Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Archives of Applied Science Research, 2012, 4 (2):1065-1073
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-508X
CODEN (USA) AASRC9

Impact of indigenous microorganisms on soil microbial and enzyme activities

T. Sumathi, A. Janardhan, A. Srilakhmi, D.V.R. Sai Gopal and G. Narasimha*

Department of Virology, Sri Venkateswara University, Tirupati, Andhra Pradesh, India

---

ABSTRACT

The effect of Indigenous microorganisms (IMO's) on the native soil was investigated in the present study. Supplementation of IMO's suspension to the soil alters the physico-chemical, biological and enzyme properties of the soil. These alternations include decreases in P^H from 7.2 to 6.8, increase in electrical conductivity 0.36 to 1.21 (µmohs/cm), water holding capacity 0.36 to 2.2ml/g of soil of control and test soils respectively. There is increase in soil texture like clay, phosphorous and potassium in the test soil. Enzyme activities such as protease and urease were assessed in both the soil samples with and without amendment of respective substrates (casein and urea). Accumulation of hydrolytic products tyrosine and ammonia from the substrates in the soil was estimated at periodic intervals. Protease and urease enzyme activities were relatively higher in soil amended with IMO's and respective substrate than control.

Key words: Indigenous microorganisms, Physico-chemical parameters, microbial biomass, enzymes activities.

INTRODUCTION

The increased use of chemical fertilizers and some organic fertilizers in agriculture helped the country in achieving self sufficiency in food grain production. However, it has also polluted the environment and caused slow deterioration of soil health. The chemical residues in the food product are also causing injury to human beings and cattle population. To combat these problems and in the light of sustainable agriculture, green technology is now being greatly used [1]. Indigenous microorganisms (IMO's) and green manures act as reserve source for all nutrients. It adds organic matter to the soil and this increases soil fertility. The importance of green manuring had been recognized as early as 5000 BC in India. IMO's inoculated plants exhibit an increased plant growth, high nutrient status including that of phosphorus besides offering resistance to pathogenic and disease causing microorganism. IMO's suspension contains a wide range of naturally chelated plant nutrients and trace elements, carbohydrates, amino acids and their growth promoting substances and these were help as a soil conditioner by stimulating microbial activity in the soil which results in improved air-water relationships in soil, improved fertility and makes soil less prone to compaction and erosion. IMO's are organisms that enrich the nutrient quality of soil. The main sources of IMO's are bacterial, fungi and cynobacteria. The most striking relationship that these having with fungal, bacterial and algal groups, the most common of which are with Mycorrhiza, Rhizobium and Cyanophyceae. These are known to deliver a number of benefits including plant nutrition, disease resistance, and tolerance to adverse soil and climatic conditions. Soil enzymes are essential for catalyzing innumerable reactions necessary for life process of microorganism in soil, decomposition of organic residues, cycling of nutrients and formation of organic matter and soil structure. Microorganisms play a major role in decomposition of several organic compounds frequently used in agriculture directly affect the synthesis and decomposition of soil organic matter. All soils contain a group of enzymes that determine soil metabolic process [2] which in turn, depend on its physical, chemical, and microbiological and biochemical properties. The enzyme levels in soil systems vary in amounts primarily due to the fact that each soil type has different amount of organic matter content, composition and activity of its living organisms and intensity of the biological process [3].These enzymes may include amylase, arylsulphatases, β - glucosidase cellulase, chitinase, dehydrogenase, phosphates, protease and urease released from

plant [4] animals [5], organic compounds and microorganisms [6, 7, 8, 9] and soil [10, 11]. Soil enzymes activities are sensors of soil degradation since they integrate information about microbial status and Physico-chemical conditions [12, 13, 14] and sensors is used in the influence of soil treatments on soil fertility [15]. Although enzymes are primarily of microbial origin it can also be originate from plants and animals. These enzymes are constantly being synthesized, could be accumulated, inactivated, and or decomposed in the soil, assuming like this, great importance for their role in the recycling of the nutrients. Soil microbial biomass, a living part of soil organic matter is an agent of transformation for added and natural organic matter and acts as a labile reservoir for plant available Nitrogen, Phosphorus, Sulphur [16]. The activity of microbial biomass is commonly used to characterize the microbial status of soil.

Microbial secreted enzymes constitute an important part of soil matrix as extracellular enzymes also called Abiontic factors influencing soil microbial activity exert control over soil enzymes production and control on nutrient availability and soil fertility [17]. The microbial enzymes involved in the mineralization of soil organic matter are cellulase, proteases, ureases, phosphatases [18, 19]. Cellulase decomposes cellulose a compound present in resides that is continuously deposited above soil the litter layer [20]. Nitrogen fertilizers are the most important management strategy for the improvement of agricultural crops. Urea is the mostly widely used organic nitrogen fertilizers and hydrolyzed to ammonium and CO_2 by urease enzyme . Organic nitrogen also affects directly the distribution and action of Proteolytic enzymes.

MATERIALS AND METHODS

Collection of IMO sample

IMO treated soil (test sample), control sample (native sample) was collected from biofarming field, Pulicherla, Chittoor (Dist), Andhra Pradesh, India.

Analytical methods for characterization of soil

The Physico-chemical properties of both test (IMO treated soil) and control sample (native soil) carried out by APHA, 2000 [21]. Due to the low cost effectiveness the present work carried out for the determination and effect of IMO's on soil Physico-chemical and enzymatic activities.

Biological properties

The biological properties including bacterial and fungal populations in the IMO' treated and control soils were enumerated by serial dilution method.

Enzyme assays

Protease assay: For assay of soil protease five grams of test sample (IMO's) and control sample were transferred to test tubes and maintained at 60% water holding capacity at room temperature in the laboratory (28±4^{o}C) at regular intervals 0, 7, 14, 21, 28 days of incubation. Duplicate soil samples of each test and control were drawn with at periodic intervals to determine the enzyme activities of protease. The effect of IMO's using different on the soil microbial enzyme activities was studied by incubating test sample 12.5,25,50 percentages with control soil sample. The soil samples were transferred to 250 ml Erlenmeyer flasks and 1 ml of toluene was added. After addition of 1% casein to soil samples containing conical flasks were plugged with cotton and incubated for 6 hours at 30^{0}C for protease activity. After desired incubation, soil extracts were passed through whattman filter paper and the filtrate was assessed by the method of Folin-Lowry, 1951 [22].

Urease assay: For urease activity in soil, 1 ml of 3% urea was added as substrate to the soil and only one ml of distilled water was added in place of urea, it was served as control. The effect of imos soil microbial enzyme activity was assessed by incubating various concentrations of test sample that is 12.5%, 25%, 50%, with control soil at different days of incubation 0, 7, 14, 21 days. Determination of urease activity in samples in the presence of buffer, 1ml of 0.1ml phosphate buffer (pH 7.1) was added to all soil samples of another set to one half of soil samples of this set, addition of 1 ml of 3% urea was made. Another half of soil samples in the set with receipt of distilled water in the place of urea served as control. After 30 min of incubation, all soil samples were shaken at 37^{0}C in a Water bath shaker. The flasks were placed in ice until ammonia was extracted with 10 ml of 2M Potassium chloride. Five milliliters of phenol sodium nitroprusside solution and 3 ml of sodium hypochlorite were added to 4 ml of 2M Potassium chloride. Extract of the mixture was shaken and incubated for 30 min in dark room and the bluish color developed was measured at 630 nm in spectrophotometer.

RESULTS AND DISCUSSION

Physico-Chemical Properties: The impact of IMO's on soil physical and chemical properties was studied and tabulated in the table No.1.The soils treated with IMO's showed altered Physico-chemical properties then the control(Table No.1)For instance improved in WHC, electrical conductivity, organic contents were observed in the test sample than the control sample. For the higher water holding capacity and electrical conductivity was observed in IMO'S treated soil than control soil. The water holding capacity and electrical conductivity increased from 0.36ml/g to2.2ml/g and 0.31umhos/cm to 1.21umhos. This increased Water holding capacity may be due to the accumulation of organic residues sample. (Table.1)

Table.1 Physico-chemical properties of soil treated (IMO)/ non treated (control)

PROPERTIES	IMO TREATED	CONTROL
P^H	6.8	7.4
Water holding capacity(ml/g of soil)	2.2	0.36
Electrical conductivity (µ mhos/cm)	1.21	0.36
Texture		
Phosphorous(kg/h)	277	35
Potassium(kg/h)	854	291
Carbon	High	Low

The results were in conformity with Sparling *et al* 2001 [23] (Dairy industry), Narasimha *et al* 1999 [24] (cotton ginning industry). Xiao *et al* 2005 [25] (Black liquor straw pulp) had increased electrical conductivity. In contrast soil polluted with cement industry had low water holding capacity and electrical conductivity [26]. The P^H of the soil was represented in (Table 1).There was slight variation in P^H from 7.4 to 6.8. Similar reports were made by Zende *et al* 1996 [27] that discharged of cane sugar residue from sugar come industry reduced the soil P^H. Higher organic content was observed in the IMO's. Higher organic content may be due to the accumulation of organic residue in soil.The total content of phosphorous in test and control sample was 277kg/h and 35kg/h respectively. Narasimha *et al* 1999 [24] (distilley) made similar reports that the discharge of effluents from cotton ginning mill enhanced the soil total phosphorus contents in the IMO'S treated soil than control soil.

Potassium content in test and control sample was 854kg/h and 291kg/h respectively. Similarly Narasimha et al 1999 [24] reported that discharge of effluents from sugar and diary industry enhanced the soil total potassium by 2 to3 folds.

Biological properties

Improved microbial populations including bacterial and fungal population were enumerated and counted in test soil than the control sample. For instance 4 and 2 fold for higher bacterial and fungal populations was observed in test soil than control and the values tabulated in table 2.

Table.2 Microbial population* in the IMO treated and control soil

Type of organism	Test sample	Control sample
Bacteria	$90x10^4$	$5x10^4$
Fungi	$12x10^4$	$6x10^4$

**Microbial population was measured in the terms of colony forming units CFU/g of soil.*

Soil microbial biomass and soil microbial activities are highly correlated which can be used as the indicators of soil fertility. The turnover and mineralization of soil substance, nutrient transformation and microbial population, affects the soil fertility Sparling *et al.,I* 2001 [23]. Micro flora of IMO's and control samples were enumerated and listed in the given table. Higher bacterial and fungal populations were observed in test soil than control soil. For test soil $90x10^4$, $5x10^4$ bacteria, and $12x10^4$, $6x10^4$ fugal colonies observed in test and control samples respectively. Higher bacterial population may be due to the favorable P^H in soil. Similarly Narasimha 1999 [24], reported that soil microbial population increased with discharge of effluents from cotton ginning mill and supplementation of animal manure, synthetic fertilizers, soil organic matter levels are simultaneously measured Jenkinson and Ladder in 1981 [16] reported that biomass generally increased by the application of organic matter which may have overcome chemical fertilizers both in terms of organic matter which may have overcome chemical fertilizers both in terms of sustainability and from an environmental conditions. In contrast irrigation with dairy effluents enhanced the soil microbial and enzyme activities [23, 28].

Fig 1. (a, b) protease activity in IMO's and control (with substrate, without substrate) samples in different incubations as by using different concentrations of soils

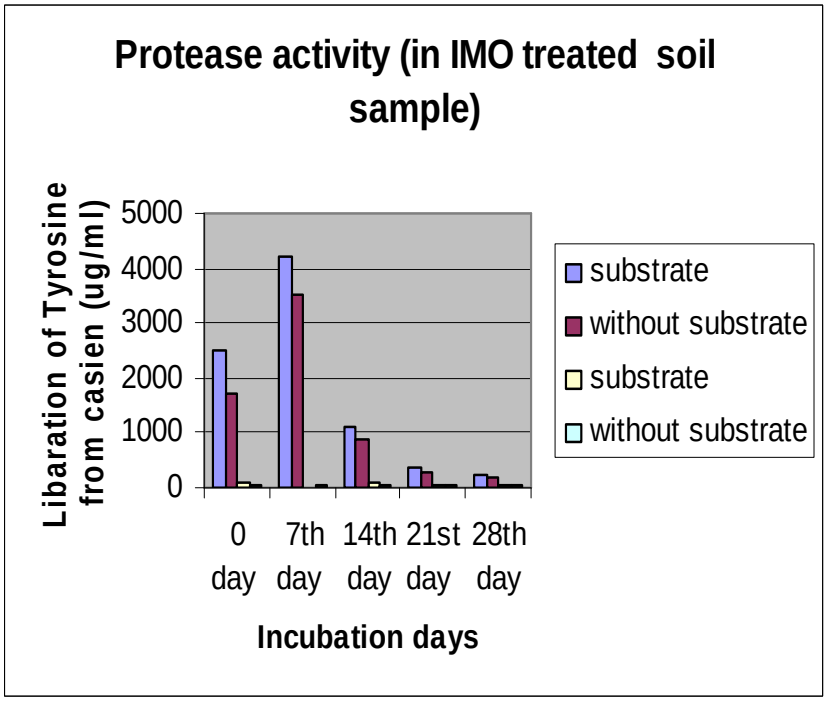

Fig. a

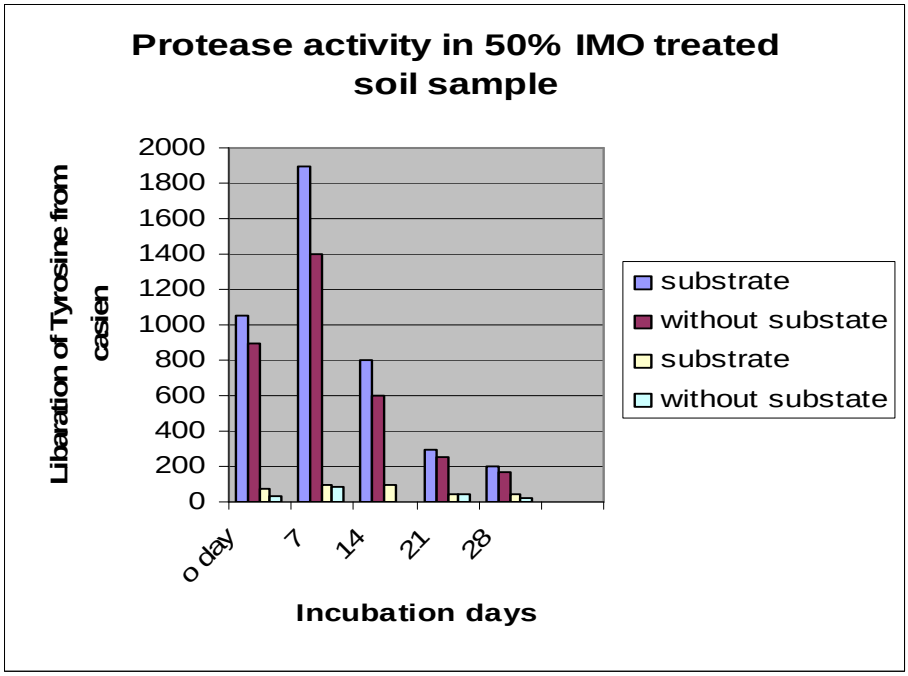

Fig.b

Fig 1. (c, d) protease activity in IMO's and control (with substrate, without substrate) samples in different incubations as by using different concentrations of soils

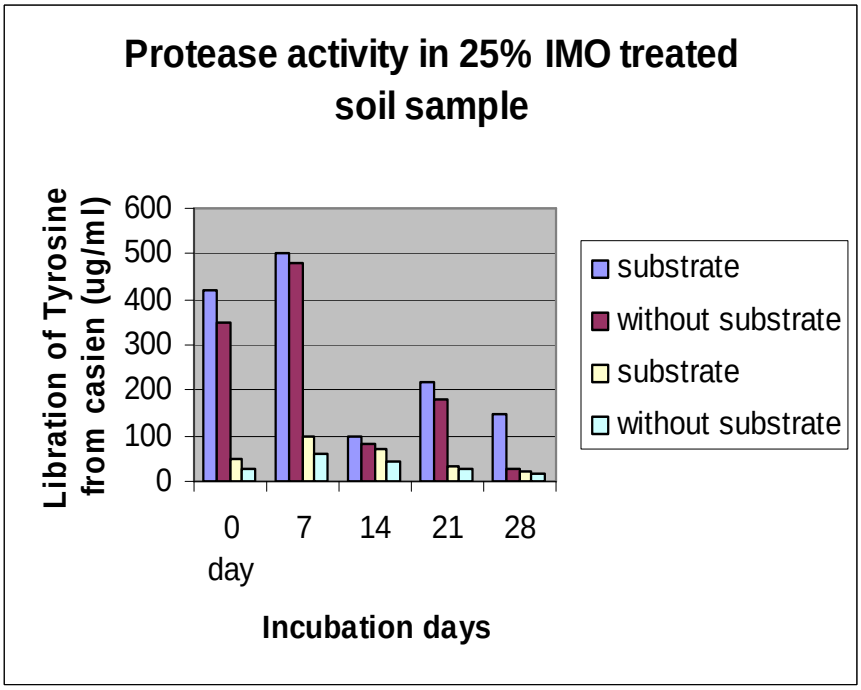

Fig.c.

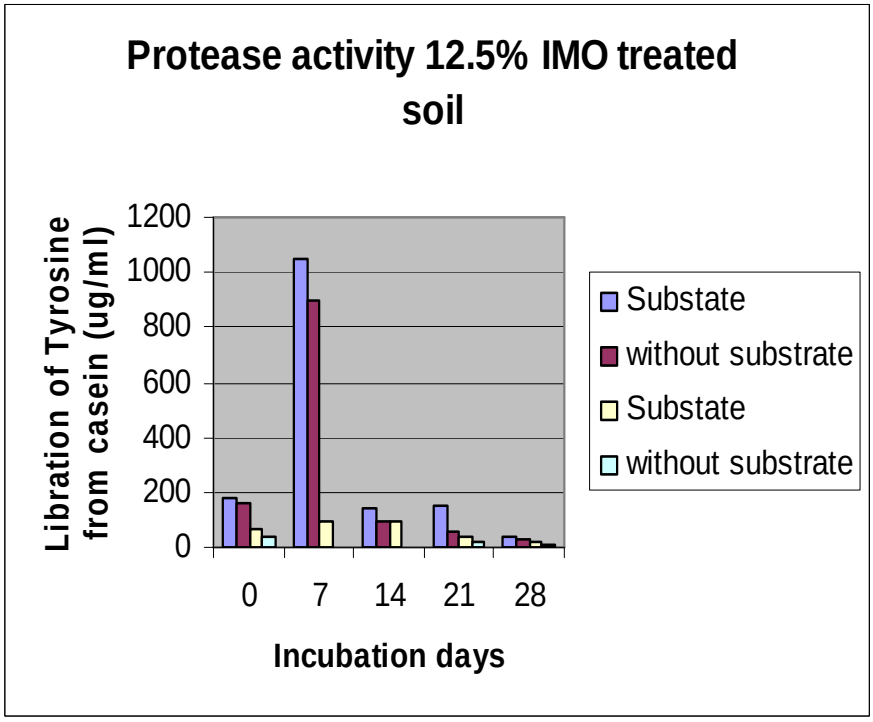

Fig.d.

Values represented in the figure are mean of duplicates ± S.D

The protease activity in soil inoculated with Indigenous microorganism (IMO's) was studied and listed in fig 1. Microorganisms and their enzymes are the indicators for the crop yield and soil fertility. Direct inoculation of

microbial population may reflect the soil fertility in terms of enzyme activities. With influence of soil incubation period protease activity was also improved up to the day interval further activities are seized at 14th to 28th day of interval in both inoculated and uninoculated soils. This trend was common in IMO-treated, and with and without substrates treated soils. Compared with the uninoculated soil widely no folds higher protease activity was observed in two treated soil than the controls. The protease activity test (IMO's treated soil) in substrate treated and untreated soils was also studied here also nearly to fold higher enzyme activity were observed in casein treated soil than untreated soil. The protease activity in soil supplemented with 12.5% was shown in fig. (1d) with increasing the soil incubation day's protease activates also increased up to 7th day declined at further incubation days. The protease activity at 7th day interval was higher than remaining intervals in both substrate and non-treated soil. For instance the protease activity in substrate soil at initial (0) day interval was 150 µg/g of soil whereas at 7th day interval tremendously higher enzyme activity was observed that is 540 µg/g of soil. Similar trend was observed at remaining days of interval. In case of control soils this trend was reduced up to 50%.The protease activity in normal soil that is without combination treated soil was recorded to have 4 fold higher enzyme activities was observed in indigenous microorganisms treated soil then control soils (fig 1 b).

The protease activity at 25% IMO's treated was also observed. Here also with increase in the concentration of IMO's to the soils slightly high protease activity was recorded in IMO's treated soil then controls soil. Per example the protease activity in IMO's at 12.5% was 150 µg where as 420µg per gram soil at 25%. Similar trend was followed at remaining days of intervals but there were no considerable higher activities in control soil (fig.1c).

The protease activity at 50% of indigenous IMO's suspension' soil was investigated and shown in fig.2b. Like previous reports with increasing in the concentration of imos the soil the protease activity also improved at 50% level concentration. For instance the protease activity in the soils treated with 50% IMO treated soil was 1080µg per gram of soil where as 420 µg, 156 µg in 25% and 12.5% soil concentration respectively with increase in the soil incubation days soil protease activity was also improved up to 7th day interval further the activity was calculated in 14 , 21 and 28 days of intervals in 50% IMO treated soils compared with control soil at different concentration of IMO there was no considerable higher activities among the various concentration of soil. Similar report was made others, Kannan and Oblisamy 1990 [29], Narasimha 1999 [24] and Discharge of effluence from agro based industries improved soil protease activity in contrast dust generated from cement industries decreased in the soil protease activity Shanti 1993, [26] the percentage of increased in the protease activity in the present study may be due to the direct inoculation of indigenous microorganisms to the soil. Increased proteolytic activity in casein treated soils may be due to the high availability of suitable substrates and increased in proteolytic microorganisms in the soils. Soil protease activity was calculated with the number of soil bacteria protease activity was enhanced not only with the addition of proteins but also with the addition of sugar . Similarly activity was decreased under alkaline conditions .

Fig 2. (a, b) Urease activity in IMO's and control (with substrate, without substrate) samples in different incubations as by using different concentrations of soil.

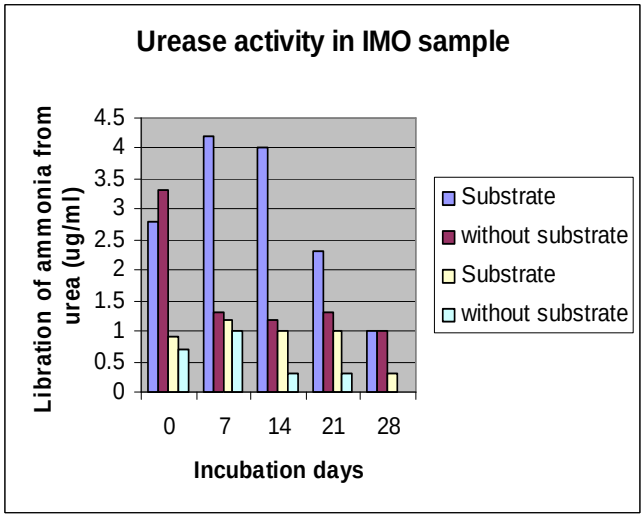

Fig.a

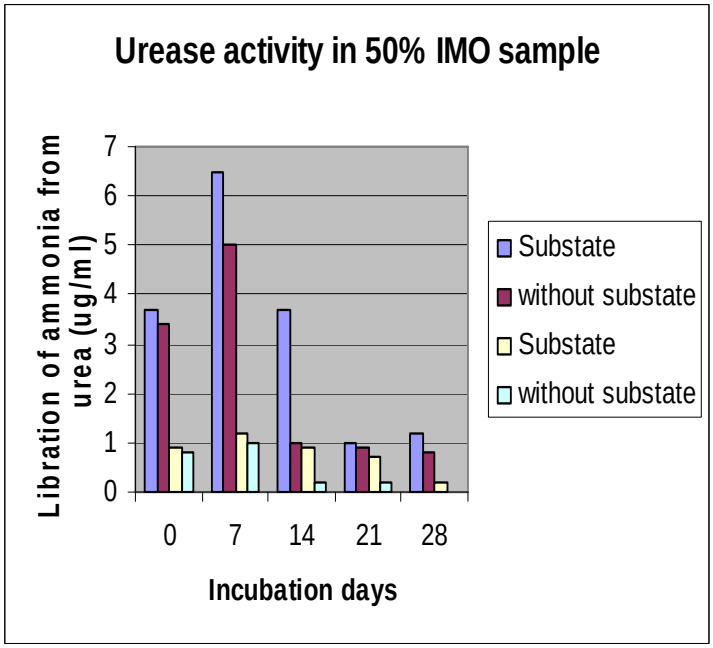

Fig.2b.
**Values represented in the figure are the ± S.D*

According to the Narasimha *et al* 1999 [24] discharged effluence from cotton ginning mill improved the soil protease activity. The urease activities of test and control soil were studied and shown in fig.2. With increasing the incubation period the urease activity improved upto 7th day interval further the activity was declined. Two fold higher urease activity was observed in test sample interval 3.9µg of ammonia /g of soil where as in control 0 .8 µg of ammonia / g of soil like other soil enzymes protease ,urease activity also increased in the first where and there after declined in both soil examples. The similar traced was observed in urease enzyme activities even in the presence of buffer in both soil ureased buffering condition soil sample treated work imos treated soil exhibited above 2-3 fold higher urease activity over control. For instance the urease activity of 0.8µ g of ammonia/g of soil in control soil where as 3.9 µ g of ammonia/ g of soil in IMO's treated soil at 7th day interval.

Fig 2. (c, d) Urease activity in IMO's and control (with substrate, without substrate) samples in different incubations as by using different concentrations of soil

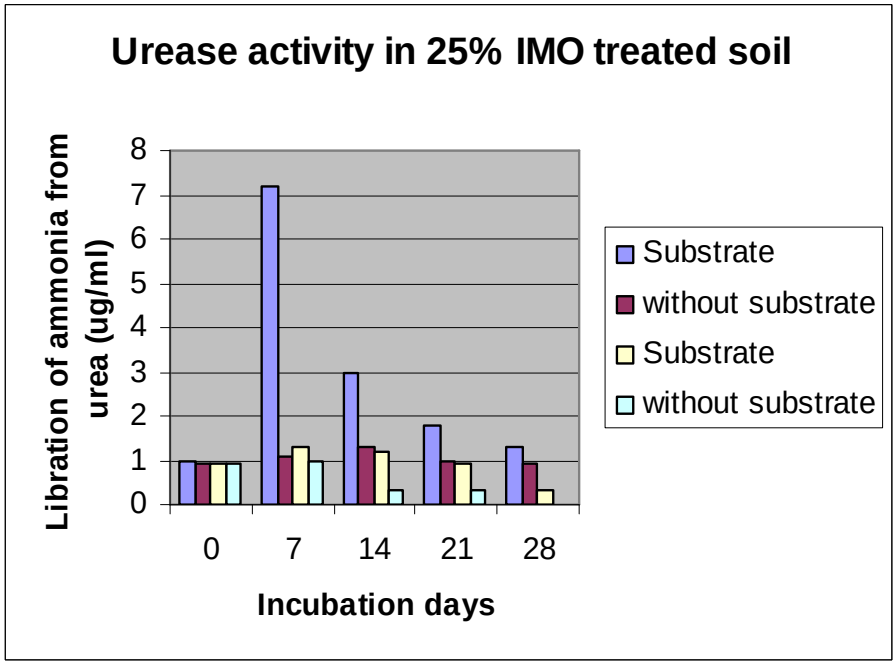

Fig.2c.

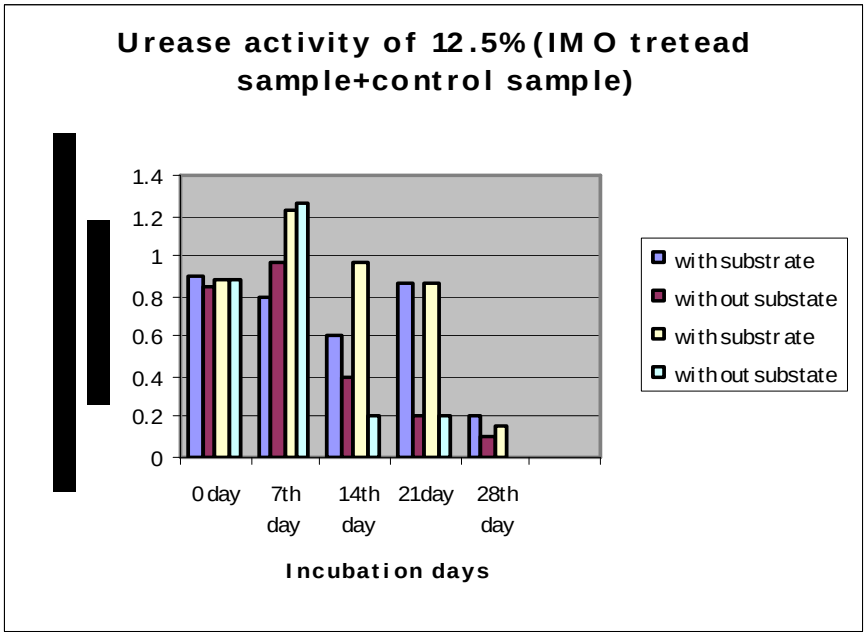

Fig.2d.
Values represented in the figure are the mean of duplicates ± S.D

Urease activity at 12.5, 25, and 50 percentage concentration of IMO's was studied and shown fig(2.d,c,b) there is a considerable lower urease activity was observed in 12.5% IMO treated soil whereas two fold higher activity was observed in 25% IMO's treated soil. Among the concentration tested in the present study higher urease activity was recorded at 50% IMO's treated soil. Similar reports were made Tabatabai and Bumhner (1971) [30]. According to their studies two fold higher urease activity in agricultural soils upon the addition of buffer solutions. The urease activity was influenced by P^H, organic matter type P^H soils. The urease was improved with supplementation organic matter content and decreased with drop of P^H. Higher urease activity in IMO's soil in the present study could be attributed to stimulation of microbial activity and favorable P^H conditions. In contrast cement dust pollution caused significant decreased in urease activity in soil samples discharged with effluents in cement industry [26]. Similarly discharges of agro based industrial effluents such as Cotton Ginning [31], Dairy [32, 33], Oil, [34] Abattoir [35] and Sugar[36] improved the soil physic-chemical, biological and enzymes activites. Improved microbial population and enzyme activites in soil treated with IMO sample was an indication of improvement of soil fertitility and soil health. According to the Narasimha *et al*, (2012) [37] the optimal values of factors influencing the production of protease under solid state fermentation were found to be moisture content 60% (v/w), incubation temperature 32±20C , inoculum level 10% (v/w), incubation period 5 days and pH 5.0.

Acknowledgements
Authors are thankful to Mrs. K.Rohini Reddy, Director of SARRA (Sounth Asian Rural Reconstruction Agency) for providing IMO samples from Pulicherla Bioforming fields of Chittor district, AP India.

REFERENCES

[1] S.P.Sharma Singh, M.V.Subhehia, S.K.Jain, P.K. Kaushal Vandna, T.S.Verma, *Research Bulletin NO.2 AICRP on Long Term Fertilizers Experiments, IISS, Bhopal (MP) and Departments of soils, CSK HPKV, Palampur, Hp* **2004,** 1-88.
[2] A.D.McLaren, *Chem. Scripta*,**1975,** 8, 97-99.
[3] G.Narasimha, A.Sridevi, G.Venkata Subba Reddy, B. Rajasekhar, *J. Chem. Pharm. Res.*, **2011**, 3(1), 128-137.
[4] T. Miwa, C.T. Ceng, M. Fujisaki, A. Toishi, *Plant Soil*, **1937**,10, 155-170.
[5] J.N. Kanfer, R.A. Mumford, S.S. Raghavan, J.Byrd, *Anal. Biochem* , **1974**, 60, 200-205.
[6] W.A.Dick, M.A.Tabatabai *Soil Sci*, **1984**, 137, 7-15.
[7] E.S.James, L.W. Russel, A. Mitrick, *Plant Soil*, **1991**, 132, 85-90.
[8] P.A. Richmond, *In: Biosynthesis and Biodegradation of Cellulose (Haigler CH and Weimer PJ Eds)* **1991**, 5-23
[9] J.G.Shawale, J. Sadana, *Archives Biochem. Biophys*, **1981**, 207, 185-196.
[10] P.J.M.Cooper, *Soil Biol. Biochem*. **1972**, 4, 333-337.
[11] A.M.Ganeshamurthy , G .Singh, N.T Singh, *J. Indian Soc. Soil Sic.* **1995**, 43, 637-641.

[12] B.Wick, R.F. Kühne, P.L.G .Vlek, *Plant Soil*, **1998**, 202, 97-107.
[13] M.A.Aon, A.C. Colaneri, *Appl. Soil Ecol*, **2001**,18, 255–270.
[14] C. Baum, P. Leinweber, A. Schlichting, *Applied soil Ecology*, **2003**, 22, 167-175.
[15] H.J.Chen, *Forest Ecology and Management*, **2003**, 178, 301-310.
[16] D.S.Jenksions, J.N. Ladd, **1981**, *In E.A.Paul and J.N. Ladd (Eds) Soil Biochemistry. Vol.5. Masrcel Dekker, New York.* 425-471.
[17] R.L. Sinsabaugh, R.K, Antibus, A.E. Linkins, C.A. McClaugherty, L. Rayburn, D. Repert, T. Weiland, *Ecol*, **1994**, 74, 1586-1593.
[18] R. Albiach, R. Canet, F. Pomares, F. Ingelmo, *Bioresour.Technol.* **2000**, 75, 43-48.
[19] T. Kunito, K. Saeki, S. Goto, H. Hayashi, H. Oyaizu, S.Matsumoto, *Bioresource Technology*, **2001**,79, 135-146.
[20] O. Dilly, P. Nannipieri, *Biol. Fertil.Soils*, **2001**, 34, 64-72.
[21] APHA-AWWA-WEF, **2000**, Standard methods for the examination of water and waste: 20th edition.
[22] O.H. Lowry, H. Rouser bough, R. Rardal, *Biochem* **1951**, 53, 265-275.
[23] G.P. Sparling, L.A. Scipper, J.M. Russel, *Aust. J. Soil. Res*., **2001**, 39, 505-518.
[24] G.Narasimha, G.V.A.K. Babu, B.R. Reddy, *J. Env. Biol*, **1999**, 20, 235-239.
[25] C. Xiao, M. Fauci, D.F. Bezdicek, W.T. Mckean, W.L. Pan, *Soil. Sci. Soc. Am. J.* 70, 72-77.
[26] M. Shanthi, Sri Krishnadevaraya University, Anantapur, India. *M. Phill thesis*, **1993**,
[27] G.K. Zende, **1996**, Sugar Industry by Product and Crop Residues in Increasing Soil Fertility and Crop Productivity in Sugar Cane Agro Industrial Alternations, 351–369.
[28] B.P. Degens, L.A. Schipper, J.J. Claydon, J.M.Russell and G.W. Yeats, *Aust. J. Soil Res.*, **2000**, 38, 25-36.
[29] A. Kannan, G. Oblisami, *Soil Biol. Biochem*, **1990**, 22, 923-927.
[30] M.A Tabatabai, J.M Bremner, *Soil Biol. Biochem.* **1971**, 3, 317-323.
[31] G**.** Narasimha, A. Sridevi, B .Viswanath, M .Subhash Chandra, B. Rajasekhar Reddy, *African Journal of Biotechnology*, **2006**, 5(4), 472-476.
[32] S. David Syam Babu, M. Ram Gopal, G. Nandana, A. Sridevi, G.Narasimha, *Asian J Microbial. Biotech.& Env. Sci*. **2009**,12, 323-327
[33] S. Nizamoddhin, A.Sridevi, G. Narasimha, *Environ. Conser. Ecol.* **2007**, 14 (1), 89-94.
[34] A. Sridevi, G. Narasimha, B. Rajasekhar reddy, *Asian J.Microbiol. Biotechnol. Environ, sciences*, **2007**, 9, 139-140.
[35] S. Radha, V.J. Nithya, A. Sridevi, G**.** Narasimha, *Archves of Appl.Microbiology*, **2011**, 3(2), 155-163.
[36] M. Nagaraju G. Narasimha V. Rangaswamy *International Biodeterioration & Biodegradation*, **2009**, 63, 1088–109.
[37] S. Radha, A. Sridevi, R. HimakiranBabu, V. J. Nithya, N. B. L. Prasad and G. Narasimha, *J. Microbiol. Biotech. Res*, **2012**, 2 (1), 6-16.