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Soil microbial activity as influenced by application of fly ash and soil amendments to maize crop in acidic alfisols

[1]Chandrakar T, [2]Jena D, [3]Dash A. K*, [4]Jena S. N, [5]Panda N and [6]Monica M

Department of Soil Science and Agricultural Chemistry, College of Agriculture, Orissa University of Agriculture & Technology, Bhubhaneswar-751003, India
*Corresponding author, E-mail: ashis_kumar05@yahoo.co.in

Abstract

Field experiments were conducted in acidic Alfisols of central research station, Orissa University Agriculture & Technology, Bhubaneswar, India during 2013-14 with maize as the test crop to study the effect of Fly ash and soil amendments (lime, gypsum) with or without addition of farm yard manure(FYM) on dehydrogenase and urease activity. The results revealed that the bacteria, fungi and actinomycetes population were maximum at 40 DAS and declined at 60 DAS. Application of lime neutralised the soil acidity and favoured bacterial growth where as the fungal population was highest in gypsum treatment under acidic environment. Integrated use of lime+flyash+FYM resulted in 66 % higher dehydrogenase activity over control (16.54 µg TPF/g soil/h). Combined application of FYM with lime, gypsum or fly ash recorded higher dehydrogenase activity as compared to their sole application. Urease activity in lime (72.5 µg urea/g soil/h) or fly ash (70.74 µg urea/g soil/h) treatment was at par, but higher than gypsum or control treatment. Application of lime alone or with FYM resulted in 20-23 % higher microbial biomass carbon (MBC) as compared to control. Integrated use of lime+fly ash+FYM recorded maximum MBC (366.22 mg C /g soil) among all treatment combinations. Maize grain yield was increased over control (38.6q/ha) by 27 % when lime was applied. Inclusion of FYM enhanced the use efficiency of lime, gypsum or fly ash. Combined application of flyash with FYM was as effective as lime treatment. Conjunctive application of lime+flyash+FYM recorded maximum grain yield (57.72 q/ha) among all treatment combinations.

Key words: Fly ash, lime, dehydrogenase activity, urease activity, microbial biomass carbon

INTRODUCTION

The thermal power plants in India consume more than 430 million-tons of coal and produces fly-ash around 145.42 million-tons out of which only 58.48% is being utilized for construction of roads and embankments, production of cement, mine-filling, reclamation of low-lying areas, agriculture, making bricks and tiles and others (CEA Annual Reports, 2012-13). Odisha generated about 24.52 million tonnes of fly ash in 2013-14 and utilizes only 61%. It is likely to touch 100 million tonnes per year by 2020 (OSPCB Annual report, 2013-14). A huge quantity of fly ash is now being dumped in ash ponds around thermal power stations which cause environmental problems. Fly ash has a great potential for use in wasteland reclamation and Agriculture.

The application of lignite fly-ash reduced the growth of several soil borne pathogenic microorganisms as reported by Karpagavalli and Ramabadran(1997), whereas the population of Rhizobium sp. and P-solubilizing bacteria were increased under the soil amended with either farmyard manure or fly-ash individually or in combination Sen, 1997).Microorganisms are the main source of enzymes in soils (Tabatabai,

1994), and thus the composition of the soil microbial communities strongly affects the potential of a soil for enzyme-mediated substrate catalysis (Kandeler et al., 1996). Soil enzymes (intracellular and extracellular) are the mediators and catalysts of biochemical processes important in soil functioning such as nutrient mineralization and cycling, decomposition and formation of soil organic matter, and decomposition of xenobiotics (i.e., pesticides). Specifically, the assessment of the activities of hydrolases can provide information on the status of key reactions that participate in rate limiting steps of the decomposition of organic matter and transformation of nutrients in soils. Thus, knowledge of several soil enzyme activities can provide information on the soil degradation potential (Tra´sar-Cepeda et al., 2000). Further, it has been reported that any change in soil management and land use is reflected in the soil enzyme activities, and that they can anticipate changes in soil quality before they are detected by other soil analyses (Ndiaye et al., 2000).

The use of amendments is a factor affecting enzymatic activities in the soil (Joergensen and Emmerling, 2006). The inclusion of organic manure, such as FYM, increases soil microbial biomass by incorporating additional microorganisms into the system and stimulating growth of autochthonous microbiota through the incorporation of new carbon sources (Ros et al., 2006).

There are little studies about the effect of fly ash on biological properties of acidic Alfisols. We hypothesized that a positive effect on soil biological properties is produced when these are amended by fly ash and FYM. Hence, the objective of this study was to determine the effect on microbial population and enzymatic activities as a result of applying fly ash, lime and gypsum alone or in combination with FYM to an Alfisol cropped with maize (Zea mays L).

MATERIAL AND METHODS

A field experiment was carried out for three seasons during 2013-14 in upland Agronomy field, Central Research Farm of Orissa University of Agriculture and Technology, Bhubaneswar, Odisha. The experiment was laid out in a randomized block design with three replications and eight treatments consist of- T_1 – control (received no amendments), T_2 - lime @ 0.2 LR, T_3- gypsum @ 2.5 q/ha, T_4 - Fly ash @ 40 t/ha, T_5 - lime @ 0.2 LR +FYM @ 10 t/ha, T_6 - gypsum @ 2.5 q/ha+ FYM @ 10 t/ha, T_7- Fly ash @ 40 t/ha + FYM @ 10 t/ha and T_8 - lime @ 0.2 LR+ Fly ash @ 40 t/ha + FYM @ 10 t/ha. The properties of the soil (0-15 cm) of the experimental site had sandy loam texture (69.2% sand, 22.3% silt and 8.5% clay), bulk density 1.63 Mg m^{-3} and water holding capacity of 26.48%. The soil was acidic in reaction (pH 4.6), low in N (82.1 mg/kg), medium in P (6.54 mg/kg) and K (58.03 mg/kg). The organic carbon content of the soil was 3.82 g/kg, ECEC 2.93 cmol(+)kg^{-1} and lime requirement value of 1633.33 kg $CaCO_3$/ha. Exchange acidity of initial surface soil was 0.44 cmol(+)kg^{-1} with 0.11 cmol(+)kg^{-1} of exchangeable Al^{3+}. The initial Ca and Mg (available) content of the soil was 1.35 and 0.92 cmol(+)/kg, respectively. The available sulphur content of initial soil was 10.5 mg/kg. The DTPA extractable Fe, Mn, Zn and Cu were 51.44, 37.5, 0.86 and 1.04 mg/kg, respectively. The DTPA extractable Cd, Pb and Cr were found 0.012, 0.14 and 0.18 mg/kg, respectively. The physical-chemical properties of the Fly ash (IMFA, Chaudar) used in study were presented in Table 1. Different size of the particles present in fly ash were; 0.02 to 2 mm - 41.2 %, 0.002 to 0.02 mm - 49.6 % and <0.002mm-6.2 % indicating that Fly ash can be easily mixed with soil since its particles sizes were comparable with size of soil separates. The bulk density is 0.98 Mg m^{-3} which was lower than the soil but its water holding capacity (47.5 %) can be compared with fine textured soil. It was neutral in reaction with pH value of 6.7 and EC of 0.16 dS m^{-1}. Organic carbon content of fly ash was 1.55 g kg^{-1}. Fly ash contains appreciable amount of available P (12.18 mg kg^{-1}), K (72.8 mg kg^{-1}) and S (39.26 mg kg^{-1}). The available Ca and Mg content in fly ash was 2.12 and 1.46 cmol(+)/kg, respectively. The DTPA extractable Fe, Mn, Zn and Cu were 105.94, 65.73, 1.92 and 1.14 mg/kg, respectively. Some of the heavy metal such as Cd, Pb and Cr were also determined. The DTPA extractable Cd, Pb and Cr were found 0.04, 0.4 and 0.2 mg/kg, respectively in fly ash. The content of total Cr, Pb and Cd was 40 ppm, 10.5 ppm and 4.0 ppm, respectively.

The maize crop was sown during mid july 2013 with recommended dose of fertilizer (NPK @120:60:60 kg/ha, respectively) through urea, SSP and MOP. Required quantities of amendments (lime, gypsum, flyash and FYM) were thoroughly mixed with soil before sowing. The crop was rainfed and received 1410 mm of rainfall during growth period. Soil samples for bio-chemical analysis were collected from the rhizosphere layer (0-15 cm) two times- at 40 and 60 days of sowing, air dried, processed and analyzed in the laboratory for pH, microbial population, soil microbial biomass carbon, soil dehydrogenase and urease activities as described below.

Enumeration of soil microbial population

Soil microbial population was determined by serial dilution and spread plate technique. One gram of the soil sample was added to test tube containing 9 ml of distilled water and then serially diluted (Wollum, 1982), spread over Nutrient Agar, Actinomyceyes Isolation Agar and Potato Dextrose Agar for enumeration of bacteria,

Table1. Physical and chemical properties of soil and fly ash used in experiment

Properties	Soil	Fly ash
Particle size distribution		
Sand (%)	69.18	41.2 (0.02- 2 mm)
Silt (%)	22.32	49.6 (0.002-0.02 mm)
Clay (%)	8.49	9.2 (< 0.002 mm)
Texture	Sandy loam	-
BD (Mg m^{-3})	1.63	0.98
WHC (%)	26.48	47.5
$pH_{(1:2.5)}$	4.6	6.7
$EC_{(1:2.5)}$ (dS/m)	0.06	0.16
OC (g kg^{-1})	3.82	1.55
ECEC (cmol(+)kg^{-1})	2.93	-
Exchange acidity ((cmol(+)kg^{-1})	0.44	-
Exchangeable Al^{3+}(cmol(+)kg^{-1})	0.33	-
Exchangeable H^{+}(cmol(+)kg^{-1})	0.11	-
LR (kg $CaCO_3$/ha)	1633.33	-
Available N (mg kg^{-1})	82.1	15.2
Available P (mg kg^{-1})	6.54	12.18
Available K (mg kg^{-1})	58.03	72.8
Available Ca (cmol(+)kg^{-1})	1.35	2.12
Available Mg (cmol(+)kg^{-1})	0.92	1.46
Available S (mg kg^{-1})	10.5	39.26
DTPA Extractable Fe (mg kg^{-1})	51.44	105.94
DTPA Extractable Mn (mg kg^{-1})	37.5	65.73
DTPA Extractable Zn (mg kg^{-1})	0.86	1.92
DTPA Extractable Cu (mg kg^{-1})	1.04	1.14
DTPA Extractable Cd (mg kg^{-1})	0.012	0.04
DTPA Extractable Pb (mg kg^{-1})	0.04	0.4
DTPA Extractable Cr (mg kg^{-1})	0.18	0.2

actinomycetes and fungal population, respectively. The plates were incubated at 30°C for 2 days for fungal isolation, 4 days for bacterial isolation and one week for actinomycetes.

Calculation

The following mathematical calculation was followed for enumeration of the microbial colony and expressed as CFU per gram of soil.

CFU/ml = No. of colony x Inverse of dilution taken/ volume of inoculums taken

Soil Microbial Biomass Carbon

Soil MBC was estimated employing fumigation and extraction procedure as described by Vance et al. (1987). The process involved collection of filtrate using Whatman filter paper No. 2 after shaking unfumigated soil (20 g) with 0.5 M K_2SO_4 for 30minutes. Similarly, another set of filtrate was collected using fumigated soil exposed to ethanol free chloroform for 24 h. Organic carbon in both the extract was analyzed using the method of digestion titration.

For digestion of organic carbon 10 ml of filtrate was transferred into a conical flask and 10 ml of $K_2Cr_2O_7$ followed by 20 ml of conc. H_2SO_4 were added and then entire content was digested for 30 minutes at 170°C. After the content in the flask cooled, 25 ml distilled water and 5 ml orthophophoric acid were added to the digested material and titrated against 0.04 M ferrous ammonium sulphate with ferroin as the indicator.

MBC = EC fumigated soil – EC unfumigated soil/ Kc

Where,

EC = Extractable carbon

Kc = 0.379 (Kc is the K_2SO_4 extract efficiency factor, Hu and Cao, 2007)

Soil dehydrogenase activity

Determination of soil dehydrogenase activity is generally done by adding alternative electron acceptors to soil samples. Water-soluble tetrazolium salts are the preferred oxidants because they form water-insoluble coloured formazans which can be measured spectrophotometrically. Dehydrogenase activity in the soil sample was determined by following the procedure as described by Klein et al. (1971). One gram of air dried soil was taken in an air tight screw capped test tubes (15 ml capacity) and 0.2 ml of 3 % solution of 2,3,5-tripHenyl tetrazolium chloride (TTC), 0.5 ml of 1 % glucose solution were added to each test tube. The bottom of the tube tapped gently to drive out all trapped oxygen, and thus a water seal is formed above the soil. Ensure that no air bubbles are formed. The tubes were incubated at 30^{o}C for 24 h. After incubation, 10 ml methanol was added and shaked vigorously. Then it was allowed to stand for 6 h. Clear pink coloured supernatant was withdrawn and readings were taken with a spectrophotometer at a wave length of 485 nm. The concentration of formazon formed in the soil sample was determined using graded concentrations of formazon. The results were expressed in microgram of triphenyl formazon (TPF) formed per gram of soil per hour (µg TPF h^{-1} g^{-1} soil).

Soil Urease Activity

The assay of urease activity in soils involves estimation of urea hydrolysis in soils by determination of the urea remaining after incubation of soil with urea solution at 37^{o}C (Pal and Chhonkar, 1981). Five gram of fresh soil sample (<2 mm, determine moisture content gravimetrically) were taken in 125 ml polypropylene (PP) bottles and treated with 5 ml of urea solution (10 mg urea). The bottles were capped and incubated at 37^{o}C. The samples were replicated four times. After 5 h, the caps were removed, added with 50 ml of 2M KCl-PMA solution, capped again and shaken for 1h.The soil suspension was filtered through suction. An aliquot of 1-2 ml of the extract containing up to 200 mg L^{-1} of urea were taken in 50 ml volumetric flask.

The volume was made to 10 ml with 2M KCl-PMA solution; 30 ml of the colouring reagent was added, swirled for few seconds and placed on a bath of boiling water. After 30 minutes the flasks were removed and cooled immediately in cold water (using ice) for 15 minutes. The volume was made to 50 ml with water and mixed thoroughly. The absorbance of red colour developed measured at 527 nm using spectrophotometer. The urea content of the extract was calculated with reference to a calibration graph plotted from the results obtained with standards containing 0, 25, 50, 100, 150 and 200 µg of urea.

Calculation

The soil urease activity was calculated from the expression (B-A)x.t where, B is the amount of urea added, A is the amount of urea found after time ‘t’ and ‘x’ is the oven dry equivalent of the amount (g) of soil taken for incubation. If time is taken in hours then urease activity can be expressed as µg urea g^{-1} soil h^{-1}.

RESULTS AND DISCUSSIONS

Effect of fly ash and soil amendments on soil reaction

The pH of the soil in control (without amendment) increased from 4.6 (initial) to 4.65 at 40 days of sowing and there after decreased to 4.61 at 60th day (Fig.1). Application of lime increased the soil pH to 6.85 at 40 day. On the other hand, application of gypsum did not alter the soil pH and behaved like control treatment. In lime treatment, as $CaCO_3$ dissolves, its reaction raise the pH, thus increases the pH dependent charge on soil colloid, which in turn retained the release Ca^{2+}, prevents its downward leaching. Further, the CO_3^{2-} ion released by $CaCO_3$ reaction forms CO_2 gas and water and deprived Ca^{2+} cations that could accompany them in leaching process. On the other hand, gypsum being a neutral salt does not raise soil pH or increase CEC. Further, the SO_4^{2-} ions released by dissolution of gypsum is available to accompany Ca^{2+} cations in leaching. Once the Ca^{2+} and SO_4^{2-} ions move down ward to sub soil, the Ca^{2+} ions replace Al^{3+} ions from the exchange sites and released Al^{3+} ions react with SO_4^{2-} to form $AlSO_4$ which are non phytotoxic. Several researchers have demonstrated that gypsum can ameliorate aluminium toxicity despite the fact that it does not increase soil pH.

Application of FYM with gypsum or lime increased the soil reaction and pH attained a value of 4.97 and 6.12, respectively. Marginal change in soil pH (increase/ decrease) with addition of FYM was resulted due to its buffering effect on soil pH.

Fly ash being neutral in pH (6.7) was better than gypsum. It increased the pH from 4.6 to 5.16 and 5.55 when applied alone or with FYM, respectively. In other words, it can be safely used in acid soils as an amendment to increase soil pH as well as provide several plant nutrients to crop. Further, integrated application of lime+FYM+Flyash was found best treatment combination for acid soils with respect to neutralising capacity associated with availability of other beneficial plant nutrients.

Microbial population

The soil bacteria have rapid reproductive potential and

Table 2. Effect of fly ash and soil amendments on microbial population

Treatment	Bacterial population (X 10^6cfu)		Fungal population (X 10^4cfu)		Actinomycetes population (X 10^4cfu)	
	40 DAS	60 DAS	40 DAS	60 DAS	40 DAS	60 DAS
Control	55.25^{c}	36.89^{cd}	40.54^{d}	30.39^{d}	56.89^{bc}	25.23^{d}
Lime (L)	70.62^{b}	40.60^{bc}	65.45^{b}	33.18^{cd}	56.75^{bc}	36.89^{bc}
Gypsum (G)	57.44^{c}	32.85^{d}	83.37^{a}	29.86^{d}	49.21^{cd}	30.00^{cd}
Fly ash (FA)	60.74^{c}	46.97^{a}	48.23^{cd}	39.01^{bc}	41.52^{d}	38.48^{b}
L+FYM	78.86^{ab}	39.01^{bcd}	50.76^{bcd}	39.34^{bc}	72.70^{a}	44.50^{ab}
G+FYM	71.17^{b}	37.42^{cd}	46.58^{d}	43.77^{ab}	62.94^{ab}	38.67^{b}
FA+FYM	83.80^{a}	40.60^{bc}	63.82^{bc}	49.07^{a}	63.48^{ab}	47.42^{a}
L+FA+FYM	80.51^{a}	43.78^{ab}	63.48^{bc}	45.36^{ab}	73.49^{a}	44.30^{ab}
CD(0.05)	9.21	6.22	16.05	6.47	12.70	8.10
CV(%)	7.53	8.94	15.86	9.54	12.17	12.11

the population increases rapidly in response to favourable changes in soil environment and food availability**.** Addition of organic-rich substances stimulates microbial growth and activity. High calcium and neutral soil pH generally resulted in largest bacterial population. Addition of Ca through lime increased the bacterial population by 28% over control treatment (55.25 x 10^6 cfu) (Table 2). Application of lime favours neutral soil pH (6.85) and add Ca which is conducive for bacterial growth. Application of gypsum and fly ash did not alter soil pH much which remained under acidic condition and the environment was not so conducive for bacterial growth although nutrients are available. This indicated that neutral pH is more important than the nutritional aspect for bacterial growth. However, application of FYM (a source of energy) with gypsum or fly ash helped to build up bacterial population as compared to their sole application. Combined application of fly ash and FYM was the best substrate among others for the bacterial growth since this treatment recorded significantly higher population as compared to lime+FYM or gypsum+FYM treatment. This might have happened since fly ash provides appreciable amount of N, P, K, S, Fe, Mn and Zn for bacterial growth and FYM stabilised the soil pH. Presence of heavy metals like Cd, Pd, Cr in fly ash did not affect the growth since, bacteria can utilise industrial wastes as substrate (Basu et al., 2011).

The bacterial population in rhizosphere soil at 60 DAS of maize crop declined drastically as compared to 40 DAS. The reduction of population in lime and gypsum treatment was higher than fly ash. Similar result was reported by Basu et al. (2011) in rhizosphere soils of peanut. The bacterial population was maxium at 50 DAS of peanut and thereafter decreased significantly at 75 and 100 DAS. Sivapalan et al. (1993) and Lee et al. (2004) reported that combined application of organics and chemical fertilizer significantly improved bacterial population over chemical fertilizer in all stages of growth. Greater proliferation of Bradyrhizobium population with increasing pH towards neutrality was also reported by Fettel et al. (1998). The enhancement of P solubilising bacteria due to liming was also reported by Barroti and Nahas (2000). Sen (1997) also observed that the populations of Rhyzobium spp. and P-solubilizing bacteria were increased under the soil amended with either FYM or FA individually or in combination.

Fungi population was lower than bacterial population and it varied between 83.37 X 10^4 cfu -40.54 X 10^4 cfu (Table 2). The lowest population was observed in control soil and increased by 61.44, 105.65 and 18.96 % in lime, gypsum and fly ash treatment, respectively. Application of gypsum resulted in higher fungal population as compared to lime and gypsum since, in addition to Ca, gypsum lowers the soil pH which is very conducive for fungal growth. Addition of FYM decreased fungal growth when it is mixed with gypsum or fly ash. This might have happened since, addition of FYM with gypsum or fly ash favours higher pH as compared to their sole application. These results were in agreement with the findings of Karpagavalli and Ramabadran (1997) who had observed that the population of soil arbuscular mycorrhizal fungi increased by the FA incorporation. In contrast, the application of lignite FA reduced the growth of soil borne pathogens. The actinomycetes population in rhizosphere soil in control was at par with lime treatment and decreased by 13.51 and 27 % with gypsum and fly ash, respectively (Table 2). On the other hand when FYM was added to lime, gypsum or fly ash, the population was significantly increased by10.63-27.79 %. High count of actinomycetes in the FYM treated plots is in accordance with the findings of Shah et al. (1990) who have observed more proliferation of these organisms in soils containing high organic matter content. Maximum population was obtained when the crop received lime+fly ash+FYM. In latter stage of growth (60 day) the population was decreased in all treatments.

Table 3. Effect of fly ash and soil amendments on dehydrogenase and urease activities

Treatment	Dehydrogenase activity (µg TPF/g soil/h)				Urease activity (µg urea/g soil/h)			
	40 DAS	% increase over control	60 DAS	% increase over control	40 DAS	% increase over control	60 DAS	% increase over control
Control	16.54^{c}	-	6.40^{b}	-	52.09^{c}	-	29.78^{d}	-
Lime (L)	19.76^{bc}	19.46	8.44^{b}	31.85	72.50^{a}	39.19	35.13^{cd}	17.97
Gypsum (G)	16.44^{c}	-0.62	7.33^{b}	14.48	65.55^{abc}	25.84	29.48^{d}	-1.01
Fly ash (FA)	15.51^{c}	-6.23	8.17^{b}	27.68	70.74^{a}	35.80	39.02^{c}	31.03
L+FYM	23.58^{ab}	42.58	15.80^{a}	146.92	67.84^{ab}	30.24	45.88^{b}	54.06
G+FYM	19.66^{bc}	18.88	16.86^{a}	163.37	52.39^{c}	0.57	48.19^{b}	61.83
FA+FYM	24.28^{ab}	46.78	15.28^{a}	138.75	56.86^{bc}	9.16	51.81^{ab}	73.99
L+FA+FYM	27.49^{a}	66.23	19.24^{a}	200.66	59.55^{abc}	14.33	55.23^{a}	85.46
CD(0.05)	5.02		4.76		13.59		6.65	
CV(%)	14.04		22.30		12.48		9.08	

Dehydrogenase activity (µg TPF/g soil/h)

Biological oxidation of soil organic compounds is generally a dehydrogenation process carried out by specific dehydrogenases involved in the oxidative energy transfer of microbial cells (Burns, 1978). The activity is a measure of microbial metabolism and thus of the oxidative microbial activity in soils. The activity of dehydrogenase enzyme in the soil system is very important as it indicates the potential of a soil to support biochemical processes which maintain soil fertility (Joychim et al., 2008). A good correlation has been reported between microbial biomass and soil dehydrogenase activity by Chander et al. (1977).

The results of our study indicated that application of lime alone or with FYM significantly increased the dehydrogenase activity over control soil (16.54 µg TPF/g soil/h) by 19 and 43%, respectively at 40 DAS of maize crop (Table 3). Application of gypsum or fly ash alone did not affect much and behaved like control soil. But, when FYM was included with these amendments, there was significant increase in dehydrogenase activity (19-47 %). Combined application of fly ash and FYM supersedes the lime+FYM treatment although the pH remained under acidic range. This might have happened due to fly ash supplied several plant nutrients towards dehydrogenase activity.

The beneficial effect of FYM enhanced the nutrient availability added through fly ash and creates a conducive environment for dehydrogenase activity. On the other hand, integrated application of lime+fly ash+FYM recorded maximum dehydrogenase activity (27.49 µg TPF/g soil/h) which was 66 % higher over control. This might have happened since, lime neutralises soil acidity, FYM act as source of energy and fly ash supports the supply of major plant nutrients. Similar results were reported by Tejada and Gonalez (2009).

The dehydrogenase activity at latter stage of maize crop (60 day) was drastically reduced in control (61%), lime (57%), gypsum (55%) and fly ash (47%) treatments as compared to that at 40 DAS. However, the intensity of activity was maintained in the treatments when amendments were mixed with FYM.

Several workers studied the effect of organic manure on dehrogenase activity. Marinari et al. (2000) reported that a higher dehydrogenase activity was observed in soil treated with vermicompst and manure compare to soil treated with chemical fertilizer. Perucci (1992) reported the application of compost caused a significant increase in dehydrogenade activity. The enzyme activity in organic amendment soil increased by an average 2-4 fold compared with the unamended soil (Martens et al., 1992).

Hinojosa et al. (2008) observed greater amount of dehydrogenase activities in non-polluted area as 71.4±5.2 µg TPF/g soil/h, average activities were reported in reclaimed area as 53.0±0.5 µg TPF/g soil/h and least value reported in polluted area as 2.9±12.1 µg TPF/g soil/h. Similar findings were reported by Pati and Sahu (2004) while studying the CO_2 evolution and enzyme activities (dehydrogenase, protease, and amylase) and found little or no inhibition of soil respiration and enzyme activities up to 2.5% fly ash amendment.

Urease activity (µg urea/g soil/h)

Urea hydrolysis in soils is an enzymatic decomposition process by the enzyme urease. Urease catalyzes the hydrolysis of urea to yield ammonia and carbamate, which spontaneously hydrolyzes to form carbonic acid and a molecule of ammonia. Soil urease is involved in nitrogen mineralization and supplying nitrogen to plants from natural and fertilizer sources. The results of the

Table4. Effect of fly ash and soil amendments on microbial biomass carbon

Treatment	MBC (µg C/g soil)			
	40 DAS	% increase over control	60 DAS	% increase over control
Control	294.29^{d}	-	187.47^{de}	-
Lime (L)	360.67^{ab}	22.56	210.13^{cd}	12.09
Gypsum (G)	345.16^{bc}	17.28	180.31^{e}	-3.82
Fly ash (FA)	339.90^{c}	15.50	189.98^{de}	1.34
L+FYM	359.04^{abc}	22.00	226.90^{abc}	21.03
G+FYM	347.19^{abc}	17.98	226.18^{bc}	20.65
FA+FYM	353.15^{abc}	20.00	240.03^{ab}	28.04
L+FA+FYM	366.22^{a}	24.44	254.25^{a}	35.62
CD(0.05)	20.68		27.50	
CV(%)	3.42		7.33	

present study indicated that after 40 days of sowing of maize crop, application of lime or fly ash alone resulted significantly higher urease activity over control but they remained at par with each other. Application of gypsum alone or in combination with FYM remained at par with each other and with control, too. The highest increase in urease activity over control was observed with lime (39.19 %) followed by fly ash (35.80 %) (Table3). Addition of FYM with lime, fly ash or gypsum did not have much impact on urease activity since the values were lower as compared to their sole application. The micro-organism derived energy and nutrient from fertilizer and amendments and did not much depend on organic manure.

After 60 days of sowing of maize crop, the urease activity was declined by 42.8-55% in lime, gypsum and fly ash treatments. But, the reduction was reduced (7-8%) when FYM was mixed with amendments. Among the treatments, combined application of fly ash + FYM + lime resulted significantly higher urease activity (85.46%) over control. This finding was corroborating with the findings of Lal et al., (1996). They reported that FA added to soil at 16% (w/w) increased urease and cellulose activities. However, acid phosphatase activity was depressed with FA application.

Microbial biomass carbon (µg C/g soil)

It is well known that organic matter in soils stimulates soil microbial populations and soil biological activity (Brady and Weil, 1999). The carbon in manure could be easily used as energy source for soil microorganisms, and resulted in increased soil microbial populations and soil biomass. The results of present study revealed that the MBC in control was 294.29 (µg C/g soil) and increased by 22.56% with lime (Table 4). Application of gypsum alone or in combination with FYM remained at par with each other but had significant effect over control. The highest increase in microbial biomass carbon over control was in lime+flyash+FYM (24.44 %) followed by fly ash+FYM (20.0 %) treatment.

Similarly, after 60 days of sowing of maize crop, The MBC was reduced by 36.3% in control and 41.7-47.8% in amendments over 40DAS. However, the reduction was minimum when FYM was mixed with amendments. This showed that FYM helped to maintain MBC content when fertilizer source was exhausted in the root zone. However combined application of fly ash + FYM (T_7) + lime treatment recorded maximum MBC at 60 DAS. These findings are in agreements with the results obtained by Goyal et al. (1992) and Hassan (1996) that addition of organic amendments increased microbial biomass and resulted in a positive correlation between MBC and soil microbial population.

Maize yield

The maize grain yield data presented in figure 2 revealed that in absolute control, the yield was 35.74, 39.94 and 40 12 q/ha during kharif 2013, rabi 2013-14 and kharif 2014, respectively. Application of lime to each crop significantly increased the pooled yield over control by 27 %. The yield in gypsum treatment was at par with control. On the other hand one time application of fly ash to first crop stabilised the yield up to third season and recorded significantly higher yield(43.12 q/ha) over control and gypsum treatment. Inclusion of FYM with amendments resulted in about 5 q/ha higher yield over their sole application. Integrated use of lime+fly ash+FYM recorded maximum yield as compared to their sole application or combination with FYM. Inclusion of FYM with amendments enhanced the activity of beneficial microbes, which play an important role in mobilization of nutrients and there by leading to better availability of

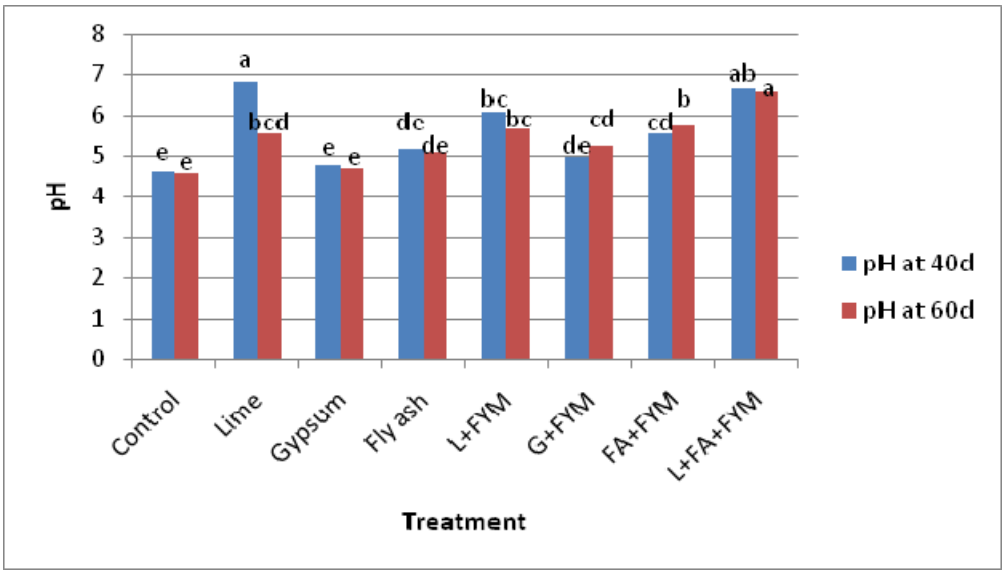

Figure1. Effect of fly ash and amendments on soil pH in the root zone of maize crop

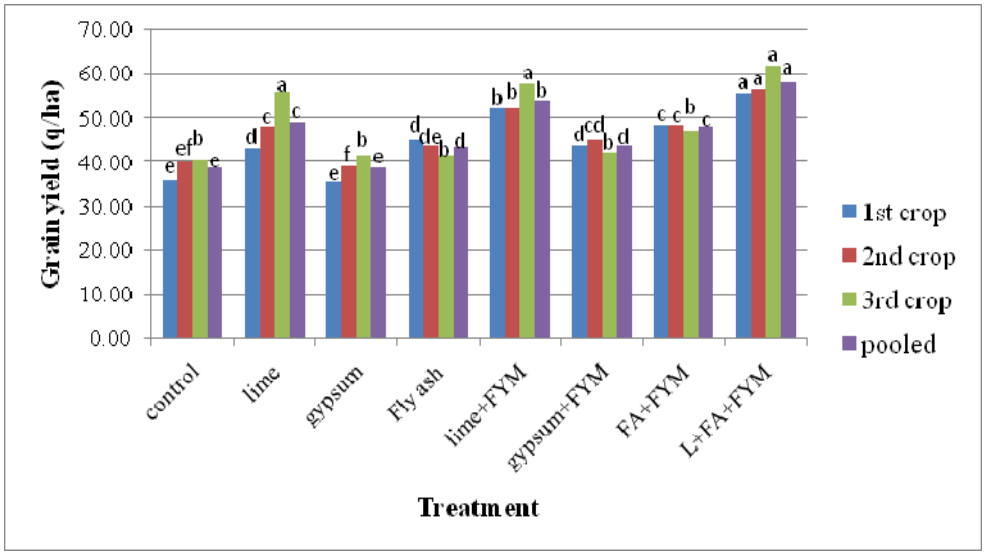

Figure 2. Effect of amendments on maize grain yield (q/ha) over the years

nutrients facilitating uptake by plants resulting in better growth and dry matter production. The data further indicated that one time application of fly ash with FYM is compared with lime treatment and can be recommended for crops in acid soils.

CONCLUSION

Overall, the impact of FA on microbial activity is thus inconsistent, but in the presence of organic manure (FYM), the effect is positive and can be compared with lime in acid soils. Integrated use of organic manure (FYM), Fly ash, lime with recommended levels of inorganic fertilizers increased the microbial population, dehydrogenase activity and urease activity, soil organic matter content, resulting in more microbial proliferation and thereby sustained soil health. The results of the present study thereby support the concept of integrated

nutrient management practices for improving the soil quality and sustained crop productivity.

REFERENCES

Barroti G, Nahas EY (2000). Total microbial and phosphate-solubilizing population in soil submitted to different cultivation systems. Pesq. Agropec. Bras. **35**: 2043-2050.

Basu M, Bhadoria PBS, Mahapatra SC (2011). Influence of Soil Ameliorants, Manures and Fertilizers on Bacterial Populations, Enzyme Activities, N Fixation and P Solubilization in Peanut Rhizosphere under Lateritic Soil. British Microbiology Resarch J. **1**(1): 11-25.

Brady NC, Weil RR (1999). Soil organic matter. In: The Nature and Properties of Soils. Upper Saddle River, New Jersey, pp. 446–490.

Burns RG (1978). Enzyme activity in soil: some theoretical and practical considerations. In: Burns RG (ed.) Soil enzymes. Academic, London, pp 295–340.

Central Electricity Authority India (2012-13). Annual Report on Fly-ash utilization.

Chander K, Goyal S, Mundra MC, Kapoor KK (1977). Organic matter, Microbial biomass and enzyme activity of soils under different crop rotations in the tropics. Biology and Fertility of Soils.24:306-310.

Fettel NA, O'Connor GE, Carpenter DJ, Evans J, Bamforth I, Oti-Boateng C, Hebb DM, Brockwell J (1998). Nodulation studies on legume exotic to Australia: the influence of soil populations and inocula of Rhizobium leguminoserum bv. Viciae and nitrogen fixation by field peas. Applied Soil Ecol. **5:** 197-310.

Goyal S, Mishra MM, Hooda IS, Singh R (1992). Organic matter-microbial biomass relatonships in field experiments under tropical conditions: effects of inorganic fertilization and organic amendments. Soil Biol. Biochem. 24: 1081–1084.

Hassan DH (1996). Effects of cadmium and sewage sludge on soil microbial biomass and enzyme activities. Bioresource Technol. 56:141–145.

Hinojosa MB, Carreira JA, Rodr´ıguez-Maroto JM, Garc´ıa-Ru´ız R (2008). Effects of pyrite sludge pollution on soil enzyme activities: Ecological dose-response model. Science of the total Environment. 396: 89-99.

Hu Chang, Cao Zhiping (2007). Size and activity of the soil microbial biomass and soil enzyme activity in long term field experiments. World Journal of Agricultural Sciences. 3(1):63-70.

Joergensen R, Emmerling C (2006). Methods for evaluating human impact on soil microorganisms based on their activity, biomass, and diversity in agricultural soils. Journal of Plant Nutrition and Soil Science. 169:295-309.

Joychim HJR, Makoi P, Ndakidemin A (2008). Selected soil enzymes: examples of their potential roles in the ecosystem. African Journal of Biochemistry. 7: 181-191.

Kandeler E, Kampichler C, Horak O (1996). Influence of heavy metals on the functional diversity of soil microbial communities. Biol. Fertil. Soils. 23: 299–306.

Karpagavalli S, Ramabadran R (1997). Effect of lignite fly ash on the growth and dry matter production (DMP) of soil borne pathogens. In: Souv and abstracts: National Sem on Bio-Utilization of Fly ash, 4–5 April 1997. Khallikote Autonomous College; Berhampur, Orissa, India: p. 11.

Klein DA, Loh TC, Goulding RL (1971). A rapid procedure to evaluate Dehydrogenase activity of soils low in organic matter. Soil Biology and Biochemistry. 3: 385-387.

Lal JK, Mishra B, Sarkar AK (1996). Effect of fly ash on soil microbial and enzymatic activity. Journal of the Indian Society of Soil Science. 44:77-80.

Lee JJ, Park RD, Kim YW, Shim JH, Chae DH, Rim YS, Kyoon B (2004). Effect of food waste compost on microbial population, soil enzyme activity and lettuce growth. Bioresource Technol. 93: 21-28.

Marinari S, Masciandaro G, Ceccanti B, Grego S (2000). Influence of organic and mineral fertilizers on soil biological and physical properties. Bioresoruce Technol. 72: 9–17.

Martens DA, Johanson JB, Frankenberger WT (1992). Production and persistence of soil enzymes with repeated addition of organic residues. Soil Sci. 153: 53–61.

Ndiaye EL, Sandeno JM, McGrath D, Dick RP (2000). Integrative biological indicators for detecting change in soil quality. American Journal of Alternative Agriculture.15: 26–36.

Odisha State Polution Control Board, Annual report (2013-14).

Pal S, Chhonkar PK (1981). Urease activity in relation to soil characteristics. Pedobiologia. 21: 152-158.

Pati SS, Sahu SK (2004). CO_2 evaluation and enzyme activities (dehydrogenase, protease and amylase) of fly ash amended soil in presence and absence of earthworms (Under laboratory condition). Geo Derma. 118:289–301.

Perucci P (1992). Enzyme activity and microbial biomass in a field soil amended with municipal refuse. Biol. Fert. Soils. 14: 54–60.

Ros MJA, Pascual C, García MT, Insam H (2006). Hydrolases activities, microbial biomass and bacterial community in a soil after long-term amendment with different compost. Soil Biology and Biochemistry. 38:3443-3452.

Sen A (1997). Microbial population dynamics in fly ash amended acid lateritic soil. B. Tech. Thesis. Kharagpur, India: Indian Institute of Technology.

Shah Z, Adans WA, Haven CDV (1990). Consumption and activity of the microbial population in an acidic upland soil and effects of liming. Soil Biology and Biochemistry. 22 (2):257–263.

Sivapalan A, Morgan WC, Franz PR (1993). Monitoring populations of soil microorganisms during conversion from a conventional to an organic system of vegetable growing. Biol. Agric. Hortic.10: 9-27.

Tabatabai MA (1994). Soil enzymes. In: Weaver, R.W., Angle, J.S., Bottomley, P.S. (Eds.), Methods of Soil Analysis: Microbiological and Biochemical Properties. Part 2. SSSA Book Ser. 5. SSSA, Madison, WI, pp. 775–833.

Tejada M, González JL (2009). Application of two vermicomposts on a rice crop: Effects on soil biological properties and rice quality and yield. Agronomy J. 101: 336-344.

Tra´sar-Cepeda C, Leiros MC, Gil-Sotres F (2000). Biochemical properties of acid soils under climax vegetation (Atlantic oakwood) in an area of the European temperate-humid zone (Galicia, NW Spain): specific parameters. Soil Biology and Biochemistry. 32: 747–755.

Vance ED, Brokes PC, Jenkinson DS (1987). An extraction method for measuring soil microbial biomass carbon. Soil Biology and Biochemistry. 19, 703-706.

Wollum AG (1982). Cultural methods for soil microorganisms. In A.L.Page, R.H. Miller and D.R. Keeney (ed.) methods of soil Analysis, part 2. Chemical and Microbiological properties, Agronomy monograph No. 9, ASA-SSSA, Madison, Wisconsin, USA, pp. 781-814.