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Recent Developments in Multi-nutrient Extractants Used in Soil Analysis

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A B S T R A C T

Universal extractant is a term used to designate reagents or procedures to extract several elements or ions to assess soil fertility status or levels of toxicity. The extraction procedure should be rapid, reproducible, inexpensive, adaptable to soils from different regions, and extract the labile forms of nutrients which might be potentially available to plants. Most of the extractants in use are fall short of these requirements. Modified M3 method for simultaneous extraction of macro and micro nutrients in arable land soils and it was found to be greatly correlated with the existing methods for NO3-N, available P, Zn, Cu and B, exchangeable K, Ca and Mg and easily reducible Mn. A new, easily applicable soil extraction method has been developed using the coffee percolator principle and the results are in close correlation with those of conventional soil testing methods and with the nutrient uptake of the sunflower and ryegrass used as test crops. Several techniques using cation or anion exchange resins which allow the simultaneous extraction of Ca, Mg, K, Al, Mn and P from soil were assessed and all the resin procedures have the potential to reduce the time required for analysis of Ca, Mg, K, Mn and P in soil. Despite of these developments, today the challenge is to select an appropriate extractant that take full advantage of multi-element analysers such as the ICP, suitable for a range of soil characteristics, such as pH, texture, organic matter content, and having an established significant relationship between elemental level and crop response. Therefore evaluation of various extraction methods for use under particular soil conditions, in various regions or within specific cropping situations is still needed.

Introduction

From 1960s to 2016, from subsistence farming to sustainable farming, from deficiency in food grain production to sufficiency in food grain production, there has been a continuous increase in fertilizer use in India and side by side there is an increase in different nutrient deficiency occurring in soil. In Marusthali 12% soils are deficient in NPKSZn, similarly 18%, 35%, 36% soils of Chhotnagpur plateau, Bengal basin and Rajasthan Bagar areas are deficient in NPKSBZn, NKB and NK respectively. So in order to get this multinutrient deficiency or sufficiency status of the soil rapidly and accurately, a multinutrient extractant is necessary. There has been continuous

research and development going on in this aspect for years but till date no perfect multinutrient extractant has been developed although many researchers have found remarkable results. Universal extractant is a term used to designate reagents or procedures to extract several elements or ions to assess soil fertility status or levels of toxicity. Universal soil extractants ideally should be: (i) adequate for the simultaneous determination of all plant nutrients; (ii) rapid; (iii) reproducible; (iv) inexpensive; (v) adaptable to all types of soils; (vi) and, overall, the best possible alternative for the evaluation of the plant available amount of the nutrient. The advantage of extracting several elements with a single solution has always been attractive for the routine laboratory work, moreover using modern equipment which allows simultaneous determination of several elements, as the inductively coupled argon plasma emission spectrometer (Raij, 1994; Jones, 1998).

Materials and Methods

Morgan's reagent

The first universal soil extracting reagent was developed by Morgan, a 0.73M sodium acetate ($NaC_2H_3O_2$) solution buffered at pH 4.8. The shaking time was 15 minutes and Soil: solution is 1:4. In 1941, the Morgan extractant procedure was described in the Connecticut Experiment Station Bulletin 450 which was followed by Bulletin 541 in 1950. These bulletins described in some detail the analysis procedures and interpretative data for the determination of 15 elements and ions. The Morgan extraction reagent was widely used in the 1950s and early 1960s, but it is in little use today. The parameters determined by this reagent are P, K, Ca, Mg, Cu, Fe, Mn, Zn, NO_3, NH_4, SO_4, Al, As, Hg, Pb. The pH of 4.8 was chosen to simulate the carbon dioxide saturated solution adjacent to the root hairs.

This pH would act as a mild solvent for iron and aluminium phosphates as well as other minerals that might release ions important in plant nutrition. The sodium acetate would be effective in replacing important soil cations and anions into the extract solution so that they could be readily measured.

The use of sodium acetate and acetic acid permitted the determination of all important soil nutrients with exception of sodium in a single extract (Morgan, 1941).

Wolf reagent (Modified Morgan's reagent)

Wolf modified Morgan's reagent in 1982 (Wolf, 1982). The Morgan-Wolf Extraction Reagent is for use with acid to neutral pH soils irrespective of texture as the soil aliquot measurement is by volume. The extraction reagent is a mixture of 0.073M sodium acetate ($NaC_2H_3O_2$), 0.52N acetic acid (CH_3COOH) and 0.001M diethylenetriarnine pentaacetic acid (DTPA) buffered at pH 4.8. The extraction reagent is best suited for the assay of well fertilized soils and most effective for monitoring their fertility level.

Mehlich no. 1 (M 1) reagent

In 1954, the Mehlich No. 1 (frequently referred to in the past as either the North Carolina or Double Acid) extraction reagent was introduced, an extractant that is still in wide use today for the determination of P, K, Ca, Mg, Na, Mn and Zn in acid sandy soils primarily from the eastern and south-eastern coastal areas of the United States.

The extraction reagent is a mixture of 0.05N HC1 in 0.025N H_2SO_4. The Soil:solution is 1:4 and Shaking time 5 min. The acid has been used here to extract elements from acid soluble fraction as well as it extracts from water soluble and exchangeable fraction from soil.

Mehlich no. 2 (M 2) reagent

Mehlich modified M 1 reagent in 1978 to allow simultaneous extraction of several plant nutrients over a wide range of soil properties. The new extractant is composed of 0.2N NH_4Cl-O.2N HOAc-0.015N NH_4F-0.012N HCl at approximately pH 2.5. The double acid (DA) extractant (0.05N HC1-0.025N H_2SO_4) meets many of the requirements of a mass analyses method for P, K, Ca, Mg, Na, Mn and Zn. However, DA is not recommended for calcareous soils or on acid soils containing recently applied rock phosphate. Under these conditions, DA extracts P in considerable excess of that obtained with Bray 1 and Olsen. In acid soils in the absence of phosphate rock improved extraction efficiency and correlation with Bray 1 were obtained by increasing the soil: extractant ratio of the DA method from 1:5 to 1:10 and by adding HF or NH4F to the reagent. With calcareous soils Smith, Ellis and Grava (1957) found that Bray 1 gave a high correlation between percentage yield of wheat and extractable P at a 1:50 soil: extractant ratio. Randall and Grava (1971) also obtained a significant decreasing curvilinear relationship between quantities of calcitic carbonates of calcareous soils and Bray 1 extractable P at 1:10, 1:50 and 1:100 soil: solution ratios. The depressing effect of $CaCO_3$, was considered due to neutralization of 0.025N HC1 and the deactivation of F ion in 0.03N NH_4F by the formation of insoluble calcium fluoride. Smillie and Syers subsequently confirmed formation of CaF_2 during a 1 minute extraction of calcite with Bray 1 and simultaneous sorption of added P. Recent observations by Mehlich showed that precipitation of CaF_2 was not restricted to calcareous soils, but may occur in neutral and acid soils. It was also shown that the advantages of the fluoride ion, when added to 0.025N HC1 to control selective extractability of P, did not apply simultaneously to Ca unless the pH of the extractant was held below about pH 2.9. To achieve this objective in calcareous soils either a wide soil: extractant ratio or a considerable higher buffer capacity is required than is inherent in Bray 1. An extractant having the composition 0.2N NH_4C1-0.2N HOAc-0.015N NH_4F-0.012N HC1 at approximately pH 2.5, was reported by Mehlich to offer the desirable buffer properties for the simultaneous extraction of P and Ca from rock phosphate and soils (Mehlich, 1978, 1984).

Mehlich no. 3 (M 3) reagent

Mehlich 2 reagent was modified to include Cu among the extractable nutrients, retain or enhance the wide range of soils for which it is suitable and minimize its corrosive properties. The substitution of nitrate for chloride anions and the addition of EDTA accomplished those objectives. The new extracting solution, already designated Mehlich 3 (M3) is composed of 0.2N CH_3COOH - 0.25N NH_4NO_3 - 0.015N NH_4F - 0.013N HNO_3 - 0.001M EDTA pH buffered at 2.5 ± 0.1.

Mylavarupu and co-workers in 2002 have been found that mean extracted concentration of K, Mg, and Zn was not significantly different between M-1 and M-3 procedures for all the samples (LSD, p=0:05). The range, standard deviation and interquartile dispersion of concentrations were also found to be very similar for K, Mg, and Zn for both the procedures. The extractable mean concentrations of M-3-P, M-3-Cu, M-3-Mn, and M-3-B were significantly higher compared to the corresponding M-1 extractable amounts. M-1-Ca was however found to be significantly higher than M-3-Ca. Since the M-3 solution contained dilute acids and EDTA, M-3 procedure was expected to extract larger amounts of micronutrients. The higher amounts of Mn, Zn, and Cu extracted by M3 could be attributed to the addition of

EDTA that resulted in solubilizing oxidized and organic forms of those nutrients. Mehlich found that addition of EDTA to the M-3 solution increased the Cu, Mn, and Zn by 170%, 50%, and 25% compared to the extracting solution without EDTA addition.

However, there was a statistical difference in the slope of the regression line between the soils having pH 4.30–7.30 and $CaCO_3$ 0–0.9 percent and the two other groups of soils (pH 7.01–8.17 and $CaCO_3$ 1–9.5 percent; pH 7.20–8.28 and $CaCO_3$ 10–48.3 percent). However, Mehlich 3 has proven to be an efficient and versatile extractant for soils containing calcium carbonates, due to the combination of acetic and nitric acids used in this solution that have a dissolving action against $CaCO_3$ (Sawyer and Mallarino, 1999).

Wang *et al.,* in 2004 found that the P ratios of the two methods are around 1 for most soils that have pH<6.5. According to Smillie and Syers (1972), acidity less than 0.1M HCl in Bray extractant would be insufficient to prevent P immobilization by CaF_2 formed during NH_4F-HCl extraction of calcareous soils. Mehlich also pointed out that the strength of extractant acidity, not fluoride, has a greater effect on P extraction in calcareous soils. These results further confirm the difference between these two extractants in P extraction in acid or calcareous soil environments. The results suggest that different conversion equations may need to be established based on soil pH. On the other hand, Bray 2 with stronger acidity appears to be able to overcome the problem associated with P extractability loss by Mehlich series and Bray 1 extractants (Wang *et al.,* 2004).

Modified Mehlich no. 3 reagent

Yanai *et al.,*in 2000 modified Mehlich 3 extractant for simultaneous extraction of macro- and micro-nutrients in arable land soil.

Composition and concentration of the new multinutrient extractant were 0.2 M CH_3COOH, 0.25 M NH_4Cl, 0.005 M C6H8O7 (citric acid), 0.05 M HCl and pH is to be maintained at 1.3. The extraction method consisted of continuous shaking for 30 min with a ratio of air-dried soil to extractant 1: 10 (w/v). Advantages of the new extractant over M-3 extractant are NO_3-N cannot be evaluated with the extractant, 0.005 M citric acid used to omit the F ions in the extractant because fluoride ions in the Mehlich 3 extractant may dissolve K from the glass bottles and EDTA in Mehlich 3 precipitates after prolonged storage so pH is decreased by HCl in the new extractant.

AB-DTPA method

A new soil test was developed for simultaneous extraction of NO_3, P, K, Zn, Fe, Cu and Mn from alkaline soils. The new extraction solution is 1 M in ammonium bicarbonate (NH_4HCO_3), 0.005 M in Diethylene Triamine Pentaacetic Acid (DTPA) and has a pH of 7.6 (Soltanpour and Schwab, 1977). The simple correlation coefficients, it is obvious that there exists a high degree of correlation between the new method and the standard methods of extraction. Since a considerable amount of calibration work for different soils and climatic conditions are available for standard methods of soil testing, the regression equations between the new and the standard methods will allow one to calculate index values of nutrients in soils for the new soil test. This was done for P, K, Zn, Fe, Cu and Mn index values used for fertilizer recommendations by the Colorado State University Soil Testing Laboratory. Trehan and Grewal (1985) experimented on different soils across various climatic regions in India to find out the suitability of AB-DTPA in Indian soils and found that for alluvial soils highest correlation was found for P and

lowest for Mg. Similarly for hilly soils highest correlation was found for Ca and lowest for Cu respectively. Pradhan *et al.,* (2015) found out the relationship of extractable Cu and Zn with important physico-chemical properties of soil. The amount of Cu extracted by the 4 extractants showed a significant positive correlation with organic C. This indicated that the extractable Cu content of the soils would increase with increasing organic C. DTPA-Cu showed a significant negative correlation with soil pH while, Mehlich 3-Cu had a significantly positive correlation with pH.

Hot Water Percolation (HWP) method

A new, easily applicable soil extraction method i.e. the hot water percolation method (HWP) has been developed using the coffee percolator principle. During hot water percolation the available, desorbable, easily soluble elements are extracted by hot water (102-105°C) at 120-150kPa pressure. Nearly every nutrient is extracted by this method in measurable quantities, and the macro-elements in appreciable quantities. The variation coefficient (CV %) of the method is in average 11%. The results are in close correlation with those of conventional soil testing methods and with the nutrient uptake of the sunflower and ryegrass used as test plants (Fuleky and Czinkota, 1993).

In addition, there is a close correlation with the K uptake of ryegrass plants, and there are also correlations with the K, Ca and Cu uptake of sunflower. There is no correlation between the Fe and Zn values measured by the HWP method and the Fe and Zn uptake of sunflower plants (Fuleky and Czinkota, 1993).

Resin extraction method

McLaughlin *et al.,* in 1994 found out the relationships between elements extracted using resin-bead & resin membrane method and conventional method which shows except for Al, there was generally good agreement between concentrations of elements extracted by resin methods and conventional methods of soil analysis. The resin procedures extracted much less Al than the method using 1 M KCl. The relationship between resin-extractable P and Olsen-P (R^2 = 0.66 for unconfined resin beads and 0.76 for resin membranes) was weaker than relationships between Ca, Mg, K and Mn extracted by resin and conventional procedures.

Results and Discussion

H3A-1 Method

Haney *et al.,* in 2006 developed a new soil extractant (H3A) with the ability to extract NH_4, NO_3, and P from soil was developed and tested against 32 soils, which varied greatly in clay content, organic carbon (C) and soil pH. The extractant (H3A) eliminates the need for separate phosphorus (P) extractants for acid and calcareous soils and maintains the extract pH, on average, within one unit of the soil pH. The extractant is composed of organic root exudates, lithium citrate, and two synthetic chelators (DTPA, EDTA).

The composition and concentration of the extractant is Lithium citrate (0.02M), Citric acid (0.0024M), Malic acid (0.004M), Oxalic acid (0.004M), 0.002 M EDTA, 0.001M DTPA, pH is to be maintained around 5.0, Soil : solution =1:10, 30 minutes shaking, 8 minutes centifugation 3000 rpm. P, NO_3-N, NH4-N can be determined by this single extractant.

Advantages of this extractant is that this would extract the nutrients near soil pH ± 1 unit, Lithium would act somewhat like K for replacing NH_4 from exchange sites, Organic acids made the extractant more flexible for use across a wider range of soil pH.

H3A-2 method

Haney *et al.,* in 2010 modified H3A-1 to reduce the extractable iron and aluminum and improve the nutrient extracting relationships with other well-known soil extractants. Correlations show improved relationships with NO_3, NH_4, PO_4, P, potassium, calcium, and zinc when compared to the original H3A-1 as well as standard soil-test methods [Olsen, potassium chloride (KCl), water, Mehlich 3, Bray 1, ammonium acetate (NH_4OAc), and diethylene triaminepentaacetic acid (DTPA)]. The Composition and concentration of the multi-nutrient extractant is 2 g/L lithium citrate (0.02M), 0.6 g/L citric acid (0.0024 M), 0.4 g/L malic acid (0.004M), 0.4 g/L oxalic acid (0.004M), pH = 4.4, Soil : solution = 1:10, Shaking time = 5 minutes and Centrifugation = 5 minutes. The modified extractant (H3A-2) averaged 12% more inorganic N (NH_4 and NO_3) than the original (H3A-1) based on the 60 NAPT samples.

In conclusion Morgan wolf, Mehlich 3 and modified mehlich 3 methods can be utilized to assess nutrient status in acid soils.AB-DTPA method is the only available method for assessing nutrient status in alkaline soils. Texture and $CaCO_3$ content should be considered while using Mehlich 3 as a multinutrientextractant. H3A-2 method may be used as a multinutrient extractant to simultaneously determine inorganic N, P, K, Ca, Zn. AB-DTPA and Mehlich-3 extractant can be more useful by use of instruments like ICP-MS/OES. Field calibration data for different extraction methods are still lacking so methods should be calibrated in specific field and crop conditions (Mehlich, 1953).

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