

Soil enzyme activity of an intensively grown rice field as influenced by long term fertilization and manuring under subtropical climatic conditions

P MAJHI, S MONDAL, KK ROUT*, M MANDAL*, M SINGH
and N SASMAL*****

**Visva-Bharati University, Department of ASEPAN, Palli Siksha Bhavana
Sriniketan, Santiniketan 731236 West Bengal, India**

***AICRP on Long Term Fertilizer Experiments**

Department of Soil Science and Agricultural Chemistry

Orissa University of Agriculture and Technology, Bhubaneswar 751003 Odisha, India

****IISS, Nabibhag, Bhopal 462038 MP, India**

*****Agro-Polytechnic (OUAT), Deogarh 768119 Odisha, India**

Email for correspondence: majhi.pradipta@yahoo.in

ABSTRACT

A long term fertilizer experiment conducted on rice-rice system since 2005 on acidic sandy loam inceptisol of Bhubaneswar in Odisha was used for studying the effect of continuous application of NPK fertilizers alone or with Zn, B and S fertilizers and FYM on activities of three important soil enzymes viz dehydrogenase, urease and phosphatase. Dehydrogenase activity was greater in control and 100 per cent NPK + FYM treatment than soils that received only NPK plus other nutrients. Lower dehydrogenase in NPK fertilizers alone or with Zn, B and S fertilizers can be attributed to negative effect of K on fungi and algae and Zn on algae. Dehydrogenase poorly correlated with SOC ($r = 0.19$). On the other hand urease and phosphatase strongly correlated with SOC content and increased substantially with fertilizer application. Addition of FYM along with NPK fertilizers recorded highest urease and phosphatase activity due to increase in SOC and microbial population. Continuous application of Zn alone or with B or S for 18 cropping cycles did not have any significant effect on urease activity. The effect was positive with Zn and S and negative with Zn and B combination. But in case of phosphatase activity all the three nutrients had positive effect which was significant with combined application of Zn and S.

Keywords: Long term manuring; rice-rice system; enzyme activity

INTRODUCTION

Rice-rice system is an important rice-based cropping system of eastern India.

Continuous mono-cropping of rice with high yielding improved varieties and application of NPK fertilizers have resulted in stagnated or declined productivity

(Ladha et al 2003) which has been ascribed to declined soil fertility with reduced nutrient supplying capacity. Long term fertilizer experiments conducted throughout the world have revealed that continuous application of NPK alone has caused many damages to physical and biological health of soil with reduced supply of N and depletion of total soil K and deficiency of nutrients like sulphur and micronutrients due to their continuous removal from soil (Subba Rao and Srivastava 1998, Singh et al 2012, Ram et al 2014). For sustainability of such a production system, improvement of soil environment is very important.

Soil enzymes play the key biochemical functions in the overall process of organic matter decomposition in soil system (Burns 1983, Sinsabaugh 1991, Mandal et al 2013). They catalyze several important biochemical reactions necessary for the life processes of microorganisms in soils and the stabilization of soil structure, the decomposition of organic matter and nutrient cycling (Dick et al 1994). These enzymes are constantly being synthesized, accumulated, inactivated and/or decomposed in the soil hence playing very important role in crop production particularly in nutrients cycling (Tabatabai 1994). The enzyme levels in soil systems may vary in amounts primarily due to the soil type and cropping effects that need to be investigated for every region. A better understanding of these soil enzymes would provide the basis in maintaining soil health

and potentially provide a unique opportunity to manage soil fertility. Therefore the present study was conducted to quantify the level of soil enzyme activities viz dehydrogenase, phosphatase and urease in different chemical fertilizers and manures in rice-rice systems.

MATERIAL and METHODS

The study was carried out under an ongoing All India Coordinated Research Project on Long Term Fertilizer Experiment funded by Indian Council Agricultural Research and initiated in 2005 in the central research farm of Orissa University of Agriculture and Technology, Bhubaneswar, Odisha on a rice-rice system. The area is located at latitude 20°16' to 20°17' N and longitude 85°48' to 85°49' E at an elevation of 30 m amsl.

The experiment was conducted with two crops of rice (*Oryza sativa* L) in each year, one in wet season (July to December) followed by another in dry season (January to May). The experimental site was a well drained medium land with an acidic (pH 5.8) sandy loam soil classified as vertic Ustochrepts. The soil had low cation exchange capacity (CEC) 3.5 cmole P⁺/kg and low organic carbon (4.3 g/kg). The available N, P and K were 187, 19.4 and 43 kg/ha respectively. The CaCl₂ extractable S was 22.2 kg/ha, Zn 1.8 ppm and hot water soluble B 0.46 ppm. The experiment was originally laid out in

randomized block design (RBD) with 12 treatments replicated 4 times. Out of these 12 treatments seven treatments viz T_1 (100% NPK), T_2 (150% NPK), T_3 (100% NPK + Zn), T_4 (100% NPK + FYM), T_5 (100% NPK + Zn + B), T_6 (100% NPK + Zn + S) and T_7 (control, no fertilizer) were selected for the present study. The 100 per cent recommended dose of NPK equaled 80:40:60 kg of N, P_2O_5 and K_2O per ha. FYM was applied @ 5 tonnes/ha in each season through incorporation before final land preparation. Sulphur was applied @ 30 kg/ha through gypsum, zinc as seedling root dipping in 0.4 per cent zinc oxide solution and boron was applied through 2 foliar sprays of borax (0.2%). For the present study soil samples were collected ten days after harvesting of wet season crop during 2014 from a depth of 0-15 cm of the soil surface with the help of a soil augur from five different spots within individual replicated plot and mixed together to prepare a composite sample for each plot. These samples were preserved, processed and analyzed for soil enzymes.

Enzyme assay

Soil enzymes viz dehydrogenase, phosphatase and urease were assayed using the procedure as per Tabatabai (1982). Twenty grams of air-dried soil (<2 mm) was mixed thoroughly with 2 g of $CaCO_3$ and 6 g of this mixture was placed in test tubes in triplicate for the analysis through triphenyl formazan (TPF) method. The amount of TPF produced was

calculated by reference to a calibration graph prepared from TPF standards at 485 nm. Soil phosphatase activity was measured by disodium phenyl phosphate method. The absorbance of color in the solution was measured at 660 nm (Tabatabai et al 1972). Urease activity was assayed using tris (hydroxymethyl) aminomethane (THAM) buffer and NH_4^+ -N was determined through distillation method (Singh et al 2007).

RESULTS and DISCUSSION

Soil enzymes activities

Soil enzymes are the biological catalysts of innumerable reactions in soils. Some are integrative indices of biological activity in soil and thus can be good indicators of soil health and environment. Data pertaining to enzyme activities viz dehydrogenase, urease and acid phosphatase are recorded in Tables 1-3.

Dehydrogenase

This enzyme is considered to exist as an integral part of intact cells but does not accumulate extracellular in the soil. Therefore it is commonly used as an indicator of biological activity in soils (Burns 1978). Any activity that affects the microbial population and their activity also affects the dehydrogenase activity. Dehydrogenase enzyme is known to oxidize soil organic matter by transferring protons and electrons from substrates to acceptors. These processes are part of respiration

Table 1. Effect of continuous manuring with inorganic fertilizers and FYM dehydrogenase activity in the surface soil of a rice-rice system

Treatment	SOC (g/kg)	Dehydrogenase activity (mg TPF/kg soil/24 h)		
		Mean	SD	CV (%)
100% NPK	4.59	190.81	8.82	4.62
150% NPK	5.19	106.24	8.52	8.02
100% NPK + Zn	5.08	152.68	6.37	4.17
100% NPK + FYM	5.84	247.26	9.06	3.66
100% NPK + Zn + B	4.89	127.12	8.66	6.81
100% NPK + Zn + S	5.17	163.96	8.06	4.91
Control	3.95	180.54	9.27	5.14
CD _{0.05}	0.74	14.54		

Table 2. Effect of continuous manuring with inorganic fertilizers and FYM urease activity in the surface soil of a rice-rice system

Treatment	SOC (g/kg)	Urease activity (mg NH ₄ ⁺ -N/kg soil/2 h)		
		Mean	SD	CV (%)
100% NPK	4.59	53.43	7.55	14.13
150% NPK	5.19	58.16	7.61	13.08
100% NPK + Zn	5.08	54.32	8.02	14.77
100% NPK + FYM	5.84	79.12	9.40	11.88
100% NPK + Zn + B	4.89	48.80	7.39	15.15
100% NPK + Zn + S	5.17	54.08	4.05	7.48
Control	3.95	30.00	4.95	16.50
CD _{0.05}	0.74	9.94		

Table 3. Effect of continuous manuring with inorganic fertilizers and FYM phosphatase activity in the surface soil of a rice-rice system

Treatment	SOC (g/kg)	Acid phosphatase activity (mg p-nitrophenol/kg soil/1 h)		
		Mean	SD	CV (%)
100% NPK	4.59	279.20	6.89	2.47
150% NPK	5.19	310.57	9.85	3.17
100% NPK + Zn	5.08	314.90	10.98	3.49
100% NPK + FYM	5.84	394.54	11.56	2.93
100% NPK + Zn + B	4.89	317.47	6.12	1.93
100% NPK + Zn + S	5.17	329.17	9.14	2.78
Control	3.95	229.22	6.15	2.68
CD _{0.05}	0.74	42.28		

pathways of soil microorganisms and are closely related to the type of soil and soil air-water conditions (Kandeler 1996). Since these processes are part of respiration pathways of soil microorganisms, studies on the activities of dehydrogenase enzyme in the soil are very important as it may give indication of the potential of the soil to support biochemical processes which are essential for maintaining soil fertility.

In the present study lower dehydrogenase activity was measured in soils that continuously received NPK fertilizers and higher in control and highest in soil that received organic manure FYM @ 10 tonnes/ha/year. The increased dehydrogenase activity in organic manure applied soil might be due to incorporation of organics and owing to increase in microbial activity of soil (Nannipieri 1994, Sridevi et al 2012). Dehydrogenase activity varied between 106.24 and 247.26 $\mu\text{g TPF/g soil 24/h}$ with the coefficient of variation (CV) ranging from 3.66 to 8.02 per cent. It weakly correlated with SOC content ($r=0.19$). Un-manured control plot also had significantly higher dehydrogenase activity than many of the fertilized treatments which either received 50 per cent more NPK or which received Zn or Zn + B or Zn + S. Between B and S the latter had greater positive impact on dehydrogenase activity. Increase in dose of NPK to 150 per cent significantly decreased the dehydrogenase activity. This might be due to overdose of K (150%) that had adverse

effect on dehydrogenase activity due to relatively high available K (Patnaik 2012). Mohanty (2015) experimenting on the same soil also reported negative impact of Zn and K on algal population which might be a reason for reduction in dehydrogenase activity. In 100 per cent NPK + FYM treatment the negative impact of K was counteracted by 10 tonnes FYM resulting in more microbes and higher dehydrogenase activity.

Urease

Urease enzyme is responsible for the hydrolysis of urea fertilizer applied to the soil into NH_3 and CO_2 with the concomitant rise in soil pH (Andrews 1989, Byrnes and Amberger 1989). This in turn results in a rapid N loss to the atmosphere through NH_3 volatilization (Fillery et al 1984, Simpson et al 1985). Thus urea hydrolysis is very vital in the regulation of N supply to plants after urea fertilization and measurement of urease activity is therefore very important.

Data on soil urease activity measured after 9 years of continuous monocropping of rice with 18 cropping cycles showed significant effect of different fertilizer treatments. The activities measured on soils of postharvest summer 2014-15 crop varied widely from a lowest of 30 mg to a highest of 79.12 mg $\text{NH}_4^+\text{-N/kg soil/24 h}$ found in 100 per cent NPK + FYM treatment with coefficient of variation of 7.48 to 16.50 per cent which was much

higher than that of phosphatase and dehydrogenase and strongly correlated with SOC ($r = 0.94$).

In contrast to dehydrogenase, application of 100 per cent NPK caused significant increase (78.1% more) in urease enzyme activity over control. Increasing the NPK dose to 150 per cent also resulted in increase in urease activity although there was non-significant enhancement. Application of Zn or B or Zn + B over 100 per cent NPK also did not have any significant effect. Results revealed that application of boron over and above 100 per cent NPK + Zn decreased the urease activity although it was not significant. Negative impact of zinc + boron on soil urease might be due to addition of lesser biomass (Ram et al 2014, Singh 2012) that did not have any significant effect on SOC which had strong correlation with urease ($r = 0.94$). Application of S had no impact on enzyme urease even after 9 cropping years. Addition of FYM @ 5 tonnes/ha per crop to 100 per cent NPK registered 163 per cent increase over control and 48.08 per cent increase over 100 per cent NPK. Thus application of FYM caused much higher increase (115%) in urease activity. This finding is supported by the work of Patnaik (2012).

Acid phosphatase

Phosphatases are a broad group of enzymes that are capable of catalysing hydrolysis of esters and anhydrides of

phosphoric acid (Schmidt and Laskowski 1961). In soil ecosystems these enzymes are believed to play critical roles in P cycles (Speir and Ross 1978).

In the present study urease and phosphatase activity was also significantly enhanced by NPK fertilizer application. In control it was 229 mg P-nitrophenol/kg soil/ 1 h as compared to 279 mg in 100 per cent NPK treatment. In all fertilizer treatments the activity varied within a relatively narrow range between 279 and 394 mg p-nitrophenol/kg soil/1 h with a coefficient of variation of 1.93 to 3.49 per cent only. Like urease highest phosphatase activity was also measured in 100 per cent NPK + FYM treatment that continuously received 10 tonnes FYM per year. Increasing the NPK dose to 150 per cent also increased the phosphatase activity but the enhancement was not significant. Unlike urease application of all the three micro and secondary nutrients Zn, B and S had positive impact on soil phosphatase activity but in each case the enhancement was not significant. Combined application of Zn and S along with 100 per cent NPK however caused significant increase in the phosphatase activity. Phosphatase activity strongly correlated with urease activity ($r = 0.93$) and SOC ($r = 0.98$).

CONCLUSION

The activity of enzyme dehydrogenase which is considered as an

index of overall microbial activity poorly correlated with SOC ($r = 0.19$). Lower dehydrogenase activity was measured in soils that continuously received NPK fertilizers and higher in control and highest ($247.26 \mu\text{g TPF/g soil/24 h}$) in soil that received organic manure in the form of FYM @ 10 tonnes/ha/year.

Urease and phosphatase were different in behavior from that of dehydrogenase and these two enzymes correlated strongly with SOC. In contrast to dehydrogenase, application of 100 per cent NPK caused significant increase in urease (78.1% more) and phosphatase (21.8%) activity over control. Increasing the NPK dose to 150 per cent also resulted in increase in their activity although the enhancement was non-significant. Application of Zn or Zn + B over 100 per cent NPK also did not have any significant effect on urease and phosphatase. Combined application of Zn and S along with 100 per cent NPK however caused significant increase in the phosphatase activity. Highest urease and phosphatase activity was measured in 100 per cent NPK + FYM treatment that continuously received 10 tonnes FYM/ha/year.

REFERENCES

- Andrews RK, Blakeley RL and Zerner B 1989. Urease: a Ni (II) metalloenzyme. In: *The bioinorganic chemistry of nickel* (JR Lancaster ed), VCH Publishers, New York, pp 141-166
- Burns RG 1978. *Soil enzymes*. Academic Press, New York, 370p.
- Burns RG 1983. Extracellular enzyme-substrate interactions in soil. In: *Microbes in their natural environment* (JH Slater, R Wittenbury and JWT Wimpenny eds), Cambridge University Press, London, pp 249-298.
- Byrnes BH and Amberger A 1989. Fate of broadcast urea in a flooded soil when treated with N-(n-butyl) thiophosphoric triamide, a urease inhibitor. *Fertilizer Research* **18**: 221-231.
- Dick RP, Sandor JA, Eash NS 1994. Soil enzyme activities after 1500 years of terrace agriculture in the Colca valley, Peru. *Agriculture, Ecosystems and Environment* **50**: 123-131.
- Fillery IRP, Simpson JR and De Datta SK 1984. Influence of field environment and fertilizer management on ammonia loss from flooded rice. *Soil Science Society of America Journal* **48**: 914-920.
- Kandeler E 1996. Nitrate. In: *Methods in soil biology* (F Schinner, R Öhlinger, E Kandeler and R Margesin eds), Springer, Berlin Heidelberg, New York, pp 408-410.
- Ladha JK, Dawe D, Pathak H, Padre AT, Yadav RL, Singh B, Singh Y, Singh Y, Singh P, Kundu AL, Sakal R, Regmi AP, Gami SK, Bhandari AL, Amin R, Yadav CR, Bhattarai EM, Das S, Aggarwal HP, Gupta RK and Hobbs PR 2003. How extensive are yield declines in long-term rice-wheat experiments in Asia? *Field Crops Research* **81**: 159-180.
- Mandal KG, Majhi P, Sahoo DK, Rout R, Kumar A, Ghosh S, Mohanty RK and Raychaudhuri M 2013. Assessing the soil environment under major cropping systems in Kuanria canal command. *Ecology, Environment and Conservation* **19(2)**: 509-513.
- Mohanty N 2015. Effect of long term manuring of an acid soil on algal activity and contribution to nitrogen nutrition of rice. MSc thesis, Orissa University of Agriculture and Technology, Bhubaneswar, odisha, India.
- Nannipieri P 1994. The potential use of soil enzymes as indicators of productivity, sustainability and pollution. In: *Soil biota management in sustainable farming systems* (CE Pankhurst, BM Double,

- VVSR Gupta and PR Grace eds). CSIRO, East Melbourne, VC, Australia, pp 238-244.
- Patnaik PK 2012. Soil enzyme activity and nutrient availability as influenced by long term manuring of a rice-rice cropping system in an acidic soil (inceptisols) of Bhubaneswar. MSc (Agric) thesis, Orissa University of Agriculture and Technology, Bhubaneswar, odisha, India.
- Ram B, Singh SK, Latore AM and Kumar O 2014. Effect of sulphur, zinc and boron application on growth and yield of hybrid rice (*Oryza sativa* L). Journal of Indian Society of Soil Science **62(2)**: 184-188.
- Schmidt G and Laskowski Sr M 1961. Phosphate ester cleavage (survey). In: The enzymes (PD Boyer, H Lardy, K Myrback eds), 2nd edn, Academic Press, New York, pp 3-35.
- Simpson JR, Freney JR, Muirhead WA and Leuning R 1985. Effects of phenylphosphoro-diamidate and dicyandiamide on nitrogen loss from flooded rice. Soil Science Society of America Journal **49**: 1426-1431.
- Singh AK, Bhusan M, Meena MK and Upadhyaya A 2012. Effect of sulphur and zinc on rice performance and nutrient dynamics in plants and soil of Indo Gangetic plains. Journal of Agricultural Science **4(11)**: 162-170.
- Singh SK, Singh AK, Sharma BK, Tarafdar JC 2007. Carbon stock and organic carbon dynamics in soils of Rajasthan, India. Journal of Arid Environment **68(3)**: 408-421.
- Sinsabaugh RL, Antibus RK and Linkins AE 1991. An enzymic approach to the analysis of microbial activity during plant litter decomposition. Agriculture, Ecosystems and Environment **34**: 43-54
- Speir TW and Ross DJ 1978. Soil phosphatase and sulphatase. In: Soil enzymes (RG Burns ed), Academic Press, London, UK, 380p.
- Sridevi S, Ramana MV and Rani SS 2012. Soil enzyme activity and nutrient availability as influenced by different nutrient management practices in maize onion cropping programme. Journal of Research, ANGRAU **39(3)**: 32-37.
- Subba Rao A and Srivastava S 1998. Role of plant nutrients in increasing crop productivity. Fertiliser News **43**: 65-75.
- Tabatabai MA 1982. Sulphur. In: Methods of soil analysis. Part 2, Chemical and microbiological properties (AL Page, RH Miller and DR Keeney eds), American Society of Agronomy, # 9 (Part 2), Agronomy Series, ASA, SSSA, Madison.
- Tabatabai MA 1994. Soil enzymes. In: Methods of soil analysis: microbiological and biochemical properties (RW Weaver, JS Angle and PS Bottomley eds), Part 2, SSSA Book Series # 5, Soil Science Society of America, Madison, WI.
- Tabatabai MA and Bremner JM 1972. Michaelis constants of soil enzymes. Soil Biology and Biochemistry **3**: 317-323.

Received: 21.7.2016

Accepted: 28.7.2016