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Effect of long-term use of imbalanced and balanced nutrients in wheat under rice-wheat cropping system through chemical fertilizers and organic manure on biological properties of soil

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Abstract
A field experiment with wheat at the Crop Research Centre, Department of Agronomy, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, under a rice–wheat system was conducted to study the long-term effect of inorganic fertilizer and FYM on biological properties of soil after 29th cycles of wheat under rice–wheat cropping system. The experiment comprised 14 treatment combinations viz., N, P and K fertilizer application as individual component and use of chemical fertilizers in conjunction with farm yard manure. The experimental treatments were replicated four times. Combined application of inorganic fertilizer and FYM found the highest microbial population in bacteria, fungi, Actinomycetes, *Azotobacter* and phosphorous solubilizing bacteria in both the surface and sub-surface soil layers was due to $N_{180}+P_{80}+K_{40}+Zn(F)+FYM$ followed by $N_{120}+P_{40}+K_{40}+Zn(F)+FYM$ and $N_{120}+P_{40}+K_{40}+FYM$ while the lowest microbial population due to control. The present investigation clearly points out the significance of balanced and imbalanced use of nutrients including FYM in rice-wheat cropping system for improving the biological soil quality of wheat crop over a long period.

Keywords: Long term fertilizer experiment, wheat, FYM, biological indices, soil quality.

Introduction
Rice and wheat are both the dominant components of the Indian food security system as they are consumed as major staple food. Presently, the rice-wheat cropping system in the Indo-Gangetic plains is showing sign of 'fatigue' due to continuous cropping of this highly nutrient and water exhaustive cereal-cereal system for the last three decades. Imbalance use of chemical fertilizers alone tends to decline soil quality and fertility over a period of years with given inputs. Therefore, the most logical way to manage long-term fertility and productivity of soil is integrated use of inorganic and organic sources of plant nutrients. For the development of sustainable food production system maintenance and management of soil fertility is pivotal (Doran *et al.* 1988) [5]. Thus, the logical way emerging to manage long-term fertility and productivity of soil is integrated use of organic and inorganic sources of plant nutrients to address the concern of excess and/ or depletion of nutrients (Aulakh and Grant, 2008) [1].

Soil quality determination also involves many biological attributes. Waksman (1927) [19] studied importance of soil microorganisms from the view point of soil fertility and stressed that besides physical and chemical factors, soil biological parameters were much needed to predict soil fertility. Soil microorganisms being an important driver of various biogeochemical cycling of nutrients, are considered to play a vital role in maintaining soil health, productivity, and sustainability (Zhao *et al.* 2014) [22]. Therefore, understanding the soil microbial community and its response to various agricultural management practices guides us to select a suitable management strategy for the establishment of more stable and sustainable agro-ecosystems (Li *et al.* 2012, Zhao *et al*. 2014) [1., 22]. It is well known that nitrogen availability often limits plant productivity in terrestrial ecosystems (Le Bauer and Treseder 2008) [9], and soil nitrogen transformations are driven directly by a diverse microbial community. For example, biological N_2-fixation is the conversion of dinitrogen to biologically available ammonium (NH_4^+) by free-living, associated and symbiotic diazotrophs from a wide range of bacterial phyla (Zhang *et al*. 2006) [21]. Changes in soil microbial community compositions are also observed after the addition of organic and inorganic amendments (Sun *et al*. 2004) [16]. Eubacterial community (Sun *et al*. 2004) [16], soil fungal population (Piao *et al*. 2008) [12], ammonium-oxidizing bacteria and denitrifying bacterial community (Enwall *et al*. 2005) [6] are some of the microbial parameters so far reported to be affected by long-term application of organic and inorganic nutrient amendments. However, only few studies have been conducted in India on the influence of long-term addition of mineral fertilizers and manures on the soil biological properties (Vineela *et al*. 2008) [18].

Materials and Methods

The present investigation was carried out in an ongoing long term experiment, after twenty nine years, located at Norman. E. Borlaug Crop Research Centre, G. B. Pant University of Agriculture and Technology, Pantnagar, U. S. Nagar, Uttarakhand which was initiated in 1984. The experimental site lies in *Tarai* plains about 30 km southward of foothill of shivalik range of Himalayas at 29^0 N latitude, 79^0 29' E longitude and at an altitude of 243.8 m above the mean sea level. The chemical analysis of top 15 cm soil showed that it was rich in organic matter and medium in phosphorus and potassium and neutral to slightly alkaline in reaction. The field experiment has been laid in Randomized Block Design (RBD) in four replications with fourteen treatments comprising control, N, NP, NPK, varying doses alone and with FYM. The N, P and K were provided as per treatment details through urea, single super phosphate and potassium, respectively. In one treatment N and P was provided using Di-ammonium phosphate. The FYM was applied @5 tonne ha^1 only in rice crop.

Collection of soil samples and processing

Individual soil samples from each plot were collected from two depths (0-15 and 15-30 cm) after harvesting of wheat crop in the year 2013-14. These individual soil samples were pooled to get one representative composite sample each for every depth from all the plots.The count of microorganisms (bacteria, fungi and actinomycetes) was carried out by using serial dilution pour plate method (Wollum 1982) [20]. The count of *Azotobacter* was estimated by using serial dilution and plate count method (Becking 1981) [2]. The population of PSB was assessed by employing serial dilution and plate count method (Pikovaskaya 1948) [13]

Statistical analysis

The experimental data were analyzed using the statistical program STPR of G. B. Pant University of Agriculture and Technology, Pantnagar in a Randomized Block Design. Analysis of Variance and critical difference (CD) between treatments was calculated at 5% level of significance. Correlation coefficients were computed using SPSS version 16.

Results and Discussions

Number of Bacteria

Effect of long-term fertilizer application alone and with FYM at varying levels on the population of soil bacteria after harvest wheat crops is illustrated in Table 1 and fig1. The population of bacteria in soil ranged from 8.14 to 8.37 log_{10} CFU g^{-1} soil in surface soil while in sub-surface soil it varied from 8.07 to 8.25 log_{10} CFU g^{-1} soil after harvest of wheat crop. The lowest number of soil bacteria was recorded in control in both the surface and sub-surface soil. While the highest number of soil bacteria (8.37 log_{10} CFU g^{-1} soil) was recorded under N_{180}+P_{80}+K_{40}+Zn(F)+FYM (T_{10}) which was statistically at par with T_9, T_8, T_7, T_{11}, T_6 and T_{12}.

Number of Fungi

An application of fertilizer alone at varying levels with or without FYM over a period of twenty-nine years significantly influenced the fungal population in surface soil while in sub-surface soil the impact of treatments was not significant (Table1 and fig2). The population of fungi in surface soil ranged from 5.47 to 5.67 log_{10} CFU g^{-1} soil, while in sub-surface soil it varied from 5.39 to 5.55 log_{10} CFU g^{-1} soil after harvest of wheat crop. The lowest number of soil fungi was supported in the absence of any extraneous supply of nutrients i.e. control which was statistically at par with all the inorganic treatments except T_7 and T_{11}. However, the highest number of soil fungi (5.67 log_{10} CFU g^{-1} soil) was recorded under N_{180}+P_{80}+K_{40}+Zn (F)+FYM (T_{10}) which was statistically at par with T_9, T_8, T_{11}, T_7 and T_6.

Number of Actinomycetes

The soil actinomycetes in surface and sub-surface soil layers were influenced significantly due to the use of fertilizer at varying levels with or without FYM for twenty-nine years (Table1and Fig 3). The balanced and imbalanced use of fertilizer and manure improved the actinomycetes population. The population of actinomycetes in soil ranged from 7.16 to 7.36 log_{10} CFU g^{-1} soil in surface layer while in sub-surface layer it varied from 7.10 to 7.27 log_{10} CFU g^{-1} soil after harvest of wheat crop. The lowest number of soil actinomycetes was recorded in the control which was statistically at par with T_{12}, T_2, T_{14} and T_5 in the surface soil, while the highest number of soil actinomycetes (7.36 log_{10} CFU g^{-1} soil) was recorded under N_{180}+P_{80}+K_{40}+Zn(F)+FYM (T_{10}) which was at par with T_9..

Total microbial population

Total microbial population (bacteria, fungi, and actinomycetes) was improved due to the application of fertilizer and FYM continuously for twenty-nine years (Table1and Fig 4). The lowest total microbial population was recorded in control which was statistically at par with T_{14}, T_4, T_2, T_{11}, T_{12} and T_{13} in the surface layer. However, the highest total microbial culturable population (9.11 log_{10} CFU g^{-1} soil) was recorded under N_{180}+P_{80}+K_{40}+Zn (F)+FYM (T_{10}) which was statistically at par with T_9, and T_8. Among the inorganic treatments, T_7 supported highest total microbial population which was statistically at par with all the treatments except T_1. In sub-surface soil the highest total microbial population (8.98 log_{10} CFU g^{-1} soil) was observed under T_{10} which was at par with T_9, T_8, T_7, T_6 and T_4 while the lowest microbial population was observed under T_1 which was at par with T_{14}, T_2, T_{11}, T_{12}, T_3 and T_{13}.

Functional groups of microbes

Azotobacter

In wheat crop the fertilizer treatments alone or in combination with FYM had significant impact on *Azotobacter* population after harvest of wheat (Table 2 and Fig 5). *Azotobacter* population was significantly improved by the continuous use of fertilizer and FYM together over control. The *Azotobacter* population in soil ranged from 4.68 to 4.80 log_{10} CFU g^{-1} soil in surface layer, while in sub-surface soil it varied from 4.41 to 4.65 log_{10} CFU g^{-1} soil after harvest of wheat crop. The lowest population of *Azotobacter* was recorded in control in both the surface and sub-surface layers. However, the highest *Azotobacter* population (4.80 log_{10} CFU g^{-1} soil) was recorded under $N_{180}+P_{80}+K_{40}$+Zn (F)+FYM (T_{10}) which was statistically at par with all the treatments except T_2, T_{13}, T_{14} and T_1. Among the inorganic treatments, T_{12} supported the higher *Azotobacter* population which was statistically at par with all the treatments receiving nutrients through inorganic source except T_{14}. Similarly, in sub-surface soil highest *Azotobacter* population (4.65 log_{10} CFU g^{-1} soil) was observed under T_{10} which was at par with T_9, while T_{12} which was inorganic treatment favored highest *Azotobacter* population compared to other treatments receiving only chemical fertilizer.

Phosphorous solubilizing bacteria

An application of fertilizers alone at varying levels and with FYM for twenty-nine years resulted in significantly higher population of PSB in soil after harvest of wheat crop over the control (Table 2 and Fig 6). The population of PSB in soil ranged from 2.17 to 2.59 log_{10} CFU g^{-1} soil in surface layer while in sub-surface layer, it varied from 2.09 to 2.44 log_{10} CFU g^{-1} soil. The lowest population of PSB was recorded in the absence of extraneous nutrients i.e. control in both the surface and sub-surface layers of soil. However, highest population of PSB (2.59 log_{10} CFU g^{-1} soil) was recorded under $N_{180}+P_{80}+K_{40}$+Zn (F)+FYM (T_{10}) which was statistically at par with T_9 and T_8. Among the inorganic treatments, T_{12} supported the higher number of PSB which was statistically at par with all the treatments except T_{14} and T_1. Similarly, in sub-surface layer highest number of PSB (2.44 log_{10} CFU g^{-1} soil) was observed under T_{10} which was at par with T_9, T_8, T_7, T_6 and T_{12}.

It can be observed that over 29 years of rice and wheat under rice- wheat cropping system, the combined use of inorganic fertilizer along with FYM supported maximum bacterial, fungal and actinomycetes population while lowest in control. This may be due to the treatments receiving organic manure along with inorganic chemical fertilizers provides higher production efficiency and system productivity along with addition of organic matter to soil which acts as source of food (carbon and energy) for microbes. Organic manure are not a substitute, but a supplement of chemical fertilizers result in build-up of organic carbon, available N, P and K; and sustain soil pH and EC as compared with chemical fertilizer alone developing the congenial environment for proliferation of microbes.

The population of culturable bacteria, fungi and actinomycetes were increased in fertilization treatments because organic manure and mineral fertilizer increases crop growth, metabolic activity resulting in more root biomass and enhanced root exudates which provide food and energy for microorganisms. In addition, organic manure could provide abundant source of substrate for the growth of bacteria, fungi and actinomycetes. Therefore, the microbial population in organic manure treatments were higher than those in inorganic fertilizer alone. Similar results of improvement of microbial population with addition of FYM have been reported by Naidu and Pillai (2001) [11]. Chouskey *et al*. (2003) [4] observed that combined use of NPK+FYM supported highest population of bacteria in the soil. Krishnamoorty and Ravikumar (1973) [8] studied the bacterial population in a permanent manurial experiment at Coimbatore. They reported that the control plots had the lowest bacterial population, while the plots treated with phosphate and cattle manure recorded the highest bacterial population. The nutrient supplied through organic source along with inorganic chemical fertilizer had higher microbial population of bacteria, fungi, actinomycetes and Azotobacter than the control and fertilizer treatments (Upadhyay *et al*. 2011) [17]. The increased bacterial population due to application of organic manures was attributed to the availability of adequate biomass as feed for the microbes (Singh *et al*. 2012) [15]. Jha *et al.* (2006) [7] reported that the addition of FYM and chemical fertilizer increased the population of bacteria, fungi, actinonomycetes and *Azotobacte*r as compared to control. This may be due to the organic manure such as FYM or vermicompost increasing the mineral nutrients, growth harmones, vitamins and improving other physical character in soil might have significant influence on microbial population. The lowest population of these microorganism in soil applied with chemical fertilizer may be due to the absence of organic fertilizer in the soil and no similar effect to increase in the microbial population.

The number of PSB was high in treatment $N_{180}+P_{80}+K_{40}$+Zn (F)+FYM (T_{10}). The similar explanations as above holds good for enhanced population of PSB due to application of inorganic fertilizer alone or with FYM. Similar results have also been reported by Bhadoria *et al.* (2011) [3] who showed that populations of PSB, soil enzyme activities, and phosphorus solubilizing power in the FYM + chemical fertilizer treated plots significantly increased compared to sole chemical fertilizer treatments under both lime and no lime application. Sharma *et al.* (2010) [14] observed long-term effect of organic residue and inorganic fertilizers on major soil microbes and on certain soil chemical properties in the rice-wheat cropping system. They also recorded a significant increase in the bacterial, fungal, actinomycetes and phosphorus solubilizing microorganism counts compared to control. This was ascribed to the continuous mineralization of nutrient from the organic and inorganic pools and in maintaining supply of food as well as energy supplies for microbial growth. Vineela *et al*. (2008) [18] also indicated that microbial populations are generally more under integrated use of organic and inorganic fertilizer.

Table 1: Effect of long-term fertilizer application at varying levels on the population of soil bacteria, fungi, actinomycetes and total microbes after twenty-ninth cycle of wheat crop at different depths under rice-wheat cropping system

Treatments		Bacteria ($\times10^8$ CFU g^{-1} soil)		Fungi ($\times10^5$ CFU g^{-1} soil)		Actinomycetes ($\times10^7$ CFU g^{-1} soil)		Total microbial population ($\times10^8$ CFU g^{-1} soil)	
		0-15 cm	15-30 cm	0-15 cm	15- 30 cm	0-15 cm	15-30 cm	0-15 cm	15-30 cm
T_1	Control	1.39 (8.14)*	1.19 (8.07)	3.01 (5.47)	2.49 (5.39)	1.43(7.16)	1.27 (7.10)	1.54 (8.86)	1.34 (8.80)
T_2	N_{120}	1.61 (8.20)	1.25 (8.09)	3.40 (5.53)	2.82 (5.43)	1.56 (7.19)	1.38 (7.16)	1.77 (8.93)	1.40 (8.82)
T_3	$N_{120}+P_{40}$	1.75 (8.24)	1.32 (8.11)	3.54 (5.54)	2.96 (5.48)	1.74 (7.24)	1.64 (7.21)	1.93 (8.97)	1.49 (8.85)
T_4	$P_{40}+K_{40}$	1.59 (8.19)	1.23 (8.08)	3.55 (5.55)	3.00 (5.49)	1.69 (7.23)	1.54 (7.20)	1.77 (8.92)	1.65 (8.89)
T_5	$N_{120}+K_{40}$	1.79 (8.24)	1.46 (8.12)	3.70 (5.56)	3.12 (5.49)	1.60 (7.20)	1.55 (7.19)	1.96 (8.97)	1.62(8.88)

T_6	$N_{120}+P_{40}+K_{40}$	1.80 (8.25)	1.51 (8.18)	3.76 (5.57)	3.22 (5.50)	1.70 (7.23)	1.56 (7.20)	1.98 (8.98)	1.67 (8.90)
T_7	$N_{120}+P_{40}+K_{40}$+Zn(F)	1.83 (8.27)	1.59 (8.20)	3.80 (5.58)	3.25 (5.51)	1.694(7.23)	1.58 (7.20)	2.01 (8.98)	1.75 (8.93)
T_8	$N_{120}+P_{40}+K_{40}$+FYM	1.92 (8.28)	1.67 (8.22)	4.23 (5.62)	3.41 (5.54)	1.75 (7.24)	1.66(7.22)	2.10 (9.01)	1.83 (8.95)
T_9	$N_{120}+P_{40}+K_{40}$+Zn(F)+FYM	2.19 (8.34)	1.65 (8.21)	4.40 (5.64)	3.31 (5.52)	2.25 (7.35)	1.81 (7.26)	2.43 (9.08)	1.84 (8.95)
T_{10}	$N_{180}+P_{80}+K_{40}$+Zn(F)+FYM	2.47 (8.37)	1.82 (8.25)	4.69 (5.67)	3.63 (5.55)	2.30(7.36)	1.90 (7.27)	2.70 (9.11)	2.01 (8.98)
T_{11}	$N_{150}+P_{40}+K_{40}$	1.82 (8.26)	1.52 (8.19)	3.91 (5.59)	3.38 (5.53)	1.70(7.23)	1.55 (7.21)	1.78 (8.93)	1.47 (8.84)
T_{12}	$N_{180}+P_{80}+K_{40}$+Zn(F)	1.80 (8.25)	1.51 (8.16)	3.36 (5.51)	2.83 (5.44)	1.52 (7.18)	1.40 (7.17)	1.78 (8.93)	1.47 (8.85)
T_{13}	$N_{180}+P_{80}$+Zn(F)	1.64 (8.21)	1.37 (8.11)	3.08 (5.49)	2.58 (5.41)	1.70 (7.23)	1.58 (7.14)	1.81 (8.94)	1.51 (8.85)
T_{14}	$N_{120}+P_{40}+K_{40}$ (DAP)	1.49 (8.17)	1.25 (8.08)	3.02 (5.48)	2.53 (5.40)	1.57 (7.19)	1.45 (7.18)	1.65 (8.89)	1.38 (8.81)
S.Em±		0.04	0.03	0.03	0.04	0.02	0.02	0.04	0.03
C.D. (5%)		0.12	0.08	0.10	NS	0.06	0.07	0.10	0.09
C.V. (%)		1.03	0.70	1.20	1.38	0.54	0.63	0.79	0.71

*Values in parentheses are log_{10} transformation

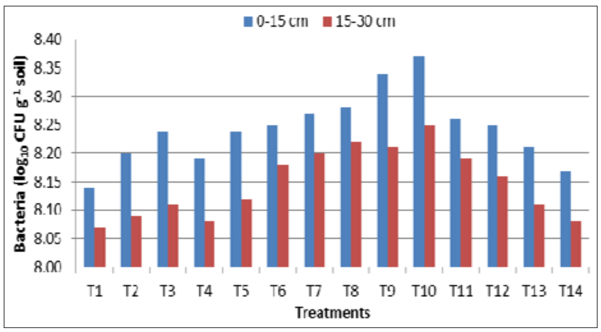

Fig 1: Effect of long-term fertilizer application at varying levels on the population of soil bacteria after twenty-ninth cycle of wheat crop at different depths under rice-wheat cropping system

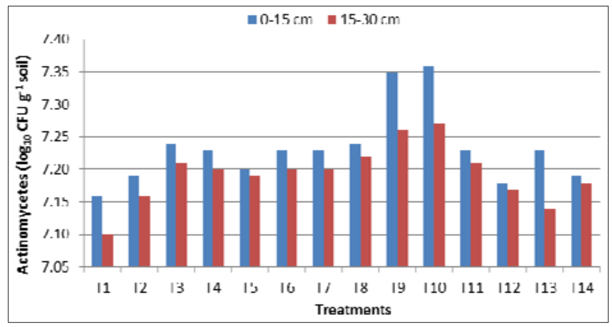

Fig 3: Effect of long-term fertilizer application at varying levels on the population of soil actinomycetes after twenty-ninth cycle of wheat crop at different depths under rice-wheat cropping system

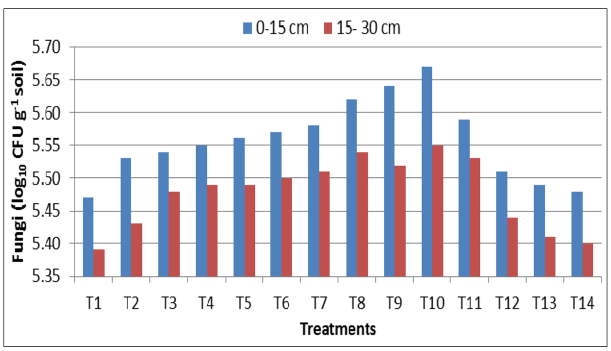

Fig 2: Effect of long-term fertilizer application at varying levels on the population of soil fungi after twenty-ninth cycle of wheat crop at different depths under rice-wheat cropping system

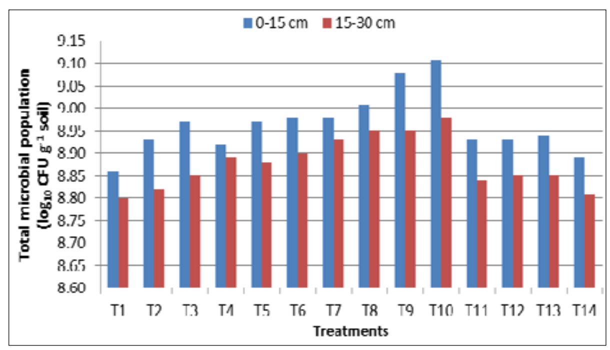

Fig 4: Effect of long-term fertilizer application at varying levels on the population of total microbes in soil after twenty-ninth cycle of wheat crop at different depths under rice-wheat cropping system.

Table 2: Effect of long-term fertilizer application at varying levels on the number of Azotobacter and phosphorous solubilizing bacteria (PSB) after twenty-ninth cycle of wheat crop at different depths under rice-wheat cropping system

Treatments		***Azotobacter* ($\times 10^4$ CFU g^{-1} soil)**		**PSB ($\times 10^2$ CFU g^{-1} soil)**	
		0-15 cm	**15-30 cm**	**0-15 cm**	**15-30 cm**
T_1	Control	4.83 (4.68)*	2.58 (4.41)	1.49 (2.17)	1.23 (2.09)
T_2	N_{120}	5.20 (4.72)	2.95 (4.46)	2.45 (2.39)	1.45 (2.14)
T_3	$N_{120}+P_{40}$	5.48 (4.73)	3.18 (4.50)	2.48 (2.40)	1.56 (2.21)
T_4	$P_{40}+K_{40}$	5.61 (4.75)	3.24 (4.51)	2.47 (2.39)	1.50 (2.18)
T_5	$N_{120}+K_{40}$	5.61 (4.75)	3.24 (4.51)	2.32 (2.33)	1.49 (2.15)
T_6	$N_{120}+P_{40}+K_{40}$	5.88 (4.76)	3.45 (4.52)	2.61 (2.41)	1.63 (2.34)
T_7	$N_{120}+P_{40}+K_{40}$+Zn(F)	5.89 (4.76)	3.56 (4.55)	2.74 (2.43)	2.49 (2.36)
T_8	$N_{120}+P_{40}+K_{40}$+FYM	5.98 (4.78)	3.62 (4.56)	2.82 (2.48)	2.54 (2.37)
T_9	$N_{120}+P_{40}+K_{40}$+Zn(F)+FYM	6.09 (4.79)	4.18 (4.61)	2.85 (2.58)	2.57 (2.41)
T_{10}	$N_{180}+P_{80}+K_{40}$+Zn(F)+FYM	6.23 (4.80)	4.45 (4.65)	3.53 (2.59)	2.73 (2.44)
T_{11}	$N_{150}+P_{40}+K_{40}$	5.89 (4.76)	3.48 (4.53)	2.67 (2.42)	1.55 (2.20)
T_{12}	$N_{180}+P_{80}+K_{40}$+Zn(F)	5.89 (4.77)	3.58 (4.55)	2.79 (2.44)	1.62 (2.34)
T_{13}	$N_{180}+P_{80}$+Zn(F)	5.13 (4.70)	3.06 (4.48)	2.12 (2.33)	1.29 (2.10)
T_{14}	$N_{120}+P_{40}+K_{40}$ (DAP)	4.90 (4.69)	2.86 (4.46)	1.60 (2.20)	1.31 (2.14)
S.Em±		0.03	0.03	0.05	0.05
C.D. (5%)		0.07	0.08	0.14	0.15
C.V. (%)		1.09	1.27	4.06	4.72

*Values in parentheses are log_{10} transformation

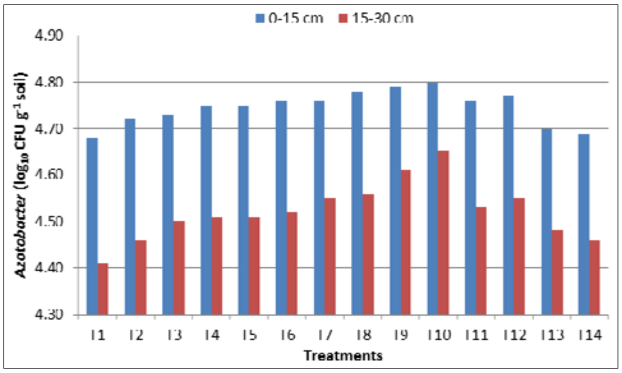

Fig 5: Effect of long-term fertilizer application at varying levels on the number of *Azotobacter* after twenty-ninth cycle of wheat crop at different depths under rice-wheat cropping system

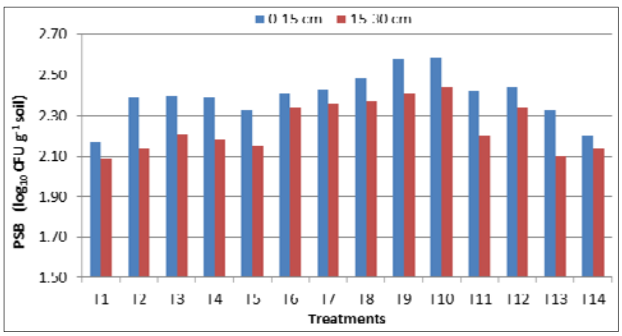

Fig 6: Effect of long-term fertilizer application at varying levels on the number of phosphorous solubilizing bacteria (PSB) after twenty-ninth cycle of wheat crop at different depths under rice-wheat cropping system

Conclusion

The biological properties such as culture-able microbial population (bacteria, fungi, actinomycetes) and functional group of microbe (*Azotobacter* and phosphorous solubilizing bacteria) of soil were improved after wheat in rice-wheat crop rotation due to use of inorganic fertilizer along with FYM over a period of twenty-nine years. Hence, the balance and imbalanced use of nutrients through chemical fertilizers and organic manures should be followed for the improvement of biological soil quality for sustainability.

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