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Evaluation of extractants and instrumental methods for determination of sodium in soils of South Gujarat

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Abstract
An experiment was conducted at Department of Soil Science and Agricultural Chemistry, Navsari Agricultural University during the year 2018-19, with the objectives of (i) to find out suitable extractant and instrument for the determination of sodium from soil and (ii) to establish the relationship of various soil properties with sodium availability. For evaluation of methods of sodium, each Taluka wise one sample; total 46 samples from south Gujarat (Bharuch, Dang, Narmada, Navsari, Surat, Tapi and Valsad) were collected from 0-15 cm depth. Soil physico-chemical properties *viz.*, pH, EC, CEC, organic C, $CaCO_3$ content and soil texture were determined from these sample soils. These soils were used to evaluate six methods of sodium. The methods of sodium was (i) Extracted through NH_4OAc and determined with FPM (Na_1), (ii) Extracted through NH_4OAc and determined with MP-AES (Na_2), (iii) Extracted through AB-DTPA and determined with FPM (Na_3), (iv) Extracted through AB-DTPA and determined with MP-AES (Na_4), (v) Extracted through Neubauer and determined with FPM (Na_5) and (vi) Extracted through Neubauer and determined with MP-AES (Na_6). The methods of sodium were evaluated through method validation parameters like linearity, sensitivity of instruments, precision and predictability. The available sodium determined from the sample soils were correlated with soil physico-chemical properties. The linear dynamic range of NH_4OAc-FPM (Na_1), AB-DTPA-FPM (Na_3) and Neubauer FPM (Na_5) extractants was between 25 to 100 ppm having Co-efficient of determination (R^2) 0.982, 0.988 and 0.994, respectively. The NH_4OAc-MP-AES (Na_2), AB-DTPA-MP-AES (Na_4) and Neubauer-MP-AES (Na_6) had linear dynamic range between 1.0 to 10 ppm with Co-efficient of determination (R^2) 0.985, 0.945 and 0.991, respectively. The sensitivity of instruments was identified by measuring limit of detection (LOD) and limit of quantification (LOQ) of FPM and MP-AES for NH_4OAc, AB-DTPA and Di-acid extractant. The LOD of FPM for NH_4OAc, AB-DTPA and Diacid extractants were 0.578, 0.382 and 0.614 ppm, respectively. The corresponding LOQ of FPM for NH_4OAc, AB-DTPA and Di-acid extractants were 1.925, 1.272 and 2.045 ppm, respectively. The LOD of MP-AES for NH_4OAc, AB-DTPA and Di-acid extractants were 1.663, 3.542 and 0.643 ppm, respectively. The corresponding LOQ of MP-AES for NH_4OAc, AB-DTPA and Di-acid extractants were 5.537, 11.79 and 2.142 ppm, respectively. Amongst the chemical methods the measured values were observed in increasing order of Na_2 (685.7 kg/ha) > Na_1 (673.8 kg/ha) > Na_4 (365.1 kg/ha) > Na_3 (255.8 kg/ha). NH_4OAc-FPM (Na_1) extractant of Na determination is accepted extractant. Sodium determined by this extractant was highly positive and significantly correlated with uptake-FPM (r=0.663**) and uptake MP-AES (r=0.674**). In extractant AB-DTPA-FPM (Na_3), highly positive and significantly correlated with uptake-FPM (r=0.630**) and uptake MP-AES (r=0.642**). Na extracted with AB-DTPAMP-AES (Na_4) was highly positive and significantly correlated with uptake FPM (r=0.645**) and uptake MP-AES (r=0.667**). In sodium determination NH_4OAc-FPM has highly positive and significantly correlation with nutrient uptake by wheat on MP-AES instruments.

Keywords: Chemical extractants, instrumental methods, sodium, wheat

Introduction
India's resounding success from its past green revolution has been followed by stagnating or declining agricultural productivity, even with increased total fertilizer use in the country over the years it might be due to imbalanced fertilizer use without considering the soil status of nutrients (Kumar *et al.* 2007) [8]. Accurate soil testing is censorious to understanding its potential fertility. If the results indicate there is an imbalance in nutrient then this can be corrected for by the application of a suitably formulated fertilizer.

Base cations are defined as the exchangeable, most prevalent and weak acid cations in the soil include ions such as potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}) and sodium (Na^+). The amount exchangeable bases and the cation exchange capacity (CEC) are important properties of soil, and responsible for cation exchange occurs due to negative charges by soil particles, in particular clay minerals, organic matter and sesquioxides. Different extractant are used for the determination of the soil nutrients because use of extractant depends on the various physico-chemical properties of soil. Basic cations like, potassium (K), calcium (Ca), magnesium (Mg) and sodium (Na) are essential elements for crop growth. These nutrients in the soil solution are taken into plants in various ionic forms, such as K^+, Ca^{+2}, Mg^{+2} and Na^+ through a combination of root interception, mass flow, and diffusion processes. Monitoring nutrient levels in soils is necessary to efficiently use fertilizers and minimize the environmental impact of fertilization practices. Various techniques can provide adequate detection range for same analytical subject. The amount of K, Ca, Mg and Na extracted from soil samples are vary from laboratory to laboratory. Major sources of variation are as follow: (1) unavailability of universal extractant. (2) Instrument's accuracy, precision and sensitivity. An array of instruments has been used to determine the quantity of these nutrients *viz*., Flame Photometer (FPM) and Microwave Plasma Atomic Emission Spectroscopy (MP-AES). An ideal method should be able to extract proportionate amount of the available form of nutrient from soils with variable properties, extract with reasonable accuracy and speed and the amount extracted should be correlated with the growth and response of crop to that nutrient under various conditions. Prior to adopting any method, standardization, validation and optimization against these criteria is essential. Many attempts have been made to find the best method for measuring plant- available K, Ca, Mg and Na using different extraction solutions. In many countries, 1 M Ammonium acetate (NH_4OAc) is considered the best option for routine soil testing purposes (Eckert and Watson, 1996; Hosseinpur and Samavati, 2008) [5, 7]. The success or failure of a soil extractor is probably related to different forms of soil K depending on the type and amount of soil minerals. The correlation between K extracted by each extractant and its uptake by plants is an important criteria for choosing an extractant is mentioned (Zarrabi and Jalali, 2008) [19]. After choosing an extractant, determination of the critical concentration of nutrients in the soil to predict plant response to chemical fertilizers and optimum fertilizer recommendation.

Various instrumental methods such as Flame Photometer (FPM) and Microwave Plasma Atomic Emission Spectroscopy (MP-AES) and extractants include chelating agents such as ammonium bicarbonate di-ethyl tri-amine penta-acetic acid (AB-DTPA) and neutral normal ammonium acetate are used for determination of K, Ca, Mg and Na from soils. These extractants and instruments commonly use in all soil testing laboratory. Correlation and regression analysis were carried out separately for each nutrient with soil properties to obtain the relationship between the amount of nutrients extracted by different methods and also with plant uptake. Taking theses point under consideration, an experiment on the following objectives had been taken up. To find out suitable extractant and instrument for the determination of basic cations from soil. To establish the relationship of various soil properties with basic cations availability.

Materials and Methods

Forty six surface soil samples from south Gujarat (Bharuch, Dang, Narmada, Navsari, Surat, Tapi and Valsad) were collected from 0-15 cm soil depth. All the soil samples are acidic, neutral and alkaline in nature and the pH ranged from 5.44 to 7.73. Electrical conductivity of samples was ranged from 0.01 to 1.2 dS/m (1:2.5 soil: water ratio). Soil salinity in south Gujarat varies from slight to strong salinity class. In Narmada, Tapiand Dang district soil salinity is moderate. The soil salinity in Surat, Navsari and Valsad belongs to slight to strong salinity class. The soil sodicity in South Gujarat in general belongs to slight sodicity class except in Navsari where soil sodicity varies from slight to moderate. Majority of samples were very low to very high in organic C, it was ranged between 0.07 to 2.53%. The $CaCO_3$ content in soil samples varied between 1.13 to 3.95%. The sample soils were clay in texture and the clay content in soils ranges from 30% in case of Waghai to 64.20% in Bharuch soil samples. Soil potassium availability indices were measured according to various methods and used different extractants.

Extract no.	Extractants	Soil-solution ratio	Equilibration time (min.)
1	0.005 M AB-DTPA	01:02	15
2	1 M NH_4OAc	01:10	30

Plant (Neubauer) study

Take 100 g soil and mix with 50 g of nutrient free quartz sand. Fill the pot and sow 100 wheat seeds. Sprinkler the distilled water to facilitate germination. Allow the seedlings to grow for 17 days and then uproot them carefully on 18th day. Dry the seedlings in oven at 60 ± 5 °C. After drying samples are ready to digest. Take 0.5 g of dried sample for digestion and determine the basic cations (K) on FPM and MP-AES (Neubauer and Schneider, 1923) [20].

Statistical Analysis

The different statistical techniques was adopted are discussed here under,

Linearity: To establish the predictability relationship between the response (Y) of different basic cations. At different levels of concentration (X). The linear equation was fitted (Y=a+bX) and coefficient of determination (R^2) was obtained.

Based on the value of slope (b), the Limit of detection (LOD) and Limit of quantification (LOQ) was worked out using following formula (Shrivastava and Gupta, 2011) [15].

LOD (ppm) =Mean/Slope×3

LOQ (ppm) =Mean/Slope×10 or LOD×3.33

Correlation: The relationship among different extractants and nutrient uptake was obtained by using Karl-pearson correlation coefficient (r) equation,

$$r = \frac{\sum(x - x)(y - y)}{\sqrt{\sum(x - \bar{x})^2 (y - \bar{y})^2}}$$

Where, $\bar{x}$ = Mean of X variable, $\bar{y}$ = Mean of Y variable

Precision: A measure of reproducibility, it is usually described by the Relative Standard Deviation (RSD). RSD was worked out by preparing the 5 samples of different extractants at different concentration with their respective elements. The respective sample is analyzed on different instruments by repeating the sample is 5 times each. The formula for calculating RSD is given below.

$$= \frac{SD}{\bar{X}} * 100$$

Results and Discussion

Method validation

Biological method particularly Neubauer is considered as an ideal method for soil fertility evaluation because plants itself used as an extractant. In case of chemical methods, NH_4OAc-FPM for basic cations is considered as standard methods of soil analysis and adopted in many analytical laboratories. Therefore, these methods are considered as a check. MP-AES is the sophisticated and newer technique of soil analysis.

Linearity

The result of linearity study in Na is depicted in Fig. 1 and 2 and also given below. The results indicated that standard curve prepared among measured and reference value in Na_1, Na_2, Na_3, Na_4, Na_5 and Na_6 was linear and all the methods recorded coefficient of determination (R^2)>0.90. Similar linear relationship between measured and reference value was observed in MP-AES by Bortolon and Gianello (2010) [2]. However, there is wide variation in detection limit of MP-AES and FPM. Vysetti *et al*. (2014) [16] and Instruction manual (PFP7) reported the detection limit of Na in both the instruments, which was 1.2×10^{-4} mg/l and 0.2 mg/l for MP-AES and FPM, respectively.

Table 1: The result of linearity study in Na

Nutrients	Methods	Linearity range (ppm)	R2
Sodium	NH_4OAc-FPM (Na_1)	1.0 to 5.0	0.981
	NH_4OAc-MP-AES (Na_2)	1.0 to 5.0	0.985
	AB-DTPA-FPM (Na_3)	1.0 to 5.0	0.988
	AB-DTPA-MP-AES (Na_4)	1.0 to 5.0	0.945
	Neubauer-FPM (Na_5)	1.0 to 5.0	0.994
	Neubauer-MP-AES (Na_6)	1.0 to 5.0	0.991

All the methods including AB-DTPA-MP-AES had good linearity however, the great advantage to MP-AES is extremely low detection limits for a wide variety of elements. Some elements can be measured down to part per quadrillion (ppq) ranges while most can be detected at part per trillion (ppt) levels, which is not possible in FPM (Vysetti *et al*. 2014) [16].

Sensitivity of instruments

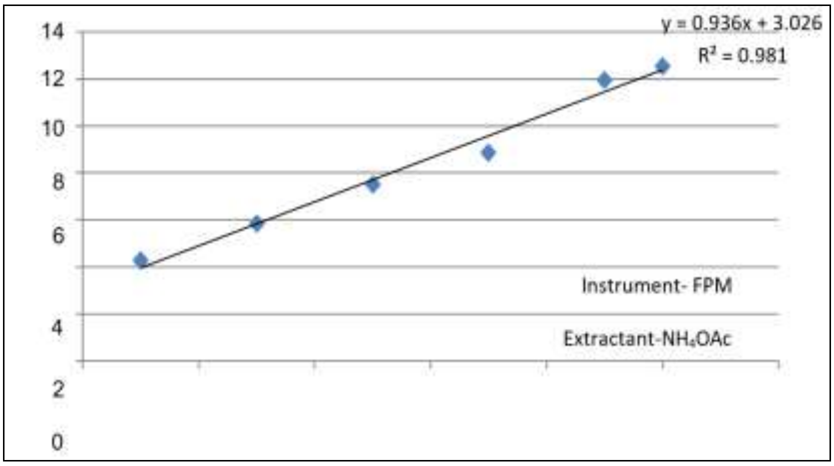
Flame Photometer (FPM)

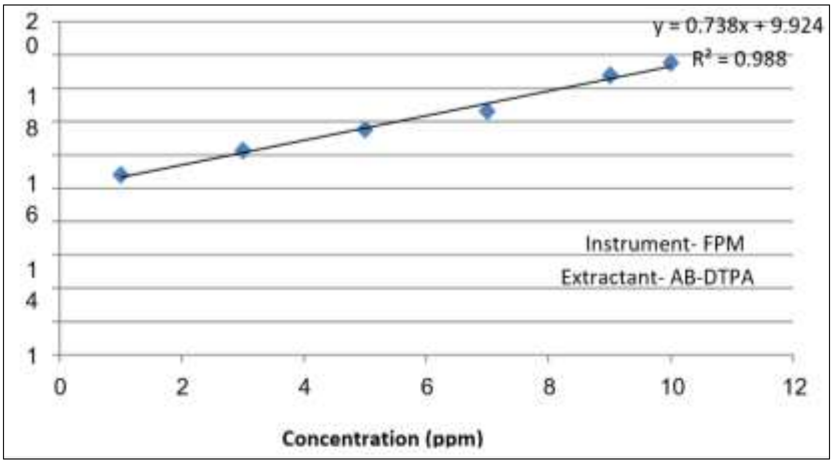
The limit of detection (LOD) and limit of quantification (LOQ) of FPM for NH_4OAc, AB-DTPA and Di-acid extractant was given in Table 2. The NH_4OAc has lower detection limit among all the extractants of FPM. This data is given as a graphical manner in Fig. 2.

Microwave Plasma Atomic Emission Spectroscopy (MP-AES)

The limit of detection (LOD) and limit of quantification (LOQ) of MP- AES for NH_4OAc, AB-DTPA and Di-acid extractant was given in Table 2. The NH_4OAc has lower detection limit among all the extractants of MP-AES.

This data is given as a graphical manner in Fig. 2.





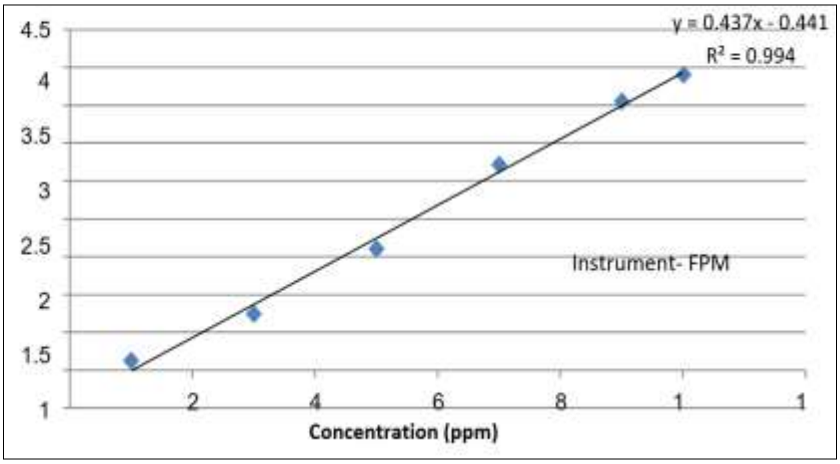


Fig 1: Response for Na determine on FPM

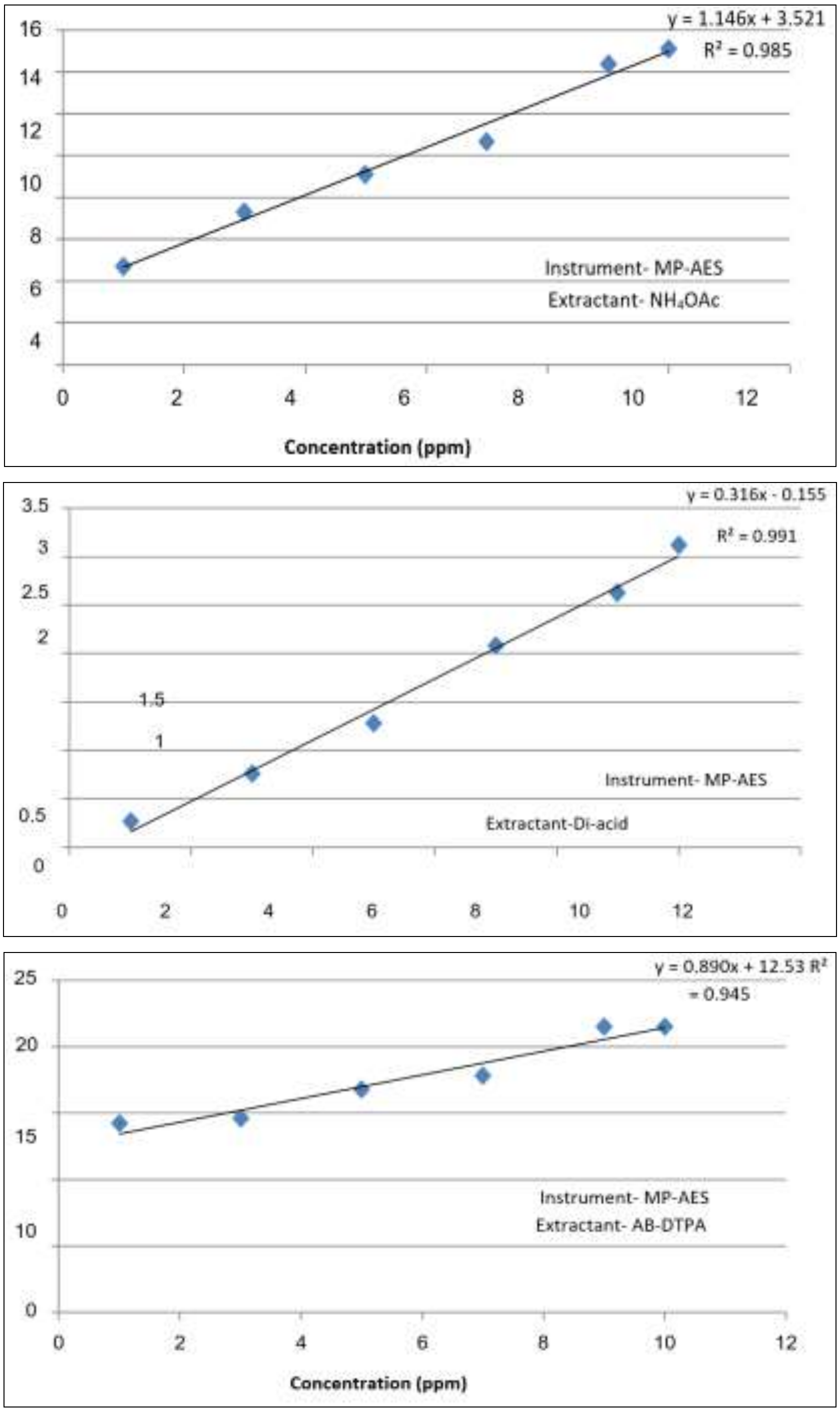


Fig 2: Response for Na determine on MP-AES

Table 2: LOD and LOQ of FPM and MP-AES for K and Na with different extractants

Elements	Instruments	Extractants	SD						Mean	Slope	LOD (ppm)	LOQ (ppm)
			1	3	5	7	9	10				
Na	FPM	NH_4OAc	0.093	0.145	0.166	0.182	0.244	0.252	0.180	0.936	0.578	1.925
		AB-DTPA	0.005	0.038	0.087	0.080	0.051	0.072	0.056	0.437	0.382	1.272
		Di-acid	0.122	0.215	0.232	0.162	0.303	0.179	0.202	0.988	0.614	2.045
	MP-AES	NH_4OAc	0.413	0.655	0.656	0.849	0.334	0.905	0.635	1.146	1.663	5.537
		AB-DTPA	1.202	0.375	1.418	1.191	0.991	1.128	1.051	0.890	3.542	11.79
		Di-acid	0.005	0.067	0.055	0.042	0.171	0.067	0.068	0.316	0.643	2.142

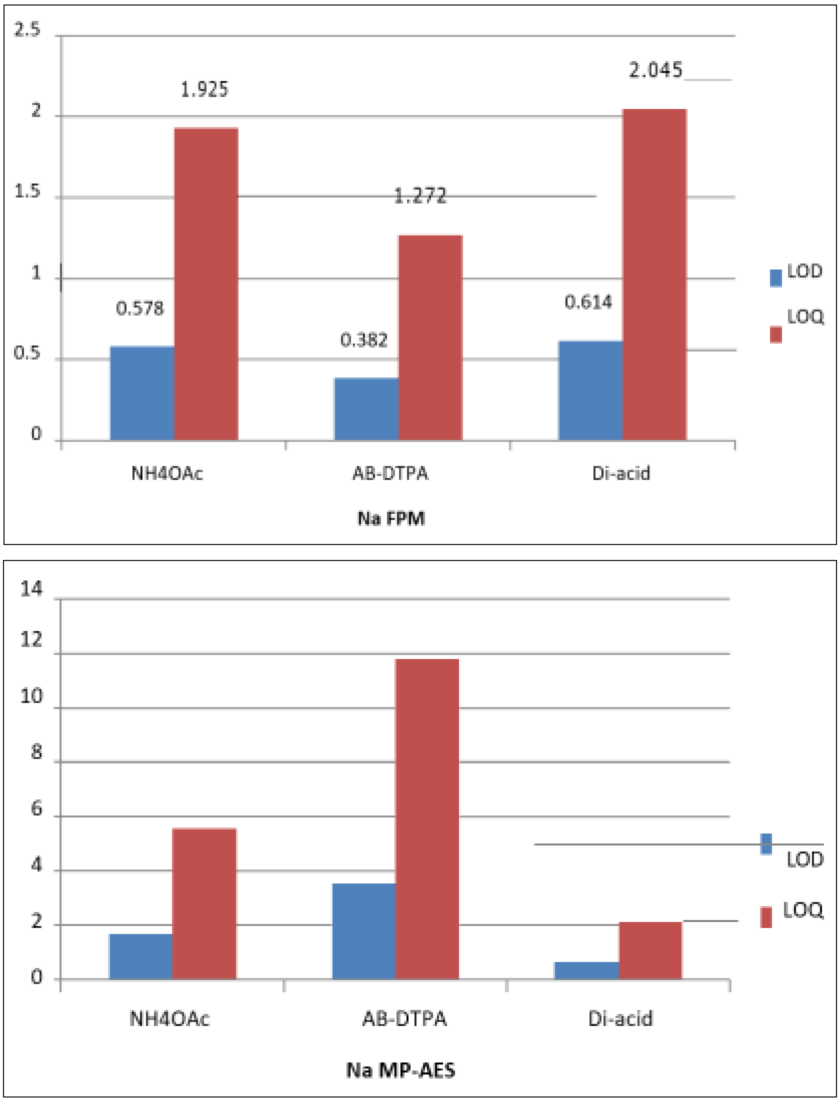


Fig 2: LOD and LOQ of FPM and MP-AES for Na with different extractant

Precision

Precision studies were carried out to ascertain the reproducibility of the proposed method. The results are presented in Table 3, as well as below given table. The RSD (%) in Na_1, Na_2, Na_3, Na_4, Na_5 and Na_6 methods of Na determination was 2.118, 10.84, 1.469, 5.753, 6.041 and 4.730, respectively. Here, the RSD (%) calculated from measured value of Na by all respective methods were within the acceptable limits (<20% for RSD). This indicates that all the methods of Na provide good precision and reproducibility. The results are akin to those reported earlier by Yang *et al.* (2014) [17].

Table 3: Precision studies were carried out to ascertain the reproducibility of the proposed method

Nutrients	Methods	Mean	RSD (%)
Sodium	NH_4OAc-FPM (Na_1)	673.8 kg/ha	2.118
	NH_4OAc-MP-AES (Na_2)	685.7 kg/ha	10.84
	AB-DTPA-FPM (Na_3)	255.8 kg/ha	1.469
	AB-DTPA-MP-AES (Na_4)	365.1 kg/ha	5.753
	Neubauer-AAS (Na_5)	0.226 %	6.041
	Neubauer-MP-AES (Na_6)	0.278 %	4.730

Amongst the above methods of Na determination value obtained by Na_2 method was higher (685.7 kg/ha) than Na determined in Na_1 (673.8 kg/ha), Na_4 (365.1 kg/ha) and Na_3 (255.8 kg/ha) methods. The lower measured value of Na in Na_3 method was mainly due to lower determination capacity of AB-DTPA that could be due to higher Ca concentration in soil. AB-DTPA cannot beusedfor the Ca rich soils. Many researchers indicated that ammonium bicarbonate after dissolution releases CO_2 (Yeh *et al*. 2005) [18] 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]. The effect of $CaCO_3$ precipitation is noticeable for acidic soils with high Ca content and for all alkaline soils. These results indicated that AB-DTPA method is a good Ca, Mg, K and Na index for both acidic and alkaline soils of low Ca concentrations (Elrashidi *et al.* 2003) [6]. AB-DTPA is not suitable for measuring Ca because of possible precipitation of $CaCO_3$during sample preparation. Similar result was obtained by Madurapperuma and Kumargamge (1995) [11], (Elrashidi *et al.* 2003) [6], Molina *et al*. (2011) [12] and Lierop and Gough (1989) [9].

Amongst the above methods of Na determination values obtained by MP-AES instrument (Na_2, Na_4 and Na_6) which had higher determination than FPM instrument. MP-AES has slowly been emerging as one of the most powerful and popular analytical tools for the analysis of different nutrients. As already described, this technique is based on the spontaneous emission of photons from atoms and ions that have been excited in magnetically excited microwave nitrogen plasma. All commonly used analytical atomic emission techniques in general suffer from various kinds of interferences but MP-AES has relatively less interferences. The nitrogen plasma is inert when compared to the chemical reactivity of various other flames used, particularly in Flame photometer. Also the high temperature of the microwave plasma helps to reduce chemical interferences. The temperature is high enough to break down most species into atoms or ions for excitation and subsequent emission. That's why MP-AES is more acceptable than FPM (Vysetti *et al*. 2014) [16].

Predictability

The result of correlation coefficient among the different methods with uptake of Na is given in Table 4. The method NH_4OAc-FPM (Na_1) is acceptable method for determination of Na. Suitability of this method was discussed many times in the literature. Therefore, uptake of nutrient content isalso considered as check method for determining the suitability of NH_4OAc-FPM, NH_4OAc-MP-AES, AB-DTPA-FPM and AB-DTPA-MP-AES methods (Fig. 3).

In method Na_3 and Na_4 multi-nutrient extractant AB-DTPA was used to extract Na from soil. This extractant is suitable for MP-AES, which can capable to determined many nutrients at a time, and reduce the determination time (Vysetti *et al*. 2014) [16]. The results of correlation determined by different methods presented in Table 4 that relationship among different extractant and uptake of wheat. Madurapperumma and Kumaragamage (2008) [10] also found that all methods showed highly significant correlation with plant content and plant uptake of Na. Significant correlations between AB-DTPA and neutral NH_4OAc methods were previously reported for acidic and alkaline upland soils Elrashidi *et al*. (2003) [6].

Table 4: Correlation between different Na extractants and uptake by wheat measured on different instruments

Extractants	NH4OAc-FPM	NH4OAcMP-AES	AB-DTPA-FPM	AB-DTPA MP-AES
Uptake- FPM (mg/100 g)	0.663**	0.653**	0.630**	0.645**
Uptake-MP-AES (mg/100 g)	0.674**	0.664**	0.642**	0.667**

* Significant at 5%; ** significant at 1%

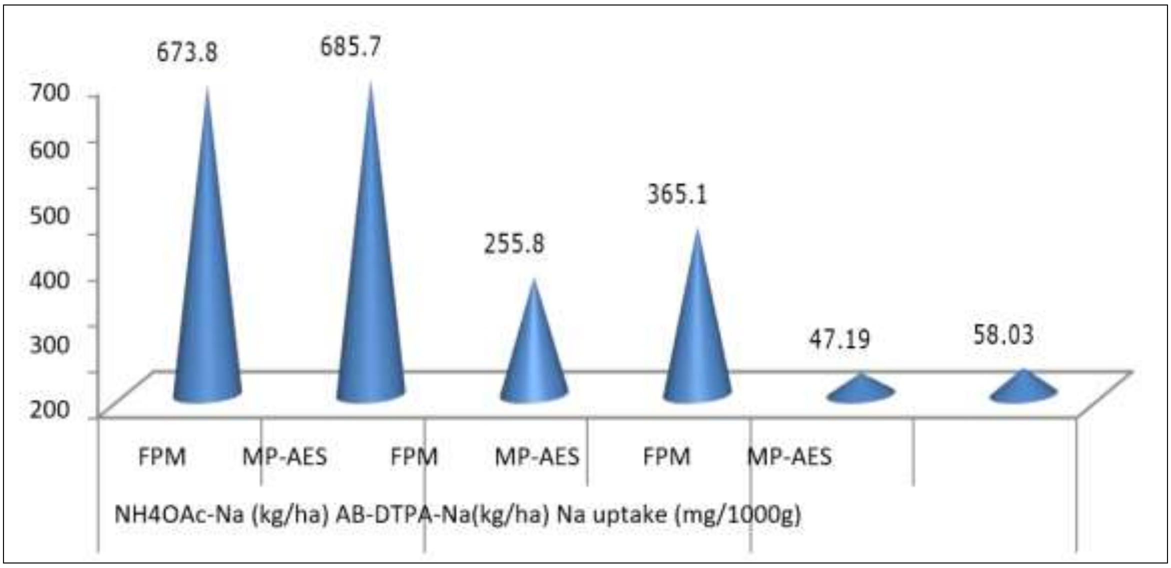


Fig 3: Uptake of Na on different instruments and extractants

Sodium availability in relation to soil properties

To establish the relation of available Na in soil with different physico- chemical properties of soil, correlation coefficient (r) was worked out and the results are presented in Table 5. The correlation coefficient showed that available Na had highly significant and positive correlation with pH (r=0.444**) and CEC (r=0.506**) because of the fact that higher content of OC in the soil leads to higher CEC resulting in higher adsorption of the cations including Na (Shankhayan *et al.*, 1996; Sharma *et. al.*, 2009) [13, 14]. Displacement of Na^+ by hydrolysis liberates hydroxide, which remains in solution, thus raising the solution pH (Busaidi and Cookson, 2003) [4]. Na has negative relation with $CaCO_3$ due to formation of sodium carbonate and bicarbonates (Busaidi and Cookson, 2003) [4].

Table 5: Correlation of Na extracted by different extractants on different instruments with physico- chemical properties of soil

Extractants	**Soil physico-chemical properties**							
	pH	**EC**	**OC (%)**	**CEC (cmol (p+) / kg)**	**$CaCO_3$ (%)**	**Sand (%)**	**Silt (%)**	**Clay (%)**
NH_4OAc-FPM	0.444**	0.515**	-0.063	0.383**	-0.129	-0.161	-0.014	0.244
NH_4OAc-MP-AES	0.416**	0.526**	-0.067	0.362*	-0.135	-0.137	-0.029	0.223
AB-DTPA-FPM	0.430**	0.512**	-0.081	0.396**	-0.129	-0.156	-0.020	0.244
AB-DTPA-MP-AES	0.368*	0.536**	-0.074	0.329*	-0.130	-0.111	-0.037	0.191
K-uptake-FPM	0.515**	0.356*	-0.220	0.262	0.104	-0.159	0.134	0.118
K-uptake-MP-AES	0.305*	0.508**	-0.031	0.242	-0.003	-0.080	0.082	0.047
Yield	0.370*	0.261	-0.124	0.204	0.157	-0.138	0.006	0.195

* Significant @ 5%; ** significant @ 1%

Conclusion

In sodium determination NH_4OAc-FPM has highly positive and significantly correlation with nutrient uptake by wheat on MP-AES instruments.

All the studied methods of sodium cations determination NH_4OAc has high predictability and the amounts measured by this extractant have significant and positive correlation with nutrient uptake by wheat on MP-AES instruments.

pH, EC, CEC and clay content of soil have positive correlation with sodium availability. While organic carbon, $CaCO_3$, sand and silt content of soil have negative correlation with available sodium.

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