

Assessment of mobile and potential mobile trace elements extractability in calcareous soils using different extracting agents

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HIGHLIGHTS

- DTPA and NH_4OAc , HNO_3 and EDTA, and MgCl_2 and NH_4NO_3 had similar behavior.
- In NH_4OAc , DTPA, and EDTA, the possibility of re-adsorption of trace elements is low.
- CaCl_2 may be more suitable than other extracts in calcareous soils.

ARTICLE INFO

Article history:

Received 12 March 2019

Revised 28 August 2019

Accepted 9 September 2019

Available online 16 November 2019

Keywords:

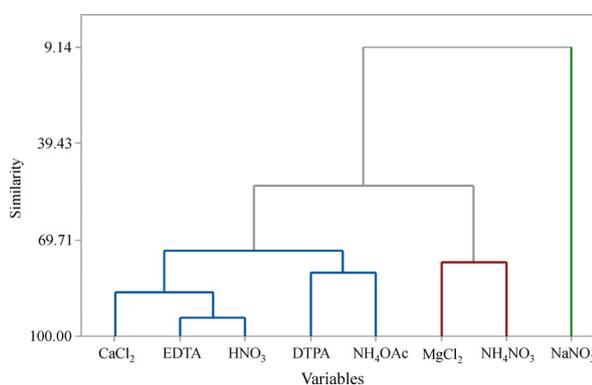
Mobility

Calcareous soils

Extracting agents

Trace elements

GRAPHIC ABSTRACT



ABSTRACT

Understanding trace elements mobility in soils, extracting agents, and their relationships with soil components, are essential for predicting their movement in soil profile and availability to plants. A laboratory study was conducted to evaluate extractability of cadmium (Cd), cobalt (Co), copper (Cu), nickel (Ni), and zinc (Zn) from calcareous soils utilizing various extracting agents to be specific CaCl_2 , DTPA, EDTA, HNO_3 , MgCl_2 , NaNO_3 , NH_4NO_3 , and NH_4OAc . Cluster analysis indicated that DTPA and NH_4OAc , HNO_3 and EDTA, and MgCl_2 and NH_4NO_3 extracting agents yielded comparative values, whereas NaNO_3 and CaCl_2 have shown different behavior than other extracting agents for all studied trace elements. The speciation of extracted trace elements in solutions indicated that in the CaCl_2 , NaNO_3 , NH_4NO_3 , and MgCl_2 extracting agents most extracted Cd, Co, Ni, Zn, and part of Cu were as free ions and may be re-adsorbed on soils, leading to lower extractability, whereas, in the case of HNO_3 extracting agent, the likelihood of re-adsorption of trace elements may be little. The results of speciation of trace elements using NH_4OAc , DTPA, and EDTA extracting agents showed that $\text{Me}(\text{Acetate})_3^-$, $\text{Me}(\text{Acetate})_2(\text{aq})$, $\text{Me}(\text{DTPA})^{3-}$, $\text{Me}(\text{EDTA})^{2-}$, and $\text{MeH}(\text{EDTA})^-$ complexes dominated in solutions indicating that the extracted trace elements may not be re-adsorbed on soils, leading to higher extractability. The results of this study are useful for short and long-term evaluations of trace elements mobility and further environmental impacts.

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1 Introduction

Soils in arid and semi-arid regions recognized for having high calcite reducing trace elements mobility. The mobility and availability pattern of trace elements in calcareous

soils varies for each trace element and are significantly dependent on soil properties such as soil texture, organic matter (OM) content, calcium carbonate (CaCO_3), clay mineralogy, concentration, and composition of trace elements in soils (Sánchez-Camazano et al., 1998; Adriano, 2001). The total trace elements content in soils can provide useful information about the accumulation of trace elements in the soil. Anyway, it does not furnish

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information concerning the mobility of trace elements in the soil (Tang et al., 2018, 2019). Thus, it may be necessary to study the trace elements availability in the soil (Ramos-Miras et al., 2011; García-Carmona et al., 2019). Trace elements' extractability from the soil will be influenced by exchange processes, complexation with OM, and sorption (Krishnamurti et al., 2000). In soil, the mobility, chemical form, and toxicity of trace elements are dependent on their reactions with soil. The mobility of trace elements is one of the most important factors in determining the short and long-term environmental impacts of trace elements (Sparks, 1995; Sahuquillo et al., 2003). Understanding trace elements mobility in soils, extracting agents and their interactions with soil components, might make advantageous in foreseeing their mobility in the soil profile and availability to plants. Various single and sequential extracting agents have been proposed to evaluate the availability of trace elements in soils (Venegas et al., 2016; Waterlot et al., 2016; Ennoukh et al., 2017; Jorge Mendoza et al., 2017; Argyraki et al., 2018). Some researchers indicated high correlations between trace elements extracted by extracting agents such as CaCl_2 and their phytoavailability (Novozamsky et al., 1993; Meers et al., 2007; Fedotov et al., 2012), but the selection of a suitable extracting agent for accurate estimation of trace elements availability in calcareous is debatable (Fedotov et al., 2012). The extracting agents are classified into several groups: acids (hydrochloric acid (HCl), nitric acid (HNO_3)), chelating agents (ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid–triethanolamine (DTPA–TEA)), buffered salt solutions (ammonium acetate (NH_4OAc)) and unbuffered (or neutral) salt solutions (calcium chloride (CaCl_2) and ammonium nitrate (NH_4NO_3)) (Kabata-Pendias, 2011). Neutral salt solutions mimic the natural soil solutions (simulate reactions of trace elements with the soil) and hence are valuable to assess the availability of trace elements (Peijnenburg et al., 2007; Kabata-Pendias, 2011). Recent studies have demonstrated that trace elements such as cadmium (Cd), zinc (Zn) can be estimated by CaCl_2 extraction (Pueyo et al., 2004; Degryse et al., 2011). de Santiago-Martín et al. (2013) estimated the mobile fraction of Cd, copper (Cu), lead, (Pb) and Zn utilizing different extracting agents in calcareous soils. The use of different chemical extracting agents with different strengths makes it could reasonably be expected to recognize mobile and potentially mobile fractions of trace elements (de Santiago-Martín et al., 2013). The EDTA is often provided a good estimate of the trace elements phytoavailability (Meers et al., 2007; Zhang et al., 2014; Kim et al., 2016; Argyraki et al., 2018). The DTPA soil test has been utilized by Lindsay and Norvell (1978) for the extraction of Cu, iron (Fe), manganese (Mn) and Zn in calcareous soils. Many numbers of chelating agents have been applied in researches, proving that DTPA might have been the most effective extracting agent for

trace elements in soils (Lindsay and Norvell, 1978; Zbíral, 2016). The efficiency of an extracting agent for assessing trace elements extractability from the soil will be identified with the extracting capability to predict the availability of trace elements (Soriano-Disla et al., 2010). A good extracting agent might foresee the mobility of trace elements under different conditions. These extracting agents release specific trace elements in soil in light of the characteristic of specific extracting agents (Krishnamurti et al., 2000). The extractability of trace elements by different extracting agents from tropical ultramafic soils was carried out in Malaysian soils by van der Ent et al., (2019). They found relationships between extracted trace elements and the physicochemical properties of the soils (van der Ent et al., 2019). But, the relationships between trace elements extracted by different extracting agents have poorly quantified in calcareous soils (Jalali et al., 2019). Trace elements extractability was tested and compared on 25 calcareous soils collected from Hamedan, Iran utilizing eight commonly used extracting agents. This study concentrated on the Cd, cobalt (Co), Cu, nickel (Ni), and Zn trace elements previously shown that related to human activity in agricultural and industrial areas of Hamedan, Iran (Jalali and Majeri, 2016.; Jalali and Khanlari, 2008). The aims of this study were (1) to evaluate extractability of Cd, Co, Cu, Ni and Zn from calcareous soils utilizing different extracting agents, to be specific CaCl_2 , DTPA, EDTA, HNO_3 , MgCl_2 , NaNO_3 , NH_4NO_3 , and NH_4OAc , (2) to find relationships between extracted trace elements and the physicochemical properties of soils, (3) to choose appropriate extracting agent to estimate the trace elements mobility from calcareous soils utilizing distinctive statistical methods.

2 Materials and methods

2.1 Soil samples analysis

Twenty-five samples selected from depth 0–30 cm of soils with different land uses in the Hamedan Province, western Iran. The soil samples were air-dried and then sieved through a 2-mm sieve. The physicochemical properties of soils analyzed by routine methods (Rowell, 1994). Particle size distribution measured by the hydrometer method (Bouyoucos, 1962). Cation exchange capacity (CEC) measured using 1 mol/L NH_4OAc (pH 7). The OM and equivalent CaCO_3 contents of soils determined using dichromate oxidation, and HCl neutralization, respectively (Rowell, 1994). Soil pH and electrical conductivity (EC) determined in a 1:5 soil to solution ratio after shaking soil suspensions for one h (Rowell, 1994). The pseudo total Cd, Co, Cu, Ni, and Zn contents extracted by 4 mol/L HNO_3 (Sposito et al., 1982) and measured by atomic absorption spectrometry (SpectrAA-220-Varian).

2.2 Extraction procedures

The air-dried and sieved soil samples utilized for the extraction procedures. In this study, 0.01 mol/L CaCl_2 , 1 mol/L MgCl_2 , 0.1 mol/L NaNO_3 , and 1 mol/L NH_4NO_3 extracting agents were utilized to estimate mobile fraction (neutral salts) and 0.005 mol/L DTPA, 0.05 mol/L EDTA, 0.5 mol/L HNO_3 , and 1 mol/L NH_4OAc extracting agents were utilized to estimate potentially mobile fraction (Table S1 in Supplementary Materials). The extracted trace elements concentrations measured by the atomic absorption spectrometer. For all soil samples, after shaking, soil samples centrifuged for 15 min and after that filtered by Whatman No. 42 filter paper. All experiments done in duplicates. A recovery test performed utilizing spiked soil samples (Cd, Co, Cu, Ni, and Zn), and recoveries were determined. The average total heavy trace elements recovery percentage was 96.8, 94.2, 98.2, 94.3, and 97.3 for Cd, Co, Cu, Ni, and Zn, respectively. Quality Assurance and Control (QA/QC) used for analytical data, standard methods used, the reagent blanks and standards carefully monitored, and replicates provided with samples. The equipment and tools calibrated using calibrations and standards. Blanks samples have been used to identify any sources of contamination.

2.3 Statistical analysis

Pearson's correlation coefficients and multiple regression analyze utilized to find the dependence of Cd, Co, Cu, Ni, and Zn contents to soil properties (Clay, pH, OM, and CEC) and to identify significant relationships between extracted trace elements by different extracting agents. The significance levels were set at $p < 0.05$, $p < 0.01$ and $p < 0.001$. The MINITAB 17.0 statistical software was employed for the calculations. A one-way analysis of variance (ANOVA) used to find significant differences. Multivariate analysis such as factor analysis (FA) and cluster analysis (CA) also used to interpret results and to find the similarities between different trace elements and extracting agents. Ward's method and the squared Euclidean distance were utilized.

2.4 Speciation

The Minteq model (Allison et al., 1991) used to calculate metal speciation and saturation indices in each extracting agent using following input parameters: measured trace elements concentration (mol/L), the soil solution pH, concentrations of each extracting agent used to extract trace elements (mol/L), atmospheric partial pressure of CO_2 ($P_{\text{CO}_2} = 3.83 \times 10^{-4}$), temperature (25°C) and measured soil CaCO_3 as solid phase. Seven soil samples that were representative of other soils chosen for speciation and saturation indices analysis.

3 Results and discussion

3.1 Soil properties

The main physicochemical properties of soils and pseudo total contents of trace elements shown in Table S2 in Supplementary Materials. The pH- H_2O value was neutral and alkaline (7.2–8.2). The average amount of CEC and OM in the soils was 15.4 cmol_c/kg and 1.3%, respectively. The pseudo total content of Cd, Co, Cu, Ni, and Zn ranged from 0.8 to 2.0, 7.9 to 11.9, 11.9 to 43.9, 30.1 to 65.8, and 23.8 to 47.1 mg/kg , respectively. Threshold trace elements detection for Varian SpectrAA 200 atomic absorption spectrometer model was (mg/L) Cd: 0.02, Co: 0.05, Cu: 0.03, Ni: 0.10, and Zn: 0.01.

3.2 Extractability of trace elements and efficiency of the extracting agents

The contents of extractable trace elements utilizing diverse extracting agents shown in Fig. 1. The highest contents of trace elements extracted by the EDTA (mean = 1.11, 4.09, 8.51, 7.56 mg/kg for Cd, Co, Cu, and Zn, respectively) and HNO_3 (2.05 mg/kg for Ni) and the lowest by the CaCl_2 (0.08, 0.15, 0.18 mg/kg for Cd, Co, and Zn, respectively), NaNO_3 (0.06 mg/kg for Cu), and NaNO_3 (0.048 mg/kg for Ni). The percentage of extractable trace elements relative to pseudo total content by different extracting agents calculated and presented in Fig. 2. There were noteworthy contrasts between EDTA and other extracting agents. There were no contrasts between EDTA and HNO_3 for Co, Ni, and Zn (Fig. 2). Generally, the mean percentages of extractable trace elements followed the order:

Cd: EDTA (84.4) > HNO_3 (55.4) > MgCl_2 (48.9) > NH_4OAc (44.3) > NaNO_3 (38.8) > NH_4NO_3 (11.0) > DTPA (9.4) > CaCl_2 (5.7).

Co: EDTA (41.9) > HNO_3 (38.0) > NH_4OAc (27.9) > MgCl_2 (12.2) > DTPA (10.4) > NaNO_3 (2.2) > CaCl_2 (1.6) > NH_4NO_3 (1.5).

Cu: EDTA (32.2) > DTPA (16.1) > HNO_3 (15.8) > NH_4OAc (9.9) > MgCl_2 (2.8) > NH_4NO_3 (1.9) > CaCl_2 (0.3) > NaNO_3 (0.3).

Ni: HNO_3 (15.0) > EDTA (14.7) > DTPA (6.2) > NH_4OAc (5.3) > MgCl_2 (4.9) > NH_4NO_3 (0.9) > NaNO_3 (0.8) > CaCl_2 (0.6).

Zn: HNO_3 (23.9) > EDTA (21.0) > DTPA (9.7) > MgCl_2 (3.3) > NH_4OAc (3.1) > NaNO_3 (0.8) > NH_4NO_3 (0.7) > CaCl_2 (0.5).

Considering EDTA extracting agent, a lower average percentage of Ni (14.7%) extracted than Cd (84.4%), Co (41.9%), Cu (32.2), and Zn (21.0%), indicate that Cd, Co, Cu, and Zn can form a complex with EDTA. The results are comparable with those obtained by Iglesias et al. (2018), who found higher Cd (20%–30%) and Cu (35%–40%) extractability than for Ni, Co, and Zn in Mediterra-

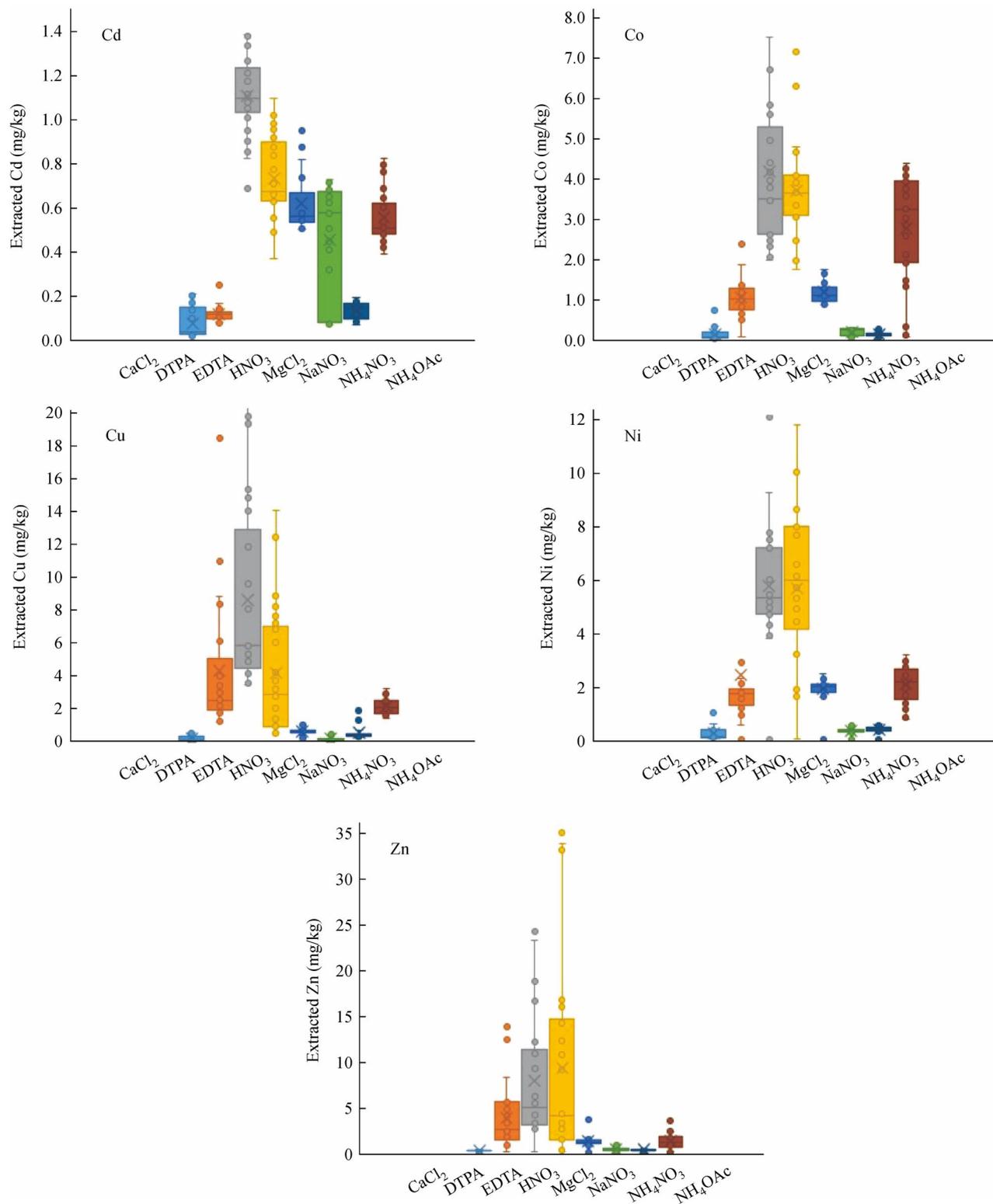


Fig. 1 Boxplot of extracted trace elements by different extracting agents (open circles denote the mean value and filled circles denote the median value).

nean agricultural soils amended with sewage sludge. Pardo et al. (2011) reported higher mobility of Cd than Ni, Cr, and Pb in amended soils. Organic acids released from the

root are also able to form a complex with trace elements and hence increasing mobility and leaching of trace elements in soil (Rajkumar et al., 2012). Thus, according

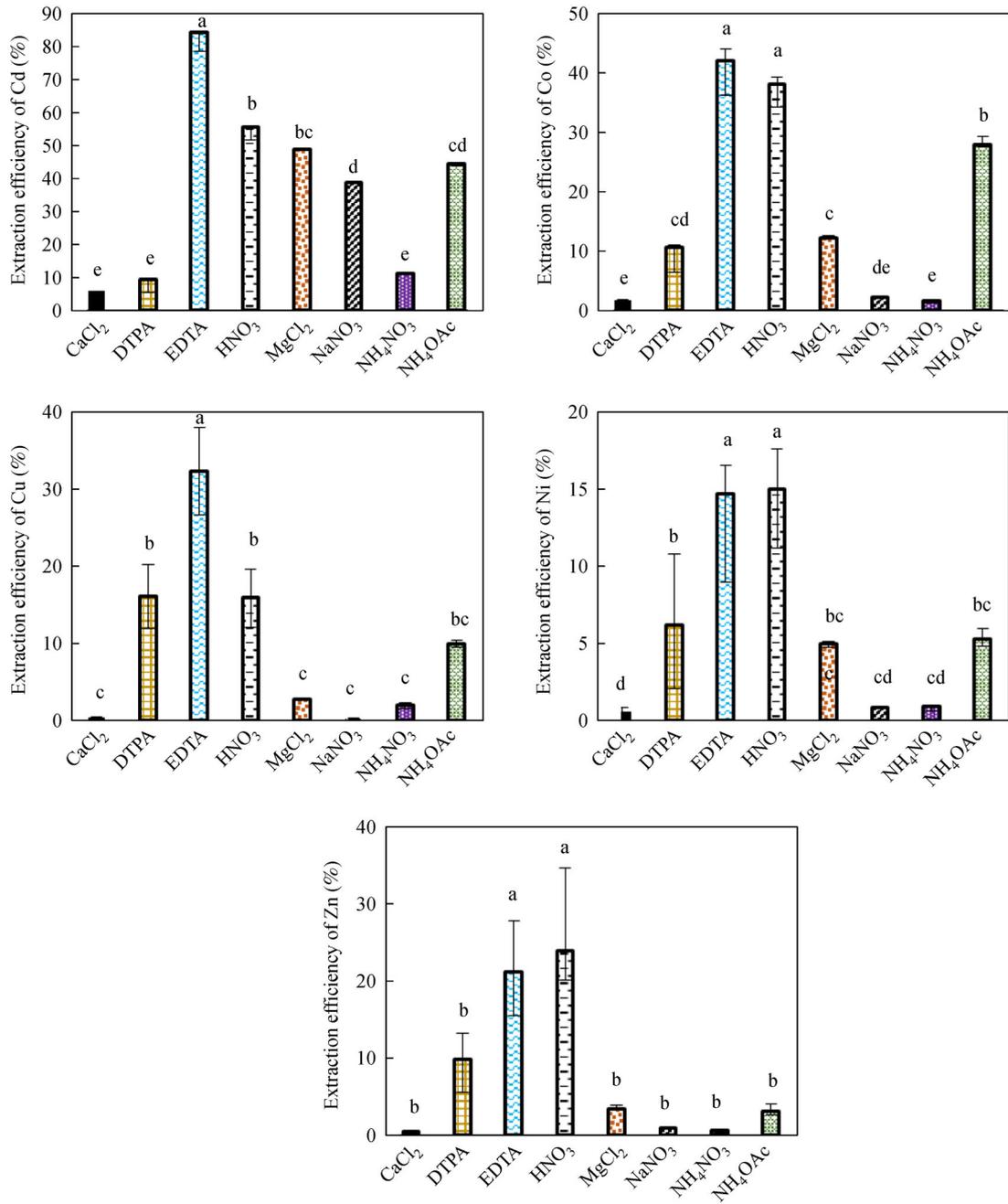


Fig. 2 Comparison between the Cd, Co, Cu, Ni, and Zn-extractable fraction of total trace element content (%) by CaCl₂, DTPA, EDTA, HNO₃, MgCl₂, NaNO₃, NH₄NO₃ and NH₄OAc. Different letters show significant differences at $p < 0.05$.

to the results in contaminated soils, EDTA extracting agent can be used to remove efficiently Cd, Co, Cu, and partly Zn.

Greater efficiency of EDTA for extracting trace elements from soils was reported by other researchers such as Zhang et al. (2010), de Santiago-Martín et al. (2013). Simple acid extracting agents (such as 2 mol/L HNO₃) is not appropriate for estimating the phytoavailability of trace elements in soils because such extracting agents extract more trace element from the soil than salt ones and is

unsatisfactory for predicting metal concentrations in plants (Gupta and Aten, 1993).

The Cd, Co, and Zn extracted by NaNO₃ extracting agents were more than CaCl₂ and NH₄NO₃ extracting agents. However, Pueyo et al. (2004) found lower contents of Cd, Cu, Pb, and Zn when using 0.1 mol/L NaNO₃ compared with 0.01 mol/L CaCl₂ or 1 mol/L NH₄NO₃. The 0.1 mol/L NaNO₃ extracting agent does not affect the solid and solution equilibrium because it does not form complexes and its cations or anions do not take part in

exchange reactions (Gupta and Aten, 1993). A higher amount of trace elements extracted by NH_4NO_3 than CaCl_2 extracting agent, which may be due to the higher ionic strength of the NH_4NO_3 extracting agent (Pueyo et al., 2004). The extracted trace elements by neutral salt solutions (CaCl_2 , MgCl_2 , NaNO_3 , and NH_4NO_3) were lower than those extracted by DTPA. It was indicated that NH_4NO_3 and CaCl_2 extracting agents could only remove trace elements from soluble and exchangeable phases (Rao et al. 2008). Some researchers (Sahuquillo et al., 2003, Li et al., 2014; Kelepertzis et al., 2015) highlighted the low ability of CaCl_2 to extract trace elements from soils. On the other hand, DTPA can remove a higher amount of trace elements from calcareous soils due to the presence of TEA, maintaining pH at 7.3 (O'Connor, 1988). Gupta and Sinha (2007) likewise acquired comparable outcomes. de Santiago-Martín et al. (2013) suggested that the low extractability of trace elements with neutral salts might be due to the specific adsorption of trace elements to the soils instead of retention on exchange positions.

3.3 Comparison between trace element contents with background contents of available trace elements in soils

Background values for Cd, Co, Cu, Ni, and Zn in agricultural soils utilizing EDTA solution in a European Mediterranean region were 0.13, 1.00, 0.80, 0.70, and 1.90 mg/kg, respectively (Ramos-Miras et al., 2011). In this study, the mean extractable Cd, Co, Cu, Ni, and Zn (1.11, 4.16, 8.51, 5.94, and 8.11 mg/kg) extracted by EDTA extracting agent were higher than those reported by Micó et al. (2007) in Spanish calcareous soils utilizing EDTA extraction. Ramos-Miras et al. (2011) reported higher concentrations of available trace elements compared with other researches. Extracted Cu and Zn by DTPA extracting agent in the most soils (72% and 64% of soils for Cu and Zn, respectively) were above the critical value of 1.75 mg/kg proposed by Zbiral (2016).

3.4 Correlation analysis between extracted trace elements by different extracting agents and soil properties

Correlation analysis is essential in analyzing trace elements in soils. Pearson correlation was utilized to investigate relationships between extractable contents (by the different extracting agents) and soil properties (Table S3 in Supplementary Materials). Particle size has a vital role in the availability of trace elements (Pinto et al., 2015). The results indicated that clay content was positively correlated with extractable Cd, Co, and Zn using NaNO_3 and extractable Cd using NH_4NO_3 . Clay and silt had a negative correlation with extractable Cd, Cu, and Ni using CaCl_2 and NH_4OAc extracting agents, whereas significant positive correlations observed between sand and extracted Cd, Cu, and Ni using both extracting agents.

Extractable Cd using NH_4NO_3 and Co using NaNO_3

extracting agents was significantly related to OM content indicating, those trace elements may be absorbed by OM (Cancela et al., 2002). Cadmium and Co, extracted by NH_4NO_3 and NaNO_3 extracting agents had a negative correlation with EC. Among soil properties, pH is viewed as the most important factor (Mühlbachová et al., 2005; Zeng et al., 2011). The negative correlation found between extractable Co and Cu using HNO_3 and pH. The pH was also significantly correlated with Ni extracted using NH_4OAc and Zn extracted by MgCl_2 .

3.5 Multiple regression analysis

Multiple regression analysis was used to find the relationships between soil properties (pH, EC, CEC, OM, CaCO_3 , clay, silt, and sand) and extracted trace elements (Table 1). For CaCl_2 and EDTA extracting agents, clay and OM were the relevant parameters. For all trace elements and extracting agents, r value > 0.40 obtained ($p < 0.05$). In the case of Cd and Ni, a higher correlation ($p < 0.001$) obtained using CaCl_2 and NH_4NO_3 than other extracting agents. Ettler et al. (2007) found that these extracting agents are also had a significant positive correlation for Cd, Cu, and Zn ($p < 0.001$). The correlation obtained by CaCl_2 -extractable Cu and Ni was generally greater than other extracting agents.

Relationships between trace elements extracted by diverse extracting agents with each other appeared in Fig. 3. According to Fig. 3, extracted Cd, Cu, Ni, and Zn by EDTA had a significant positive correlation with these trace elements extracted by the HNO_3 extracting agent. For Cd and Zn, a significant correlation obtained between neutral salt solutions (NaNO_3 with NH_4NO_3 and NH_4NO_3 with MgCl_2 , respectively).

3.6 Multivariate analysis

The FA used to find the similarity between trace elements utilizing mean values of all extracting agents for each trace element and to recognize the similarity between different extracting agents utilizing mean values of trace elements extracted by each extracting agent. Two factors extracted, accounting for over 70.3% of the total variation of soil trace elements (Cd, Co, Cu, Ni, and Zn). The factors 1 and 2 for the trace elements described 39% and 31.3% of the total sample variance, respectively. The component loadings of Cu (0.970) and Zn (0.974) were higher on factor 1 while, on factor 2, Co (0.858) and Ni (0.853) had higher loading. Hierarchical cluster analysis used on the Euclidean distances and the dendrogram of the trace elements and extracting agents shown in Fig. 4 and Fig. 5, respectively.

The dendrogram used to examine the relationships between different trace elements using extracting agents separately. Each of the eight dendrograms had two main large clusters. When the trace elements extracted with the

Table 1 Multiple regressions between Cd, Co, Cu, Ni, and Zn extracted by different extracting agents and soil properties

Metal	Method	Equation	<i>r</i>
Cd	CaCl ₂	0.192 – 0.025 OM – 0.004 Clay	0.85***
	DTPA	–	–
	EDTA	1.267 – 0.007 Clay	0.42*
	HNO ₃	0.494 + 1.441 EC	0.54**
	MgCl ₂	0.642 + 0.004 Silt	0.68***
	NaNO ₃	–0.142 + 0.017 CEC + 0.119 OM + 0.014 Clay – 0.004 Silt	0.86***
	NH ₄ NO ₃	0.093 – 0.192 EC + 0.004 CEC + 0.011 OM	0.89***
	NH ₄ OAc	1.137 + 0.018 CaCO ₃ –0.008 Clay–0.009 Silt	0.75***
Co	CaCl ₂	0.32 – 0.01 Clay	0.54**
	DTPA	–	–
	EDTA	–	–
	HNO ₃	4.52 – 0.09 CaCO ₃	0.65***
	MgCl ₂	1.31 – 0.01 Clay	0.47*
	NaNO ₃	0.29 – 0.68 EC + 0.002 Silt	0.82***
	NH ₄ NO ₃	0.11 + 0.001 Silt	0.40*
	NH ₄ OAc	3.49 + 0.07 CaCO ₃ – 0.05 Silt	0.72***
Cu	CaCl ₂	0.18 + 0.85 EC – 0.06 OM – 0.01 Clay	0.90***
	DTPA	18.78 – 2.64 pH + 37.86 EC – 0.92 OM	0.80***
	EDTA	17.40 – 1.50 OM – 0.33 Clay	0.71***
	HNO ₃	38.81 – 4.23 pH – 0.16 Clay	0.71***
	MgCl ₂	–	–
	NaNO ₃	0.67- 0.09 pH + 0.03 OM	0.47*
	NH ₄ NO ₃	–0.03 + 2.76 EC	0.57**
	NH ₄ OAc	1.81 + 3.37 EC – 0.01 Silt	0.68***
Ni	CaCl ₂	0.597 – 0.070 OM – 0.014 Clay	0.75***
	DTPA	3.690 – 0.461 CEC + 0.280 Clay	0.49*
	EDTA	7.814 – 0.780 OM – 0.265 CaCO ₃ + 0.079 Silt	0.59**
	HNO ₃	8.786 – 0.253 CaCO ₃	0.57**
	MgCl ₂	–	–
	NaNO ₃	0.269 + 0.003 Clay	0.44*
	NH ₄ NO ₃	0.938 – 0.090 pH + 0.004 Silt	0.73***
	NH ₄ OAc	2.60 – 0.020 Silt	0.59**
Zn	CaCl ₂	–	–
	DTPA	11.63 – 0.47 CEC	0.42*
	EDTA	24.84 – 0.67 Clay	0.53**
	HNO ₃	101.00 – 10.27 pH – 0.66 Clay	0.68***
	MgCl ₂	9.20 – 1.08 pH – 0.038 CEC + 0.06 CaCO ₃	0.67***
	NaNO ₃	0.01 + 0.02 CEC	0.58**
	NH ₄ NO ₃	1.12 – 0.12 pH	0.47*
	NH ₄ OAc	–	–

Note: **p* < 0.05, ** *p* < 0.01, *** *p* < 0.001.

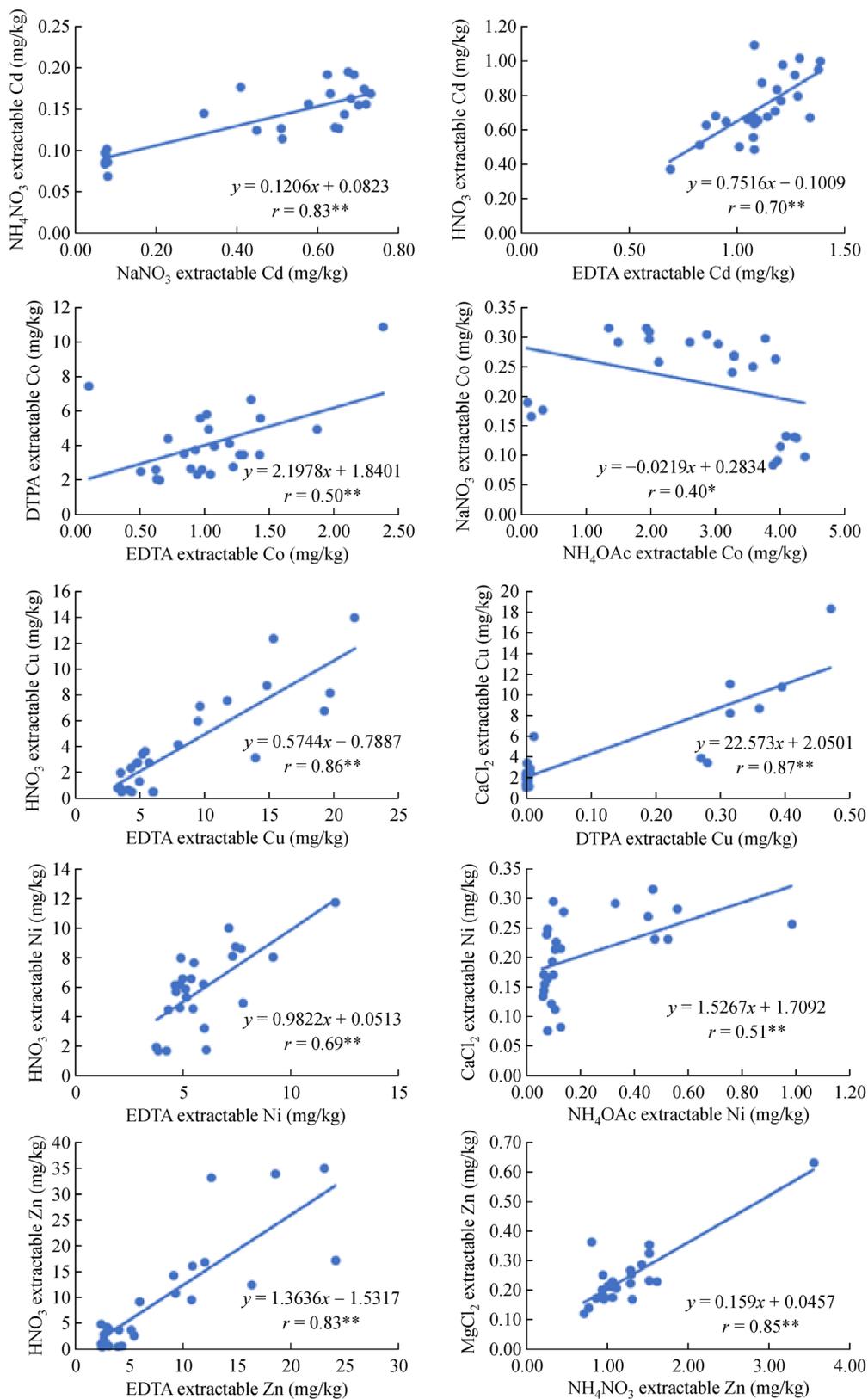


Fig. 3 Results of the linear regression analysis between extractable Cd, Co, Cu, Ni and Zn by different extracting agents ($*p < 0.05$, $**p < 0.01$).

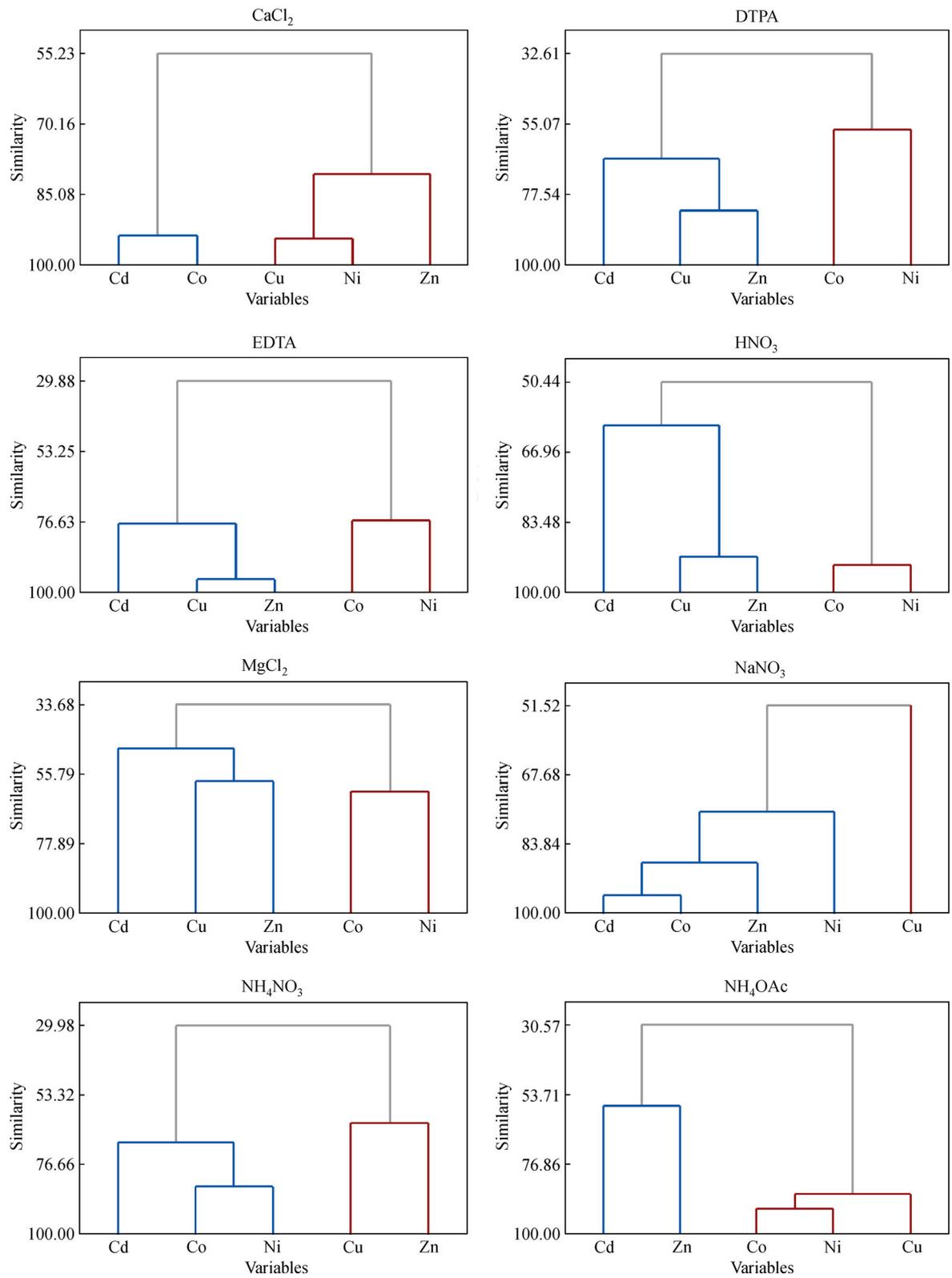


Fig. 4 Dendrogram of trace elements extracted by different extracting agents.

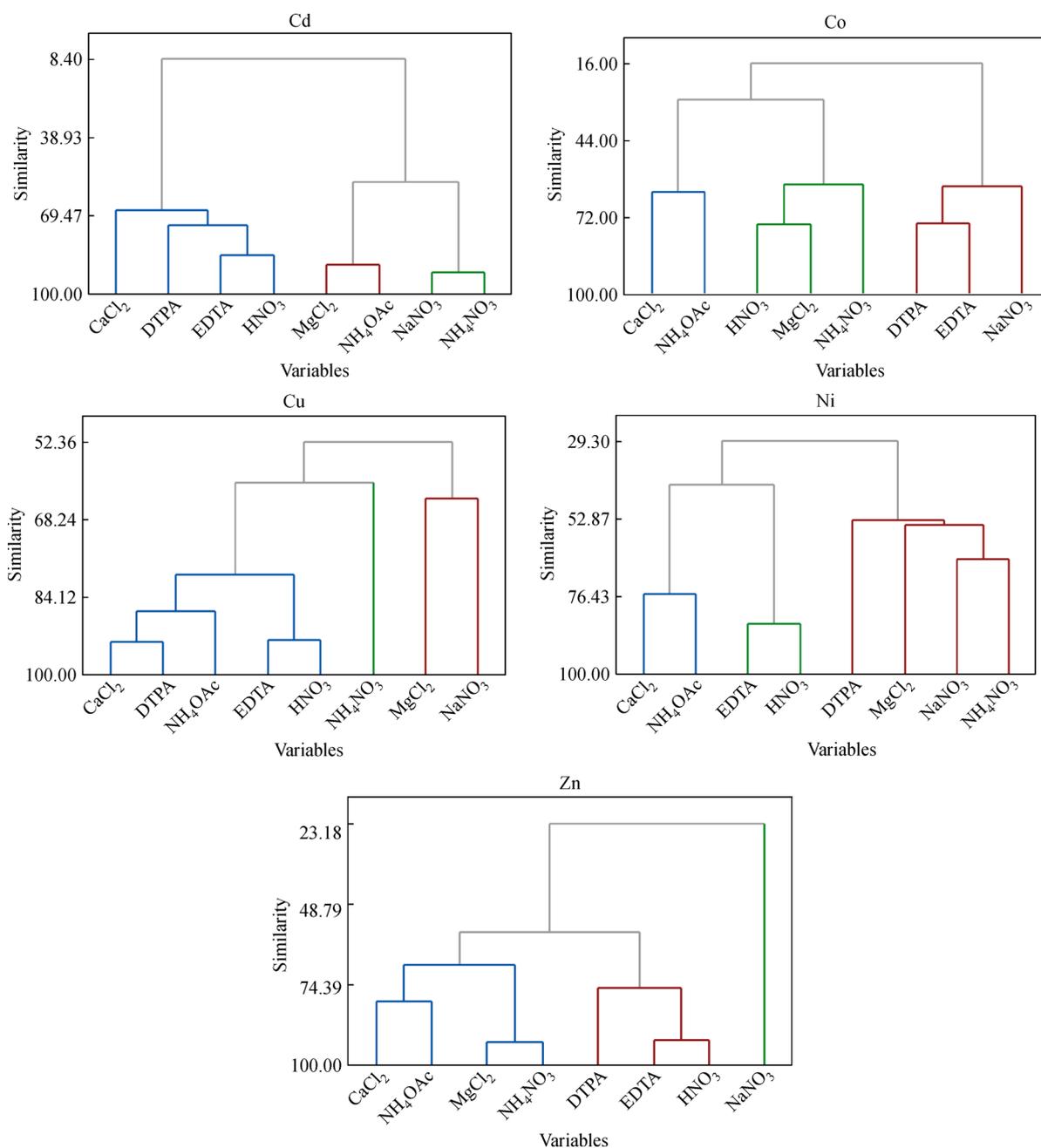


Fig. 5 Dendrograms of extracting agents for each trace element.

EDTA, HNO₃, MgCl₂, NH₄NO₃, and DTPA extracting agents used in clustering (Fig. 4), the first cluster contained a pair of Co-Ni and the second cluster contained a pair of Cu-Zn and Cd variables. In the case of CaCl₂, the pairs of Cd-Co, Cu-Ni and Zn variables included in the first and second clusters, respectively. Clustering the trace elements extracted by NaNO₃ extracting agent showed a pair of Cd-Co and Zn, Ni, Cu included in the first and second clusters respectively, while in the case of NH₄OAc a pair of Cd-Co and a pair of Zn-Ni and Cu included in the first and second

cluster, respectively. Finally, cluster analysis performed to identify the similarity between trace elements released by different extracting agents using the mean values of all extracting agents for each trace element. The dendrogram of the trace elements as variables showed two main clusters of trace elements. The first cluster included a pair of Co-Ni, while cluster 2 contained a pair of Cu-Zn, and Cd (Fig. 6(a)). There were high correlations between Cu and Zn, and between Ni and Co, indicating that their sources may be comparative, but Cd was relatively poorly

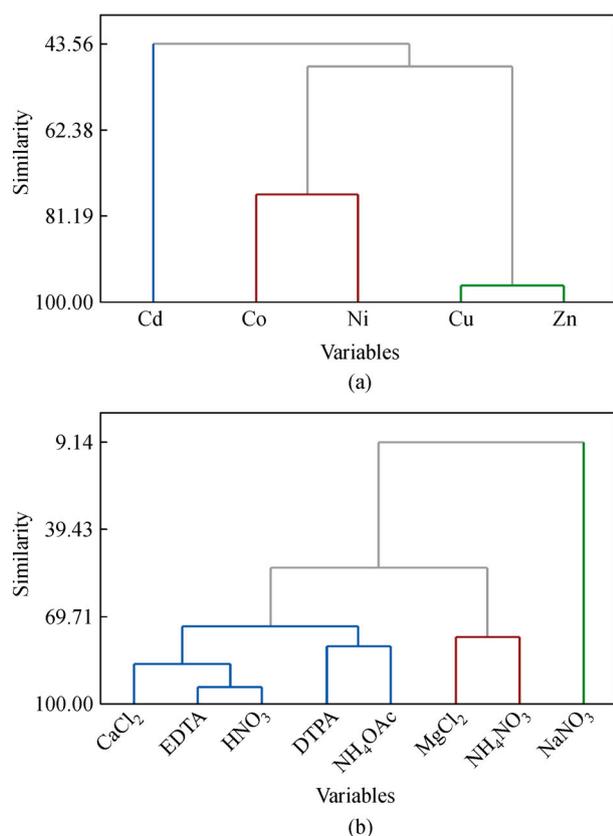


Fig. 6 (a) Dendrograms of extracted trace elements (mean of extracting agents) and (b) extracting agents (mean of extracted trace elements).

correlated to the Cu and Zn, and Ni and Co, indicating the sources of Cd may be diverse.

The factors 1 and 2 for the extracting agents described 54.2% and 19.0% of the total sample variance, respectively. The component loadings of CaCl₂ (0.894), NaNO₃ (-0.722), NH₄OAc (0.748), HNO₃ (0.876), DTPA (0.747), and EDTA (0.747) were higher on the factor 1 whereas, on the factor 2, MgCl₂ (-0.617) and NH₄NO₃ (-0.785) had higher loading.

The dendrogram used to examine the relationships between different extracting agents using trace elements separately. Each of the five dendrograms had two main large clusters. When the extracting agents extracted Cd utilized in clustering (Fig. 5) the first cluster contained a pair of EDTA-HNO₃, and CaCl₂ and DTPA and the second cluster contained pairs of MgCl₂-NH₄OAc and NaNO₃-NH₄NO₃ extracting agents. In the case of Co (Fig. 5) pairs of CaCl₂-NH₄OAc, HNO₃-MgCl₂, and NH₄NO₃ included in the first cluster and in the second cluster comprised a pair of DTPA-EDTA, and NaNO₃ extracting agent. Clustering extracting agents extracted Cu (Fig. 5) showed that the first cluster contained a pair of CaCl₂-DTPA, and NH₄OAc and EDTA- HNO₃, and NH₄NO₃ extracting agent, and a pair of MgCl₂-NaNO₃ extracting agent

included in the second cluster. The first and second clusters for Ni (Fig. 5) comprised pairs of CaCl₂-NH₄OAc and EDTA-HNO₃ extracting agents and a pair of NaNO₃-NH₄NO₃, and DTPA and MgCl₂, respectively. When the extracting agents extracted Zn utilized in clustering (Fig. 5), the first cluster contained pairs of CaCl₂-NH₄OAc and NH₄NO₃-MgCl₂, and the second cluster contained a pair of HNO₃-EDTA, DTPA, and NaNO₃ extracting agents.

Finally, cluster analysis performed to identify the similarity between extractants utilizing mean values of all trace elements for each extracting agent. The dendrogram of the extracting agents showed two clusters of extracting agents (Fig. 6(b)). The first cluster included pairs of EDTA-HNO₃, CaCl₂ and DTPA-NH₄OAc, and the second cluster contained pairs of MgCl₂-NH₄NO₃ and NaNO₃ variables.

In general, EDTA and HNO₃ extracted a higher percentage of trace elements than other extracting agents. They may be valuable for soils contaminated with trace elements, but CaCl₂, NH₄OAc, MgCl₂, and NH₄NO₃ extracting agents can be utilized to extract mobile trace elements. Gupta and Sinha (2007) applied cluster analysis on trace elements extracted by diverse extracting agents and found two clusters, including NH₄NO₃, CaCl₂, and DTPA and EDTA and NaNO₃. They indicated that the first group had a close relationship. On the contrary, EDTA and NaNO₃ extracting agents showed a dispersed relationship. Ettler et al. (2007) indicated that 1 mol/kg NH₄NO₃ has high ionic strength for speciation of trace elements in the extracts.

Gupta and Sinha (2007) reported that EDTA had the best correlation with trace elements accumulated by *Sesamum indicum* grown on soils amended with tannery sludge. They also suggested that 0.01 mol/kg CaCl₂ was appropriate for extracting trace elements such as Cd, Zn, Ni, and Cu. The use of CaCl₂ extraction is justified for the following reasons: (1) some authors (Gupta and Aten, 1993; Hammer and Keller, 2002) reported that CaCl₂ might be more useful for estimating trace elements availability than DTPA. (2) 0.01 mol/kg CaCl₂ solution has the same ionic strength in the soil solution (Novozamsky et al., 1993; Gupta and Aten, 1993). (3) The CaCl₂-extractable trace elements remove trace elements from the solution and exchange phases (Gupta and Sinha, 2007). It indicated that extractable trace elements with CaCl₂ extraction agent might be a good indication of the mobility of various trace elements. Vázquez et al. (2008) aimed to evaluate different extractions and found that CaCl₂-extractable Cu and Zn were well correlated with their concentrations in white lupin. It suggested that the CaCl₂ extracting agent might be more suitable than other extracting agents in calcareous soils.

3.7 Metal speciation and saturation indices

Results of trace elements speciation and saturation indices

in each extracting agent appeared in Table 2 and Table S4 in Supplementary Materials, respectively. The speciation of the trace elements in the CaCl_2 extracting agent was as follows: Co (95%), Ni (93%), and Zn (93%) were mainly present as the free ionic form, while Cd and Cu were mostly present as Cd^{2+} (49%), CdCl^+ (47%) and Cu^{2+} (32%), CuCO_3 (aq) (57%), respectively. The pH of the suspension of CaCl_2 extracting agent was near 7.2 (average of 7 soils) and high percentage of negatively charged was dominated, indicating that most extracted Cd, Co, Ni, Zn, and part of Cu may be re-adsorbed on soils, resulting in lower extractability (Ettler et al., 2007; Peters, 1999).

Cadmium in the MgCl_2 extracting agent was mainly present in the CdCl_2 (aq) (74%) and CdCl^+ (26%) forms, Co was mainly present as Co^{2+} (66%) and CoCl^+ (23%), Cu as CuCO_3 (aq) (59%), CuCl^+ (19%), and Cu^{2+} (12%); Ni as Ni^{2+} (64%), NiCl^+ (18%), and NiHCO_3^+ (15%); and Zn as ZnCl_3^- (29%), ZnCl_4^{2-} (23%), ZnCl^+ (22%), ZnCl_2 (aq) (15%), and Zn^{2+} (10%). In the case of this extracting agent and considering pH of the soil suspension (7.1), the possibility of re-adsorption of Co, Ni, and Zn is higher than Cd and Cu.

In the case of the HNO_3 extracting agent, the dominate Cd species were Cd^{2+} (68%) and CdNO_3^+ (29%). Cobalt was mainly present as Co^{2+} (76%) and CoNO_3^+ (16%). Copper was present as Cu^{2+} (69%) and CuNO_3^+ (30%). Nickel was present as Ni^{2+} (75%) and NiNO_3^+ (25%), whereas Zn was present as Zn^{2+} (74%) and ZnNO_3^+ (25%). The pH of the suspension of the HNO_3 extracting agent was lower than 2, and positive charges may be dominated. Thus, the possibility of re-adsorption of trace elements is low. The dominance of free ion species indicates that the dissolution of soil minerals or the exchange of H^+ with cations may be important mechanisms affecting trace element extraction using HNO_3 (Vidal et al., 2004).

The speciation of Cd, Co, Ni, and Zn in the NaNO_3 extracting agent dominated by the free ionic form, accounting for 84%, 86%, 80%, and 72%, respectively, with CdNO_3^+ (10%), CONO_3^+ (5%), NiNO_3^+ (7%), NiCO_3 (aq) (7%), ZnCO_3 (aq) (10%), and ZnNO_3^+ (7%). Copper was mainly present as CuCO_3 (aq) (77%) and CuOH^+ (11%). Considering the pH of the extractions (8.1), the possibility of re-adsorption of Cd, Co, Ni, and Zn was higher than Cu. The speciation of trace elements in the NH_4NO_3 extracting agent dominated by CdNO_3^+ (47%), Cd^{2+} (38%), $\text{Cd}(\text{NO}_3)_2$ (aq) (12%); Co^{2+} (44%), $\text{Co}(\text{NO}_3)_2$ (aq) (28%), CoNO_3^+ (27%); CuNH_3^{2+} (38%), CuNO_3^+ (22%), $\text{Cu}(\text{NH}_3)_2^{2+}$ (18%), Cu^{2+} (18%); Ni^{2+} (48%), NiNO_3^+ (47%); Zn^{2+} (47%), ZnNO_3^+ (46%). Considering the pH of the extractions (6.2), the possibility of re-adsorption of all trace elements was high.

The speciation of Co, Ni, and Zn in the NH_4OAc extracting agent dominated by $\text{Me}-(\text{Acetate})^+$ and $\text{Me}-(\text{Acetate})_2$ (aq), while Cu was mainly present

as $\text{Cu}-(\text{Acetate})_3^-$, $\text{Cu}(\text{NH}_3)_3^{2+}$, $\text{Cu}(\text{NH}_3)_4^{2+}$, and $\text{Cu}-(\text{Acetate})_2$ (aq) and Cd was mainly present as $\text{Cd}-(\text{Acetate})_3^-$ and $\text{Cd}-(\text{Acetate})_2$ (aq). As the results showed the pH of the suspension of NH_4OAc was 7.3, thus, about 60% and 40% of extracted Cd and Cu may be re-adsorbed on soils, resulting in lower extractability (Peters, 1999; Ettler et al., 2007).

The speciation of trace elements in the DTPA extracting agent dominated by $\text{Me}(\text{DTPA})^{3-}$ and in the EDTA extracting agent dominated by $\text{Me}(\text{EDTA})^{2-}$ and $\text{MeH}(\text{EDTA})^-$ complexes. The formation of soluble compounds between trace elements and chelating agents caused the highest extraction efficiencies of these extracting agents.

Regarding the pH of the suspension of DTPA (≈ 8.5) and EDTA (≈ 5.5), the formation of soluble complexes with trace elements in the DTPA and EDTA extracting agents was expected (de Santiago-Martín et al. 2013). Thus, the extracted trace elements may not be re-adsorbed on soils, resulting in higher extractability (Peters, 1999; Ettler et al., 2007).

Saturation indices of minerals controlling trace elements solubility extracted by different extracting agents were calculated (Table S4 in Supplementary Materials). The extracts were under saturation with respect to Cd, Co, Cu, Ni, and Zn minerals in CaCl_2 , DTPA, EDTA, HNO_3 , NaNO_3 , NH_4NO_3 and NH_4OAc extracting agents, whereas Cu was near equilibrium with respect to malachite and tenorite(c), Co and Ni with regard to CoCO_3 (s), and NiCO_3 (s), respectively, in MgCl_2 extracting agent. Cobalt, Cu, Ni, and Zn were near equilibrium with respect to CoCO_3 (s), malachite and tenorite(am), NiCO_3 (s), and ZnCO_3 (s) and smithsonite, respectively, in NaNO_3 extracting agent.

4 Conclusions

In this study, diverse extracting agents utilized to extract trace elements from calcareous soils. The content of extractable trace elements varied widely among the calcareous soils. The amount of trace elements extracted by different extracting agents also varied among diverse extracting agents. In this study, factor analysis performed to group the trace elements and extracting agents. Cluster analysis applied to trace elements extracted with diverse extracting agents showed different grouping of trace elements (i) Co and Ni (ii) Cu and Zn, and (iii) Cd, whereas cluster analysis applied to find the similarity between extracting agents also exhibited different grouping of variables (i) EDTA- HNO_3 and CaCl_2 (ii) DTPA- NH_4OAc (iii) MgCl_2 - NH_4NO_3 (iv) NaNO_3 . Overall, based on cluster analysis and mobility, it suggested that the CaCl_2 extracting agent might be more suitable than other extracting agents in calcareous soils.

Electronic Supplementary Material Supplementary material is available in the online version of this article at <https://doi.org/10.1007/s11783-019-1186-4> and is accessible for authorized users.

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