ISSN 0970-7050
R. No. 48407/88

Journal of Hill Research

Volume 19, Number 1, June 2006

Sikkim Science Society

Journal of Hill Research
19(1) : 13-23, 2006

Effect of Fertilizers Treatment on Soil Microbial Population Numbers and Enzyme Activities under Leguminous Cultivation

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(Received 9 July 2005; revised version received 18 February 2006; accepted 21 March 2006)

ABSTRACT

Microbial population numbers and their enzyme activities in terms of different enzymes, namely dehydrogenase, urease and phosphatase were estimated in inorganic, organic and organic fertilizers treatment. The population of fungi and bacteria increased from pre-fertilizers treatment (April) to post treatment (May). The highest fungal population was recorded in NPK treatment whereas the maximum bacterial population was recorded in FYM fertilizer treatment. A positive correlation was established between dehydrogenase activity and fungal population ($P \leq 0.001$) in NPK and FYM treatment in the both soil layer ($P \leq 0.001$). Maximum Urease activity was observed in NPK treatment in May. However, maximum phosphatase activity was obtained in September where it markedly increased from August to September. The results of the investigation indicated that fertilizers treatment has an impact on microbial population numbers and microbial enzyme activities.

INTRODUCTION

Enzymes are very responsive to different agricultural soil conservation practices such as non-tillage, organic amendments, crop rotation (Miller and Dick, 1995; Banerjee *et al.* 1997; Bergstrom *et al.*, 1998) and organic cultivation (Beyer *et al.*, 1992). Soil dehydrogenase is an extra-cellular enzyme which is considered to be a good tool to measure microbial oxidative activity (Ross, 1971) as an indicator of any disruption caused by pesticide application, trace element discharge and soil management practices (Reddy and Faza, 1989), as a measure of microbial biomass (Ladd, 1978) and measure of soil respiration.

Urease is a hydrolyse enzyme responsible for hydrolytic conversion of the substrate, urea into carbon dioxide and ammonia. Owing to this property, it has an applied importance in the N-economy of soil. Phosphatase enzyme mediates the release of inorganic phosphorous from organically bound phosphorous and can be inhibited by inorganic phosphate, which produces a feedback inhibition of this enzyme (Nannipieri *et al.*, 1979). Soil enzyme activity is often used as an index of microbial activity in soils as well as their fertility. Therefore, an attempt has been made to assess the microbial population numbers and activity under fertilizers treatment of leguminous cultivation by measuring the enzymes activities.

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MATERIALS AND METHODS

LOCATION OF THE STUDY AREA

The study was carried out at an upland experimental block at North Eastern Hill region complex, Barapani, Shillong; Meghalaya, India on Groundnut (*Arachis hypogaea* L.). The geographical position of the study site is at 25^0 38' N latitude and 91^0 52' E longitudes and is situated at an altitude of 850 msl. The soil of the experimental site was sandy loam (54.50%) with moderate permeability, silt (30.80%) and clay (14.45%) and soil pH ranged from pH 4.9 to pH 6.0.The climate of the study area is humid and sub-tropical. The area receives heavy rainfall along the Cherapunjee range through long peninsular belt. The rain starts from middle of April and it continues till late October.

High yielding variety of groundnut ICGS-76 from International Crop Research Institute for Semi-Arid Tropics, Hyderabad India was sown in the year 2001 and 2002. The experimental field was divided into four blocks with three replicates, each for different treatments and levels of fertilizer. The optimum fertilizers dosage for groundnut was applied as *Inorganic fertilizers* (N = urea = 20kg/ha; P = single phosphate = 60kg/ha; K = muriate of potash = 40kg/ha; *Organic fertilizer* (Farmyard Manure = 10t/ha and *Combination of inorganic and organic fertilizers* (N = 10kg/ha; P = 30kg/ha; K = 20kg/ha; FYM = 5t/ha)]. According to the types of fertilizer treatment, each of the experimental plots viz., controls, inorganic, organic and combinations of inorganic and organic were designated as CTRL, NPK, FYM and NPK+FYM respectively. The experimental block was set up in a slope land terrace, with a good drainage system. Each triplicate plot had a size of 3 X 4 m^2 and groundnut was sown in a 10 X 30 cm spacing rows.

SOIL SAMPLING AND SOIL ANALYSES

Soil samples were collected randomly at monthly intervals from each treatment at 0-10 cm and 10-20 cm from April (pre-sowing) to October (post harvest).

ENUMERATION OF FUNGI AND BACTERIA IN SOIL

Serial dilution plate method (Parkinson *et al.,* 1971) was followed for the isolation of fungal and bacterial populations. Bacteria were enumerated using nutrient agar (Difco manual, 1953) and fungi on rose Bengal agar (Martin, 1950) and incubated at 25 ± 1° C for 5 days for fungi and at 30±1° C for 24 hours for bacteria.

DETERMINATION OF ENZYME ACTIVITIES IN SOIL

Casida's (1977) 2-3-5-Triphenyl tetrazolium chloride (TTC) reduction technique was used to assay dehydrogenase activity. Urease activity was estimated by McGarity and Myer's (1967) method and phosphatase activity by the method of Tabatabai and Bremner (1969).

RESULTS AND DISCUSSION

Effect of fertilizers on population of fungi and bacteria in soil

The colony form unit of bacteria was markedly higher than that of fungi as expected since within the majority of soil microorganisms, bacteria accounts up to 99% of the total microbiota. The increase in fungal and bacterial population after the addition of fertilizers (Fig. 1 and 2) might be due to the increased agricultural management practices such as cropping systems, fertilizer application, cultivation practices and soil organic amendments (Tilak *et al.,* 1995). In the first year, at surface soil, the fungal population ranged from 7.3 x 10^3 to 1.1 x 10^5 cfu/ g of soil in FYM and NPK plots in October and May respectively whereas, at subsurface soil layer it ranged from 8.8 x 10^2 to 4.2 x 10^4 cfu/ g of soil in NPK plot in October and May respectively. In second year at surface soil layer, fungal population ranged from 1.3 x 10^4 to 8.4 x 10^4 cfu/ g of soil in FYM and NPK plots in April and August respectively whereas, at subsurface soil layer it ranged from 3.7 X 10^3 to 2.7 x 10^4 cfu/ g of soil in NPK plot in April and May respectively. The maximum fungal population at NPK treated plot (Fig. 1) is in agreement with the observation of Upadhayay and Rai (1979), which could be attributed that higher fertility and aeration of soil favoured wider spectrum of fungal genera and species.

In 2001, bacterial population ranged between 0.5 x 10^5 cfu/ g of soil in April in CTRL plot and 13.3 x 10^5 cfu/ g of soil in September in FYM plot at the surface layer. Whereas, at subsurface soil layer, it ranged between 0.2 x 10^5 cfu/ g of soil in April in FYM plot and 8.8 x 10^5 cfu/ g of soil in September in NPK plot. In 2002 at surface soil layer, the bacterial population ranged between 3.8 x 10^5 cfu/ g of soil in September in NPK+FYM plot and 17 x 10^5 cfu/ g of soil in July in NPK plot. At 10-20 it ranged between 1 x 10^5 cfu/ g of soil in October in CTRL plot and 5.8 x 10^5 cfu/ g of soil in June in FYM plot. The peak in bacterial population in FYM and NPH+FYM treatments could be due to the increased in nutrient supply and cation exchange capacity by farmyard manures (Nambiar, 1994).

Effect of fertilizers on dehydrogenase enzyme activity

The increasing trends in dehydrogenase activity from pre-fertilizers treatment to post fertilizers treatment could be due to the increase in soil microbial population or due to the effects of soil management practices i.e. fertilization and plowing of soil for seedbed preparation (Jenkinson and Powlson, 1976). In the first year, the enzyme activity dropped remarkable from May to June onward, whereas such activity was not observed in the second year (Fig. 3). At the surface soil layer in the first year, dehydrogenase activity ranged from 0.30 to 1.33 mg TPF/ g dry soil/ 24 h in NPK and NPK+FYM plots in September and May respectively. In the second year, it ranged between 0.065 and 0.63 mg TPF/ g dry soil/ 24 h in NPK+FYM and FYM plots in April and August respectively. At the subsurface soil layer in the first year, dehydrogenase activity ranged from 0.07 to 1.28 mg TPF/ g dry soil/ 24 h in NPK and NPK+FYM plots in September and May and respectively. In the second year, it ranged between 0.01 and 0.33 mg TPF/ g dry soil/ 24 h in NPK+FYM and FYM plots in April

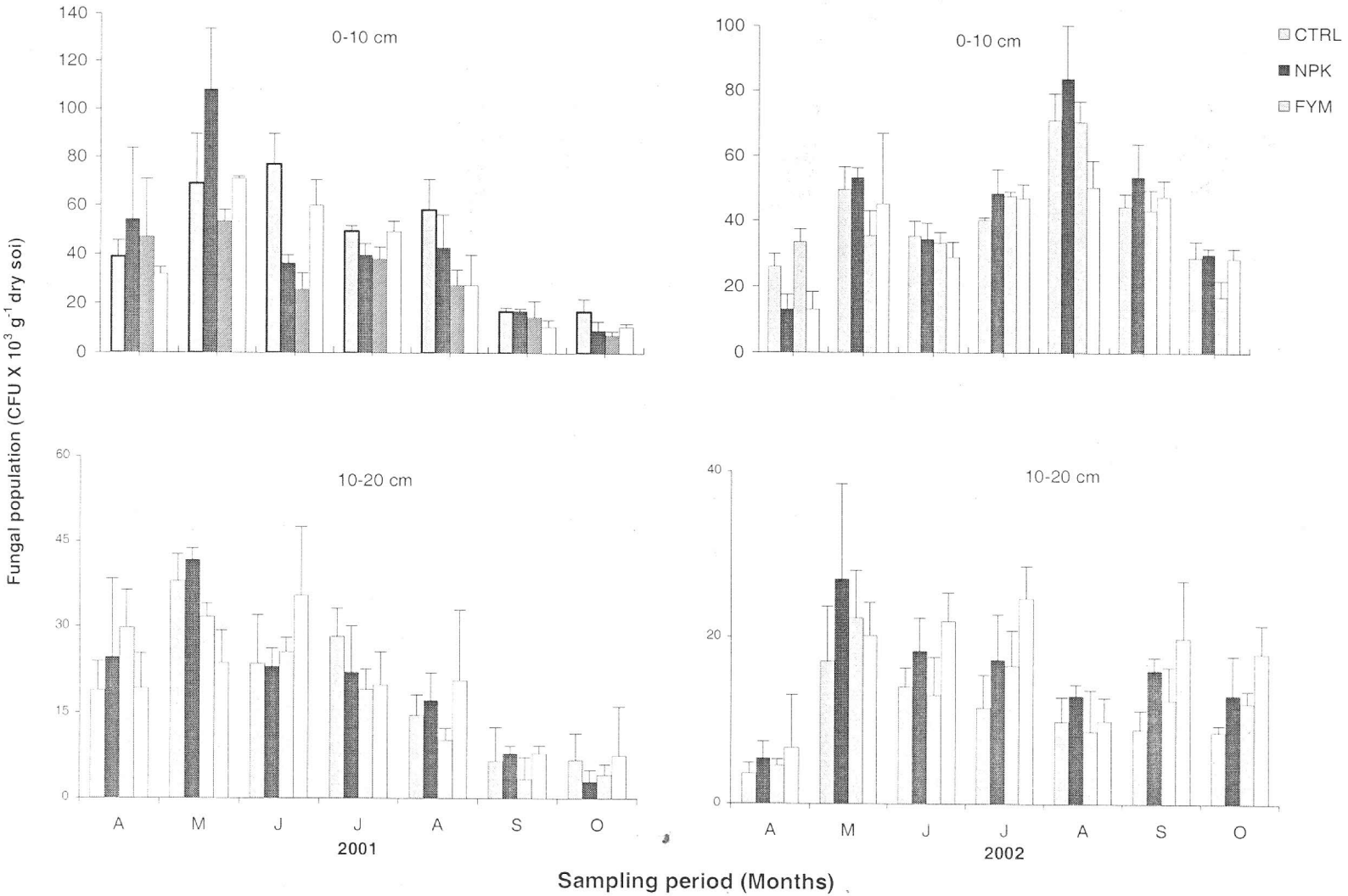

Fig 1. Fungal population in groundnut field soil at 0-10 cm and 10-20 cm depths.

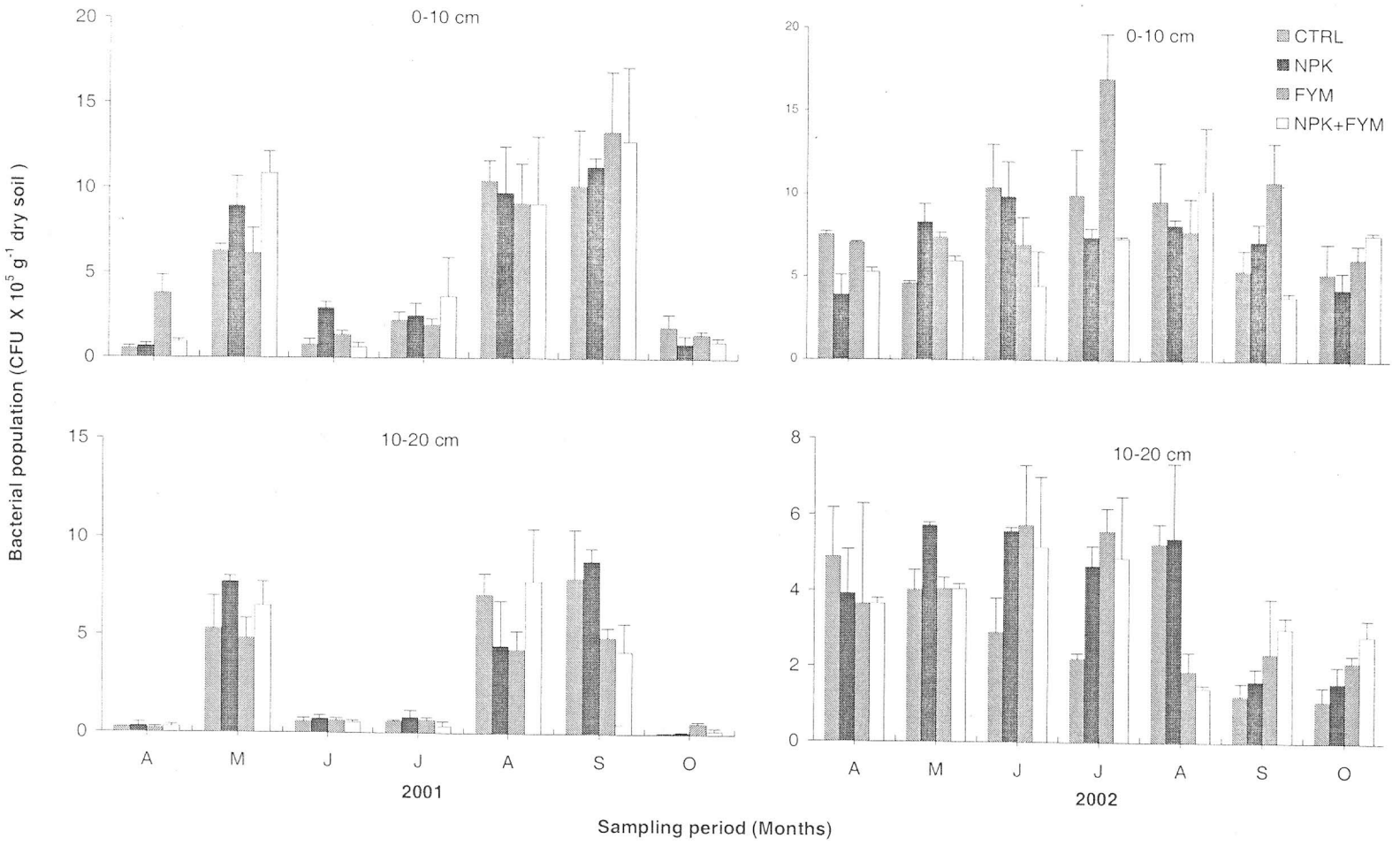

Fig 2 Bacterial population in groundnut field soil at 0-10 cm and 10-20 cm depths.

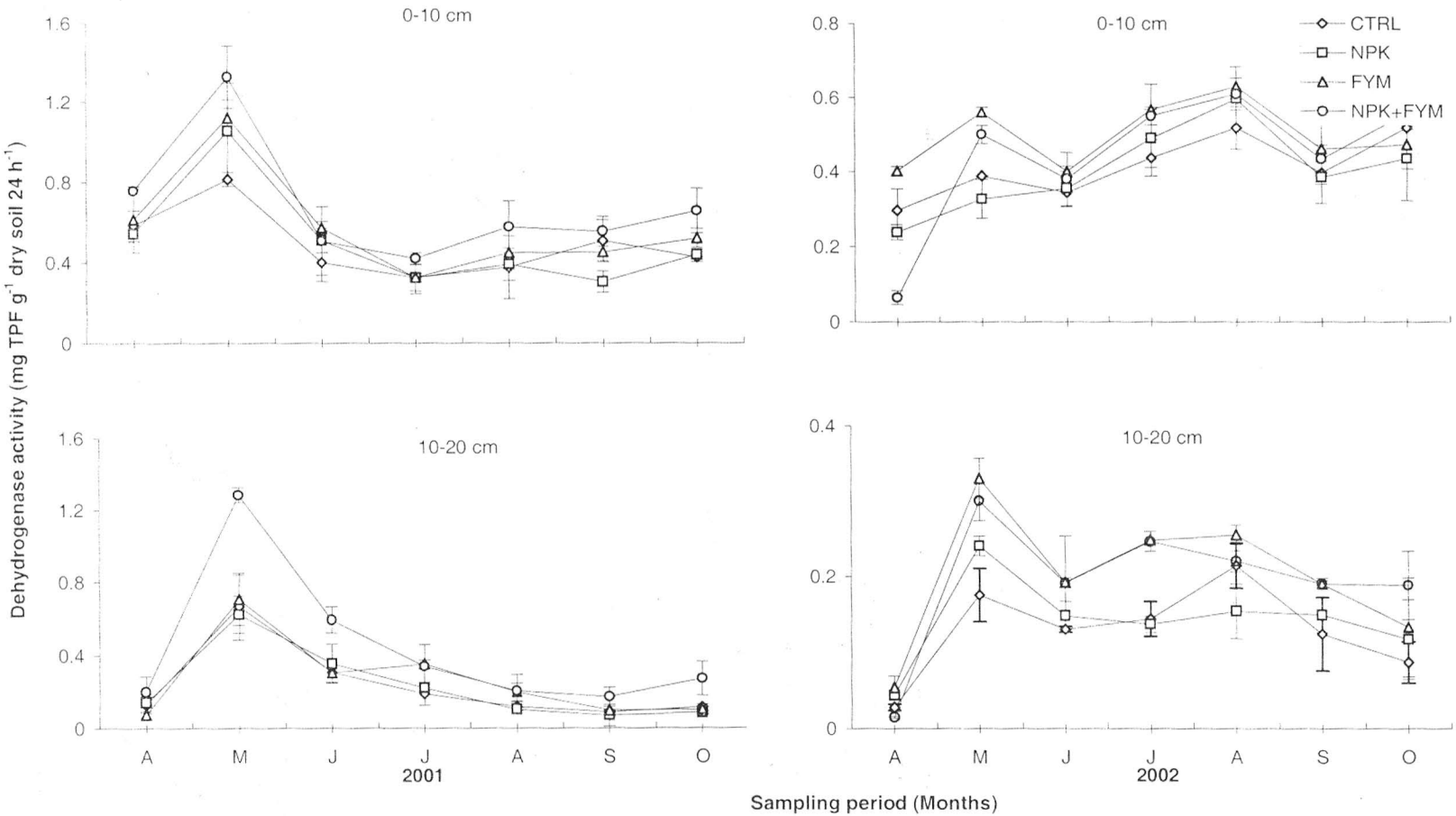

Fig 3. Dehydrogenase activity in groundnut field soil at 0-10 cm and 10-20 cm depths.

and May respectively. Therefore, it can be concluded that the dissimilar activity of dehydrogenase in the first and second year could be due to the effect of soil microbial population, seasonal conditions and soil management which were strongly linked to the activity of soil enzyme (Deng and Tabatabai, 1997; Klose *et al.*, 1999).

The one-way analysis of variance (ANOVA) showed insignificant variation ($P \leq 0.05$) of dehydrogenase activity among treatments at two soil depths, and a significant variation ($P \leq 0.001$) of enzyme activity between surface and subsurface soil layers was observed except in NPK+FYM plot. Dehydrogenase activity was positively correlated with soil temperature ($P \leq 0.001$) in all plots. Correlation coefficient values ($P \leq 0.001$) showed that the enzyme activity at surface soil layer was positively correlated with enzyme activity at subsurface soil layer.

Maximum dehydrogenase activity in FYM and NPK+FYM plots coincided with the observation of Simek *et al.* (1999), that dehydrogenase activity was lower in soil that had received the largest amount of fertilizers; which suggests that it was highly sensitive to the inhibitory effects associated with large amount of fertilizer additions. Another possibility of higher enzyme activity in the manure treated plot is that manure promoted biological and microbial activities, which accelerated the breakdown of organic substances in the added manure (Parham *et al.*, 2002) and that within each sampling period, the highest dehydrogenase activity was in those soil treated with animal manure, while lowest was in the CTRL or NP-treated plots. Thus, the addition of FYM and NPK+FYM enhanced and promoted dehydrogenase enzyme activity (Ross, 1971, Tiwari, 1996). It can be hypothesized that FYM treatment was thus expected to influence more on the activity of dehydrogenase than that of NPK+FYM treatment (James *et al.*, 1996). Since soil microbial population control the soil dehydrogenase activity, the fertilizers treatment that affect on the microbial population was believed to be more effective on the dehydrogenase activity rather than plant factors (Asiegbu, 1984).

The surface soil layer showed a higher enzyme activity than the subsurface soil layer. The insignificant variation of dehydrogenase activity within treatments coincided with the finding of Tiwari (1996) that no significant impact of individual treatments of the fertilizers on the dehydrogenase activity. It was well documented that some soil microbial activity was affected by plant through root exudation, but the present result showed that the plant factors might not influence dehydrogenase activity, because it was observed that the enzyme activity in FYM and NPK+FYM plots was not dropped even after post harvest. The analysis of variance that showed a significant variation of dehydrogenase activity between surface and sub-surface layers could be due to the differences in soil microbial population and environmental factors at these layers.

It can be concluded that addition of fertilizers i.e. FYM (organic fertilizers) and NPK+FYM (combination of inorganic and organic) as well as soil management practices have an impact on the size and activity of soil dehydrogenase enzyme and the removal of plant from the plot does not show significant effect on enzyme activity.

Effect of fertilizers on urease enzyme activity

The urease activity showed consistent trends of distribution within sampling month in the first and second years. Maximum urease activity was displayed in NPK plot in May (Fig. 4). The increased urease activity from pre-fertilizers treatment to post treatment was due to the increased in soil microbial population (Tiwari *et al.*, 1988). In general, greater urease activity was observed at fertilizers treated plots than the control plot which was in agreement with the finding of Garcia-Gil *et al.* (2000) that mineral fertilization plot had the greatest urease activity than unfertilized plot. So, the increased activity of enzyme in treated plots than the control could be due to the presence of higher soil microbial population in treated plots (Asiegbu, 1984).

In the first year at surface soil layer, urease activity ranged between 0.02 and 0.07 NH^+_4-N/ g dry soil/ 3 h in CTRL and NPK plots in June and May respectively. At subsurface soil layer, enzyme activity ranged from 0.01 to 0.07 NH^+_4-N/ g dry soil/ 3 h in NPK plot in May and June. In the second year at surface soil layer, the enzyme activity ranged between 0.04 and 0.11 NH^+_4-N/ g dry soil/ 3 h in NPK+FYM and NPK in October and May respectively. At subsurface soil layer, the enzyme activity ranged between 0.03 and 0.10 and NH^+_4-N/ g dry soil/ 3 h in NPK+FYM and NPK in October and May respectively.

The one-way analysis of variance (ANOVA) showed insignificant variation ($P \leq 0.05$) of urease activity among treatments and between surface and subsurface soil layers. Correlation coefficient values ($P \leq 0.001$) showed that the enzyme activity at surface soil layer was positively correlated with enzyme activity at subsurface soil layer. In CTRL plot at both the soil layer, urease activity was positively correlated with pH ($P \leq 0.01$). The consistent distribution of urease activity within sampling period i.e. the enzyme activity drastically dropped in June coincided with the reports of Schiner *et al.* (1980), that compound fertilization inhibited urease activity after six weeks of application.

Within each sampling months, urease enzyme showed a fluctuation activity among treated and untreated plots, where it was expected to show higher enzyme activity only at treated plots. It was observed that in some months, CTRL plot exhibited higher activity than fertilized plot and the enzyme activity dropped at post harvest. So, the fluctuation in the enzyme activity could be due the effects of groundnut plant through its rhizosphere effect

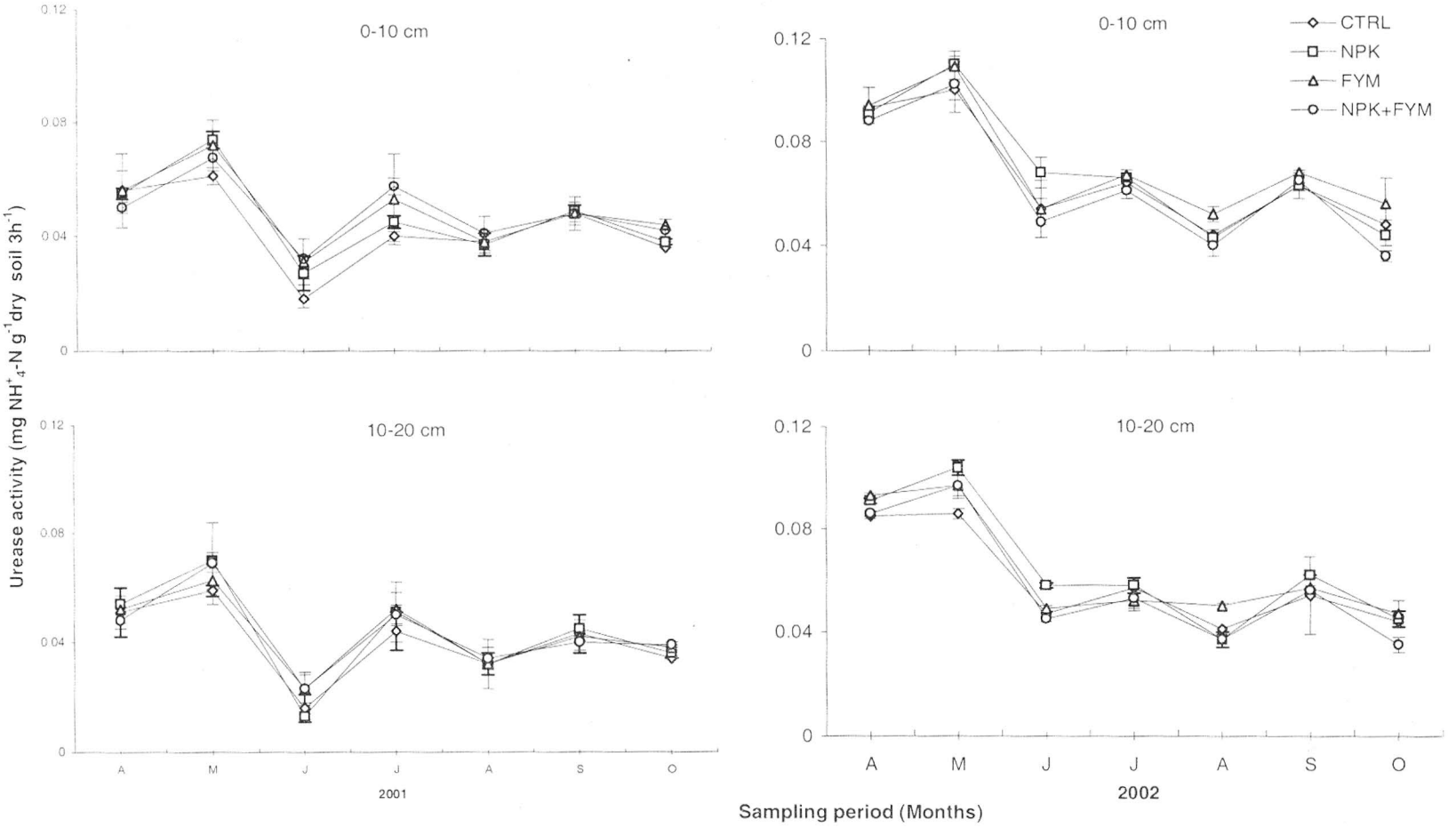

Fig 4. Urease activity in groundnut field soil at 0-10 cm and 10-20 cm depths.

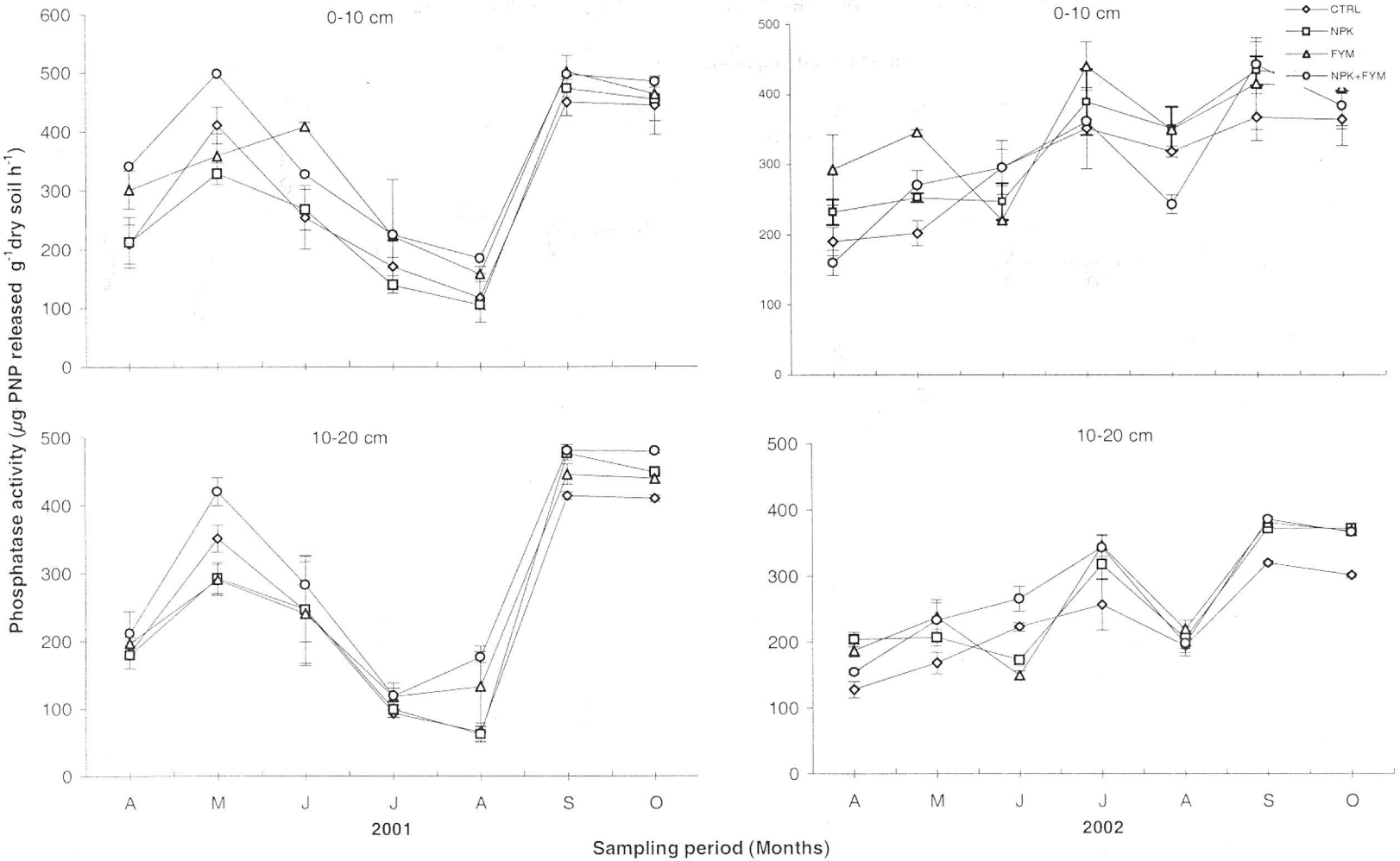

Fig 5. Acid phosphatase activity in groundnut field soil at 0-10 cm and 10-20 cm depths.

(Burns, 1978) and due to the retention of fertilizers. The analysis of variance (ANOVA), which indicated the insignificance variation ($P \leq 0.005$) of soil urease activity between treatments, at both the soil layers (0-10 cm and 10-20 cm) may be due to the fluctuation in enzyme activity, which was led by the retention of fertilizers and the effect of plant. Thus, it can be concluded that the application of fertilizer as well as retention of fertilizers and host legume plant could affect the soil urease activity and its activity was higher in NPK treated plot throughout the entire investigation.

Effects of fertilizers on phosphatase enzyme activity

Generally, higher enzyme activity was observed at surface soil than the subsurface soil layer. The increased phosphatase activity from pre-fertilizers treatment to post treatment coincided with the report of Speir and Ross (1990), that phosphatase activity is originated from microorganisms, wherein, it showed an increased fungal and bacterial population number in May. In the first year at surface soil layer, phosphatase activity ranged between 104.96 and 501.80 µg PNP released/ g dry soil/1 h in NPK and FYM plots in August and September respectively. At subsurface soil layer, it ranged between 62.20 and 480.0 µg PNP released/ g dry soil/ 1 h in NPK and NPK+FYM plots in August and September respectively. In second year at surface soil layer, the enzyme activity ranged between 160.06 and 442.0 µg PNP released/ g dry soil/1 h in NPK+FYM in April and July respectively. At subsurface soil layer, it ranged between 128.46 and 385.73 µg PNP released/ g dry soil/1 h in CTRL and NPK+FYM plots in April and September respectively.

The one-way analysis of variance showed significant variation ($P \leq 0.05$) of phosphatase activity between CTRL and FYM plots at surface layer, whereas at subsurface soil layer, it was observed between CTRL and NPK+FYM plots. Correlation coefficient values ($P \leq 0.001$) showed that the enzyme activity at surface soil layer was positively correlated with enzyme activity at subsurface soil layer. At both the soil layer in CTRL, FYM and NPK plot, phosphatase activity was positively correlated with soil temperature and pH ($P \leq 0.01$).

A consistent trend in distribution of phosphatase activity i.e. higher enzyme activity in NPK+FYM and FYM treated plots and maximum enzyme activity in NPK+FYM plot in May was mainly due to higher organic matter contents and microbial population (Spiers and McGill (1979). The soil treated with NPK+FYM and FYM fertilizers, which were highly bound unavailable form of organic P likely exhibited more enzyme activity which indicated that soil organic matter level and soil microbial activities vital for the nutrients turnover and long-term productivity of the soil were enhanced by use of organic amendments along with inorganic fertilizers (Goyal *et al.*, 1999). This enhancement of biological activities in the manure treated soil was evidenced by relatively high phosphatase activity (Parham *et al.*, 2002) and suggested that the possible reason of higher enzyme activity in a manure treatment was that manure promoted biological and microbial activities, which accelerated the breakdown of organic substances in the added manure. A remarkable increase in activity was observed from August to September (Fig. 5). So, it can be hypothesized that the result, which showed maximum enzyme activity in September, wherein the groundnut plant attained its maximum growth (120 Day After Sowing) could be due to the rhizosphere effect (Burns, 1978). It was well documented that the root exudation of plant enhanced the microbial population and the activity of soil microorganisms was strongly linked to the activity of enzymes (Deng and Tabatabai, 1997; Klose *et al.*, 1999). The legumes could also enrich their immediate soil environment with rhizobia through rhizosphere effect (Thies *et al.*, 1995). The decreased enzyme activity from September towards post harvest (October) could be due to the removal of plant, which in turn affects the soil microbial population.

The correlation coefficient value that showed a positive correlation of phosphatase activity with temperature coincided with the finding of Sinsabaugh *et al.* (1991) who stated that temperature is a controlling factor of the enzyme activity and it effects the enzyme activity indirectly through influencing microbial proliferation, and also directly, by modifying enzyme kinetics (Chrost, 1991).

Thus, it can be concluded that higher phosphatase enzyme activity was noted in NPK+FYM and FYM treated plots and microbial population was one of the controlling factors for the activity. Plant has a great beneficial influence on the enzyme activity through its root exudation, where the enzyme was strongly correlated with soil temperature.

ACKNOWLEDGEMENTS

The authors are grateful to the Head, Department of Botany for providing laboratory facilities and North Eastern Council, Shillong for providing financial support in the form of project to North Eastern Biodiversity Research Cell, NEHU, Shillong and the Division of Agronomy, ICAR, Barapani, Shillong, for their cooperation and support in setting up the experimental plots.

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