Relative Suitability of Phosphorus Extraction Procedures for F Tropical Ferralsol

J. S. Tenywa1*, E. Odama1 and A. K. Amoding1

1Department of Agricultural Production, College of Agricultural and Environmental Sciences, Makerere University, P.O.Box 7062, Kampala, Uganda.

ABSTRACT

Purpose: To evaluate the predictive capacity common procedures for soil P extraction and testing in laboratories in the region.

Materials and Methods: A pot study with treatments viz. soil phosphorus extraction methods (Bray I, Bray II and Mehlich 3), and six P application rates (0, 20, 40, 60, 80 and 100 kg P ha⁻¹). Maize (Zea mays L.) variety Longe IV was the test crop. A Ferralsol from northwestern Uganda (West Nile) was used in this study.

Results and Conclusion: Mehlich 3 correlated most with plant P uptake by presenting the highest correlation coefficient with plant P content (r = 0.254) and a number of leaves per plant (r = 0.733). A strong positive correlation existed between Bray I and Mehlich 3 extractable P values (r = 0.975), suggesting lack of a marked difference between them; implying that either of the two procedures could be applied for soil P extraction in Ferralsols. However, Mehlich 3, being a multi-nutrient extractant, was recommended as the most suitable for P extraction for the Ferralsol used in this study.

Keywords: Bray; extractant; Mehlich 3; Uganda; Zea mays.
ABBREVIATIONS

SSA : Sub-Saharan Africa
ZARDI : Zonal Agricultural Research and Development Institute
XRD : X-ray Diffraction
WAP : Weeks after Planting
LSD : Least Significant Differences
NARO : National Agricultural Research Organization, Uganda

1. INTRODUCTION

Phosphorus deficiency is a fundamental impediment to sustainable crop production on more than 40% of the world’s arable lands [1,2], and most especially in Sub-Saharan Africa (SSA) [3]. Most of the soils in the SSA region are heavily weathered and laden with oxides and hydroxides of Al and Fe, which have high P sorption potentials to extents that complicate P management [4]. Besides, there is evidence of P depletion in soils of most Sub-Saharan countries, often averaging 20 kg P ha\(^{-1}\) per year [5].

A major challenge in achieving reliable information on which sound soil P management strategies can be designed lies in the unavailability of widely accredited soil test data from related laboratories. Although soil analytical laboratories in SSA have strived to achieve this accreditation, use of dissimilar P extraction methods, most of which were developed specifically for temperate soil conditions, makes it difficult for to share information and attain consistent recommendations for the farming communities. As such, laboratories in the region cannot easily share quality control mechanisms for soil P testing as is done elsewhere [6]. Consequently, soil phosphorus analysts provide widely varying results and recommendations for a soil sample sourced from the same site.

The most widely used soil P extractants in SSA are Bray I [7], Mehlich 3 [8] and Olsen [9]. Other reagents rarely used include Bray II, Mehlich I and II and Resin [10]. Unfortunately, none of these soil P extraction agents have been evaluated deliberately for suitability for testing the physicochemically unique tropical soils of the SSA. This study was, thus conducted to evaluate the predictive capacity of some of the most common extraction reagents for soil P in laboratories in SSA.

2. MATERIALS AND METHODS

A greenhouse experiment was conducted at Abi Zonal Agricultural Research and Development Institute (ZARDI) in north-western Uganda, from July 2008 to October 2009. Bulk soil was collected from a field in Arua district in Uganda at 3°45.8' N and 30°56.74' E, from 0–30 cm depth. The field had been cultivated continuously without fertilising use reportedly (by farmers) for more than 10 years. Visible debris such as roots, litter and stones were manually removed, and the soil air-dried for seven days prior to use in the study. A sub-sample of approximately 500 g of soil was taken and analysed for extractable P using Bray I and II, and Mehlich 3 procedures (Table 1).

Table 1. Pre-study soil pH and extractable phosphorus of the soil from Abi Agricultural Research Centre in North-Western Uganda

| Soil properties          | Test value |
|--------------------------|------------|
| pH(H\(_2\)O)              | 5.6        |
| Bray I P (mg kg\(^{-1}\)) | 10.18      |
| Bray II P (mg kg\(^{-1}\)) | 10.61      |
| Mehlich 3 P (mg kg\(^{-1}\)) | 7.05       |
| Textural name            | Sandy loam |

2.1 X-ray Diffraction

Mineralogical analysis of the clay fraction of the soil was done using an X-ray diffractometer (XRD) at the University of California, Davis in U.S.A. Three peaks emerged prominently for the dominant clay minerals in the study soil; these included mica at 10 Å, and kaolinite (7.0 Å and 3.5 Å) (Fig. 1).

Mica peaks remained stable regardless of heat or saturation treatment. In contrast, kaolinite generated peaks that disappeared with 550°C heat treatment, but was not affected by glycerol solvation. The fourth and shorter peak at 3.3 Å indicated detection of quartz (Fig. 1).

2.2 Experimental Set Up

Treatments included (a) three soil P extraction methods namely, Bray I, Bray II and Mehlich 3; and (b) six P rates of 0, 80, 160, 240, 320 and 400 mg P per pot containing 8 kg of soil; equivalent to 0, 20, 40, 60, 80 and 100 kg P ha\(^{-1}\). Weighed triple super phosphate, the fertiliser source of P, was thoroughly mixed with the potting soil on a distilled water-cleaned and air-dried polythene sheet. Each bucket also received a blanket application of N and K at 60 and 40 kg ha\(^{-1}\), respectively, in the form of urea and muriate of potash, to obviate their possible plant growth limitation. The experiment was laid out in a completely randomised design with 4...
replications. The experiment was repeated 3 times.

Maize (Zea mays L.), Longe IV variety was the experimental crop. Four seeds were sown per pot, at 2 – 3 cm depth. The pots were watered with distilled water to field capacity (25%, determined by hand feel) using a plastic watering can. Five days after emergence, the seedlings were thinned to 3 plants per pot. Hand weeding was done to maintain the experiment weed free.

Parameters measured included plant P content and biomass yield per plant. These were evaluated at eight weeks after planting (WAP). For plant P determination, the three plants per pot were cut at soil surface level, using a kitchen knife. They were then chopped into 3-5 cm pieces, oven-dried at 65°C for 48 hours and weighed for dry weight per pot. The plant samples were dried further at 105°C and then ground into fine powder using a milling machine (CycroTech. Sample Mill made by FOSS). Sub-samples were extracted using a digestion mixture and P tested using the colorimetry procedure [11].

At the same stage of plant sampling, soil samples were collected from the pots for determination of extractable P. Soil from the pots was first emptied on polythene sheets, each pot separately, and mixed thoroughly. Approximately 500 g samples were taken for air-drying for 7 days under room temperature (25-27°C), before the samples were extracted for available P prediction using Bray I, Bray II and Mehlich 3 extraction reagents and the colorimetry testing procedure [11].

2.3 Data Analysis

Data collected were subjected to analysis of variance using GenStat software (Version 12). Where treatment effects were significant, Fisher’s Protected Least Significant Differences (LSD) at 5% probability level was used to separate the means. The Watson and Mullen [12] procedure for determining the best method for P extraction by running correlations between the amount of P extracted by the extracting agent and the amount of P taken up by the plant in question was adopted for this study. Thus, correlations were run between P for each extraction procedure against plant growth parameters. The magnitude of the correlation coefficient was the considered the basis for identifying the most suitable extraction procedure for the Ferralsol used in this study.

![Fig. 1. Sequence of X-ray diffraction patterns for the clay fraction of a Ferralsol from Arua district in North Western Uganda](image)
3. RESULTS AND DISCUSSION

3.1 Pre Experiment Soil Analytical Data

The soil in the study had a favourable pH for P availability to plants. There was no significant difference in the P extracted by Bray I and Bray II but both Bray 1 and Bray 11 extracted >30% more P than Mehlich 3 (Table 1). The difference in extractible P fractions obtained using the three P extraction procedures demonstrates the varied P extracting powers of the three reagents on a Ferralsol. This seems to be linked to the varying acid concentrations in the Bray-based extracting agents. For example, Bray II (0.03 M NH₄F + 0.1 M HCl) is more acidic than Bray I (0.03 M NH₄F + 0.025 M HCl) because of relatively higher concentrations of HCl in the former [13]; thus it extracted the greatest amount of available P (Table 2). In contrast, in Mehlich 3 procedure, phosphorus is extracted by reaction with acetic acid and fluoride compounds [14]. It is also possible that the differences in the amount of P extracted by the three extractants are due to their selectivity in solubilising different P fractions to varied extents [15]. For example, NH₄F selects to solubilise only Al-bound P [16].

3.2 Relationships of P Extraction Procedures with Number of Leaves

There was evidence of a relationship between P extraction agents and number of leaves (Fig. 2). The relationship was in the order of Mehlich 3 (r=0.733) > Bray 1 (r = 0.614) >Bray 2 P (r=0.34). The strong relationship exhibited by Mehlich 3 with number of leaves (r = 0.733, Fig. 2) is a good indication that Mehlich 3 was the best extractant in mimicking P uptake by plants. The gradual increment in number of leaves observed, which was limited to about 8 leaves per plant, could be explained by the fact that this is largely determined genetically. In a similar effort, Ali [17] reported that phosphorus level did not significantly affect maize foliage per plant.

The superior correlation coefficient of Mehlich 3 observed over Bray I and II could be so because Mehlich 3 closely simulated plant P uptake under the natural soil conditions. The fact that Bray I correlated relatively better with number of leaves than Bray II (Figs. 2 and 3), could be as a result of the less acidic nature of the former (0.025 M HCl), which better simulated plant P uptake. Bray II, with a higher acid content (0.1 M HCl), could have extracted more P thus, overestimating the available P content, resulting into the low correlation with plant growth parameters. The fluoride in the Bray I extractant enhances P release from aluminium phosphates by decreasing Al³⁺ activity in solution through the formation of various Al-F complexes [18]. Fluoride is also effective at suppressing the re-adsorption of solubilised P by soil colloids [18].

3.3 Correlation of Extractable Soil P with Plant P

In general, there were weak correlations among the P extracted using the three extraction procedures and plant P content in the plants (Fig. 3). Nevertheless, Mehlich 3 procedure tended to present the highest correlation coefficient (r = 0.254). Bray II method had the lowest and negative correlation coefficient (r = - 0.191) (Fig. 3).

| Treatment (kg P ha⁻¹) | Bray I P | Bray II P | Mehlich 3 P |
|----------------------|---------|----------|-------------|
|                      | (mg kg⁻¹) | (mg kg⁻¹) | (mg kg⁻¹)   |
| 0                    | 10.18   | 10.61    | 7.05        |
| 20                   | 20.00   | 18.54    | 12.08       |
| 40                   | 18.10   | 14.10    | 10.35       |
| 60                   | 15.43   | 15.82    | 9.10        |
| 80                   | 24.08   | 22.02    | 14.30       |
| 100                  | 23.23   | 16.43    | 17.58       |
| L.S.D (0.05)         | 10.98   | 9.29     | 7.04        |
Fig. 2. Correlations between number of leaves and (A) Bray I, (B) Bray II and (C) Mehlich 3 extractants for obtained Ferralsol from Arua District in Uganda

Mehlich 3 correlated more strongly with Bray I than Bray II (Fig. 4). This implies that the two reagents differ minimally in simulating plant P uptake in the Ferralsol. This is in tandem with work done by Tran et al. [19] in Quebec, Canada who noted that the amount of Mehlich 3 P was similar to that extracted by the Bray I method on most non-calcareous soils. Mallarino [20], also while working in Iowa, USA noted that Mehlich 3 and Bray I soil test results were highly correlated in neutral to acid soils. The only contrast was that Mehlich 3 extracted slightly more P than Bray I in
their case, while Bray I extracted more P in the present study (Table 2). Similar work done by Hailin et al. [21] on inter-laboratory validation of the Mehlich 3 procedure for extraction of plant-available phosphorus, indicated that Mehlich 3 was both accurate and precise when standardised procedures were used. Conclusions drawn after the work done by Kleinman et al. [22] in Nebraska, USA also indicated that Mehlich 3 solution, a multi-element extractant, was suitable for removing P and other ionic species in both acidic and neutral soils, a factor that has boosted its popularity in University and commercial soil laboratories as a standard soil test. Furthermore, Watson and Mullen [12] asserted that phosphorus extracted using the Mehlich 3 mimicked P uptake by plants. This means that Mehlich 3 would be a better predictor of the amount of P available for plant uptake under natural conditions.

**Fig. 3.** Correlations between plant P and (A) Bray I, (B) Bray II and (C) Mehlich 3 extractants for obtained Ferralsol from Arua District in Uganda
In some soils, Mehlich 3 has been found to have a detection limit of 1.0 mg kg$^{-1}$ for P [23]. Therefore, the weak coefficients could be a result of the biomass dilution effect that might have confounded the plant P concentration response. Nutrient uptake and internal mobility affect the nutrient concentrations in plant tissues [24]. Often, there is a mismatch between rate of plant growth and that of nutrient uptake, coupled with movement of the nutrients within and between plant parts [24]. Under normal growing conditions, nutrient uptake and plant growth are closely parallel during most of the vegetative growth period [24].

4. CONCLUSION

This study has revealed that Mehlich 3 and Bray I are the most suitable for predicting plant available P in Ferralsols with mica, kaolinite and quartz mineralogy; and pH 5.6. Despite their near equal competence in the extraction of P from the study Ferralsols, Bray 1 is a single-nutrient extractant, while Mehlich 3 is a multi-element extractant. Mehlich 3, being a multi-nutrient extractant, is recommended for plant available P extraction for the Ferralsol used in this study.
COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Vance CP. Symbiotic nitrogen fixation and phosphorus acquisition. Plant nutrition in a world of declining renewable resources. Plant Physiology. 2001;127:390-397.
2. Hofman G, Cleemput OV. Soil and plant nitrogen. International Fertiliser Industry Association (IFA), Paris, France. 2004:48.
3. Henao J, Baanante C. Agricultural production and soil nutrient mining in Africa. Summary of IFDC Technical Bulletin, IFDC, Muscle Shoals, Alabama. USA; 2006.
4. Penn Chad J, Jason G. Warren. Soil Science Society of America Journal. 2008;73:560-568. DOI: 10.2136/sssaj2008.0198
5. Stoornvogel JJ, Smaling EMA, Janssen BH. Calculating soil nutrient balances in Africa at different scales. Fertilizer Research. 1993;35(3):227–235.
6. Van Reeuwijk LP, Houba V.JG. External quality control of data. WEPAL, Dept. of Soil, Science and Plant Nutrition, Wageningen Agricultural University, P.O.Box 8005, 6700 EC Wageningen, the Netherlands; 1998. Available:http://www.benp.wau.nl/wepal
7. Bray RH, Kurtz LT. Determination of total, organic and available forms of phosphorus in soils. Soil Science. 1945;59:39-45.
8. Nelson WL, Mehlich A, Winters E. The development, evaluation, and use of soil tests for phosphorus availability. Agronomy Journal. 1953;4:153-158.
9. Olsen SR, Cole CV, Watanabe FS, Dean LA. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Circular 939. U.S. Government Printing Office, Washington D.C., USA; 1954.
10. Fixen PE, Grove JN. Testing soils for phosphorus. In: Westerman (Ed). Soil testing and plant analysis, 3rd Edition. Soil Science Society of American, Madison, WI. 1990:141-180.
11. Anderson JM, Ingram JSI. Tropical soil biology and fertility. Oxford University Press, 2nd Edition. 1993;240. ISBN: 10 0851988210.
12. Watson M, Mullen R. Understanding plant-available phosphorus. Fact Sheet 3373. The Ohio State University Cooperative Extension, Columbus, Ohio, USA; 2007.
13. Frank K, Beegle D, Denning J. Recommended phosphorus tests. In: Brown J. R. (ed.) Recommended chemical soil test procedures for the north central region. North Central Regional Publication No. 221 (revised). Missouri Agricultural Experiment Station SB 1001. Columbia, Missouri, USA. 1998;21-23.
14. Mehlich A. Mehlich 3 soil test extractant: A modification of Mehlich II extractant. Communications in Soil Science and Plant Analysis. 1984;15:1409-1416.
15. Dhillon NS, Handal HS, Dev G. Studies on P nutrition of rice-wheat cropping sequence in a samana sandy loam. Indian Society of Soil Science Journal. 1993;41:784-786.
16. Soinne Helena OAI. Extraction methods in soil phosphorus characterization - Limitations and applications. Pro Terra No. 47. Academic dissertation, Faculty of Agriculture and Forestry of the University of Helsinki, University of Helsinki; 2009. Available:https://www.researchgate.net/publication/47932382_Extraction_methods_in_soil_phosphorus_characterisation_Limitations_and_applications
17. Ali A. Effect of nitrogen and magnesium on growth, yield and quality of hybrid maize (Zea mays L.). M.Sc. Thesis, Department of Agronomy, University of Agriculture Faisalabad, Pakistan. 1998;81.
18. Kleinman PJA, Sharpley AN. Estimating soil phosphorus sorption saturation from Mehlich-3 data. Communications in Soil and Plant Analysis. 2002:33:1825-1839.
19. Tran TS, Giroux M, Guilbeault J, Audesse P. Evaluation of Mehlich-III extractant to estimate the available P in Quebec soils. Communications in Soil Science and Plant Analysis. 1990;21(1-2): 1-28.
20. Mallarino AP. Field calibration for corn of the Mehlich 3 soil phosphorus test with colorimetric and inductively coupled plasma emission spectroscopy determination methods. Soil Science Society of America Journal. 2003;67:1928-1934.
21. Hailin Z, Kariuki S, Schroder JL, Payton ME, Focht C. Interlaboratory validation of the Mehlich 3 method for extraction of plant-available phosphorus. Journal of AOAC International. 2009; 92(1):91-10.
22. Kleinman PJA, Sharpley AN, Gartley K, Jarrell WM, Kuo S, Menon RG, Myers R, Reddy KR, Skogley O. Interlaboratory comparison of soil phosphorus extracted by various soil test methods. Publications from USDA-ARS / UNL Faculty. 2001; 544. Available: http://digitalcommons.unl.edu/usdaarsfacpub/544

23. Zhang H, Kariuki S, Schroder S, Payton ME, Focht C. Interlaboratory validation of the Mehlich 3 method for extraction of plant-available phosphorus. Journal of AOAC International. 2009; 92(1). Available: https://www.researchgate.net/publication/24344769

24. Scheffe L. How to collect and interpret plant tissue samples; 2008. Available: http://www.nm.nrcs.usda.gov/technical/handbooks/iwm/NM_IWM_Field_Manual/Section05/5d-How_to_Collect_and_Interpret_Plant_Tissue_Samples.pdf (Visited on 18th December 2010)