

Division - Soil Processes and Properties | Commission - Soil Chemistry

# Comparing Methods for Extracting Heavy Metals from Histosols for Establishing Quality Reference Values

**Erica Souto Abreu Lima<sup>(1)\*</sup>, Nelson Moura Brasil do Amaral Sobrinho<sup>(1)</sup>, Daniel Vidal Pérez<sup>(2)</sup> and Izabella Bezerra Coutinho<sup>(1)</sup>**<sup>(1)</sup> Universidade Federal Rural do Rio de Janeiro, Departamento de Solos, Seropédica, Rio de Janeiro, Brasil.<sup>(2)</sup> Empresa Brasileira de Pesquisa Agropecuária, Embrapa Solos, Rio de Janeiro, Rio de Janeiro, Brasil.

**ABSTRACT:** The diversity of methods for heavy metal extraction from soils complicates comparison of results used by environmental agencies to establish quality reference values. The aim of this study was to evaluate three soil digestion methods regarding the solubilizing capacity of Ba, Cr, Cu, Fe, Mn, Ni, Pb and Zn, and to propose a standard method to establish Quality Reference Values (QRVs) for Brazilian Histosols. Twenty soil samples were selected to evaluate the USEPA 3051, USEPA 3051A and Aqua Regia methods in a closed system. The methods tested were statistically similar regarding Cu, Fe and Mn extraction from soils. However, the methods can not be considered similar for Ba, Cr, Ni, Pb and Zn; Aqua Regia recorded the highest levels, except for Ba. The Aqua Regia method proved suitable for metal extraction from soils with organic characteristics and can be used to establish QRVs.

**Keywords:** organic horizons, pseudo-total content, soil digestion methods.

\* **Corresponding author:**

E-mail: [ericaabreulima@gmail.com](mailto:ericaabreulima@gmail.com)

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## INTRODUCTION

Histosols are poorly evolved and fragile soils that formed under restricted drainage conditions or under humid, cold and water-saturated environments. They have low geographical representativeness (approximately 1 %) compared to the other Brazilian soil orders; however, they play important economic, social and environmental roles (Valladares et al., 2008). They are extensively used by family farmers in subsistence production systems or even olericulture. Furthermore, given their high organic matter content, they may accumulate pollutants and/or be degraded depending on the development of anthropic activities.

Those soils usually have low pH values and high organic C content, which strongly affect their chemical and physical properties (Santos et al., 2013). Therefore, assessing the natural content of heavy metals in soils with low anthropic impact and proposing Quality Reference Values (QRVs) of specific heavy metals for Histosols is essential for monitoring these areas.

Achieving reliable laboratory results starts with choosing the appropriate sample digestion method for subsequent analytical determinations. Considering the lack of analytical methods specific for organic soils, valuation and standardization of soil digestion methods is necessary. Currently, various acid digestion methods can be identified in the national and international literature, with varying solubilizing solutions, acid ratios, times, temperatures and digestion systems (open or closed), which result in different solubilizing capacities of organic and mineral soil fractions (Pelozato et al., 2011). The methods proposed by the United States Environmental Protection Agency (USEPA) are extensively used in the US and internationally by environmental agencies. In Brazil, the National Environmental Council (Conselho Nacional do Meio Ambiente - Conama, 2009), through resolution 420/2009 Annex I, recommends using the USEPA 3050 and 3051 methods or their updates to establish Quality Reference Values of inorganic substances. However, such recommendations overlook the peculiarities of organic soils.

The Aqua Regia (AR) method is also widely used and effective for extracting heavy metals from soils. This method has been used by the International Organization for Standardization (ISO) as the standard for soil certification in Europe.

Various studies have indicated significant differences in the quantity of metals recovered after using each method (Saldanha et al., 1997; Chen and Ma, 2001; Chander et al., 2008; Melo and Silva, 2008; Caires, 2009; Pérez et al., 2013; Santos and Alleoni, 2013), this situation requires choosing only one standard digestion method for purposes of comparison.

Significant differences in heavy metal extraction capacity between the AR and USEPA 3051 methods, wherein AR showed a better recovery rate for most metals analyzed upon testing different soils from the southwestern Amazon region (Santos and Alleoni, 2013). Pérez et al. (2013) also showed that the AR method performed better than the USEPA 3051 and 3051A methods in soils from Mato Grosso do Sul. However, Melo and Silva (2008) observed that the digestion method using AR yielded the lowest recovery rate when evaluating the effects of the digestion method on Cu, Mn and Zn recovery from organic waste.

Based on the assumption that the quantities of heavy metals extracted from Histosol samples would be different after using different soil digestion methods, the present study aimed, to evaluate the EPA 3051, EPA 3051A and AR methods regarding the solubilizing capacities of Ba, Cr, Cu, Fe, Mn, Ni, Pb and Zn present in organic horizons and to propose a standard digestion method to determine the QRVs in Histosols and soil horizons with high organic matter content.

## MATERIALS AND METHODS

Twenty samples from organic horizons derived from Histosols collected in areas of minimal anthropic activity in different Brazilian edaphoclimatic regions were used. The samples came from a soil collection of the Soil Genesis and Classification Laboratory of the Federal Rural University of Rio de Janeiro (Universidade Federal Rural do Rio de Janeiro), which were classified and characterized by Valladares (2003), Fontana (2009) and Ebeling (2010).

The pH in water; P, K, and Na, extracted by Mehlich-1;  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , and  $\text{Al}^{3+}$ , extracted by 1 mol L<sup>-1</sup> KCl; potential acidity (H+Al), determined by calcium acetate 0.5 mol L<sup>-1</sup>; cation exchange capacity at pH 7.0 (CEC); and sum of bases (SB) of the soil samples were assessed according to Donagema et al. (2011).

The mineral matter (MM) and organic matter (OM) contents were determined according to the Brazilian Soil Classification System (*Sistema Brasileiro de Classificação de Solos - SiBCS*) (Santos et al., 2013). The method for determining OM consisted of weighing a specific quantity of a previously oven-dried sample (105 °C for 24 h) and placing it in a muffle furnace for 6 h at 600 °C. The OM content was assessed by calculating the difference between the oven-dried sample and the weight of the sample removed from the muffle. The percentage of MM was also assessed by furnace combustion, wherein [MM% = (dry weight at 600 °C/6 h) / (dry weight at 105 °C/24 h) × 100].

The total carbon content was determined by dry combustion of 5.0 ± 0.1 mg samples using an elemental analyzer Perkin Elmer 2400 CHNS. The reference standard used was acetanilide (C = 71.09 %, H = 6.71 %, and N = 10.36 %).

Chemical fractionation was performed to determine the fulvic acid, humic acid and humin contents in the samples, according to the protocol described by Benites et al. (2003).

The chemical characterization and organic matter fractionation of the organic horizons are shown in table 1.

Three soil digestion methods were evaluated to define the most suitable digestion method to study the organic horizons: AR (ISO 12914, 2012), USEPA 3051 (USEPA, 1994) and USEPA 3051A (USEPA, 2007), which were conducted in a closed system using microwave radiation in a MARS Xpress® device (Table 2). All analyses were performed in triplicates and used high purity acids (P.A.) and Milli-Q water for dilution. The concentrations of Ba, Cr, Cu, Fe, Mn, Ni, Pb and Zn in the extracts resulting from digestion were determined by atomic absorption spectrometry using a Varian SpectrAA 55B device.

Standard Reference Materials (SRM 2709, San Joaquin Soil and SRM 2782, Industrial Sludge) from the National Institute of Standards and Technology (NIST) were used to validate the methods. The levels assessed were compared to the leachable concentrations, and the values recommended by the NIST as the content of reference NIST samples were determined based on total determination methods, that is, methods that use hydrofluoric acid (HF) to dissolve soil silicates or by X ray fluorescence (NIST, 2002).

Linear regression was used for the organic horizon results to compare the methods; to satisfy the null hypothesis that the two methods are similar, the slope should not differ from 1 and the y-intercept should not differ from 0 (Miller and Miller, 2005). For that purpose, 99 % confidence intervals were calculated for the respective coefficients.

Principal Component Analysis (PCA) was performed to assess the interaction between the soil variables and their effects on the variation rates of the methods. Therefore, the data were standardized to mean 0 and variance 1 to avoid the effects of the measurement units on the analyses.

**Table 1.** Identification, chemical characterization and organic matter fractionation of the organic horizons

| Sample | Location/<br>Horizon | pH(H <sub>2</sub> O) | P    | K <sup>+</sup> | Ca <sup>2+</sup> | Mg <sup>2+</sup> | SB   | Al <sup>3+</sup> | H+Al | CEC  | MM   | OM    | C     | C-HAF | C-FAF | C-HUM |
|--------|----------------------|----------------------|------|----------------|------------------|------------------|------|------------------|------|------|------|-------|-------|-------|-------|-------|
|        |                      |                      |      |                |                  |                  |      |                  |      |      |      |       |       |       |       |       |
| 1      | BA02/<br>Hdoj3       | 2.5                  | 0.0  | 0.1            | 3.8              | 4.2              | 8.2  | 8.5              | 88.6 | 96.9 | 26.0 | 740.0 | 369.9 | 189.5 | 16.7  | 175.8 |
| 2      | BA03/<br>Hdopj1      | 3.6                  | 54.0 | 0.4            | 5.9              | 5.7              | 12.3 | 1.6              | 44.9 | 57.2 | 19.0 | 807.0 | 387.6 | 159.5 | 36.5  | 184.3 |
| 3      | BA03/<br>Hdopj2      | 3.5                  | 0.0  | 0.1            | 1.7              | 3.1              | 5.2  | 2.6              | 43.6 | 48.7 | 6.0  | 939.0 | 555.4 | 198.9 | 16.2  | 281.1 |
| 4      | MA05/<br>Hdo1        | 4.3                  | 15.0 | 1.3            | 9.0              | 9.2              | 20.3 | 2.7              | 19.3 | 39.6 | 64.0 | 355.0 | 154.1 | 34.2  | 12.7  | 96.5  |
| 5      | MA05/<br>Hdo2        | 4.4                  | 14.0 | 1.0            | 7.1              | 7.9              | 16.4 | 5.0              | 28.9 | 45.3 | 79.0 | 208.0 | 110.4 | 23.5  | 9.0   | 64.5  |
| 6      | MG01/<br>Hd          | 4.6                  | 13.0 | 0.1            | 3.2              | 1.6              | 5.8  | 1.9              | 45.5 | 51.3 | 59.0 | 412.0 | 213.7 | 95.5  | 23.6  | 77.6  |
| 7      | MG01/<br>Hdo         | 4.5                  | 1.0  | 0.4            | 0.6              | 0.9              | 3.0  | 7.9              | 29.9 | 32.9 | 38.0 | 615.0 | 279.0 | 65.9  | 40.0  | 147.4 |
| 8      | PR02/<br>Hdp1        | 4.4                  | 40.0 | 0.4            | 5.6              | 3.3              | 9.4  | 0.7              | 34.8 | 44.2 | 72.0 | 279.0 | 144.1 | 89.5  | 13.1  | 36.5  |
| 9      | PR02/<br>Hdp2        | 4.1                  | 7.0  | 0.2            | 3.5              | 1.8              | 5.6  | 4.5              | 63.2 | 68.8 | 62.0 | 376.0 | 192.8 | 143.4 | 10.0  | 30.7  |
| 10     | RJ02/<br>Ho1         | 4.1                  | 5.0  | 0.3            | 3.4              | 4.8              | 10.3 | 4.4              | 51.2 | 61.5 | 16.0 | 837.0 | 426.1 | 87.1  | 11.6  | 335.0 |
| 11     | RJ03/<br>2Hbd        | 4.8                  | 41.0 | 0.2            | 4.5              | 2.2              | 7.1  | 1.5              | 25.4 | 32.5 | 66.0 | 335.0 | 178.2 | 50.5  | 14.3  | 78.8  |
| 12     | RJ03/<br>Hdo1        | 5.3                  | 2.0  | 0.3            | 12.1             | 14.8             | 27.8 | 1.1              | 20.0 | 47.7 | 28.0 | 718.0 | 314.9 | 121.2 | 42.6  | 139.2 |
| 13     | RJ03/<br>Hdp2        | 4.9                  | 31.0 | 0.2            | 5.7              | 2.8              | 9.4  | 1.4              | 18.2 | 27.6 | 75.0 | 254.0 | 125.9 | 43.8  | 14.1  | 54.1  |
| 14     | RJ 04/<br>Hdp1       | 5.5                  | 23.0 | 0.1            | 2.5              | 4.7              | 7.6  | 0.1              | 20.3 | 27.9 | 81.0 | 186.0 | 91.7  | 30.5  | 15.6  | 20.9  |
| 15     | RS 04/<br>Hdj        | 3.5                  | 27.0 | 0.2            | 4.5              | 13.4             | 18.2 | 3.8              | 70.3 | 88.5 | 32.0 | 678.0 | 482.2 | 153.7 | 16.6  | 240.1 |
| 16     | RS 05/<br>Hdj        | 3.5                  | 27.0 | 0.1            | 4.0              | 6.0              | 10.2 | 2.2              | 47.2 | 57.4 | 51.0 | 488.0 | 291.6 | 134.0 | 24.4  | 111.4 |
| 17     | SC 01/<br>H2         | 4.6                  | N.D  | 0.7            | 13.9             | 8.6              | 23.3 | 0.6              | 16.4 | 39.7 | N.D  | N.D   | N.D   | 108.5 | 26.0  | 224.5 |
| 18     | SC 02/<br>Hdoj2      | 3.3                  | 6.0  | 0.2            | 4.4              | 3.1              | 7.9  | 2.1              | 44.2 | 52.1 | 6.0  | 940.0 | 528.1 | 188.4 | 24.5  | 260.3 |
| 19     | SP 01/<br>Hp1        | 5.2                  | 4.0  | 0.5            | 0.6              | 1.5              | 3.2  | 0.4              | 58.2 | 61.4 | 61.0 | 391.0 | 207.4 | 112.8 | 23.7  | 53.7  |
| 20     | SP 01/<br>Hp2        | 5.1                  | 1.0  | 0.1            | 0.3              | 1.1              | 1.5  | 0.3              | 41.7 | 43.3 | 69.0 | 313.0 | 144.4 | 95.1  | 13.2  | 17.7  |

pH in water (1:2.5, v/v); P, K, and Na: Mehlich-1; Ca<sup>2+</sup>, Mg<sup>2+</sup>, and Al<sup>3+</sup>: 1 mol L<sup>-1</sup> KCl; H+Al: acidity potential, calcium acetate 0.5 mol L<sup>-1</sup>; CEC: cation exchange capacity at pH 7.0; MM: mineral matter; OM: organic matter: Walkley-Black method; C-CHN: carbon determined by CHN analysis; N-CHN: nitrogen determined by CHN analysis; C-FAF: carbon in the fulvic acid fraction; C-HAF: carbon in the humic acid fraction; C-HUM: carbon in the humin fraction. Sources: Valladares (2003), Fontana (2009), and Ebeling (2010).

**Table 2.** Description of the digestion methods analyzed

| Digestion      | Method          | Proportion (v/v)           | System                       |
|----------------|-----------------|----------------------------|------------------------------|
| Pseudo - Total | Aqua Regia (AR) | 1 HNO <sub>3</sub> : 3 HCl | Closed system<br>(microwave) |
|                | EPA 3051 (NIT)  | HNO <sub>3</sub>           |                              |
|                | EPA 3051A (RAR) | 3 HNO <sub>3</sub> :1 HCl  |                              |

The variation rates of the methods was determined from the equation 1:

$$VR (\%) = \frac{(Vd - VMB) \times 100}{VMB} \quad \text{Eq. 1}$$

where VR is variation rates, in percentage; Vd - metal value extracted by the subject method; and VMB - metal value extracted by the method based.

The statistical procedures were performed using Statistical Analysis Software (SAS, 2010) and Excel® in the Microsoft Office® package.

## RESULTS AND DISCUSSION

### Heavy metal recovery from reference soil samples

The heavy metal contents in reference samples SRM 2709 and SRM 2782 recovered using the soil digestion methods are shown in table 3. The recovery rates of the reference samples were satisfactory (over 60 % for all soil digestion methods) when compared with the leachable values, except for Pb in the SRM 2709 sample, indicating the efficiency of the methods.

### Comparison of digestion methods for determination of metals in organic horizons

Figure 1 shows the regression plots of the heavy metals extracted using the USEPA 3051 method and the contents of the heavy metals extracted using the AR and Reversed Aqua Regia (RAR) methods (USEPA 3051A). Table 4 shows the parameters associated with the linear regressions ( $\hat{y} = a + b x$ ) of the three digestion methods studied for Ba, Cr, Cu, Fe, Mn, Ni, Pb and Zn and their respective confidence intervals (upper and lower) at a 99 % significance level.

Cooper, Fe and Mn were statistically similar among the methods because the slopes were not different from 1 and the y-intercepts were not different from 0, considering a 99 % confidence interval (Miller and Miller, 2005). The digestions for Pb using AR and USEPA 3051 performed noticeably better than the USEPA 3051A digestion method.

An analysis of Ni and Zn showed that the AR method performed better than nitric digestion and therefore better than the USEPA 3051A method, which can be considered statistically similar to the USEPA3051 method. Similar results were observed in mineral soils in the state of Mato Grosso do Sul, in which the highest metal contents were extracted using the AR method (Pérez et al., 2013).

Statistical similarities were also observed between the AR and USEPA 3051 methods and between the AR and the USEPA 3051A methods for Cr. However, the USEPA 3051A method can not be considered similar to USEPA 3051 because the y-intercept was higher than zero, although the slope was close to 1.

Nitric digestion showed clearly higher solubilizing capacity than the other digestion methods for Ba, in contrast to the results for the other metals analyzed, for which the

**Table 3.** Recovery of heavy metal in reference soils (NIST SRM 2709 and SRM 2782) by three digestion methods

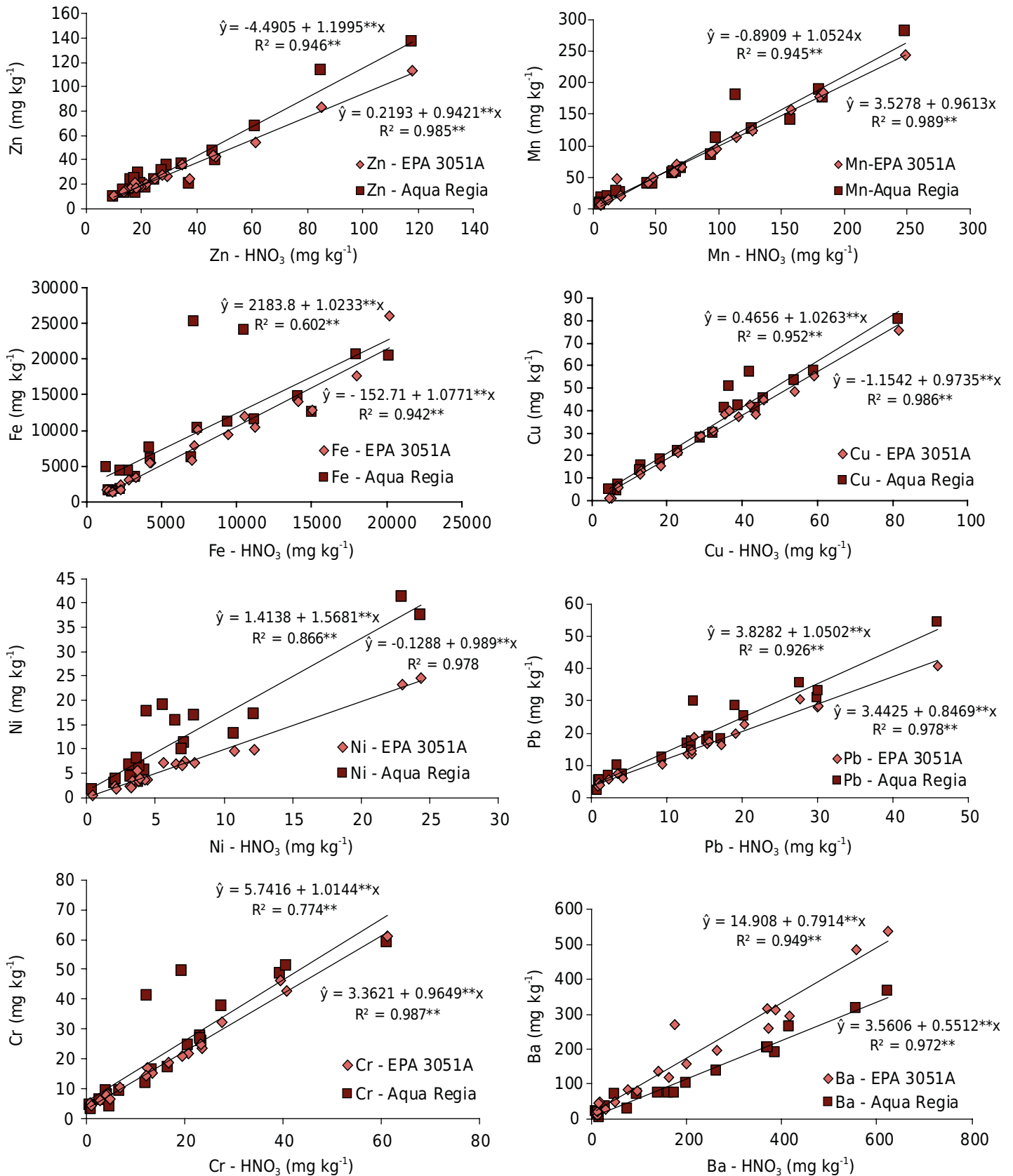
| Sample NIST | Certified value     | Leach Recovery NIST | EPA 3051            |                                      | EPA 3051A           |                                      | Aqua Regia          |                                      |
|-------------|---------------------|---------------------|---------------------|--------------------------------------|---------------------|--------------------------------------|---------------------|--------------------------------------|
|             |                     |                     | Determined value    | Recovery (determined) <sup>(1)</sup> | Determined value    | Recovery (determined) <sup>(1)</sup> | Determined value    | Recovery (determined) <sup>(1)</sup> |
|             | mg kg <sup>-1</sup> | %                   | mg kg <sup>-1</sup> | %                                    | mg kg <sup>-1</sup> | %                                    | mg kg <sup>-1</sup> | %                                    |
| Zn          |                     |                     |                     |                                      |                     |                                      |                     |                                      |
| 2709        | 106±3               | 94                  | 91.6                | 86                                   | 98.5                | 99                                   | 104.4               | 105                                  |
| 2782        | 1254±196            | 93                  | 1183                | 101                                  | 1290                | 111                                  | 1125                | 96                                   |
| Fe          |                     |                     |                     |                                      |                     |                                      |                     |                                      |
| 2709        | 35000±1100          | 86                  | 28270               | 94                                   | 33157               | 111                                  | 40205               | 134                                  |
| 2782        | 269000±7000         | 94                  | 320939              | 126                                  | 304219              | 120                                  | 278927              | 110                                  |
| Mn          |                     |                     |                     |                                      |                     |                                      |                     |                                      |
| 2709        | 538±17              | 87                  | 417.1               | 89                                   | 468.9               | 100                                  | 467.1               | 100                                  |
| 2782        | 300                 | 86                  | 197.1               | 76                                   | 245.0               | 95                                   | 246.1               | 96                                   |
| Cu          |                     |                     |                     |                                      |                     |                                      |                     |                                      |
| 2709        | 34.6±0.7            | 92                  | 34.4                | 108                                  | 33.3                | 104                                  | 31.0                | 97                                   |
| 2782        | 2594±52             | 94                  | 2429                | 100                                  | 2439                | 100                                  | 2327                | 96                                   |
| Ni          |                     |                     |                     |                                      |                     |                                      |                     |                                      |
| 2709        | 88±5                | 89                  | 72.7                | 93                                   | 78.1                | 100                                  | 75.3                | 96                                   |
| 2782        | 154.1±3.1           | 62                  | 109.9               | 115                                  | 117.5               | 122                                  | 101.8               | 106                                  |
| Pb          |                     |                     |                     |                                      |                     |                                      |                     |                                      |
| 2709        | 18.9±0.5            | 69                  | 5.5                 | 42                                   | 8.6                 | 66                                   | 14.5                | 112                                  |
| 2782        | 574±11              | 97                  | 482.5               | 87                                   | 500.5               | 90                                   | 551.5               | 100                                  |
| Cr          |                     |                     |                     |                                      |                     |                                      |                     |                                      |
| 2709        | 130±4               | 61                  | 48.5                | 61                                   | 54.7                | 69                                   | 66.7                | 84                                   |
| 2782        | 109±6.0             | 61                  | 50.1                | 76                                   | 58.5                | 89                                   | 72.0                | 109                                  |
| Ba          |                     |                     |                     |                                      |                     |                                      |                     |                                      |
| 2709        | 968±40              | 41                  | 460.5               | 116                                  | 376.2               | 95                                   | 343.8               | 86                                   |
| 2782        | 254±24              | 60                  | 170.5               | 112                                  | 168.7               | 111                                  | 146.8               | 97                                   |

<sup>(1)</sup> Percentage of metals recovered in relation to the leachate.

AR method had the highest extraction capacity. The reduced Ba recovery by methods using hydrochloric acid may be associated with the formation of BaCl<sub>2</sub> precipitates.

The methods were effective for all the elements studied, and the respective slopes and y-intercepts fell within the confidence interval when assessed at a probability level of 0.01 (Table 4).

Accordingly, the AR method can be recommended as the most suitable for heavy metal extraction from soils with high organic matter content because that method showed the



**Figure 1.** Contents of Zn, Mn, Fe, Cu, Ni, Pb, Cr, and Ba extracted using the EPA 3051, Aqua Regia and EPA 3051A methods for Histosols. \*\*: significant at 1 % probability.

best performance for most of the metals analyzed among the methods tested. Similar results were observed in the state of Mato Grosso do Sul, in which the contents of the heavy metals extracted using the AR method were higher than those using the USEPA 3051 and 3051A methods (Pérez et al., 2013). A contrasting performance was described

**Table 4.** Parameters associated with the linear regressions of the three digestion methods studied for Ba, Cr, Cu, Fe, Mn, Ni, Pb and Zn

| y   | x                | a       | b    | Confidence interval (1 %) |         |       |       |
|-----|------------------|---------|------|---------------------------|---------|-------|-------|
|     |                  |         |      | a                         |         | b     |       |
|     |                  |         |      | Upper                     | Lower   | Upper | Lower |
| Zn  |                  |         |      |                           |         |       |       |
| RAR | HNO <sub>3</sub> | 0.22    | 0.94 | -3.12                     | 3.56    | 0.86  | 1.02  |
| AR  | HNO <sub>3</sub> | -4.50   | 1.20 | -12.89                    | 3.91    | 1.01  | 1.39  |
| AR  | RAR              | -5.01   | 1.28 | -11.14                    | 1.11    | 1.13  | 1.43  |
| Mn  |                  |         |      |                           |         |       |       |
| RAR | HNO <sub>3</sub> | 3.53    | 0.96 | -3.74                     | 10.80   | 0.89  | 1.02  |
| AR  | HNO <sub>3</sub> | -0.89   | 1.05 | -19.07                    | 17.29   | 0.88  | 1.22  |
| AR  | RAR              | -4.02   | 1.09 | -23.46                    | 15.41   | 0.90  | 1.27  |
| Fe  |                  |         |      |                           |         |       |       |
| RAR | HNO <sub>3</sub> | -152.71 | 1.08 | -1836                     | 1530.26 | 0.89  | 1.26  |
| AR  | HNO <sub>3</sub> | 2183.79 | 1.02 | -3040                     | 7407.91 | 0.46  | 1.59  |
| AR  | RAR              | 2436.55 | 0.94 | -2513                     | 7386.38 | 0.44  | 1.43  |
| Cu  |                  |         |      |                           |         |       |       |
| RAR | HNO <sub>3</sub> | -1.15   | 0.97 | -4.04                     | 1.73    | 0.89  | 1.05  |
| AR  | HNO <sub>3</sub> | 0.46    | 1.02 | -5.18                     | 6.11    | 0.87  | 1.18  |
| AR  | RAR              | 1.58    | 1.05 | -2.55                     | 5.71    | 0.93  | 1.18  |
| Ni  |                  |         |      |                           |         |       |       |
| RAR | HNO <sub>3</sub> | -0.13   | 0.99 | -1.06                     | 0.80    | 0.89  | 1.09  |
| AR  | HNO <sub>3</sub> | 1.41    | 1.57 | -2.49                     | 5.32    | 1.15  | 1.98  |
| AR  | RAR              | 1.61    | 1.59 | -1.91                     | 5.13    | 1.20  | 1.97  |
| Pb  |                  |         |      |                           |         |       |       |
| RAR | HNO <sub>3</sub> | 3.44    | 0.85 | 1.81                      | 5.07    | 0.76  | 0.93  |
| AR  | HNO <sub>3</sub> | 3.83    | 1.05 | 0.03                      | 7.63    | 0.85  | 1.25  |
| AR  | RAR              | -0.57   | 1.25 | -3.85                     | 2.70    | 1.07  | 1.42  |
| Cr  |                  |         |      |                           |         |       |       |
| RAR | HNO <sub>3</sub> | 3.36    | 0.96 | 1.63                      | 5.09    | 0.89  | 1.04  |
| AR  | HNO <sub>3</sub> | 5.74    | 1.01 | -3.03                     | 14.51   | 0.64  | 1.39  |
| AR  | RAR              | 2.13    | 1.06 | -7.29                     | 11.54   | 0.68  | 1.42  |
| Ba  |                  |         |      |                           |         |       |       |
| RAR | HNO <sub>3</sub> | 14.91   | 0.79 | -19.07                    | 48.88   | 0.67  | 0.91  |
| AR  | HNO <sub>3</sub> | 3.56    | 0.55 | -13.80                    | 20.92   | 0.49  | 0.61  |
| AR  | RAR              | 0.47    | 0.65 | -32.96                    | 33.91   | 0.51  | 0.80  |

RAR: Reversed Aqua Regia (EPA 3051A); AR: Aqua Regia; HNO<sub>3</sub>: EPA 3051.

by Melo and Silva (2008), who observed that the digestion method using AR yielded the lowest rate of metal recovery from organic waste.

The differences in the metal recovery rates using the different methods may be associated with the nature of the organic compounds and, especially, the soil mineral composition. Histosols still have a significant ratio of mineral material, despite having a higher quantity of



organic material than mineral soils (Table 1). This characteristic therefore explains the better performance of digestion using AR, which employs a higher concentration of hydrochloric acid in the digestion, which promotes higher dissolution of the soil mineral fraction.

The results reported confirm the importance of specifically standardizing the method for soils with organic characteristics because the use of standard methods for mineral soils or organic waste may lead to unreliable results and incorrect interpretations.

### Relationship between rates of variation of the methods and soil traits

The rates of variation between the methods were calculated for the metals that showed differences in the quantity extracted using different digestion methods

**Table 5.** Variation rates among digestion methods EPA 3051, EPA 3051A and Aqua Regia obtained in the different organic horizons

| Sample              | Zn          |            |            | Ni          |            |            | Pb          |            |            | Cr          |            |            | Ba          |            |            |
|---------------------|-------------|------------|------------|-------------|------------|------------|-------------|------------|------------|-------------|------------|------------|-------------|------------|------------|
|                     | RAR:<br>NIT | AR:<br>NIT | AR:<br>RAR | RAR:<br>NIT | AR:<br>NIT | AR:<br>RAR | RAR:<br>NIT | AR:<br>NIT | AR:<br>RAR | RAR:<br>NIT | AR:<br>NIT | AR:<br>RAR | RAR:<br>NIT | AR:<br>NIT | AR:<br>RAR |
|                     | %           |            |            |             |            |            |             |            |            |             |            |            |             |            |            |
| 1                   | -6          | -29        | -25        | -36         | 28         | 99         | 136         | 186        | 21         | 34          | -13        | -35        | 11          | -65        | -69        |
| 2                   | -11         | 10         | 24         | -20         | 39         | 74         | -11         | 18         | 33         | -1          | 9          | 10         | -22         | -49        | -34        |
| 3                   | 0           | 5          | 5          | -25         | 109        | 179        | 3           | 48         | 44         | 7           | 14         | 7          | 63          | 38         | -15        |
| 4                   | -8          | -17        | -9         | 5           | 140        | 129        | 8           | 15         | 7          | 17          | 35         | 16         | -7          | 22         | 32         |
| 5                   | -4          | 15         | 20         | 1           | 53         | 52         | 114         | 186        | 33         | 38          | 231        | 140        | -13         | -43        | -35        |
| 6                   | 2           | 2          | 0          | -10         | -21        | -12        | 11          | 20         | 9          | 124         | 113        | -5         | -18         | -28        | -13        |
| 7                   | -5          | 2          | 8          | -11         | 111        | 136        | -7          | 2          | 10         | 5           | 25         | 19         | -4          | -47        | -45        |
| 8                   | 7           | -6         | -12        | -23         | 69         | 119        | 350         | 417        | 15         | 57          | 32         | -16        | 145         | -91        | -96        |
| 9                   | 1           | 45         | 43         | -16         | 301        | 377        | 10          | 28         | 16         | 17          | 22         | 4          | -17         | 8          | 31         |
| 10                  | -34         | -47        | -19        | 52          | 112        | 40         | -6          | 9          | 16         | 0           | -4         | -4         | 52          | -58        | -72        |
| 11                  | 2           | 10         | 8          | 3           | 57         | 51         | 4           | 29         | 24         | 15          | 23         | 6          | -26         | -48        | -30        |
| 12                  | -1          | -5         | -4         | -11         | 22         | 38         | -6          | 4          | 10         | 13          | 1          | -10        | -15         | -45        | -36        |
| 13                  | -11         | 0          | 12         | -4          | 43         | 50         | 0           | 15         | 15         | 20          | -2         | -18        | -19         | -51        | -39        |
| 14                  | 5           | -9         | -13        | -12         | 31         | 48         | 44          | 72         | 19         | 88          | 83         | -2         | -28         | -55        | -38        |
| 15                  | -6          | -22        | -17        | 13          | 32         | 16         | 320         | 160        | -38        | 585         | 445        | -20        | -30         | -37        | -10        |
| 16                  | -1          | -11        | -10        | 0           | 80         | 80         | 11          | 24         | 12         | 5           | 17         | 11         | 37          | 67         | 22         |
| 17                  | 19          | 36         | 14         | 3           | 65         | 60         | 8           | 31         | 22         | 89          | 119        | 16         | -30         | -46        | -22        |
| 18                  | -4          | 48         | 54         | 12          | 195        | 163        | 229         | 343        | 35         | 381         | 230        | -31        | 197         | 14         | -62        |
| 19                  | -2          | 33         | 36         | 1           | 80         | 78         | 7           | 30         | 21         | 7           | 151        | 135        | -14         | -41        | -32        |
| 20                  | -10         | 19         | 32         | 26          | 239        | 168        | 37          | 117        | 59         | 6           | 18         | 12         | -1          | 40         | 42         |
| Mean <sup>(1)</sup> | 7           | 19         | 18         | 14          | 91         | 98         | 66          | 88         | 23         | 75          | 79         | 26         | 37          | 45         | 39         |
| Max <sup>(1)</sup>  | 34          | 48         | 54         | 52          | 301        | 377        | 350         | 417        | 59         | 585         | 445        | 140        | 197         | 91         | 96         |
| Min <sup>(1)</sup>  | 0           | 0          | 0          | 0           | 21         | 12         | 0           | 2          | 7          | 0           | 1          | 2          | 1           | 8          | 10         |
| SD <sup>(1)</sup>   | 8           | 16         | 14         | 13          | 76         | 82         | 109         | 117        | 14         | 147         | 112        | 39         | 49          | 19         | 22         |

NIT: EPA 3051 (concentrated HNO<sub>3</sub>); RAR: EPA 3051A (Reversed Aqua Regia); AR: Aqua Regia. Max: maximum; Min: minimum; SD: standard deviation.  
<sup>(1)</sup> Values calculated from the data in module.

(Table 5): Ba, Cr, Ni, Pb and Zn. This enabled calculation of the magnitude of that difference in each horizon studied. The simplest method, which uses nitric acid only (USEPA 3051), was chosen as the basis for comparison because a total digestion method was not used. The USEPA 3051A (RAR) digestion method was used as the basis for comparing the AR:RAR.

The variation rates with negative values indicate that the method used as the basis for comparison had the highest extraction capacity (highest value of metal concentration). However, variation rates with positive values indicate that the method to which the base method was compared was better (Table 5). Thus, the variation may be larger or smaller depending on the element analyzed and the specific characteristics of each horizon. Sample 09 can be used as an example: for Zn and Ni, this sample showed high rates of variation between AR and the USEPA 3051 method and between AR and USEPA 3051A, although it showed no significant variation between the USEPA 3051A and the USEPA 3051 methods (concentrated HNO<sub>3</sub>). No significant variations were observed among the methods for Ba, Cr and Pb. Conversely, no significant variations were observed among the methods for any tested metal when analyzing the rates of variation of sample 12. Sample 15 showed significant variations in the Cr and Pb levels for the AR and RAR methods compared with the nitric digestion method. Thus, the results clearly show that the difference in variation rates and element recovery efficiency was directly associated with the dynamics of the chemical element, the method used and the ratio of the mineral fraction (composition) of the horizon.

The highest variations in Ni and Zn were observed when comparing the AR method with the others. However, the highest variations in Cr and Pb were observed for the AR and/or RAR methods in comparison to the method using nitric acid only. The highest variations in the case of Ba occurred between the RAR and the nitric acid methods.

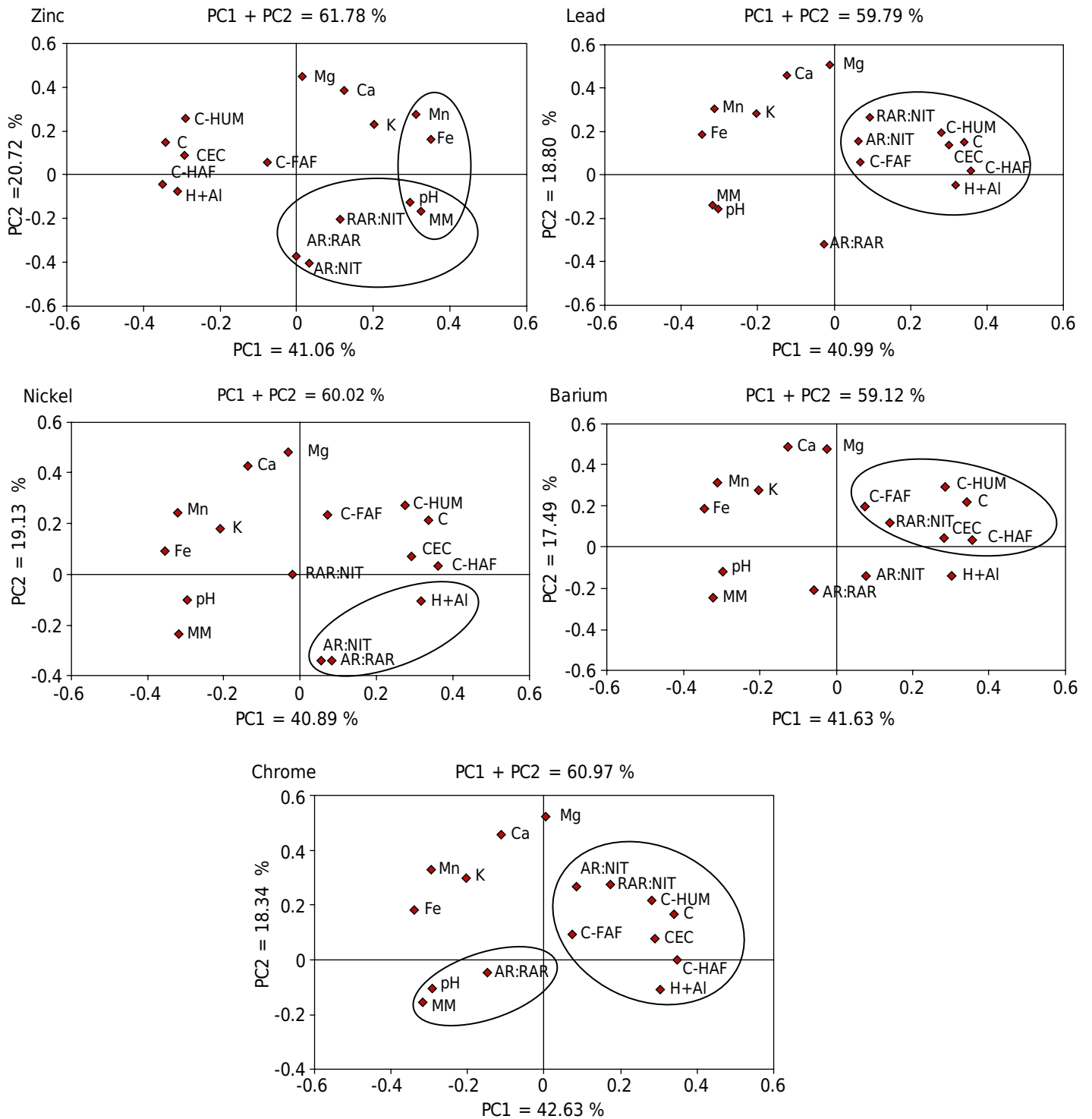
Multivariate analyses were performed using Principal Component Analysis (PCA) to best understand which soil traits most affected the variations in the levels of Ba, Cr, Ni, Pb and Zn metals extracted by the methods. For this analysis, it should be noted that the data were standardized to a mean of 0 and a variance of 1 to avoid the effects of different variable units. Values higher than 0.30 were established for the weights of the significant variables for the present study.

Principal components 1 and 2 were plotted from the weights to visualize and best explain the results because the two components accounted for more than 50 % of the cumulative variance in all cases (Figure 2).

Figure 2 can be used to relate the metal variation rates with the soil traits. The position of the markers relative to the origin (0,0) represents significance, that is, the farther the distance from the origin, the more significant the marker will be, and a greater proximity between markers represents a greater contribution of the traits.

Approximately 62 % of the cumulative variance in the Zn variation rates and the traits of organic horizons were explained by the first two components; principal component 1 (PC1) explained 41.1 %, and principal component 2 (PC2) explained 20.7 % of the total variance (Figure 2).

The rates of variation between the methods for Zn showed a closer relationship with the MM content and pH value, which are directly related to the Fe and Mn contents (Figure 2). Although this contribution was not as close, the results clearly show that the increase in mineral content of the organic horizons may lead to a higher variation in Zn extraction between the AR and other methods. This is due to the increased solubilizing capacity of the mineral fraction of the AR method because it used a higher HCl ratio in the protocol, which has solubilizing characteristics. In a study that sought to establish QRVs for heavy metals in soils from three river basins in the state of Espírito Santo using multivariate



**Figure 2.** Principal components 1 and 2, for variation rates of the levels of metals between the methods and the properties of organic horizons. H+Al: acidity potential; CEC: cation exchange capacity at pH 7.0; MM: mineral matter, C: carbon determined by CHN analysis; C-FAF: carbon in the fulvic acid fraction; C-HAF: carbon in the humic acid fraction; C-HUM: carbon in the humin fraction.

analysis methods, significant correlations were also found between the Zn levels and the pH values and Fe levels (Paye et al., 2012).

Principal components 1 and 2, which were extracted according to the rates of variation of the Ni and soil traits, accounted for 60.0 % of the cumulative variance - PC1 accounted for 40.9 % and PC2, 19.1 % of the total variance (Figure 2). The rates of variation of AR:NIT and AR:RAR for Ni showed no significant relationship with the selected soil traits, and potential acidity was the variable that most contributed to the variation rates in

comparison to the other traits analyzed. However, though Ni is also related to soil organic content, which is directly linked to potential acidity, the contribution of those traits to the rates of variation of the methods was smaller for this element than that for the rates assessed for Ba, Cr and Pb.

Principal components 1 and 2 together accounted for 59.8 % of the cumulative variance for Pb; PC1 accounted for 41.0 %, and PC2 accounted for 18.8 % of the total variation (Figure 2). The rates of variation of AR:NIT and ARI:NIT were closely and significantly related to the variables that represented the organic content of the horizons, and C-HAF had the highest significance among the properties. Cu and Pb have high affinities for the carboxylic and phenolic groups of organic matter and are therefore usually associated with the soil organic fraction (Sparks, 2002).

Principal components 1 and 2 accounted for 59.1 % (41.6 % PC1 and 17.5 % PC2) of the cumulative variance for Ba, and 61.0 % (42.6 % PC1 and 18.3 % PC2) of the cumulative variance for Cr. The relationship clearly showed that the highest variation in those elements (ARI:NIT) was closely linked to soil organic matter content, especially total C content, followed by C in the humin fraction and humic acid fraction, which were the most significant traits. Conversely, the variations between the AR and RAR methods were mainly related to MM and pH.

## CONCLUSIONS

The digestion methods showed different heavy metal solubilizing capacities that varied according to the element and chemical and physical characteristics of the Histosol samples.

The concentrations of extracted Cu, Fe and Mn were not different among the three soil digestion methods. The digestion methods should not be considered similar regarding Cr, Ni, Pb and Zn, and the AR method had the highest content of metals in the Histosols. Ba performed contrary to the other metals, and the USEPA 3051 method was the most effective for extraction.

The highest Ni and Zn variation rates in the Histosols were observed between AR and the USEPA 3051 method and between AR and the USEPA 3051A method due to the variation, primarily of potential acidity and mineral matter, respectively. However, the highest variations in Cr and Pb were observed between the AR:NIT and ARI:NIT ratios, and those variations were predominantly associated with the organic content of those soils. Ba showed the highest variation between the RAR and the nitric digestion method.

The AR method proved to be suitable for heavy metal extraction from organic soils and can therefore be used as a standard method to determine quality reference values.

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