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Long-term effects of organic manures and fertilizers on soil enzymes activity

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Abstract
A field experiment was conducted during 2014-15 at Agronomy Research Farm of CCS Haryana Agricultural University, Hisar (India) on an ongoing long term (29 years) experiment which was initiated in 1985. Seven treatments were laid out in randomized block design with three replications. The seven treatments were control (T1), 50% RDF through NPK (T2), 100% RDF through NPK (T3), 50% RDF through NPK+ 50% N through FYM (T4), 50% RDF through NPK + 50 % N through wheat straw (T5), 50% RDF through NPK+ 50% N through green manure (T6) and farmer's practice (T7). The cropping system adopted was of pearl millet and wheat. Enzymes activity was found higher in organic manure amended treatments. The maximum urease (89 µg NH_4^+/g soil/hr) and alkaline phosphatse (711 µg PNP/g soil/hr) activity were found in wheat straw amended treatment. The dehydrogensae activity was higher in FYM applied plots which was 78 µg TPF/g soil/24 hr.

Keywords: Urease, alkaline phosphatase, dehydrogenase, organic manures, soil enzymes activity

1. Introduction
Many intensive cereal based cropping systems are under practice in India based on their respective agro-climatic regions. The pearl millet (*Pennisetum glaucum* L.) – wheat (*Triticum aestivum* L.) is the most important cropping system adopted in arid and semi-arid ecological regions of Haryana. Soil enzymes are considered to be the contributors of soil microbial activity as well as soil quality in soil environment (Liu *et al.,* 2010) [6]. It also plays an important role in organic matter decomposition and in the dynamics of nutrient transformations in the soil. Some enzymes only facilitate the breakdown of organic matter *e.g*., hydrolase, glucosidase, while others are involved in nutrient mineralization *e.g*., amidase, urease, phosphatase *etc*. Addition of organic manures significantly increases the urease, alkaline phosphatase and dehydrogenase activity in the soil as compared to chemical fertilizers and also very little attention has been paid on pearl millet – wheat cropping system. Hence a long term experiment was planned to study the effects of organic manures and chemical fertilizers on the soil enzymes.

2. Materials and methods
2.1 Experimental site
The study entitled "Long-term effects of organic manures and fertilizers on soil enzymes activity" was conducted during 2014-15 at Agronomy Research Farm of CCS Haryana Agricultural University, Hisar (India). The details of the materials used and methods adopted for carrying out the present study are described as below. A long term field experiment was started in *Rabi* 1985 to study the effect of application of chemical fertilizers alone and there 50% substitution with organic sources, on crop yield and soil properties. The soil texture of the experimental plot was classified as sandy loam. The experimental site is located at 29^o 16'N latitude and 75^o 7'E longitude in North-West part of India. The climate of the experimental area is semi arid with a mean annual precipitation of 445 mm and mean annual temperature of 24.9^{o}C. The physio-chemical properties of surface soil (0-15 cm) analyzed at the start of experiment (1985) had a pH (1:2 soil: water) of 8.1, EC (1:2 soil: water) value of 0.36 dS m^{-1}, SOC content of 3.9 g kg^{-1}, Available N content of 98.0 mg kg^{-1}, Available P content of 12.6 mg kg^{-1} and available K content of 217.0 mg $kg^{-1.}$ The average nutrient composition of FYM, green manure and wheat straw applied in the experiment during 2013 are presented in Table 1.

Table 1: Mean nutrient composition of various organic manures used

Organic manure	OC (%)	N (%)	P (%)	K (%)	C:N
Farm yard manure (FYM)	38.1	1.2	0.97	1.87	31.8
Green manure (GM)	51.6	2.4	0.33	2.03	21.5
Wheat straw (WS)	50.8	0.5	0.06	1.71	101.6

2.2 Experimental design and treatments
The experiment commenced in 1985 which had been cropped in pearl millet - wheat sequence included 7 treatments comprising of different levels of inorganic fertilizers with and without organic manures arranged in a randomized block design with three replications. The varieties used were PBW – 502 and HHB – 197 in wheat and pearl millet respectively. The experimental plot size was 10 m × 8 m = 80 m^2. The treatment details are depicted in Table 2.

Table 2: Treatment details applied in *Kharif* and *Rabi* season

Treat. No.	Treatments applied during season	
	Kharif	***Rabi***
T1	Control	Control
T2	50% RDF through NPK	50% RDF through NPK
T3	100% RDF through NPK	100% RDF through NPK
T4	50% RDF through NPK+ 50% N through FYM	100% RDF through NPK
T5	50% RDF through NPK+ 50% N through WS*	100% RDF through NPK
T6	50% RDF through NPK+ 50% N through GM*	100% RDF through NPK
T7	Farmer's Practice	Farmer's Practice

*WS-Wheat Straw, GM-Green Manure

Farmer's Practice:
For pearl-millet: N - 50 kg/ha, P_2O_5 – 20 kg/ha, FYM @ 40 q/ha
For wheat : N - 155 kg/ha, P_2O_5 - 60 kg/ha, FYM @ 25 q/ha, $ZnSO_4$-10 kg/ha

2.3 Soil sampling
Soil samples were collected from 0-15 cm soil depth after the harvest of wheat crop on 12th April, 2014 using a 5 cm diameter auger. Five samples per plot were taken and the composite sample was obtained by mixing the collected soil samples thoroughly by hand on a clean thick polythene sheet. The bulk sample was reduced to about 500 g by quartering process. The composite samples obtained were further divided into two parts. One part was air dried then grinded, sieved (2 mm) and finally stored in cloth bags after proper labelling. These samples were kept in the laboratory for further soil analysis. The second part of the composite sample was stored at the moist field conditions at 0°C. These samples stored at 0°C were used to analyse enzyme activity. The soil samples were analyzed for different enzymes activity by adopting standard procedures as described below:

2.3.1 Urease Activity
Urease activity was measured following the method of Tabatabai and Bremner (1972) [15]. Five gm of soil was incubated with 9 ml THAM buffer, 0.2 ml toluene and 1 ml of 0.2% of urea solution at 37°C for 2 hours. Then 50 ml KCl-$AgSO_4$ solution was added and shaking was done for 30 minutes. Soil suspension was filtered. Taking 20 ml aliquot from filtrate, NH_4-N was determined by steam distillation method (Keeney and Nelson, 1982) [4].

2.3.2 Alkaline phosphatase Activity
Alkaline phosphatase activity was assessed by method described by Tabatabai and Bremner, 1969 [14]. Enzyme activity was estimated by taking 1 g of soil with 0.2 ml toluene, 4 ml of modified universal buffer (MUB, pH 11) and 1 ml of p-nitrophenyl phosphate (PNP). After incubation for 1 hour at 37°C, the enzyme reaction was stopped by adding 4 ml of 0.5 M NaOH and 1 ml of 0.5 M $CaCl_2$. Soil suspension was filtered and absorbance was measured at 420 nm.

2.3.3 Dehydrogenase Activity
Soil dehydrogenase activity was determined by estimating the rate of production of tri-phenyl formazan (TPF) from tri-phenyl tetrazolium (Casida *et al*., 1964) [3]. Distilled water and 1 ml TTC (3%) were added to soil sample (3 g) and incubated at 37°C for 24 hours. Soil solution was washed with methanol (50 ml) to remove reddish colour. The red colour intensity was measured at 485 nm.

3. Results
3.1 Soil enzymes
Enzymatic activities are critical for various biological and biochemical processes such as organic matter degradation, litter mineralization and recycling of nutrients. Further, enzyme activities are potential indicators of soil quality because they are sensitive, rapid and inexpensive representatives of the potential metabolic activity of the soil (Liang *et. al*., 2014) [7]. The effect of various treatments on the activity of three different enzymes, *i.e.* urease, alkaline phosphatase and dehydrogenase were studied (Table 3). Minimum enzyme activity was recorded in the control as compared to all other treatments. However, among fertilized treatments *viz*. T4, T5 and T6, which received organic manures exhibited more enzymatic activity than T2 and T3 to which RDF 50% and 100%, respectively were applied.

3.1.1 Urease activity
Urease activity ranged from 40 µg NH_4^+/g soil/hr in control to 89 µg NH_4^+/g soil/hr in wheat straw amended treatment. As far as urease activity was concerned, the maximum urease activity observed in T5 (89 µg NH_4^+ /g soil/hr) in which wheat straw was found to be the source of OC followed by T6 (79 µg NH_4^+/g soil/hr) and T4 (71 µg NH_4^+/g soil/hr) to which green manure and FYM was added respectively. Urease activity was found to have increase of 31, 49 and 39 µg NH_4^+/g soil/hr in farmyard (T4), wheat straw (T5) and green manure (T6), respectively over control treatment.

3.1.2 Alkaline phosphatase activity
Alkaline phosphatase activity ranged from 474 µg PNP/g soil/hr in control to 711µg PNP/g soil/hr in wheat straw amended treatment. The highest activity of alkaline phosphatase enzyme was recorded in T5 (711 µg PNP/g

soil/hr) followed by T4 (685 µg PNP/g soil/hr) and T6 (606 µg PNP/g soil/hr). All these treatments were amended with different organic carbon sources. Although significantly lower than organically amended treatments, but T3 (100% RDF) also reported appreciable activity of alkaline phosphatase (576 µg PNP/g soil/hr). The percent increase in alkaline phosphatase activity due to farmyard manure (T4), wheat straw (T5) and green manure (T6) incorporation over control were 44.5, 50 and 27.8% respectively.

3.1.3 Dehydrogenase activity

Dehydrogenase activity ranged from 36 µg TPF/g soil/24 hr in control to 78 µg TPF/g soil/24 hr in farmyard amended treatment. The values and trend observed in the activity of dehydrogenase enzyme in the soil after application of different treatments were as follows : 78 µg TPF/g soil/24 hr (50% RDF through NPK+ 50% N through FYM) > 64 µg TPF/ g soil/24 hr (50% RDF through NPK+ 50% N through WS) > 59 µg TPF / g soil/24 hr (50% RDF through NPK+ 50% N through GM) > 48 µg TPF / g soil/24 hr (100% RDF through NPK) > 42 µg TPF / g soil/ 24 hr (50% RDF through NPK) > 41 µg TPF/ g soil/24 hr. (Farmer's practice) > 36 µg TPF / g soil/24 hr (Control). Maximum activity observed in the treatments which received organic manure. T3 also reported significant dehydrogenase activity as compared to control. The percent increase in dehydrogenase activity due to farmyard manure (T4), wheat straw (T5) and green manure (T6) incorporation over control were 116.7, 77.7 and 63.8%, respectively.

Table 3: Long-term effects of various treatments on soil enzymes in surface (0-15 cm) soil at wheat harvest after 29 cycles of pearl millet-wheat cropping sequence

T. No.	Treatments	Soil Enzymes		
		Urease Activity (µg NH_4^+/gsoil/hr)	**Alkaline Phosphatase Activity (µg PNP/g soil/hr)**	**Dehydrogenase Activity (µgTPF/g soil/24hr)**
T1	Control	40	474	36
T2	50% RDF through NPK	63	525	42
T3	100% RDF through NPK	68	576	48
T4	50% RDF through NPK+ 50% N through FYM	71	685	78
T5	50% RDF through NPK+ 50% N through WS	89	711	64
T6	50% RDF through NPK+ 50% N through GM	79	606	59
T7	Farmer's Practice	52	489	41
CD (p= 0.05)		8.0	46.0	4.0

4. Discussion

The treatments receiving FYM and wheat straw showed greater enzyme activity as compared to mineral fertilizers that were added to the soil. However, based on the obtained data, it can be interpreted that activity of urease, alkaline phosphatase and dehydrogenase increased by 1.7, 1.2 and 1.3 times respectively on addition of 100% RDF through NPK as compared to control. This can be attributed to greater input of root biomass due to better crop productivity (Liu *et al*., 2010) [6]. However, this observation was not consistent with the findings of Saha *et al*. (2008) [13] and Bohme and Bhome (2006) [2] who reported that P fertilizer had a negative effect on alkaline phosphatase activity and dehydrogenase activity is highly sensitive to inhibiting effect associated with mineral fertilizer addition.

Among organic amendments, dehydrogenase activity was increased by FYM as compared to wheat straw and green manure. The results are in accordance with the results of Liu *et al*., (2010) [6]. It can be explained based on the fact that dehydrogenase activity is influence rather by the quality than by quantity of organic manure incorporated into the soil. Since FYM is easily decomposable, it is strongly influenced by the activity of dehydrogenase (Pancholy and Rice, 1973) [11]. Generally, the enzyme activities in the soil are closely related to organic matter content. Application of balanced amount of nutrients and manures improve the MBC status of soils which corresponds to higher enzyme activity (Mandal *et al*., 2007) [8]. Increase in urease activity (Raju *et al*., 2013; Lakshmi *et al*., 2014) [12, 5], alkaline phosphates activity (Mohammadi *et al*., 2011; Akca *et al*., 2015) [9, 1] and dehydrogenase activity (Pancholy and Rice., 1973; Liu *et al*., 2010 and Moharana *et al*., 2014) [11, 6, 10] with addition of organic manure with or without mineral fertilizer is well documented and we have received same set of results.

5. Conclusion

The enzymes activity was higher in organic manure amended treatment as compare to inorganic fertilizers only. The urease activity ranged from 40 to 89 µg NH_4^+/g soil/hr. The highest urease activity was observed in 50% RDF through NPK+ 50% N through wheat straw. The alkaline phosphatase ranged from 474 to 711 µg PNP/g soil/hr. The highest activity of alkaline phosphatase enzyme was recorded in 50% RDF through NPK+ 50% N through wheat straw. The dehydrogenase activity ranged from 36 to 78 µg TPF/g soil/24hr. The highest dehydrogenase activity was recorded in 50% RDF through NPK+ 50% N through FYM.

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