International Journal of Chemical Studies

Comparative assessment of nutrient availability in soils of pomegranate orchards using universal extractant and traditional extractants

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P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(4): 1818-1822
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Received: 09-05-2018
Accepted: 18-06-2018

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Abstract

A study was conducted to examine the reliability of Ammonium bicarbonate diethylene triamine penta aceticacid (AB-DTPA) extractant in determining the nutrient status of soils of pomegranate orchards as compared to traditional methods. AB-DTPA is used for extracting available P_2O_5, K_2O, Zn, Cu Fe and Mn simultaneously from soils and estimated using microwave plasma atomic emission spectrophotometer (MPAES). This was compared against the nutrient values determined using the traditional methods *viz.*, colorimetric estimation of available P_2O_5 using Olsen's extractant, flame photometry technique for available K_2O using neutral normal ammonium acetate and DTPA extractant for estimation of Zn, Fe, Mn and from soil. The estimated values using different extractants showed significant difference for availability of P_2O_5, Zn, Cu, Fe and Mn in pomegranate orchard soils while, K values did not differ. To assess the probable effect of Ca, the nutrient values were further categorized as low, medium and high Ca containing soil. The results indicated higher correlation between two methods for availability of P_2O_5 (0.783), Zn (0.780), Cu (0.838), Fe (0.721) and Mn (0.858) in low Ca containing soils. However, the linear relationship can't be established for medium and high Ca containing soils, signifying limitation of using AB-DTPA extractant for assessing soil nutrient variables using MP-AES instrument for Ca rich soils.

Keywords: AB-DTPA, MP-AES, Olsen's extractant, neutral normal ammonium acetate

1. Introduction

Soil analysis provides information about actual status of nutrient availability for the assimilation by the plant and it gives an idea on amount of supplemental nutrients needed. It helps to monitor the quantity of available nutrients present over time, which is useful in fertilizer management practices. Soil and plant analysis is an important tool for the nutrient management practices, the tedious and time consuming analytical protocols followed at present fails to provide results in time. Therefore improved instruments have been designed and procured by many laboratories for quick mineral estimation in the sample.

One such instrument is Micro wave Plasma-Atomic Emission Spectroscopy that works on emission spectroscopy by creating plasma using nitrogen as fuel and creating microwaves around the plasma to improve the efficacy of emission of electromagnetic waves from the sample. This instrument quantifies most of the nutrients except variable elements *viz.*, N and Cl in single feed. However, it needs to be standardized and requires development of analytical protocols that suits both the scientific measurement and practical applicability for the field condition.

In the traditional methods of soil analysis, different extractacts are used for extracting various nutrients from soil. This restricts availing benefit of the instrument. Hence, universal extractant can be used which serve as single extractant to extract many of the nutrients from soil. Mehlich No. 3, Morgan-Wolf and ammonium bicarbonate diethylene triamine penta acetic acid (AB-DTPA) are some of the extractants that can be used as universal extractants (Rodriguez *et al.* 1989, Molina *et al.* 2012, Madurapperuma and Kumaragamage, 1999, Mesfin *et al.* 2015) [13, 11, 9, 10] to determine multi nutrients from the soil and assay of the obtained extractant makes it more advantageous without the sample manipulation.

Soltanpour and Schuwab (1977) [15] introduced AB-DTPA for simultaneous extraction of P, K, Zn, Cu, Fe and Mn from alkaline soils of Colorado State. Ammonium in this solution acts as a displacing agent for cations adsorbed onto clay minerals, and therefore is used for the extraction of exchangeable cations.

Bicarbonate ion is used for the extraction of soil phosphorus while, DTPA is used to extract micro nutrients. In the present study, the feasibility of AB-DTPA extractant in determining available P, K, Zn, Cu, Fe and Mn in soils of pomegranate orchards was examined in comparison to traditional extractants that is being routinely used for assessing the nutrient status of soils in different villages of Bagalkot Taluka.

2. Material and Methods

Collection of soil samples: Soil samples were collected from one hundred and fifty pomegranate orchards of seven villages *viz.*, Junnur, Seemikeri, Govinakoppa, Kaladagi, Sokanadagi, Chikkasamshi and Hiresamshi. The soil samples were collected from the vicinity of plants for 0-15 cm depth and appropriately 45 cm away from the dripper position using post hole auger. All samples were air dried ground and made to pass through 2 mm sieve for the laboratory analysis.

Estimation of nutrients using traditional methods: The available phosphorus from soil was extracted using Olsen's extractant. The blue colour was developed by ascorbic acid method and the intensity was read at 660 nm using spectrophotometer (Thermo scientific Spectronic 200) and calculated referring to P-standard curve in terms of P_2O_5 kg ha^{-1} (Jackson, 1973) [4]. The available potassium was extracted from soil using neutral normal ammonium acetate in 1:5 soil to extractant ratio and the concentration of potassium in the extract was determined using Flame photometer (Thermo fisher 128) by calibrating with standards and calculated in terms of K_2O kg ha^{-1} (Jackson 1973) [4]. The method developed by Lindsay and Norvell (1978) [8] using DTPA (Diethylene Triamine Penta Acetic Acid) was adopted for the estimation of Zn, Fe, Mn and Cu. The micronutrient cations were extracted with DTPA buffer at 1:2 soil to extractant ratio and measured using Microwave plasma atomic emission spectrophotometer (Agilent Technology 4200).

Estimation of nutrients using universal extractant: Ammonium bicarbonate di ethylene triamine penta aceticacid (AB-DTPA), universal extractant was used to extract the soil nutrients *viz*., available P_2O_5, K_2O, Zn, Cu, Fe and Mn. AB-DTPA solution was prepared by dissolving 1.97 g of DTPA in 800-ml distilled water to which 2 ml of 1:1 NH_4OH was added. To the above solution, 79.06 g NH_4HCO_3 was added and stirred gently until complete dissolution. The pH of the solution was adjusted to 7.6 (Soltanpour and Schuwab, 1977) [15]. The nutrients were extracted from soil using AB-DTPA at 1:2 soil to extractant ratio by shaking for 15 minutes and filtered. Then 0.25 ml conc. HNO_3 was added to each 2 ml of filtrate to remove excess bi carbonates. Then the nutrient content was estimated using Micro wave Plasma Atomic Emission Spectroscopy (Agilent Technology 4200) by calibrating with multiple standards.

Statistical Analysis: To understand similarity and deviation with regard to nutrient content in both AB-DTPA and traditional extractant, simple correlation between each nutrient were calculated using Pearson product moment correlation coefficient. The MS-Office excel programme was used for calculating the simple correlation matrix. The perfect linear correlation was attained when r = ± 1 and r = 0 implies that X & Y tend to have no linear relationship. The table r value 2.60 and 1.97 at 0.01 and 0.05 probabilities respectively were used to determine the significance of relationship between two variables (Snedecor and Cochran, 1981) [14].

Further, the soils were grouped into three categories depending on exchangeable Ca content in the soil. The soil that contain exchangeable Ca < 24 Cmol (p+)/kg were categorized as low, 24-32 Cmol (p+)/kg as medium and > 32 Cmol (p+)/kg as high Ca containing soil. The nutrient content of these respective soils were separated, their range and mean were calculated and one way ANOVA and simple correlation matrix were studied to understand the significance difference between the categories and to establish the linear relationship.

3. Results and Discussion

Estimation of macronutrients availability in soil using traditional and universal extractant

The concentration of macronutrient by traditional and universal extractant methods showed a significant difference in pomegranate orchard soils. Phosphorus recorded significantly lower mean P_2O_5 content of 39.52 kg ha^{-1} in ABDTPA- method as compared to the Olsen's method (47.19 kg ha^{-1}) (Table 1) while, potassium availability estimated using both the methods content showed on par values. Correlation matrix between the two methods revealed higher correlation (r = 0.849) for K_2O but, less linear relationship for P_2O_5 (r= 0.720) (Table 2). Similarly, the scattered diagram potted for available P_2O_5 content using both the extractant showed relatively higher R^2 value (0.5489) for K_2O but, very low value for P_2O_5 (R^2=0.7212) (Fig 1&2). The NH_4^+ ion in both the extractant has the capacity to replace potassium from the soil exchangeable sites owing to their similar ionic radii (Buurman *et al.* 1985) [2]. However, the extent of correlation obtained in the present study is low compared to earlier reports from various workers (Liu and Bates, 1990; Molina *et al.* 2012: Madhurapperuma and Kumaragamage, 2008; Iatrou *et al.* 2015 and Zhue *et al.* 2016) [7, 11, 5, 17] indicating its limitation for estimating nutrients from calcium rich alkaline soils.

Table 1: Estimation of available nutrient content using traditional and universal extractant

Nutrient	Traditional extractant		Universal extractant	
	Range	Average	Range	Average
Phosphorus (P_2O_5 kg ha^{-1})	18.55-64.95	47.19 ± 14.16^{a}	16.29-64.48	39.52 ± 12.92^{b}
Potassium (K_2O kg ha^{-1})	139.9-284.2	218.0 ± 36.1^{ns}	145.6-286.9	237.5 ± 35.3^{ns}
Zinc (mg kg $^{-1}$)	0.44-3.62	1.07 ± 0.98^{b}	0.39-4.23	1.68 ± 2.34^{a}
Copper (mg kg $^{-1}$)	2.17-8.49	5.44 ± 1.93^{b}	2.52-12.59	7.33 ± 2.27^{a}
Iron (mg kg $^{-1}$)	2.44-5.94	4.45 ± 1.1^{b}	3.56-12.56	6.98 ± 2.01^{a}
Manganese (mg kg $^{-1}$)	11.13-23.30	17.63 ± 3.04^{b}	12.3-36.8	20.82 ± 5.09^{a}

Ns indicates non-significant difference means of same parameter with different letters are statistically significant at p<0.05 among various methods

Table 2: Correlation index for various nutrients estimated using AB-DTPA and Traditional extractants

Nutrients	Correlation index (r)
Phosphorus	0.720**
Potassium	0.849**
Zinc	0.499*
Copper	0.631**
Iron	0.535*
Manganese	0.320*

Unit of expression of Macro nutrients P and K is in kg ha^{-1} and micronutrient Mn, Fe, Zn and Cu in mg kg^{-1}, *$p<0.05$ and **$p<0.0$

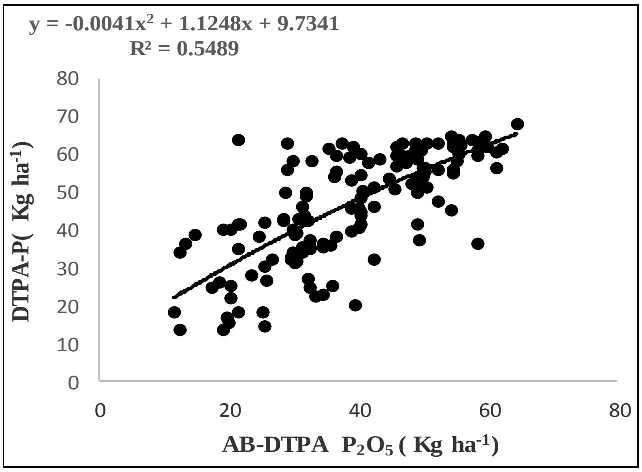

Fig 1: Scatter diagram of available P_2O_5 estimated by Olsen's and AB- DTPA extractants

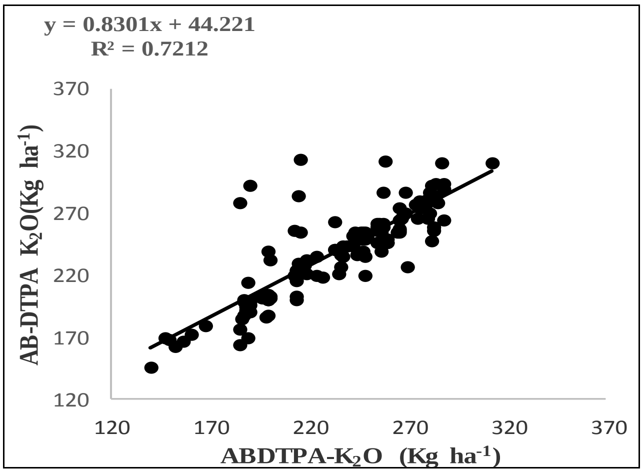

Fig 2: Scatter diagram of available K_2O estimated by neutral normal ammonium acetate and AB- DTPA extractants

Similarly, the earlier literature suggested few lacunas with regard to estimation of phosphorus by AB-DTPA using emission spectroscopy technique. Rodriguez *et al*. (1989) [13] indicated co-precipitation of P along with Ca in higher temperature either in flame or plasma that fails to estimate distinct emission lines for these nutrients. Hence the data were further categorized based on the exchangeable Ca content in soil into low (< 28 cmol (p+) kg^{-1}), medium (28-32 cmol (p+)/kg) and high (>32 cmol (p+)/kg) and accordingly, 98, 37 and 15 soils were grouped in respective categories.

The mean values of soil available P_2O_5 in low Ca content was on par between the traditional method (46.68 kg ha^{-1}) and universal extractant method (41.23 kg ha^{-1}) (Table 3) and the correlation coefficient (0.783) indicated significant positive linear relationship (Table 5). But, the P_2O_5 content showed significant variation among medium and high Ca containing soil recording higher availability in traditional method (40.47 & 51.25 kg P_2O_5 respectively) as compared to AB-DTPA method (23.36 & 41.81 kg P_2O_5 respectively) (Table 2). The linear relationship could not be established between two methods that recorded r values of 0.543 and 0.198 in medium and high Ca soil respectively (Table 5). This indicate the limitation of P estimation using AB-DTPA extractant using MP-AES instrument for Ca dominant alkaline soil, Soltanpour, (1985) also indicated anomalies in quantitative detection of Ca in flame due to anionic interference such as P and S.

Estimation of micronutrients availability in soil using traditional and universal extractant

Micro nutrients recorded a significantly higher mean content of Zn, Cu, Fe and Mn in AB-DTPA (1.68, 7.33, 6.98 and 20.82 mg kg^{-1} respectively) method as compared to DTPA method (1.07, 5.44, 4.45 and 17.63 mg kg^{-1} respectively) (Table 1). The correlation index recorded r value of 0.499, 0.631, 0.535 and 0.320 for Zn, Cu, Fe and Mn respectively, indicating limited linear relationship (Table 2) between two methods. The scatter plot also very low R^2 values 0.2497 (Fig.3), 0.3993(Fig. 4), 0.2869 (Fig.5) and 0.5309 (Fig.6) for Zn, Cu, Fe and Mn respectively indicating poor linear relationship between two extractants.

Further, when the nutrient values were categorized based on Ca content in soil, the variation were non-significant between two methods in soil that contained low Ca content. But significantly higher mean concentration of 3.20, 6.79, 6.64 and 23.65 mg kg^{-1} respectively was recorded in universal extractant method as compared to traditional DTPA method (2.15, 4.82,)

Table 3: Estimation of available P_2O_5 and K_2O content using traditional and universal extractant under varied levels of exchangeable Ca content

Exchangeable Ca content in soil	Traditional method		Universal extractant method	
	Range	Average	Range	Average
Available phosphorus (P_2O_5 kg ha^{-1})				
Low Ca (n=98)	18.55-64.15	46.68 ± 16.15 ^{ns}	16.29-64.48	41.23 ± 12.0^{ns}
Medium Ca (n =37)	31.60-63.48	51.25 ± 8.52^{a}	28.49-58.28	41.81 ± 8.19^{b}
High Ca (15)	18.55-50.36	40.47 ± 12.19^{a}	11.29-40.62	23.36 ± 11.19^{b}
Available potassium (K_2O kg ha^{-1})				
Potassium	**Range**	**Average**	**Range**	**Average**
Low Ca (n=98)	139.9-278.2	202.0 ± 33.7^{ns}	145.6-312.9	229.4 ± 36.5 ^{ns}
Medium Ca (n =37)	189.1-284.2	228.4 ± 24.9^{ns}	168.8-294.3	232.9 ± 27.0 ^{ns}
High Ca (15)	186.3-282.3	258.4 ± 26.2 ^{ns}	187.4-294.3	260.6 ± 26.8 ^{ns}

Ns indicates non-significant difference means of same parameter with different letters are statistically significant at $p<0.05$ among various methods

Table 4: Estimation of micro nutrients in soil using traditional and universal extractants under varied levels of exchangeable Ca

Exchangeable Ca content in soil	**DTPA**		**Universal extractant**	
	Range	**Average**	**Range**	**Average**
	Zn content in soil (mg kg $^{-1}$)			
Low Ca (n=98)	0.44-1.82	1.12 ± 0.92^{ns}	0.39-3.21	1.45 ± 1.42^{ns}
Medium Ca (n =37)	1.72-3.62	2.15 ± 1.08^{b}	3.18-4.88	3.20 ± 1.44^{a}
High Ca (15)	0.84-1.96	1.21 ± 0.80^{b}	4.89-5.73	4.80 ± 1.98^{a}
	Cu content in soil (mg kg $^{-1}$)			
Low Ca (n=98)	2.66-8.49	5.75 ± 1.87^{ns}	3.26-12.54	7.30 ± 2.09^{ns}
Medium Ca (n =37)	2.17-8.36	4.82 ± 1.95^{b}	2.22-10.94	6.79 ± 2.17^{a}
High Ca (15)	2.64-7.80	4.96 ± 1.76^{b}	2.52-12.59	8.88 ± 2.77^{a}
	Fe content in soil (mg kg $^{-1}$)			
Low Ca (n=98)	2.44-5.94	4.29 ± 1.15^{ns}	3.56-8.96	6.0 ± 1.23^{ns}
Medium Ca (n =37)	2.64-5.88	4.38 ± 0.91^{b}	5.19-11.76	6.64 ± 1.66^{a}
High Ca (15)	2.98-4.96	3.86 ± 0.86^{b}	5.12-12.65	9.63 ± 2.19^{a}
	Mn content in soil (mg kg $^{-1}$)			
Low Ca (n=98)	11.13-20.56	16.51 ± 2.89^{ns}	12.3-28.4	18.61 ± 3.38^{ns}
Medium Ca (n =37)	14.43-22.12	19.43 ± 2.00^{b}	15.3-32.6	23.65 ± 4.20^{a}
High Ca (15)	17.42-23.30	20.52 ± 1.90^{b}	15.6-36.8	28.32 ± 5.64^{a}

Ns indicates non-significant difference, means of same parameter with different letters are statistically significant at $p<0.05$ among various methods

Table 5: Correlation index for various nutrients under varied levels of exchangeable Ca estimated using AB-DTPA and Traditional extractants

Correlation index (r)	**Low Ca**	**Medium Ca**	**High Ca**
Phosphorus	0.783**	0.543*	0.198
Zinc	0.780**	0.336*	0.101
Copper	0.838**	0.517*	0.133
Iron	0.721**	0.439*	0.197
Manganese	0.858**	0.310*	0.115

Unit of expression of Macro nutrients P and K is in kg ha^{-1} and micronutrient Mn, Fe, Zn and Cu in mg kg^{-1}* $p<0.05$ and **$p<0.01$

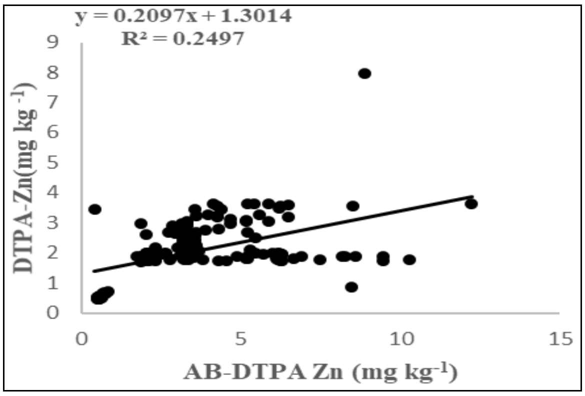

Fig 3: Scatter diagram of Zn estimated by DTPA and AB-DTPA extractant

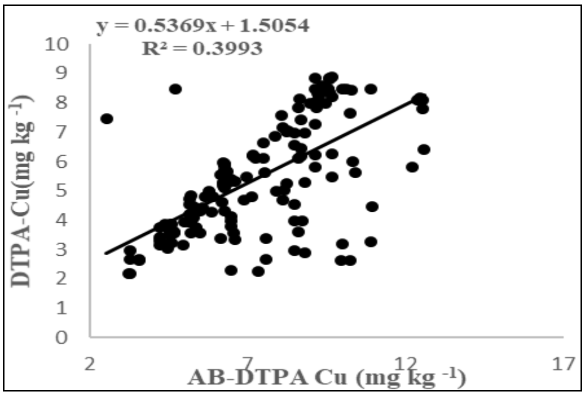

Fig 4: Scatter diagram of Cu estimated by DTPA and AB-DTPA extractant

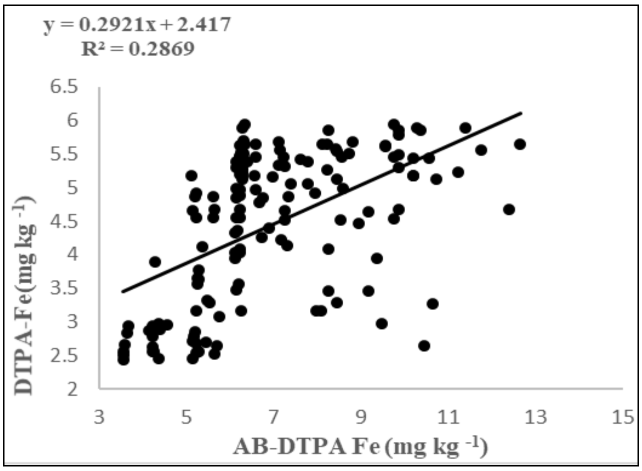

Fig 5: Scatter diagram of Fe estimated by DTPA and AB-DTPA extractant

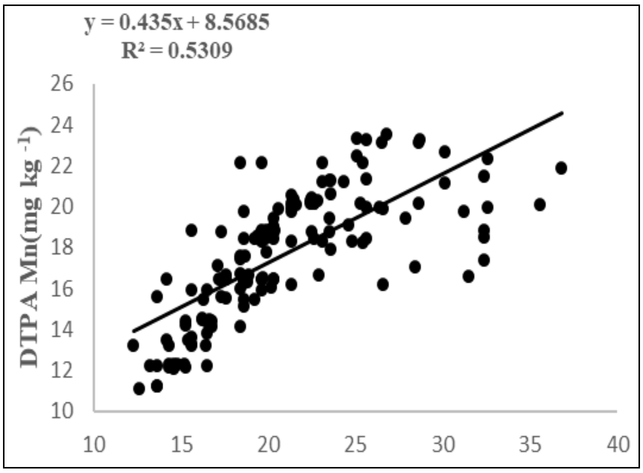

Fig 6: Scatter diagram of Mn estimated by DTPA and AB-DTPA extractant

4.38 And 19.43 mg kg^{-1}) in medium Ca containing soils. Similarly in high Ca containing soils, micro nutrients *viz.*, Zn, Cu, Fe and Mn showed a significantly higher mean content of 4.80, 8.88, 9.63 and 28.32 mg kg^{-1} respectively in universal extractant method as compared to traditional method (1.21, 4.96, 3.86 and 20.52 mg kg^{-1}) (Table 4). The correlation r value also increased recording, 0.780, 0.838, 0.721 and 0.858

for Zn, Cu Fe and Mn respectively in low Ca containing soils (Table 5). However the medium and high Ca containing soil could not establish linear correlation between the methods.

The results indicated that AB-DTPA cannot be used for the ca rich soils. Many researchers indicated that ammonium bicarbonate after dissolution releases CO_2 (Yeh *et al.* 2005) [16] that combines with water to form carbonic acid (Brucato *et al.* 1997) [3]. The carbonic acid dissolves appreciable amounts of calcium carbonate (Al-Hosny and Grassian, 2004) [1]. Thus, solubilizing the unavailable micronutrients sorbed on $CaCO_3$ surfaces resulting in over estimation of micro nutrient as compared to DTPA extracatant (Karima *et al.* 2008) [6].

4. Conclusion

The soil nutrient analysis using traditional and universal extractant showed significant difference in the mean values of available P_2O_5, Zn, Cu, Fe and Mn while K values did not differ. Further categorizing the nutrient values based on Ca content in soil indicated relatively higher linear association between two methods for P (0.783), Zn (0.780), Cu (0.838), Fe (0.721) and Mn (0.858) in low Ca containing soils. The linear relationship can't be established for medium and high Ca containing soils signifying limitation of using AB-DTPA extractant for assessing soil nutrient variables using MP-AES instrument for Ca rich soils.

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