African Journal of Agronomy Vol. 2 (7), pp. 188-193, July, 2014. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Microbial dynamics as subjective by inorganic fertilizer and concentrate manure in alluvia soil of Varanasi, India

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Accepted 28 June, 2014

A field experiment was conducted during rabi season, 2009-2010 at Agricultural Research Farm, BHU, Varanasi, on alluvial soils to determine the effects of concentrate organic manure (wellgrow formulations) with levels of inorganic fertilizers. The results reveal that the higher soil microbial population was seen with the application of 100% NPK + 300 kg wellgrow soil ha^{-1}. Soil enzymes varied with the production systems. The urease, phosphatase and dehydrogenase activities were higher in wellgrow dose application with recommended dose of NPK. The soil enzymes and microbial population (bacteria, fungi and actinomycetes) were very responsive to organic manure application, but their levels and activities were not reflected in wheat crop under alluvial soils. Enzymatic activities were positively and significantly correlated with content of organic carbon.

Key words: Dehydrogenase, phosphatase, urease, bacteria, fungi, actinomycetes, wellgrow.

INTRODUCTION

The role that microbial activity play 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 that regulate 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, orga-nic 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 organic matter and is often used as an indi-

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cator of soil quality and health (Ge et al., 2010). The addition of organics and management practices greatly influence the microbial populations which is expected to cause changes in the soil enzymatic activities.

Incorporation of organic manures influenced soil enzymatic activity either because of the composition of the added materials themselves or because they increased micro-bial activity of the soil (DeForest et al., 2012). Improve-ment of the soil structure due to FYM application leads to a better environment for root development (Dejene and Lemlem, 2012) . FYM also improves soil water holding capacity (Tadesse et al., 2013). The fact that the use of organic fertilizers maintains soil health has raised interests in organic farming (Khan et al., 2010). 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 reac-tivates the biogeochemical cycles (Watts et al., 2010). Microbial population can also be increased through rhizosphere inoculation of bio-agents. Soil enzymatic activity is responsible for farming stable organic molecules that contributes to the per-manence of the soil ecosystem. Urease and phosphatase are two important enzymes involved in the N and P cycles, respectively (Badiane et al., 2001). Combined use of organic manures improved the microbial load of the soil rather than single organic manure application (Krishnakumar et al., 2005). The objective of this study was to evaluate the microbial population and enzymatic activities as influenced by 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). In the case of wellgrow soil (certified organic input), organic manure (powder) from plant products with better nutritional value 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 (Table 1). Wellgrow grain is pelleted organic manure. It is also a certified organic input from plant products originating from non-timber forest product enhancing efficiency of nitrogenous fertilizers, improving soil fertility and crop vigor, especially targeted towards cereal crop (Meena et al., 2013)

Site description and field experiment

This study was conducted at the Agricultural Research Farm, Institute of Agricultural Sciences, BHU, Varanasi (25° 18′ N latitude, 83° 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°C and 7.1 to 29.7°C, respectively. Soil samples were collected from the experimental field and analyzed for phyco-chemical and biological properties. Some of the initial soil properties (0 to 15 cm) are 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 consisting of nine treatments of wellgrow formulations and different levels of recommended dose of fertilizers (120:60:60 kg ha^{-1}); viz. (i) 100% NPK (e.g. nitrogen, phosphorous and potassium) (control), (ii) 50% NPK + 300 kg wellgrow soil ha^{-1}, (iii) 50% NPK + 300 kg wellgrow grain ha^{-1}, (iv) 75% NPK + 200 kg wellgrow soil ha^{-1}, (v) 75% NPK + 200 kg wellgrow grain ha^{-1}, (vi) 100% NPK + 200 kg wellgrow soil ha^{-1}, (vii) 100% NPK + 200 kg wellgrow grain ha^{-1}, (viii) 100% NPK + 300 kg wellgrow soil ha^{-1} and (ix) 100% NPK + 300 kg wellgrow grain ha^{-1}. 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 for chemical and microbial analysis and preparation

Initial soil samples were collected in August 2009 prior to the start of the experiment. After harvesting of rice, soil samples were taken from the surface layer (0 to 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. The moist soil samples were sieved (2 mm) after removing plant material and roots. Half of the soil samples were air-dried and stored at room temperature until chemical analysis. All chemical results are means of triplicate analyses and are expressed on an oven-dry basis. The rest of the sieved soil (2 mm) was immediately transferred to the laboratory for microbiological analysis. Soil samples were kept at 4°C in plastic bags for a few days to stabilize the microbiological activity disturbed during soil sampling and handling, and then analysed.

Microbiological analyses

Total bacteria, fungi and actinomycetes were estimated by the serial dilution and plating technique as described by Rolf and Bakken (1987). Total bacterial counts specific medium [1.0 g Di-potassium phosphate (K_2HPO_4), 0.2 g magnesium sulfate ($MgSO_4$), 0.1 g calcium chloride ($CaCl_2$), 0.1 g sodium chloride (NaCl), trace amount of ferric chloride ($FeCl_3$), 0.5 g potassium nitrate (KNO_3), 1.0 g aspargine, 0.1 g mannitol, 15 g agar-agar and 1000 mL distilled water], actinomycetes specific medium [1.0 g Dextrose, 0.10 g Di-potassium phosphate (K_2HPO_4), 0.10 g sodium nitrate, 0.10 g magnesium sulfate ($MgSO_4$), 15 g agar and 1000 mL distilled water] and fungal specific medium [10 g glucose, 5.0 g peptone, 1.0 g di-potassium phosphate (K_2HPO_4), 0.5 g magne-sium sulfate ($MgSO_4$), 15 g agar and 1000 mL distilled water], streptomycin @ 30 mg mL^{-1} was added to melted medium after it has cooled to 50°C, then prepared and plated on the glass Petri dish. 0.1 mL from 10^1 to 10^5 dilutions was poured on the surface of Petri dish with the help of a micropipette and was evenly spread with the help of a sterile spreader and incubated at 30±2°C. Colony count was carried out daily up to 10 days.

Soil dehydrogenase activity was estimated by reducing

Table 1. Characteristics of wellgrow soil and grain.

Parameter	Wellgrow soil	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

2,3,5- triphenyltetrazolium chloride (Casida et al., 1964). Five grams of soil sample were mixed with 50 mg of $CaCO_3$ and 1 mL of 3% (w/v) 2,3,5-triphenyltetrazolium chloride (TTC) and incubated for 24 h at 37 ± 1°C. Dehydrogenase enzyme converts TTC to 2,3,5-triphenylformazan (TPF). The TPF formed was extracted with acetone (3 × 15 mL), the extracts were filtered through Whatman No. 42 and absorption was measured at 485 nm with a spectro-photometer (Analytik Jena, Germany).

Urease activity was measured following the method of Tabatabai and Bremner (1969). Five grams of soil were incubated with 5 mL of 0.05 M THAM buffer (pH 9.0) and 1 mL of 0.2% of urea solution at 37 ± 1°C for 2 h. Excess urea was extracted with KCl-PMA solution and estimated colorimetrically at 527 nm.

Alkaline phosphatase activity was assayed using 1 g of soil (wet equivalent), 4 mL of 0.1 M modified universal buffer (pH 11 for alkaline phosphatase) and 1 mL of 25 m M p-nitrophenyl phosphate (Tabatabai and Bremner, 1969). After incubation for 1 h at 37 ± 1°C, the enzyme reaction was stopped by adding 4 mL of 0.5 M NaOH and 1 mL of 0.5 M $CaCl_2$ to prevent dispersion of humic substances. After centrifugation at 4000 rpm for 10 min, the absorbance was measured in the supernatant at 400 nm; enzyme activity was expressed as mg p-nitrophenol g^{-1} soil h^{-1}.

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 in the experiment, the microbial population was significantly increased in all the treat-ments. In the data of bacterial population before sowing of wheat crop, maximum bacterial population (58×10^5 g^{-1} soil) was observed with 100% NPK with + 200 kg wellgrow grain ha^{-1}, it may be due to the residual effect of rice crop at flowering stage of wheat, bacterial population was significantly influenced by wellgrow 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). Maximum population of bacteria was recorded with 100% NPK + 300 kg wellgrow grain/soil ha^{-1}. At harvest, lower bacterial population was observed in comparison with flowering stage of wheat, but at harvest, the maximum bacterial population was also observed in T_9 (68×10^5 g^{-1} soil) treatment (Table 3). This treatment significantly increased over all the treatments except T_8 ($66 \times 10^5 g^{-1}$ soil), at harvest decreasing bacterial population which was due to decrease in organic carbon. This finding is in accordance with the finding of Watts et al. (2010). This clearly revealed that organic material significantly increases the bacterial population. The soil microbial population in 100% NPK +300 kg wellgrow grain ha^{-1}, soil microbial biomass has been used as an index of soil fertility which depends on nutrient fluxes (Krishnakumar et al., 2005; Meena et al., 2013).

Fungi population increased with advancement of growth stages of crop with all treatments. Before sowing of wheat crop maximum fungal population was observed (50×10^4 g^{-1} soils). At flowering stage of wheat, highest population of fungi was registered (70×10^4 g^{-1} soil) with 100% NPK + 300 kg wellgrow soil ha^{-1} followed by 100% NPK + 300 kg wellgrow grain ha^{-1} (67×10^4 g^{-1} soil) (Table 3). Maximum fungal population was observed at flowering stage as compared to at harvest of wheat. Application of 100% NPK + 300 kg wellgrow soil ha^{-1} significantly increased in terms of percent over control which was 33%. At harvest, fungal population was maximum in T_9 (58×10^4 g^{-1} soil) which showed significant superiority over all the treatments and at par with T_8 (100% NPK + 300 kg wellgrow soil ha^{-1}). Fungal population decreased at harvest due to lack of availability of nutrients and organic matter as compared to flowering stage of wheat. Fungi population increased with advance-ment growth stages of crop. It might be possible that increased total root biomass with the passage of time, might be instrumental to supporting higher fungi popu-lation. Similar results have been recently described in a study on wheat, in which total bacteria and fungi were evaluated (Vujanovic et al., 2012; Nedunchezhiyan et al., 2013).

Actinomycetes population varied significantly with application of concentrate manure. Before sowing of crop, highest population of actinomycetes was registered with 100% NPK + 200 kg wellgrow grain ha^{-1} (57×10^4 g^{-1} soil), this treatment is at par with (T_8 and T_9). This finding is in accordance with the findings of Zak et al. (2011). At flowering stage of wheat, significantly superior actinomy-cetes population was recorded with 100% NPK + 300 kg wellgrow soil ha^{-1}, followed by treatment having 100% NPK+ 200 kg wellgrow grain/soil formulations at harvest of crop population of actinomycetes decreased, signifi-cant maximum actinomycetes population was registered

Table 2. Initial soil Biochemical properties of experimental site (0-15 cm).

Properties	Value	Properties	Value
pH (soil:water, 1:2.5) EC (dS m^{-1})	7.40	Bacteria (cfu×10^5 g^{-1} soil)	27
	0.27	Fungi (cfu×10^4 g^{-1} soil)	20
Organic C (g kg^{-1} soil) Available	2.4	Actinomycetes (cfu×10^4 g^{-1} soil)	16
nitrogen (mg kg^{-1} soil)	88	Dehydrogenase (µg TPF g^{-1} soil day^{-1})	59
Available phosphorous (mg kg^{-1} soil)	5.65	Urease (µg UH g^{-1} soil h^{-1})	206
Available potassium (mg kg^{-1} soil)	57	Alkaline Phosphatase (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.

Treatment	Bacteria (cfu×10^5 g^{-1} soil)			Mean	Fungi (cfu×10^4 g^{-1} soil)			Mean	Actinomycetes (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}	56de	56	36^{d}	49^{d}	35^{e}	40	37^{ef}	50de	43^{g}	43
T4	48^{cd}	66^{abc}	54^{ef}	56	38^{cd}	52^{d}	41^{d}	44	43^{d}	55^{cde}	49de	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	

T_1: 100% NPK (120:60:60 kg ha^{-1}), T_2: 50% NPK + 300 kg wellgrow soil ha^{-1}, T_3: 50% NPK + 300 kg wellgrow grain ha^{-1}, T_4: 75% NPK + 200 kg wellgrow soil ha^{-1},T_5: 75% NPK + 200 kg wellgrow grain ha^{-1}, T_6: 100% NPK + 200 kg wellgrow soil ha^{-1}, T_7: 100% NPK + 200 kg wellgrow grain ha^{-1}, T_8: 100% NPK + 300 kg wellgrow soil ha^{-1}, T_9: 100% NPK + 300 kg wellgrow grain ha^{-1}. S_1: Before sowing, S_2: flowering stage, S_3: after harvest.

registered (62 × 10^4 g^{-1} soil) with 100% NPK + 300 kg wellgrow grain/soil formulations. 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 of 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 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; Meena et al., 2013).

Urease activity

Urease is an important enzyme responsible for the hydrolysis of urea fertilizer applied to the soil, NH_3 and CO_2 with the concomitant rise in soil pH (Byrnes and Amberger, 1989). Before sowing of wheat crop, maxi-mum urease activates registered 100% NPK + 200 kg wellgrow grain ha^{-1} (320 µg UH g^{-1} soil hr^{-1}), this treatment was followed by 100% NPK + 300 kg wellgrow soil ha^{-1} and 100% NPK + 300 kg wellgrow grain ha^{-1} (306 and 316 µg UH g^{-1} soil hr^{-1}, respectively). At flowering stage of wheat, highest urease activities was registered with 100% NPK + 300 kg wellgrow soil (327 µg UH g^{-1} soil hr^{-1}) followed by 100% NPK + 300 kg wellgrow grain ha^{-1} (324 µg UH g^{-1} soil hr^{-1}). This could be attributed to their higher N content and faster decomposition and release

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.

Treatment	UA (µg UH g^{-1}soil h^{-1})			Mean	DHA (µg TPF g^{-1} soil 24 h^{1})			Mean	APA (µg 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: 100% NPK (120:60:60 kg ha^{-1}), T_2: 50% NPK + 300 kg wellgrow soil ha^{-1}, T_3: 50% NPK + 300 kg wellgrow grain ha^{-1}, T_4: 75% NPK + 200 kg wellgrow soil ha^{-1},T_5: 75% NPK + 200 kg wellgrow grain ha^{-1}, T_6: 100% NPK + 200 kg wellgrow soil ha^{-1}, T_7: 100% NPK + 200 kg wellgrow grain ha^{-1}, T_8: 100% NPK + 300 kg wellgrow soil ha^{-1}, T_9: 100% NPK + 300 kg wellgrow grain ha^{-1}. 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 harvest of crop as influenced by concentrate organic manure and inorganic fertilization.

Soil properties		Urease		Dehydrogenase		Phosphatase		Organic carbon	
		S2	S3	S2	S3	S2	S3	S2	S3
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).

of NH_4-N (Saha et al., 2008; Meena *et al.,* 2013)

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 with at flowering stage of wheat, maximum urease activity was registered (264 µg UH g^{-1} soil hr^{-1}) with treatment 100% NPK + 300 kg wellgrow soil ha^{-1} and at par with 100% NPK + 300 kg wellgrow grain soil ha^{-1} (261 µg UH g^{-1} soil hr^{-1}), 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 oforganic 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 before sowing of crop maximum observed with 100% NPK + 300 kg wellgrow grain ha^{-1} (188 µg TPF g^{-1} soil day^{-1}) followed by T_8 and T_7 may be due to higher organic matter content and relatively higher organic carbon (Wlodarczyk et al., 2002). Similar trend was also observed in flowering stage of wheat and maximum dehydrogenase activities was registered (313 µg TPF g^{-1} soil day^{-1}) with 100% NPK + 300 kg wellgrow grain ha^{-1} at flowering stage of wheat. Crop growth stage also greatly impacted dehydrogenase activity. Dehydrogenase activity measured during flowering stage was almost double that measured 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 was seen in comparison with at flowering stage of crop, highest dehydrogenase activity was registered as 252 µg TPF g^{-1} soil day^{-1} with treatment 100% NPK + 300 kg wellgrow soil ha^{-1} and it is at par with T_9, T_7 and T_6 (225, 225 and 215 µg TPF g^{-1} soil day^{-1} respectively), the increase in activity during the flowering stage compared well with harvest stage, suggesting 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 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 on alkaline phosphatase tend to be lowest in the control treatment before sowing, flowering and at harvest of crop (Table 4), and highest (86 µg p-NP g^{-1} soil h^{-1}) with the application of 100% NPK + 200 kg wellgrow grain ha^{-1} before sowing of crop. At flowering stage, maximum alkaline phosphatase activates (83 µg p-NP g^{-1} soil h^{-1}) 100% NPK + 300 kg wellgrow grain ha^{-1}. Sriramachandrasekharan and Ravichandran (2011) 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 and positively correlated at flowering and 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, 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 (Table 5). Several studies have observed inverse relationships between inorganic P availability and phosphatase activity although this depends on initial bioavailable P (DeForest et al., 2012).

Conclusions

Integrated use of organic manure (wellgrow) and inorganic fertilizers improved the enzymatic activities as well as microbial population of bacterial, fungal and actinomycetes with the best application at 100% NPK + 300 kg wellgrow grain/soil ha^{-1}. Decomposition of organic matter and recycling of carbon have substantial effect on the activity of enzyme evolved in mineralization of nutrients. Soil enzymes significantly contribute to soil health. The activities of urease, dehydrogenase and phosphatase were significantly influenced by the crop growth stages. Enzymatic activity increased up to flowering stage of wheat and declined thereafter. Hence judicious application of 100% NPK + 300 kg wellgrow grain/soil ha^{-1} emerged as the best treatment for both flowering and harvest stage for enzymatic activity as well as microbial population. Manure together with reduced

dose of nutrient levels not only improved crop growth but also significantly buildup nutrient in the soil; it also maintained a balanced enzymatic activity with a lesser pollution potential then high dose of nutritional levels.

ACKNOWLEDGEMENT

The authors wish to acknowledge the Head, Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, BHU Varanasi, for providing the necessary facilities to conduct this research work. We also pay sincere thanks to Prof. Dr. Stefan Schmidt, Editor of African Journal of Microbiology Research to the modification in manuscript

REFERENCES

Badiane NNY, Chotte L, Pate E, Masse D, Rouland C (2001). Use of soil enzyme activities to monitor soil quality in natural and improved fallows in semi-arid tropical regions. Appl. Soil Ecol. 18:229-238.

Bohem L, Langer U, Bohem F (2005). Microbial biomass, enzyme activities and microbial community structure in two European long-term field experiments. Agric. Ecosyst. Environ 109:141-152.

Burns RG (1982). Enzyme activity in soil: location and a possible role in microbial ecology. Soil Biol. Biochem. 14:423-427.

Burns RG, DeForest JL, Marxsen J, Sinsabaugh RL, Stromberger ME, Wallenstein MD, Michael NW, Zoppini A (2013). Soil enzymes in a changing environment: Current knowledge and future directions. Soil Biol. Biochem. 58:216-234.

Byrnes BH, Amberger A (1989). Fate of broadcast urea in a flooded soil when treated with N-(n-butyl) thio-phospheric triamide, a urease inhibitor. Fertil. Res. 18:221-231.

Casida LE, Klein D, Santoro T (1964). Soil dehydrogenase activity, Soil Sci. 98:371-376.

DeForest JL, Smemo KA, Burke DJ, Elliott HL, Becker JC (2012). Soil microbial responses to elevated phosphorus and pH in acidic temperate deciduous forests. Biogeochem. 109:189-202.

Dejene M, Lemlem M (2012). Integrated agronomic crop managements to i mprove Tef productivity under terminal drought. In: I. Md. M. Rahman and H. Hasegawa, Eds., *Water Stress*, In Tech Open Sci. 235- 254.

Dick RP, Sandor JA, Eash NS (1994). Soil enzyme activities after 1500 years of terrace agriculture in the Colca Valley, Peru. Agric. Ecosyst. Environ. 50:123-131.

Duncan DM (1955). Multiple range and multiple F-test. Biometric. 11:1-42.

Ge G, Li Z, Fan F, Chu G, Hou Z, Liang Y (2010). Soil biological activity and their seasonal variations in response to long-term application of organic and inorganic fertilizers. Plant Soil. 326: 31-44.

Khan NI, Malik AU, Umer F, Bodla MI (2010). Effect of tillage and farm yard manure on physical properties of soil. Int. Res. J. Plant Sci. 1(4):75-82.

Krishnakumar S, Saravanan A, Natarajan SK, Veerabadran V, Mani S (2005). Microbial population and enzymatic activity as influenced by organic farming. Res. J. Agric. Biol. Sci. 1(1):85-88.

Maestre FT, Puche MD, Guerrero C, Escudero A (2011). Shrub encroachment does not reduce the activity of some soil enzymes in Mediterranean semiarid grasslands. Soil Biol. Biochem. 43:1746-1749

Masto RE, Chhonkar PK, Singh D, Patra AK (2006). Changes in soil biological and biochemical characteristics in a long-term field trial on a sub-tropical Inceptisol. Soil Biol. Biochem. 38:1577-1582.

Maurya BR, Vimal S, Dhyani PP (2011). Enzymatic activities and microbial population in agri-soils of Almora district of central Himalaya as influenced by altitudes. Int. J. Soil Sci. 6:238-248.

Meena VS, Maurya BR, Bohra JS, Verma R, Meena MD (2013). Effect of concentrate manure and nutrient levels on enzymatic activities and microbial population under submerged rice in alluvium soil of Varanasi. Crop Res. Int. J. 45(3): 1-9.

Meena VS, Maurya BR, Verma R, Meena MD (2013). Effect of concentrate m anure and different levels of nutrients on growth a nd yield of rice in Eastern Uttar Pradesh. Annals Biol. 29(2):158-163.

Nannipieri P, Badalucco L (2003). Biological processes, In: Benbi, D.K., Nieder, R. (Eds.), Handbook of Processes and Modelling in the Soil-Plant System. Haworth Press, Binghamton, NY, pp. 57-82.

Nannipieri P, Kandeler E, Ruggiero P (2002). Enzyme activities and microbial and biochemical processes in soil. In: Burns, RG and Dick RP (Eds.), Enzymes in the Environment: Activity, Ecology and Applications. Marcel Dekker, New York, pp. 1-33.

Ndubuisi-Nnaji UU, Adegoke AA, Ogbu HI, Ezenobi NO, Okoh AI (2011). Effect of long-term organic fertilizer application on soil microbial dynamics. Afr. J. Biotechnol. 10(4):556-559.

Nedunchezhiyan M, Byju G, Dash SN, Ranasingh N (2013). Selected Soil Enzyme Activities, Soil microbial biomass carbon and root yield as influenced by organic production systems in sweet potato. Commun. Soil Sci. Plant Analy. DOI:10.1080/00103624.2012.756506.

Rolf AO, Bakken LR (1987). Vibility of soil bacteria: optimization of plate counting technique and comparison between total counts and plate counts within different size groups. Micro. Ecol. 13:59-74.

Saha S, Prakash V, Kundu S, Kumar N, Mina BL (2008). Soil enzymatic activity as affected by long term application of farm yard manure and mineral fertilizer under a rainfed soybean-wheat system in N-W Himalaya. Euro. J. Soil Biol. 44:309-315.

Sriramachandrasekharan MV, Ravichandran M (2011). Enzyme dynamics and soil properties in paddy soil fertilized with mineral and green manure n sources. Agric. Segm. 2(2) AGS/1558.

Tabatabai MA (1994). Soil enzymes In: Weaver RW, Angle JS and Bottomley PS (Eds.) Methods of soil analysis, part 2. Microbiological and biochemical properties. SSSA Book Series No. 5. Soil Sci. Soc. Am. Madison, Wis., pp. 775-833.

Tabatabai MA, Bremner JM (1969). Use of p-nitrophenylphosphate for assay of soil phosphatase activity: Soil Biol. Biochem. 1:301-307.

Tadesse T, Dechassa N, Bayu W, Gebeyehu S (2013). Effects of farmyard manure and inorganic fertilizer application on soil physico-chemical properties and nutrient balance in Rain-Fed Lowland Rice Ecosystem. Am. J. Plant Sci. 4:309-316.

Tejada M, Benítez C, Gómez I, Parrado J (2011). Use of bio stimulants on soil restoration: Effects on soil biochemical properties and microbial community. Appl. Soil Ecol. 49:11-17.

Vujanovic V, Mavragani D, Hamel C (2012). Fungal communities associated with durum wheat production system: A characterization by growth stage, plant organ and preceding crop. Crop Prot. 37:26-34.

Watts DB, Allen TH, Feng Y, Prior SA (2010). Soil microbial community dynamics as influenced by composted dairy manure, soil properties, and landscape position" Soil Sci. 175:474- 486.

Wlodarczyk T, Stepniewski W, Brzezinska M (2002). Dehydrogenase activity, redox potential, and emissions of carbon dioxide and nitrous oxide from Cambisols under flooding conditions. Biol. Fert. Soils. 36:200-206.

Zak DR, Pregitzer KS, Burton AJ, Edwards IP, Kellner H (2011). Microbial responses to a changing environment: implications for the future functioning of terrestrial ecosystems. Fungal Ecol. 4:386-395.