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Microbial Population and Soil Enzymatic Activities under Long Term Rice-Fallow and Uncultivated Soils of Nalbari District, Assam, India.

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ABSTRACT

Microorganisms in soils and their enzymatic activities play a critical role in maintaining soil fertility and sustaining crop productivity by nutrient transformation and organic matter decomposition. The present investigation was carried out to assess the effect of long term continuous cultivation of rice on microbial population and soil enzymatic activities that was compared with adjacent uncultivated soils. For this Geo-referenced (N:26^{0}31.882'to26^{0}18.224'and E:091^{0}30.536'to091^{0}15.750') soil samples (0-15cm depth) were collected after harvest of rice from rice-fallow system and adjacent uncultivated sites.A total of 120 numbers of soil samples were collected and microbial counts of general bacteria, fungi, *Azotobacter, Azospirillum,* Phosphate solubilizing bacteria (PSB) and soil enzymatic activities viz. Dehydrogenase (DHA), Fluroscence diacetate hydrolases (FDA), Phosphomonoesterase (PME) and Arylsulphatase (ARYL) were determined following standard procedures.The results revealed that population of bacteria (6.49 $\log_{10}$cfu g^{-1}), *Azotobacter* (3.77$\log_{10}$cfu g^{-1}), *Azospirillum* (3.75$\log_{10}$cfu g^{-1}), Phosphate solubilizing bacteria (3.71$\log_{10}$cfu g^{-1}), registered higher in rice-fallow cultivated soils compared to adjacent uncultivated soils (bacteria-6.36 $\log_{10}$cfu g^{-1}, *Azotobacter*-3.62 $\log_{10}$cfu g^{-1},*Azospirillum*-3.54 $\log_{10}$cfu g^{-1}, PSB- 3.69 $\log_{10}$cfu g^{-1}and differed significantly ($p<0.05$),whereas the population of fungi was found more in uncultivated (5.38$\log_{10}$cfu g^{-1}) soils compared to rice cultivated soils (5.30 $\log_{10}$cfu g^{-1}).The soil enzymatic activities viz. DHA , FDA, PME and ARYL were recorded significantly ($p<0.05$) higher in rice - fallow soils (DHA-79.01µg TPF g^{-1} 24 h^{-1}, FDA-12.16µg fluorescein g^{-1} h^{-1}, PME-114.06µg *p*-nitro phenol g^{-1} h^{-1}, ARYL-21.43 µg *p* -nitrophenol g^{-1} h^{-1}) as compared to uncultivated soils (DHA-66.26µg TPF g^{-1} 24 h^{-1}, FDA-9.49 µg fluorescein g^{-1} h^{-1}, PME-92.14µg *p*-nitro phenol g^{-1} h^{-1}, ARYL-15.72µg *p*-nitrophenol g^{-1} h^{-1}). The results indicated that continuous cultivation of rice crop followed by a fallow period enhanced the biological properties of soils including microbial population count and enzymatic activities in soils.

Key Words: Microorganisms, Population, Rice- fallow, Soil enzymes, Uncultivated

INTRODUCTION

Beneficial soil microbes are essential and integral component of soil, directly related to soil fertility and plant growth. Soil microorganisms play pivotal role by performing various functions in soils that includes mineralization of organic matter, nitrogen fixation, phosphorous availability, disease control, degradation of pesticides. Microorganisms play a crucial part in soil nutrient cycling by decomposing the organic material into plant-available elements, maintenance of soil structure, degradation of agrochemicals and pollutants, and plant pest control and maintain the soil health (Kuht *et al*, 2022: Stockdale and Brookes, 2006), hence it has often been indicated as an important component of soil fertility (Nogueira *et al*, 2006).

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Soil enzyme is a kind of biologically active protein and enzymes having catalytic ability released by soil microorganisms, and closely related to the organic matter content in soil and play a key role in decomposing organic matter, nutrients availability to plants hence important in Agriculture (Rao *et al,* 2017). Although enzymes are primarily of microbial origin, it can also be originated from plants and animals in soil. Enzymes catalyses all biochemical reactions and are integral part of nutrient cycling in soil (Nivitha and Vimalan, 2022) and these are sensitive indicators of soil ecological stress or other environmental changes (Marinari *et al*, 2006).Soil enzymatic activities are the potential indicator of soil quality that responds to management and environmental induced changes (Mohammadi, 2011). Both activities of soil enzyme and diversity of microbial community depend on land use patterns. Therefore, investigating soil physical, chemical, and biological properties under diverse land use patterns is vital to conserve and rejuvenate the soil's ability to provide ecological services (Van Leeuwen *et al*, 2017).

Nalbari district is one of the agriculturally important district of Assam lies between 26^0 N Latitude and 91^0 E Longitude with mean elevation 89 m above msl and about 80% of the population directly or indirectly dependent on agriculture and allied activities. Though the district comprises 2.6% of state's geographical area, it contributes 5.46% and 4.96% of the state's net and gross cropped area, respectively. Rice is the major crop covering an area of 65 thousand hectare and rice based cropping systems are predominantly practiced by the farmers in the district and majority of the area comes under rice -fallow system. The present study was undertaken with an objective to assess the impact of long term continuous cultivation of rice crop under rice –fallow system on soil microbial population and enzymatic activities in soils comparing with adjacent uncultivated soils.

MATERIALS AND METHODS

Geo-referenced (N: $26^0$31.882 ' to $26^0$18.224' and E: $091^0$30.536' to $091^0$15.750') soil samples (0-15cm) from rice -fallow cultivated soils were collected after harvest of rice crop during the year 2015-16. For comparison, the soil samples from adjacent uncultivated sites were also collected. The representativeness and uniformity of the fields were taken into consideration while collecting the soil samples. The sampling is focused on the plough layer because; this is where most soil quality changes are expected to occur due to long -term land use and soil management practices. All total 120 soil samples, 60 from cultivated rice-fallow and 60 from adjacent uncultivated soils, were collected covering 23 villages of the district. At the time of collection of soil samples, the crop history including management practices was recorded from respective farmer. The soil samples were mixed uniformly, kept in zipped poly pouches with proper labelling and store at 4^0C for analysis of biological parameters.

Enumeration of microbial population

The classical serial dilution technique was used for enumeration of bacteria, fungi, *Azotobacter*, *Azospirillum* and phosphate solubilizing bacteria (PSB) from the soil by spread plate technique on appropriate media. Nutrient agar (NA) and Martin Rose Bengal (MRB) media were used for enumeration of bacteria and fungi respectively. The soil sample of 1 g was suspended in 9 ml water blank followed by serial dilution up to 10^{-5}. Aliquot of 10 µl from 10^{-3}, 10^{-4} and 10^{-5} dilution were spread over solidified media in triplicates and plates were incubated at 30 ± 1^0C for bacteria and fungi population.

For enumeration of *Azotobacter*, *Azospirillum* and PSB the media used were that of Burk's, nitrogen free bromothymol blue (NFb) and Pikovskaya's media respectively. 100 µl aliquot of 10^{-4} and 10^{-5} dilutions were spread over the solidified media in triplicates and plates were incubated at 30 ± 1^0C for *Azotobacter* and PSB while NFb plates were incubated at 35 ± 1^0C for 3-5 days. The microbial numbers were estimated as colony forming unit per gram(cfu g^{-1}) soil on dry weight basis and transformed to $\log_{10}$cfu g^{-1}.

Dehydrogenase activity (DHA)

Dehydrogenase activity (DHA) was determined by the reduction of triphenyl tetrazolium chloride (TTC) to triphenyl formazan

(TPF) as described by Casida*et al* (1964) with modifications. Moist soil (10 g) was treated with 10 ml of 3% TTC, and then incubated at 32^{0}C for 7 days in screw cap test tube (30 ml). After incubation period, the soil was extracted by addition of 10 ml of extractant (methanol) following incubation in dark and agitated for 1 h. The mixture was then filtered using Whatman No.42 filter paper. After the filtration, 1 ml of filtrate was transferred to 1.5 ml micro centrifuge tube and centrifuged at 5000 rpm for 5 min. Absorbance of the supernatant was measured in Nanodrop 1000 spectrophotometer at OD_{485nm}. To account for any abiotic TTC reductions, sterile controls consisted of autoclaved soil (121^{0}C, 20 min. for three consecutive days) to which 10 mL of TTC was added. Spectrophotometer blanks consisted of 10 g soil and TTC replaced with 10 mL Millipore water. Except for the addition of Millipore water in blank and autoclaving in control, they were treated like samples for the rest of the procedure. A calibration curve was constructed by determining OD_{485nm} values for the working standard of TPF (20, 40, 80, 120, 200, 300 and 500 µ ml^{-1}). The OD_{485nm} values was compared to that of TPF standards. DH activities was expressed on dry weight as µg TPF g^{-1}24 h^{-1} on dry weight basis as

$$\text{DH activity } (\mu g \text{ TPF } g^{-1} 24\ h^{-1}) = \frac{[(TPFs) - (TPFc)] \times 20}{edw}$$

Where, TPFs = TPF conc. (µg ml^{-1}) in the sample; TPFc = TPF conc. (µg ml^{-1}) in the sterile control; edw is the equivalent dry weight of 1 gm soil; 20 is the volume of solution added in the assay (TTC + Extractant). All samples were replicated three times.

Phosphomonoesterase activity (PME)

Phosphomonoesterase (PME) activity involves the use of an artificial substrate, *p*-nitrophenyl phosphate (*p*-NPP). The product of PME activity, *p*-nitrophenyl, is a yellow chromophore under alkaline conditions and can be detected colorimetrically. The method of Tabatabai and Bremner (1969) was followed to estimate the PME activity. Moist soil (5 g) was taken in 30 ml screw cap test tubes and 10 ml modified universal buffer (pH 6.5), 0.25 ml toluene and 1 ml of *p*-NPP (115 mM) solution were added to it. After properly mixing and vortexing for 30 seconds, the samples were incubated at 37^{0}C for 1 hour. At the end of the incubation, 1 ml $CaCl_2$ (0.5M) and 4 ml of NaOH (0.5M) were added and mixed again for 5 min. in a vortex mixture, and allowed to settle for 5 min. 1 ml of the mixture was taken in a 1.5 ml of microcentrifuge tube, and the soils were removed by centrifugation at 5000 rpm for 5 min. The absorbance of the supernatant was determined at OD_{400nm} using Nanodrop 1000 spectrophotometer. The control was prepared in a similar manner except for the fact that 1 ml of *p*-NPP (115 mM) solution was added only after the addition of $CaCl_2$ (1 ml) and NaOH (4 mL) but immediately before centrifugation. Spectrophotometer blank consisted of similar to that of sample but *p*-NPP replaced with 1 ml Millipore water. *p*-nitrophenyl content of sample was calculated by referring to the calibration curve obtained with standards containing 0, 50, 100, 150, 200 and 250 µg of *p*-nitrophenyl. PME activity was expressed as µg *p*-nitrophenyl g^{-1} h^{-1} on dry weight basis. All samples were replicated three times.

Fluorescein di acetate hydrolysis (FDA)

Fluorescein di acetate (FDA), hydrolysis activity was carried out following the method described by Adam and Duncan (2001). Moist soil (1 gm) weighed in 30 ml sterile screw cap test tube and added 7.5 ml potassium phosphate buffer (pH 7.6, 60 mM) and allowed to equilibrate at 25^{0}C on an Environmental shaker. The reaction was started by addition of 0.1 mL FDA solution (1000 µg ml^{-1}) and incubated at 25^{0}C for 1 hour. Spectrophotometer blanks consisted of the soil and buffer mixture with the FDA solution replaced by 0.1 ml acetone and incubated like the sample.After completion of 1 hour incubation, the reaction was stopped immediately by adding 7.5 ml of chloroform: methanol (2:1) and mixing the content thoroughly in vortex for 30 seconds and allowed to settle for 30 min. After settling down 1.0 ml of upper phase was transferred to 1.5 ml microcentrifuge tube and centrifuged (5000 rpm

for 5 min.) to remove suspended particles and absorbance of the supernatant was measured at OD_{490nm} using Nanodrop 1000 spectrophotometer. The calibration curve was prepared with standards 0, 2, 4, 6, 8, 10 µg fluorescein mL^{-1}. The mass of fluorescein produced in each assay was determined from the corresponding optical density (OD_{490nm}) value divided by the equivalent dry weight of soil (determined by loss of weight of field- moist sub –samples after heating at 105^0C until constant weight). FDA hydrolysis activity was expressed as µg fluorescein g^{-1} h^{-1}. All samples were replicated three times.

Arylsulphatase activity (ARYL)

The assay for Arylsulphatase (ARYL) activity was carried out by using *p*-nitrophenyl sulphate (*p* -NPS) as substrate (Tabatabai and Bremner, 1970). Soil (2.0 g) was placed in 15 ml screw cap test tube and amended with 4.0 ml of acetate buffer (0.5M, pH 5.8), 1.0 ml of 20mM *p* –NPS and 0.5 ml toluene. The sample was vortexed and incubated at 20^0C for 5 h. On completion of incubation, 2.0 ml of 1.0 M NaOH and 1.0 ml of $CaCl_2$ was added. The sample was mixed and the amount of *p*-nitrophenyl, the hydrolysis product produced in the supernatant was measured at OD_{400nm} (Nanodrop 1000 Spectrophotometer). The ARYL activity was calculated from *p*-nitrophenol standard curve and expressed on dry weight basis (µg *p*-nitrophenol g^{-1} h^{-1}). All samples were replicated three times.

RESULTS AND DISCUSSION

Population of Bacteria

The mean bacterial population in cultivated sites of rice-fallow system was found to be of 6.49(±0.13) log_{10}cfu g^{-1} compared to the 6.36(±0.22) log_{10}cfu g^{-1} in uncultivated sites (Table1, Fig 1). Both rice-fallow and uncultivated soils contained higher population of bacteria (>5log_{10}cfu g^{-1}) and exhibited significant difference between cultivated and uncultivated soils (Table 1). Higher bacterial population in rice-fallow soils might be attributed to root exudation in rice cultivation, since 40-90% of the 30-60% root allocated substrates in rice translocate into the soil (Lynch and Whipps, 1990). Srivastava *et al* (2004) reported a higher bacterial population (5.46 log_{10}cfu g^{-1}) in sub-humid moist bioclimate followed by semi-arid (5.34 log_{10}cfu g^{-1}) soil in different Indo-Gangetic Plains under rice based crop sequence. A previous report indicates that the relative abundance of soil bacteria increases under high soil moisture conditions and is confirmed by the presence of high microbial density in the sub-humid (moist) IGP, whereas fungi dominate in dry soil (Collins *et al*, 2008).

Population of Fungi

The mean population counts of fungi in the soils of cultivated sites of rice-fallow, sequences found to be of 5.30(±0.14) log_{10}cfu g^{-1} compared to the 5.38(±0.12) log_{10}cfu g^{-1} in uncultivated site (Table 1, Fig1) and exhibited significant difference between rice-fallow and uncultivated soils (Table 1). Comparatively the lower population sizes in cultivated soils might be due to the tillage practices which could damage the fungal hyphae as well as metabolic and physiological requirement of fungi, since fungi prefers low water content for growth (Griffin, 1969; Lopes *et al*, 2011).

Population of *Azotobacter*

The population count of *Azotobacter* in the soils of rice-fallow system were found to be of 3.77(±0.22), log_{10}cfu g^{-1} compared to the 3.62(±0.17), log_{10}cfu g^{-1} in uncultivated sites (Table 1, Fig1). In rice-fallow and uncultivated sites, the *Azotobacter* population was found low (<4 log_{10}cfu g^{-1}) but, exhibited significant difference between rice-fallow and uncultivated soils (Table 1). Palleroni (1984), reported that the free- living non-symbiotic nitrogen-fixing bacteria and those belonging to genus *Azotobacter* sp. which is heterotrophic, aerobic microorganism being broadly dispersed in different environments, such as soil, water and sediments. The existence of *Azotobacter* in the soils of the present investigation is also attributed to the findings of Garg *et al*. (2001), who indicated that the strains of *Azotobacter* could be usefully employed in aquaculture systems.

Population of *Azospirillum*

With reference to the *Azospirillum* population, the mean population counts in the

Table1. Descriptive statistics of population of microorganisms (log_{10}cfu g^{-1}) in Rice –fallow and Uncultivated soils

	Descriptive values	Bacteria	Fungi	*Azotobacter*	*Azospirillum*	PSB
Rice-Fallow	Minimum	6.17	4.95	3.30	3.12	3.30
	Maximum	6.79	5.78	4.17	4.25	4.05
	Mean (±SD)	6.49 (0.13)	5.30 (0.14)	3.77(0.22)	3.75(0.22)	3.71(0.18)
Uncultivated	Minimum	5.45	5.07	3.30	3.30	3.30
	Maximum	6.70	5.70	3.92	3.86	3.93
	Mean (±SD)	6.36(0.22)	5.38 (0.12)	3.62 (0.17)	3.57(0.15)	3.57(0.15)
Paired t -test		4.94*	4.40*	4.00*	6.31*	4.39*

*Significant at p=0.05 level

Table 2. Descriptive statistics of enzymatic activities in Rice –fallow and Uncultivated soils

	Descriptive values	DHA	FDA	PME	ARYL
Rice - Fallow	Minimum	17.85	3.16	36.54	17.46
	Maximum	257.35	19.85	180.96	24.49
	Mean (±SD)	79.01 (±38.51)	12.16 (±4.37)	114.06 (±34.74)	21.43 (±1.89)
Uncultivated	Minimum	11.52	5.24	46.76	1.22
	Maximum	121.72	19.23	132.76	21.45
	Mean (±SD)	66.26 (±27.84)	9.49 (±2.49)	92.14 (±22.78)	15.72 (±3.03)
Paired t -test		4.79*	6.26*	9.53*	15.46*

*Significant at p=0.05 level

cultivated soils of rice-fallow were found to be of 3.75(±0.22), log_{10}cfu g^{-1} compared to the 3.54(±0.13), log_{10}cfu g^{-1} in uncultivated sites (Table 1, Fig1). Both in rice-fallow and uncultivated sites, the *Azospirillum* population was found low (<4 log_{10}cfu g^{-1}) and differed significantly. As *Azospirillum* is being microaerophilic in nature (Tengsingh Baliah and Rajalakshmi, 2015) can be able to colonize the rhizosphere of rice for fixing N_2 in waterlogged situation (Roper and Ladha, 1995). Similar reports of occurrence of *Azospirillum* in rice cultivation were also available from findings of extensive research works (Choudhury and Kennedy, 2004).

Population of phosphate solubilizing bacteria (PSB)

Likewise, in case of phosphate solubilizing bacteria (PSB) the mean population count in the cultivated soils of rice-fallow, were found to be of 3.71(±0.18), log_{10}cfu g^{-1} compared to the 3.57(±0.15), log_{10}cfu g^{-1} in uncultivated sites (Table 1, Fig1). In both cultivated and uncultivated sites the PSB population was found low (<4 log_{10}cfu g^{-1}) and differed significantly. The occurrence of PSB in the range of 3.33-8.33 log_{10}cfu g^{-1} in rice soils were reported from rice rhizosphere of Assam (Nath *et al*., 2010).

Dehydrogenase activity

The mean value of dehydrogenase (DHA) enzyme activity in the cultivated soils of rice-fallow was recorded as 79.01(±38.51), µg TPF g^{-1} 24 h^{-1} as compared to the mean value of 66.26(±27.84), µg TPF g^{-1} 24 h^{-1}in uncultivated sites (Table 2,Fig 2). In cultivated sites 76.67% soils belonged to low level (<100 µg TPF g^{-1} 24 h^{-1}) and rest 23.33% soils in medium category (100-400 µg TPF g^{-1} 24 h^{-1})of DHA activity whereas in uncultivated sites 90% soils showed low level (<100 µg TPF g^{-1} 24 h^{-1}) and only 10% showed medium level(100-400 µg TPF g^{-1} 24 h^{-1}) of DHA

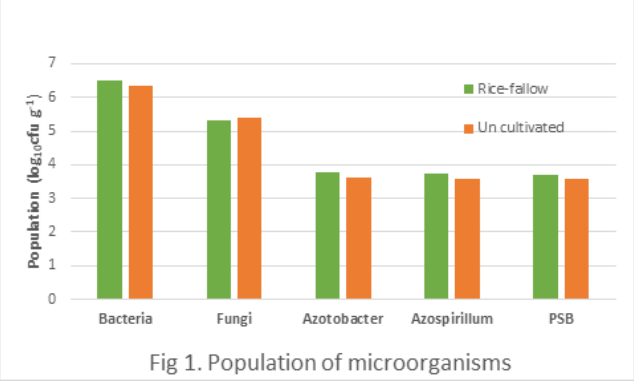

Fig 1. Population of microorganisms

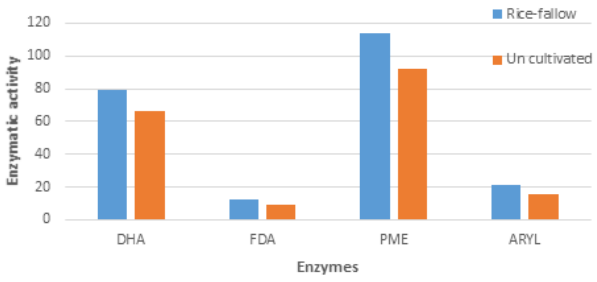

Fig2. Soil Enzymatic activities

activity. (Table 2). Natural decomposition of crop stubbles might stimulate DHA because the organic material on decomposition may provide intra and extracellular enzymes and may also stimulate microbial activity in the soil (Bhattacharyya *et al*., 2005).

Phosphomonoesterase activity

The mean value of phosphomonoesterase (PME) activity in cultivated soils of rice-fallow sequences were recorded as 114.06(±34.74), µg *p*-nitro phenol g^{-1} h^{-1}as compared to the mean value of 92.14(±22.78) µg *p*-nitro phenol g^{-1} h^{-1} in uncultivated sites (Table 2, Fig 2) . In rice-fallow 43.33% soils belonged to low level (<100 µg *p*-nitro phenol g^{-1} h^{-1}) and 56.67% soils belonged to medium level(100-200 *p*-nitro phenol g^{-1} h^{-1}) of PME activity whereas in uncultivated sites 68.33% soils showed low level (<100 µg *p*-nitro phenol g^{-1} h-1) and 31.67% soils showed medium level (100-200 µg *p*-nitro phenol g^{-1} h^{-1}).(Table 2). The mean value differed significantly between the rice-fallow and uncultivated sites. The persistence of elevated and active PME in cultivated soils might be due to their adsorption and protection against proteolysis, once released as extracellular enzymes or following cell death or during humification of organic residues (Nannipieri *et al*, 1996). Comparatively, higher activity of the enzyme PME in cultivated soils might also be due to the higher levels of microbial status maintained in the cultivated sites.

Fluorescein diacetate activity

The fluorescein diacetate (FDA)activity in the cultivated soils in rice-fallow, were estimated and the mean values were recorded as 12.16(±4.37) µg fluorescein g^{-1} h^{-1}as compared to the mean value of 9.43(±2.49) µg fluorescein g^{-1} h^{-1} in uncultivated sites (Table 2, Fig 2). In rice-fallow 68.33% soils belonged to medium level (10-31 µg fluorescein g^{-1} h^{-1})and 31.67% soils belonged to low level(<10 µg fluorescein g^{-1} h^{-1}) of FDA activity whereas in uncultivated sites 63.33% soils showed medium level (10-30 µg fluorescein g^{-1} h^{-1}) and 36.67% soils showed low level(<10 µg fluorescein g^{-1} h^{-1}) of FDA activity (Table 2). The mean value differed significantly between the rice-fallow and uncultivated sites. (Table 2). Nayak *et al* (2007) also illustrated the hydrolase activity of FDA in tropical rice soils. A similar result on FDA activity to the tune of 10.52 µg fluorescein g^{-1} h^{-1} was reported from the paddy soils of Assam (Nath *et al*, 2012).

Arylsulphatase activity

In cultivated soils under rice-fallow sequences the mean values of Arylsulphatase (ARYL) activity was 21.43(±1.89) µg *p* -nitrophenol g^{-1} h^{-1} while in the uncultivated sites, the mean values was 15.72(±3.03), µg *p* -nitrophenol g^{-1} h^{-1} (Table 2, Fig 2). Both in rice-fallow and uncultivated sites 100% soils belonged to low level (<30µg *p* -nitrophenol g^{-1} h^{-1})(Table 2). The mean value differed significantly between the cultivated and uncultivated sites. (Table 2). The enzyme ARYL catalyzes the hydrolysis of aromatic sulphate esters and releasing sulphate for plants uptake. Significantly higher ARYL in the rice-fallow soils might be due to the accretion of rhizodeposits, decomposed roots and leftover rice stubbles after harvest of rice (Lopes *et al*, 2011). In addition, ARYL being extracellular soil enzyme make complexes with humid colloids and get

stabilized on clay surfaces and organic matter, thereby increasing its content in cultivated soils (Bandick and Dick, 1999)

CONCLUSION

The present investigation indicated that due to the continuous cultivation of rice crops, there was an increase in population of bacteria, *Azotobacter, Azospirillum* and PSB in soils as compared to the adjacent uncultivated soils. Similarly, the enzymatic activities in soil also showed a higher value than the uncultivated soils. Continuous cultivation of rice crops in the long term increases microbial population and enzymatic activities as compared to the uncultivated soils. Therefore, the cultivation of crops with proper soil management practices like balanced use of chemical fertilizers based on soil test values, use of organic manures, and inclusion of legume crops in crop sequence etc. has to be adopted for sustaining soil health and crop productivity.

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