UNIVERSIDADE FEDERAL DE UBERLÂNDIA
MESTRADO ACADÊMICO EM AGRONOMIA

CAIO CÉSAR SALES GOMES

SENSITIVITY OF CATIONIC MICRONUTRIENT EXTRACTORS TO pH ELEVATION IN CERRADO SOILS

UBERLÂNDIA
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Dissertação apresentada à Universidade Federal de Uberlândia, como parte das exigências do Programa de Pós-Graduação em Agronomia.

Orientador: Prof. Dr. Wedisson Oliveira Santos

UBERLÂNDIA
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UNIVERSIDADE FEDERAL DE UBERLÂNDIA
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RESUMO

A aplicação de corretivos de acidez nos solos redistribui espécies geoquímicas de Zn, Cu, Mn e Fe entre frações disponíveis e indisponíveis para as plantas, por meio de processos afetados pela variação de pH, como precipitação por hidrólise, dispersão de matéria orgânica, dinâmica de adsorção-dessorção e alterações nas propriedades interfaciais dos coloides. No entanto, a sensibilidade dos extratores químicos tradicionais a essas mudanças geoquímicas não está suficientemente elucidada, especialmente em sistemas altamente intemperizados. Este estudo teve como objetivo avaliar a sensibilidade de diferentes extratores ao aumento do pH do solo induzido pela calagem e identificar mudanças nas espécies químicas de micronutrientes catiônicos. O delineamento experimental seguiu um esquema fatorial 2 × 7, envolvendo dois tipos de solo representantes das principais classes pedogenéticas do Brasil (Latossolo e Neossolo) e sete níveis de correção com $CaCO_3$ (0-, 0,25-, 0,50-, 1,0-, 1,5-, 2,0- e 2,5-vezes o teor de H+Al dos solos). Os tratamentos foram dispostos em blocos casualizados com três repetições. Após a estabilização da acidez ativa do solo (pH), os teores de Cu, Fe, Mn e Zn foram determinados utilizando-se quatro extratores (Mehlich-1, Mehlich-3, KCl e DTPA). O comportamento dos extratores variou devido aos seus distintos mecanismos de acesso às espécies químicas. O Mehlich-1 mostrou-se insensível ao aumento do pH do solo pois é ácido e o pH da solução extratora não sofre mudanças expressivas após contato com o solo, levando à dissolução das espécies hidrolisados, enquanto o DTPA foi sensível a elevação do pH provavelmente porque suas constantes de complexação com os nutrientes não superaram as constantes de precipitação-dissolução, limitando a dissolução das espécies precipitadas. O KCl apresentou baixa eficiência na recuperação de Zn, Cu, Mn e Fe, provavelmente devido à predominância de espécies não trocáveis, o que o torna aparentemente insensível às mudanças de pH. O comportamento variável do Mehlich-3 decorre de seus múltiplos mecanismos de extração. Para avaliar a disponibilidade de nutrientes em curto prazo, o DTPA destacou-se como o extrator mais adequado para quantificar teores disponíveis de Zn, Cu, Mn e Fe para as plantas.

Palavras chave: fracionamento; métodos; micronutrientes; ph do solo.

ABSTRACT

The application of acidity correctors to soils redistributes geochemical species of Zn, Cu, Mn, and Fe between fractions available and unavailable to plants, through processes affected by pH variation, such as precipitation by hydrolysis, dispersion of organic matter, adsorption desorption dynamics and changes in the interfacial properties of colloids. However, the sensitivity of traditional chemical extractors to these geochemical changes has not been sufficiently elucidated, especially in highly weathered systems. The aim of this study was to evaluate the sensitivity of different extractors to the increase in soil pH induced by liming and to identify changes in the chemical species of cationic micronutrients. The experimental design followed a 2 × 7 factorial scheme, involving two types of soil representing the main pedogenetic classes in Brazil (Latossolo and Neossolo) and seven levels of correction with $CaCO_3$ (0-, 0.25-, 0.50-, 1.0-, 1.5-, 2.0- and 2.5-times the H+Al content of the soils). The treatments were arranged in randomized blocks with three replications. After stabilizing the active acidity of the soil (pH), the Cu, Fe, Mn and Zn contents were determined using four extractants (Mehlich-1, Mehlich-3, KCl and DTPA). The behavior of the extractants varied due to their different mechanisms for accessing the chemical species. Mehlich-1 proved to be insensitive to an increase in soil pH because it is acidic and the pH of the extracting solution does not undergo significant changes after contact with the soil, leading to the dissolution of hydrolyzed species, while DTPA was sensitive to an increase in pH, probably because its complexation constants with nutrients did not exceed the precipitation-dissolution constants, limiting the dissolution of precipitated species. KCl showed low efficiency in recovering Zn, Cu, Mn and Fe, probably due to the predominance of non-exchangeable species, which makes it apparently insensitive to pH changes. The variable behavior of Mehlich-3 stems from its multiple extraction mechanisms. To assess the availability of nutrients in the short term, DTPA stood out as the most suitable extractant for quantifying the available levels of Zn, Cu, Mn and Fe for plants.

Keywords: fractionation; methods; micronutrients; soil ph.

SUMÁRIO

1. INTRODUCTION

Liming is a cornerstone agronomic practice in tropical soil management, used to increase soil pH, neutralize phytotoxic aluminum (Al^{3+}) and hydrogen (H^+) ions, and supplement calcium (Ca^{2+}) and magnesium (Mg^{2+}). However, this alkaline perturbation induces profound geochemical shifts in available micronutrients (Fe, Zn, Cu, Mn), altering their speciation through hydrolysis (Alloway, 1990; Nascimento, 2001), redox reactions, and ligand complexation with hydroxyl, carbonate, or organic matter. Simultaneously, liming modifies colloidal charge density, disrupts organo-mineral aggregates, and re-equilibrates organometallic complexes. These physicochemical interactions—adsorption-desorption, precipitation-dissolution, and ligand displacement—collectively control nutrient phytoavailability and require precise analytical methods to assess their bioavailability (Nascimento, 2001).

Prediction of nutrient availability relies on correlations between soil extractables levels and plant uptake, but their low content and dynamic soil interactions complicate calibration (Bortolon, 2005). Traditional chemical extractants—dilute acids, chelating agents, and ion-exchange salts—target different nutrient pools via acid dissolution (e.g., Mehlich-1, HCl), ligand complexation (DTPA, EDTA), or cation displacement (KCl). Dilute acids solubilize exchangeable and weakly bound fractions (De Abreu et al., 1995; Haq; Miller, 1972; Silviera; Sommers, 1977), while chelators mimic root exudates by forming soluble complexes that desorb nutrients from solid phases (Haynes; Swift, 1983; Lantman, 1982; Lindsay; Norvell, 1978; Shuman; Anderson, 1974). Neutral salts selectively extract exchangeable cations without altering pH. Despite their widespread use, extractant efficacy varies with soil conditions; for example, Mehlich-1 is favored in Brazil for multi-element analysis (P, K, Zn, Cu, Mn and Fe) due to operational practicality, although its theoretical basis for cationic micronutrients remains controversial (Nascimento, 2001; Pickering; Shuman, 1981).

Critical limitations remain: acid-based methods run the risk of overestimating bioavailability by dissolving non-labile phases; when chelating agents are used, the relationship between equilibrium constants for the complexes formed and the solubility of chemical species remains poorly established (Haynes; Swift, 1983; Lantman; EJ, 1982; Lindsay & Norvell, 1978). DTPA may be of limited use in acidic soils because its alkaline buffering chelating capacity may be inadequate. The 0.1 M TEA buffer is nearly

80% neutralized at pH 7.3 and has little resistance to pH reduction. In highly acidic soils, the titratable acidity can neutralize almost all the conjugate base of the buffer, affecting the pH and compromising the extract value (Norvell, 1984). Extractants based on ion exchangers, such as KCl, may have limited extraction of cationic micronutrients because their available forms also include highly reactive precipitated species and chemically adsorbed forms (Santos, 2020).

The distribution of nutrients among soil fractions-soluble, exchangeable, adsorbed, or organically bound-requires advanced fractionation approaches. Sequential extraction protocols (Barreto et al., 2024; Tessier; Campbell; Bisson, 1979) classify nutrients into four operational pools: F1 (soluble/exchangeable), F2 (mobilizable by adsorption), F3 (bound to amorphous oxides), and F4 (organic complexes). Such fractionation methods are valuable for elucidating liming-induced changes in nutrient speciation and for validating the sensitivity of extractors to pH-driven transformations.

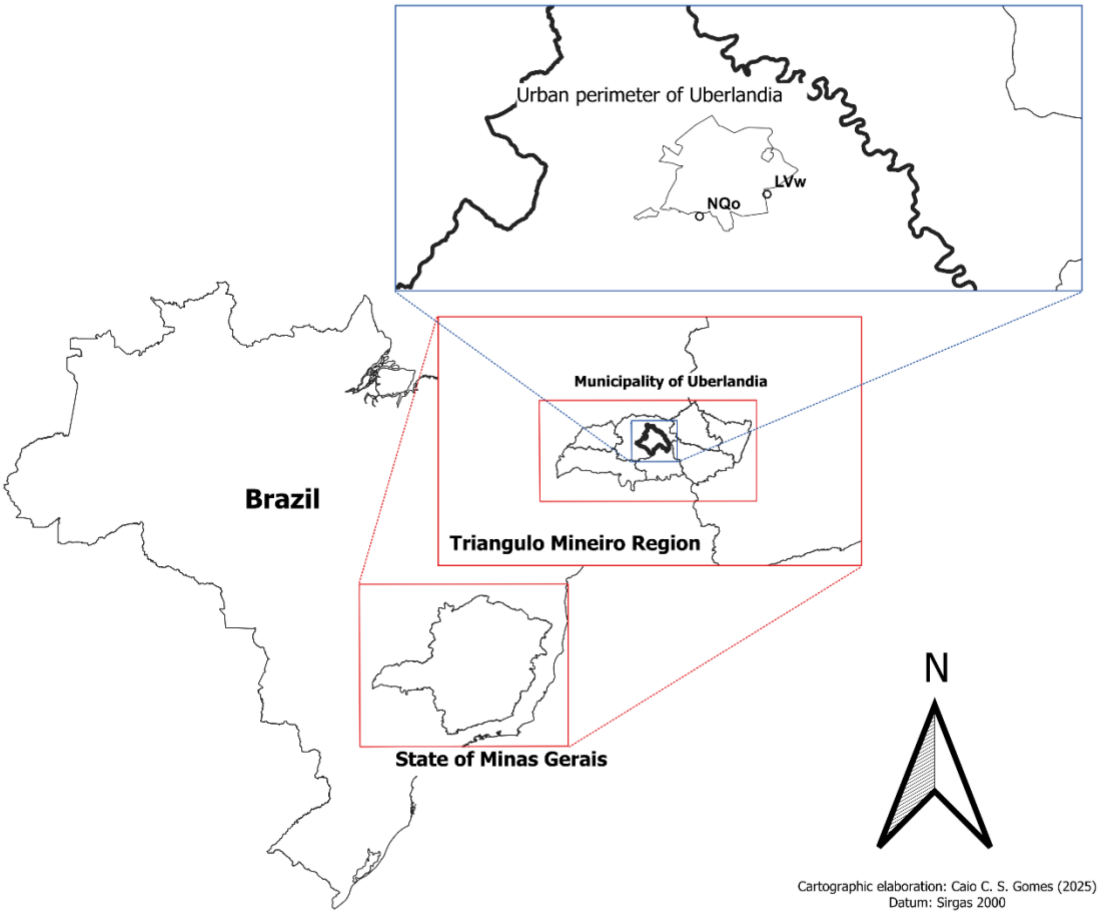
Despite these advances, the responsiveness of routine extractants to elevated pH in tropical soils remains poorly characterized. Misinterpretation of nutrient availability under liming can lead to incorrect fertilization strategies, exacerbating deficiencies or toxicities. This study bridges this gap by evaluating conventional extractants (Mehlich-1, DTPA, EDTA, KCl) under pH-altered conditions and correlating their extractability with geochemical speciation via sequential fractionation. By clarifying method limitations and mechanistic responses to alkalinity, this work aims to refine extractant selection or inspire novel protocols tailored to evolving soil management practices.

2. MATERIAL AND METHODS

2.1 Soil sampling and sample characterization

For the research, a representative amount of two typical soils of the Brazilian savannah, widely used for agriculture, was collected from the top layer 0-20 cm considering ten random sampling points. As shown in Figure 1, the selected areas are located in the municipality of Uberlândia, in the Triângulo Mineiro region, State of Minas Gerais, Brazil (Figure 1).

Figure 1- Location of sampling points for top samples (0-20 cm) of an Ferralsol (18°56'44.4"S 48°12'54.9"W) and an Arenosol (18°58'33.8"S 48°18'29.4"W) in the municipally of Uberlândia, Triângulo Mineiro region, State of Minas Gerais, Brazil.



Cartographic elaboration: Caio C. S. Gomes (2025)
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Note: Soil classification according to the World Reference Base for Soil Resources (WRB) – FR: Ferralsol and AR – Arenosol; Coordinates in geographic coordinate reference system.
Fonte: O próprio autor.

Soil samples were air-dried and sieved to obtain < 2 mm particles prior to chemical-physical characterization (Tables 1 and 2) and testing. The analyses were carried out at the facility of Soil and Plant Tissue Analysis Lab (LABAS) of the Federal University of Uberlândia, State of Minas Gerais, Brazil.

Table 1- Chemical characteristics of soil samples from the 0-20 cm layer in the Triângulo Mineiro region, Minas Gerais, Brazil.

Soil	**pH**	**P**	**K**	**Ca**	**Mg**	**Al**	**H+Al**	**rem-P**	**MO**
		....mg dm^{-3}...		...........cmol$_c$ dm^{-3}...............				mg L^{-1}	dag kg $^{-1}$
FR	4.7	3.3	115.2	2.8	1.1	0.0	2.8	20.9	1.6
AR	4.6	0.6	6.4	0.4	0.1	0.3	2.0	35.7	0.9

pH in water, ratio v/v 1/2.5; P, K- Mehlich-1 extractor; Ca^{2+}, Mg^{2+} and Al^{3+} (1.0 mol/L KCl extractor); H+Al in 0.5 mol/L calcium acetate, pH 7.0; MO (Organic Matter) - Colorimetric Method; S= Monocalcium Phosphate; rem-P= remaining P concentration of a 60 mg/L P solution in 0.01 mol/L $CaCl_2$; Soil classification according to the World Reference Base for Soil Resources (WRB) – FR: Ferralsol and AR - Arenosol.

Fonte: O próprio autor.

Table 2- Granulometric composition of soil samples from the 0-20 cm layer in the Triângulo Mineiro region, Minas Gerais, Brazil.

Soil	**Coarse Sand**	**Fine Sand**	**Silt**	**Clay**	**Textural classification***
	....g/kg.............................				
FR	118	67	179	636	Very Clayey
AR	316	480	58	146	Sandy

FR: Ferralsol and AR - Arenosol.

Fonte: O próprio autor.

FR has a history of pasture management, while the AR remains in its native state, with no history of agricultural intervention.

2.2 Experimentation

To promote variation in soil pH, a 2 × 7 factorial design was used with two soils (FR and AR) and seven liming levels based on their content of potential acidity content (0, 0.25, 0.5, 1.0, 1.5, 2.0 and 2.5 H+Al). Pure calcium carbonate salt was used as the liming material.

In triplicate, 1.5 kg soil samples were mixed with the corresponding $CaCO_3$ rates in plastic bags and kept at 80% of their water holding capacity until pH stabilization. During incubation, the bags were opened every three days to release the CO_2 produced by the neutralization reaction.

2.2.1 Chemical Characterization

The effect of soil pH on extractable nutrient content was measured using several methods. Air-dried and sieved soil samples (< 2 mm) were analyzed for available Zn, Cu, Mn and Fe contents using the Mehlich-1 (Mehlich, 1978), Mehlich-3 (Mehlich, 1984), DTPA (Lindsay & Norvell, 1978) and 1.0 mol/L KCl methods. In additon, to investigate the buffering effect of the soil on the extraction solution, pH measurements were taken for each extractor before and after interaction with the soil. Analytical procedures were performed as described by da Silva (2008), including chemical dosing by atomic absorption spectrophotometry.

In order to investigate the relationship between the nutrient contents of the different methods and their chemical species over pH variations, a subset of samples was speciated (Table 7), following the procedures developed by Tessier et al. (1979) and modified by Barreto et al. (2024). After a sequential extraction, four chemical species of the elements were obtained:

F1 - Soluble and exchangeable fraction;

F2 - Potentially mobilizable fraction, possibly adsorbed/associated with Fe, Al and Ca species;

F3 - Fraction bound to low crystallinity Al-Fe-Mn oxyhydroxides; and

F4 – Fraction associated with soil organic matter.

2.2.2 Statistical Approaches

The data were tested for a simple linear correlation analysis (Pearson's correlation) was performed to evaluate the relationships between:

Soil pH and the extractable nutrient content determined by the Mehlich-1 (M-1), Mehlich-3 (M-3), DTPA, and KCl methods;

The extractable nutrient content between the different methods; and

The extractable nutrient content obtained by these methods and the geochemical fractions (F1–F4).

Significance levels were set at 1% and 5% using a t-test. All analyses were conducted using R software and Microsoft Excel, while graphical representations were generated using SigmaPlot software.

3. RESULTS

3.1 Soil pH- Induced Changes in Extractable Micronutrients

Correlation data between the extractable Zn, Cu, Mn and Fe contents using the M-1, M-3, DTPA and KCl methods, and soil pH are presented in Figures 2 and 3. These operational extractors showed variable sensitivity to changes in soil pH. Specifically, a lack of correlation between extractable nutrient content and pH - indicating insensitivity - was observed in the Ferralsol (Figure 2) for DTPA (for Mn), KCl (for Mn, Zn and Fe), and M-3 (for Cu, Fe and Mn). In the Arenosol (Figure 3), M-1 and KCl showed no sensitivity to any of the nutrients, while DTPA was insensitive to Mn, and M-3 was insensitive to Fe, Mn, and Zn.

The extractant sensitivities showed clear trends and variable correlation strengths (Figures 2 and 3). In fact, significant negative correlations were observed between Cu, Zn and Fe DTPA contents and pH in Ferralsol, and between Cu and Fe and pH in Arenosol. In contrast, Zn-DTPA content in Arenosol showed a positive correlation with soil pH. For M-3, Zn in Ferralsol showed a negative correlation with pH, while Cu in Arenosol showed a positive correlation. A positive correlation between Cu-KCl contents and pH was observed in Ferralsol.

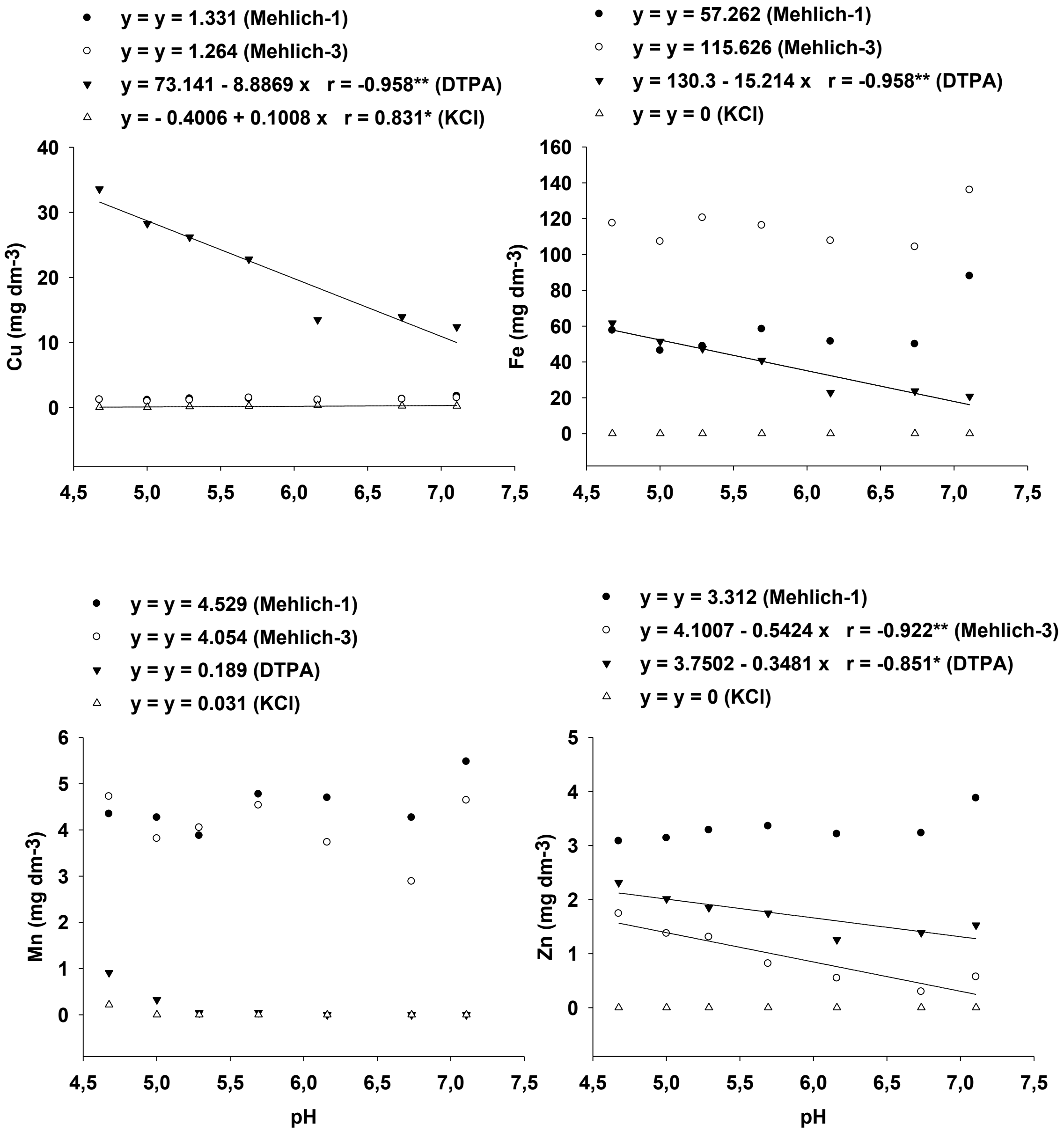
Observing the pH measurement results of the extractants before and after interaction with the soil (Table 8), it is evident that M-1, M-3 and DTPA were not affected by the buffering capacity of the soil, as their extracts showed a consistent behavior with minimal pH variation. In contrast, KCl was affected by soil pH, ranging from 4.71 to 7.08 in Ferralsol and from 4.63 to 7.46 in Arenosol, with the latter showing a wider range of values due to its lower buffering effect.

Disregarding the sensitivity of the extractants to soil pH variations, the extractability analysis showed that in clayey soil the overall range of Cu extracted was from <1 to almost 34 mg kg^{-1}, with DTPA standing out. As expected, the extraction range for Fe was even wider, varying from 57 to almost 117 mg kg^{-1} following the order: KCl < M-1 < DTPA < M-3. The Mn extraction capacity of M-1 was similar to M-3, while DTPA was comparable to KCl. For Zn, DTPA and M-3 recovered similar amounts, while KCl extracted none and M-1 had the highest recovery.

In the sandy soil, the extracted Cu followed a similar pattern to that observed in Ferralsol, ranging from <1 to almost 31 mg kg^{-1}, also highlighting DTPA. For Fe, considering the lower buffering capacity, the elemental recoveries reached up to 124 mg kg^{-1}, following the order: KCl < DTPA < M-1 < M-3, with M-1 recovering more than DTPA compared to the clayey soil. As in the Ferralsol, the Mn extraction capacity of M-1 was comparable to M-3, while both DTPA and KCl extracted none. Regarding Zn, DTPA extracted almost forty times less than M-1, while M-3 and KCl showed no extraction.

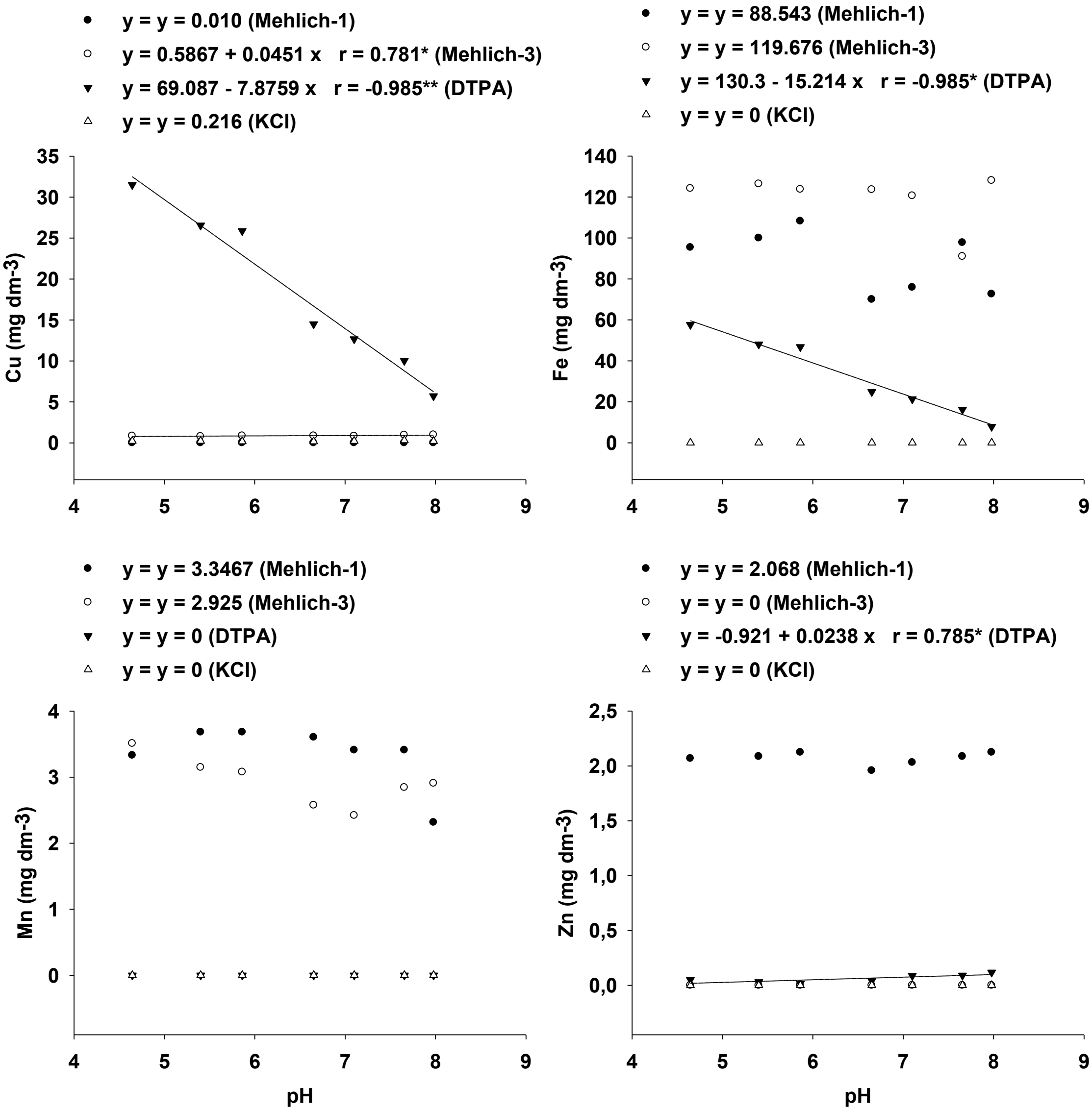
KCl showed the lowest nutrient recovery capacity among the extractants analyzed, while M-3 and M-1 showed higher recovery rates. DTPA showed average recovery rates among the extractants. Despite their similar extraction capacity, M-1 extracted slightly higher amounts of Mn and Zn in both soils. However, while Mehlich-3 exhibited some sensitivity, M-1 did not. On average, the nutrient extraction capacity in both soils followed the sequence: M-1 > M-3 > DTPA > KCl.

Figure 2- Pearson correlation between Micronutrient contents extracted by different Methods and Ferralsol pH.



Increasing or decreasing lines indicate positive or negative correlations between the variables (pH and nutrient contents), significant at $p < 0.05$ (*) or $p < 0.01$ (**) by t-test. Scattered data points indicate a lack of correlation.

Figure 3- Pearson correlation between micronutrient contents extracted by different methods and Arenosol pH.

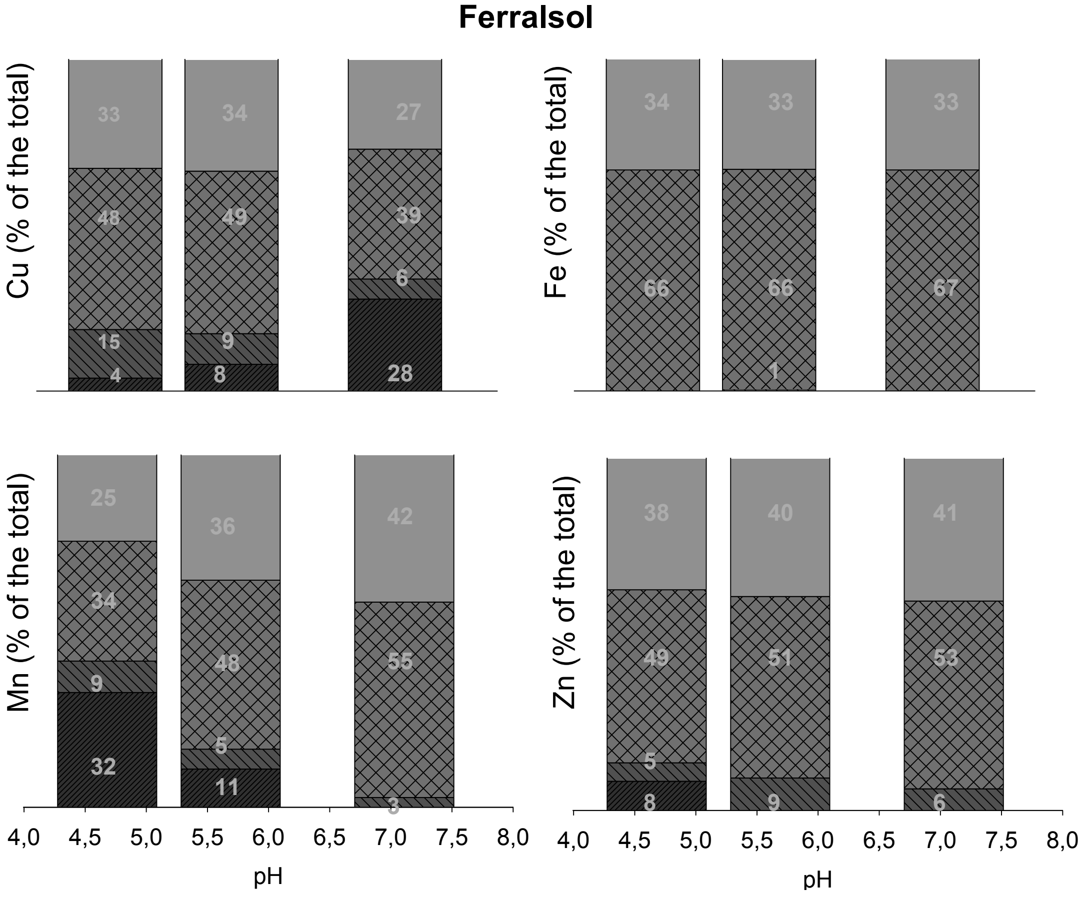


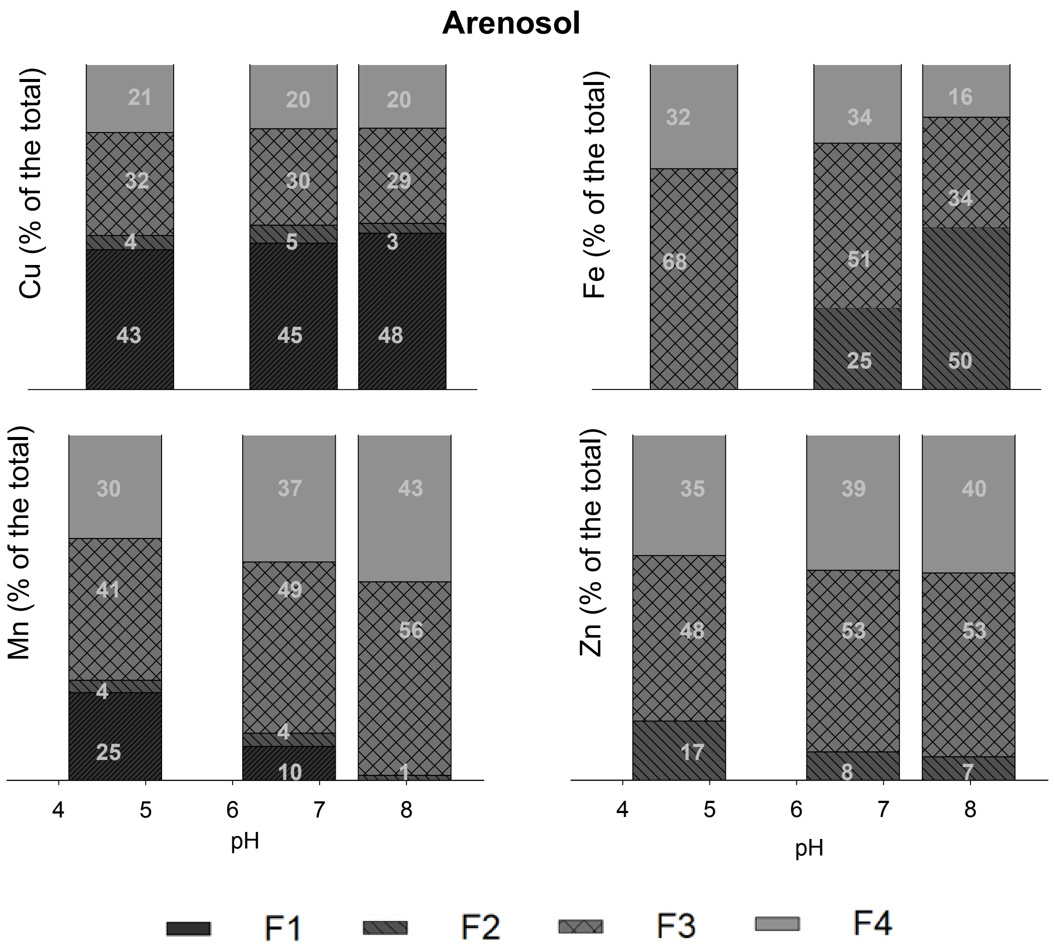
Increasing or decreasing lines indicate positive or negative correlations between the variables (pH and nutrient contents), significant at $p < 0.05$ (*) or $p < 0.01$ (**) by the t-test. Scattered data points indicate a lack of correlation.

3.2 Soil pH- Induced Changes in Micronutrients Species

Figure 4 illustrates the distribution of Cu, Fe, Mn and Zn nutrients among the soil fractions in Ferralsol and Arenosol subjected to sequential extraction. Most of the nutrients were associated with oxyhydroxides, followed by organic matter, exchangeable and potentially mobilized forms. Soil pH and soil class influenced the trends observed for these geochemical species. In fact, at Ferralsol, increasing pH elevated the exchangeable Cu fraction while reducing exchangeable Fe, Mn, and Zn. At the same time, the organic matter and oxyhydroxides bound fractions increased for Mn and Zn, but decreased for Cu, while Fe remained stable. The mobilizable fraction showed no change for Fe and Zn, but decreased for Cu and Mn. Already in the Arenosol, the Cu fractions associated with organic matter, oxyhydroxides and potentially mobilizable remained largely constant with increasing pH, although its exchangeable fraction increased slightly. For Fe, oxyhydroxides-bound and mobilizable fractions increase with pH, while Mn and Zn showed increased organic matter-bound fractions but decreased mobilizable and exchangeable fractions. Oxyhydroxides bound retention also increased for Mn and Zn at higher pH. Notably, the maximum pH- associated retention of Fe decreased.

Figure 4- pH- Dependent Fractionation of Cu, Fe, Mn, and Zn in Ferralsol and Arenosol: Geochemical partitioning across a sequential fraction (F1, F2, F3 and F4).





F1: Soluble and Exchangeable Fraction; F2: Potentially mobilizable Fraction; F3: Low Crystallinity Al-Fe-Mn oxyhydroxides Fraction, and F4: Associated to Soil Organic Matter.

3.3 Relationships Between Soil Extractants and Geochemical Species

3.3.1 Correlations between extractable nutrient contents by M-1, M-3, DTPA, and KCl extraction methods

Significant Pearson’s correlations were observed between specific extractants, exclusively in the Ferralsol, as detailed in Table 3. A negative strong correlation was observed between DTPA and KCl extraction methods. On the other hand, positive correlations were observed between M-1 and M-3; DTPA and KCl, and M-3 and DTPA for Mn, Fe and Zn, respectively.

Table 3- Pearson’s linear correlation between chemical extractants per micronutrient in Ferralsol.

Nutrient	Correlation	r
Cu	DTPA / KCl	-0.91**
Fe	M-1 / M-3	0.87*
Mn	DTPA / KCl	0.94**
Zn	M-3 / DTPA	0.95**

* and ** means significant at 5 or 1%, respectively according to t-test. Mehlich-1 (M-1); Mehlich-3 (M-3)

3.3.2 Correlations between extractable nutrient contents and geochemical species

The correlations between Zn, Cu, Mn, and Fe contents (determined by the different extraction methods) and their respective soil geochemical fractions, as well as soil pH, are presented in Tables 4 and 5.

In the Ferralsol (Table 4), the Cu-DTPA contents showed negative correlations with both the more readily available fraction (F1) and the more recalcitrant ones (F3 and F4). Interestingly, the contents extracted by the KCl method showed positive correlations with the two most recalcitrant Cu fractions. F1, F3 and F4 correlated strongly with soil pH. Among Cu species, F3 correlated positively with F1, F4 with F1 and F4 with F3.

For Fe, there was a correlation only between the F3 and F4 fractions. For Mn, its DTPA content correlated positively with F1 and F2 species and negatively with F3 and F4 species. All fractions correlated with each other as well as with soil pH, being positive between F1 and F2, F3 and F4, F3 and F3-F4 with soil pH. The remaining interactions were negatively correlated.

Zinc correlation was positively significant between F1 with M-3 and with DTPA extractors, and between F3 and F4. A negative correlation was observed between F1 and soil pH.

Table 4- Linear correlation between chemical extractants, soil pH and relative percentage obtained in sequential extraction in Ferralsol.

Cu	M-1	M-3	DTPA	KCl	F1	F2	F3	F4	Soil pH
F1	0.60	0.46	-0.88**	0.61	1.00				
F2	-0.38	-0.28	0.39	-0.47	-0.48	1.00			
F3	0.62	0.48	-0.83**	0.73*	0.68*	-0.49	1.00		
F4	0.62	0.48	-0.83**	0.73*	0.68*	-0.49	1.00**	1.00	
Soil pH	-	-	-	-	0.94**	-0.60	0.83*	0.83**	1.00

Fe	M-1	M-3	DTPA	KCl	F1	F2	F3	F4	Soil pH
F1					1.00				
F2	-0.14	0.08	0.08			1.00			
F3	-0.52	-0.01	0.08			0.28	1.00		
F4	-0.52	-0.01	0.08			0.28	1.00**	1.00	
Soil pH	-	-	-	-		-0.05	0.05	0.05	1.00

Mn	M-1	M-3	DTPA	KCl	F1	F2	F3	F4	Soil pH
F1	-0.48	0.06	0.93**	0.65	1.00				
F2	-0.08	0.17	0.80**	0.30	0.88**	1.00			
F3	-0.51	-0.07	-0.78*	-0.57	-0.89**	-0.79*	1.00		
F4	-0.51	-0.07	-0.78**	-0.57	-0.89**	-0.79*	1.00**	1.00	
Soil pH	-	-	-	-	-0.94**	-0.89**	0.91**	0.91**	1.00

Zn	M-1	M-3	DTPA	KCl	F1	F2	F3	F4	Soil pH
F1	-0.62	0.93**	0.84**		1.00				
F2	-0.10	-0.23	-0.41		-0.16	1.00			
F3	-0.40	-0.27	-0.29		-0.24	0.64	1.00		
F4	-0.40	-0.27	-0.29		-0.24	0.64	1.00**	1.00	
Soil pH	-	-	-	-	-0.79*	-0.02	-0.04	-0.04	1.00

* and ** denote statistical significant at the 5 and 1% according to Pearson test (t-test). Absence of correlation coefficient (r values) indicates that the content of the species was below the quantification limits of the analytical

methods employed. Data represented by dash (-) were not included in the table as are already presented in Figures 2 and 3. Mehlich-1 (M-1), Mehlich-3 (M-3). F1: Soluble and Exchangeable Fraction; F2: Potentially mobilizable Fraction; F3: Low Crystallinity Al-Fe-Mn oxyhydroxides Fraction, and F4: Associated to Soil Organic Matter.

In the Arenosol, extractable nutrient contents showed surprising correlations with geochemical species, while they were more common among geochemical species (Table 5). For Cu, a negative correlation was observed between DTPA and the F1 fraction for Cu, Fe. Positive correlation was found between F3 and F4 fractions and between F1 and soil pH. For Fe, DTPA content was negatively correlated with F2 fraction, while positive correlation was observed between F3 and F4, and among soil pH with F2, F3 and F4. For Mn, a negative correlation was observed between F1 fraction and soil pH, as well as with F3 and F4 fractions. For Zn, positive correlations were found between F3 and F4 and soil pH, as well as among each other and negative correlations with F2.

Table 5- Linear correlation between chemical extractants, soil pH and relative percentage obtained in sequential extraction in Arenosol.

Cu	M-1	M-3	DTPA	KCl	F1	F2	F3	F4	Soil pH
F1	-0.54	0.49	-0.93**	-0.14	1.00				
F2	-0.41	-0.03	0.10	-0.23	-0.31	1.00			
F3	-0.11	0.29	-0.56	0.05	0.40	-0.15	1.00		
F4	-0.11	0.29	-0.56	0.05	0.40	-0.15	1.00**	1.00	
Soil pH	-	-	-	-	0.94**	-0.18	0.51	0.51	1.00

Fe	M-1	M-3	DTPA	KCl	F1	F2	F3	F4	Soil pH
F1					1.00				
F2	-0.56	0.01	-0.92**			1.00			
F3	-0.24	0.10	-0.63			0.66	1.00		
F4	-0.24	0.10	-0.63			0.66	1.00**	1.00	
Soil pH	-	-	-	-		0.90**	0.75*	0.75*	1.00

Mn	M-1	M-3	DTPA	KCl	F1	F2	F3	F4	Soil pH
F1	0.52	0.52			1.00				
F2	0.17	-0.54			0.28	1.00			
F3	-0.30	0.05			-0.68*	-0.40	1.00		
F4	-0.30	0.05			-0.68*	-0.40	1.00**	1.00	
Soil pH	-	-	-	-	-0.99**	-0.30	0.69*	0.69*	1.00

Zn	M-1	M-3	DTPA	KCl	F1	F2	F3	F4	Soil pH
F1					1.00				
F2	0.03		-0.09			1.00			
F3	0.19		0.36			-0.72*	1.00		
F4	0.19		0.36			-0.72*	1.00**	1.00	

Soil pH	-	-	-	-		-0.85**	0.90**	0.90**	1.00

* and ** denote statistical significant at the 5 and 1% according to Pearson test (t-test). Absence of correlation coefficient (r values) indicates that the content of the species was below the quantification limits of the analytical methods employed. Data represented by dash (-) were not included in the table as are already presented in Figures 2 and 3. Mehlich-1 (M-1), Mehlich-3 (M-3).

4. DISCUSSION

The extraction of micronutrients from soil depends on the mechanisms of action of the extractants and the chemical forms in which these elements are present. The extractability of the methods in response to changes in soil pH should be investigated from several perspectives based on our results: cases where there is no correlation, cases with a positive correlation, and cases with a negative correlation.

4.1 Cases in Which the Extractor Shows Insensitivity to Soil pH Changes

Even with an increase in pH, which typically favors the precipitation of Zn, Cu, Mn, and Fe (Gondal et al., 2021), extractants may (in the absence of correlation) continue to extract significant amounts due to their ability to access precipitated nutrient species. This perspective is particularly robust for acid extractors, such as M-1, which was found to be insensitive to soil pH for all nutrients. In fact, even in soils with high pH, the acidification induced by the M-1 extractant can solubilize the precipitated forms of the nutrients (De Abreu et al., 1995; Fontes et al., 2021; Galrao, 1995), especially when the buffering capacity of the soil does not cause significant changes in the pH of the extractant during extraction (Pegoraro; Reis; Souza, 2017; Silva, 2003). In our study, the initial pH of M-1, even in the more buffered Ferralsol, shifted to a value very close (from 1.36 to 1.44) to that observed at the highest liming rates.

For the M-3 extractant, which operates through a complex extraction mechanism involving acidity, complexation, and ion exchange, its insensitivity to soil pH in certain situations (e.g., Cu, Fe, and Mn in Ferralsol, and Fe, Mn, and Zn in Arenosol) is supported by studies describing its ability to extract precipitated forms due to its ability to dissolve oxydroxides formed at high soil pH (Mehlich, 1984; Zhu; Ozoros-Hampton; Li, 2016) as observed for Zn (García et al., 1997; Menezes; Dias; Lima, 2010), Cu, Fe and Mn (Garcia et al., 1997). The pH of its extract varied slightly more than M-1, while still remaining acidic.

However, for the KCl extractant, a neutral salt, this behavior may seem counterintuitive at first, since its expected extraction mechanism is primarily by ionic exchange (Valladares et al., 2001). However, several explanations can be proposed: given its very low recovery of nutrients, the apparent insensitivity of the extractant may be confused with the low extraction levels. This is consistent with the fact that a significant portion of the available forms of Zn, Cu, Mn, and Fe in the soil may not be exchangeable - existing instead as highly reactive precipitates, complexes, or chemically adsorbed species - forms that are inaccessible to the cationic exchanger K^+; the increase in pH reduces hydrogen ion competition in cation exchange by increasing negative charges, which could enhance the retention of Zn, Cu, Fe, and Mn ions, thereby protecting these elements from hydrolysis. In addition, as a saline solution, the high ionic strength of the extractant ($I = 1.0$ mol/L) decreases the ionic activity of the ions (Wang et al., 2004), potentially promoting the dissolution of more reactive precipitated species (salting-in effect). Only the extraction pH was affected by the buffering capacity of the soil.

DTPA is a chelating agent that forms stable complexes with metals such as Zn, Cu, Mn, and Fe (Rogers; Dari; Schroeder, 2019; Seth et al., 2017). These complexes are generally affected by soil pH, as DTPA has shown sensitivity to hydrolysis in most cases. However, in certain situations where its strong complexing capacity predominates, the influence of soil pH on metal availability may be reduced, allowing extractant capacity to overcome the tendency of the elements to precipitate at high pH. Studies have shown that DTPA is effective in soils with a pH of 6 to 8.5 (Lindsay & Norvell, 1978). Similar to the acidic extractants, DTPA showed a minimal pH variation but maintained extraction under alkaline conditions.

As mentioned above, the acidic behavior of M-1, reinforced by its ability to dissolve precipitated nutrient species regardless of soil pH, suggests that the extractant does not reflect the actual availability of micronutrients to plants, but rather the total solubilizable fraction, which may include forms that are not easily accessible under natural soil conditions.

Although acid extractants did not show a correlation with increasing soil pH for Fe, it is not expected that DTPA would correlate well with Fe since higher pH values are typically associated with Fe deficiency conditions (Sillampa, 1982). Under these circumstances, even a complexing and saline extractant method faces greater difficulty in accessing Fe, which explains why it would not be consistent with the behavior of acid extractants.

It was observed that for both soils in Mn, the results obtained in F1 were strongly and negatively correlated with pH. This indicates that although DTPA (-0.72) and M-3 (-0.71) in the Ferralsol and Arenosol, respectively, showed insensitivity with soil pH, a negative trend in the Mn recovery by these extractants was evident, particularly when supported by speciation results for F1 and F2. This behavior was more pronounced in Ferralsol, where F1 is positively correlated with F2 (0.88**) and negatively correlated with F3 (-0.89**) and F4 (-0.89**), in agreement with Lindsay (1979), who found that Mn availability in soil is influenced by the balance between different Mn oxide forms.

4.2 Cases Where Extractors Increase Extraction with Rising Soil pH

Positive correlations, such as those observed for Cu-KCl in Ferralsol and Cu-M-3 and Zn-DTPA in Arenosol, suggest that although an increase in soil pH generally promotes the precipitation of these nutrients, there are cases where extractants such as KCl, Mehlich-3, and DTPA exhibit greater extraction efficiencies at higher pH levels. This increase suggests that these extractants may have access to higher nutrient concentrations than those precipitated by hydrolysis. In addition to the arguments presented in the attempt to explain the insensitivity of the methods to the formation of precipitated species (previous topic), the possible dispersion of soils, the instability of organomineral or organometallic compounds with increasing soil pH, or even extraction time and higher temperature (Lindsay; Martens, 1990), which may favor extraction, should also be considered.

In fact, only DTPA and M-3 were sensitive to Cu extractions, with DTPA showing the highest recovery. This can be attributed to the high stability of the DTPA-Cu complex, as described by Moreira (2003). However, this behavior was not observed for Zn. Although M-3 had a lower overall sensitivity, it showed a stronger correlation than DTPA in Ferralsol. In Arenosol, its positive correlation with soil pH suggested Zn dissolution, although M-3 (pH = 2.3), which contains EDTA, forms a medium where the binding of the chelator with the micronutrient is almost zero (due to acidic conditions), the higher concentration of DTPA (0.005 mol/L) compared to EDTA in M-3 (0.001

mol/L) may also contribute to this difference. Furthermore, de Abreu et al. (1996) observed that due to the ability of soil organic matter to retain up to 45% of Cu in complexed forms, DTPA was more effective in recovering the element compared to M-1. This can be attributed to the superior ability and preference of DTPA to solubilze Cu in labile solid forms through complexation in this fraction.

Overall, all extractants showed a similar pattern, except for Zn, which varied between soils. Also, studies on Zn availability reported a similar trend: M-1 > M-3 > DTPA (Menezes; Dias; Lima, 2010) and M-1 > M-3 > HCl > DTPA (Moreira et al., 2016). Although a weak correlation (r= 0.29, 5% significance), Sharma et al. (2018) observed that Zn was positively correlated with soil pH. This consistent extractant behavior in both soils indicates that there was little buffering effect from soil to soil. This fact indicates that the soil pH effect was greater than the soil buffering effect. Furthermore, since most of the extractants did not correlate with pH, there may have been some resolubilization of micronutrients according to each mechanism.

F3 and F4 behavior among them was expected since higher pH levels promote chemical precipitation (Zang *et al*., 2017) and the nutrients are retained these unavailable fractions and potentially accessed showing positive correlations with soil pH. Sims (1986); Shuman (1988); Miller; Martens; Zelazny (1986), generally suggest that metals are more likely to be retained in Fe and Mn oxyhydroxides in clayey soils and in the organic-bound fraction in sandy soils, both of which have low organic matter contents. This trend is consistent with the low organic matter content presented in both soil classes.

4.3 Cases Where Extractors Decrease Extraction as Soil pH Increases

Negative correlations**,** as often observed for DTPA (for Cu, Fe and Zn in Ferralsol), and for Zn-M-3 (Ferralsol), indicate that these extractors are sensitive to the chemically precipitated forms of nutrients. This effect occurs because the alkaline reaction of DTPA maintains a buffered soil pH, limiting the solubilization of precipitated nutrient forms (De Abreu et al., 1995). As observed with Zn, DTPA extraction showed a significant negative correlation with liming, reinforcing its superior performance in higher pH soils (Menezes; Dias; Lima, 2010).

The Cu species showed a positive correlation between the available Cu-bound fractions (F1 and F2) and soil pH, suggesting that the extractors used to recover the available fractions were not suitable for this nutrient. This is supported by the behavior of DTPA compared to the more recalcitrant fractions (F3 and F4), which again behave as expected. This context does not indicate the solubilization of the precipitated contents and ensures an accurate representation of the available fractions by the extractant.

A similar trend was observed for Fe, further highlighting that the extractant used for F1 and F2 was also inadequate for exchangeable forms. Walna et al. (2010) reported a low available Fe content when analyzing Mn and Fe fractionation in soils affected by prolonged acid rain, demonstrating the strong capacity of soils to retain Fe. This finding is consistent with the Fe speciation from the present study, mainly in the Arenosol, where F3 and F4 showed comparable behavior to Cu. These results reinforce the understanding that increased precipitation and environmental factors contribute to the retention of Fe in less available forms.

For Zn in Arenosol, although there was no extraction in F1 and DTPA showed a positive correlation with soil pH, it was negatively correlated with F2 - a less labile fraction and probably available as outer-sphere complexes or even occluded forms - and positively correlated with F3 and F4. In Ferralsol, it is noteworthy to observe that F1 correlated more strongly with M-3 (0.93**) than with DTPA (0.84**), supporting the effectiveness of these extractants in reflecting Zn content over soil pH. However, it also suggests that M-3, with its acid mechanism, may have targeted more stable forms of Zn, probably associated with oxyhydroxides (Fontes et al., 2021), highlighting DTPA as a potentially more suitable alternative.

4.4 Geochemical Species Across Soil Pools: Recalcitrant fractions

If chemical precipitation is assumed to have occurred, a higher percentage of oxyhydroxides would be expected since the increase in soil pH reduces nutrient availability. The retention of heavy metals on oxide surfaces with variable charges, is inversely proportional to the degree of crystallinity (Okasaki; Takamidoh; Yamane, 1986), and it is worth noting that the extractor used was designed to target low-crystallinity oxides, which helps explain the results obtained, because the native behavior of our soils, favors the retention of nutrients in this fraction. In addition, it’s important to consider that the oxyhydroxide forms are not only influenced by pH changes, but also by mineralogy and soil redox conditions, which can further affect the mobility and bioavailability of micronutrients (Sharma et al., 2022).

Nascimento (2001) identified higher Zn levels in the residual fraction, followed by the fraction bound to oxyhydroxides, in a Ferralsol with natural pH and high organic matter content, without a history of intensive agricultural activity. The author observed that the organic fraction exceeded the fraction bound to oxyhydroxides, contrary to what was found in our soils evaluated. Furthermore, it was observed that, in general, increasing doses led to the retention of the element in the exchangeable and organic fractions. In contrast, the organic fraction (F3) presented a lower relative percentage than the oxyhydroxide fraction in all soils, pH ranges and nutrients analyzed. This behavior is related to the low content of organic matter in the studied soils, 1.7 and 0.9 dag kg^{-1} (Table 1) in the Ferralsol and Arenosol, respectively.

4.5 Relationship between Extraction Methods

De Abreu et al. (1995) demonstrated that DTPA is effective in extracting Cu and Mn, with pH being the most critical factor influencing their correlation. This suggests that DTPA and KCl share a mechanistic relationship, as both are minimally affected by soil pH. Furthermore, the correlation between DTPA and M-3 for Zn can be attributed to their similar behavior with increasing soil pH. Similarly, M-3 and M-1 show a similar mechanism for solubilizing Fe species in Ferralsol.

5. CONCLUSION

The behavior of the extractants Mehlich-1, Mehlich-3, DTPA, and KCl reveals specificities in the extraction of Cu, Mn, Fe, and Zn from the soil due to their distinct extraction mechanisms and the variations in the chemical species of these elements. Nevertheless, some generalizations can be made:

- Mehlich-1 (M-1) is insensitive to increases in soil pH during the extraction of Zn, Cu, Mn, and Fe, as it can solubilize species precipitated by hydrolysis.
- DTPA is generally sensitive to the formation of precipitated species because the nutrient-DTPA complexation constants are insufficient to exceed the precipitation-dissolution constants of the precipitated species of Zn, Cu, Mn, and Fe, thereby limiting their dissolution.
- The low recovery efficiency of Zn, Cu, Mn, and Fe by the KCl extractant is likely due to the predominance of non-exchangeable species of these elements in the soil. Consequently, the apparent insensitivity of this extractant to increases in soil pH may be due to its inherently low extraction rates.
- The variable behavior of the Mehlich-3 extractant is related to its multiple extraction mechanisms, which allow it to interact differently with various soil conditions.
- When assessing the short-term availability of Zn, Cu, Mn, and Fe, the DTPA extractant appears to be the most suitable for evaluating plant-available nutrients.

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SUPLEMENTARY MATERIAL

Table 6- Pearson's linear correlation between chemical extractants per micronutrient in Ferralsol and Arenosol.

Cu_Ferralsol	M-1	M-3	DTPA	KCl
M-1	1			
M-3	0.70	1		
DTPA	-0.40	-0.46	1	
KCl	0.28	0.56	-0.91**	1

Fe_ Ferralsol	M-1	M-3	DTPA	KCl
M-1	1			
M-3	0.87*	1		
DTPA	-0.40	-0.09	1	
KCl				1

Mn_ Ferralsol	M-1	M-3	DTPA	KCl
M-1	1			
M-3	0.43	1		
DTPA	-0.26	0.42	1	
KCl	-0.16	0.45	0.94**	1

Zn_ Ferralsol	M-1	M-3	DTPA	KCl
M-1	1			
M-3	-0.46	1		
DTPA	-0.39	0.95**	1	
KCl				1

Cu_Arenosol	M-1	M-3	DTPA	KCl
M-1	1			
M-3	0.17	1		
DTPA	0.21	-0.75	1	
KCl	0.27	0.22	-0.18	1

Fe_Arenosol	M-1	M-3	DTPA	KCl
M-1	1			
M-3	0.59	1		
DTPA	0.10	0.45	1	
KCl				1

Mn_Arenosol	M-1	M-3	DTPA	KCl
M-1	1			
M-3	0.01	1		

DTPA			1	
KCl	0.0	0.0	0.0	1

Zn_Arenosol	M-1	M-3	DTPA	KCl
M-1	1			
M-3		1		
DTPA	0.14		1	
KCl				1

* and ** denote statistical significance at 5 and 1% according to Pearson's t-test. The absence of a correlation coefficient (r values) indicates that the species content was below the quantification limits of the analytical methods employed.

Table 7- Scheme of sequential extraction procedures based on Barreto et al. (2024) approach and targeted metal forms.

Fraction	Targeted forms	Extractant	Equilibration
F1	Soluble and exchangeable fraction	10 mL 0.1M $MgCl_2$	Shake for 1 h at room temperature. Separate extract from the solid residue by centrifugation for 5 min at 1060*g*[1].
F2	Potentially mobilizable fraction, possibly adsorbed and associated with Fe, Al and Ca	10 mL 0.11M NaOAc	Shake for 8 h at room temperature. Separate extract from the solid residue by centrifugation for 5 min at 1060*g*[1].
F3	Fraction bound to low crystallinity Al-Fe-Mn oxyhydroxides	20 ml of solution (0.175 M $NH_4C_2O_4$ + 0.1 $H_2C_2O_4$), pH 3.25	Shake for 2h in room temperature at dark. Separate extract from the solid residue by centrifugation for 5 min at 1060*g*[1].
F4	Fraction associated with soil organic matter	2 mL 0.02M HNO_3 + 8 mL of 30% H_2O_2 (pH 2)	Pre-digestion for 4h, at room temperature, eventually manual shake Heat to 80ºC for 2h, eventually manual shake.
		After cooling, 10 mL 0.02M HNO_3	Shake for 30 min Separate extract from the solid residue by centrifugation for 5 min at 1060*g*[1].

[1]: Adapted

Table 8- pH measurement of different extractants beofere and after interaction with soil.

Extract pH	Ferralsol_M-1	Arenosol_M-1	Ferralsol_KCl	Arenosol _KCl	Ferralsol_DTPA	Arenosol _DTPA	Ferralsol_M-3	Arenosol _M-3
0%[1]	1.41	1.30	4.71	4.63	7.21	7.29	3.11	2.83
0%	1.36	1.32	4.76	4.51	7.20	7.29	2.96	2.64
0%	1.58	1.32	4.86	4.55	7.20	7.29	2.83	2.59
25%	1.43	1.36	5.14	4.85	7.24	7.30	2.70	2.51
25%	1.38	1.30	5.18	4.87	7.25	7.30	2.72	2.51
25%	1.38	1.31	5.09	5.08	7.24	7.31	2.74	2.62
50%	1.41	1.31	5.35	5.63	7.24	7.31	2.79	2.69
50%	1.36	1.30	5.39	5.87	7.26	7.32	2.84	2.89
50%	1.39	1.30	5.44	5.75	7.25	7.31	2.96	2.97
100%	1.46	1.31	5.94	6.27	7.27	7.33	3.05	2.89
100%	1.50	1.32	5.82	6.27	7.27	7.33	3.18	2.72
100%	1.43	1.36	6.16	6.50	7.28	7.33	3.05	2.65
150%	1.40	1.31	6.24	6.67	7.30	7.35	2.87	2.66
150%	1.51	1.30	6.36	6.69	7.30	7.34	2.95	2.62
150%	1.38	1.31	6.46	6.66	7.30	7.35	2.81	2.63
200%	1.39	1.31	6.78	7.00	7.32	7.36	3.05	2.68
200%	1.42	1.36	6.89	6.84	7.32	7.36	2.92	2.74
200%	1.39	1.31	6.53	7.06	7.33	7.35	3.02	2.87
250%	1.41	1.33	6.80	7.3	7.34	7.37	3.23	3.03
250%	1.44	1.33	7.08	7.46	7.34	7.36	3.20	2.93
250%	1.44	1.35	6.94	7.37	7.34	7.37	3.50	2.73
Average	1.42	1.32	5.37	5.22	7.27	7.33	2.94	2.71
Extractant pH	1.22		6.17		7.35		2.61	

[1]: Percentage for neutralization of potential acidity