat{Y} = -0.10 + 1.47^{***}OM + 0.37^{***}MnOx - 0.09^{**}AFeOx + 0.09^{*}CFeOx$ | 0.79 |
| Cu - 2 | $\hat{Y} = -2.06 + 0.94^{***}OM + 0.31^{***}MnOx - 0.07^{*}CFeOx + 0.39^{**}pH$ | 0.75 |
| Cu - 3 | $\hat{Y} = 0.64 + 0.69^{***}MnOx + 0.02^{***}Res - 0.22^{**}pH$ | 0.63 |
| Fe - 0 | $\hat{Y} = -337 + 0.83^{*}Exch + 1.61^{***}OM + 1.51^{***}MnOx - 0.01^{*}AFeOx - 0.0009^{***}CFeOx - 0.001^{***}Res + 72^{***}pH$ | 0.92 |
| Fe - 1 | $\hat{Y} = -269 + 1.08^{**}Exch + 1.34^{***}MnOx - 0.001^{***}CFeOx - 0.001^{***}Res + 59^{***}pH$ | 0.89 |
| Fe - 2 | $\hat{Y} = -133 + 1.05^{***}Exch + 0.79^{***}OM + 0.92^{***}MnOx - 0.001^{***}CFeOx - 0.0004^{***}Res - 30^{***}pH$ | 0.95 |
| Fe - 3 | $\hat{Y} = -83 + 1.01^{***}Exch + 1.14^{***}OM + 0.95^{***}MnOx - 0.001^{***}CFeOx - 0.0004^{***}Res - 20^{***}pH$ | 0.92 |
| Mn - 0 | $\hat{Y} = -1.87 + 0.50^{***}Exch + 0.97^{***}MnOx - 0.01^{***}Res$ | 0.93 |
| Mn - 1 | $\hat{Y} = -1.51 + 0.34^{***}Exch - 0.69^{***}MnOx + 0.06^{\circ}AFeOx - 0.004^{***}Res$ | 0.94 |

continue...

contuation...

| | | |
|--------|---|------|
| Mn - 2 | $\hat{Y} = -2.175 + 0.488^{***} \text{Exch} + 0.896^{***} \text{MnOx} + 0.075^{\circ} \text{AFeOx} - 0.003^{\circ} \text{Res}$ | 0.95 |
| Mn - 3 | $\hat{Y} = -2.13 + 0.48^{***} \text{Exch} + 0.45^{***} \text{MnOx} + 0.16^{***} \text{AFeOx} - 0.002^{\circ} \text{Res}$ | 0.97 |
| Zn - 0 | $\hat{Y} = -20.46 + 3.65^{***} \text{Exch} + 1.71^{\circ} \text{OM} + 2.24^{***} \text{MnOx} + 0.43^{\circ} \text{CFeOx} + 3.61^{**} \text{pH}$ | 0.89 |
| Zn - 1 | $\hat{Y} = -9.48 + 2.01^{***} \text{Exch} + 0.65^{***} \text{MnOx} + 0.26^{***} \text{CFeOx} + 0.01^{\circ} \text{Res} + 1.60^{***} \text{pH}$ | 0.96 |
| Zn - 2 | $\hat{Y} = -5.13 + 1.76^{***} \text{Exch} + 0.48^{***} \text{MnOx} + 0.16^{**} \text{CFeOx} + 0.91^{**} \text{pH}$ | 0.94 |
| Zn - 3 | $\hat{Y} = -5.54 + 1.49^{***} \text{Exch} + 0.40^{***} \text{MnOx} + 0.23^{\circ} \text{AFeOx} + 0.14^{**} \text{CFeOx} + 1.03^{**} \text{pH}$ | 0.91 |

(¹) Exch = exchangeable fraction, OM = linked to organic matter fraction, MnOx = occluded in Mn oxides fraction, AFeOx = occluded in amorphous Fe oxides fraction, CFEOx = occluded in crystalline Fe oxides fraction, Res = occluded in residual Fe oxides, Al, caulinite fraction. (²) Metal = metal extracted from soil; (³) Cycle: 0 = before corn growth; 1, 2, 3 = after 1st, 2nd and 3rd growth cycles, respectively; (⁴) ^o, ^{*}, ^{**}, ^{***} = significant at the 10, 5, 1 and 0,1 % levels; Stepwise Regression Analysis.

Table 5

Total and partial R² values for the coefficients of the regression equations between the concentrations of Cu, Fe, Mn and Zn extracted with DTPA and Mehlich-1 and the Cu, Fe, Mn and Zn concentrations in soil fractions⁽¹⁾ and soil pH, before and after each corn growth cycle

| Metal ⁽²⁾ | Cycle ⁽³⁾ | R ² (total) ⁽⁴⁾ | R ² (parcial) ⁽⁴⁾ | | | | | | |
|---|----------------------|---------------------------------------|---|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| | | | pH-H ₂ O | Exch | OM | MnOx | AFeOx | CFEOx | Res |
| ----- Extraction with DTPA pH 7.3 ----- | | | | | | | | | |
| Cu | 0 | 0.83 | - | - | 0.79 ^{***} | - | - | 0.03 ^{**} | 0.02 ^{***} |
| | 1 | 0.75 | - | - | 0.73 ^{***} | - | - | - | 0.02 [*] |
| | 2 | 0.84 | - | - | 0.72 ^{***} | 0.11 ^{***} | - | 0.02 ^{**} | - |
| | 3 | 0.88 | - | - | 0.77 ^{***} | 0.10 ^{***} | <0.01 ^o | 0.01 ^{**} | - |
| Fe | 0 | 0.90 | 0.26 ^{***} | 0.11 ^{***} | 0.02 ^{**} | 0.05 ^{***} | 0.03 ^{***} | - | 0.42 ^{***} |
| | 1 | 0.87 | 0.55 ^{***} | 0.10 ^{***} | 0.05 ^{***} | 0.07 ^{***} | 0.09 ^{***} | - | - |
| | 2 | 0.82 | 0.49 ^{***} | 0.10 ^{***} | 0.07 ^{***} | 0.13 ^{***} | 0.03 ^{**} | - | - |
| | 3 | 0.91 | 0.58 ^{***} | 0.09 ^{***} | 0.05 ^{***} | 0.13 ^{***} | 0.04 ^{***} | - | 0.02 ^{***} |
| Mn | 0 | 0.92 | 0.03 ^{***} | 0.87 ^{***} | <0.01 ^o | - | - | - | 0.01 ^{**} |
| | 1 | 0.97 | 0.01 ^{***} | 0.93 ^{***} | <0.01 ^o | 0.03 ^{***} | - | <0.01 ^o | <0.01 ^o |
| | 2 | 0.97 | 0.01 ^{***} | 0.93 ^{***} | - | 0.02 ^{***} | - | - | <0.01 ^{**} |
| | 3 | 0.96 | 0.02 ^{***} | 0.92 ^{***} | - | - | 0.01 ^{***} | - | - |
| Zn | 0 | 0.97 | <0.01 ^{**} | 0.93 ^{***} | - | 0.03 ^{***} | <0.01 [*] | <0.01 [*] | - |
| | 1 | 0.96 | <0.01 ^o | 0.92 ^{***} | - | 0.03 ^{***} | - | <0.01 ^{**} | - |
| | 2 | 0.97 | - | 0.96 ^{***} | - | 0.01 ^{***} | <0.01 ^o | - | - |
| | 3 | 0.87 | - | 0.80 ^{***} | - | 0.05 ^{***} | - | - | 0.02 ^{**} |

continue...

contuation...

| ----- Extraction with Mehlich ¹ ----- | | | | | | | | | |
|--|---|------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| Cu | 0 | 0.68 | - | - | 0.40 ^{***} | 0.21 ^{***} | - | 0.05 ^{***} | 0.02 [*] |
| | 1 | 0.79 | - | - | 0.53 ^{***} | 0.22 ^{***} | 0.02 ^{**} | 0.02 [*] | - |
| | 2 | 0.75 | 0.04 ^{**} | - | 0.55 ^{***} | 0.15 ^{***} | - | 0.02 [*] | - |
| | 3 | 0.63 | - | - | 0.36 ^{***} | 0.23 ^{***} | 0.02 ^o | 0.02 ^o | - |
| Fe | 0 | 0.92 | 0.05 ^{***} | 0.01 ^{**} | 0.01 [*] | 0.32 ^{***} | 0.01 [*] | 0.40 ^{***} | 0.12 ^{***} |
| | 1 | 0.89 | 0.05 ^{***} | 0.01 ^{**} | - | 0.38 ^{***} | - | 0.34 ^{***} | 0.10 ^{***} |
| | 2 | 0.95 | 0.04 ^{***} | 0.04 ^{***} | 0.01 ^{***} | 0.36 ^{***} | - | 0.43 ^{***} | 0.06 ^{***} |
| | 3 | 0.92 | 0.02 ^{***} | 0.02 ^{***} | 0.05 ^{***} | 0.37 ^{***} | - | 0.42 ^{***} | 0.05 ^{***} |
| Mn | 0 | 0.93 | - | 0.83 ^{***} | - | 0.08 ^{***} | - | - | 0.03 ^{***} |
| | 1 | 0.94 | - | 0.82 ^{***} | - | 0.10 ^{***} | <0.01 ^o | - | 0.02 ^{***} |
| | 2 | 0.95 | - | 0.85 ^{***} | - | 0.09 ^{***} | <0.01 ^o | - | <0.01 [*] |
| | 3 | 0.97 | - | 0.90 ^{***} | - | 0.05 ^{***} | 0.01 ^{***} | - | <0.01 ^o |
| Zn | 0 | 0.89 | 0.01 ^{***} | 0.74 ^{***} | <0.01 ^o | 0.13 ^{***} | - | 0.01 [*] | - |
| | 1 | 0.96 | 0.01 ^{***} | 0.86 ^{***} | - | 0.07 ^{***} | - | 0.01 ^{***} | <0.01 [*] |
| | 2 | 0.94 | 0.01 ^{**} | 0.88 ^{***} | - | 0.05 ^{***} | - | <0.01 [*] | - |
| | 3 | 0.91 | 0.01 [*] | 0.82 ^{***} | - | 0.07 ^{***} | 0.02 ^{***} | 0.01 ^{**} | - |

⁽¹⁾Exch = exchangeable fraction, OM = linked to organic matter fraction, MnOx = occluded in Mn oxides fraction, AFeOx = occluded in amorphous Fe oxides fraction, CFeOx = occluded in crystalline Fe oxides fraction, Res = occluded in residual Fe oxides, Al, caulinite fraction. ⁽²⁾ Metal = soil metal; ⁽³⁾ Cycles: 0 = before corn growth; 1, 2, 3 = after 1st, 2nd and 3rd growth cycles, respectively. ⁽⁴⁾ ^o, ^{*}, ^{**}, ^{***} = significant at the 10, 5, 1 and 0,1 % levels; Stepwise Regression Analysis.

The soil acidity affected more the Fe extracted with DTPA and Mehlich-1 as compared to Mn, Zn and Cu (Tables 4 and 5). Agreeing with findings of Shuman (1986), the variation of soil pH influenced more the Fe DTPA extraction (26 to 58%) than the Mehlich-1 extraction (2 to 5 %) (Table 5). Furthermore, the DTPA action on more labile fractions (Fe-Exch, Fe-OM, Fe-MnOx and Fe-AFe-Ox) extracted smaller amounts (maximum of 13 %), with very close values among the fractions (Table 5). This confirms the DTPA's slighter action and lesser aggressiveness when attacking more labile fractions (Lindsay & Norvell, 1978). Differently, Mehlich-1 is more aggressive

(Rajj & Bataglia, 1991; Oliveira, Novais, Neves, Vasconcelos, & Alves, 1999; Menezes, Dias, Neves, & Silva, 2010), attacking more stable fractions (MnOx, CFeOx and Res) and extracting bioavailable Fe at values reaching 40 % (Table 5). It is worth mentioning that the intense acidity of Mehlich-1 solubilizes significant parts of the MnOx and FeOx fractions, in such a way that some of the cationic micronutrients extracted from these fractions may not be available for plants (Shuman, 1986). The highest concentration of Fe in the residual fraction, unavailable to plants in a nutrient poor forest soil, shows the strong retention of Fe by the soil (Walna et al., 2010).

For Mn, both DTPA and Mehlich-1 attacked the exchangeable (Mn-Ex) and occluded in Mn oxides (Mn-MnOx) fractions (Tables 4 and 5), suggesting that Mn availability depends on the equilibrium involving soil Mn oxides, in crystalline or amorphous forms (Lindsay, 1979). However, in accordance with Shuman (1986) and Nascimento, Fontes and Neves (2002a), it was observed that Mn availability predominated in the exchangeable fraction (Mn-Exch), reaching 87 to 93 % (DTPA) and 82 to 90 % (Mehlich-1) (Table 5). The Mn-MnOx fraction had lower contribution to Mn availability (2 to 3 % for DTPA and 5 to 10 % for Mehlich-1) (Table 5).

Both DTPA and Mehlich-1 extracted Zn similarly, attacking mainly the element in the exchangeable form (Tables 5 and 6). The DTPA extracted 80 to 96 % of available Zn from the exchangeable fraction, whereas Mehlich-1 extracted 74 to 88 % (Table 5). Secondly, the available Zn came, to a smaller extent, from the forms occluded in the Mn oxides (1 to 5 % with DTPA and 5 to 13 % with Mehlich-1) (Table 5), showing the stronger action of Mehlich-1 than DTPA over the Mn-Ox fraction. Those results show that the exchangeable Zn is the main component for the soil Zn availability, confirming data from Shuman (1986), Sims (1986), Oliveira et al. (1999) and Nascimento, Fontes and Neves (2002b). The retention of Zn in the soil-clay exchange complex is the main contributor for the maintenance of Zn in the exchangeable fraction. The soil clay content is the characteristic having the best correlation with the Zn adsorption processes in soils (Nascimento & Fontes, 2004). Although Shuman

(1985) reported that Zn is located mainly in the residual fraction and in the crystalline Fe oxides fraction, the Zn extracted with DTPA came mainly from the exchangeable fraction (Tables 5 and 6). Probably, the DTPA does not attack the more stable forms of soil Zn, which does not contribute for Zn availability. What happens is the chelation of soil Zn^{2+} by DTPA, decreasing the Zn^{2+} concentration in soil solution to very low rates (Lindsay & Norvell, 1978). This causes an equilibrium displacement with Zn moving from the exchangeable fraction to the soil solution and from the less labile fractions to the more labile fractions, until it is established a saturation equilibrium with the excess of DTPA in the suspension. However, this equilibrium is reached only with 10 mmol dm^{-3} Zn in the soil (Lindsay & Norvell, 1978), a concentration much higher than the total amount of Zn found in most soils since the range reported in the literature has an overall mean of 0.85 mmol dm^{-3} for total Zn concentration (Alloway, 2008). The extraction with DTPA does not attack the more stable forms of Zn, not extracted by the extractor. Differently, Mehlich-1 (pH 1.2) has an acidic strength that can solubilize forms of Zn strongly bound to the soil amorphous fractions. The more important fractions solubilized for Zn liberation were the exchangeable (Exch-Zn) and Mn oxides (Zn-MnOx), probably, confirming the high affinity between Zn and those soil fractions reported by Abreu, Ferreira, and Borkert (2001).

Correlations between the corn Cu, Fe, Mn, Zn contents and the available soil concentrations

The mean of available Cu for all soils was 0.64 mg/dm³ for DTPA extraction and 0.63 mg/dm³ for Mehlich-1 extraction (Table 6). According to Rajj, Cantarella, Quaggio and Furlani (1996), above 0.8 mg/dm³ Cu (DTPA extraction) is considered high availability, whereas Alvarez and Ribeiro (1999) describe 1.2 mg/dm³ Cu extracted by Mehlich-1 as the critical level of Cu for fertilizer recommendation. There was a moderate linear relationship ($r =$

0.48) between the Cu in the plant shoots and Cu in DTPA soil extracts. On the other hand, with Mehlich-1, the relationship was stronger ($r = 0.80$) (Table 7). In spite of available Cu being lower than the plant requirement, there was correlation between the bioavailable Cu (DTPA and Mehlich-1) and its content in the corn shoots. Plants absorbed Cu, mainly, from the MnOx and OM fractions (Tables 7 and 8), showing the importance of the soil organic matter as a Cu supplier for the plant.

Table 6
Concentrations of Cu, Fe, Mn and Zn in soil ⁽¹⁾: DTPA and Mehlich-1 extractions (mean from the four determinations for extractions made after the growth cycles ⁽²⁾)

| | | Soil ⁽¹⁾ | | | | | | | | | | | | Mean |
|-------------------------------|--------|---------------------|--------|--------|-------|--------|-------|-------|-------|-------|-------|-------|-------|--------------|
| RYO-1 | INC-1 | RYO-2 | oINC | INC-2 | RYO-3 | FENT | RYO-4 | RO-1 | RO-2 | RO-3 | RYO-5 | RO-4 | dyRO | |
| -----mg/dm ³ ----- | | | | | | | | | | | | | | |
| <u>Cu - DTPA</u> | | | | | | | | | | | | | | |
| 0.39 | 0.40 | 0.22 | 0.22 | 0.49 | 0.45 | 1.99 | 0.26 | 0.57 | 0.77 | 0.23 | 0.44 | 0.70 | 4.25 | 0.64 |
| <u>Cu - Mehlich-1</u> | | | | | | | | | | | | | | |
| 0.34 | 0.38 | 0.16 | 0.15 | 0.79 | 0.52 | 2.60 | 0.06 | 0.48 | 0.67 | 0.06 | 0.37 | 0.34 | 6.38 | 0.63 |
| <u>Fe - DTPA</u> | | | | | | | | | | | | | | |
| 60.83 | 57.15 | 52.35 | 51.45 | 43.81 | 28.68 | 18.46 | 32.45 | 50.87 | 63.00 | 31.78 | 69.73 | 40.00 | 20.25 | 41.28 |
| <u>Fe - Mehlich-1</u> | | | | | | | | | | | | | | |
| 82.03 | 101.88 | 116.18 | 116.03 | 134.18 | 46.14 | 20.70 | 77.20 | 77.13 | 54.72 | 39.47 | 94.70 | 30.13 | 38.87 | 64.46 |
| <u>Mn - DTPA</u> | | | | | | | | | | | | | | |
| 0.91 | 1.46 | 3.71 | 1.81 | 1.99h | 5.21d | 109.02 | 1.05 | 2.98 | 2.74 | 1.31 | 2.99 | 13.98 | 24.92 | 4.30 |
| <u>Mn - Mehlich-1</u> | | | | | | | | | | | | | | |
| 0.79 | 1.53 | 4.01 | 2.23 | 2.54 | 7.58 | 121.41 | 1.06 | 3.87 | 3.37 | 1.37 | 4.12 | 24.05 | 35.11 | 5.23 |
| <u>Zn - DTPA</u> | | | | | | | | | | | | | | |
| 2.49 | 1.42 | 0.70 | 0.56 f | 1.42 | 0.87 | 1.76 | 0.60 | 0.56 | 0.76 | 0.49 | 0.59 | 0.54 | 0.54 | 0.88 |
| <u>Zn - Mehlich-1</u> | | | | | | | | | | | | | | |
| 5.44 | 3.02 | 1.43 | 1.50 | 2.98 | 2.00 | 4.16 | 2.11 | 1.34 | 2.04 | 1.21 | 1.14 | 1.72 | 1.28 | 2.06 |

⁽¹⁾ RYO-1 = Red-Yellow Oxisol; INC-1 = Inceptisol; RYO-2 = Red-Yellow Oxisol; oINC = Oxisolic Inceptisol; INC-2 = Inceptisol; RYO-3 = Red-Yellow Oxisol; FENT = Fluvic Entisol; RYO-4 = Red-Yellow Oxisol; RO-1 = Red Oxisol; RO-2 = Red Oxisol; RO-3 = Red Oxisol; RYO-5 = Red-Yellow Oxisol; RO-4 = Red Oxisol; dyRO = Dystriferic Red Oxisol.

⁽²⁾ Soil extractions: 1st: before corn cultivation; 2nd: after the 1st corn cultivation; 3rd: after the 2nd cultivation; 4th: after 3rd cultivation.

Table 7

Linear correlation coefficients⁽¹⁾ of the regression equations between the contents ($\mu\text{g}/\text{pot}$) of Cu, Fe, Mn, and Zn in corn plants in three consecutive growth cycles and the concentrations of Cu, Fe, Mn and Zn in the soil fractions (sequential extraction) and in soils by extraction with DTPA and M⁻¹

| Variable ⁽³⁾ | Linear correlation coefficients | | | |
|-------------------------|---------------------------------|------------------|------------------|----------------------|
| | 1 ⁽⁴⁾ | 2 ⁽⁵⁾ | 3 ⁽⁶⁾ | Total ⁽⁷⁾ |
| ----- Cu ----- | | | | |
| Cu-OM | 0.31* | 0.39*** | 0.35*** | 0.35*** |
| Cu-MnOx | 0.65** | 0.66*** | 0.65*** | 0.67*** |
| Cu-AFeOx | -0.06 | -0.06 | -0.04 | -0.05 |
| Cu-CFeOxFe | -0.04 | -0.04 | -0.04 | -0.04 |
| Cu-Res | -0.16° | -0.17° | 0.16° | 0.17° |
| Cu - DTPA | 0.42*** | 0.51*** | 0.71*** | 0.48*** |
| Cu - M-1 | 0.74*** | 0.84*** | 0.79*** | 0.80*** |
| ----- Fe ----- | | | | |
| Fe-Exch | 0.17° | 0.37*** | 0.08 | 0.26* |
| Fe-OM | 0.04 | 0.67*** | 0.37*** | 0.39*** |
| Fe-MnOx | 0.13 | 0.05 | 0.06 | 0.11 |
| Fe-AFeOx | 0.45*** | 0.46*** | 0.58*** | 0.60*** |
| Fe-CFeOx | 0.43*** | 0.24* | 0.64*** | 0.53*** |
| Fe-Res | -0.06 | 0.21* | 0.42*** | 0.16° |
| Fe - DTPA | -0.17° | 0.16° | -0.16° | 0.05 |
| Fe - M-1 | 0.01 | -0.07 | -0.32** | -0.16° |
| ----- Mn ----- | | | | |
| Mn-Exch | 0.73*** | 0.79*** | 0.78*** | 0.80*** |
| Mn-OM | 0.26* | 0.37*** | 0.46*** | 0.38*** |
| Mn-MnOx | 0.59*** | 0.76*** | 0.77*** | 0.74*** |
| Mn-AFeOx | 0.49*** | 0.69*** | 0.68*** | 0.65*** |
| Mn-CFeOx | 0.43*** | 0.55*** | 0.49*** | 0.51*** |
| Mn-Res | -0.05 | 0.10 | 0.21* | 0.10 |
| Mn-DTPA | 0.77*** | 0.86*** | 0.84*** | 0.84*** |
| Mn-M-1 | 0.86*** | 0.94*** | 0.89*** | 0.95*** |

continue...

contuation...

| | -----Zn----- | | | |
|----------|--------------------|---------|---------|--------------------|
| Zn-Exch | 0.89*** | 0.79*** | 0.89*** | 0.91*** |
| Zn-MO | 0.71*** | 0.72*** | 0.77*** | 0.76*** |
| Zn-MnOx | 0.56*** | 0.68*** | 0.59*** | 0.62*** |
| Zn-AFeOx | -0.09 | 0.00 | -0.11 | -0.08 |
| Zn-CFeOx | -0.21 [†] | 0.05 | -0.12 | -0.15 [°] |
| Zn-Res | 0.53*** | 0.67*** | 0.60*** | 0.60*** |
| Zn-DTPA | 0.88*** | 0.84*** | 0.90*** | 0.92*** |
| Zn-M-1 | 0.81*** | 0.87*** | 0.86*** | 0.87*** |

⁽¹⁾ °, †, ** and ***: significant at 10, 5, 1 and 0,1% levels, respectively, by the F test; Stepwise Regression Analysis. ⁽²⁾ Excluding FENT, RO-4, and dyRO soils. ⁽³⁾ Metal-soil fraction: Exch = exchangeable; OM = linked to organic matter; MnOx = occluded in Mn oxides; AFeOx = occluded in amorphous Fe oxides; CFeOx = occluded in crystalline Fe oxides; Res = occluded in residual Fe oxides, in Al, and in caulinite; Metal-DTPA = DTPA extraction; Metal-MEHLICH-1 = Mehlich-1 extraction. ⁽⁴⁾ ⁽⁵⁾ ⁽⁶⁾ ⁽⁷⁾ Metals in shoots: 1, 2, 3 = 1st, 2nd, and 3rd cycles, respectively; Total = summation of 1, 2, and 3.

The relationships between Fe in the plant shoots and in soil extracts from DTPA and Mehlich-1 extractions were very weak ($r = 0.05$ and -0.16 respectively) (Table 7). The mean of available Fe was 41.28 mg/dm^3 (DTPA extraction) and 64.46 mg/dm^3 (Mehlich-1 extraction) (Table 6). According Raij et al. (1996), above 12 mg/dm^3 in soil (DTPA) the Fe availability is high, whereas for Alvarez and Ribeiro (1999) 30 mg/dm^3 is the critical level (Mehlich-1). This confirms that most Brazilian soils have adequate available Fe and deficiency is rare or does not exist (Borkert, Pavan, & Bataglia, 2001). In the fractions, Fe was present mainly as Fe-AFeOx, Fe-CFeOx and Fe-OM (Table 7). Although there was no clear pattern for the relationship between Fe in the corn shoots and Fe in soil fractions, the root uptake suggests that Fe is originated mainly from the AFeOx and CFeOx fractions (Tables 7 and 8).

The relationship for Mn content in shoots and concentration in DTPA (pH 7.3) extracts ($r = 0.84$) and M-1 soil extracts ($r = 0.95$) were strong (Table 7). DTPA-pH 7.3 extracted 4.30 mg/dm^3 Mn, 86 % of the limit (5.0 mg/dm^3) for fertilizer recommendation (Raij et al., 1996) and M-1 extracted 5.23 mg/dm^3 Mn, 66 % of the critical level for plants (8.0 mg/dm^3) (Alvarez & Ribeiro, 1999). However, according to Joshi et al. (2017), DTPA-pH 7.3 was not satisfactory to determine Mn availability in acidic soils, indicating that DTPA-pH 5.3 is better. In the present work, the plants absorbed Mn mainly from Exch and MnOx fractions (Tables 7 and 8). Joshi et al. (2017) found that in acidic soils, the retention of Mn occurred predominantly in the residual fraction, but a significant and positive correlation with Mn plant uptake and Mn concentration in maize plant tissues occurred in the water soluble and exchangeable Mn fractions.

Table 8

Regression equations between the contents of Cu, Fe, Mn, and Zn in corn shoots, in µg/pot, and the concentrations of Cu, Fe, Mn, and Zn in the soil fractions, and soil pH, in the corn growth cycles

| Metal ⁽¹⁾ -Cycle ⁽²⁾ | Regression equation ⁽³⁾ | R ² |
|---|---|----------------|
| ----- Metal in corn plants (µg/pot) as a function of metal in soil chemical fractions (mg/kg) ----- | | |
| Cu - 1 | $\hat{Y} = -90.10 + 27.60^{***}OM + 18.37^{***}MnOx - 3.33^{*}AFeOx + 18.67^{*}pH$ | 0.55 |
| Cu - 2 | $\hat{Y} = 7.15 + 28.90^{***}OM + 5.10^{***}MnOx - 3.16^{***}AFeOx - 2.47^{**}CFeOx - 0.20^{**}Res$ | 0.73 |
| Cu - 3 | $\hat{Y} = -57.66 + 19.20^{***}OM + 11.27^{***}MnOx - 2.30^{*}AFeOx + 11.63^{*}pH$ | 0.61 |
| Cu - total | $\hat{Y} = -192.26 + 68.43^{***}OM + 38.24^{***}MnOx - 0.69^{**}AFeOx + 39.43^{**}pH$ | 0.65 |
| Fe - 1 | $\hat{Y} = 657 + 37.28^{*} + 0.51^{***}AFeOx + 0.03^{***}CFeOx - 0.03^{***}Res$ | 0.46 |
| Fe - 2 | $\hat{Y} = 5638 + 47.15^{***}Exch + 88.52^{***}OM - 12.15^{**}MnOx + 0.54^{***}AFeOx - 0.01^{*}Res - 1096^{**}pH$ | 0.66 |
| Fe - 3 | $\hat{Y} = 303 + 50.61^{***}OM + 0.02^{***}CFeOx$ | 0.53 |
| Fe - total | $\hat{Y} = 13120 + 110.77^{**}Exch - 44.58^{***}MnOx + 2.33^{***}AFeOx - 0.02^{*}Res - 2017^{**}pH$ | 0.57 |
| Mn - 1 | $\hat{Y} = -52.90 + 151.73^{***}Exch + 39.17^{***}CFeOx - 3.26^{**}Res$ | 0.63 |
| Mn - 2 | $\hat{Y} = -401.25 + 75.79^{*}Exch + 224.53^{***}MnOx$ | 0.76 |
| Mn - 3 | $\hat{Y} = -686.36 + 118.51^{***}Exch + 397.73^{***}MnOx$ | 0.75 |
| Mn - total | $\hat{Y} = -1043.50 + 322.10^{***}Exch + 915.02^{***}MnOx - 5.05^{*}Res$ | 0.77 |
| Zn - 1 | $\hat{Y} = 141.16 + 638.17^{***}Exch$ | 0.79 |
| Zn - 2 | $\hat{Y} = -121.08 + 45.18^{*}Exch + 129.96^{**}OM + 38.25^{**}MnOx + 3.34^{***}Res$ | 0.74 |
| Zn - 3 | $\hat{Y} = -33.90 + 161.62^{***}Exch + 131.76^{**}OM + 2.27^{**}Res$ | 0.82 |
| Zn - total | $\hat{Y} = 219.79 + 959.54^{***}Exch + 98.47^{*}MnOx$ | 0.84 |

| Metal | Cycle | R ² (total) | R ² (partial) | | | | | | |
|---------------------------------|-------|---------------------------|--------------------------|---------------------|---------------------|---------------------|----------------------|----------------------|--------------------|
| | | | pH - H ₂ O | Exch ⁽⁴⁾ | OM ⁽⁵⁾ | MnOx ⁽⁶⁾ | AFeOx ⁽⁷⁾ | CFeOx ⁽⁸⁾ | Res ⁽⁹⁾ |
| ----- Plant metal content ----- | | | | | | | | | |
| Cu | 1 | 0.58 | 0.07 ^{**} | - | 0.06 ^{***} | 0.42 ^{***} | 0.03 [*] | - | - |
| | 2 | 0.74 | - | - | 0.11 ^{***} | 0.44 ^{***} | 0.14 ^{***} | 0.02 [*] | 0.03 ^{**} |
| | 3 | 0.61 | 0.07 ^{**} | - | 0.08 ^{***} | 0.42 ^{***} | 0.03 [*] | - | - |
| | Total | 0.65 | 0.04 ^{**} | - | 0.08 ^{***} | 0.45 ^{***} | 0.08 ^{**} | - | - |
| Fe | 1 | 0.46 | - | 0.02 [°] | - | - | 0.20 ^{**} | 0.11 ^{**} | 0.13 ^{**} |
| | 2 | 0.66 | 0.02 [*] | 0.07 ^{**} | 0.45 ^{***} | 0.02 [*] | 0.06 ^{**} | - | 0.03 [*] |
| | 3 | 0.53 | - | - | 0.12 ^{***} | - | - | 0.41 ^{***} | - |
| | Total | 0.57 | 0.02 [°] | 0.06 ^{**} | - | 0.09 ^{***} | 0.37 ^{***} | - | 0.03 [°] |

continue...

contuation...

| | | | | | | | | | |
|----|-------|------|---|---------------------|---|---------------------|---|--------------------|--------------------|
| Mn | 1 | 0.63 | - | 0.53 ^{***} | - | - | - | 0.04 ^{**} | 0.06 ^{**} |
| | 2 | 0.76 | - | 0.62 ^{***} | - | 0.13 ^{***} | - | - | - |
| | 3 | 0.75 | - | 0.60 ^{***} | - | 0.15 ^{***} | - | - | - |
| | Total | 0.77 | - | 0.64 ^{***} | - | 0.11 ^{***} | - | - | 0.02 [*] |

| | | | | | | | | | |
|----|-------|------|---|---------------------|---------------------|--------------------|---|---|---------------------|
| Zn | 1 | 0.79 | - | 0.79 ^{***} | - | - | - | - | - |
| | 2 | 0.77 | - | 0.63 ^{***} | 0.05 ^{***} | 0.03 ^{**} | - | - | 0.06 ^{***} |
| | 3 | 0.82 | - | 0.79 ^{***} | 0.01 [*] | - | - | - | 0.02 ^{**} |
| | Total | 0.84 | - | 0.84 ^{***} | - | 0.01 [°] | - | - | - |

(1) Metal in shoots; (2) 1, 2, 3 = 1st, 2nd, and 3rd growth cycles, respectively and total = summation of 1, 2, and 3. (3) °, *, ** and *** = significant at 10, 5, 1 and 0,1% levels by the F test, respectively, Stepwise Regression Analysis. (4) Exchangeable; (5) Linked to organic matter; (7) Occluded in Mn oxides; (8) Occluded in amorphous Fe oxides; (9) Occluded in crystalline Fe oxides; (10) Occluded in residual Fe oxides, Al, and kaolinite.

Zinc showed strong relationship between the content in corn shoots and concentrations in DTPA ($r = 0.92$) and Mehlich-1 extracts ($r = 0.87$) (Table 7). The mean of Zn concentration in DTPA extracts was 0.88 mg dm^{-3} , 73 % of 1.2 mg/dm^3 , which is a reference limit for high soil concentrations (Rajj et al., 1996). With Mehlich-1, the mean Zn concentration for all soils was 2.06 mg/dm^3 , which reaches 137 % of 1.5 mg/dm^3 , the reported critical level for fertilizer application (Alvarez & Ribeiro, 1999). Zinc uptake by corn occurred mainly from the forms present in the exchangeable fraction (Tables 7 and 8). In rice and soybean, increased Zn concentration in the labile fraction exchangeable Zn showed high positive correlation with total Zn accumulated in the plants (Leite et al., 2019).

For Cu and Mn, the correlation between the available metal extracted from soil and the metal content in the plant was higher with Mehlich-1 than with DTPA, whereas for Zn the opposite relationship occurred (Table 7). This suggests that Mehlich-1 is better for predicting the Cu and Mn availability for plants and DTPA

is better for the prediction of Zn availability. For Fe there was no correlation.

There is a dynamic system keeping the cationic available forms in equilibrium with the soil solution soluble forms, from which the plants absorb the nutrients (Barber, 1995; Marschner, 2011). The soil fractions retaining the cationic micronutrients may constitute a reservoir for feeding the plant roots and contribute to plant growth.

Concentrations of Cu, Fe, Mn, and Zn in the corn shoots and dry matter yield as related the growth cycles

Going from the 1st cultivation cycle to the 2nd and the 3rd, the micronutrient concentrations in the corn shoots decreased in all treatments, except for Fe (Table 9). Only for Cu, shoot concentrations were below the range considered adequate for corn, according to criteria described by Bull (1993). This is in agreement with the observed means of available Cu for all soils, 0.64 mg dm^{-3} (DTPA)

and 0.63 mg dm^{-3} (Mehlich-1) (Table 6), which are below 0.8 mg dm^{-3} Cu (DTPA extraction) and 1.2 mg dm^{-3} (Mehlich-1 extraction), described as reference levels for fertilizer recommendation (Raji, Cantarella, and Furlani, 1996); Alvarez & Ribeiro, 1999). Based on the Bull's criterion, Fe, Mn and Zn concentrations in shoots were within, or above, the adequate range for corn (Table 9), indicating that, for them, the soil availability was satisfactory, with shoot concentration sufficiency maintained along the consecutive cultivation cycles. Contrary to what happened for Cu, the soil available forms of Fe, Mn, and Zn, were enough for supplying them along the growth cycles, keeping the shoot concentrations at adequate levels. Due to small differences found between the Mehlich-1 and DTPA extractions, it is suggested that both are efficient in the prognosis of the soil fertility status for corn in the studied soils confirming data from Raji and Bataglia (1991), Sims and Johnson (1991), Lopes and Abreu (2000), Abreu et al. (2001), and Borkert et al. (2001).

The mean shoot dry matter yield for the corn plants without micronutrient addition was 9.3 g, similar to that with addition of Cu, Fe, Mn, and Zn together (10.3 g) and to the dry matter produced with Cu, Fe, Mn, and Zn added individually, without the others (9.6 g). The variation among the growth cycles showed a pattern with the mean dry matter in the 1st cycle (9.3-10.6 g) decreasing to 7.1-8.3 g in the 2nd, and increasing again to 10.5-12.9 g in the 3rd cycle (Table 10). The variation in dry weight was due to the adjustment of P, K, S and Mg supplying to the plants along the three growth cycles. The initial concentrations of Fe, Mn, and Zn in the soils were enough for keeping the concentrations in the corn shoots at sufficient levels. Although Cu concentrations were below the adequate range, the dry matter production with treatments with Cu was not clearly affected as compared to the observed for treatments with Fe, Mn, and Zn.

Table 9

Concentrations (mg kg^{-1}) and contents ($\mu\text{g/vaso}$) of Cu, Fe, Mn, and Zn in shoots of corn plants (means from plants in all soils, in each treatment), after cultivation in three consecutive growth cycles

| Plant Analysis | Treatment 1 (without micronutrient addition to soil) | | | |
|---|--|-------------------------------|------------------------------|------------------------------|
| | Micronutrient concentration in corn shoots | | | |
| | Cu | Fe | Mn | Zn |
| | ----- mg kg^{-1} ($\mu\text{g/vaso}$) ----- | | | |
| After 1 st Cycle | 1.5 (12) | 200 (1,842) | 152 (1,440) | 30 (283) |
| After 2 nd Cycle | 0.9 (5.2) | 203 (1,454) | 110 (782) | 23 (163) |
| After 3 rd Cycle | 0.8 (6.7) | 123 (1,356) | 104 (1,133) | 17(183) |
| | Treatments with addition of a single micronutrient | | | |
| | Treatment 3 | Treatment 4 | Treatment 5 | Treatment 6 |
| | (2.0 mg dm^{-3} Cu) | (10.0 mg dm^{-3} Fe) | (5.0 mg dm^{-3} Mn) | (5.0 mg dm^{-3} Zn) |
| | Micronutrient concentration in corn shoots | | | |
| | Cu | Fe | Mn | Zn |
| | ----- mg kg^{-1} ($\mu\text{g/vaso}$) ----- | | | |
| After 1 st Cycle | 3.2 (31.7) | 284 (2,635) | 232 (2,443) | 80 (844) |
| After 2 nd Cycle | 2.1 (14.7) | 269 (2,073) | 168 (1,210) | 49 (347) |
| After 3 rd Cycle | 1.9 (22.00) | 138 (1,437) | 165 (1,735) | 37 (160) |
| | Treatment 7 - with addition of all micronutrient | | | |
| | Micronutrient concentration in corn shoots | | | |
| | Cu | Fe | Mn | Zn |
| | ----- mg kg^{-1} ($\mu\text{g/vaso}$) ----- | | | |
| After 1 st Cycle | 2.69 (25) | 210 (2,040) | 237 (2,307) | 61 (598) |
| After 2 nd Cycle | 1.80 (13.1) | 217 (1,805) | 170 (1,410) | 38 (315) |
| After 3 rd Cycle | 1.43 (16.1) | 102 (1,311) | 168(2.160) | 26 (333) |
| Adequate concentration for corn (Bull, 1993) | 6-20 | 50-250 | 42-150 | 15-50 |

Table 10

Shoot dry matter (means from plants in all soils, in each treatment) of plants grown in soils with addition of Cu (2.0 mg dm⁻³), Fe (10.0 mg dm⁻³), Mn (5.0 mg dm⁻³), and Zn (5.0 mg dm⁻³), after cultivation in three consecutive growth cycles

| Plant Analysis | Treatment 1 (No micronutrient added) | | | |
|-----------------------------|--|-----------------------------|----------------------------|----------------------------|
| | Dry matter (g) | | | |
| After 1 st Cycle | 9.5 | | | |
| After 2 nd Cycle | 7.2 | | | |
| After 3 rd Cycle | 11.1 | | | |
| | Treatments with addition of a single micronutrient | | | |
| | Treatment 3 | Treatment 4 | Treatment 5 | Treatment 6 |
| | 2.0 mg dm ⁻³ Cu | 10.0 mg dm ⁻³ Fe | 5.0 mg dm ⁻³ Mn | 5.0 mg dm ⁻³ Zn |
| | Dry matter (g) | | | |
| After 1 st Cycle | 10.3 | 9.3 | 10.6 | 10.5 |
| After 2 nd Cycle | 7.85 | 7.7 | 7.2 | 7.1 |
| After 3 rd Cycle | 12.71 | 10.5 | 10.6 | 10.6 |
| | Treatment 7 (All micronutrient added - mg dm ⁻³ - 2.0 Cu 10.0 Fe 5.0 Mn 5.0 Zn) | | | |
| | Dry matter (g) | | | |
| After 1 st Cycle | 9.8 | | | |
| After 2 nd Cycle | 8.3 | | | |
| After 3 rd Cycle | 12.9 | | | |

Conclusions

In the studied weathered soils, the soil organic matter is the main contributor for the Cu available for plants. For Fe and Mn, the cations available for plant uptake are supplied mainly for the forms bound to the exchangeable fraction which is the main contributor for the readily available pool of them. Zinc also has its available forms for the plants provided mainly by the exchangeable fraction.

Prediction of cationic micronutrients supplying for plants was better for Cu and Mn with Mehlich-1 extraction, and, for Zn, with DTPA extraction, whereas for Fe there

was no correlation. Correlations of available concentrations of Cu, Mn, and Zn extracted with Mehlich-1 and DTPA were positive with the contents of Cu, Mn and Zn determined in plants.

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