

A Comparative Study on the use of Calcium Acetate Lactate, Calcium Chloride and Acidic Ammonium Acetate- Ethylene Diaminetetra Acetic Acid (AAAc-EDTA) for the Quantification of Extractable, P, K and Mg from Acidic Soils

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Authors' contributions

All the authors participated in preparing the protocol for sample collection. The samples were collected by author NJN who equally drafted the manuscript which was corrected by authors EEE and EGN. Author ES managed the analysis of soil samples in the laboratory. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Soils need to be tested before and after the establishments of plantations. This is because the results of a soil test gives a relative measure of the amount of nutrients available in the soil and thus are use as a base for nutrient diagnosis. The Calcium Acetate lactate (CAL) method for the extraction of available soil K and P and the CaCl₂ method for the extraction of available Mg were compared to the Acidic ammonium acetate- ethylene diaminetetraacetic acid (AAAc-EDTA) method for the determination of

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these elements in soil. A total of 130 samples were collected during a survey and analysed for K, P and Mg content after using the different extracting solutions. According to the results obtained, CAL and AAAC-EDTA methods were similar in their extracting power for P. In the case of K, the CAL method extracted only about half of the amount of K extracted using the AAAC-EDTA method. On the other hand, CaCl_2 extracted about twice the amount of Mg extracted using the AAAC-EDTA method. These extraction methods were poorly correlated to each other except the methods for P. This shows that P data obtained by the two methods can be interpreted in the same way. The difference in the quantities of nutrients extracted was associated to the differences in the reaction mechanisms between the soil nutrients and the extracting solutions. There was an influence of pH and organic carbon content on the amount of K and Mg extracted. These preliminary studies indicate that the choice of the extraction procedure may depend on the resources available as well as the soil properties. However, the AAAC-EDTA extracting solution offers the advantage of extracting many nutrients and can save time and energy.

Keywords: Calcium acetate lactate; calcium chloride; acidic ammonium acetate-ethylenediaminetetraacetic acid; potassium; magnesium and phosphorus.

1. INTRODUCTION

Besides nitrogen, P, K and Mg are the essential nutrients needed by most plants. Their levels in soils are often analysed because if their levels are too low, they will negatively affect plant growth or production. A relative measure of the amount of nutrients in the soil is obtained by soil testing [1]. The advantage of a soil test is that plant nutrient disorder resulting from the soil nutrient deficiency can be detected before they are visible in the plant. The most common methods used to diagnose the nutrient status of soils are through chemical testing [2]. A number of methods have been used for the extraction of available P, K and Mg in soils. Some commonly used methods for the extraction of plant available K and P are the Bray 2 method for available P and neutral ammonium acetate method for available K [3,4]. Studies found that the Bray 2 [5] extractable P in some soils diverges considerably depending on the extraction time and soil texture [6]. A comparison of Mehlich 3, ammonium acetate and water as multi nutrient extractants for the determination of the bioavailability of K, Mg, P, Mn and B was carried out by Matula in 2009 [2]. The results showed that there were no marked differences in correlations between soil tests and the plant in K, Mg and P. For the determination of soil available Mg, several methods have been used namely; Ammonium Lactate (Egner-Riehm-Domingo), calcium chloride (Schachtschabel, 0.0125 M CaCl_2 1:20), potassium chloride (1 M KCl 1:10), ammonium acetate (1 M NH_4OAc 1:10), Mehlich 3 [7] Olsen [8] methods and water soluble magnesium (1:5). Very strong correlations ($r = 0.96-0.99$) were found between magnesium content determined by calcium chloride, potassium chloride, ammonium acetate and Mehlich 3 methods [9]. Another study on soils in Ethiopia showed that the magnitude of soil available P extracted using the Truog method [10] (0.001M H_2SO_4 buffered with $(\text{NH}_4)_2\text{SO}_4$) was greater than that extracted by CAL, Olsen, Bray II and greater than the amount extracted by Warren and Cooke method [11].

Using distilled water, NaHCO_3 (pH 8.5), CaCl_2 , HNO_3 and NH_4OAc (pH 7) as extractants for available K showed that the amount of extractable K by NH_4OAc (pH 7) and HNO_3 showed the highest significant linear correlation with K uptake and dry matter yield in maize [12]. Traditionally, Calcium and potassium chloride extracts are used only for magnesium determination, and available phosphorus and potassium are measured by other methods.

The drawback is that it is time-consuming and requires higher reagent and labour input; therefore time and money will be saved by using one extract for the determination of all these nutrients [13]. Although the amount of nutrients extracted varies with the extractant used, research evidence has shown that soil nutrients also depend on soil pH, and humus or organic carbon content. The amount of Mg extracted by the ammonium lactate method was affected more by soil pH when compared to Mg extracted by CaCl₂, KCl, NH₄OAc, Mehlich and H₂O methods [9]. The advantage of using AAAC-EDTA as soil nutrient extractant is that it is a multi nutrient extractant. It has been used for the analysis of cotton soils in Egypt [14].

There are therefore a multitude of extractants in use however, the choice of an extractant depends on the following qualities; simultaneous extraction of all important nutrients from the soil, efficient in all soil types, accurate and reproducible, simple, not expensive, good for expeditious detection and relate soil available nutrients to plant nutrients [2]. It is true that no soil test method can fulfill all these roles; the suitability of a method can be based on how close the method fulfills these conditions. The objective of this work was to determine the content of P, K and Mg in acidic soils using two methods of extraction, compare and correlate the methods to each other as well as correlate extracted nutrients to soil pH and organic carbon.

2. MATERIALS AND METHODS

2.1 Soil Sampling and Analysis

One hundred and thirty (130) composite soil samples were collected from rubber estates belonging to the Cameroon Development Corporation (CDC) situated between latitude 4° 05 ' - 4° 42 ' N and longitude 9° 22 ' - 9°40 ' E and the Société Forestière Agricole du Cameroon (SAFACAM) situated between latitude 3° 46 ' - 3°51 ' N and longitude 9° 7 ' - 9°9 ' E. Organic debris on the soil surface were removed and samples collected after augering to a depth of 0-15 cm. Each sample consisted of at least 10 sub-samples from the entire sampled area. The collected samples were stored in properly labeled polythene bags and taken to the laboratory for analyses. On arrival at the laboratory, samples were air-dried (temperature of 25°C) separately on shelves in the soil drying room in order to avoid contamination. The dried samples were passed through 2-mm sieves in preparation for laboratory analyses. The laboratory procedures used are summarised in Table 1.

Table 1. Analytical methods used for the determination of soil parameters

Parameter	Analytical method		
	Extractant	Method of assay	Reference
pH	1+2.5 soil+CaCl ₂ suspension	pH meter reading	DIN ISO 10390, 2005 [15]
Total C	none	vario MAX CNS	Tchienkoua and Zech, 2004 [16]
Available P and K	Calcium Acetate Lactate (CAL) at ratio 1:20 (pH 4.1)	ICP-OES	Schüller, 1969 [17]
Available Mg	0.0125M CaCl ₂	ICP-OES	Schachtschabel, 1954 [18]
Available P, K and Mg	0.5M ammonium acetate + 0.5M acetic acid + 0.02M Na ₂ EDTA (pH 4.65)	ICP-OES	Lakanen and Erviö, 1971 [19]

2.2 Statistical Analysis

An analysis of variance was carried out to investigate the statistical differences between the methods of analysis for each element using the JMP statistical software (SAS 2002) [20]. Simple linear correlations were carried out to study the relationship between the methods of analysis for each nutrient element and the method with soil pH and organic carbon.

3. RESULTS AND DISCUSSION

3.1 Soil Nutrient Concentrations

The soils in the studied site were all acidic with pH < 6 (Table 2). Average K, P and Mg extracted by the selected methods varied. This is because the nutrient extracted from soils depends on the soil and extractants characteristics [21]. Soil organic carbon varied between 1 and 14% and this variation could be attributed to the varying nature of the cover crops and tree sizes found in the fields sampled [22].

Table 2. Mean, minimum, and maximum values of soil pH, Organic carbon and P, K and Mg concentrations

Parameter	pH in CaCl ₂	% organic C	K (mg/kg)		P (mg/kg)		Mg (mg/kg)	
			AAAc-EDTA	CAL	AAAc-EDTA	CAL	AAAc-EDTA	CaCl ₂
Mean	4.3	3	219.7	120.5	8.8	8.6	38.5	76.0
Max	5.7	14.1	1753.3	941.3	68.5	84.2	615.5	566.6
Min	3.7	1	93.0	43.7	2.0	1.0	7.4	4.5
STDEV	0.5	1.6	163.6	96.4	11.2	14.3	64.8	91.1

Max = Maximum, Min = Minimum, STDEV = Standard deviation

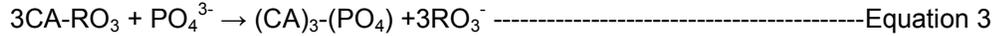
3.2 A Comparison of the Extraction Methods

The results of the analysis of variance showed that there was a significant difference between the amount of K extracted from the soil using the CAL and the AAAC-EDTA methods (Fig. 1) as well as the amount of Mg extracted by the CaCl₂ and the AAAC-EDTA methods. The AAAC-EDTA extracted about twice the amount of K extracted by CAL and only about half the amount of Mg extracted by CaCl₂. AAAC-EDTA has a high extracting power [23] compared to CAL. The high extracting power of AAAC-EDTA could be attributed to the multidentate nature of the EDTA portion of the extractant. In addition to the EDTA fraction, the ammonium ion (NH₄⁺) is also available which can be displaced and replaced. This shows that there are two possible reactions involve when AAAC-EDTA is used as an extractant.

In the extraction of P the possible mechanism involves the neutralization reaction between the phosphate and the Ca²⁺ in CAL and with NH₄⁺ in AAAC-EDTA with the reactions below.

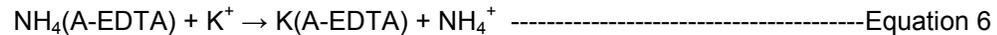


Considering equations 1 and 2, the mole ratio of P extracted by CAL and AAAC-EDTA is 1:1. Another possible mechanism involves the replacement of lactate ion (RO_3^-) in CAL and EDTA ion in AAAC-EDTA which is represented by the equations below.



Considering equations 3 and 4, the mole ratio of P extracted by CAL and AAAC-EDTA is 1:4. Due to the fact that the amount of P extracted by both methods was not significantly different we can conclude that the first mechanism is the most important mechanism for P extraction. It is worth noting that these methods are both acetate based and of similar pH. The lack of differences in the amounts of P extracted by the two methods suggests that the two extractants can be used for the determination of extractable P in these soils with similar interpretations.

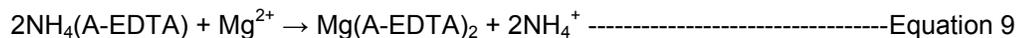
The mechanism of the extraction of K and Mg ions by the extractants involves the displacement of cations by cations of different sizes. The displacing cation in CAL and CaCl_2 extractants is Ca^{2+} (ionic radius = 0.099 nm) which is smaller than K^+ ion (ionic radius = 0.133 nm) but larger than the Mg^{2+} ion (0.065 nm). In the case of AAAC-EDTA, it contains NH_4^+ , whose ionic radius is similar to K^+ [21]. The displacement reactions taking place are depicted below;



From this analogy it shows that Ca^{2+} can easily displace and replace K^+ thus the higher number of moles of K product produced in eq. 5 compared to equation 6. However, the fact that more K was extracted by the AAAC-EDTA method than the CAL method shows that another mechanism was involved. Most probably a neutralisation reaction between K^+ and EDTA ion which is depicted in equation 7.



Due to the differences in ionic sizes, Ca^{2+} ion may not easily displace Mg^{2+} ion. Since the ionic radius of NH_4^+ is smaller than Mg^{2+} , it is expected that NH_4^+ will easily displace the Mg^{2+} thus making it more available. From the cation exchange reactions shown in equations 8 and 9, the moles of Mg extracted by CaCl_2 and AAAC-EDTA were the same.



However, the higher amount of Mg extracted using CaCl_2 compared to AAAC-EDTA suggests that Mg^{2+} ions were involved in a complexation reaction with the EDTA fraction of the AAAC-EDTA extractant. The possible reaction of EDTA with Mg^{2+} is shown in equation 10. The stable complex formed with the Mg^{2+} [24] right at the centre of the molecule makes the Mg^{2+} less available for detection in solution.



The mechanism of P removal can be by dissolution of sorbing components or by desorption depending on the pH of the extractant [24].

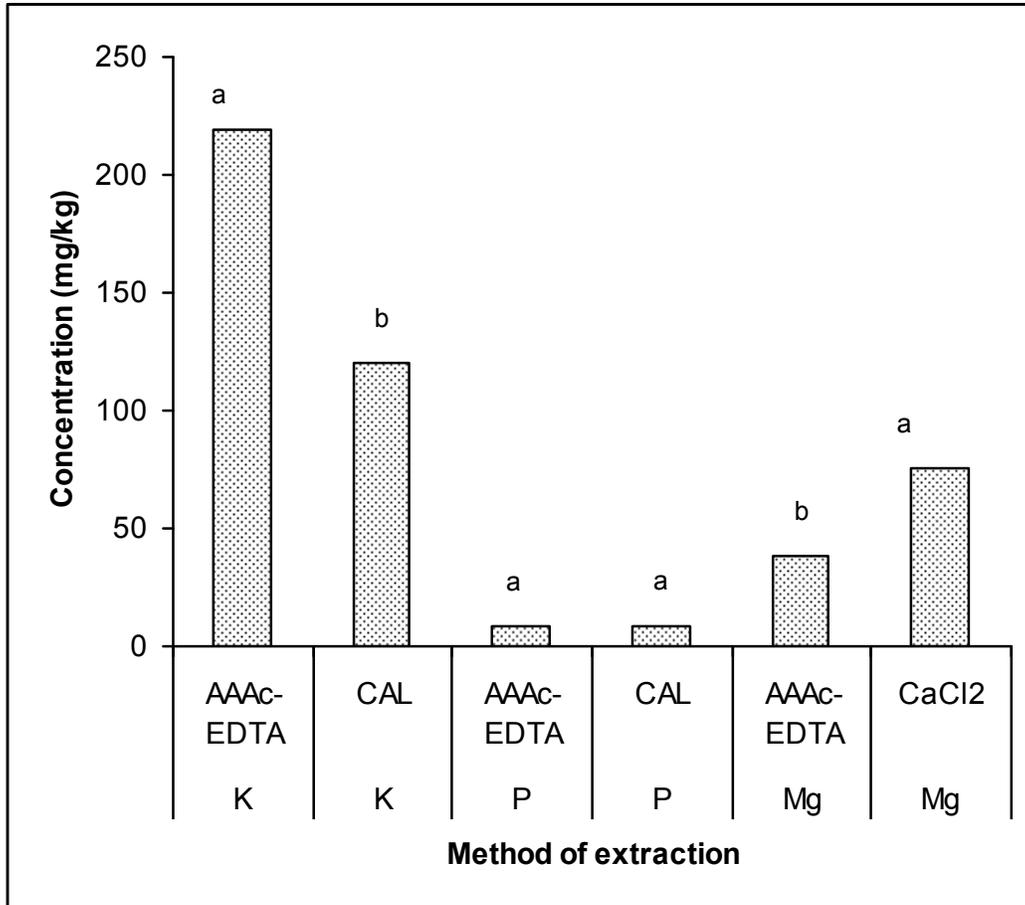


Fig. 1. An analysis of variance showing the difference between P, K and Mg values analysed by different methods

Levels not connected by same letter are significantly different at $\alpha = .01$

3.3 Correlation Studies

3.3.1 Correlations between methods of nutrient extraction

In order to know how well the extraction methods correlate with each other, each nutrient element was considered in turn. There was a highly significant correlation between the AAac-EDTA and CAL methods for the extraction of soil P with a coefficient of determination (R^2) of 0.72 (Table 3). This means that CAL extractable P accounts for 72% variation in AAac-EDTA extractable P which confirms the fact that the obtained values were not different using the both methods. In the case of K and Mg, non significant correlations were obtained between both methods of extraction. This shows that the methods of nutrient extraction were independent of each other.

Table 3. Coefficient of determination (R^2) and the corresponding P -values for the relationships between the two extraction solutions for K, P and Mg

Parameter	K (mg/kg)		P (mg/kg)		Mg (mg/kg)	
	AAAc-EDTA	-v- CAL	AAAc-EDTA	-v- CAL	AAAc-EDTA	-v- CAL
R^2	0.05		0.72		0.12	
P	.02		<.0001		<.0001	

Although there was a significant relationship between the methods used for extracting soil Mg, only 12% variation in the amount of soil Mg extracted by CaCl_2 could be accounted for by the variation in AAAC-EDTA extracted Mg.

3.2.2 Correlations between soil nutrients, pH and Organic carbon

The correlations between soil nutrients and pH varied with the method of extraction as well as the nutrient element involved (Table 4). This was similar to the correlation between soil nutrients and organic carbon. Significant correlations between two parameters show that a change in one parameter affects another with positive correlation meaning increasing one parameter increases another and negative correlation meaning increasing one parameter decreases another.

Table 4. Correlation coefficient between soil nutrients obtained by different extraction methods with pH and organic carbon

Parameter	K (mg/kg)		P (mg/kg)		Mg (mg/kg)	
	AAAc-EDTA	CAL	AAAc-EDTA	CAL	AAAc-EDTA	CaCl_2
pH	0.22 (0.01)	0.28 (0.001)	0.14 (0.12)	-0.22 (0.014)	0.25 (0.004)	0.72 (0.0000)
% organic C	0.1 (0.23)	0.54 (0.000)	0.1 (0.39)	-0.1 (0.32)	0.17 (0.05)	0.57 (0.000)

P-values are in brackets

Significant positive correlations were obtained between soil pH, organic carbon and K extracted by the CAL method as well as Mg by the CaCl_2 solution. There was also a significant positive correlation between pH and Mg which could be attributed to the fact that a high concentration of H^+ activity at low pH impedes the availability of Mg in soil [25]. This positive significant correlation is in confirmatory with the findings of Bacchewa and Gajbhiye in 2011 [26]. These significant correlations show that the amount of nutrients extracted will vary with change in soil pH or organic carbon. This means the method may not be suitable in many soil types.

In order to get a deeper understanding of the influence of the various soil parameters on the nutrient contents, the data was divided into three categories (Table 5). Soil P increases as the pH of the solution decreases. This confirms the negative relationship between CAL extracted P and pH and suggests that the pH range was still suitable for P. On the other hand, no matter the method of extraction, soil K and Mg decreased with a decrease in soil pH this confirms the fact that at low pH the K^+ and Mg^{2+} ions become less available. This could be attributed to competition between these cations and the H^+ ion in solution.

Similar results for the effect of soil pH on Mg content extracted by CaCl₂ and other methods have been shown [9]. Osemwota in 2007 found that pH was positively and significantly correlated with available K but concluded that the relationship with pH may vary as soil parameters vary because the pH of any soil is influenced by many other factors [27]. In this study, the classification of data into categories confirms the relationship between soil pH and K content.

Table 5. Mean values of soil P, K and Mg in different categories of physico-chemical properties

Available nutrients	pH			Organic C		
	5.0-6.0	4.0-4.99	3.0-3.99	4.6-8.5%	3-4.6%	0.5-2.9%
P (AAAc-EDTA)	6.5	7.0	12.0	3.8	8.8	8.9
P (CAL)	5.2	5.8	14.0	3.4	6.7	8.8
K (AAAc-EDTA)	244.5	247.3	172.2	104.6	282.6	215.9
K (CAL)	177.7	105.6	114.7	352.6	244.4	109.3
Mg(AAAc-EDTA)	55.5	50.3	15.4	17.8	78.7	35.7
Mg (CaCl ₂)	188.8	84.9	13.8	250.0	186.5	66.2

The average amount of soil P increased with a decrease in the soil organic carbon content confirming the negative correlation obtained (Table 4). The variation in K and Mg with organic carbon content was dependent on the method of extraction. Using the AAAC-EDTA extraction solution yielded maximum K and Mg when the organic carbon content was between 3 and 4.6 %. On the other hand, there was a decrease in soil extractable K and Mg with a decrease in soil organic carbon. This confirms the fact that the methods are not very correlated and the results obtained using the two methods may not be similarly interpreted.

4. CONCLUSION

The soils studied were all acidic. There was a significant difference between the amounts of K and Mg obtained following the two methods of extraction. On the other hand no difference was obtained for P following the two methods of extraction. Consequently, a significant correlation was found between the two methods of P extraction. The pH of the soil had no influence on the amounts of nutrients extracted by the different methods. However, the soil organic carbon content exerted a strong influence on the amounts of K and Mg extracted by the two methods, with P content being an exception. The variation was attributed to differences in the mechanisms of reactions between the soil nutrients and the extractants. These preliminary results show that the choice of the method to be used may depend on the elements to be analysed the soil physical properties and the resources available. The AAAC-EDTA extractant offers the advantage of being a multi nutrient extractant whose usage may save time and energy.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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