

Journal of Soil Science and Environmental Management

Full Length Research Paper

Applicability of the Mehlich-3 method for the sitespecific soil nutrient management in Northern Mozambique

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Received 19 June, 2018; Accepted 15 August, 2018

Mehlich 3 (M-3) is a multi-nutrient extractant which provides a rapid and cost-effective soil test for various soils. This study investigated the applicability of M-3 for cation and micronutrient determination under the specific soil fertilization and crop rotation given in northern Mozambique. Cations and micronutrients extracted with M-3 were compared with the classical methods; neutral ammonium acetate (NH₄OAc) for calcium (Ca), potassium (K), magnesium (Mg), and sodium (Na); diethylene triamine penta-acetic acid (DTPA) for copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn); and Bray I for phosphorus (P). Results indicated that M-3 was useful for Ca, K, Mg, P and Zn determination. Various types of soil fertilization or crop rotations did not alter the accuracy and precision of cation determination using M-3 and NH₄OAc. Addition of organic and chemical fertilizers was thought to adversely affect the quantity of M-3 and DTPA extractable micronutrients and their correlations. However, levels of M-3 micronutrients in soils were well above those considered to be critical levels as same as that of DTPA. This suggested that M-3 was an acceptable procedure for determining cations and micronutrients in the routine analysis and could be advantageous for soil nutrient management in the specific conditions given.

Key words: Bray I, crop rotation, diethylene triamine penta-acetic acid (DTPA), Mehlich 3, Mozambique, Nacala, neutral ammonium acetate (NH₄OAc)

INTRODUCTION

Soil analysis is important to recognize the status of soil fertility in crop production system. A proper soil testing method would also facilitate the precise use of fertilizer and economize on fertilizer management in the world.

The M-3 can be considered as one of the precise and high throughput procedure, which was widely acknowledged as an effective multi-nutrient extracting procedure for determining soil cations (Mehlich, 1984; Hanlon and Johnson, 1984; Eckert and Watson, 1996; Mamo et al., 1996; Wang et al., 2004; Paz-Ferreiro et al., 2012; Carter and Gregorich 2014) and micronutrients (Mehlich, 1984; Lindsay and Norvell, 1978; García et al., 1997).

Previously, authors made a success of using the M-3 to determine exchangeable potassium (K), calcium (Ca), magnesium (Mg) and phosphorus (P) in a broad range of soils along the Nacala corridor, northern Mozambique (Fukuda et al., 2017). So far, M-3 has proved to be useful as high throughput procedure for soil diagnosis in regional scale, in determining soil major elements in northern region.

However, the studies have not yet evaluated the applicability of M-3 on the soil fertility status particularly cations and micronutrients in a specific cropping system in Mozambique. Although high throughput soil diagnosis contributes to decision on farmer's management, more accurate and precise analysis would be required for scientific activity to identify very small difference among several experimental treatments. And in scientific activity, the determination needs to be conducted not only for major element, but also for minor element such as micronutrients. Furthermore, there is the necessity of acquiring the site-specific level of soil information in order to make better decisions on soil nutrient management for ensuring food security and to improve soil database (Maria and Yost, 2006).

Therefore, this study aimed to evaluate the applicability of M-3 for determination of major elements and micronutrients, for scientific purpose focusing on application effect of several fertilizers under the sitespecific conditions.

MATERIALS AND METHODS

Characteristics of study sites

The experiments were conducted at the Instituto de Investigação Agrária de Moçambique (IIAM) in Nampula (S15° 09' and E39° 18') in two consecutive rainy seasons in 2012/2013 and 2013/2014. Nampula province is located on a plateau with an altitude of 200-500 m above sea level and is part of the Nacala corridor, northern Mozambique. The climate is tropical, with a rainy season from December to May and a dry season from June to November. Average precipitations in the dry and rainy seasons are 106 and 967 mm, and mean temperatures are 24.1 and 25.5°C, respectively (Tsujimoto et al., 2011). The soil was classified as Oxisols; predicted USDA soil taxonomy class (12th edition, 2014) obtained from SOILGRIDS-global gridded soil information powered by ISRIC World Soil Information.

Experimental design

The experiment was laid out as a two-factor randomized complete

block design with three replications. Each sub-plot was 12 m x 8 m. There were two treatments in terms of crop rotation; 1) maize (*Zea mays* L. var. Matuba)-soybean (*Glycine max* (L.) Merrill. var. TGX-1835-10E) (growing maize in the first, and soybean in the second seasons) and 2) maize-maize (growing maize for two consecutive seasons). There were seven fertilization treatments; 1) Control (without fertilizer application), 2) N (nitrogen) PK, 3) PK, 4) NK, 5) NP, 6) Poultry manure (PM), and 7) PM + NPK.

Fertilizers were applied as 100 kg and 24 kg N ha⁻¹ as urea for maize and soybean, respectively; 60 kg and 48 kg P_2O_5 ha⁻¹ as triple superphosphate for maize and soybean, respectively; 30 kg and 24 kg K₂O ha⁻¹ as potassium sulfate (K₂SO₄) for maize and soybean, respectively, and 5 Mg ha⁻¹ PM for both crops. These fertilizing materials were incorporated into the soils each year before planting. Fertilizer treatments and nutrient contents of PM used in this study are summarized in Tables 1 and 2, respectively.

Soil sampling and measurement

Soil samples were collected from each plot at a depth of 0 - 20 cm after 2 years of cultivation. A total of 42 soil samples were taken from the experimental plots. Soil samples were air-dried, ground, and sieved with a 2 mm-sieve prior to chemical soil analysis. A mixture of soil and distilled water in a ratio of 1.5 was used to measure soil pH (LAQUA pH/ION pH meter, F-72, Horiba, Japan) and electrical conductivity; EC (COND meter ES-51, Horiba, Japan). Exchangeable acidity (sum of exchangeable H^+ and Al^{3+}) was determined by titration of 1 M potassium chloride (KCI) extract with 0.01 mol L⁻¹ (M) sodium hydroxide (NaOH) solution using phenolphthalein as indicator, and by back-titration, after acidification with 4% (w/v) sodium fluoride (NaF), with 0.01 M hydrochloric acid (HCI) solution according to McLean (1965). Total nitrogen (T-N) and carbon (T-C) were determined by dry combustion method using a Sumigraph NC-220 (Sumika Chemical Analysis Service, Ltd., Japan). P fixation was determined, according to ECAMS (1997). Briefly, 12.5 g air-dried soil was filled with 25 mL of 13,440 mg P₂O₅ L^{-1} diammonium phosphate ((NH₄)₂HPO₄) extracting solution (pH 7.0), and left to stand for 24 h with occasional swirling, and filtrated (Advantec, Japan). The concentrations of P in the filtrate were determined by a colorimetric method (Murphy and Riley, 1962) using a UV-1800 spectrophotometer (Shimadzu, Japan). The effective cation exchange capacity (ECEC) was calculated as the total of ammonium acetate (NH4OAc) Ca, Mg, K, and Na, and exchangeable acidity (Robertson et al., 1999). The base saturation ratio (BSR) was calculated as a sum of the exchangeable cations divided by the ECEC (USDA, 2004).

The M-3 extraction for cations and micronutrients

Exchangeable cations (Ca, Mg, K, and Na), micronutrients (Cu, Fe, Mn, and Zn), and available P were extracted using M-3 solution (0.2 N acetic acid (CH₃COOH), 0.25 N ammonium nitrate (NH₄NO₃), 0.015 N ammonium fluoride (NH₄F), 0.013 N nitric acid (HNO₃), and 0.001 M ethylene diamine tetra-acetic acid (EDTA)), according to Mehlich (1984) and USDA (2004). Briefly, air-dried soil samples (2.0 g) were weighed into 50-mL test tubes, and 20 mL of M-3 solution was added. Samples were shaken for 5 min using a reciprocating shaker. The sample solution was filtered through ash-free filter paper (Advantec, Japan). The concentrations of exchangeable cations, P, and micronutrients in the filtrates were determined using

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Author(s) agree that this article remain permanently open access under the terms of the <u>Creative Commons Attribution</u> <u>License 4.0 International License</u> Table 1. Fertilizer application for maize-soybean and maize-maize cultivation.

Tuanturant	N	P ₂ O ₅	K₂O	Poultry manure	
		(PM) (Mg ha⁻¹)			
Maize in 2	012/2013 - Soybea	n in 2013/2014			
T1) Control	0 [0-0] ^{††}	0 [0-0]	0 [0-0]	0 [0-0]	
T2) NPK	124 [100-24]	108 [60-48]	54 [30-24]	0 [0-0]	
T3) PK	0 [0-0]	108 [60-48]	54 [30-24]	0 [0-0]	
T4) NK	124 [100-24]	0 [0-0]	54 [30-24]	0 [0-0]	
T5) NP	124 [100-24]	108 [60-48]	0 [0-0]	0 [0-0]	
T6) PM	0 [0-0]	0 [0-0]	0 [0-0]	10 [5-5]	
T7) NPK+PM	124 [100-24]	108 [60-48]	54 [30-24]	10 [5-5]	

Maize in	2012/2013 - Maize	in 2013/2014		
T1) Control	0 [0-0]	0 [0-0]	0 [0-0]	0 [0-0]
T2) NPK	200 [100-100]	120 [60-60]	60 [30-30]	0 [0-0]
T3) PK	0 [0-0]	120 [60-60]	60 [30-30]	0 [0-0]
T4) NK	200 [100-100]	0 [0-0]	60 [30-30]	0 [0-0]
T5) NP	200 [100-100]	120 [60-60]	0 [0-0]	0 [0-0]
T6) PM	0 [0-0]	0 [0-0]	0 [0-0]	10 [5-5]
T7) NPK+PM	200 [100-100]	120 [60-60]	60 [30-30]	10 [5-5]

[†]Control; with no fertilizers, N; Nitrogen, P; Phosphorus, K; Potassium, PM; Poultry manure. ^{††}Values in brackets indicate fertilizer application rates in years 2012/2013 and 2013/2014, respectively.

Table 2. Nutrient contents of poultry manure.

T-N	T-C	0.00	Р	К	Ca	Mg	Na	Zn	Cu	Mn	Fe
(g kg	⁻¹ DM)	C/N ratio		(mg g ⁻¹ DM)			(mg kg ⁻¹ DM)				
9.0	82.0	9.1	12	1.5	51	5.3	1.3	173	84	394	2,756

T-N; Total Nitrogen, T-C; Total Carbon, P; Phosphorus, K; Potassium, Ca; Calcium, Mg; Magnesium, Na; Sodium, Zn; Zinc, Cu; Copper, Mn; Manganese, and Fe; Iron. T-N and T-C were analyzed by Sumigraph NC-220 (Sumika Chemical Analysis Service, Ltd). Contents of macro- and micro-nutrients dissolved in 0.5 M hydrochloric acid (HCI) extracts after dry ashing were analyzed by inductively coupled plasma atomic emission spectrophotometry (ICPE-9000, Shimadzu, Japan.).

inductively coupled plasma atomic emission spectrophotometry (ICP-AES; ICPE-9000, Shimadzu, Japan).

Classical methods for a comparative purpose

Soil samples were extracted for exchangeable Ca, K, Mg, and Na using 1 mol L^{-1} (M) ammonium acetate (NH₄OAc) at pH 7.0 as follows: air dried soil samples (5.0 g) were weighed into 50-mL test tubes, and 25 mL of 1 M NH₄OAc was added. Samples were shaken for 30 min. After centrifugation at 3,000 rpm for 5 min, the supernatant was filtered through 5C filter paper into a 100-mL volumetric flask. The soil was washed four times in total with 25 mL aliquots of 1 M NH₄OAc using the same procedure, and the filtrates were combined and brought up to a final volume of 100 mL. Cation determination was conducted by ICP-AES in the same manner as for the M-3 extracts.

Available P in soils was extracted using Bray I solution, which consists of 0.025 M HCl and 0.03 M NH₄F (Bray and Kurtz, 1945). The concentrations of P in the Bray I extracts were determined by a colorimetric method (Murphy and Riley, 1962) using a UV-1800 spectrophotometer (Shimadzu, Japan).

The DTPA method, a classical method for determining multielements of micronutrients (that is, Cu, Fe, Mn, and Zn) was carried out according to Lindsay and Norvell (1978). Ten gram air-dried soil was shaken by 20 mL of DTPA extracting solution (0.005 M diethylenetriaminepentaacetic acid (DTPA), 0.1 M triethanolamine (TEA), and 0.01 M calcium chloride (CaCl₂), with a pH of 7.3 for 2 h, and filtrated through No. 5C filter paper (Advantec, Japan). Concentrations of DTPA Fe, Zn, Mn, and Cu in filtrates were determined using ICP-AES.

Statistical analysis

Linear regression analysis was conducted to evaluate accuracy and precision of M-3 against the classical soil extraction procedures. Determination coefficient (r^2) and root mean square error (RMSE) from linear regression analysis were calculated and used for accuracy evaluation. Significant differences in soil nutrient concentrations among fertilizer treatments of both methods were evaluated via Tukey's HSD. Significant differences between both methods within each treatment were compared by using the Student's *t*-test (Draper and Smith, 1998).

Table 3. Selected properties of cultivated soils.

Demonster		EO (m. O , m ⁻¹)	T-N	T-C	Bray I-P	P fixation	Ex. Acidity	ECEC [†]	BSR [‡]
Parameter	рп (н20)	EC(msm)	(g k	(g⁻¹)	(mg kg⁻¹)	(g P₂O₅ kg⁻¹)	(cmol _c	kg⁻¹)	(%)
Mean (<i>n=42</i>)	5.63	4.0	0.46	5.21	20.4	1.0	0.32	1.80	77.1
Max	6.07	15.6	0.67	7.66	77.8	3.5	0.57	6.17	98.8
Min	4.90	1.6	0.33	3.39	2.3	0.1	0.07	1.02	53.7
SD	0.26	2.2	0.07	0.81	17.9	0.6	0.15	0.93	14.3

EC: Electrical conductivity, T-N: Total Nitrogen, T-C: Total Carbon, Ex. Acidity: Exchangeable acidity. $ECEC^{\dagger}$ is effective cation exchangeable capacity which is calculated as the sum of exchangeable cations (Ca, Mg, K, and Na) extracted by 1 M NH₄OAc (pH7.0) and exchangeable acidity. BSR^{\dagger} is base saturation ratio which is calculated by the sum of exchangeable cations divided by ECEC.

RESULTS AND DISCUSSION

Some chemical properties

Some properties of the soils taken from experimental plots are listed in Table 3. Soil pH values ranged from 4.90 to 6.07 with a mean value of 5.63. Other mean values were 4.0 mS m⁻¹ EC, 0.46 g kg⁻¹ T-N, 5.21 g kg⁻¹ T-C, 20.4 mg kg⁻¹ Bray I-P, 1.0 g P_2O_5 kg⁻¹ P fixation, 0.32 cmol_c kg⁻¹ exchangeable acidity, 1.80 cmol_c kg⁻¹ ECEC, and 77.1% BSR.

At this site, soils could be classified as slightly acid to very strong acid (USDA, 1998), non-saline (USDA, 2011), very low to very high available P (ISU, 2013), very low to low ECEC and medium to very high BSR (Maria and Yost, 2006).

Based on these results, mean values of soil pH, ECEC, and T-C were lower than values obtained from a broad range of soils around the Nampula area reported by Tsujimoto et al. (2011). It seems that the soils have been acidified and some exchangeable cations and total carbon have been exploited under current experimental treatments. As well as the soil acidification due to a natural process of leaching over time. Major exchangeable bases in soils could be utilized by crops through the growing season. Moreover, when the crop biomass has been harvested, transferring this biomass off the fields could result in a decrease in soil organic matter.

Evaluation of M-3 solution as soil cation extractant compared with 1 M NH_4OAc

Table 4 shows linear regression and determination coefficients (r^2) of exchangeable K, Ca, Mg, and Na extracted by M-3 and NH₄OAc. Determination coefficients were 0.939 (p < 0.001) for K, 0.917 (p < 0.001) for Ca, 0.936 (p < 0.001) for Mg, and 0.552 (p < 0.001) for Na. RMSEs were 0.021 for K, 0.177 for Ca, and 0.027 for Mg, and 0.006 for Na, respectively. This indicated that M-3 was as applicable for scientific purpose determination of K, Ca, and Mg as the NH₄OAc method, and had high

accuracy and precision, except for the determination of Na.

In some previous studies comparing M-3 and NH₄OAc methods for measuring exchangeable cations, Na has been neglected and left undiscussed (Mehlich, 1984; Michaelson et al., 1987; Eckert and Watson, 1996). However, Wang et al. (2004) reported high correlation of Na extracted by M-3 and NH₄OAc ($r^2 = 0.923$, p < 0.01) in Louisiana soils. Furthermore, Mamo et al. (1996) found a significant correlation of Na extracted by both methods on Ethiopian soil ($r^2 = 0.84$, p < 0.001), but not in German soils $(r^2 = 0.34)$ included in the same study. In these reports, mean values of Na extracted by NH₄OAc and M-3 ranged between 0.30 - 0.40 cmol_c kg⁻¹ (Mamo et al., 1996) and 0.4-0.6 cmol_c kg⁻¹ (Wang et al., 2004). In this study, approximately one-fifth to one-tenth of such Na concentrations (that is, trace to 0.06 cmol_c kg⁻¹) were observed. It was thought that very low of Na values probably resulted in the inability to predict M-3 Na from NH₄OAc Na and vice versa. This result was accorded with the study of Mylavarapu et al. (2014) which implied that M-3 has dissatisfied performance on low cation exchange capacity soil (that is, less than 10 cmol_c kg⁻¹). According to ECEC, values obtained in this study was only 1.80 cmol_c kg⁻¹ or approximately 5-times lower than the threshold value given above.

Evaluation of M-3 solution as available P extractant compared with Bray I.

Table 4 shows the linear regression of available P extracted by M-3 and Bray I methods. A high determination coefficient (r^2) between both methods was found ($r^2 = 0.936$, p < 0.001) with low RMSE (4.923). This suggests that the M-3 method was comparable to the Bray I method for P determination.

The slope value of the linear regression of Bray I-P (xaxis) with M-3 P (y-axis) was 1.038 (Table 4), indicating that M-3 P values determined by the ICP were slightly higher than by the colorimetric Bray I-P. This is because M-3 solution is more acidic than Bray I solution. In addition, M-3 P determined by ICP often provides higher

Devenenter	Linear ı	regression [†]	Det. Coef. [‡]	DMCC §	Sig. [¶]
Parameter	Slope	Intercept	(r ²)	RNISE °	
M-3 vs Bray 1-P	1.038	3.288	0.936	4.923	***
M-3 vs NH₄OAc K	0.929	0.014	0.939	0.021	***
M-3 vs NH₄OAc Ca	0.677	0.127	0.917	0.177	***
M-3 vs NH₄OAc Mg	0.867	0.029	0.936	0.027	***
M-3 vs NH₄OAc Na	1.310	0.016	0.552	0.006	***

Table 4. Linear regression of soil macronutrient concentrations extracted by Mehlich 3 (M-3) and ammonium acetate (NH_4OAc) for Ca, K, Mg, and Na, and by M-3 and Bray I for P.

[†] Mehlich 3 Cations (P, K, Ca, Mg) = a x (Bray I-P or NH₄OAc K, Ca, Mg, and Na) + b, where, a is the slope, and b is the intercept, respectively. Det. Coef.[‡]: the determination of coefficient (r^2). RMSE [§]: root mean square error. Sig.[¶]: significant difference between two extracting methods (M-3 vs classical) at p < 0.001 (Student's *t* test).

values than those of P determined by colorimetry (Paz-Ferreiro et al., 2012). However, Bray I-P values may be adapted to M-3 extractable P values by directly performing a single linear conversion. The ISU (2013) interpreted the classes of M-3 P as very low (0 to 15 mg kg⁻¹), low (16 to 25 mg kg⁻¹), optimum (26 to 35 mg kg⁻¹), high (36 to 45 mg kg⁻¹), and very high (>46 mg kg⁻¹), compared with the Bray I-P as very low (0 to 8 mg kg⁻¹), low (9 to 15 mg kg⁻¹), optimum (16 to 20 mg kg⁻¹), high (21 to 30 mg kg⁻¹), and very high (>31 mg kg⁻¹), respectively.

It should be noted that the availability of P in soils is greatly affected by soil pH; some soils in which the pH values were below 5.5 or above 7.8 may have a low availability of P (USDA, 1998). Therefore, classification of soil pH range may be included when the variation in soil P values and decreases of the determination coefficient between M-3 and Bray I methods were clearly observed.

Evaluation of M-3 solution as soil micronutrient extractant compared with DTPA

Figure 1 shows the correlation of M-3 Zn, Cu, Mn, and Fe with DTPA extraction. The determination coefficient (r^2) of Zn extracted by M-3 and DTPA was 0.936 (p < 0.001) with 0.159 RMSE. Other determination coefficients between the two methods were 0.585 (p < 0.001, RMSE = 0.141) for Cu, 0.263 (p < 0.001, RMSE = 33.85) for Mn, and 0.071 (p < 0.089, RMSE = 5.76) for Fe, respectively.

Zn extracted by M-3 and DTPA were highly correlated, suggesting that M-3 could be as applicable for determining soil Zn as DTPA. Similar to this current study, Wang et al. (2004) reported poor correlations between two extracting methods for Mn ($r^2 = 0.420$) and Fe ($r^2 =$ 0.380) in Louisiana soils. Wendt (1995) reported a poor correlation for Fe ($r^2 = 0.47$) and Mn ($r^2 = 0.28$) in Malawian soils. In addition, Vocasek and Friedericks (1994) revealed a poor correlation of Mn in acidic to alkaline soils. Wang et al. (2004) also stated that a fundamental difference in a chemical reaction of M-3 and DTPA with soils such metal-complex stability, soil of specific regions, soil pH and the competition from other cations could be related factors that contributed to the quantities of M-3 and DTPA extracted nutrients and in turn affected the correlations of two methods. In this

study, it was thought that typical soils and newly-added ions from soil fertilization could have influenced the chemical reaction of both extractants with soils.

For the present, there was no information on critical levels of micronutrients in Mozambigue. Our measured values of M-3 micronutrients were compared with ranges of critical levels for micronutrients extracted by various extractants (acid and alkaline based-extractants but without M-3) in many tropical countries (Lindsay and Cox, 1985) in which was once summarized and cited by Maria and Yost (2006) as follows; 0.5 to10 mg kg⁻¹ for Zn, and 0.2 to 10 mg kg⁻¹ for Cu, 1 to 20 mg kg⁻¹ for Mn, and 0.4 to 10 mg kg⁻¹ for Fe. It was found that values of M-3 extractable Zn, Cu, Mn, and Fe were in or above ranges of critical values as well as DTPA (Figure 1). This suggested that M-3 was an acceptable soil test. In this study site, soils had adequate Zn and Cu, and excess of Mn and Fe levels determined with M-3 and DTPA methods. Similarly, levels of M-3 Zn, Cu, and Mn were rated for very low to optimum, while M-3 Fe level was low to optimum according to critical levels for micronutrients established by Ethiopian Soil Information System (EthioSIS) cited by Bulta et al. (2016).

This suggested that the M-3 method could replace the DTPA in determining micronutrients if needed in this sitespecific level. Measured values could provide an acceptable range of Zn, Cu, Mn, and Fe levels and it could be accompanied with a general soil micronutrient decision and management. In the future, the field calibration and yield response to micronutrient application essential for M-3 extraction. were Furthermore, micronutrient soil tests with various extracting solutions should be calibrated on a broad range of soils, particularly in tropics; including Mozambique, where the soils and nutrient reserves are variable due to its parent material's age and highly weathered conditions (Lindsay



Figure 1. Comparison of Mehlich 3 extractable micronutrients against DTPA; (a) Zinc (Zn), (b) copper (Cu), (c) manganese (Mn), (d) iron (Fe). Bars indicated standard errors (SE). *** Significant at p < 0.001 (Student's *t*-test).

and Cox, 1985).

Evaluation of M-3 against classical procedures under soil fertilization and crop rotation

Levels of soil Ca, K, Mg, P, Zn, Cu, Mn, and Fe extracted by M-3 and classical methods are presented in Figures 2 and 3 for maize-soybean and maize-maize cultivations, respectively.

On both types of crop rotation, levels of Ca, Mg, K and P extracted by M-3 and classical methods had similar trends in all fertilizer treatments and there were no significant differences of two methods within treatments (Figures 2(a) to (d) and 3 (a) to (d)). This pointed that M-3 had comparative advantage in determining soil cations with the classical methods and the accuracy and precision of the determination seemed not to be influenced by types of soil fertilization and crop rotation.

In general, M-3 micronutrients showed higher values

than that of DTPA, regardless of soil fertilization and crop rotation. It was possible due to higher strength of M-3 acidity (Figures 2 (e) to (h) and 3 (e) to (h)). Shuman (1988a) depicted that metals extracted with M-3 increased with increases in organic matter application, but the DTPA did not consistently show this pattern under incubation study. On the other hand, sole M-3 Zn seemed to increase with additional manure application, while M-3 Cu, Mn, and Fe did not increase in this study. Moreover, Shuman (1988b) reported that increased P fertilization increased M-3 and DTPA Mn and Fe, but our results were in contradiction to the finding of Shuman (1998b). It appeared that M-3 and DTPA Mn and Fe were not significant differences among non-P and P applied soils. So far, it was thought that application of organic matter (that is, cattle manure) to soils could alter soil environment through enhanced Ca concentration and increased soil pH, and other organic metal complexes (Alloush, 2003), and including the newly-added ions from organic and chemical fertilizers should have influenced



T1) Control, T2) NPK, T3) PK, T4) NK, T5) NP, T6) Poultry Manure (PM), T7) NPK+PM

Figure 2. Nutrient concentrations of maize-soybean-cultivated soils extracted by Mehlich 3 (White column) and classical methods (Filled column) with respect to exchangeable calcium; Ca (a), magnesium; Mg (b), potassium; K (c), phosphorus; P (d), extractable zinc; Zn (e), copper; Cu (f), manganese; Mn (g), and iron; Fe (h). Classical methods were 1 M NH₄OAc (pH 7) for Ca, Mg, K; Bray I for P; and DTPA for Zn, Cu, Mn, and Fe. Data are shown as means of three replicates ± SE. Error bars indicated standard errors (SE). Different letters among treatments indicate significant differences (Tukey's HSD, p < 0.05). Capital letters compared Mehlich 3 extraction among treatments. Small letters compared classical extraction among treatments. Asterisk (*) indicates significant differences (Student's t-test, p < 0.05) between the Mehlich 3 and classical extraction within each treatment.

the chemical reaction of M-3 and DTPA with soils.

Conclusions

The M-3 has proved to be a useful extractant for Ca, K,

Mg, P, and Zn under the site-specific conditions given. Hitherto, information on critical levels of micronutrients in Mozambique has not been established. This study compared levels of M-3 micronutrient with ranges of critical levels for micronutrients of tropical countries. The M-3 Zn, Cu, Mn, and Fe were well above those



Figure 3. Nutrient concentrations of maize-maize-cultivated soils extracted by Mehlich 3 (White column) and classical methods (Filled column) with respect to exchangeable calcium; Ca (a), magnesium; Mg (b), potassium; K (c), phosphorus; P (d), extractable zinc; Zn (e), copper; Cu (f), manganese; Mn (g), and iron; Fe (h). Classical methods were 1 M NH₄OAc (pH 7) for Ca, Mg, K; Bray I for P; and DTPA for Zn, Cu, Mn, and Fe. Data are shown as means of three replicates ± SE. Error bars indicated standard errors (SE). Different letters among treatments indicate significant differences (Tukey's HSD, p < 0.05). Capital letters compared Mehlich 3 extraction among treatments. Small letters compared classical extraction among treatments. Asterisk (*) indicates significant differences (Student's t-test, p < 0.05) between the Mehlich 3 and classical extraction within each treatment.

considered to be critical level as same as DTPA extraction. It suggested that M-3 was an acceptable soil test method and M-3 was advantageous on routine analysis and general soil nutrient management. However, the field calibration and yield response to micronutrient

application were required for M-3 extraction. In the future, micronutrient soil tests with various extracting solutions should be calibrated under intensive cropping systems and on a broad range of soils, particularly in the tropics where the soils and nutrient reserves are variable due to its parent material's age and highly weathering conditions.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors gratefully appreciate the staff members of the Instituto de Investigação Agrária de Moçambique (IIAM) and the Japan International Research Center for Agricultural Sciences (JIRCAS) for their technical support. This study was conducted as a part of the project "Improving Research and Technology Transfer Capacity for Nacala Corridor Agriculture Development,

Mozambique" funded by Japan International Cooperation Agency (JICA). Soil samples imported from Mozambique to Japan were permitted by the Minister of Agriculture, Forestry, and Fisheries of Japan under Plant Quarantine Law.

REFERENCES

- Alloush GA (2003). Dissolution and effectiveness of phosphate rock in acidic soil amended with cattle manure. Plant Soil 251:37-46. (https://doi.org/10.1023/A:1022987915057).
- Bray RH, Kurtz LT (1945). Determination of total, organic, and available forms of phosphorus in soils. Soil Science 59:39-45.
- Bulta AL, Assefa TM, Woldeyohannes WH, Desta HS (2016). Soil micronutrients status assessment, mapping and spatial distribution of Damboya, Kedida Gamela, and Kecha Bira Districts, Kambata Tambaro zone, Southern Ethiopia. African Journal of Agricultural Research 11(44):4504-4516.

https://doi.org/10.5897/AJAR2016.11494

Carter MR, Gregorich EG (2014). Soil sampling and methods of analysis.

http://aesl.ces.uga.edu/sera6/PUB/MethodsManualFinalSERA6.pdf

- Draper NR, Smith H (1998). Applied regression analysis (3rd ed.)-Wiley series in probability and statistics. A Wiley-Interscience publication/John Wiley and Sons Inc, NY, P 736.
- Eckert DJ, Watson ME (1996). Integrating the Mehlich-3 extractant into existing soil test interpretation schemes. Communications in Soil Science and Plant Analysis 27:1237-1249.
- Editorial Committee of the Analytical Method of Soil Environment (1997). The analytical method of soil environment. Hakuyusya, Tokyo, Japan.
- Fukuda M, Nakamura S, da Conceição Lopes Fonseca A, Nasukawa H, Ibraimo MM, Naruo K, Kobayashi K, Oya T (2017). Evaluation of the Mehlich 3 reagent as an extractant for cations and available phosphorus for soils in Mozambique. Communications in Soil Science and Plant Analysis 48(12):462-1472. (https://doi.org/10.1080/00103624.2017.1373789)
- García A, de Iorio AF, Barros M, Bargiela M, Rendina A (1997). Comparison of soil tests to determine micronutrients status in Argentina soils. Communications in Soil Science and Plant Analysis 28(19-20):1777-1792. (https://doi.org/10.1080/00103629709369915)
- Hanlon EA, Johnson GV (1984). Bray/Kurtz, Mehlich 3, AB/DTPA and ammonium acetate extraction of P, K, and Mg in four Oklahoma soils. Communications in Soil Science and Plant Analysis 15(3):277-294.
- Iowa State University (ISU) (2013). A general guide for crop nutrient and limestone recommendations in Iowa. PM1688, Iowa State University Extension and Outreach, IA. P 18.
- Lindsay WL, Cox FR (1985). Micronutrient soil testing for the tropics. Fertilizer Research 7:169-200.

- Lindsay WL, Norvell WA (1978). Development of a DTPA soil test for zinc, iron, manganese, and copper. Soil Science Society of America Journal 42:421-428.
- Mamo T, Richter C, Heiligtag B (1996). Comparison of extractants for the determination of available phosphorus, potassium, calcium, magnesium and sodium in some Ethiopian and German soils. Communications in Soil Science and Plant Analysis 27(9-10):2197-2212. (https://doi.org/10.1080/00103629609369697)
- Maria RM, Yost R (2006). A survey of soil fertility status of four agroecological zones of Mozambique. Soil Science 171(11):902-914. (doi: 10.1097/01.ss.0000228058.38581.de)
- McLean EO (1965). Aluminum, pp.978-998, in Black CA ed., Methods of soil analysis, Part 2, American Society of Agronomy, Madison, Wisconsin.

(https://doi.org/10.1080/00103628409367568)

Michaelson GJ, Ping CL, Mitchell GA (1987). Correlation of Mehlich 3, Bray 1, and Ammonium acetate extractable P, K, Ca, and Mg for Alaska agricultural soils. Communications in Soil Science and Plant Analysis 27(9):2197-2212.

(https://doi.org/10.1080/00103628709367877)

- Murphy J, Riley JP (1962). A modified single solution method for the determination of phosphate in natural waters. Analytica Chimica Acta 27:31-36. (https://doi.org/10.1016/S0003-2670(00)88444-5)
- Mylavarapu R, Obreza T, Morgan K, Hochmuth G, Nair V, Wright A (2014). Extraction of soil nutrients using Mehlich-3 for acid-mineral soils of Florida. Original publication SL407, Department of soil and water sciences, UF/IFAS Extension, University of Florida, FL, P 5.
- Paz-Ferreiro J, Vázquez EV, de Abreu CA (2012). Phosphorus determination after Mehlich 3 extraction and anion exchange resin in an agriculture soil of northwestern Spain. Communications in Soil Science and Plant Analysis 43(1-2):102-111. (https://doi.org/10.1080/00103624.2012.634688)
- Robertson GPP, Sollins P, Ellis BG, Lajtha K (1999). Exchangeable ions, pH, and cation exchange capacity, pp. 104-114, in Roberson G, Bledsoe PCS, Coleman DC, Sollins P eds., Standard soil method, Oxford University Press, NY.
- Shuman LM (1988a). Effect of organic matter on the distribution of manganese, copper, iron, and zinc in soil fractionation. Soil Science. 146:3. (DOI 10.1097/00010694-198809000-00007)
- Shuman LM (1988b). Effect of phosphorus level on extractable micronutrients and their distribution among soil fractions. Soil Science Society of America Journal 52(1):136-141.
- Tsujimoto Y, Tobita S, Fukuda M, Nakamura S, Omae H, Osamu I, Martinho C, Chitio F, Hitsuda K, da Rocha CMC (2011). Characterisation of agro-environments for optimizing cropping systems along the Nacala corridor, Mozambique. African Crop Science Conference Proceedings 10:279-282.
- United States Department of Agriculture (1998). Soil quality indicator: pH. Soil quality information sheet. Natural Resources Conservation Service, DC. P 2.
- United States Department of Agriculture (2004). Soil survey laboratory methods manual. Soil survey investigations Report No. 42 (version 4.0). Natural Resources Conservation Service, DC. P 700.
- United States Department of Agriculture (2011). Soil quality indicators: Soil electrical conductivity. Soil quality information sheet. Natural Resources Conservation Service, DC. P 3.
- Vocasek FF, Friedericks JB (1994). Soil micronutrient extraction by Mehlich 3 compared to CaCl₂-DTPA. Communications in Soil Science and Plant Analysis 25(9-10):1583-1593.
- Wang JJ, Harrell DL, Henderson RE, Bell PF (2004). Comparison of soil-test extractants for phosphorus, potassium, calcium, magnesium, sodium, zinc, copper, manganese, and iron in Louisiana soils. Communications in Soil Science and Plant Analysis 35(1-2):145-160.
- Wendt JW (1995). Evaluation of the Mehlich 3 soil extractant for upland Malawi. Communications in Soil Science and Plant Analysis 26(5-6):687-702.