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# Dynamics of microbial communities associated in the rhizosphere profile of aerobic rice

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#### Abstract

An experiment was conducted to assess the microbial communities present in the rhizosphere soil of aerobic rice at different depths under glass house condition. The 3 feet length 4 inches diameter PVC pipes filled with soil were placed in battery boxes and seeds of aerobic rice varieties BI-33 (renamed as ARB-6) and AM-72 were sown and watered as and when required. After germination, three plants were maintained in each pipe with three replications. Soil samples were collected on 40<sup>th</sup> and 80<sup>th</sup> day of sowing by cutting the rhizosphere soil profile at 5 cm, 10 cm, 20 cm, 40 cm, 60 cm, 80 cm and 100 cm depth. Soil was thoroughly mixed and random soil sample was collected at each mentioned depth. The microbial population of rhizosphere present at different depths was enumerated by serial dilution plate method using suitable agar media. The highest population of bacteria including free living nitrogen fixers, phosphate solubilizers and fungi were present at 5 to 10 cm depths and the populations of the communities decreased as the depth of soil increased. Contrary to this, the population of actinomycetes increased as the depth of soil increased. This concludes that microbial population varies significantly with different soil depths and further, several factors could greatly influence the microbial communities that improve crop production.

Keywords: Microbial communities, soil depth, rhizosphere

#### Introduction

Microorganisms are ubiquitous to nature and soil is a reservoir of almost all kinds of microorganisms. The numbers and kinds of microorganisms present in soil are governed by soil type, moisture content, soil pH, soil temperature, aeration and plant root system. Microbiological decomposition of organic matter is an essential step to release the bound nutrients in organic residues in to an available form and the inorganic minerals come from rocks through mineral decomposition. In addition, microorganisms play an important role in N<sub>2</sub>- fixation, phosphate solubilisation, plant growth promotion, mineralization and control of root pathogens. Fungi, bacteria, actinomycetes, algae, protozoa and viruses are the different groups of microorganisms present in soil. The rhizosphere refers to the region of soil subjected to influence of plant roots. It is characterized by immense microbiological activity than the soil away from plant roots (non-rhizosphere). The rhizosphere is a densely populated area in which roots must compete with the invading root systems of neighbouring plant species for space, water and mineral nutrients. The intensity of such activity depends upon migration of the root exudates. The overall influence of plant root exudates on soil microorganisms is termed as rhizosphere effect.

Microorganisms present in rhizosphere soil play an important role in ecological fitness of their plant host. The important microbial process that are expected to occur in rhizosphere include pathogenesis and its counterpart plant protection, growth promotion as well as production of antibiotics, geochemical cycling of minerals and the plant colonization (Kent and Triplett, 2002) <sup>[5]</sup>. Plant microbe interactions may thus be considered beneficial, neutral or harmful to the plant depending on specific microorganism and plant involved on prevailing environmental conditions (Bais *et al.*, 2006) <sup>[1]</sup>. Exploring these microorganisms by unrevealing their possible relationships with plants has launched a new and fascinating area of investigations in the rhizosphere research. In the light of the above, the present investigation was carried out to understand microbial communities inhabited in the rice rhizosphere at different depths.

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### Materials and Methods

An experiment was conducted to enumerate the microbial communities present in the rhizosphere soil of aerobic rice at different depths under glass house conditions in the Department of Agricultural Microbiology, UAS, GKVK, Bengaluru - 560065. The 3 feet length 4 inches diameter PVC pipes filled with soil were placed in battery boxes and seeds of aerobic rice varieties BI-33 (renamed as ARB-6) and AM-72 were sown and watered as and when required. After germination, three plants were maintained in each pipe with three replications. Soil samples were collected on 40<sup>th</sup> and 80<sup>th</sup> day of sowing by cutting the rhizosphere soil profile at 5 cm, 10 cm, 20 cm, 40 cm, 60 cm, 80 cm and 100 cm depth. Soil was thoroughly mixed and random soil sample was collected at each depth. The rhizosphere microflora viz., bacteria, fungi, actinomycetes, free living nitrogen fixer and phosphate solubilizers present at different depths (5 cm, 10 cm, 20 cm, 40 cm, 60 cm, 80 cm and 100 cm) were enumerated by serial dilution plate method using nutrient agar, Martin's Rose Bengal agar, Kuster's agar, Waksman No.77 agar and Pikovaskaya's agar respectively using appropriate dilutions.

### Statistical analysis

The data obtained was subjected for statistical analysis by one way analysis of variance using WASP: 1.0 (Web Agri Stat Package) statistical tool. (www.icargoa.res.in/wasp/index.php).

### Results

The data obtained for microbial populations at different depths of aerobic rice variety BI-33 is presented in Table 1 and Fig. 1. The total microbial population was significantly differed with increasing depths at 40 days after sowing (DAS). Maximum population of bacteria and fungi was recorded at 5 cm and 10 cm soil depths. The population of bacteria and fungi was found to decrease as the depth of soil increased and the least population of bacteria and fungi was observed at 100 cm depth. On contrary, actinomycetes population was increased as the depth increased. Maximum population of actinomycetes was recorded at 100 cm depth which linearly increased as the depth increased. Similarly, the highest population of nitrogen fixers and PSB was observed at 5 cm and 10 cm depth which were closely followed by 20 cm and 40 cm depths. The population of both the groups decreased as the depth of soil increased and the least population of both was observed at 100 cm depth.

The samples analysed at 80 DAS recorded significantly increased population of bacteria, fungi, actinomycetes, nitrogen fixers and P solubilizers as compared to the population at 40 DAS. The highest population of bacteria, fungi, nitrogen fixers and P solubilizers was observed in the surface soil (5 cm depth and 10 cm depth) compared to deeper soil profile. The population of bacteria, fungi, nitrogen fixer and phosphate solubilizers decreased as the depth increased and the least population was observed at 100 cm depth. Whereas, in case of actinomycetes the least population was observed at 5 cm depth (27 x  $10^3$ Cfu/g soil) when compared to 100 cm depth (41 x  $10^3$ cfu/g soil). In general, the actinomycetes population increased as the soil depth increased.

The data on the microbial populations at different depths for aerobic rice variety AM-72 is presented in Table 2 and Fig. 2. The bacterial population at 40 DAS increased at the top 5 and 10 cm and decreased as the depth increased. However, highest bacterial population was observed at 5 cm ( $31.67 \times 10^5$ cfu/g soil) and 10 cm depths ( $28.0 \times 10^5$ cfu/g soil) followed by subsequent depths. Significantly, least bacterial population was observed at 100 cm depth ( $15.34 \times 10^5$ cfu/g soil). In case of fungi, high population was noticed at 5cm ( $15.66 \times 10^3$  cfu/g soil) and 10cm ( $14.0 \times 10^3$ cfu/g soil). The lowest fungal population was observed at 100cm ( $5.0\times 10^3$ cfu/g soil) depth which was on par with 80, 60 and 40 cm depths. The actinomycetes population increased as the depth increased and the least number of actinomycetes were observed at 5 cm ( $12.0 \times 10^3$ cfu/g soil) and 10 cm ( $12.33 \times 10^3$ cfu/g soil) depths, which were on par with each other and significantly less compare to 100 cm depth ( $28.0 \times 10^3$ cfu/g soil).

Correspondingly, the population of nitrogen fixers was highest at 10 cm (0.52 x  $10^{3}$ cfu/g soil) and 5 cm (0.47 x  $10^{3}$ cfu/g soil) depths which are statistically on par with each other followed by 20 cm (0.33 x  $10^{3}$ cfu/g soil) and 40 cm (0.36 x  $10^{3}$ cfu/g soil) depths. The least number of nitrogen fixers was recorded at 80 cm and 100 cm depths. Similarly, the population of PSB increased significantly at both 5 cm and 10 cm depths. As the depth increased, the population of phosphate solubilising bacteria decreased and was found to be least at 100 cm depth.

In general, at 80 DAS there was an increase in the population of bacteria, fungi, actinomycetes, nitrogen fixers and PSB compared to 40 DAS. Significantly highest bacterial population was observed at 5 cm depth (119.00 x 10<sup>5</sup>cfu/g) followed by 10 cm depth. The population of bacteria decreased as the depth increased and the least was found at 100 cm depth. The fungal population also decreased as the depth of soil increased and the highest population of fungi was observed at 5 cm depth which was closely followed by 10 cm depth. At 80th day the actinomycetes population increased as the depth increased. The highest population of actinomycetes was recorded at100 cm depth (40.0 x 10<sup>3</sup>cfu/g soil) and the least was at 5 cm (28.0x 10<sup>3</sup>cfu/g soil) depth. The population of nitrogen fixer and phosphate solubilizers also decreased with increased soil depths. Significantly highest population of nitrogen fixers and phosphate solubilizers were recorded in the top soil at 5 cm and 10 cm depths and the least was recorded at 100cm depth (11.0 x  $10^{3}$ cfu/g soil).

### Discussion

Two varieties of aerobic rice namely, BI-33 and AM-72 were raised in 1 meter long PVC pipes (Plate 1 and 2) for 80 days. The microbial communities such as bacteria, fungi, actinomycetes, free living nitrogen fixers and phosphate solubilizers associated at different depths (5, 10, 20, 40, 60, 80, 100 cm) were estimated at 40 and 80 days after sowing (DAS) using appropriate agar media.

At 40 days after sowing the population of bacteria, fungi, free-living nitrogen fixers and phosphate solubilizers significantly increased at 5 cm depth followed by 10 cm depth. The bacterial population was higher at all depths compared to fungi, nitrogen fixers and phosphate solubilizers. This could be due to the fact that the surface soils are rich in organic substrates which could serve as energy source for microbial communities. Further, these organic substrates upon mineralization may have released N, P, K and other nutrients in inorganic form required for the growth and multiplication of microorganisms. Roper and Halsall (1986) <sup>[10]</sup> also reported increased microorganisms in soil incorporated with straw an organic material that could serve as a carbon source. Further, the factors like aeration, availability of carbon source and the

root exudates could have been responsible for increased populations in the surface soil. The higher organic carbon matter content, sufficient moisture, low pH and narrow C/N ratio must have favoured the bacterial population (Ram et al., 2013) [7]. Jackson and Illamurugu (2014) [4] reported that microbial populations occurred at various growth stages of rice and that higher population of bacteria followed by fungal and actinomycetes were found at the flowering stage. Even in the present study the bacterial population was higher at 80 days which closely correspond to flowering stage. Therefore the results of the present study are in confirmation with the above studies. The population of bacteria and fungi decreased with the depth of soil. It is quite obvious that the carbon and available nutrients decrease with increase in depth of the soil. This could be the reason for the decrease in the populations of different microbial communities in this study.

In the present study, the population of actinomycetes was found to be significantly high as the soil depth increased. Highest population of actinomycetes was recorded at 100 cm depth. This is in contrast with bacteria and fungi whose populations have decreased as the soil depth increased. Griffiths et al. (1999)<sup>[3]</sup> reported that the variation in carbon content of soil plays a key role in the abundance of soil microorganisms, as addition of carbon source could raise the population of fungi and Gram negative bacteria and reduce actinomycetes population due to completion for nutrients as bacteria and fungi can multiply and grow much faster than the actinomycetes (Griffiths et al., 1999)<sup>[3]</sup>. Margaret et al. (2003) <sup>[6]</sup> also reported that at deeper layers of soil, the available carbon decreases and hence more number of actinomycetes prevails. This is quite true because, it is well known that even in compost piles, the population of actinomycetes increase during maturity stage, when all the simple carbohydrates are exhausted due to the activity of bacteria and fungi in the initial stages of composting. Probably the same analogy holds well in this study where the available nutrients decrease at deeper layers of soil. Further, since actinomycetes are slow growers, they may not compete with bacteria and fungi at top soil to build-up their population. They form spores and sustain their population in deeper soils. Fierer *et al.* (2003) <sup>[2]</sup> also made similar observations of greater abundance of actinomycetes and gram positive bacteria at deeper soil. These subsurface microbes like actinomycetes may have a critical influence on longer term soil carbon sequestration given that sub-surface horizons can harbor substantial quantities of organic carbon with long turnover times.

The microbial populations almost doubled at 80 days after sowing when the crop was at panicle initiation stage. This suggests that the stage of the crop growth also influences the microbial communities in the rhizosphere for active colonization (Reichardt et al., 1997)<sup>[8]</sup>. Jackson and Illamurugu (2014)<sup>[4]</sup> also reported that the microbial populations occurred at various growth stages of rice and that higher population of bacteria followed by fungal and actinomycetes were found at the flowering stage. However, the population of bacteria, fungi, nitrogen fixers and PO<sub>4</sub>solubilizers decreased as the depth of the soil increased as in case of 40 days after sowing. The microbial communities in the rhizosphere of the variety AM-72 also showed similar trend as in case of the variety BI-33. However, the population of bacteria in this variety was significantly increased at both the intervals. But, this is not in agreement with the observations made by Roesti et al. (2006)<sup>[9]</sup> for wheat. In wheat, the population of bacteria in the rhizosphere soil was higher at booting stage but decreased at harvesting stage in the field experiment. This suggests that the crop may probably play a significant role in determining the populations of different communities of microorganisms at different stages of growth.

Soil depth (cm)		Microl	bial population at	40 DAS		Microbial population at 80 DAS					
	Bacteria	Fungi	Actinomycetes	N <sub>2</sub> - fixer	PSB	Bacteria	Fungi	Actinomycetes	N <sub>2</sub> - fixer	PSB	
		(	(x10 <sup>3</sup> CFU/g of soi	l)		Bacteria (x10 <sup>5</sup> CFU/g)	(x10 <sup>3</sup> CFU/g of soil)				
5	133.67 <sup>a</sup>	10.67 <sup>a</sup>	11.00 <sup>f</sup>	0.29 <sup>a</sup>	33.00 <sup>a</sup>	150.00 <sup>a</sup>	40.00 <sup>a</sup>	27.00 <sup>f</sup>	2.80 <sup>a</sup>	33.67 <sup>a</sup>	
10	70.00 <sup>b</sup>	9.00 <sup>a</sup>	12.00 <sup>f</sup>	0.28 <sup>a</sup>	33.34 <sup>a</sup>	100.00 <sup>b</sup>	37.00 <sup>a</sup>	30.00 <sup>e</sup>	2.44 <sup>b</sup>	35.00 <sup>a</sup>	
20	59.67°	5.00 <sup>b</sup>	14.33 <sup>e</sup>	0.29 <sup>a</sup>	20.00 <sup>b</sup>	91.67 <sup>b</sup>	33.00 <sup>b</sup>	32.00 <sup>d</sup>	1.70 <sup>c</sup>	26.00 <sup>b</sup>	
40	32.67 <sup>d</sup>	5.70 <sup>b</sup>	16.33 <sup>d</sup>	0.26 <sup>ab</sup>	10.00 <sup>cd</sup>	35.67°	30.00 <sup>b</sup>	35.33°	0.58 <sup>d</sup>	23.67 <sup>b</sup>	
60	28.34 <sup>de</sup>	5.00 <sup>b</sup>	20.00 <sup>c</sup>	0.19 <sup>bc</sup>	11.67 <sup>c</sup>	38.67°	24.00 <sup>c</sup>	37.33 <sup>b</sup>	0.34 <sup>e</sup>	19.00 <sup>c</sup>	
80	24.34 <sup>e</sup>	6.00 <sup>b</sup>	22.33 <sup>b</sup>	0.14 <sup>c</sup>	7.00 <sup>cd</sup>	39.00 <sup>c</sup>	17.00 <sup>d</sup>	38.00 <sup>b</sup>	0.35 <sup>e</sup>	13.34 <sup>d</sup>	
100	12.67 <sup>f</sup>	4.34 <sup>c</sup>	24.66 <sup>a</sup>	0.13 <sup>c</sup>	2.67 <sup>d</sup>	27.00 <sup>c</sup>	12.67 <sup>e</sup>	41.00 <sup>a</sup>	0.36 <sup>e</sup>	9.67 <sup>e</sup>	
S. Em	2.69	0.69	0.54	0.02	2.65	4.58	1.18	0.48	0.06	1.00	
LSD@ 1%	11.36	2.90	2.31	0.10	11.17	19.29	4.98	2.05	0.29	4.25	

Table 1: Microbial populations at different depths in the rhizosphere soil of aerobic rice (Variety BI-33)

**Note:** Means followed by the same letters in the column do not differ significantly at p=<0.01. **DAS:** Days after sowing; CFU: Colony forming units; PSB: Phosphate solubilising bacteria.

 Table 2: Microbial populations at different depths of aerobic rice rhizosphere soil (Variety AM-72)

Soil depth (cm)		l populations at 40	DAS	Microbial populations at 80DAS						
	Bacteria	Fungi	Actinomycetes	N <sub>2</sub> - fixers	PSB	Bacteria	Fungi	Actinomycetes	N <sub>2</sub> -fixers	PSB
	(x10 <sup>5</sup> CFU/g)	(x10 <sup>3</sup> CFU/g of soil)				(x10 <sup>5</sup> CFU/g)	FU/g) (x10 <sup>3</sup> CFU/g of soil)			
5	31.67 <sup>a</sup>	15.66 <sup>a</sup>	12.00 <sup>f</sup>	0.47 <sup>a</sup>	27.67 <sup>a</sup>	119.00 <sup>a</sup>	28.34 <sup>a</sup>	28.00 <sup>e</sup>	2.24 <sup>a</sup>	33.66 <sup>a</sup>
10	28.00 <sup>a</sup>	14.00 <sup>a</sup>	12.33 <sup>f</sup>	0.52 <sup>a</sup>	28.00 <sup>a</sup>	89.67 <sup>b</sup>	23.00 <sup>b</sup>	30.33 <sup>d</sup>	1.95 <sup>b</sup>	33.67 <sup>a</sup>
20	25.00 <sup>b</sup>	8.00 <sup>b</sup>	15.00 <sup>e</sup>	0.33 <sup>b</sup>	22.34 <sup>b</sup>	39.34°	18.34 <sup>c</sup>	31.66 <sup>d</sup>	1.83 <sup>b</sup>	27.00 <sup>b</sup>
40	25.00 <sup>b</sup>	6.33 <sup>bc</sup>	18.00 <sup>d</sup>	0.36 <sup>b</sup>	18.00 <sup>c</sup>	29.34 <sup>cd</sup>	17.00 <sup>c</sup>	33.33°	0.58°	23.34 <sup>c</sup>
60	21.33°	5.33°	22.00 <sup>c</sup>	0.25 <sup>c</sup>	13.67 <sup>d</sup>	25.00 <sup>d</sup>	13.00 <sup>d</sup>	35.00 <sup>b</sup>	0.55°	20.34 <sup>c</sup>
80	20.00 <sup>c</sup>	6.67 <sup>bc</sup>	25.33 <sup>b</sup>	0.17 <sup>cd</sup>	10.67 <sup>e</sup>	26.34 <sup>d</sup>	6.34 <sup>e</sup>	36.00 <sup>b</sup>	0.54 <sup>c</sup>	15.34 <sup>d</sup>
100	15.34 <sup>d</sup>	5.00 <sup>c</sup>	28.00 <sup>a</sup>	0.21d	7.00 <sup>f</sup>	12.34 <sup>e</sup>	5.34 <sup>e</sup>	40.00 <sup>a</sup>	0.35 <sup>d</sup>	11.00 <sup>e</sup>
S. Em	1.13	0.85	0.70	0.02	0.85	4.04	0.59	0.53	0.05	1.03
LSD@ 1%	4.78	3.59	2.95	0.09	3.59	17.03	2.49	2.25	0.25	4.38

Note: Means followed by the same letters in the column do not differ significantly at p = < 0.01.

DAS: Days after sowing; CFU: Colony forming units; PSB: Phosphate solubilising bacteria.

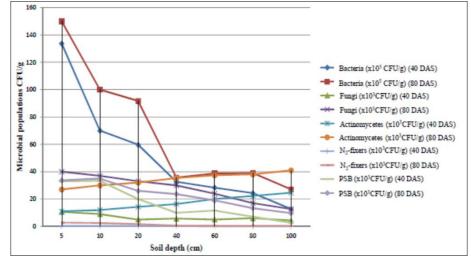


Fig 1: Microbial populations at different in the rhizosphere soil of aerobic rice (variety BI-33)

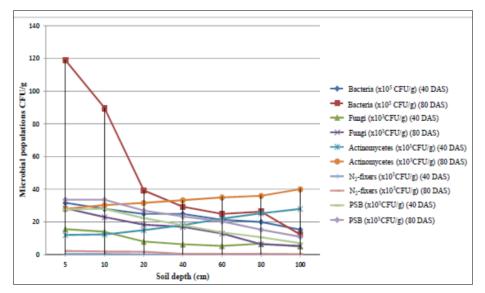


Fig 2: Microbial populations at different depths in the rhizosphere soil of aerobic rice (variety AM-72)

### Conclusion

Microbial communities present at different depths of rhizosphere soil were enumerated and found that the bacteria, fungi and actinomycetes recorded at different depths with varied microbial population. The various factors like soil type, moisture content, soil pH, soil temperature, aeration and plant root system influence microbial communities. The highest population of bacteria including the free living nitrogen fixers and phosphate solubilizers and fungi was present at 5 to 10 cm depths and the populations of the communities decreased as the depth of soil increased. Contrary to this, the population of actinomycetes increased as the depth of soil increased.

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