International Journal of Science and Research (IJSR)
ISSN: 2319-7064
ResearchGate Impact Factor (2018): 0.28 | SJIF (2019): 7.583

Development of Semi-Quantitative Method for Primary Detection of Soil-Phosphate Levels

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Abstract: ***Phosphorus plays an important role in key biological mechanisms like photosynthesis, respiration and has major part in plant growth and reproduction. It is important to estimate soil phosphate level and its regular management in farms to ensure optimal outcome. Uses of in-situ methods have various advantages as these procedures are less labor-intensive, low cost, less time consuming and fairly accurate. Present study focuses on developing a semi-quantitative soil phosphorus estimation method which shall predict the phosphate deficiency or high concentration of phosphates in soil using modified colorimetric procedure. This study can help to device a suitable cost effective fertilization usage strategy ensuring efficient fertilizing practices and aid farmers to use more specific soil testing methods if required.***

Keywords: soil testing, phosphate concentration, semi-quantitative, fertilizers

1. Introduction

Soil testing was initiated in the country in the beginning of planning era by setting up of 16 soil testing laboratories during 1955 [1]. Soil macro/micro nutrient testing has great importance for farmers to increase productivity/ hectare of the land available. The benefits of soil testing have been established through scientific research, extensive field demonstrations and on the basis of actual fertilizer use by the farmers on soil test based fertilizer use recommendations [2].

Phosphorus is found in all biological forms as an integral part of the building blocks of biomolecules like phospholipids, nucleic acids, etc. In the soil, phosphorus is found in two forms viz. organic and inorganic (with low solubility) in an equilibrium state and its concentration depends on various factors like pH, temperature, moisture content and presence of other macro/micro-nutrients. Plants can take-up only organic form of the phosphorus mainly phosphates ($-PO_3^{-4}$). Phosphorus makes 0.1% to 0.5% of the total dry weight of the plant and play essential role in some specific processes like nitrogen fixation, seed fruit and legume formation etc. [2]. Phosphorus estimation from farm-soil is mainly aimed towards three major areas to identify the optimal phosphorus concentration required by the soil/plants, amount of phosphorus to be added in bioavailable form (fertilizers) and to minimize the loss of excess phosphorus addition resulting in economic return [3]. Poor phosphorus management is not only responsible for economic loss of farmers but also results in eutrophication ofnearby water-bodies [4]. There are various qualitative and quantitative methods available for estimation of plant-available phosphorus such as inductively coupled plasma (ICP) emission spectroscopy, optical emission spectroscopy (OES) and mass spectroscopy (MS), Nuclear magnetic resonance (NMR), X-ray spectroscopy, secondary ion mass spectroscopy (SIMS) etc. [5-7]. These are labor-intensive. Current study is aiming towards the rapid, *in-situ* and reliable method of soil Phosphorus testing which enabled authors to use photometric methods providing a visible indication of available phosphorus in real time.

2. Materials and methods

Soil samples were collected from different regions from Nashik and stored as per the standard procedures mentioned in soil testing manual of India [2] to check the efficiency of the method. Two soil samples viz. Sample-A and Sample-B obtained through Shrambhoomi Innovations, were sent for standard soil testing procedure provided by (Nation Agro Foundation) NAF within standard time for comparative soil testing [2]. Phosphorus extraction procedures were coupled with Bray's and Morgan's photometric detection methods and modified for this study [8, 9]. Nine different extraction methods (I to IX) were studied viz. I: Wein'sextractant-distilled water, II: Wein's extractant-1M HCl, III: Wein's extractant-0.1 M $CaCl_2$, IV: Bray's extractant no.1-0.003M NH_4F- 0.023M HCl, V: Bray's extractant no.2-0.003 M NH_4F - 0.09 M HCl, VI: Olsen's extractant-0.0499 M sodium bicarbonate, VII: Morgan's extractant-acetate buffer pH 4.8, VIII: Egner's extractant- sodium lactate buffer pH 3.7 and IX: William's extractant-0.4353 M acetic acid. Phosphorus standards were prepared by using potassium di-hydrogen phosphate (Merck: 7778-77-0) in 0 ppm to 80 ppm in respective extractants to obtain the standard cure for each extraction-detection method [10-12]. The tests were done in triplicates and the results analyzed statistically. Student's T-test was performed to ensure the coefficient of determination (R^2) obtained by the test have significant difference [13].

3. Results and Discussion

Both Bray's and Morgan's method for phosphorus estimation were studied individually for all nine extractants aiming primary screening of the combination of extractants and methods. Thestatistically significant combinations were studied further for confirmation of results. Limit of detection (LOD) and limit of quantitation (LOQ) were determined for both the methods.

3.1 Bray's method

Two extractants were showing optimal results; Extractant-XI (William's extractant) with coefficient of determination (R^2) 0.9693 followed by Extractant VI (Olsen's extractant) coefficient of determination (R^2) 0.9648.Combination of Bray's method for William's extractant is further studied for statistically significant range of standards for confirmation, it was found that,mentioned combination showing limit of detection (LOD) 14 ppm and limit of quantitation (LOQ) 47 ppm, with coefficient of determination (R^2) 0.9693 as displayed in figure 1.

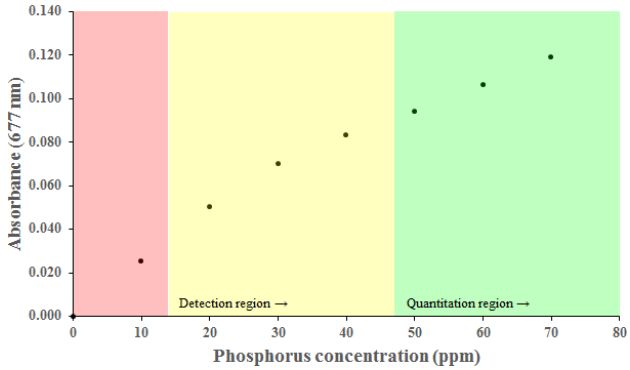

Figure 1: Estimation of phosphorus by Bray's method with William's extraction procedure

3.2 Morgan's method

In the Morgan's estimation method, three of the extractants exhibited statistically significant results viz. Extractant-VIII (Egner's extractant), Extractant-I (Wein'sextractant) and Extractant-VII (Morgan's extractant). Extractant-VIII (Egner's extractant) shown highest coefficient of determination (R^2) 0.9240 followed by Extractant-VII (Morgan's extractant) i.e. 0.9176 and Extractant-I (Wein's extractant) i.e. 0.9011. Morgan's method in combination with Egner's extractant was further studied. It wasfound that above combination was showing limit of detection (LOD) 11 ppm and limit of quantitation (LOQ) 38 ppm, with coefficient of determination (R^2) 0.9803 as displayed in figure 2; which was also highest among both theexperimental methods when results were compared with Bray's method.

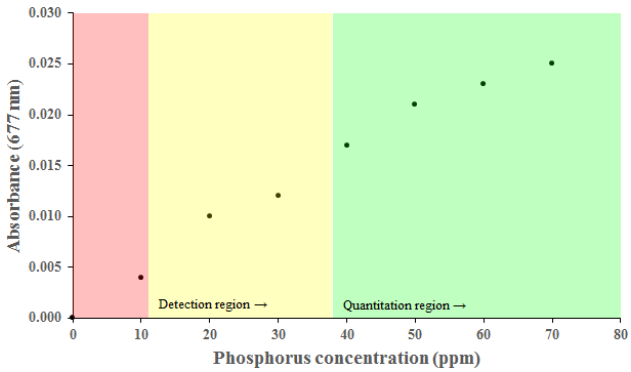

Figure 2: Estimation of phosphorus by Morgan's method with Egner's extraction procedure

3.3 Soil sample testing

Acidic extraction of the soil-phosphorus is commonly practiced in combination with various detection methods. Bio-available forms of soil phosphorus that can be extracted using acidic extraction environment are major quanta of available phosphorus in soil and play important role in plant growth and development [14, 15].Similar observations were obtained in our studies for acidic extractant VIII & IX. William and Stewart highlighted the role of diluted acetic acid which can be used to extract soil phosphates with concentration as low as 50 ppm [16]. Our observations highlights efficiency of acetic acid when used in extraction procedures for soil-phosphorus.

Both the tests were performed in triplicates and coefficient of determination (R^2) obtained were tested for significant difference using student's T-test. It was found that *p-value* (0.019) is less than level of significance ($\alpha = 0.050$) highlighting the significant difference between the two methods.

Since this study is aimed towards designing a suitable *in-situ* method for semi-quantitative detection of phosphorus it is important to consider the highest range of detection, lower limit of detection and quantitation with optimal accuracy. Considering above requirements, Egner's extractant (lactate buffer) with Morgan's method of detection was found to be the most suitable for the purpose followed by William's extractant (acetic acid) with Bray's method.

This method was used for testing soil phosphate of two lab-tested soil samples for determination of available phosphorus by Morgan's method of detection paired with Egner's extractant and compared with standard results provided by NAF. Sample-A and Sample-B were found showing available phosphorus as 60.78 ppm and 90.12 ppm respectively by standard NAF method whereas available phosphorus was observed to be 59.2 ppm and 91.54 respectively when tested with optimized method with average of 97.92% accuracy.

4. Conclusion

Morgan's method, when used with Egner'sextractant,was found to be very effective in devising an *in-situ* model for on field testing of macronutrient phosphorus with statistically considerable accuracy and repeatability. Current study revealed the potential of non-conventional colorimetric methods to be used as in-situ methods for preliminary semi-quantitative estimation of phosphorus in very cost effective and less labor intensive way. Methods followed in current study can be optimized for various soil types and broader range detection of the phosphorus. It also opens the window for various other macronutrient estimations by considering biochemical factors which may enhance the outcome.

5. Acknowledgements

The authors acknowledge the financial support of, National Agro Foundation and Shrambhoomi Innovations. The authors thank the Principal and the staff of Department of

Zoology, The D.G. Ruparel College of Arts, Science and Commerce for their support to carry out this work.

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