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SOIL MICROBIAL POPULATION AND SELECTED ENZYME ACTIVITIES AS INFLUENCED BY CONCENTRATE MANURE AND INORGANIC FERTILIZER IN ALLUVIUM SOIL OF VARANASI

V. S. MEENA, B. R. MAURYA, R. VERMA*, R. S. MEENA[1], G. K. JATAV, SUNITA KUMARI MEENA, R. MEENA AND S. K. MEENA

Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences,
Banaras Hindu University, Varanasi - 221 005, INDIA
[1]Department of Agronomy, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi - 221 005, INDIA
e-mail: rsmeenaagro@gmail.com

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***Corresponding author**

ABSTRACT

A field experiment was conducted rabi season 2009-10 at Agricultural Research Farm, BHU, Varanasi, on alluvial soils to assessed the effects of concentrate organic manure and levels of inorganic fertilizers on microbial dynamics in alluvium soil. The maximum bacterial, fungal and actinomycetes population were recorded The total bacterial counts ranged from 42 $\times$ 10^5 cfu/g to 67$\times$$10^6$ cfu/g, while the fungal density varied from 35 $\times$ 10^5 cfu/g to 70 $\times$$10^5$ cfu/g and actinomycetes range 43$\times$ 10^5 cfu/g to 62$\times$$10^5$ cfu/g. The highest microbial population were recorded during flowering stage of wheat with 100% NPK + 300 kg wellgrow soil/ha, while the lowest counts were recorded after harvest of wheat with 100% RDF. Highest soil enzymatic activity such as urease 300 μg UH g^{-1}soil/h, dehydrogenase 235 μg TPF g^{-1} soil/day and phosphatase 78 ìg p-NP g^{-1} soil/h were observed with 100% NPK + 300 kg wellgrow grain/ha. Enzymatic activities were positively and significantly correlated with content of organic carbon.

INTRODUCTION

The role that microbial activity plays in ecosystem processes is significant because approximately 80% to 90% of soil processes are mediated by microorganisms (Nannipieri and Badalucco, 2003). Soil microbial population are the driving force behind regulating soil processes such as organic matter decomposition and nutrient cycling, it is imperative to have a better understanding of the factors that regulate its size, activity, and structure (Masto *et al.,* 2006). Soils containing a high microbial diversity are characteristic of a healthy soil-plant relationship, whereas those with low microbial diversity are characterized as an unhealthy soil that often hardly responds to environmental changes (Tejada *et al.,* 2011).

Soil enzymatic activities can be used as an index of soil fertility and microbial functional diversity (Nannipieri *et al.,* 2002; Maurya *et al.,* 2011) in catalyzing several biochemical reactions which are necessary for the life processes of soil micro-organisms, organic wastes decomposition, organic matter formation and nutrients cycling (Tabatabai, 1994). The microbial population dynamics is governed by interactions between plant type, climate, and management practices. In addition, the soil microbial biomass in soil system responds more quickly to management practices than OM and is often used as an indicator of soil quality and health (Ge *et al.,* 2010).

The addition of organic manure greatly influences the microbial populations which expected to cause changes in the organic matter content of soil that directly influenced microbial dynamics of soil (DeForest *et al.,* 2012). The microbiological and biochemical conditions of a soil can serve as a marker of the soil status and is closely linked to its natural soil fertility. Addition of the organic fraction stimulates the natural soil micro organisms and reactivates the biogeochemical cycles (Watts *et al.,* 2010). Urease and phosphatase are two important enzymes involved in the N and P cycles, respectively (Badiane *et al.,* 2001). The objective of this study was to evaluate the microbial dynamics as influenced by concentrate organic manure and inorganic fertilizer in alluvium soil.

MATERIALS AND METHODS

Wellgrow is a plant product formulation in grain and in powder forms produced by an Indian Tobacco Company (ITC). Composition of wellgrow soil and grain presented in Table 1. In case of wellgrow soil (Certified organic input) is organic manure (powder) from plant products with better nutritional value made from non-timber forest product enhances efficiency of nitrogenous fertilizers and acts as a good nutritional media for the growth of bio-fertilizers and bio-pesticides to increase their performance.

Site description and field experiment

The study was conducted at the Agricultural Research Farm,

Institute of Agricultural Sciences, BHU, Varanasi (25^0 18$^¢$ N latitude, 83^0 03¢ E longitude and 128.93 m above MSL) The weekly mean maximum and minimum temperature during the experimentation ranged from 15.1 to 42.3 ^{o}C and 7.1 to 29.7 ^{o}C, respectively. Soil samples were collected from the experimental field and analyze for phyco-chemical and biological properties. Some of the initial soil properties (0–15 cm) were present in Table 2.

Experimental design and treatments

The field experiment was laid out in a randomized block design with three replications having a plot size of 4 x 3.35 m^2 experiment consisted nine treatments of wellgrow formulations and different levels of recommended dose of fertilizers (120:60:60 kg/ha) viz.,(i) 100% NPK (control), (ii) 50% NPK + 300 kg wellgrow soil/ha, (iii) 50% NPK + 300 kg wellgrow grain/ha, (iv) 75% NPK + 200 kg wellgrow soil/ha, (v) 75% NPK + 200 kg wellgrow grain/ha, (vi) 100% NPK + 200 kg wellgrow soil/ha, (vii) 100% NPK + 200 kg wellgrow grain/ ha, (viii) 100% NPK + 300 kg wellgrow soil/ha, (ix) 100% NPK + 300 kg wellgrow grain/ha. Recommended doses of phosphorous and potassium was applied as basal doses before sowing of wheat through di-ammonium phosphate and muriate of potash. Nitrogen was applied through urea in three equal splits at basal, tillering and flowering stages of wheat. Wheat variety HUW-234 used as a test crop.

Soil sampling

Initial soil samples were collected in August 2009 prior to the start of the experiment. After harvest of rice, soil samples were taken from the surface layer (0–15 cm) of nine treatments with three replications, second soil sampling at the time of flowering stage of wheat and third soil sampling was done after the harvest of wheat crop in May, 2010. For microbial analysis soil samples were kept at 4 ^{o}C in plastic bags for a few days to stabilize the microbiological activity disturbed during soil sampling and handling, and then analysed. Total bacteria, fungi and actinomycetes were estimated by following the standard procedure of Rolf and Bakken (1987) and dehydrogenase and urease (Tabatabai, 1982) and alkaline phosphatase activity measured by Tabatabai and Bremner, (1986).

Statistical analysis: Data were assessed by Duncan's multiple range tests (Duncan 1955) with a probability P = 0.05. Least significant difference (LSD) between the mean values was evaluated by a one-way analysis of variance by using SPSS version 10.0

RESULTS AND DISCUSSION

Microbial Population of Bacteria, Fungi and Actinomycetes

Table 3 indicates that over the experiment the microbial population was significantly increased in all the treatments. Data of bacterial population at before sowing of wheat crop maximum bacterial population observed with 100% NPK with + 200 kg wellgrow grain/ha it may be due to the residual effect of rice crop, at flowering stage of wheat bacterial population was significantly influenced by wellgrow

Table 1: Characteristics of wellgrow soil and grain

Parameters	Wellgrow soil Range	Wellgrow grain Range
Organic carbon (%)	20-25	18-20
Total nitrogen (%)	1.6 -2.6	1.3 -1.4
Phosphorus (as P_2O_5) (%)	0.25-1.2	1.1-1.2
Potash (as K_2O) (%)	0.89-1.47	1.3-1.4
C/N ratio	10-16:1	13-15:1
Colour	Brown	Black
Moisture (%)	9 -10	8.2-8.4

formulation with doses of inorganic fertilization and significant increase in population of bacteria over all other treatments (viz. control, 50, 75% NPK with wellgrow levels). At harvest lower bacterial population was observed in comparison to flowering stage of wheat, but at the harvest the maximum bacterial population is also observed with T_9 treatment (Table 3). This treatment significantly increased over all the treatments except T_8, at harvest decreasing bacterial population was due to decrease in organic carbon. This finding is in accordance to the finding of Watts *et al.,* (2010). This clearly revealed that organic material significantly increases the bacterial population; soil microbial biomass has been used as an index of soil fertility which depends on nutrient fluxes (Krishnakumar *et al*. 2005).

Fungi population increased with advancement growth stages of crop with all treatments. At flowering stage of wheat highest population of fungi registered with 100% NPK +300 kg wellgrow soil/ha followed by 100% NPK +300 kg wellgrow grain/ha (Table 3). More fungal population was observed at flowering stage compared to at harvest of wheat. Application of 100% NPK +300 kg wellgrow soil/ha significant increased in term of per cent over control was 33%. At harvested fungal population maximum observed with T_9 which showed significant superior all over the treatments and at par with T_8. Fungal population decreased at harvest due to lack availability of nutrients and organic matter compared to flowering stage of wheat. Similar finding were also had been reported by Nedunchezhiyan *et al*. (2013).

Actinomycetes population varied significantly with application of concentrate manure. At before sowing of crop highest population of actinomycetes was registered with 100% NPK +200 kg wellgrow grain/ha, this treatment at par with (T_8 and T_9). This finding is in accordance to the finding of Zak et *al*. (2011). At flowering stage of wheat significantly superior actinomycetes population recorded with 100% NPK + 300 kg wellgrow soil/ha, maximum significantly actinomycetes population was registered with 100% NPK+ 300 kg wellgrow grain/ha. This is consistent with the finding of Bohme *et al*. (2005) who reported that microbial biomass was greater in soil after the application of farmyard manure.

Soil enzymatic activities

Soil enzyme activity is an indirect indication on the activities of microbes which is directly correlated with soil microbial dynamics. Enzyme activity in the soil environment is considered to be a major contributor of overall soil microbial activity (Burns *et al.,* 2013). In the present investigation, significantly increased enzyme activity of urease, dehydrogenase, and alkaline phosphatase was noticed due to application of wellgrow soil, wellgrow grain and nutrient

Table 2: Initial soil Bio-chemical properties of experimental site (0-15 cm)

Properties	Value	Properties	Value
pH (soil:water, 1:2.5)	7.40	Bacteria 10^5	27
EC (dS m^{-1})	0.27	Fungi 10^4 cfu g^{-1} soil	20
Organic C (g kg^{-1} soil)	2.4	Actinomycetes 10^4	16
Available N (mg kg^{-1} soil)	88	DHA (ìg TPF g^{-1} soil day^{-1})	59
Available P	5.65	UH (ìg UH g^{-1} Soil h^{-1})	206
Available K	57	APA (μg PNP g^{-1} soil h^{-1})	37

Table 3: Soil microbial population (Bacteria, Fungi and Actinomycetes) at different growth stages (cfu g^{-1} of soil) of wheat as influenced by concentrate manure and inorganic fertilizers

Treatments	Bacteria (cfu × 10^5 g^{-1} soil)			Mean	Fungi (cfu × 10^4 g^{-1} soil)			Mean	Actinomyceties (cfu × 10^4 g^{-1} soil)			Mean
	S_1	S_2	S_3		S_1	S_2	S_3		S_1	S_2	S_3	
T1	42e	60^{c}	51^{f}	51	35^{d}	47^{d}	36^{e}	39	37f	48^{e}	44^{fg}	43
T2	46d	62^{bc}	53^{ef}	54	37^{cd}	49^{d}	40^{d}	42	39e	54^{cde}	47^{ef}	47
T3	45^{d}	66^{abc}	56^{de}	56	36^{d}	49^{d}	35^{e}	40	37^{ef}	50^{de}	43^{g}	43
T4	48^{cd}	66^{abc}	54^{ef}	56	38^{cd}	52^{d}	41^{d}	44	43^{d}	55^{cde}	49^{de}	49
T5	49^{c}	72^{ab}	59^{c}	60	39^{c}	53^{d}	44^{c}	45	47^{c}	59^{bcd}	52^{cd}	53
T6	53^{b}	74^{ab}	64^{b}	64	44^{b}	60^{c}	48^{b}	51	50^{a}	62^{abc}	57^{b}	56
T7	58^{a}	70^{abc}	59^{bcd}	62	48^{a}	63^{bc}	50^{b}	54	57^{a}	63^{abc}	55^{bc}	58
T8	56^{a}	76^{a}	66^{ab}	66	47^{a}	70^{a}	56^{a}	58	56^{a}	70^{a}	62^{a}	63
T9	57^{a}	76^{a}	68^{a}	67	50^{a}	67^{ab}	58^{a}	58	56^{a}	67^{ab}	62^{a}	62
Mean	50	69	59	-	42	57	45		47	59	52	
LSD (p = 0.05)	2.8	10.90	3.14		3.0	6.31	3.07		3.2	9.17	3.50	

Table 4: Soil enzymes activities [Urease (UA) Dehydrogenase (DHA) and alkaline phosphatase (APA)] at different growth stages of wheat in the study soils as influenced by concentrate manure and inorganic fertilizers

Treatments	UA (μg UH g^{-1}soil h^{-1})			Mean	DHA (μg TPF g^{-1} soil 24 h^{1})			Mean	APA (ìg p-NP g^{-1} soil h^{-1})			Mean
	S_1	S_2	S_3		S_1	S_2	S_3		S_1	S_2	S_3	
T1	213^{d}	257^{e}	224^{e}	231	191^{f}	151^{e}	126^{c}	156	26^{f}	46e	35^{e}	36
T2	243^{cd}	266^{d}	237^{d}	249	124^{e}	154^{e}	139^{c}	139	39^{e}	53de	43^{d}	45
T3	251^{c}	269^{cd}	237^{d}	252	118^{e}	172^{d}	140^{c}	143	38^{e}	55cd	46^{cd}	46
T4	271^{bc}	275^{c}	245^{c}	264	136^{d}	178^{d}	147^{c}	154	49^{d}	62c	48^{c}	53
T5	274^{bc}	275^{c}	247^{c}	265	143^{cd}	218^{c}	146^{c}	169	54^{d}	59cd	50^{c}	54
T6	289^{ab}	290^{b}	256^{b}	278	162^{bc}	285^{b}	215^{b}	221	64^{c}	69b	57^{b}	63
T7	320^{a}	296^{b}	257^{b}	291	157^{a}	291^{b}	225^{b}	224	86^{a}	72b	57^{b}	72
T8	306^{a}	327a	264^{a}	299	159^{ab}	295^{b}	252^{a}	235	77^{b}	84a	70^{a}	77
T9	316^{a}	324^{a}	261^{ab}	300	138^{ab}	313^{a}	225^{b}	225	82^{ab}	83a	70^{a}	78
Mean	276	287	248	-	148	229	179	-	57	65	53	-
LSD (p = 0.05)	30.0	6.39	5.22		8.6	13.84	22.50		7.2	7.03	4.50	

T_1-T_9 treatment details were given in materials and method section, S_1: Before sowing, S_2: Flowering stage, S_3 After harvest

Table 5: Correlations of organic carbon with enzyme activities in wheat at flowering and at harvest of crop as influenced by concentrate organic manure and inorganic fertilization

Soil properties		Urease		Dehydrogenase		Phosphatase		Organic carbon	
		S_2	S_3	S_2	S_3	S_2	S_3	S_2	S_3
Urease	S_2	-	0.890^{**}	0.893^{**}	0.913^{**}	0.932^{**}	0.952^{**}	0.511^{**}	0.901^{**}
	S_3		-	0.910^{**}	0.881^{**}	0.900^{**}	0.925^{**}	0.423^{*}	0.859^{**}
Dehydrogenase	S_2			-	0.932^{**}	0.895^{**}	0.902^{**}	0.464^{*}	0.873^{**}
	S_3				-	0.889^{**}	0.875^{**}	0.447^{*}	0.825^{**}
Phosphatase	S_2					-	0.931^{**}	0.635^{**}	0.919^{**}
	S_3						-	0.496^{**}	0.918^{**}
Organic carbon	S_2							-	0.561^{**}
	S_3								-

S_2: Flowering stage, S_3 After harvest, **. Correlation is significant at the 0.01 level (2-tailed), *. Correlation is significant at the 0.05 level (2-tailed).

levels (Table 4). Due to the effects of external disturbance on their activity, enzymes can serve as sensitive indicators of soil quality (Dick *et al.,* 1994: Nedunchezhiyan *et al.,* 2013).

Urease activity

Urease is an important 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 (Byrnes and Amberger, 1989). Before sowing of wheat crop maximum urease activates registered with 100% NPK + 200 kg wellgrow grain/ha, followed by 100% NPK + 300 kg wellgrow soil/ha and 100% NPK + 300 kg wellgrow grain/ha. At flowering stage of wheat highest urease activities was registered with 100% NPK + 300 kg wellgrow soil/ha, followed by 100% NPK + 300 kg wellgrow

grain/ha. This could be attributed to their higher N content and faster decomposition and release of NH_4-N (Saha *et al.,* 2008). Soil enzymes regulate the transformation process of elements required for plant growth in soil (Burns, 1982). Crop growth stages also influenced the urease activity. Under field conditions, urease activity was highest at flowering stage but under greenhouse conditions the activity was more pronounced at tillering stages (Watts *et al.,* 2010).

At harvest lower urease activity in comparison to at flowering stage of wheat, maximum urease activity registered with treatment 100% NPK +300 kg wellgrow soil/ha and at par with 100% NPK +300 kg wellgrow grain soil/ha, the lower activity of urease at harvest of crop could be related to lower microbial biomass and decreasing content of soil organic carbon. The correlation between urease and organic carbon at flowering and at harvest stages (r=0.511 and r=0.901, respectively) were positively significant (Table 5). Maestre *et al,* (2011) reported a decrease in the urease activity with addition of inorganic N whereas crop residues and organic manure additions increased it. Enzyme activities of soils are usually correlated with their organic carbon and available N contents (Ndubuisi-Nnaji *et al.,* 2011). Higher levels of organic carbon stimulate microbial activity, and therefore enzyme synthesis.

Dehydrogenase activity

Dehydrogenase is an enzyme that occurs in all intact viable microbial cells. These soil enzymes function as a measurement of the metabolic state of soil microorganisms by relating it to the presence of viable microorganisms and their oxidative capacity. Therefore, dehydrogenase can be used as a measure of microbial respiration and a reliable index of microbial activity in soil (Tejada *et al.,* 2011). Data on dehydrogenase activities at before sowing of crop maximum observed with 100% NPK + 300 kg wellgrow grain/ha, followed by T_8 and T_7 it may be due to higher organic matter content (Wlodarczyk *et al.,* 2002). Similar trained also observed in flowering stage of wheat and maximum dehydrogenase activities registered with 100% NPK + 300 kg wellgrow grain/ha. Crop growth stage also greatly impacted dehydrogenase activity. Activity of dehydrogenase measured during flowering stage was almost double that measured at before sowing of the crop (Table 4). The higher dehydrogenase activity after addition of concentrate manure could be due to increased microbial activity, which is known to stimulate the dehydrogenase activity (Watts *et al.,* 2010). At harvest of wheat lower urease activity in comparison to at flowering stage of crop, highest dehydrogenase activity registered with 100% NPK +300 kg wellgrow soil/ha and at par with T_9, T_7 and T_6, the increase in activity during the flowering stage compared with harvest stage suggests that greater microbial biomass occurred with a change in growth stage. These results suggest that changes in the size of microbial populations and respiratory activity occurred in response to the increase in available substrate. In addition, an increase in available substrate corresponds to more readily available C and N pools, which were most likely disproportionally enhanced as a result of manure addition.

This was confirmed by the significant positive correlation between dehydrogenase and organic carbon at flowering and at harvest (r=0.464, 0.873, respectively Table 5). In the present study, lowest dehydrogenase activity measured after harvest can be attributed to oxidation status of the soil as water was drained at maturity

Alkaline Phosphatase activity

Alkaline phosphatase is an enzyme of great agronomic value because it hydrolyses compounds of organic phosphorus and transforms them into different forms of inorganic phosphorus that are assimilated by plants (Maestre *et al.,* 2011). Data of alkaline phosphatase tended to be lowest in the control treatment in before sowing, flowering and at harvest of crop (Table 4), and highest with the application of 100% NPK + 200 kg wellgrow grain/ha at before sowing of crop. At flowering stage maximum alkaline phosphatase activates with 100% NPK + 300 kg wellgrow grain/ha. Sriramachandrasekharan and Ravichandran (2011) similar reported that the addition of organic substances to the soil served as a carbon source that enhanced microbial biomass and phosphatase activity, showing that these enzymes are of microbiological origin and crop growth stage also significantly influenced soil enzyme activities (Bohem *et al.,* 2005). This hypothesis is also supported by the alkaline phosphatase activity and organic carbon highly significant positively correlated at flowering and at harvest stages (r=0.635 and r=0.919, respectively), at the 0.01 level (2-tailed). The importance of organic carbon in nutrient cycling was evident that fact that the enzyme activity quantified in the present study showed positive correlation with organic carbon. This indicates that organic material significantly increases the enzymatic activity in soil. Several studies have observed inverse relationships between inorganic P availability and phosphatase activity e although this depends on initial bio-available P (DeForest *et al.,* 2012).

Soil microbial dynamics significantly contribute to soil health; the enzymatic activities were significantly influenced by the crop growth stages. Hence judicious application of 100% NPK + 300 kg wellgrow grain/ha emerged out as the best treatment. Manure along with reduced dose of nutrient levels not only improved crop growth but also significantly nutrient buildup in soil; it also maintained a balanced enzymatic activity with a lesser pollution potential then high dose of nutritional levels.

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