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LONG TERM EFFECTS OF ORGANIC MANURES (FYM, SEWAGE SLUDGE) AMENDMENTS ON SOIL ENZYMATIC ACTIVITIES IN AN ALLUVIAL SOIL OF ALIGARH DISTRICT: A 20 YEARS STUDY

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ABSTRACT

The aim of the present study was to evaluate long term effect (20 Years) of FYM and sewage sludge on changes in microbial biomass, microbial population and enzymatic activities (DHA, acid, alkaline phosphatase, catalase, invertase and urease) under a wheat/ maize cropping system in the alluvial soil of Aligarh district. The results showed that application of FYM or sewage sludge @5t ha^{-1} enhances microbial population, biomass and enzymatic activities as compared to control. The microbial population, biomass and enzymatic activities decreased in presence of 10 t ha^{-1} of sewage sludge than control, which may be due to adverse effects of heavy metals present in sewage sludge. The microbial population, biomass and enzymatic activities in presence of 10 t ha^{-1} of FYM were lower than 5 t ha^{-1} of FYM but higher than control. From these results it can be concluded that long term application of FYM at the lower rate of amendment is the best management option for improving soil quality and microbial activities

KEY WORDS: Organic manure, FYM, Sewage sludge, enzymatic activities, alluvial soil

INTRODUCTION:

Soil fertility can be defined as the capacity of soil to provide physical, chemical and biological needs for the growth of plants for productivity[1]. Recently soil microbiological and biochemical properties such as microbial biomass, soil respiration, microbial activity, soil enzyme activities etc. have been defined as important soil quality indicators. A soil is biologically active when biological processes precede rapidly[2]. Enzymatic activities are crucial for biological and biochemical processes such as organic matter degradation, litter mineralization and recycling of macronutrients (N, P, S) and some micronutrients[3, 4]. Furthermore, enzyme activities have been suggested as potential indicators of cropping and management history and organic matter change, soil degradation and contamination and climate change[5]

The utilization of bio solids/sewage sludge in agriculture is gaining popularity as a source of waste disposal. Sewage sludge consists of by-products of wastewater treatment. It is a mixture of water, inorganic and organic materials removed from wastewater coming from various sources (domestic sewage, industries), storm water runoff from roads and other paved area, through physical, biological, and/or chemical treatments. The organic matter content in sludge can improve soil physical, chemical, and biological properties with ensuring better cultivation and aquifers capacity of soil[6].Long term field experiments are the primary source of information to determine the effects of crop management on soil productivity.

The work reported here was carried out with the objective of evaluating the effects of sewage sludge and farm yield manure (FYM) over 20 years on microbial biomass, microbial population and enzyme activities of dehydrogenase, acid and alkaline phosphatase, catalase, invertase and urease under a wheat/ maize cropping system in the alluvial soil of Aligarh district.

MATERIALS AND METHODS:

The study site is located in Aligarh, India (27^{o}54'30"N, 78^{o}4'26" E). The experimental area belongs to semi- arid climate with an average annual temperature of 24.6^{o}C and annual rainfall of 600 mm. The soils were classified as alluvial typicustochrept. The prevalent cropping system in this region is winter wheat (middle October to May) and maize (early June to late September).Three treatments were selected for the study: (i) control (CK); (ii) sewage sludge (5 t ha^{-1}and10 t ha^{-1} for each crop); (iii) farmyard manure (FYM: 5 t ha^{-1}and10 t ha^{-1} for each crop). Each treatment was replicated three times (2x2 m size marked by wooden pegs) in a randomized block design. Annual doses of mineral nitrogen fertilizers were applied. The entire doses of mineral fertilizers and manure were homogenously surface broadcast by hand right before the wheat and maize sowing. Three irrigation events for wheat and two for maize) were applied annually. After harvest all the above ground residues were pulverized and incorporated into soil in sewage sludge and FYM. Soil sampling was carried out annually before mineral fertilization at the beginning of May in the period 1993 to 2013 from topsoil in the depths of 0–20 cm at four sites of each individual plot. The average physico-chemical properties of soil are given in Table 1. The soil samples were drawn at different time intervals (0, 7, 14, 21, 35, 56, 70 and 91 d) after incubation and stored in plastic vials at 4 ^{0}C to evaluate the enzyme activities.

Dehydrogenase activity (DHA) activity was measured according to Casida *et al.*[7].Air dried 20 g soil was mixed with 0.2 g of $CaCO_3$ and 6 g of this mixture was placed in three test tubes separately. After adding 1 mL of 3 % aqueous solution of TTC (Triphenyltetrazolium chloride) and 2.5 mL of deionized water, samples were then incubated at 37^{0}C for 24 hr. After incubation Triphenyl formazon (TPF) formed was extracted with 10 mL of methanol by shaking vigorously for 2 h and filtered. Intensity of developed red color was measured spectrophotometerically at 485nm. The DHA activity was expressed as ug TPF d^{-1} g^{-1} soil.

Acid/alkaline phosphatase activity was measured according to Tabatabai and Bremner[8].Air dried 1 g soil was mixed with 0.2 mL of toluene, 4 mL of modified universal buffer (pH 6.5 for acid phosphatase and pH 11for alkaline phosphatase) and 1 mL of *p*-nitro phenyl phosphate solution. After incubation at 37^{0}C for 1 h, the extinction of the PNP (*p*-nitro phenol) was measured spectrophotometerically at 420 nm. The activity was expressed as ug of PNP hydrolyzed h^{-1} g^{-1} soil.

Urease activity was measured as per the method described by Tabatabai and Bremner[9].Five g of soil was incubated with 5 mL of 10% urea solution and 20 mL of citric acid buffer (pH 6.7) at 37^0 C for 24 h. Amount of $N\text{-}NH_4$ produced was estimated in the filtrate at 580 nm spectrophotometrically using mixed reagent (phenol+ NaOH) and sodium hypochlorite. The results were expressed as ug NH_4^+ d^{-1} g^{-1} soil.

Invertase activity was measured as per the method described byGuan[10]. Five g air-dried soil was placed in a50 ml Erlenmeyer flask with 0.1 ml of toluene, 5 ml of phosphate buffer (pH 5.5) and 15 ml of 8% sucrose. The samples were mixed on a vortex and incubated at 37^0 C for 24 h. The glucose released by invertase was then reacted with 3, 5-dinitrosalicylic acid (DNS) and was measured by colorimetric method at 510 nm.

Catalase activity was measured by back-titrating residual H_2O_2 with $KMnO_4$[11]. Two g soil was added to 40 mL distilled water with 5 mL of 0.3% hydrogen peroxide solution. The mixture was shaken for 20 min and then 5 mL of 1.5 M H_2SO_4 was added. Afterwards the solution was filtered and titrated using 0.02 M $KMnO_4$.The reacted amount of 0.02 M $KMnO_4$was calculated per g of dry soil. Catalase activity was expressed as mL of 0.1 mol/L $KMnO_4$ g^{-1} soil.

Microbial biomass carbon (MBC) was determined by chloroform fumigation method[12]. Out of two sets of moist soil one set was subjected to chloroform fumigation for 24 h in a desiccator. Both the fumigated and un-fumigated soil samples were extracted with 0.5 M K_2SO_4 by shaking for 30 min on an end-to-end shaker. The extracts were filtered through a Whatman filter paper. Organic C was quantified by potassium dichromate oxidation method[13]. All the results were evaluated on an oven-dry sample basis and are the mean of six replicates. The microbial biomass (C) was calculated as the difference in soluble organic C between the fumigated and un-fumigated samples using a k_{EC} factor of 0.45 to account for the non-extractable microbial biomass C.

Quantification of bacteria, fungus, Actinomycetes in soil was made using dilution plate techniques. Dilution was made up to 10^{-7} for bacteria, 10^{-3} for fungus and 10^{-3} for actinomycetes.

All the chemicals used were of analytical grade and all the experiments were done in triplicate.

Results and Discussion

Microbial biomass carbon (MBC): Soil organic carbon plays an important role in soil biological, chemical and physical properties. Application of farm yield manure (FYM) resulted in a significant boost in the microbial biomass carbon level over the control at both the amended level. In presence of sewage sludge the MBC increased at lower dose (5 t ha^{-1}). At higher dose (10 t ha^{-1}) of sewage sludge the microbial biomass decreased (Table 1).Decrease in MBC at higher dose of sewage sludge might be due adverse effects of heavy metals present in sewage sludge[14].In addition, the heavy metals might impose resistant to microbial community through mutation to spread their generations.

Microbial population: Data of the study ((Table 1) denote that the population of microbes (Bacteria, fungi and actinomycetes) increased by addition of FYM (at both the levels) and by addition of lower dose (5 t ha^{-1}) of sewage sludge over the control. At higher concentration of sewage sludge (10 t ha^{-1}) the microbial population level decreases over the control. These results suggest that the promotional effect of organic matter on microbial population was diminished (Table 1) due to higher heavy metals contamination. Similar results are also reported by Epelde *etal*[15]. Giri *et al*.,[16]. Stoven *etal*[14],observed a significant decrease in microbial population due to heavy metals. .

Dehydrogenase activity: Dehydrogenase enzyme occurs intracellular in the all living microbial cells and it is linked with microbial respiratory process. Dehydrogenase activity of soil representing metabolic oxidative activity of soil organisms was significantly influenced by FYM and sewage sludge applications. The activity of dehydrogenase increased by 58% for FYM and 34% for sewage sludge over control in presence of 5 t $ha^{-1}$of manure, while in presence of10 t ha^{-1}ofmanure the DHA activity was 8% higher than control for FYM and 20% lower for sewage sludge (Fig. 1). The results of present study denote that DHA activity which regulate nutrient transformation is influenced by FYM or sewage sludge. The stronger effects of FYM on DHA activity might be due to more easily decomposable components of organic matter on the metabolism of soil microorganisms. The decrease in DHA activity in presence of higher doses of sewage sludge might be due to adverse effects of heavy metals on microorganisms. The study also showed that dehydrogenase activity is significantly correlated with microbial population and soil organic matter content, as organic matter is the seat of microbial population and activity. Similar results are also reported by other workers[17, 18].

Acid and alkaline phosphatase activities: Phosphates are inducible enzymes excreted by plant roots and soil organisms, which can be stimulated by P starvation[19]. Therefore, phosphatase activities have been regarded as an important factor in maintaining and controlling mineralization rate of soil organic P and a good indicator of P deficiency[20].Acid phosphatase activity increased significantly due to application of FYM or sewage sludge @ 5 t ha^{-1} than control. The increase was 60% for FYM and 39% for sewage sludge; while at higher dose of manure i.e. 10 t ha^{-1} the activity of acid phosphatase was higher by 8% over control for FYM and lower by 12% for sewage sludge. Activity of alkaline phosphatase (Fig. 1) enhanced by 45% for FYM and 16% for sewage sludge over control in presence of lower dose of manure (5 t ha^{-1}), while activity retarded by 7% for FYM and 13% for sewage sludge in presence of 10 t $ha^{-1}$of manure than control. The data denote that phosphatase activity increases when the sources of nutrients have an equilibrated balance between C and N. The results also denote that phosphate solubilising microorganisms not only solubilize inorganic phosphate compounds but also mineralize organic phosphorous.

Urease activity: The urease enzyme plays an important role in the efficient use of urea fertilizer and some environmental risk assessments. The activity of enzyme urease in presence of 5 t $ha^{-1}$of manure increased by 32% for FYM and 28% for sewage sludge over control. The activity of urease in presence of 10 t ha^{-1} of sewage sludge retarded by 3.4% than control, while it increased by 5.0% over control in presence of 10 t ha^{-1} of FYM (Fig 1). These data indicate that higher organic matter levels provide a more favorable environment for the accumulation of enzymes in the soil matrix, since soil organic constituents are thought to be important in forming stable complexes with free enzymes. Similar results are also reported by other workers[21, 22].

*Invertase activity:*Invertasecatalyses the hydrolysis of sucrose into glucose and fructose. Enzyme activity was expressed as the amount of glucose formed from the substrate. The results denote that the introduction of manure (FYM or sewage sludge) @ 5 t ha^{-1} to the soil samples led to enhance in the enzymatic activity (Fig.1), the increase was 89% for FYM and 32% for sewage sludge in comparison to control. The activity of invertase in presence of 10 t ha^{-1} of sewage sludge retarded by 23% than control, while it increased by 14% over control in presence of 10 t ha^{-1} of FYM. The study also showed that invertase activity is significantly correlated with soil organic carbon, as organic matter is the seat of microbial and enzymatic activity. The higher values of enzymatic activity in presence of manure might be associated with biotic and abiotic processes which take place in soil.

Catalase activity: The catalase can split hydrogen peroxide into molecular oxygen and water and thus prevent cells from damage by reactive oxygen species[23]. This enzyme is reported in all the aerobic microorganisms, plant and animal cells. Response of catalase activity to manure amendment to Aligarh soil is given in Fig. 1. The results denote that the introduction of manure (FYM or sewage sludge) @ 5 t $ha^{-1}$to the soil samples led to enhance in the enzymatic activity, the increase was 85% for FYM and 52% for sewage sludge in comparison to control. The higher values of enzymatic activity in presence of manure might be associated with biotic and abiotic processes which take place in soil. The catalase activity in presence of 10 t ha^{-1} of FYM was 23% higher than control and 21% lower than control in presence of 10 t ha^{-1} of sewage sludge. These data suggests that the presence of a minimum amount of fresh organic matter is needed to activate soil catalase activity[24].

CONCLUSIONS: The results of the present study suggests that application of organic manure to the native soil @5t ha^{-1} improved the microbial population, biomass and stimulate the activity of DHA, acid, alkaline phosphatase, catalase, invertase and urease. At higher concentration (10t ha^{-1}) of sewage sludge the microbial population, biomass and stimulate the activity of DHA, acid, alkaline phosphatase, urease and catalase decreased in comparison to control which might be due to adverse effects of heavy metals present in sewage sludge. Thus it can be concluded from the present studies that FYM or sewage sludge at lower rate could be used as an alternate nutrient source for maintaining soil nutrient availability and enzyme activities. From the present long term study it can also be concluded that long term application of FYM is the best management option for improving soil quality and microbial activities.

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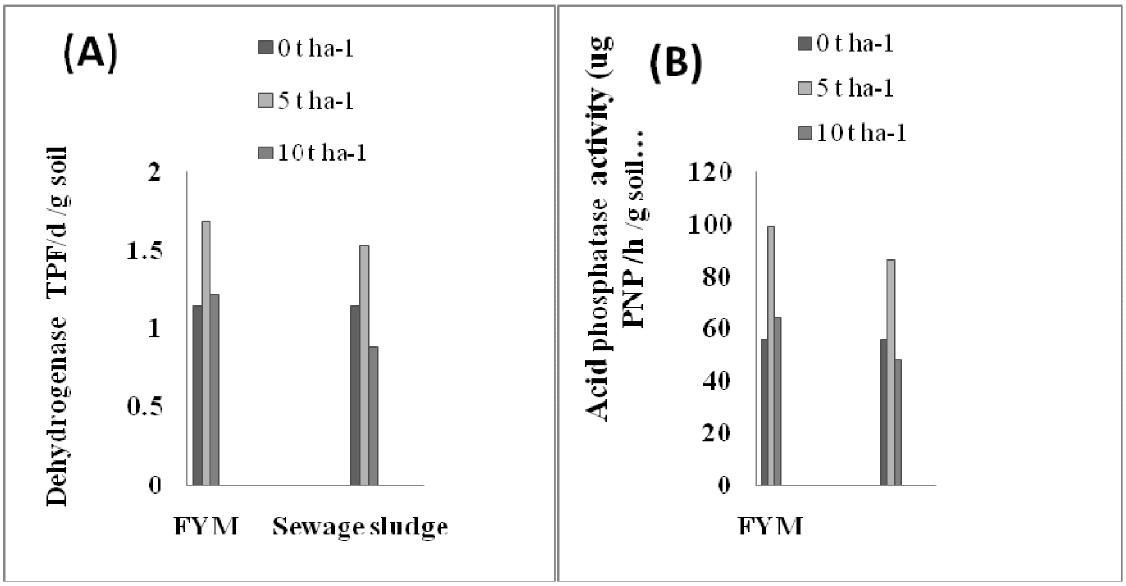
Table 1 Physicochemical characterization of the soil (1993-2013)

Parameter	Blank	FYM		Sewage Sludge	
		5t ha^{-1}	10t ha^{-1}	5t ha^{-1}	10t ha^{-1}
pH (1:2.5)	8.82 ± 0.24	8.74 ± 0.24	8.58 ± 0.24	8.70 ± 0.24	8.52± 0.24
EC	**3.04±0.28**	**3.26± 0.42**	**3.36±0.32**	**3.28± 0.34**	**3.38± 0.32**
Total Nitrogen (%)	**0.234±0.106**	**0.335±0.094**	**0.425±0.118**	**0.312±0.124**	**0.396±0.128**
Organic matter (%)	**1.75±0.08**	**2.08±0.16**	**2.32±0.18**	**2.02±0.12**	**2.28±0.14**
Microbial biomass-C (mg kg^{-1})	**314 ± 28**	484 ± 58	318 ± 22	476 ± 44	288 ± 32
Total P (%)	**0.058±0.01**	**0.066± 0.008**	**0.074± 0.012**	**0.062± 0.012**	**0.072± 0.016**
Na (mg kg^{-1})	545± 28	636±22	689±31	666±42	658± 34
K (mg kg^{-1})	764±34	828± 44	882± 38	814± 30	846± 28

CEC(meq/100g)	11.4± 1.2	13.4± 1.6	16.4± 1.4	12.8±1.0	14.8± 1.4
Total Zn (mg kg^{-1})	34± 2.8	39.5± 2.2	42.5± 2.6	44.6± 3.4	62.4±3. 2
Total Cr (mg kg^{-1})	10.34± 1.44	14.44±1.36	17.86± 1.94	16.44± 1.76	22.44± 2.48
Total Cu (mg kg^{-1})	9.94± 1.14	12.32± 1.38	15.42± 2.04	13.14± 1.66	19.76± 1.98
Total Ni (mg kg^{-1})	10.54± 1.08	12.58± 1.88	14.12± 2.04	12.68± 1.34	17.28± 2.04
Total Cd (mg kg^{-1})	0.152±0.036	0.196±0.048	0.214±0.068	0.208±0.074	0.286± 0.088
Total Pb(mg kg^{-1})	9.48± 0.94	12.14± 1.24	13.48± 1.36	13.45± 1.16	17.54± 1.02
Bacteria ($x10^6 g^{-1}$ dry soil)	94 ± 16	134 ± 22	126 ± 18	118 ± 12	90± 28
Fungi ($x10^3$ g^{-1} dry soil)	**11±1.2**	**18± 0.9**	**15±1.4**	**16± 1.1**	**10± 1.6**
Actinomycetes ($x10^3$ g^{-1} dry soil)	**14±0.8**	**17±1.1**	**16±0.9**	**16±1.1**	**15±1.0**



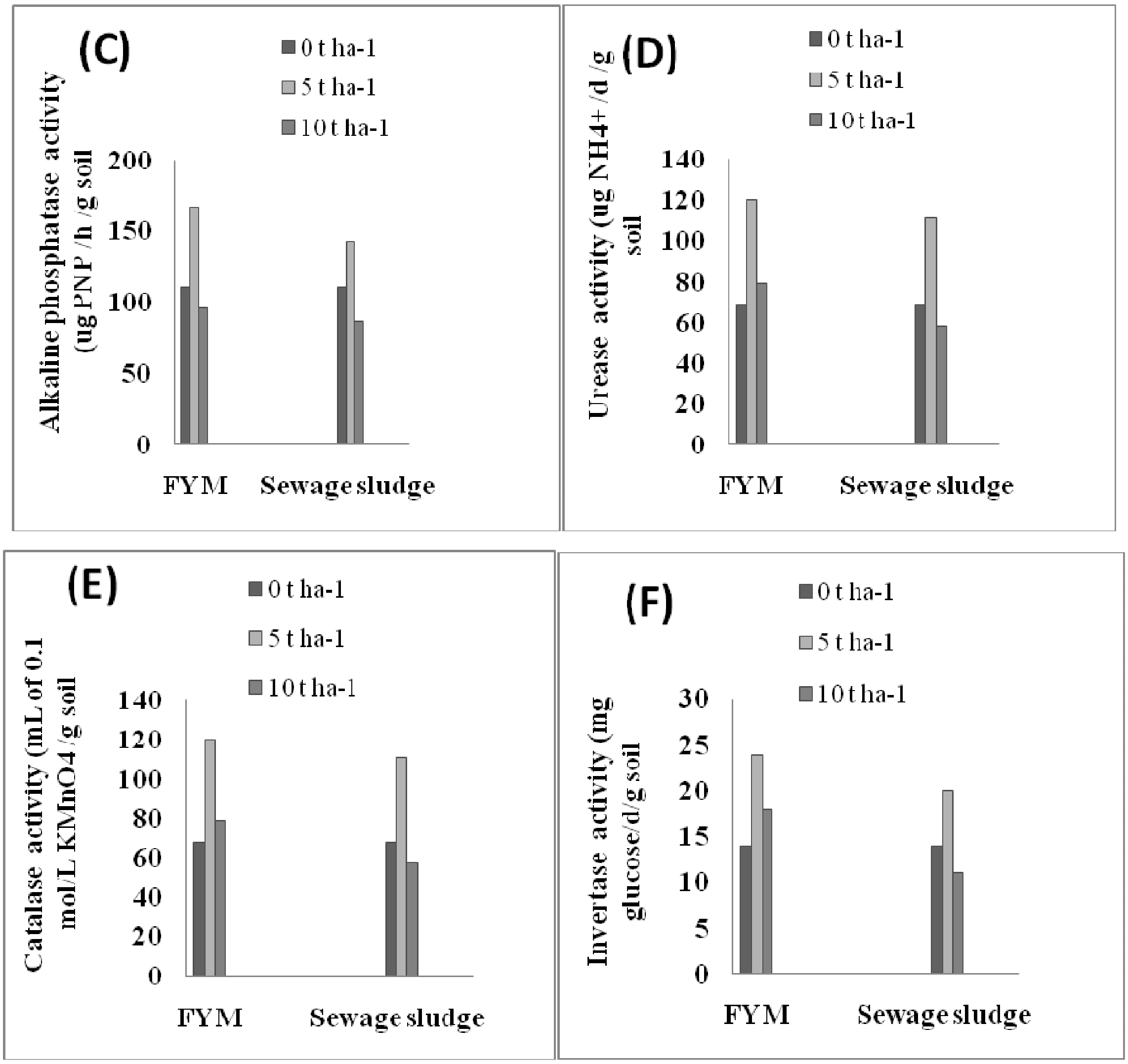


Fig.1.Effect of low and high concentration of FYM and sewage sludge on enzymatic activities (A) dehydrogenase, (B) acid phosphatase (C) alkaline phosphatase (D) urease (E) catalase and (F) invertase in alluvial soil of Aligarh .
