Evaluation of Extractants for determination of Micronutrients in Soils of South Gujarat, India

Abstract:

An experiment was conducted at Department of Soil Science and Agricultural Chemistry, Navsari Agricultural University during the year 2018-19. The methods of micronutrients determination were (i) extracted through DTPA (ii) extracted through AB-DTPA (iii) extracted through Neubauer.The methods of micronutrients were evaluated through method validation parameters like linearity, sensitivity of instruments, precision and predictability. The available micronutrients determined from the sample soils were correlated with soil physico-chemical properties to know the relationship of these properties with available micronutrients. The linearity study of the series of micronutrients standard were measured through different methods of micronutrient determination. These measured values showed linear relationship with reference values. The coefficient of determination (R^2) for all the methods was > 0.90. The precision was determined by RSD (%) of different micronutrient methods which were in the range of 1.834 to 19.11 %. All the studied methods of copper, iron, zinc and manganese determination, AB-DTPA extractant method has highly significant and positive correlation with nutrient uptake by wheat on MP-AES instrument. Organic C and sand content of soil have positive correlation with micronutrients availability. While pH, EC, CEC, $CaCO_3$, silt and clay content of soil have negative correlation with available micronutrients.

Keywords: Chemical extractants,micronutrients,DTPA, MP-AES.

Introduction :

India has achieved self-sufficiency in food production but intensive cropping has resulted in the marked depletion of native fertility of soils. Micronutrient deficiency in Indian soils has emerged as one of the major constraints to crop productivity. Role of micronutrients in balanced plant nutrition is well established. However, exploitive nature of modern agriculture involving use of high analysis NPK fertilizers coupled with limited use of organic manures and less recycling of crop residues are important factors contributing accelerated exhaustion of micronutrients from soil. The macro and micronutrient govern the fertility of the soils and control the yields of the crops. Soil characterization in relation to evaluation of micronutrient status of the soils of an area is an important aspect in context of sustainable agriculture production. The stagnation in crop productivity cannot be boosted without judicious use of micronutrient fertilizers to overcome deficiency imbalances. Enhanced removal of micronutrients resulted in the depletion of micronutrient cations from the soil reserves (Dhane and Shukla 1995). The micronutrient deficiencies which were sparse and sporadic initially (Takkar and Randhawa, 1980) are now widespread. Analysis of 2.52 lakhs surface soil samples collected from different parts of the country revealed the predominance of zinc deficiency in divergent soils. Of these samples 49, 12, 4, 3, 33% and 41% soils are tested to be deficient in available zinc (Zn), iron (Fe), manganese (Mn), copper (Cu), boron (B) and sulphur (S), respectively (Rattan *et al.* 2009). In Madhya Pradesh, deficiency of zinc was observed in about 58% of soil samples. Deficiency of micronutrients may either be primary, due to their low total content or secondary, caused by soil factors reducing their availability to plants. The emergence of micronutrients deficiency has generally been considered as secondary. Soils with finer particles and with higher organic matter can generally provide a greater reserve of these elements whereas, coarse textured soils such as sand have fewer reserve and tend to get depleted rather quickly. The availability of micronutrient cations are influenced by several factors such as pH, $CaCO_3$, organic matter, soluble salts, cation exchange capacity and texture of soils. For an effective correction of a micronutrient deficiency in the field, it is necessary to understand the reasons of its deficiency in the soil. Inventory of the available micronutrient status of the soil helps in demarcating areas where application of particular micronutrient is needed for profitable crop production. Micronutrients are needful elements for normal growth of plants, that are needed at little amount. If these elements are not available sufficiently, plants will suffer from physiological stresses that are caused by inefficiency of several enzymatic systems and other related metabolic functions. It widely accepted that the behavior of micronutrients in soils cannot be assessed by measuring only the total metal concentration. This is because of the complexity of metal ion dynamics in the soil system and the interactive role of plant and environmental factors on the whole process. The deficiencies of micronutrients have become major restrictions to productivity, stability and sustainability of soils (Bibiso *et al.* 2015). Universal soil extractant is the term that has been adopted to designate a reagent that can be used to extract more than one class of elements and /or ions from a soil with the concentration found being a means for assessing the soil's fertility status or levels of toxicity. The advantages of universal extractants in soil testing methods include increasing the reliability of soil test, increasing accuracy and precision of the tests and it saves time and increase the efficiency of the methods in routine soil analysis. Extractants include chelating agents such as Di-ethyl tri-amine penta-acetic acid (DTPA) and Ammonium bicarbonate Di-ethyl tri-amine penta-acetic acid (AB-DTPA) are used for determination of micronutrients from soils. Considering the above facts in view, the present investigation has been planned to study the "Evaluation of extractants for determination of micronutrients in soils of south Gujarat" to find out suitable extractant for the determination of micronutrients from soil and to establish the relationship of various soil properties with micronutrients 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 either acidic, neutral or alkaline in nature and the pH ranged from5.44to7.73.Electricalconductivityofsampleswasrangedfrom0.01to1.2 dS/m (1:2.5 soil: water ratio). Soil salinity in south Gujarat varies from slight to strong salinity class. In Narmada, Tapi and 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 micronutrient availability indices were measured according to various used different extractants DTPA and AB-DTPA.

List 1: Plant (Neubauer)study

Extract no.	Extractants	Soil-solution ratio	Equilibration time (min.)
1	DTPA	01:02	2 hrs
2	AB-DTPA	01:02	15 min at 180 rpm

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 micronutrients on AAS and MP-AES (Neubauer and Schneider, 1923).

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).
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,

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

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. Therespective sample is analyzed on different instruments by repeating the sample is 5 times each. The formula for calculating RSD is givenbelow.

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

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, DTPA-AAS for micronutrients is considered as standard method of soil analysis and adopted in many analytical laboratories. Therefore, this method is considered as a check. MP-AES is the sophisticated and newer technique of soil analysis. This technique is compared with check and discussed in this chapter.

Linearity

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well defined mathematical transformation, proportional to the concentration of analyze in samples within a given range. Therefore, here it is considered as parameter of accuracy. Because of accuracy is the measure of exactness of an analytical method or the closeness of agreement between the measured value and the reference value. Linearity is one of the parameter to test the accuracy of the any method.

The result of linearity study in micronutrients is depicted in **Table 1.0**. The results indicated that standard curve prepared among measured and reference value in Cu, Fe, Zn and Mn were linear and all the methods recorded coefficient of determination (R_2) >0.95. 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 AAS (Vysetti *et al.* 2014).

Table 1. The result of linearity study in micronutrients

Nutrients	**Methods**	**Linearity range (ppm)**	R^2
Copper	DTPA-AAS (Cu_1)	0.4 to 2.0	0.994
	DTPA-MP-AES (Cu_2)	0.5 to 3.5	0.980
	AB-DTPA-AAS (Cu_3)	0.4 to 2.0	0.985
	AB-DTPA-MP-AES (Cu_4)	0.5 to 3.5	0.989
	Neubauer-AAS (Cu_5)	0.4 to 2.0	0.985
	Neubauer-MP-AES (Cu_6)	0.5 to 3.5	0.989
Iron	DTPA-AAS (Fe_1)	1.0 to 5.0	0.985
	DTPA-MP-AES (Fe_2)	0.5 to 3.5	0.997
	AB-DTPA-AAS (Fe_3)	1.0 to 5.0	0.995
	AB-DTPA-MP-AES (Fe_4)	0.5 to 3.5	0.923
	Neubauer-AAS (Fe_5)	1.0 to 5.0	0.988
	Neubauer-MP-AES (Fe_6)	0.5 to 3.5	0.923
Zinc	DTPA-AAS (Zn_1)	0.3 to 1.5	0.989
	DTPA-MP-AES (Zn_2)	0.5 to 3.5	0.990
	AB-DTPA-AAS (Zn_3)	0.3 to 1.5	0.979
	AB-DTPA-MP-AES (Zn_4)	0.5 to 3.5	0.900
	Neubauer-AAS (Zn_5)	0.3 to 1.5	0.979
	Neubauer-MP-AES (Zn_6)	0.5 to 3.5	0.961
Manganese	DTPA-AAS (Mn_1)	0.5 to 4.0	0.959
	DTPA-MP-AES (Mn_2)	0.5 to 3.5	0.998
	AB-DTPA-AAS (Mn_3)	0.5 to 4.0	0.996
	AB-DTPA-MP-AES (Mn_4)	0.5 to 3.5	0.973
	Neubauer-AAS (Mn_5)	0.5 to 2.0	0.996
	Neubauer-MP-AES (Mn_6)	0.5 to 3.5	0.973

Precision

Precision studies were carried out to ascertain the reproducibility of the proposed method. The % RSD in methods of micronutrients determination, the results are presented in below given **Table 2.**. Here, the % RSD calculated from measured value of Cu, Fe, Zn and Mn by all respective methods were within the acceptable limits (<20% for RSD). This indicates that all methods provide good precision and reproducibility. The results are akin to those reported earlier by Sante (2017) and Yang *et al.* (2014).

Amongst the above methods for micronutrients determination value obtained by AB-DTPA-MP-AES method was higher than in AB-DTPA-AAS, DTPA-MP-AES and DTPA-AAS methods. The higher measured value of Cu, Fe, Zn and Mn in AB-DTPA-MP-AES method was mainly due to higher determination capacity of AB-DTPA. Higher determination capacity of AB-DTPA could be due to higher Ca concentration in soil. 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) that combines with water to form carbonic acid (Brucato *et al.* 1997). The carbonic acid dissolves appreciable amounts of calcium carbonate (Al-Hosny and Grassian, 2004). Thus, solubilizing the unavailable micronutrients sorbed on $CaCO_3$ surfaces resulting in higher estimation of micro nutrient as compared to DTPA extractant (Karimian *et al.* 1999). Similar result was obtained by Anusree *et al.* (2018) and Khan *et al.* (2007).

Table 2.: Available micronutrients extracted by different extractants from soils on different instruments

Nutrients	Methods	Mean	RSD (%)
Copper	DTPA-AAS (Cu_1)	573.3 kg/ha	1.178
	DTPA-MP-AES (Cu_2)	626.9 kg/ha	5.920
	AB-DTPA-AAS (Cu_3)	462.4 kg/ha	2.188
	AB-DTPA-MP-AES (Cu_4)	507.4 kg/ha	7.268
	Neubauer-AAS (Cu_5)	1.304 %	1.779
	Neubauer-MP-AES (Cu_6)	1.466 %	2.563
Iron	DTPA-AAS (Fe_1)	6.977 ppm	2.200
	DTPA-MP-AES (Fe_2)	11.964 ppm	16.85
	AB-DTPA-AAS (Fe_3)	9.286 ppm	11.18
	AB-DTPA-MP-AES (Fe_4)	20.354 ppm	8.798
	Neubauer-AAS (Fe_5)	0.203 %	3.711
	Neubauer-MP-AES (Fe_6)	0.323 %	7.918
Zinc	DTPA-AAS (Zn_1)	1.445 ppm	3.017
	DTPA-MP-AES (Zn_2)	2.019 ppm	19.21
	AB-DTPA-AAS (Zn_3)	1.836 ppm	7.883
	AB-DTPA-MP-AES (Zn_4)	2.605 ppm	12.23
	Neubauer-AAS (Zn_5)	0.008 %	4.730
	Neubauer-MP-AES (Zn_6)	0.012 %	12.234
Manganese	DTPA-AAS (Mn_1)	15.85 ppm	3.311
	DTPA-MP-AES (Mn_2)	28.29 ppm	18.90
	AB-DTPA-AAS (Mn_3)	22.23 ppm	12.69
	AB-DTPA-MP-AES (Mn_4)	32.97 ppm	5.768
	Neubauer-AAS (Mn_5)	0.009 %	10.15
	Neubauer-MP-AES (Mn_6)	0.013 %	4.615

Predictability

Micronutrients

The result of correlation coefficient among the different methods with uptake of micronutrients are given in **Table 3**in preceding chapter. The method DTPAAAS is acceptable method for determination of Cu, Fe, Zn and Mn. Suitability of this method was discussed many times in the literature. Therefore, uptake of nutrient content is also considered as check method for determining the suitability of DTPA-AAS, DTPA-MP-AES, AB-DTPA-AAS and AB-DTPAMP-AES methods. In method AB-DTPA-AAS and AB-DTPA-MP-AES multi-nutrient extractant AB-DTPA was used to extract these micronutrients 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). However, for better predictability, any method should have good correlation between measured value with yield and uptake. The results of correlation determined by different methods presented in **Table 3**that shows relationship among different extractant and uptake of wheat. Madurapperumma and Kumaragamage (2008) also found that Cu, Fe, Zn and Mn measured by AB-DTPA-MP-AES was significantly and positively correlated with Cu, Fe, Zn and Mn measured by DTPA, Cu, Fe, Zn and Mn uptake by rice and rice yield.

Table 3: Correlation between different micronutrients extractants and uptake by wheat measured on different instruments

Extractants		DTPA -AAS	DTPA MP-AES	AB-DTPA -AAS	AB-DTPA MP-AES
Copper	Uptake- AAS (mg/100g)	0.310*	0.330*	0.288*	0.372**
	Uptake-MP-AES (mg/100g)	0.311*	0.319*	0.294*	0.384**
Iron	Uptake- AAS (mg/100g)	0.313*	0.290*	0.317*	0.378**
	Uptake-MP-AES (mg/100g)	0.306*	0.305*	0.312*	0.441**
Zinc	Uptake- AAS (mg/100g)	0.303*	0.309*	0.366*	0.387**
	Uptake-MP-AES (mg/100g)	0.289*	0.290*	0.389**	0.391**
Manganese	Uptake- AAS (mg/100g)	0.342*	0.286*	0.345*	0.379**
	Uptake-MP-AES (mg/100g)	0.377**	0.297*	0.399**	0.409**

*** Significant at 5%; ** significant at1%**

Micronutrients availability in relation to soil properties

To establish the relation of available Cu, Fe, Zn and Mn in soil with different physicochemical properties of soil, correlation coefficient (r) was worked out and the results are presented in **Table 4**. The correlation coefficient showed that available these micronutrients had significant and positive correlation with sand content in soil (r=0.361*) and positive correlation with organic C, while highly significant and negative correlation with pH (r=0.408**). This can be explained by considering that organic matter increases the availability of micronutrients by forming soluble complexing agents which decreases the formation of insoluble micronutrients complexes. Cu, Fe, Zn and Mn have negative correlation with EC and $CaCO_3$, silt and clay content of soil. This relationship can be explained by considering the fact that at higher pH, copper precipitates as copper hydroxide $Cu(OH)_2$, which is not readily available to the plants. However, at higher $CaCO_3$ content copper is precipitates into $CuCO_3$ the will reduce the copper availability for the plants. In fact that most readily available form of iron is Fe^{2+} ions, which convert into less soluble form (Fe^{3+}ions) after oxidation. High pH is responsible for its oxidation. However, at higher $CaCO_3$ content iron is precipitates into Fe_2CO_3 the will reduce the iron availability for the plants.In case of Zinc that the formation of insoluble zinc hydroxide $Zn(OH)_2$ which is not soluble in soil solution, hence not available for the take up by plants. High pH is responsible for its oxidation. However, at higher $CaCO_3$ content zinc is precipitates into $ZnCO_3$ that will reduce the Zn availability for the plants. Manganese convert in to insoluble manganese hydroxide $Mn(OH)_2$ which is not soluble in soil solution, hence not available for the take up by plants. High pH is responsible for its oxidation. However, at higher $CaCO_3$ content manganese is precipitates into $MnCO_3$ that will reduce the Mn availability for the plants. The similar results were also reported earlier by Mandal *et al.* (2019), Meena and Mathur (2017), Tisdale *et al.* (1997) and Meena *et al.* (2006).

Table 4: Correlation of K 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 (%)**
Copper								
DTPA-AAS	-0.408**	-0.158	0.161	-0.308*	-0.051	0.361*	-0.352*	-0.232
DTPA-MP-AES	-0.515	-0.141	0.084	-0.138	0.071	0.201	-0.227	-0.103
AB-DTPA-AAS	-0.575**	-0.037	0.253	-0.457*	-0.024	0.509**	-0.415**	-0.394**
AB-DTPA-MP-AES	-0.512**	0.017	0.244	-0.277	-0.073	0.521**	-0.445**	-0.385**
Cu-uptake-AAS	0.158	-0.133	-0.137	0.066	0.241	-0.097	-0.085	0.211
Cu-uptake-MP-AES	0.120	-0.104	-0.089	0.046	0.230	-0.086	-0.087	0.198
Iron								
DTPA-AAS	-0.599**	-0.345*	0.362*	-0.472**	0.171	0.349*	-0.211	-0.332**
DTPA-MP-AES	-0.608**	-0.339*	0.373*	-0.495**	0.136	0.394*	-0.238	-0.374**
AB-DTPA-AAS	-0.586**	-0.330*	0.362*	-0.433**	0.159	0.319*	-0.190	-0.306**
AB-DTPA-MP-AES	-0.550**	-0.338*	0.385*	-0.430**	0.149	0.279*	-0.177	-0.259*
Fe-uptake-AAS	-0.006	-0.005	0.082	0.101	0.200	0.079	-0.140	0.002
Fe-uptake-MP-AES	-0.101	-0.089	0.067	-0.185	0.200	0.141	-0.168	-0.064
Zinc								
DTPA-AAS	-0.402**	0.232	0.393**	-0.136	-0.191	0.298*	-0.267	-0.211
DTPA-MP-AES	-0.396**	0.229	0.390**	-0.132	-0.190	0.297*	-0.267	-0.209
AB-DTPA-AAS	-0.450**	0.162	0.377**	-0.229	-0.132	0.328*	-0.281	-0.242
AB-DTPA-MP-AES	-0.342*	0.301*	0.305**	-0.101	-0.231	0.299	-0.278	-0.202
Zn-uptake-AAS	0.184	0.065	-0.131	0.184	-0.193	0.136	-0.236	0.001
Zn-uptake-MP-AES	0.199	0.008	-0.219	0.156	-0.037	0.147	-0.259	0.006
Manganese								
DTPA-AAS	-0.510**	0.083	0.362**	-0.221	-0.194	0.407**	-0.411**	-0.251
DTPA-MP-AES	-0.577**	0.113	0.392**	-0.207	-0.275	0.465**	-0.457**	-0.296
AB-DTPA-AAS	-0.274	0.199	-0.011	-0.125	-0.121	0.341**	-0.344**	-0.207
AB-DTPA-MP-AES	-0.352**	0.187	0.051	-0.187	-0.146	0.419**	-0.404**	-0.271
Mn-uptake-AAS	-0.208	-0.112	0.024	-0.270	0.139	0.291	-0.278	-0.190
Mn-uptake-MP-AES	-0.166	-0.031	0.064	-0.219	0.168	0.225	-0.234	-0.133
Yield	0.009	-0.011	0.055	-0.010	0.184	-0.055	-0.083	0.148

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

Conclusion:

Based on the results of present study following conclusions may be drawn. All the studied methods of copper, iron, zinc and manganese determination, AB-DTPA-MP-AES method has highly significant. Organic C and sand content of soil have positive correlation with¬ micronutrients availability. While pH, EC, CEC, CaCO$_3$, silt and clay content of soil have negative correlation with available micronutrients.

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