Methodology Article *Journal of Plantation Crops, 2013, 41(2): 219-226*

A different approach to soil analysis: Indicative studies

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(Manuscript Received: 01-02-13, Revised: 18-04-13, Accepted: 02-05-13)

Abstract

Soil analysis is a tool that has been employed with the primary goal of providing recommendations for soil rectification, crop productivity and for soil health management. Time tested methods like ammonium acetate extraction and diethylene triamine penta acetic acid (DTPA) are commonly used for analysis of bioavailable nutrients. However, there are some limitations to these methods as both extraction fluids are buffered to neutral or near-neutral pH. Hence extracted nutrients represent a "potential or ideal-case" fertility status of soil instead of an "actual" field status. In the 'Regular methods', we are overlooking the role of pH, the master variable, in determining the availability of nutrients. Hence, in 'Modified methods', the extraction fluid is buffered to actual soil pH. Results obtained with over 150 random samples representing a range of pH, have indicated a difference in values between regular and modified extraction methods. The modified methods (MM) of ammonium acetate and DTPA extraction adjusted to soil pH were found to be better than regular method (RM) for estimation of calcium, magnesium with ammonium acetate and iron and manganese with DTPA in alkaline soils above pH 8.0. For a complete picture of soil health, productivity and fertility, microbiological and enzymatic analysis of soils were included in the present study. Soil solution equivalent medium (SSE) was found to be the appropriate culture medium for microbial counts. A linear relationship was found between urease activity and available nitrogen of soil.

Keywords: Ammonium acetate extract, DTPA extract, pH, soil enzymes, soil solution equivalent (SSE) medium, soil testing

Introduction

Agriculture has been an integral part of Indian civilization since time immemorial. From subsistence farming to commercial agriculture and from the Green Revolution to the Gene Revolution, farmers have been known to embrace change and adapt to the need of the day. The three F's - food, fertilizer and finance have played havoc in the last few years. Added to this are the issues of depleted soils, cost of production, population growth, environmental changes, decrease in arable lands, amongst other problems which seem to threaten agriculture. With these problems on hand, we will have to modify and reevaluate our methodology of agriculture. In our analytical laboratories, we have been conducting analysis of soils, plant tissues and irrigation water for more than four decades and concurrently providing recommendations for use of various soil amendments and fertilizers at the grass root level.

Recently, we have been getting feedback from farmers that soil tests are not necessarily in-sync with field level data. The factor that seems most plausible for this misalliance is the method of buffering extractants to neutral or near neutral pH. This has been supported by Suarez (1996), who states that, "Typically, the exchange reaction is conducted under buffered or constant pH conditions. This serves to provide a reference base for comparison between soils but may result in extractions that are different than those conducted at the pH of the soil in its field condition". This led

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us to modify our methodology so as to give a more realistic picture about availability of cations in soil.

Materials and Methods

150 composite soil samples from surface soil (0-22.5 cm) from various agro-climatic zones varying in soil texture from sandy loam to clay soils and varying soil pH from strongly acidic to strongly alkaline were collected and analysed by regular method (RM) and modified method (MM) of ammonium acetate extraction and diethylene triamine penta acetic acid (DTPA) extraction, in which extractants were adjusted to actual soil pH. Microbiological and enzymatic analysis of important soil enzymes like urease, phosphomonoesterase and hydrolytic enzymes (FDA assay) were also analysed to determine the soil health status. In microbiological analysis, soil solution equivalent (SSE) medium described by Angle *et al*., (1991) adjusted to actual soil pH was used. The details of methods of analysis are given below:

Soil pH: pH was measured in 1:2.5 :: soil:water suspension with glass electrode pH meter as described by Dewis and Freitas (1970).

Regular ammonium acetate method (RM): Soil was extracted with 1:5 :: soil:1N ammonium acetate solution adjusted to pH 7.0. (Knudsen *et al*., 1982). Calcium was determined by flame photometer while magnesium was estimated by atomic absorption spectrophotometer (AAS).

Modified ammonium acetate method (MM): The ammonium acetate extractant was adjusted to the actual soil pH in acidic range, by using dilute acetic acid and to alkaline range by dilute ammonia solution.

Regular DTPA method (RM): Soil was extracted with 1:2 :: soil:DTPA extractant solution adjusted to pH 7.3 (Lindsay and Norvell, 1978) and iron, manganese, zinc and copper were estimated by AAS.

Modified DTPA method (MM): The pH of the DTPA extractant was adjusted to pH of soil by using dilute hydrochloric acid (in acidic soils) and by using dilute sodium hydroxide (in alkaline soils).

Microbiological analysis of soil: Soil solution equivalent (SSE) medium (Angle *et al*., 1991) was used for culturing micro-organisms. The SSE closely simulates the soil solution that microorganisms would be exposed to in their native habitat. SSE medium contains the following components (millimolar): NO_3^- = 2.5; NH_4^+ = 2.5; HPO_4^{2-} = 0.005; Na^+ = 2.5; Ca^{2+} = 4.0; Mg^{2+} = 2.0; K^+ = 0.503; Cl^- = 4.0; SO_4^{2-} = 5.0 and ethylenediamine-di (o-hydroxyphenylacetic acid) = 0.02. The media solution was then adjusted to actual pH of the soil that was being analysed. The pH was adjusted to alkaline range using a mixture of KOH and NaOH and to acidic range by using 1N HCl. This SSE medium was further modified and made selective for growth of bacteria and fungi by using either antifungal or antibacterial agents respectively.

Enzymatic analysis of soils: Urease activity was estimated by incubating the soil with aqueous urea solution, extracting ammonium with 1M KCl and 0.01N HCl and spectrophotometrically quantifying indophenol reaction at 690 nm. (Kandeler and Gerber, 1988).

Phosphomonoesterase activity was analysed by colorimetric estimation of p-nitrophenol released when soil was incubated with sodium p-nitrophenyl phosphate solution and toluene at 37 °C for one hour. The released p-nitrophenol developed a stable colour and was estimated spectrophotometrically at 400 nm. (Tabatabai and Bremner, 1969).

Lipases, proteases and esterases are enzymes involved in the microbial decomposition of organic matter in soil. These enzymes hydrolyze fluorescein diacetate (FDA) to the end-product fluorescein, which was measured spectrophotometrically at 490 nm. (Green *et al*., 2006).

Results and discussion

Levels of calcium estimated by ammonium acetate extraction

Calcium levels extracted by regular ammonium acetate method (RM) buffered at pH 7.0 were found appropriate than modified ammonium acetate method (MM), where the extractant was adjusted to soil pH in moderately acidic to strongly acidic soil pH (below pH 6.0) (Fig. 1) and this is corroborated by pH- nutrient availability chart. In medium alkaline to strongly alkaline pH (pH 8.3-9.3), MM extracted lower amounts of calcium than

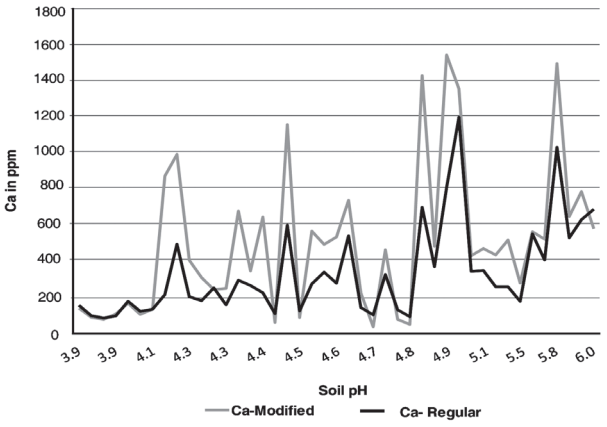

Fig. 1. Calcium extracted by regular & modified ammonium acetate methods in acidic soils

RM (Fig. 2). As per pH chart, the availability of calcium becomes marginal above pH 8.5 and declines with the increase in pH. Hence, in alkaline pH range above pH 8.3, MM might be better method for calcium estimation than RM. Suarez (1996) reported that at higher pH, field condition limits $CaCO_3$ solubility and the extracted values mask potential calcium deficiency or ionic imbalance.

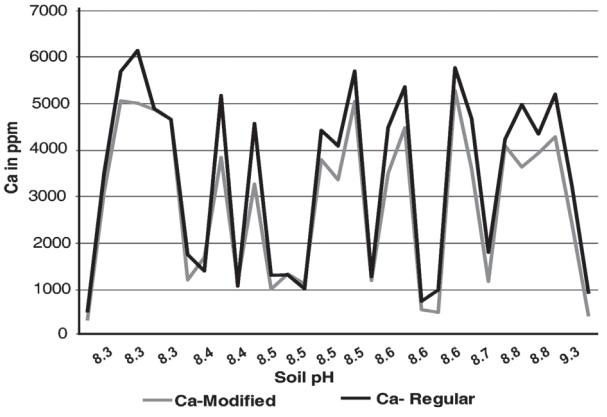

Fig. 2. Calcium extracted by regular and modified ammonium acetate methods in alkaline soils

Levels of magnesium estimated by ammonium acetate extraction

Magnesium levels extracted by regular ammonium acetate method (RM) buffered at pH 7.0 and modified ammonium acetate method (MM) buffered at soil pH were found to be similar in strongly acidic to near neutral pH (pH 3.9-6.7). It seems that either of the methods can be used in this pH range. Grove *et al*. (1982) and Gillman and Hallman (1988) have reported that the ammonium acetate method is widely used in acid soils, which results in higher values of alkaline earth metals. Magnesium extracted by RM was found appropriate than MM in near neutral to medium alkaline pH (pH 6.8-8.5) (Fig. 3 and 4) and it corroborates with pH-nutrient availability chart. In medium alkaline pH (above pH 8.5), magnesium levels extracted by MM were found to be lower than RM (Fig. 4). As per pH - nutrient availability chart, availability of Mg becomes marginal above pH 8.5. Thus, MM seems to be a more appropriate method of estimating magnesium in this pH (above pH 8.5) than RM.

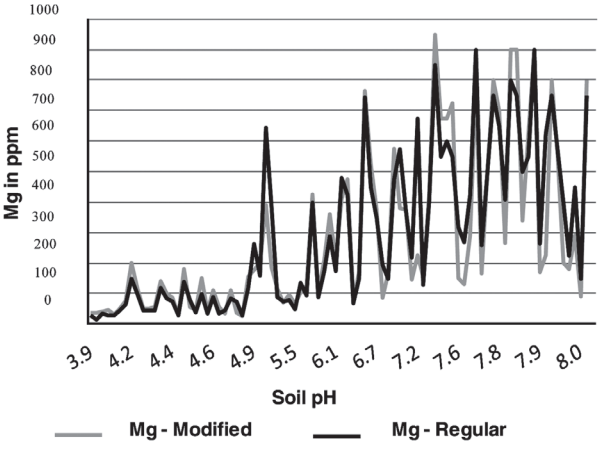

Fig. 3. Magnesium extracted by regular and modified ammonium acetate methods in acidic to alkaline soils

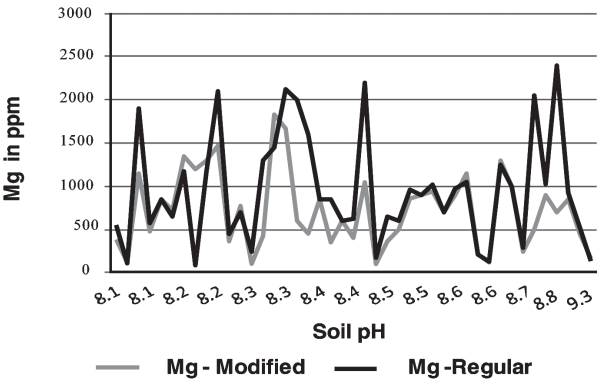

Fig. 4. Magnesium extracted by regular and modified ammonium acetate methods in alkaline soils

Levels of iron extracted by diethylene-triamine penta acetic acid (DTPA) method

Iron levels extracted by modified method of DTPA extraction (MM) were found to be lower than regular method of DTPA extraction (RM) in medium acidic to strongly acidic pH (below pH 5.7) (Fig. 5). As per the pH-nutrient availability chart, very little iron is available in the pH range below 5.0.

Loeppert and Inskeep (1996) have reported that DTPA soil test method is appropriate for determining iron levels in neutral to calcareous soils and may not be suitable for acidic soils below pH 6.0. In the medium acidic to medium alkaline pH (pH 5.7-8.1), both RM and MM extracted almost similar amounts of iron (Fig. 5). Thus, either of the two methods can be used for estimating iron in this pH. In medium alkaline to strongly alkaline pH (above pH 8.1), MM has given very low values of extractable iron than RM. (Fig. 5). As per pH-nutrient availability chart, iron is marginally available above pH 8.1. Haynes and Swift (1983) found significant variability in extractable iron as a function of pH of extracting solution.

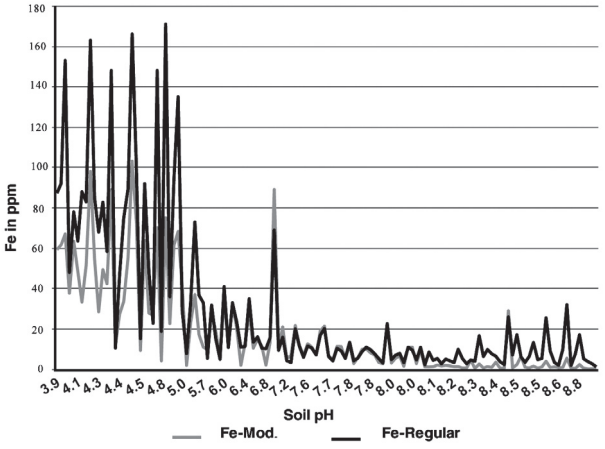

Fig. 5. Iron extracted by regular and modified DTPA methods in acidic to alkaline soils

Levels of manganese extracted by diethylene-triamine penta acetic acid (DTPA) method

Manganese levels were found to be lower in the regular method of DTPA extraction (RM) than modified method (MM) in strongly acidic to near neutral pH (below pH 6.8) (Fig. 6). In the pH range neutral to medium alkaline (pH 6.8- 8.0), both RM and MM extract almost similar amounts of Mn. In this pH range, either of the methods can be used to estimate Mn levels in soil. In the pH range medium alkaline to strongly alkaline (above pH 8.0), MM extracted lower levels of Mn compared to RM (Fig 6). As per the pH-nutrient availability chart, the availability of Mn is very low above pH 8.0. Hence MM seems to be a better method of extraction of Mn in this pH range than RM. Adams (1965) has reported that Mn availability is greatly influenced by soil pH.

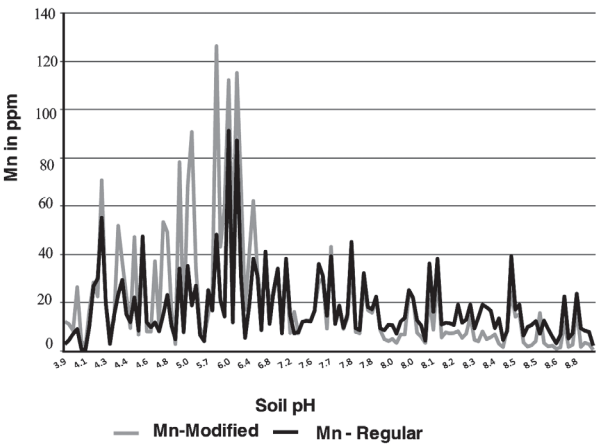

Fig. 6. Manganese extracted by regular and modified DTPA methods in acidic to alkaline soils

Levels of zinc and copper extracted by diethylene-triamine penta acetic acid (DTPA) method

Zinc levels extracted by regular DTPA method (RM) were found to be almost similar to modified DTPA method (MM) in acidic, neutral as well as in alkaline pH range (pH 3.9-8.8) (Fig. 7) and not influenced by variation in soil pH. Hence, either of the methods can be used for the estimation of Zn levels in soil.

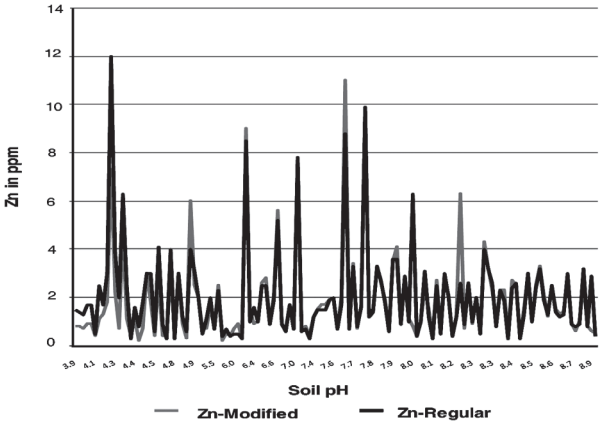

Fig. 7. Zinc extracted by regular and modified DTPA methods in acidic to alkaline soils

Copper levels estimated by modified method of DTPA extraction (MM) were found to be lower than the regular method of DTPA extraction (RM) in slightly acidic to strongly acidic pH (below pH 6.0) (Fig. 8). As per the pH- nutrient availability chart, copper availability is marginally low below pH 5.0. Hence MM of DTPA extraction seems to be more appropriate than the RM. From slightly acidic

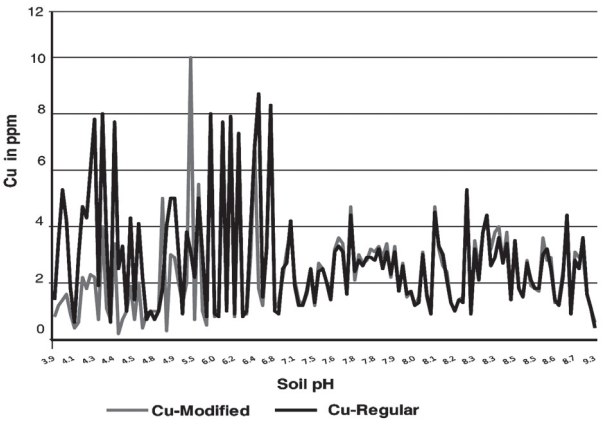

Fig. 8. Copper extracted by regular and modified methods of DTPA in acidic to alkaline soils

to strongly alkaline pH (pH 6.0-9.3), copper extracted by RM and MM of DTPA extraction was found to be almost similar and hence either of the methods can be adopted for copper estimation (Fig. 8).

Microbiological analysis using nutrient agar medium and soil solution equivalent medium (SSE)

The goal of this part of the experiment was to understand the importance of micro-organisms in nutrient cycling and bio-geochemical reactions. Current microbiological methods examine the growth of certain micro-organisms on selective medium. Selective medium allows maximum growth of a particular micro-organism. However, selective medium will give only potential case scenario, wherein all conditions are ideal for the growth of that micro-organism. Since we were interested in real case scenario, we have used the soil solution equivalent (SSE) medium, which was adjusted to be selective for certain bacteria and further adjusted to actual soil pH. (Zuberer, 1996). The advantage of using SSE medium is that it closely simulates the soil solution that microorganisms would be exposed to in their native habitat. By using this medium, we got a good picture of the micro-organisms that a particular soil can support.

There is little doubt that the growth rate of microorganisms in soil is dramatically different from the growth rate of the same microorganisms on artificial medium. (Nannipieri *et al*., 1990). It is a known fact that fungal colonies proliferate more under acidic conditions while bacteria grow better under alkaline conditions. Rousk *et al*. (2009) have shown that growth-based measurements reveal a five fold decrease in bacterial growth and a five fold increase in fungal growth under lower pH levels. Current data also show that SSE extracts more fungus in the acidic medium than in alkaline medium (Fig. 9). SSE medium tends to extract more bacterial colonies compared to regular nutrient agar medium across a range of pH. Bacterial counts were almost 100 fold higher in SSE medium compared to regular (nutrient agar) medium suggesting that SSE medium may be a more indicative medium to use in microbiological analysis (Fig. 10).

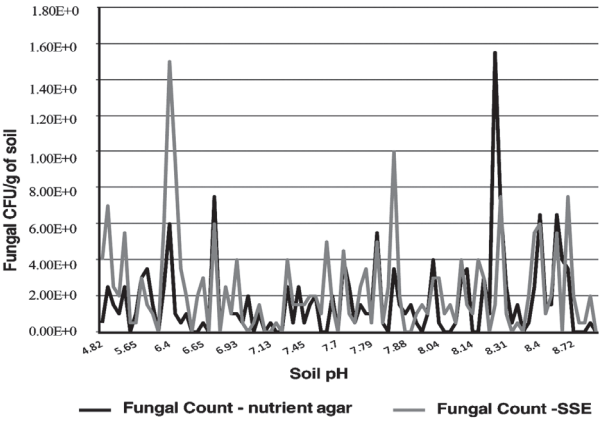

Fig. 9. Fungal CFU g^{-1} of soil under regular nutrient agar medium and SSE medium

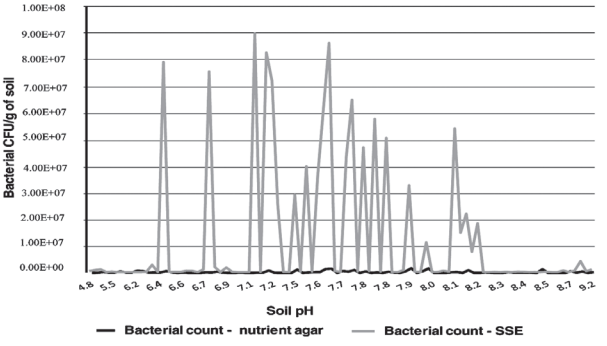

Fig. 10. Bacterial CFU g^{-1} of soil under regular nutrient agar and SSE medium

Enzymatic analysis with relation to microbial count and availability of nutrients in soil

Enzymatic analysis of key enzymes like urease, phosphomonoesterase and group of hydrolytic enzymes like protease, esterase and lipase (FDA assay) will be meaningful to determine the

soil health status and hence they have been included in the current study.

Urease activity with relation to availability of nitrogen

Urease is the enzyme that catalyses the hydrolysis of urea into ammonium ion which can be held on the exchange sites of soil clay, absorbed by plant roots or converted into nitrite (NO_2^-) by *Nitrosomonas* and finally to nitrate (NO_3^-) by *Nitrobacter*. Plants can utilize either ammonium or nitrate or both. Large number of bacteria, fungi and actinomycetes in soil possess urease. Activity of urease increases in proportion with the size of soil microbial population and organic matter content. Urease is also a good indicator of N-cycling in soil. Urease enzyme is sensitive to heavy metal contamination and hence a decrease in urease over time can indicate pollution of soils. The samples analysed in the present study showed that as the urease activity in the soil increases, more nitrogen (N) becomes available. In other words, a linear relationship was found between urease activity in soil and available nitrogen (Fig. 11).

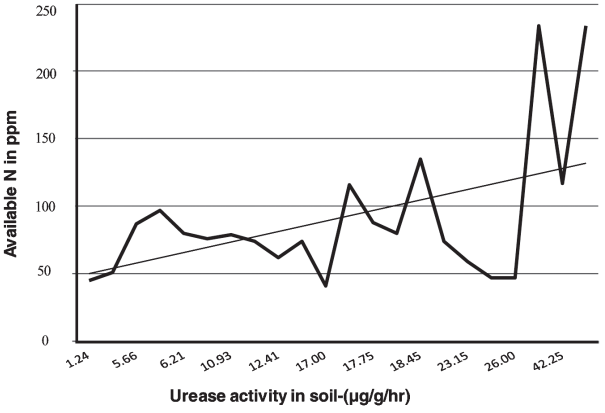

Fig. 11. Available N in ppm as a function of urease activity in soil

Phosphomonoesterase activity in relation to total microbial population and phosphorus availability in soil

Acid and alkaline phosphatases are important in the mineralization of soil organic phosphorus. They are extracellular enzymes that catalyze the hydrolysis of organic phosphates to inorganic orthophosphates. Eivazi and Tabatabai (1977) and Juma and Tabatabai (1977, 1978) have reported that acid phosphatase is predominant in acid soils and alkaline phosphatase in alkaline soils. Higher plants are devoid of alkaline phosphatase activity and hence the alkaline phosphatase activity in soil is derived totally from micro-organisms.

Phosphorus solubilising bacteria and fungi secrete phosphatases. It includes a variety of microorganisms like *Pseudomonas, Arthrobacter, Bacillus, Sphingomonas, Streptomyces, Azospirillum, Enterobacter* and *Erwinia*, amongst others. These enzymes are sensitive to drought and heavy metal contamination. In our study, mild linear trend was found between total microbial population and phosphomonoesterase activity (Fig. 12). This may be due to the fact that many genera of fungi and bacteria are involved in secretion of phosphomonoesterases and their activities are influenced by soil pH and other environmental factors.

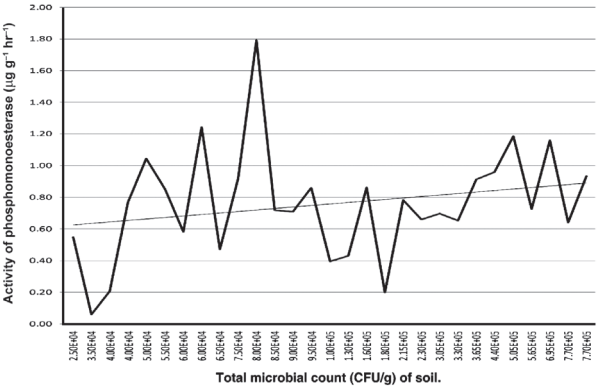

Fig. 12. Phosphomonoesterase activity in soil as a function of Total microbial count

Phosphomonoesterases are an important link between organic phosphorus and available phosphorus in soil. In our study, a mild linear trend was found between phosphomonoesterase activity and available phosphorus in soil (Fig. 13). The effect of soil pH and presence of orthophosphate, a competitive inhibitor of phosphatases in soils should also be ascertained (Juma and Tabatabai, 1978).

Fluorescein diacetate activity (FDA) with relation to total microbial count

FDA is a substrate that is hydrolysed by multiple enzymes like lipases, proteases and esterases, which are widely found in soil borne microorganisms and hence is a direct indicator of

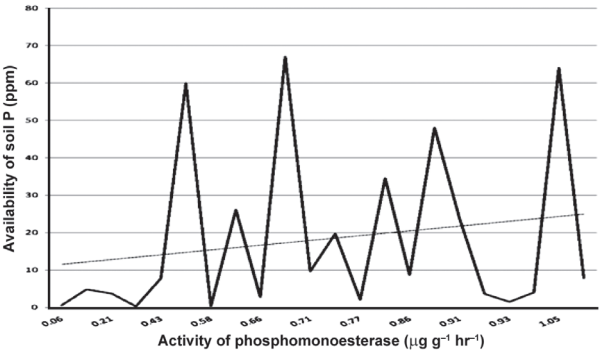

Fig. 13. Available P (ppm) as a function of phosphomonoesterase activity in soil

organic matter turnover in soil. The FDA assay has been suggested as a measure of total hydrolytic capacity of the soils and a broad spectrum indicator of soil biological activity (Bandick *et al*., 1999). FDA is a relatively non-polar compound and so it easily diffuses through the cell membrane, where it gets hydrolysed to form the fluorescent fluorescein compound (Rothman and Papermaster, 1966). FDA activity in soil is sensitive to pesticide contamination. In the present study, a mild linear trend was found between the total microbial counts (CFU g^{-1}) and FDA activity in soil (Fig. 14).

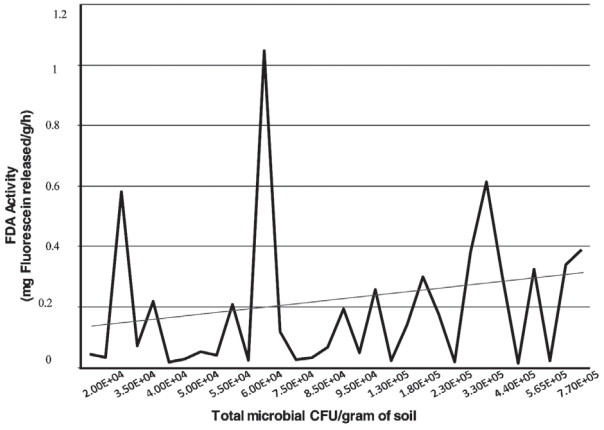

Fig. 14. FDA activity as a function of soil microbial count (CFU g^{-1} soil)

Conclusions

I. The modified method of ammonium acetate extraction (MM) adjusted to soil pH was found to be better than the regular method (RM) buffered at neutral pH (pH- 7.0) for estimation of calcium and magnesium in alkaline soils (pH above 8.3).

II. The modified method of DTPA extraction (MM) adjusted to soil pH was found to be better than the regular method (RM) buffered at pH 7.3 for estimation of iron and manganese in alkaline soils (pH above 8.0) as well as for estimation of iron and copper in acidic soils (pH below 5.7).

III. The soil solution equivalent (SSE) medium adjusted to soil pH was found to be better culture medium than regular nutrient agar medium for culturing fungal and bacterial colonies in acidic as well as in alkaline soils simulating field conditions indicative of soil health status.

IV. A linear relationship was found between urease activity and available nitrogen of soil.

Future Line of work

i. In future, data need to be generated on various crops at multi-locations to obtain a relationship between nutrients concentration / uptake with soil test values extracted by regular methods of ammonium acetate and DTPA extract as well as with modified method of both extractants for selecting the appropriate extractant.

ii. For culturing bacteria and fungi, more data are required with respect to regular nutrient agar medium and soil solution equivalent (SSE) medium adjusted to soil pH simulating field conditions.

iii. More work needs to be done to examine the relationship between phosphomonoesterase activity with total microbial counts and available phosphorus (P) of soil. Fluorescein diacetate (FDA) activity with total microbial counts of soils may also be examined further.

References

Adams, F. 1965. Manganese. In: *Methods of Soil Analysis*. Part 2, (Ed.) C.A Black ASA, Madison, W1. pp. 1011-1018.

Angle, J.S., McGrath, S.P. and Chaney, R.L. 1991. New culture Medium containing ionic concentrations of nutrients similar to concentrations found in the soil solution. *Applied Microbiology* **57**: 3674-3676.

Bandick, A.K. and Dick, R.P. 1999. Field management effects on soil enzyme activities. *Soil Biology and Biochemistry* **31**: 1471-1479.

Dewis, J. and Freitas, F. 1970. pH values. In: *Physical and Chemical Methods of Soil and Water Analysis*. Soil Bulletin 10, Food and Agriculture Organisation of the United Nations, Rome. pp. 65-67.

Eivazi, F. and Tabatabai, M.A. 1977. Phophatases in soils. *Soil Biology Biochemistry* **9**: 167-172.

Gillman, G.P and Hallman, M.J.1988. Measurement of exchange properties of Andisols by the compulsive exchange method. *Soil Science Society of America Journal* **52**: 1196-1198.

Green, V.S., Stott, D.E., Diack, M. 2006. *Soil Biology and Biochemistry* **38**: 693-701.

Grove, J.H., Fowler, C.S. and Sumner, M.E. 1982. Determination of the charge character of selective acid soils. *Soil Science Society of America Journal* **46**: 32-38.

Haynes, R.J., and Swift, R.S. 1983. An evaluation of the use of DTPA and EDTA as extractants for micronutrients in moderately acid soils. *Plant and Soil* **74**: 111-122.

Juma, N.G. and Tabatabai, M.A. 1977. Effects of trace elements on phosphatase activity in soils. *Soil Science Society of America Journal* **41**: 343-346.

Juma, N.G. and Tabatabai, M.A. 1978. Distribution of phosphomonoesterases in soils. *Soil Science* **126**: 101-108.

Kandeler, E. and Gerber, H. 1988. Short term assay of soil urease activity using colorimetric determination of ammonium. *Biology and Fertility of Soils* **6**: 68-72.

Knudsen, D., Peterson, G.A. and Pratt, P.F. 1982. Lithium, sodium and potassium (13). In: *Methods of Soil Analysis, Part 2,* 2nd edition. *Chemical and Microbiological properties.* (Eds.) Page, A.L, Miller, R.H. and Keeney, D.R. American Society of Agronomy, Inc. Madison, Wisconsin, USA. pp. 225-246.

Lindsay, W.I. and Norvell, W.A. 1978. Development of DTPA test for zinc, iron, manganese and copper. *Soil Science Society of America Journal* **42**: 421-428.

Loeppert, R.H. and Inskeep, W.P. 1996. *Methods of Soil Analysis, Part 3. Chemical methods.* SSSA Book Series No 5. USDA-ARS, Soil Science society of America and American Society of Agronomy, USA. 655 pp.

Nannipieri, P., Greco, S. and Ceccanti, B. 1990. Ecological significance of the biological activity in soil. In: *Soil Biochemistry,* **(**Eds.) Bollag, J.M. and Stotzky, G. Marcel Dekker Inc., New York. pp. 293-356.

Rothman, B and Papermaster, B.W. 1966. Membrane properties of living mammalian cells as studied by enzymatic hydrolysis of fluorogenic esters. In: *Proceedings of the National Academy of Science*, USA. **55**: 134-141.

Rousk, J., Brookes, P.C. and Baath, E. 2009. Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. *Applied Environmental Microbiology* **75**(6): 1589-1596.

Suarez, D.L. 1996. *Methods of Soil Analysis, part 3. Chemical methods*-SSSA Book Series No. 5. USDA-ARS, Soil Science Society of America and American society of Agronomy, USA. pp. 582-585.

Tabatabai, M.A. and Bremner, J.M. 1969. Use of p-nitrophenol phosphate for the assay of soil phosphatase activity. *Soil Biology Biochemistry* 1: 301-307.

Zuberer, D.A. 1996. *Methods of Soil Analysis, part 2. Microbiological and Biochemical Properties*- SSSA Book Series No. 5. USDA- ARS, Soil Science society of America and American Society of Agronomy, 135 pp.