



## Evaluating soil extraction methods for chemical characterization of ultramafic soils in Kinabalu Park (Malaysia)



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### ABSTRACT

Soils derived from ultramafic bedrock are known for hosting distinct vegetation types as a consequence of atypical soil chemistries consisting of high trace elements concentrations (Ni, Cr, Co) and exchangeable cation imbalances (high Mg:Ca quotients); the high Mg:Ca ratio causes Ca infertility in plant species not adapted to ultramafic soils. Ecological studies use a range of single-stage extraction methods for chemical characterization of such soils in order to be able to interpret plant response, and ultimately to explain plant community composition. Few studies to date have compared different soil extraction methods in relation to tropical ultramafic soils. This study compares eight commonly used extraction methods on a large number of ultramafic soil samples collected from Kinabalu Park (Malaysia). The tested methods were: for trace elements:  $\text{NH}_4\text{Ac}$ , DTPA,  $\text{CaCl}_2$ , Sr ( $\text{NO}_3$ )<sub>2</sub> and Mehlich-3, for exchangeable cations:  $\text{NH}_4\text{Ac}$  and silverthiorea, and for plant-available phosphorus: Mehlich-3 and Olsen-P. These single-stage extraction methods were compared and evaluated for predictive power for chemically characterizing soils, interrelatedness and ecological application. The methods were also contrasted with a sequential extraction scheme. Finally, several operational parameters including molar ratio (0.01 and 0.1 M  $\text{CaCl}_2$ , Sr( $\text{NO}_3$ )<sub>2</sub>) and pH buffering (DTPA-TEA) were also evaluated. The majority of single-stage extraction methods are highly inter-correlated and predictive power could be improved by including independent soil parameters (pH, CEC, pseudo-total element concentration) in the multivariate regression equation. Ecological interpretation remains difficult because of lack of experimental studies in relation to plant uptake response and potential phytotoxicity effects on tropical native plants from ultramafic soils.

### 1. Introduction

Ultramafic rocks are widespread on earth, particularly in tropical countries (Cuba, New Caledonia, Indonesia, Philippines and Malaysia). These rocks are parts of the upper mantle and consist largely of magnesium-iron-silicate minerals. Soils derived from such bedrock are relatively high in the trace elements nickel (Ni), cobalt (Co) and chromium (Cr), but concomitantly have cation imbalances as a result of high magnesium (Mg) but low calcium (Ca) (Proctor et al., 1981; Echevarria, 2018). This atypical soil chemistry has caused the occurrence of distinct vegetation types characterized by relatively low stature and high levels of endemism (Brooks, 1987; Proctor, 2003; van der Ent et al., 2018). The main soil-edaphic factors that are most often cited to be important in relation to the ecology of ultramafic soils are the (potential) phytotoxicity induced by Ni, and possibly by Co and Cr, and nutrient deficiency as a result of low Ca (and high Mg) and very low potassium (K)

and phosphorus (P) (Vlams and Jenny, 1948; Walker, 1954; Proctor et al., 1981; Brooks, 1987; Proctor, 2003; Brady et al., 2005). Ecological studies have used single-stage soil extraction methods for: (i) Quantifying potential phytotoxic trace elements mainly Ni; (ii) Demonstrating (high) Mg:Ca quotients in the cation exchange capacity (CEC); and (iii) Estimating potentially plant-available P (Vlams and Jenny, 1948; Proctor et al., 1981). Together these extractions aim to characterize the chemical properties of ultramafic soils under investigation with the ultimate objective of linking such information to ecological attributes, such as vegetation stunting, species-diversity per unit area or functional traits of individual species. However, none of the commonly used methods has been specifically developed for ultramafic soils, or for the use with regards to ecological parameters. Rather, most methods were originally developed for agricultural soils in relation to (trace element) nutrition and deficiency, or phytotoxicity risk assessments. Few studies have compared different methods in use in ecological studies on

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(tropical) ultramafic soils and evaluated the usefulness of such methods for the characterization of ultramafic soils.

### 1.1. Chemical extraction methods for estimating potential soil trace element phytotoxicity

Potentially phytoavailable trace element soil fractions can be estimated with a range of different methods, including: (a) Single-stage chemical extractants; (b) Sequential extractants schemes; (c) Ion exchange resin methods (IER); (d) Isotopic exchange kinetics (IEK); and (e) Diffusive Gradients in Thin-films (DGT) (Echevarria et al., 1998; Robinson et al., 1999). None of these methods, however, can exactly simulate trace elements transfer to plants. Single-stage chemical extractants can be grouped in: (i) neutral salt-based extracts; (ii) chelator-based extracts; (iii) acid-based extracts; or (iv) synthetic root exudate-based extracts. Important parameters for all extraction methods are the molar concentration of the extract, the liquid to solid ratio, pH, and the equilibration time (Meers et al., 2007a,b).

Neutral salt-based extracts include  $\text{CaCl}_2$ ,  $\text{Ca}(\text{NO}_3)_2$ ,  $\text{KCl}$ ,  $\text{NaNO}_3$ , and  $\text{NH}_4\text{Ac}$  (ammonium acetate) in various molar concentrations. Initially, the aim for using such solutions was to assess the pool of exchangeable cations that are sorbed onto the Cation Exchange Capacity (CEC) (Ciesielski and Sterckeman, 1997). The ion replacement power in such extract solutions (with identical anions, such as  $\text{NO}_3^-$ ) decreases according to the Z number of the element in the order:  $\text{Ba}^{2+} > \text{Sr}^{2+} > \text{Ca}^{2+} > \text{Mg}^{2+} > \text{NH}_4^+ > \text{K}^+ > \text{Na}^+$  (Mengel and Kirkby, 2001). The specific chemical adsorption of divalent macronutrient cations by the soil phases is mainly related to metal ion hydrolysis and increases with increasing pH with pK values of the metal ion hydrolysis, for example 9.9 (Ni) and 9.7 (Co) (Brummer et al., 1986). However, ion exchange in soils is also indirectly influenced by soil pH because of the competition with  $\text{H}^+$  ions for sorption onto the CEC (Tiller et al., 1984). Neutral salt extracts based on monovalent and divalent cations are essentially pH dependent (Anderson and Christensen, 1988; Echevarria et al., 2006), and have been reported to perform better than methods using chelators or methods that significantly acidify or buffer the soil, altering the pH at which the extraction actually occurs (Menziez et al., 2007). Frequently used are 0.01 or 0.1 M  $\text{CaCl}_2$  solutions; and at the ionic strength of 0.01 M the extractant solution has a similar ionic strength to that of most soil solutions (Novozamsky et al., 1993; Houba et al., 2000). However, although in most 'normal' soils  $\text{Ca}^{2+}$  is the dominant cation, in ultramafic soils  $\text{Mg}^{2+}$  is generally the dominant cation, and hence an extractant based on  $\text{Mg}^{2+}$  might be more appropriate. Because  $\text{Cl}^-$  is a complexing anion to some metals as opposed to the  $\text{NO}_3^-$  counter ion (Gommy et al., 1998), and because the pH can be shifted by high  $\text{Ca}^{2+}$  levels, 0.1 M  $\text{Sr}(\text{NO}_3)_2$  (Wang et al., 2003) and 0.01 M  $\text{Sr}(\text{NO}_3)_2$  (Kukier et al., 2004) have also been proposed. In both the  $\text{CaCl}_2$  and  $\text{Sr}(\text{NO}_3)_2$  extracts, the divalent cations also promote coagulation of the colloidal material in the suspension, making higher molar concentrations that are required for monovalent cations ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{NH}_4^+$ ) unnecessary (Meers et al., 2007b). The 0.01 M  $\text{Sr}(\text{NO}_3)_2$  extraction has proven to be effective in predicting extractable Ni and plant Ni uptake across a range of soils (Siebielec et al., 2007). In many studies on ultramafic soils, 1 M  $\text{NH}_4\text{Ac}$  (pH 7.0) has been used for phytoavailable trace elements, and concomitantly for exchangeable cations (Proctor et al., 2000; Robinson et al., 2003; Brearley, 2005). Compared to divalent cations,  $\text{NH}_4^+$  is less competitive for desorption of metals from the solid soil phases, but performs similar to  $\text{K}^+$  on account of its nearly identical ion radius (Gryschko et al., 2004; Gommy et al., 1998). The extractant is commonly buffered at pH 7 to prevent carbonate dissolution, but due to the increasing formation of  $\text{NH}_3$  from  $\text{NH}_4^+$  by dissociation with rising pH, metal ammine complexes can form (Gryschko et al., 2004). This might be important (but has not been studied) in the case of ultramafic soils, with high concentrations of  $\text{Ni}^{2+}$ , and the use of  $\text{NH}_4\text{Ac}$  has strong effect on soil extract pH and might also result in the formation of

soluble Ni-hexammine complexes, which can potentially result in overestimation of exchangeable soil Ni. Also, in acidified ultramafic soils, buffering the extraction solution at pH 7 (2–3 units higher than soil pH) can generate CEC of Fe-oxides and change the retention of metal cations by these minerals, which dominate the soil composition (Becquer et al., 2001).

Chelator-based extractants based on synthetic amino-polycarboxylic acids include EDTA and DTPA. The DTPA (Diethylene triamine pentaacetic acid) method was originally developed to diagnose deficiency of micronutrients in soils (Lindsay and Norvell, 1978), but has been widely used for studies with ultramafic soils (L'Huillier and Edighoffer, 1996; Echevarria et al., 1998, 2006; Lazarus et al., 2011; Chardot-Jacques et al., 2013; Ünver et al., 2013). The DTPA-extract is made up of 0.005 M DTPA with 0.01 M  $\text{CaCl}_2$  and is buffered at pH 7.3 with 0.01 M triethanolamine (TEA). The extraction of trace elements in this extract is promoted by the chelation action of DTPA and the  $\text{Ca}^{2+}$  exchange with other cations, as well as  $\text{Cl}^-$  complexation (Lindsay and Norvell, 1978; Hsiao et al., 2009). The buffer (TEA) was designed to prevent carbonate dissolution (Lindsay and Norvell, 1978), but carbonates are not likely to be important in ultramafic soils with pH 4–5.8, and Becquer et al. (1995) proposed unbuffered (excluding TEA) DTPA adjusted to pH 5.3. It is, however, important to keep the Ca:DTPA ratio intact because Ca:DTPA binding is necessary to control the exchangeability and chelation of DTPA (Lindsay and Norvell, 1978). The DTPA method was developed for soil deficient in trace elements, whereas ultramafic soils have a surplus of Ni, hence the DTPA-method risks over-saturation and the soil-extractant ratio needs to be adjusted accordingly (Kukier and Chaney, 2001). Although some studies found (weak) correlation between soil Ni-DTPA and uptake in non-accumulating plants (L'Huillier and Edighoffer, 1996), such correlations are mostly restricted to comparing similar soils with a narrow pH range (Sukkariyah et al., 2005), and when applied to a variety of soils, poor prediction of phytoavailability has been reported (Menziez et al., 2007). This can be explained by the rather high chelation stability constants of Ni-DTPA and Co-DTPA at  $\log K > 20.2$  and 19.3 respectively (Anderegg et al., 2005), which is unlikely representative for the chelation capacities of carboxylic acids (for example citrate-Ni  $\log K$  5.4) in the plant rhizosphere. However, DTPA-extractable Ni can, in some cases, be strongly correlated to Ni uptake by hyperaccumulators in a limited range of pH (i.e. 4.2 to 5.6) in temperate ultramafic soils (Chardot et al., 2007), whereas it is not the case in circum-neutral Mediterranean ultramafic soils (Bani et al., 2009). However, in all these soils, the Ni-DTPA appears to be a useful extractant of the isotopically-exchangeable pools from which all plants take up Ni (Massoura et al., 2004; Chardot et al., 2007; Estrade et al., 2015): i.e. high-activity clays and hydrous Fe oxides (Massoura et al., 2006; Chardot et al., 2007; Bani et al., 2009, 2014).

Acid-based extracts include a digest, normally microwave-pressure-aided or in a hot block with mineral acids such as  $\text{HCl}$  and  $\text{HNO}_3$ . These extracts are used to provide 'pseudo-total' levels by dissolution of oxides, hydroxides, carbonates, organic matter and, if HF is also added to mix, to provide 'near-total' values by also breaking down silicate matrices. Such a digest gives a measure for virtually all trace elements present in the soil. In more dilute form,  $\text{HNO}_3$  (0.1 M) can also be used to leach metals from the soils, and this has the benefit that the  $\text{NO}_3^-$  counter ion is not complexing. Alternatively (very) dilute organic acids (such as acetic, citric, formic, lactic and malic acid) can be used to mimic plant root exudates as these acids are the most abundant Low Molecular Weight Organic Acids (LMWOA) present in the rhizosphere of many plants (Meers et al., 2007b). Wang et al. (2003) and Feng et al. (2005) proposed 10 mM LMWOA extraction solutions, consisting of formic acid, citric acid and malic acids, and reported good correlations with plant metal uptake. The acidity of these organic acids dissolves hydroxides and carbonates, and the citrate and malate counter ions complex Ni and other trace elements. However, the evidence for dilute solutions of carboxylic acids being suitable extractants for metals

remains weak.

Selective sequential extractions (SSE) provide operationally defined solid-phase fractionations of metals over soil pools (Quantin et al., 2002). Various schemes have been proposed based on the (much simpler) BCR-protocol (Quevauviller et al., 1994), but for trace elements (Ni, Co, Cr) in ultramafic soils, the most reported is a 7-step program that consists of the following stages: (1) Water soluble; (2) Exchangeable; (3) bound to Mn oxides; (4) Bound to amorphous Fe oxides; (5) Bound to crystalline Fe oxides; (6) Bound to organic matter; (7) and Residual (Quantin et al., 2002). Of these fractions, the water soluble and exchangeable are immediately phytoavailable, the fraction bound to Mn oxides, amorphous Fe oxides and bound to organic matter are potentially phytoavailable, whereas the residual fraction is not phytoavailable. Together these labile and non-labile pools determine the mobility and phytoavailability of Ni, Cr and Co in ultramafic soils. Some of these labile fractions extracted individually (and not through a complete sequential procedure) from a wide range of ultramafic soils have been proved to be strongly correlated to isotopically-exchangeable Ni pools, which is the principal source of Ni available to plants in soils: e.g. amorphous Fe oxides (Massoura et al., 2004, 2006).

In this study, ultramafic soil samples were collected from a 700-km<sup>2</sup> area encompassing Kinabalu Park in Malaysia. These soil samples were analysed with the aims of characterizing soil chemical properties, in particular those of ecological relevance to plants, such as the cation exchange complex, availability of macro-nutrients, and concentrations of extractable trace elements. To that end, we compared and evaluated the results of a range of extraction methods commonly used in the field of tropical ecology with a focus on predictive power for characterizing soils, interrelatedness and ecological application.

## 2. Materials and methods

### 2.1. Study area and sample collection

Mount Kinabalu Park is located in Sabah (Malaysia) on the island of Borneo (6°5' N and 160°33' E), covering an area of 754 km<sup>2</sup> including two mountains: Kinabalu (4095 m) and Tambuyukon (2579 m). Although Mount Kinabalu itself is a granite pluton (Cottam et al., 2010), the lower slopes are covered with sedimentary rocks. Ultramafic rock outcrops appear like a collar around the massif on mid-elevation, and also outcrops on Mount Tambuyukon. In total, ultramafic outcrops cover 142 km<sup>2</sup> within the Park boundaries (Collenette, 1964). Kinabalu Park is covered with intact rainforest and has a humid tropical climate with a mean monthly air temperature of 20 °C throughout the year at 1680 m, with a daily fluctuation of 7–9 °C (Kitayama, 1991).

During 2010–2014, a large ecological research project was conducted in Kinabalu Park, and in the nearby Bidu-Bidu Hills and Trus Madi Forest Reserves, all in the Malaysian state of Sabah. The project was wide-ranging, but focused on the plant-soil relationships of the vegetation on ultramafic soils at these localities. Data from these soil samples has been previously reported (Van der Ent et al., 2015a, 2015b, 2016a, 2016b, 2018b) and we refer to these publications for full details on the sample collection. Briefly, the soil samples were collected from 14 different ultramafic localities (474–2950 masl) in Kinabalu Park, including in total 95 discrete sample sites. At each site, 3 soil samples (1–2 kg) were collected in the A/B mineral horizon, and care was taken not to include organic surface layers. All soil samples were packed, brought to the local field station, air-dried at room temperature to constant weight (2–3 weeks), sieved to < 2 mm, shipped to Australia, and gamma irradiated at Steritech Pty. Ltd. in Brisbane following Australian Quarantine Regulations.

### 2.2. Laboratory analyses

The chemical analysis of the soil samples took place in the laboratory of the Centre for Mined Land Rehabilitation (CMLR) at The

University of Queensland in Australia. The soil samples (0.3 g) were digested using freshly prepared Aqua Regia (4 mL 70% nitric acid and 3 mL 37% hydrochloric acid per sample) in a digestion microwave for a 1-hour program and diluted to 45 mL before analysis (Rayment and Higginson, 1992; method 17B1). Soil pH and electrical conductivity (EC) was obtained in a 1:2.5 soil:water mixture after 1-h equilibrium time on an end-over-end shaker and 1-hour settling time. Plant-available phosphorus as Olsen-P (Olsen et al., 1954) was extracted with 1.0 g soil extracted with 20 mL 0.5 M NaHCO<sub>3</sub> (pH 8.5) for 30 min (Rayment and Higginson, 1992; method 9C1). Plant-available phosphorus ('ML-3') was also extracted with Mehlich-3 solution consisting of (0.2 M CH<sub>3</sub>COOH + 0.25 M NH<sub>4</sub>NO<sub>3</sub> + 0.015 M NH<sub>4</sub>F + 0.013 M HNO<sub>3</sub> + 0.001 M EDTA at pH 2.50 ± 0.05), according to Mehlich (1984). This method is also used for phytoavailable trace elements, and as such provides a 'multi-functional' extract. Exchangeable trace elements (Ni, Co, Cr, Mn) were extracted in 0.1 M Sr(NO<sub>3</sub>)<sub>2</sub> at a soil:solution ratio of 1:4 (10 g:40 mL) and 2-h equilibrium time (Wang et al., 2003). This method was repeated on a selection of 25 samples using 0.01 M Sr(NO<sub>3</sub>)<sub>2</sub> for a comparison. In addition, a second method for exchangeable trace elements was used with 0.01 M CaCl<sub>2</sub> separately with 2-h equilibrium time (Houba et al., 2000; Meers et al., 2007a). This method was also repeated on the same selection of 25 samples used in the Sr(NO<sub>3</sub>)<sub>2</sub> method using 0.1 M CaCl<sub>2</sub>.

Potentially phytoavailable trace elements (Ni, Co, Cr, Mn) were extracted with standard Diethylene triamine pentaacetic acid (DTPA) according to Lindsay and Norvell (1978), and also (separately) using an adaptation by Becquer et al. (1995), which excluding TEA, was adjusted to pH 5.3 and had an equilibrium time of 2 h (instead of 1 h). Another method for potentially phytoavailable trace elements was also used, by extraction with a mixture of carboxylic acids (acetic, malic and citrate acid in molar ratio of 1:2:2 at 2, 4 and 4 mM, respectively) at a soil:solution ratio of 1:4 (10 g:40 mL) and 2-h shaking equilibrium time (method loosely based on Feng et al., 2005). Exchangeable cations were extracted with silver-thiorea (Dohrmann, 2006) over a 16-h equilibrium time on an end-over-end shaker in the dark (to prevent silver precipitation). In addition, a second method for exchangeable cations, the traditional 1 M pH 7.0 ammonium acetate (NH<sub>4</sub>Ac) was used with 2-h equilibrium time (Meers et al., 2007a).

All soil extractions were undertaken in disposable 50 mL polypropylene (PP) centrifuge tubes. Soil samples were weighed using a 4-decimal balance and weights recorded for correction of the precise weights in the mass balance calculations. All samples were agitated ('equilibrated') for method-specific times using an end-over-end shaker at 60 rpm and subsequent centrifuged (10 min at 4000 rpm) and the supernatant was collected in 10 mL polyethylene tubes. The extraction methods and operational conditions are given in Table 1.

Nickel, Co and Cr partitioning was evaluated with a 5-step selective sequential extraction scheme to provide operationally defined solid-phase trace element (Ni, Cr, Co, Mn) fractionation. This scheme is based on Quantin et al. (2002), which was in turn modified mainly from Leleyter and Probst (1999). Adaptations were made here by combining step 1 and step 2, and by using HNO<sub>3</sub>/HF high-pressure microwave digests for the residual fraction (step 5) instead of an alkaline fusion as in Quantin et al. (2002). The step for the 'organic bound phase' was also omitted because the tested soils were extremely low in organic matter. As such the fractions were: water soluble and exchangeable (i), bound to Mn oxides (ii), bound to amorphous Fe oxides (iii), bound to crystalline Fe oxides (iv), and residual (v). After each extraction step, the tubes were centrifuged for 10 min at 4000 rpm and the supernatants were then filtered through 0.45 μm membranes. The residues were washed with 20 mL of TDI water, centrifuged again for 10 min at 4000 rpm, the water decanted, and the residue dried at 40 °C prior to the next extraction step. The different extraction phases and operational conditions are presented in Table 2.

All soil extracts samples were analysed with Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) (Varian Vista Pro II)

**Table 1**  
Chemical soil extractants used in this study.

Extraction medium	Liquid:soil ratio	Equilibrium time	References
Neutral-salt extracts			
0.1 M strontium nitrate	4:1	1 h	Wang et al. (2003)
0.01 M strontium nitrate	4:1	1 h	Kukier et al. (2004)
0.1 M calcium chloride	4:1	1 h	Meers et al. (2007a,b)
0.01 M calcium chloride	4:1	1 h	Meers et al. (2007a,b)
Chelator extracts			
DTPA-TEA pH 7.3	5:1	2 h	Lindsay and Norvell (1978)
DTPA pH 5.3	5:1	2 h	Becquer et al. (1995)
Acid based extracts			
70% nitric acid, 37% hydrochloric acid	12:0.3	1 h	Rayment and Higginson (1992)
Carboxylic acid mix (acetic, malic, citric acid)	4:1	2 h	Feng et al. (2005)
Exchangeable cations			
1 M ammonium acetate	10:1	2 h	DIN (1995)
0.01 M silverthioarea	40:0.8	16 h	Pleysier and Juo (1980)
Phosphorus extracts			
Olsen-P (sodium hydrogen carbonate)	20:1	30 min.	Olsen et al. (1954)
Mehlich-3 (ammonium fluoride based)	10:1	5 min.	Mehlich (1984)

for Ni, Co, Cu, Zn, Mn, Fe, Mg, Ca, Na, K, S and P. Each method included 3 sample blanks, 2 NIST standards, 2 ASPAC reference soils, 3 random sample duplicates and 3 multi-element standards as part of the quality control. The ICP-AES instrument was calibrated using a 6-point multi-element standard (which included all measured elements) prepared in each extraction solution.

### 2.3. Statistical analysis

The soil chemistry data was analysed using the software package STATISTICA Version 9.0 (StatSoft), Excel for Mac version 2011 (Microsoft) and PRIMER Version 6 (PRIMER-E).

## 3. Results

### 3.1. Extraction methods

In the context of ultramafic soils, the most frequently used extraction methods to estimate potentially phytoavailable Ni, Co and other trace elements are the DTPA-extract,  $\text{NH}_4\text{Ac}$  extract,  $\text{CaCl}_2$  extract and the  $\text{Sr}(\text{NO}_3)_2$  extract (McLaughlin et al., 2000; Kukier and Chaney, 2001; Wang et al., 2003). Of these DTPA-TEA, DTPA, 1 M  $\text{NH}_4\text{Ac}$ , 0.01 M and 0.1 M  $\text{CaCl}_2$ , 0.1 M and 0.01 M  $\text{Sr}(\text{NO}_3)_2$ , were tested here, in addition to the Mehlich-3 extract and a 0.01 M mix of carboxylic acids. Fig. 1 compares the different extraction methods for the amounts of Ni, Cr and Co extracted. Extractable amounts of Cr are extremely low for all extractants, but the acid-based extractants released the most Cr. The greatest amounts of Ni and Co were extracted with acid-based extracts, and relatively large amounts were also extracted with DTPA. Nickel is moderately extractable (3.5% of the mean pseudo-total soil Ni), but Cr was almost completely unavailable (0.009% of the mean pseudo-total soil Cr) in the DTPA extracts (Table 3). Fig. 2 shows correlation for Co and Ni between DTPA extract and other extracts (0.1 M  $\text{CaCl}_2$ , 0.1 M  $\text{Sr}(\text{NO}_3)_2$  and Mehlich-3). For the Co and Ni, relatively

high correlation exists between the DTPA and Mehlich-3 extracts, followed by  $\text{CaCl}_2$ , but low correlation with the  $\text{Sr}(\text{NO}_3)_2$  extract (Supplementary Fig. 1).

### 3.2. Buffered and unbuffered DTPA extractions

Unbuffered DTPA (excluding TEA, pH 5.3) was used for all soils ( $n = 343$ ) and buffered DTPA (including TEA, pH 7.3) on a subset of soils ( $n = 93$ ). Both methods correlate well ( $r = 0.75$  for Co, 0.59 for Cr, 0.84 for Mn, and 0.80 for Ni; at  $p < 0.01$ ), but the buffered DTPA extracts had considerably more Ni, Co and Mn compared to unbuffered DTPA, indicating the role of extraction solution pH in the extraction method.

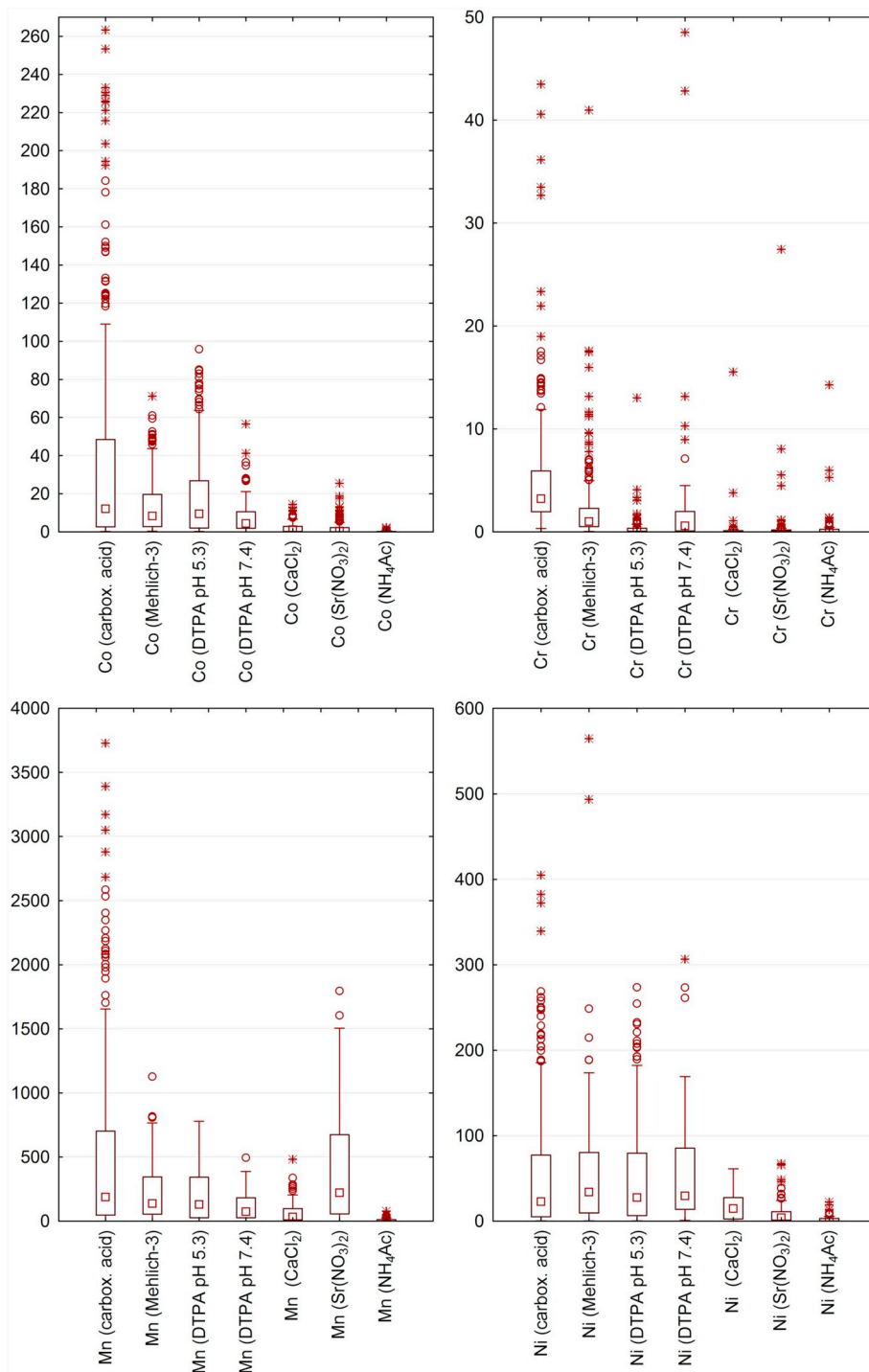
### 3.3. $\text{Sr}(\text{NO}_3)_2$ and $\text{CaCl}_2$ (0.1 and 0.01 M) extractions

The 0.1 M and 0.01 M  $\text{Sr}(\text{NO}_3)_2$  and 0.1 M and 0.01 M  $\text{CaCl}_2$  extractants were tested on a selection of 25 samples, as it was predicted that the higher molarity would result in higher extractable levels of Ni and Co (hence better detection precision during ICP-AES analysis), but would also induce a stronger drop in pH due to displacement of  $\text{H}^+$ . Comparing 0.01 M  $\text{CaCl}_2$  with 0.1 M  $\text{Sr}(\text{NO}_3)_2$  shows a very good correlation ( $r = 0.99$ ), and a good correlation ( $r = 0.82$  at  $p < 0.01$ ) was obtained for 0.1 M  $\text{CaCl}_2$  versus 0.1 M  $\text{Sr}(\text{NO}_3)_2$  (Fig. 3). In both cases, the  $\text{Sr}(\text{NO}_3)_2$  extracts had greater amounts of Ni (up to a factor 3), which can be explained by the greater cation displacement power for  $\text{Sr}^{2+}$  compared to  $\text{Ca}^{2+}$  at the same molar concentration. The change in pH in both extracts is somewhat erratic (in relation to the extracted amount of Ni) but vary little between the extractants. Calcium or Sr concentration does affect competition with sorption and pH of the extraction fluid which clearly affects extractable Ni (Fig. 3).

**Table 2**  
Selective sequential extraction scheme.

Fraction	Reagents	Conditions
Water soluble and exchangeable	0.01 M $\text{Sr}(\text{NO}_3)_2$	2 h at 20 °C
Bound to Mn-oxides	0.1 M hydroxylammonium chloride	30 min at 20 °C
Bound to amorphous Fe-oxides	0.2 M ammonium oxalate + 0.2 M oxalic acid	4 h in the dark at 20 °C
Bound to crystalline Fe-oxides	0.2 M ammonium oxalate + 0.2 M oxalic acid + 0.1 M ascorbic acid	30 min at 90 °C in water bath
Residual	70% $\text{HNO}_3$ + 32% $\text{HCl}$ + 37% HF	1 h at 115 °C in digestion microwave





**Fig. 1.** Boxplots of Ni, Co, Cr, Mn extracted using various soil extraction methods ( $\mu\text{g g}^{-1}$ ). Key to symbols: open squares are the  $\pm$  mean, whiskers are  $\pm$  standard deviation, circles are outliers and asterisks are extreme outliers.

**3.4. Multivariate regression of independent parameters**

Soil extraction methods generally benefit from having factors for prediction of plant response such as soil pH and CEC incorporated in the multivariate regression model to increase accuracy (Haq et al., 1980; Meers et al., 2007b; Siebielec et al., 2007; Römken et al., 2009). Here we use a multivariate regression expressing extractable Ni or Co contents as a function of independent factors using the following equation (after Meers et al., 2007b):

$$\log(M_{\text{extraction}}) = \alpha \log(M_{\text{total}}) + \beta \text{pH} + \gamma \log(\text{CEC}) + \delta$$

- $M_{\text{extraction}}$  Metal extracted in  $\mu\text{g g}^{-1}$
- $M_{\text{total}}$  Pseudo-total metal concentration (HNO<sub>3</sub>/HCl-digest) in  $\mu\text{g g}^{-1}$
- pH Measured soil pH
- CEC Soil CEC in  $\text{mmol}^{(+)}\text{kg}^{-1}$
- $\alpha, \beta, \gamma$  and  $\delta$  are constants

All independent input variables were log-transformed (except for pH, which is already log-transformed) prior to regression analysis because values covered several orders of magnitude with log-normal distribution fits. The regression equation provides empirical

**Table 3**  
Elemental concentrations (ranges and means) in different extracts ( $\mu\text{g g}^{-1}$ ),  $n = 343$ .

	DTPA extract $\mu\text{g g}^{-1}$		Mehlich-3 extract $\mu\text{g g}^{-1}$		Sr(NO <sub>3</sub> ) <sub>2</sub> extract $\mu\text{g g}^{-1}$		Carboxylic extract $\mu\text{g g}^{-1}$		HNO <sub>3</sub> /HCl digest $\mu\text{g g}^{-1}$	
Al	0.3–485	55	17–1750	415	0.1–220	15	5.8–4250	265	1200–118,000	19,600
Ca	–	–	3.1–2600	230	1.8–1770	210	2.1–1550	125	205–24,500	2100
Co	0.1–95	20	0.5–70	15	0.1–25	1.8	0.2–275	40	0.5–1500	250
Cr	0.1–15	0.5	0.04–40	2.5	0.1–25	0.3	0.3–85	5.8	225–21,800	4000
Cu	0.1–25	1.5	0.3–125	5.0	0.1–25	0.2	0.1–8.7	0.5	2.5–325	50
Fe	0.6–875	95	25–1750	210	0.1–35	1.1	12.1–2350	410	21,500–535,000	157,000
K	1.0–110	30	< 0.01–440	35	0.1–135	30	0.1–95	25	0.1–1060	90
Mg	2.9–1100	315	5.1–6850	980	5.0–3150	510	4.0–7850	620	270–235,000	26,700
Mn	0.4–780	190	0.7–1150	215	0.3–11,800	445	0.4–3700	550	45–33,500	3250
Na	1.9–25	10	6.6–345	25	0.5–30	7.1	0.1–65	5.8	0.1–360	105
Ni	0.2–275	50	0.4–620	55	0.1–65	7.9	0.2–405	55	15–7000	1440
P	0.1–15	0.7	< 0.01–30	2.0	0.1–735	60	0.1–45	1.6	20–485	130
S	1.0–215	17	< 0.01–115	16.5	0.1–20	1.2	0.8–80	9.6	85–755	350
Zn	0.1–4.8	0.7	0.1–5.0	1.0	0.1–15	3.0	0.1–3.1	0.8	15–375	115

information on relevant factors influencing Ni or Co extractability using various extractants, thus allowing for the identification of the most influencing of these factors. We tested various factors, including pH, EC, CEC, pseudo-total metal concentrations and extractable metal concentrations (other than Ni or Co), but after evaluating the fits only pH, CEC and pseudo-total metal concentrations provided improved predictability. Tables 4 and 5 lists the calculated factors and constants for the multivariate analysis, and it is clear that in most cases pseudo-total Ni or Co is the single most important factor predicting extractability in the extracts.

3.5. Sequential extraction for trace element partitioning

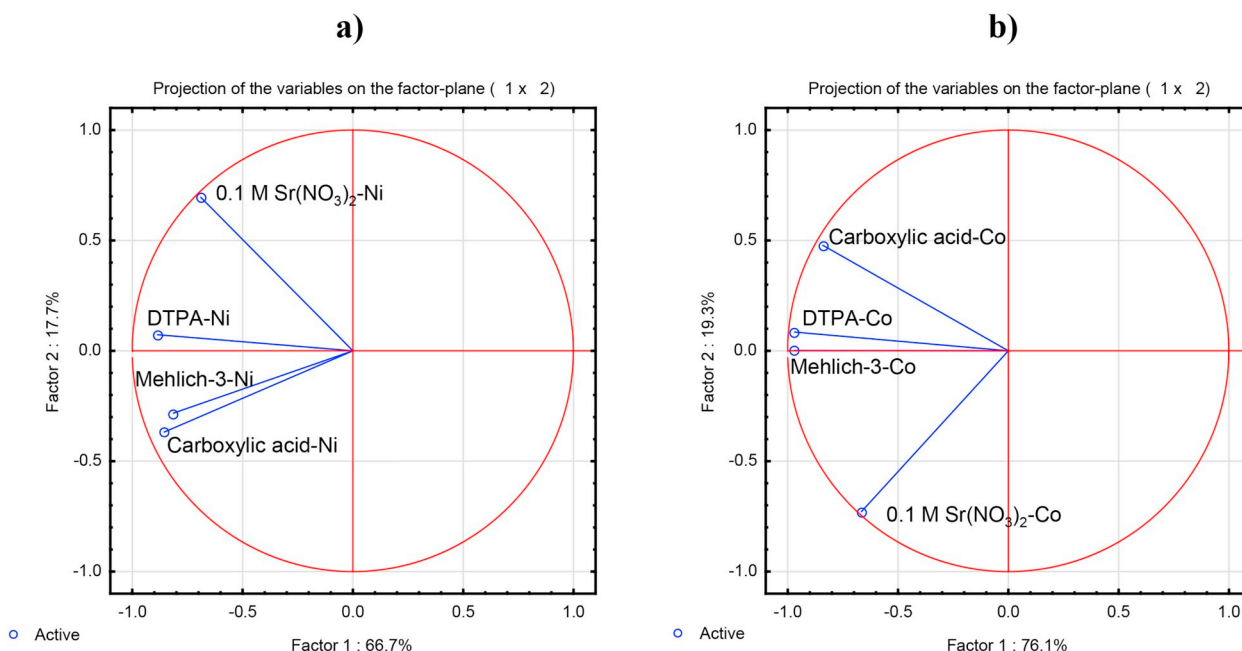
Selective sequential extractions show that Cr is mainly associated with 'crystalline Fe-oxides', explaining its low extractability in the Sr (NO<sub>3</sub>)<sub>2</sub> and DTPA extracts. However, Ni and Co are mainly bound in the 'Mn-oxide' and most of all in 'amorphous Fe-oxide' fractions and hence are more phytoavailable, as is evident from higher concentrations in the Sr(NO<sub>3</sub>)<sub>2</sub> and DTPA extracts. Table 6 shows relative portions of Ni, Co and Cr over the 5 fractions of the selective extraction procedure.

3.6. Cation Exchange Capacity (CEC) and component ions

Fig. 4 shows K extractability in silverthiorea (AgTU) extract versus K in Mehlich-3 (ML-3), 0.1 M Sr(NO<sub>3</sub>)<sub>2</sub> and carboxylic acid (CA) extracts. These extraction methods are highly intercorrelated for K, and for Ca and Mg (data not shown), and therefore all could be used for measuring Cation Exchange Capacity and component ions. This has major benefits, as combining methods (for example the carboxylic acid extract) for both cations and trace elements reduces time and costs.

3.7. Plant available phosphorus

Olsen-P and Mehlich-3 extractions are relatively poorly correlated ( $r = 0.41$  at  $p < 0.01$ ), but given that the majority of the ultramafic soils are between pH 3.7–5.5, the Mehlich-3 method appears more appropriate. However, for near-neutral pH ultramafic soils, the Olsen-method would be more likely to be related to plant available P than the Mehlich-method.



**Fig. 2.** Plot of principal component analysis (PCA) of a) Ni extractability in DTPA, 0.1 M CaCl<sub>2</sub>, 0.1 M Sr(NO<sub>3</sub>)<sub>2</sub> and Mehlich-3 extracts and b) Co extractability in DTPA, 0.1 M CaCl<sub>2</sub>, 0.1 M Sr(NO<sub>3</sub>)<sub>2</sub> and Mehlich-3 extracts.

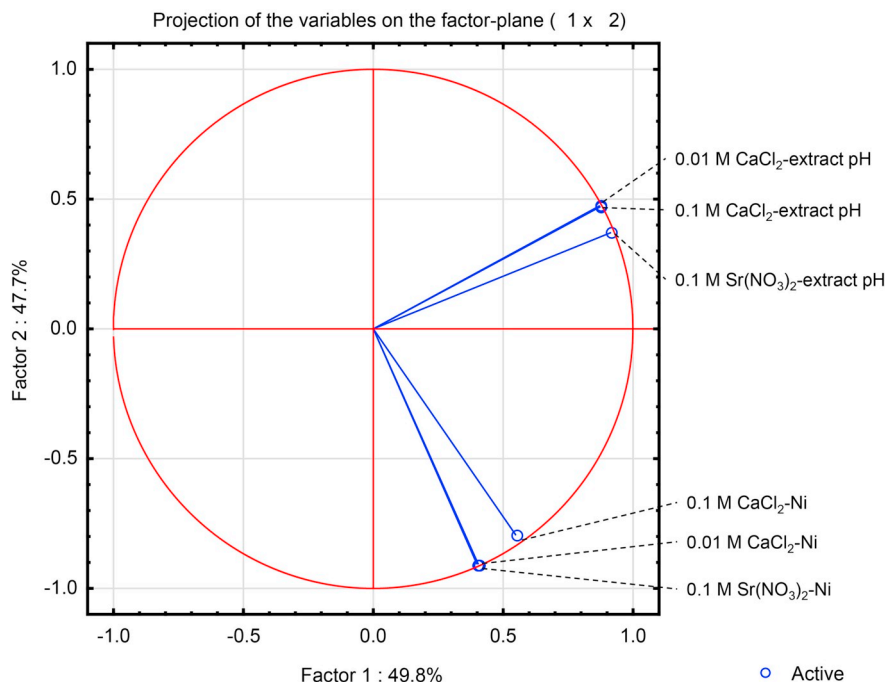


Fig. 3. Principal component analysis of nickel extractability in extracts of 0.1 M  $\text{Sr}(\text{NO}_3)_2$ , 0.01 M and 0.1 M  $\text{CaCl}_2$ , versus extraction solution pH.

Table 4

Multivariate regression analysis of Ni-extractions and multivariate regression analysis of co-extractions.

Extraction method	$\delta$	$\alpha$	$\beta$	$\gamma$
Carboxylic acid Ni	-3.45	0.70	0.40	0.30
DTPA Ni	-2.50	0.85	0.10	0.50
0.1 M $\text{Sr}(\text{NO}_3)_2$ Ni	-0.90	0.70	-0.25	0.45
Mehlich-3 Ni	-2.80	0.65	0.30	0.40
Carboxylic acid Co	-0.85	0.95	-0.07	0.19
DTPA Co	-0.025	0.90	-0.25	0.20
0.1 M $\text{Sr}(\text{NO}_3)_2$ Co	0.80	0.70	-0.45	0.05
Mehlich-3 Co	-0.40	0.65	-0.05	0.15

#### 4. Discussion

Although ultramafic soils are enriched in the trace elements Ni, Co, Cr, Fe, and Mn, phytoavailable concentrations of these elements are only a fraction of the pseudo-total concentrations present in the soil. Nevertheless, these elements are potentially present at phytotoxic concentrations and are widely seen as important factors contributing to the adversity of strongly acidic ultramafic soils to plants (Cooke and Inkson, 1955; Kukier and Chaney, 2003). In addition, major cation imbalance towards Mg and the deficit in Ca, N, P and K are also thought to be major factors affecting plants growing in these soils (Walker et al., 1955; Brooks, 1987; Nagy and Proctor, 1997; Echevarria, 2018). The high geodiversity of Mount Kinabalu Park, a result of complex geology, paleo-history, topography and climate, has created ultramafic soils that are extremely diverse in their pedology and chemical properties (van der Ent et al., 2018). Appropriate chemical characterization of these soils is therefore a challenge. Unfortunately, most chemical extractants used for characterizing soils (trace elements, nutrient status and cation exchange complex) were historically developed for agricultural soils with restricted ranges of main properties, or within ecotoxicological frameworks, and the appropriateness for using such methods for estimating ecological effects on plants growing in tropical ultramafic soils is questionable. Although there is only limited knowledge of the precise chemical interactions of these extractions in ultramafic soils, most extraction methods tested here perform well in characterizing different

types of ultramafic soils. In ultramafic soils, Ni is mainly associated with low-charge (serpentine, talc) and high-charge (smectite) clays, with Fe-(Mn) oxides and with Layered Double Hydroxide (LDH) phase minerals (Massoura et al., 2006; Siebecker and Sparks, 2010; Siebecker et al., 2018), but there is no extraction method specific for these phases altogether, although Ni may be (partly) co-extracted in existing methods. However, exchangeable Al and Mg are more important in the case of the carboxylic and DTPA extractable Ni. This can be explained by the presence of Layered Double Hydroxide (LDH) phase minerals that can adsorb Ni, and from which the carboxylic acid and DTPA extractants can desorb (part of the) Ni. In the unbuffered salt extracts ( $\text{Sr}(\text{NO}_3)_2$ ), soil pH is an important factor. This pH-dependence is not surprising because this extractant essentially represents solubility of Ni at soil pH. Regarding Cr, strong chelating agents (as anions) can competitively desorb CrVI anions but can also better complex  $\text{Cr}^{3+}$  than divalent ions. In the case of DTPA, it is interesting to note that acid pH mobilizes less Cr than higher pH. This fits well with the fact that anions are more easily desorbed at higher pH. When Mn is highly available,  $\text{Cr}^{3+}$  can be oxidized by reducing MnIV into MnII. CrVI is then complexed onto the surface of Fe-oxides, and therefore desorbed by DTPA or carboxylates such as citrate, which in turn adsorb onto the surface of Fe-oxides such as other anionic extractants, e.g. phosphate (Raous et al., 2013).

Exchangeable cations are fundamental to buffering soil pH and hence directly and indirectly influence many soil processes. CEC soil extraction methods rely on attempting to displace all exchangeable cations. Exchangeable cations are often measured with the 1 M pH 7.0 ammonium acetate ( $\text{NH}_4\text{Ac}$ ) method (DIN, 1995), with cobalt(III) hexamine trichloride method (Ciesielski and Sterckeman, 1997; in the case of ultramafic soils: Raous et al., 2013) or the silverthioarea method (Pleysier and Juo, 1980; Searle, 1986; Dohrmann, 2006). The second method is not adapted to evaluate exchangeable Co in ultramafic soils although it has been shown to be a reliable method for Ni in ultramafic soils (Raous et al., 2013; Bani et al., 2014). The last is, however, not frequently used for ultramafic soils, but has the benefit over  $\text{NH}_4\text{Ac}$  as it adopts the pH of the soil solution, has a greater cation displacement power, and exchange takes place at low ionic strength (0.01 M) (Pleysier and Juo, 1980; Proctor et al., 1981). As is typical for

**Table 5**  
Correlations of extractability of different elements in extracts (major cations),  
n = 343 \*\* denotes  $p < 0.0001$ .

	HNO <sub>3</sub> /HCl	Sr(NO <sub>3</sub> ) <sub>2</sub>	Carboxylic acid	Mehlich-3	DTPA
<b>Na</b>					
Sr(NO <sub>3</sub> ) <sub>2</sub>	-0.07				
Carboxylic acid	-0.08	0.90**			
Mehlich-3	0.10	0.30**	0.35**		
DTPA	-0.10	0.90**	0.95**	0.30**	
Silverthiorea	0.15	0.25**	0.25**	-0.10	0.25**
<b>Mg</b>					
Sr(NO <sub>3</sub> ) <sub>2</sub>	0.50**				
Carboxylic acid	0.70**	0.55**			
Mehlich-3	0.70**	0.80**	0.80**		
DTPA	0.60**	0.85**	0.65**	0.90**	
Silverthiorea	0.40**	0.50**	0.65**	0.60**	0.60**
<b>K</b>					
Sr(NO <sub>3</sub> ) <sub>2</sub>	0.20				
Carboxylic acid	0.25**	0.90**			
Mehlich-3	0.25**	0.75**	0.80**		
DTPA	0.25**	0.90**	0.95**	0.75**	
Silverthiorea	0.30**	0.85**	0.90**	0.75**	0.90**
<b>Al</b>					
Sr(NO <sub>3</sub> ) <sub>2</sub>	0.50**				
Carboxylic acid	0.70**	0.50**			
Mehlich-3	0.70**	0.55**	0.9**		
DTPA	0.50**	0.50**	0.70**	0.80**	
Silverthiorea	0.50**	0.55**	0.65**	0.65**	0.75**
<b>Ca</b>					
Sr(NO <sub>3</sub> ) <sub>2</sub>	0.55**				
Carboxylic acid	0.55**	0.85**			
Mehlich-3	0.55**	0.85**	0.95**		
Silverthiorea	0.55**	0.85**	0.95**	0.95**	
<b>Co</b>					
	HNO <sub>3</sub> /HCl	Carboxylic acid	Sr(NO <sub>3</sub> ) <sub>2</sub>	Mehlich-3	
Carboxylic acid	0.80**				
Sr(NO <sub>3</sub> ) <sub>2</sub>	0.30**	0.20**			
Mehlich-3	0.70**	0.75**	0.60**		
DTPA	0.70**	0.80**	0.55**	0.95**	
<b>Cr</b>					
Carboxylic acid	0.09				
Sr(NO <sub>3</sub> ) <sub>2</sub>	-0.04	0.35**			
Mehlich-3	-0.10	0.00	-0.05		
DTPA	0.10	0.50**	0.80**	-0.05	
<b>Fe</b>					
Carboxylic acid	-0.10				
Sr(NO <sub>3</sub> ) <sub>2</sub>	-0.05	0.015			
Mehlich-3	-0.40**	0.60**	0.25**		
DTPA	-0.10	0.25**	0.60**	0.45**	
<b>Mn</b>					
Carboxylic acid	0.75**				
Sr(NO <sub>3</sub> ) <sub>2</sub>	0.05	0.05			
Mehlich-3	0.55**	0.75**	0.005		
DTPA	0.55**	0.75**	0.025	0.90**	
<b>Ni</b>					
Carboxylic acid	0.5**				
Sr(NO <sub>3</sub> ) <sub>2</sub>	0.25**	0.30**			
Mehlich-3	0.30**	0.65**	0.40**		
DTPA	0.50**	0.50**	0.55**	0.50**	

ultramafic soils, the cation exchange complex is saturated with Mg<sup>2+</sup>, but absolute concentrations of Ca<sup>2+</sup> are not always low. The total cation exchangeable capacity (CEC) is lowest in the strongly leached Ferralsols, and high to extremely high in hypermagnesian Leptosols and serpentinitic Cambisols (Echevarria, 2018; van der Ent et al., 2018).

Apart from pseudo-total P (acid digest), P was extracted with the Olsen-P and Mehlich-3 methods, aimed at quantifying approximate plant available P concentrations. The Olsen-P method was initially

developed for calcareous soils as the extraction solution is pH 8.5 (Sharpley et al., 2008), whereas the Mehlich-3 method was developed for non-calcareous soils with an extractant solution of pH 2.5 (Mehlich, 1984). The Mehlich-3 method is similar to the Bray-1 method as both employ a dilute NH<sub>4</sub>F extraction medium (Lucero et al., 1998). Olsen P was proved to selectively extract isotopically-exchangeable P in a wide array of soils (Fardeau et al., 1988) which makes it a more universal method than initially thought by its developers.

A soil extraction method should be able to explain potential phytotoxicity effects as a result of trace elements, and should have predictive power for plant-uptake of these elements or at least a correlation with plant-uptake. However, plant uptake usually depends on the plant status and needs and may substantially vary between species. Even more, with high supply, plant uptake can respond without any correlation with the chemically mobile pool of an element (e.g. Ni for *Alyssum murale* in low pH soils: see Kukier et al., 2004; Bani et al., 2014). Ideally, the extractant provides simultaneous information about trace elements, exchangeable cations (major and trace) and macronutrients. Of the tested method, only Mehlich-3 method was specifically designed for such a 'multi-functional' purpose (Mehlich, 1984). This method uses dilute acetic acid combined with low concentration of EDTA for release and chelation of trace elements. Also present in the extraction solution are NH<sub>4</sub>NO<sub>3</sub> to exchange cations from the exchange complex, and further NH<sub>4</sub>F to extract P (hence essentially identical to the Bray-1 method of phytoavailable P). The 0.01 and 0.1 M Sr(NO<sub>3</sub>)<sub>2</sub> extracts appear to achieve more consistent results compared to other dilute neutral salt extracts (0.01 and 0.1 M CaCl<sub>2</sub>), and have been shown to correlate with immediate Ni phytotoxicity (Kukier and Chaney, 2001; Siebielec et al., 2007). The buffered and unbuffered DTPA methods are highly correlated, despite the fact that the unbuffered version is more adaptable to the soil pH. The high correlation of the carboxylic acid method not only with DTPA, Mehlich-3, CaCl<sub>2</sub> and Sr(NO<sub>3</sub>)<sub>2</sub> extractable trace elements (Ni, Cr, Co), but also with silverthiorea-CEC and silverthiorea exchangeable cations (Ca, Mg, K) means that this method is well-suited for general characterization of tropical ultramafic soils. It also shows that available Ni is composed of CEC-associated and surface-complexed pools. Finally, these data confirm that the knowledge on the precise speciation of Ni within bearing phases is the key to describing and quantifying its availability in soils (Massoura et al., 2006; Siebecker et al., 2018). However, as with all methods tested here, ecological implications for tropical vegetation or elemental uptake in native plants growing in tropical ultramafic soils remains unstudied. Without plant uptake data, the predictive values of the soil extractions should be interpreted cautiously. For instance, the DTPA extractable Ni can, in some cases, be inversely correlated with Ni accumulation by *Alyssum* species (Kukier et al., 2004; Nkrumah et al., 2016), whereas it is positively correlated with Ni uptake by *Noccaea caerulea* (Chardot et al., 2007). Future research is required to assess suitable extraction methods for ultramafic soils by evaluating the correlation of extraction results with plant composition or deficiency, adequacy and toxicity of specific elements. There is the need to test whether the displaced soil solution extraction method (Proctor et al., 1981; Kukier et al., 2004; Coinchelin et al., 2012) could be useful in predicting short-term phytoavailability of trace elements in local ultramafic soils as it was shown that hyperaccumulators can concentrate Ni during active root uptake (up to five times) from the initial concentration in the displaced soil solution, that means, depletion of the labile pools is strongly active (Coinchelin et al., 2012). Furthermore, tropical ultramafic soils present suitable opportunity for use in Ni agromining (a technology that extracts strategic metals from the biomass of selected 'metal crops') (van der Ent et al., 2013, 2015b). Hence, it is imperative to develop robust but simple Ni phytoavailability assays to predict Ni yield in 'metal crops' as Ni accumulation by 'hypernickelophores' has little evident relationship with single soil extraction methods including displaced soil solution (Nkrumah et al., 2016). The fact that plant species native to (tropical) ultramafic soils have evolved



**Table 6**

Sequential and non-sequential extraction of a) Ni, b) Co, c) Cr and d) Mn in 14 representative soils (as percentage of AR digest). The pH of the representative soils ranges from 5.11 to 9.19. Sequential extraction: exchangeable (Exch.), oxidisable Mn-oxides (Mn-OX), amorphous Fe-oxides (AM-Fe) or crystalline Fe-oxides (CR-Fe); non-sequential extraction: carboxylic acid (Carbox.), 0.1 M strontium nitrate (SrNO<sub>3</sub>)<sub>2</sub> or Melhich-3 (ML-3); and digest: digest with either HNO<sub>3</sub> + HCl (AR) or HNO<sub>3</sub> + HCl + HF (HF). < LOD denotes below detection limit.

a										
Samples	pH	Sequential extraction				Non-sequential extraction			Digest	
		Exch.	Mn-OX	AM-Fe	CR-Fe	Carbox.	DTPA	ML-3	AR	HF
		%				%			µg g <sup>-1</sup>	
1	5.66	3.83	0.35	12.2	21.1	5.6	10.0	11.9	420	630
2	5.67	2.76	0.12	3.2	9.2	1.3	3.0	3.9	1250	1400
3	5.82	2.09	0.39	4.3	42.7	3.9	3.9	5.5	595	855
4	5.11	1.69	0.13	2.8	15.6	1.9	2.6	3.5	305	615
5	6.48	0.31	0.22	34.2	11.4	7.9	10.0	7.0	1650	1650
6	7.21	0.02	1.02	15.6	4.1	10.6	3.3	4.2	1900	2100
7	6.94	0.81	0.23	6.9	4.9	7.0	6.2	4.5	2550	1950
8	5.59	0.10	0.01	0.2	6.0	0.1	0.2	0.2	2500	2950
9	9.19	0.00	0.66	13.0	2.6	5.2	0.4	26.8	2300	1250
10	9.19	0.05	1.09	19.6	5.2	15.2	0.8	43.1	1300	1300
11	6.86	0.17	0.06	16.1	9.1	3.6	2.0	4.1	3400	3400
12	6.44	0.28	1.02	12.6	7.2	6.2	10.9	4.0	1600	1600
13	7.15	0.11	0.55	16.0	8.4	1.9	2.0	1.9	2850	2350
14	6.82	0.44	0.88	29.7	11.5	9.2	17.5	8.7	2500	2300

b										
Samples	Sequential extraction				Non-sequential extraction			Digest		
	Exch.	Mn-OX	AM-Fe	CR-Fe	Carbox.	DTPA	ML-3	AR	HF	
	%				%			µg g <sup>-1</sup>		
1	0.006	0.001	2.7	22.8	0.25	0.25	0.09	2390	2740	
2	0.012	0.003	5.8	13.8	0.76	0.76	1.46	425	1290	
3	0.007	0.002	1.1	33.5	0.13	0.13	0.12	625	3040	
4	0.006	0.001	1.2	24.1	0.13	0.13	0.05	4050	4800	
5	0.000	0.001	2.4	17.8	0.14	0.14	0.24	2030	3730	
6	0.017	0.001	1.8	18.4	0.13	0.13	0.04	1520	2440	
7	0.002	0.001	1.3	14.6	0.04	0.04	0.03	3000	5600	
8	0.001	0.003	0.3	8.4	0.09	0.09	0.01	12,600	10,600	
9	0.000	0.004	6.1	6.3	1.22	1.22	0.02	2960	630	
10	0.004	0.014	14.7	14.8	6.16	6.16	0.06	1280	600	
11	0.006	0.001	1.0	14.6	0.06	0.06	0.05	1980	2360	
12	0.001	0.000	0.4	10.5	0.03	0.03	0.02	6520	8660	
13	0.001	0.000	1.4	13.9	0.04	0.04	0.04	2490	2030	
14	0.003	0.000	0.5	8.5	0.02	0.02	0.04	5420	4610	

c										
Samples	Sequential extraction				Non-sequential extraction			Digest		
	Exch.	Mn-OX	AM-Fe	CR-Fe	Carbox.	DTPA	ML-3	AR	HF	
	%				%			µg g <sup>-1</sup>		
1	1.57	1.3	13.6	19.0	7.5	8.8	9.02	25	7	
2	2.35	1.0	2.9	7.0	1.8	3.3	3.29	100	9	
3	2.01	5.6	13.2	6.8	36.1	21.7	26.80	75	8	
4	7.74	2.7	7.4	4.1	17.1	16.9	14.80	160	10	
5	0.00	1.2	34.5	2.2	17.0	3.4	3.85	280	16	
6	0.04	2.3	22.7	0.3	31.9	3.1	7.44	330	18	
7	1.17	3.5	25.6	0.4	20.4	4.8	7.11	480	25	
8	25.70	9.4	66.7	0.5	78.2	64.3	90.60	4	0	
9	0.02	0.1	0.4	9.3	1.0	0.4	5.64	140	7	
10	0.23	0.2	0.2	21.9	1.8	1.2	13.10	60	7	
11	0.02	0.6	29.1	2.0	12.2	0.7	6.61	360	17	
12	0.01	3.6	15.6	1.1	15.7	2.9	3.97	560	27	
13	0.10	2.0	24.3	1.2	3.8	5.3	2.44	260	14	
14	0.02	2.6	23.4	1.9	13.6	5.9	2.55	410	18	

(continued on next page)

Table 6 (continued)

Samples	Sequential extraction				Non-sequential extraction			Digest	
	Exch.	Mn-OX	AM-Fe	CR-Fe	Carbox.	DTPA	ML-3	AR	HF
	%				%			$\mu\text{g g}^{-1}$	
1	2.4	1.2	14.8	7.9	3.8	3.5	3.9	320	305
2	10.4	2.8	20.3	4.8	4.9	7.3	9.8	340	635
3	6.3	5.7	6.8	2.5	32.3	18.0	25.0	1330	1220
4	19.6	3.9	7.0	2.8	20.9	17.1	21.1	2000	1520
5	0.1	4.2	26.8	1.4	22.4	4.7	4.7	3120	3090
6	0.0	7.0	17.9	0.2	46.6	5.6	12.2	3300	4150
7	3.5	3.9	10.5	0.4	21.6	5.9	8.1	7540	4570
8	3.2	0.4	0.8	0.7	2.6	1.2	3.3	1560	1590
9	< LOD	0.5	0.4	0.4	1.3	< LOD	6.7	1950	575
10	0.0	1.4	1.1	1.1	6.1	0.6	18.3	655	540
11	0.1	2.3	22.4	1.9	14.3	1.5	9.2	4380	3030
12	< LOD	4.3	10.6	0.8	14.2	5.3	4.5	7330	5760
13	< LOD	3.1	21.2	0.5	4.1	4.4	2.8	3640	2230
14	< LOD	3.8	17.7	1.4	12.9	7.8	3.1	5460	3880

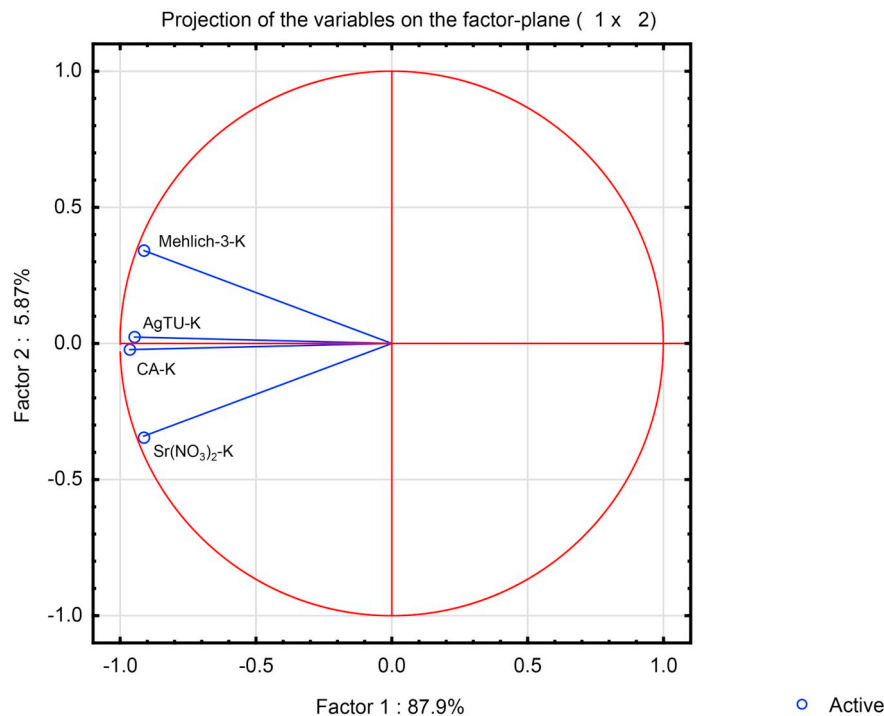


Fig. 4. Principal component analysis of extractable K in AgTU, carboxylic acid (CA),  $\text{Sr}(\text{NO}_3)_2$  (0.1 M) and Mehlich-3 extracts.

while confronted with extreme soil chemistry means that they must be highly tolerant, and therefore the results from experimental trials using ‘normal’ plants are not immediately applicable. Finally, plants vary widely not only in their tolerance to soil chemistry, but also in their uptake characteristics of trace elements (Ni, Co, Cr, Mn) depending on genotypic and phenotypic controlled ecophysiologicals (Ernst, 2006; Ünver et al., 2013), therefore aiming for a soil extraction method that accurately predicts the response of all plant species on ultramafic soils is futile. However, it was shown that despite these strong differences in Ni uptake, the available pool explored and ‘mined’ by plant roots was the same in an ultramafic soil (Massoura et al., 2004), thus metal availability is a necessary, but not in itself sufficient, information for the prediction of uptake. The method of choice needs to be inexpensive and simple, comparable to existing literature, as well as applicable to a wide range of different ultramafic soils. These conditions are met specifically with the carboxylic acid method. For more complex hyperaccumulator

crops, both a mild extraction that is related to soil chemistry and a more intense DTPA extractions might be relevant to assess both the initial pool in the soil solution (*i.e.* intensity) and the entire pool that replenishes the soil solution (*i.e.* quantity) under significant depletion exerted by hyperaccumulators (Echevarria et al., 1998, 2006; Massoura et al., 2004, 2006; Coinchelin et al., 2012).

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