



## Effect of Long-Term Fertilization and Hybrid Maize on Seasonal and Temporal Changes in Soil Enzyme Activity

Pragyan Paramita Rout<sup>1\*</sup>, N. Chandrasekaran<sup>2</sup> and Dhaneshwar Padhan<sup>3</sup>

<sup>1,2</sup>Department of Soil Science and Agricultural Chemistry, TNAU, Coimbatore-641003

<sup>3</sup>Department of Agricultural Chemistry & Soil Science, BCKVV, Nadia, West Bengal-741235

**Soil enzymes play vital role in nutrient mineralization and their activity in soil is an excellent sensor in predicting the nutrient supplying capacity to plants. A field experiment was conducted to study the effect of long-term fertilization and maize hybrid on seasonal and temporal changes in soil enzyme activities (phosphatase and dehydrogenase). There were ten treatments each replicated four times viz., T<sub>1</sub> - 50 % NPK, T<sub>2</sub> - 100% NPK, T<sub>3</sub> - 150% NPK, T<sub>4</sub> - 100% NPK + hand weeding, T<sub>5</sub> - 100% NPK + ZnSO<sub>4</sub>, T<sub>6</sub> - 100% NP, T<sub>7</sub> - 100% N alone, T<sub>8</sub> - 100% NPK + FYM, T<sub>9</sub> - 100% NPK (-S) and T<sub>10</sub> - Control. The results showed that during monsoon fallow period, dehydrogenase activity increased, while phosphatase activity recorded lower values after monsoon rainfall. Unlike phosphatase activity, dehydrogenase activity followed a declining trend as growth stages of hybrid maize (Co-6) proceeds. Among the treatments, 100 % NPK + FYM @ 10 t ha<sup>-1</sup> (T<sub>8</sub>) recorded the highest activities of these enzymes emphasising the importance of integrated nutrient management in improving biochemical properties of soil, thereby sustaining the soil health over long run.**

**Key words:** Long-term fertilization, Maize hybrid, Soil enzyme activity

Enzymes play key role in the cycling of nutrients in soil and their activity is considered as a sensor of soil fertility (Yao *et al.*, 2006). Soil phosphatase hydrolyses the ester bonds (C-O-P) in organic matter, thereby releasing inorganic P from organically bound P (Rodriguez and Fraga, 1999). Singh and Walker (2006) reported that under phosphorus deficiency conditions, both plants and microorganisms release phosphatase enzymes into the soil, which have potential to mobilize P reserve. Similarly, dehydrogenase enzyme oxidizes soil organic matter and is considered as an indicator of overall microbial activity in soil (Stepniewska and Wolinska, 2005).

The activity of soil enzymes is greatly affected by various abiotic factors such as temperature, moisture, pH, and oxygen content (Nannipieri *et al.*, 2002). Pavel *et al.* (2004) reported that temperature and moisture influence enzyme activities indirectly through microbial growth and substrate availability. Similarly, soil enzyme activity also exhibits temporal variation as growth stages of crop proceeds. Yang *et al.* (2008) observed higher activities of phosphatase in soil during vigorous growth stages of cucumber, while lower activity during the early and late growth stages. Besides, soil enzyme activities are also influenced by fertilization, agricultural management practices such as crop rotation, amendments and tillage under intensive cropping system (Eivazi *et al.*, 2003). Hence, quantifying such changes in enzyme activity provide an index for nutrient transformation reactions in soil (Masto *et al.*, 2006).

In contrast, information on the biological processes, such as soil enzymatic activities, which mediate nutrients cycling and influence their acquisition during active crop growth stages under LTFEs are limited (Barnard *et al.*, 2006). With this background a study has been conducted to quantify the effect of long-term fertilization and hybrid maize (Co-6) cultivation on seasonal and temporal variations in soil enzyme activity during monsoon fallow period followed by cropping period.

### Material and Methods

#### Experimental site

The present study was conducted during the year 2013-14 is a part of ongoing All India Coordinated Research Project (AICRP) on Long term Fertilizer Experiment (LTFE) at Eastern Block farm of Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu to record the seasonal changes in soil enzyme activity during monsoon fallow period after harvest of 100th crop (finger millet) when rainfall occurs due to North East monsoon (during October to November) followed by temporal changes during cropping period with hybrid maize (Co-6) (101th crop). The experimental site is situated at 11° North latitude, 77° East longitude with an altitude of 426.7 meter above MSL.

#### Weather and soil type

During the study period (October 2013 - May 2014), the experimental location recorded a maximum and minimum temperature of 34.5°C and 17.2°C, respectively with a total rainfall of 8.1 mm. The soil of experimental site belongs to Inceptisol order,

\*Corresponding author's email: parimita.pragyan00@gmail.com

having calcareous mixed black soil with sandy clay loam texture and comes under Perianaickenpalayam series of Vertic Ustropept. The initial estimate of soil properties of experimental site is given in Table 1.

### Treatments

Ten treatments were imposed for the present study, each replicated four times in randomized block design viz., T<sub>1</sub>- 50 % NPK, T<sub>2</sub>- 100% NPK, T<sub>3</sub>- 150% NPK, T<sub>4</sub>- 100% NPK + hand weeding, T<sub>5</sub>- 100% NPK + ZnSO<sub>4</sub>, T<sub>6</sub>- 100 % NP, T<sub>7</sub>- 100% N alone, T<sub>8</sub>- 100% NPK + FYM, T<sub>9</sub>- 100% NPK (-S) and T<sub>10</sub>- Absolute control. The hybrid maize CO-6 was used as test crop. The recommended dose of N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O based on initial soil test was 250:75:75 kg ha<sup>-1</sup>. The sources of N, P and K used were urea, single super phosphate (SSP) and muriate of potash. In sulphur free treatment (T<sub>9</sub>), diammonium phosphate (DAP) was used instead of SSP as a source of P.

### Biochemical and statistical analysis

Composite surface (0-15 cm) soil samples were collected from each plot both during monsoon fallow (at three stages viz., Before Rain Fall (BRF), After Rain Fall (ARF) and End of Fallow Period (EFP) followed by cropping period (at three critical growth stages of hybrid maize viz., knee high, tasseling, milky and harvest). The soil phosphatase and dehydrogenase activities were determined by *p*-nitro phenol method (Tabatabai and Bremner, 1969) and TTZ (tri phenyl tetrazolium chloride) reduction method (Casida *et al.*, 1964), respectively. All other soil parameters were analysed following standard protocols. The data are statistically analysed by using analysis of variance (ANOVA) and correlation statistics as suggested by Panse and Sukhatme (1985). For statistical analysis of data, tools such as Microsoft Excel (Microsoft Corporation, USA) and AGRES window version 7.0 packages were used.

## Results and Discussion

### Acid phosphatase activity

The soil phosphatase enzyme plays a key role in P mineralization and its activity is highly correlated with the magnitude of soil available P (Dick, 1994). The activity of soil phosphatase was evaluated in terms of  $\mu\text{g } p\text{-nitro phenol released per gram of soil per hour}$ .

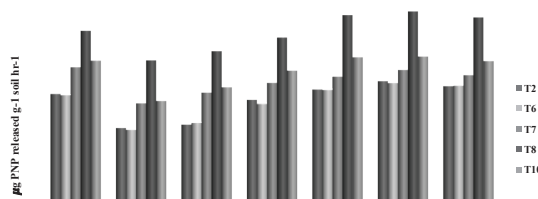
**Table 1. Initial properties of the experimental soil**

Properties	Initial (1972)
Textural class	Sandy clay loam
pH	8.20
Electrical conductivity (dSm <sup>-1</sup> )	0.20
Organic carbon (g kg <sup>-1</sup> )	3.0
Available nitrogen (kg ha <sup>-1</sup> )	178.0
Available phosphorus (kg ha <sup>-1</sup> )	11.0
Available potassium (kg ha <sup>-1</sup> )	810.0
Available Zn (mg kg <sup>-1</sup> )	2.58
Available Mn (mg kg <sup>-1</sup> )	2.74
Available Cu (mg kg <sup>-1</sup> )	4.20
Available Fe (mg kg <sup>-1</sup> )	2.74

The results obtained on the activity of acid phosphatase (Fig. 1) during monsoon fallow period

showed that before rainfall stage (BRF), its activity was higher, ranging from 24.6 to 42.3  $\mu\text{g } p\text{-nitro phenol released g}^{-1} \text{ soil hr}^{-1}$  followed by a decline after rainfall stage. The lowering in acid phosphatase activity during monsoon fallow period than cropping period coincides with the findings of Masto *et al.* (2006), showing significant seasonal effects on soil phosphatase activity and a sharp increase in acid phosphatase due to wheat cultivation

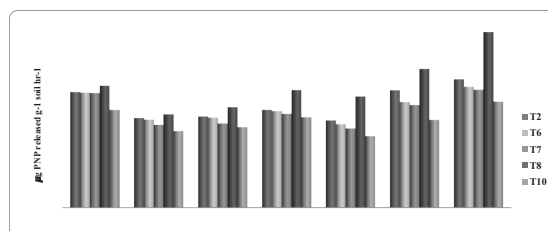
**Fig 1. Effect of long term fertilization on seasonal and temporal changes on soil acid phosphatase activity ( $\mu\text{g } p\text{-nitro phenol released g}^{-1} \text{ soil hr}^{-1}$ )**



S<sub>1</sub>-before rain fall, S<sub>2</sub>-after rain fall, S<sub>3</sub>-end of fallow period, S<sub>4</sub>-knee high, S<sub>5</sub>-tasseling, S<sub>6</sub>-milky, S<sub>7</sub>-harvest

compared to fallow field, which indicates the strong rhizospheric effects on phosphatase activity. The acid phosphatase activity was also found to be influenced by the growth stages of hybrid maize. The increase in acid phosphatase activity with the age of hybrid maize may be attributed to the development of root system with age and increase in total root surface area (Yadav and Tarafdar, 2001) followed by a decrease in activity at harvest stage.

**Fig 2. Effect of long term fertilization on seasonal and temporal changes on soil alkaline phosphatase activity ( $\mu\text{g } p\text{-nitro phenol released g}^{-1} \text{ soil hr}^{-1}$ )**



S<sub>1</sub>-before rain fall, S<sub>2</sub>-after rain fall, S<sub>3</sub>-end of fallow period, S<sub>4</sub>-knee high, S<sub>5</sub>-tasseling, S<sub>6</sub>-milky, S<sub>7</sub>-harvest

Among treatments imposed, markedly higher acid phosphatase activity was observed in control (T<sub>10</sub>) irrespective of growth stages of hybrid maize. Such rise in activity might be caused due to the P stressed condition that had induced plant roots and microorganisms to release phosphatase enzyme to mobilize the native soil P reserve (Singh and Walker, 2006). The profound influence of organic manures on acid phosphatase activity was evident from increased activity observed in the treatment receiving FYM at 10 t ha<sup>-1</sup> along with 100% NPK (T<sub>8</sub>). Such stimulation in activity upon addition of organic manure may largely be due to the increased microbial population, resulting in the build-up of enzyme level in soil (Mohammadi, 2011). Similarly, soil enzyme activity was found to be low in treatment receiving 100 % NP (T<sub>6</sub>) when

compared to treatment receiving 100% N ( $T_7$ ). Such decline might be due to the exclusion of P that in turn would have triggered the release of acid phosphatase in soil under P deficient conditions as reported by Wen-Hui *et al.* (2007).

#### Alkaline phosphatase activity

The results on alkaline phosphatase activity (Fig. 2)

showed that its activity was much higher than acid phosphatase, irrespective of the treatments imposed, which might be due to the alkaline reaction of the soil (pH=8.2). This is in consistent with earlier findings that phosphatase activity is strongly influenced by soil pH (Dick, 1994). Initially, before rain fall (BRF) stage, the alkaline phosphatase activity ranged from 24.6 to 42.3  $\mu\text{g } p\text{-nitro phenol released g}^{-1} \text{ soil hr}^{-1}$ .

**Table 2. Effect of seasonal and temporal changes on soil dehydrogenase activity ( $\mu\text{g TPF, tri phenyl formazan released g}^{-1} \text{ soil day}^{-1}$ )**

Treatments	Monsoon fallow period			Cropping Period			
	BRF	ARF	EFP	knee high	tasseling	milky	harvest
$T_1$	4.8	5.5	5.3	4.8	4.0	4.3	3.8
$T_2$	4.6	5.7	5.4	4.7	4.5	4.5	3.7
$T_3$	5.1	6.0	5.7	5.2	4.8	5.1	4.1
$T_4$	4.6	5.3	5.1	4.7	4.4	4.6	3.8
$T_5$	4.0	5.1	4.9	4.3	4.0	4.2	4.0
$T_6$	4.1	5.6	5.4	5.0	4.7	4.9	4.1
$T_7$	4.4	5.6	5.2	4.8	4.4	4.6	3.9
$T_8$	6.7	8.1	7.2	7.0	6.6	6.9	6.3
$T_9$	3.8	4.2	3.9	3.7	3.5	3.9	3.6
$T_{10}$	3.5	3.7	3.6	3.3	3.0	3.4	2.7
SEd	0.36	0.20	0.22	0.21	0.26	0.22	0.19
CD(P=0.05)	0.74	0.41	0.45	0.44	0.52	0.44	0.39

BRF: Before rain fall, ARF: After rain fall, EFP: End of fallow period

The reduced alkaline phosphatase activity in monsoon fallow period was similar to that of acid phosphatase. This may be attributed to the increased moisture conditions that prevailed after rainfall leading to enhanced organic matter decomposition resulting in improved available P in soil which caused a reduction in alkaline phosphatase activity (Bell *et al.*, 2006). However, among the various stages compared, higher values were recorded at the harvest stage of maize irrespective of the treatments.

Among treatments, alkaline phosphatase activity was found to increase with application of 100% NPK fertilizers and FYM. This may be due to the fact that in general, the enzyme activity in the soil is closely related to the organic matter build up (Beyer *et al.*, 1993). A proportionate increase in the alkaline phosphatase activity was observed following the additions of nutrients with exception of the super optimal level of fertilizers (150% NPK) indicating the sensitivities of alkaline phosphatase to nutrient additions (Kanchikerimath and Singh, 2001).

#### Dehydrogenase activity

The activity of soil dehydrogenase (Table 2) was assessed in terms of  $\mu\text{g tri phenyl formazone (TPF)}$  released per gram soil per day. At before rain fall (BRF) stage, dehydrogenase activity ranged from 3.5 to 6.7  $\mu\text{g g}^{-1} \text{ soil day}^{-1}$ . Unlike phosphatase activity, dehydrogenase activity increased irrespective of treatments after monsoon rainfall (ARF). This may be reasoned to the increased soil moisture after rainfall, which favours the microbial population and decomposition of organic matter, resulting in increased activity of dehydrogenase (Garcia *et al.*, 2002). At the

end of monsoon fallow period (EFP), a sharp decline in dehydrogenase activity was recorded.

The dehydrogenase activity was found to be strongly affected by the crop growth stages. Among all growth stages of hybrid maize compared, knee high stage ( $S_4$ ) showed the highest dehydrogenase activity and lower values were recorded during milky and harvest stage irrespective of the treatments, which was consistent with the findings of Gu *et al.* (2009) who reported temporal fluctuations in the activity of dehydrogenase at different growth stages of two rice varieties and found higher activities at seedling stage than tillering, but decreased as rice crop matures.

A significant increase in dehydrogenase activity was evident due to combined application of FYM along with NPK fertilizers. The overall dehydrogenase activity ranged from 2.7 to 8.1  $\mu\text{g g}^{-1} \text{ soil day}^{-1}$ . The high organic matter levels in the FYM supplied treatment ( $T_8$ ) might have provided a more favourable environment for the accumulation of enzymes in the soil matrix, since organic constituents are thought to be important in forming stable complexes with free enzymes. This was in line with the finding of Liu *et al.* (2010), who reported that dehydrogenase activity was increased by FYM applications as compared to straw treatments. The findings also suggested that exclusion of S from fertilizer schedule *viz.*,  $T_9$ -100% NPK (S free) caused a drastic reduction in dehydrogenase activity. According to Pancholy and Rice (1973), stronger effect of sulphur on dehydrogenase activity might be due to the more easily decomposable components of crop residues on the metabolism of soil microorganisms.

## Conclusion

The results emanated from this study clearly ascertain that the soil phosphatase and dehydrogenase activity is closely related with the C inputs, crop growth stages and abiotic soil environment. The enhanced activity of such enzymes due to the long term application of manure and fertilizer promoted the recycling of nutrients and transformation of added nutrients into different labile pools under continuous intensive cropping system and thereby maintain the soil fertility in order to sustain soil productivity and biological health over long run.

## References

- Barnard, R., X. Le Roux, B.A. Hungate, E.E. Clel and J.C. Blankinship, L. Barthes, P.W. Leadley. 2006. Several components of global change alter nitrifying and denitrifying activities in an annual grassland. *Functional Ecology*, **20**: 557-564.
- Bell, J.M., C.A. Robinson and R.C. Schwartz. 2006. Changes in soil properties and enzymatic activities following manure applications to a rangeland. *Rangeland Ecology and Management*, **59(3)**: 314-320.
- Beyer, L., C. Wachendorf, C. Elsner and R. Knabe. 1993. Suitability of dehydrogenase activity assay as an index of soil biological activity. *Biology and Fertility of Soils*, **16**: 52-56.
- Blaise, D., J.V. Singh, A.N. Bonde, K.U. Tekale and C.D. Mayee. 2005. Effects of farmyard manure and fertilizers on yield, fibre quality and nutrient balance of rainfed cotton (*Gossypium hirsutum*). *Bioresource Technology*, **96**: 345-349.
- Casida, J.E., D.A. Klein and T. Santoro. 1964. Soil dehydrogenase activity. *Soil Science*, **98**: 371-376.
- Dick, R.P. 1994. Soil enzyme activities as indicators of soil quality. In *Defining Soil Quality for a Sustainable Environment*, J.W. Doran, D.C. Coleman, D.F. Bezdicek and B.A. Stewart (Eds.), Soil Science Society of America, Madison, WI, pp: 108-123.
- Ebhin Masto, R., P. K. Chhonkar, D. Singh, and A. K. Patra. 2006. Changes in soil biological and biochemical characteristics in a long-term field trial on a sub-tropical inceptisol. *Soil Biology and Biochemistry*, **38**: 1577-1582.
- Garcia, C., T. Hernandez, A. Roldan and A. Martin. 2002. Effect of plant cover decline on chemical and microbiological parameters under Mediterranean climate. *Soil Biology and Biochemistry*, **34**: 635-642.
- Gu, Y., P. Wang and C.H. Kong. 2009. Urease, invertase, dehydrogenase and polyphenol oxidase activities in paddy soil influenced by allelopathic rice variety. *European Journal of Soil Biology*, **45**: 436-441.
- Kanchikerimath, M., and D. Singh. 2001. Soil organic matter and biological properties after 26 years of maize-wheat-cowpea as affected by manure and fertilization in a Cambisol in semiarid region of India. *Agriculture, Ecosystems and Environment*, **86**: 155-162.
- Liu, E., C. Yan, X. Mei, W. He, S.H. Bing, L. Ding, Q. Liu, S. Liu and T. Fan. 2010. Long-term effect of chemical fertilizer, straw, and manure on soil chemical and biological properties in northwest China. *Geoderma*, **158** (3-4): 173-180.
- Mandal, A., A.K. Patra, D. Singh, A. Swarup and R.E. Masto. 2007. Effect of long-term application of manure and fertilizer on biological and biochemical activities in soil during crop development stages. *Bioresource Technology*, **98**: 3585-3592.
- Masto, R.E., P.K. Chhonkar, D. Singh and A.K. Patra. 2006. Changes in soil biological and biochemical characteristics in a long-term field trial on a sub-tropical inceptisol. *Soil Biology and Biochemistry*, **38**: 1577-1582.
- Mohammadi, K. 2011. Soil microbial activity and biomass as influenced by tillage and fertilization in wheat production. *American and Eurasian Journal of Agricultural and environmental Science*, **10(3)**: 330-337.
- Nannipieri, P., E. Kandeler and P. Ruggiero. 2002. Enzyme activities and microbiological and biochemical processes in soil. In *Enzymes in the environment: activity, ecology and applications*, R.G. Burns and R.P. Dick (Eds), Marcel Dekker, New York, USA.
- Pancholy, S.L. and E.L. Rice. 1973. Soil enzymes in relation to old field succession: amylase, cellulase, invertase, dehydrogenase, and urease. *Soil Science Society of America Proceedings*, **37**: 47-50.
- Panase, V.G. and P.V. Sukhatme. 1985. *Statistical Methods for Agricultural Workers*. Publication and information division. ICAR, New Delhi.
- Pavel, R., J. Doyle and Y. Steinberger. 2004. Seasonal pattern of Cellulase concentration in desert soil. *Soil Biology and Biochemistry*, **36**: 549-554.
- Rodriguez, H. and R. Fraga. 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. *Advances in Biotechnology*, **17**: 319-339.
- Singh, B. K and A. Walker. 2006. Microbial degradation of organo-phosphorus compounds. *FEMS Microbiology Reviews*, **30**: 428-471.
- Stepniewska, Z. and A. Wolinska. 2005. Soil dehydrogenase activity in the presence of chromium (III) and (VI). *International Agrophysics*, **19**: 79-83.
- Tabatabai, M.A and J.M. Bremner. 1969. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biology and Biochemistry*, **1**: 301-307.
- Wen-Hui, Z., C. Zu-Cong, and Z. He. 2007. Effects of long-term application of inorganic fertilizers on biochemical properties of a rice-planting red soil. *Pedosphere*, **17**: 419-428.
- Yadav, R. and J. Tarafdar. 2001. Influence of organic and inorganic phosphorus supply on the maximum secretion of acid phosphatase by plants. *Biology and Fertility of Soils*, **34(3)**: 140-143.
- Yang, L., T. Li, F. Li, J.H. Lemcoff and S. Cohen. 2008. Fertilization regulates soil enzymatic activity and fertility dynamics in a cucumber field. *Scientia Horticulturae*, **116**: 21-26.
- Yao, X.H., M. Huang, Z.H. Lu, and H.P. Yuan. 2006. Influence of acetamiprid on soil enzymatic activities and respiration. *European Journal of Soil Biology*, **42**: 120-126.