Diversity of Nitrogen Fixing and Phosphate Solubilising Bacteria in Rhizosphere and Non Rhizosphere Soils of Chickpea and Sorghum

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Abstract

Nitrogen fixing and phosphate solubilising bacteria developed elsewhere have not been very consistent in their performance everywhere, owing to their poor ability to the changing soil and agro climatic conditions. There is a wide gap in understanding the relationship between various soil properties and these potential bacteria or their activities especially in vertisols. Thus, in the present investigation an attempt was made to study the diversity of efficient nitrogen fixing and phosphate solubilising bacterial population in the rhizosphere and non rhizosphere, chickpea and sorghum, black and red soils. Rhizobium population was recorded to be more in the rhizosphere of chickpea (black soil) and azotobacter population was recorded to be more in the rhizopshere of sorghum (red soil). However, black soils have revealed relatively more number of rhizobia and azotobacter, when compared to the red soils. Phosphate solubilising bacteria were reported to be more in red soils, when compared to black soils, irrespective of rhizosphere and non rhizosphere of both chickpea and sorghum.

KEYWORDS: Diversity, Nitrogen fixing, Phosphate solubilizing, Rhizosphere

1. INTRODUCTION

Soil productivity is one of the major environmental concerns for the worlds poor. Several National and international forums reemphasized the need to establish sustainable livelihoods for the poorer who are worst affected by soil fertility and crop productivity [1]. This is a critical issue for both human care and environmental well being. In view of the threats and demands currently placed upon our soil eco-systems, there is an increasing interest in the factors governing soil fertility and microbial diversity. Many of the functions of the soil including the productivity and health of agricultural processes are being carried out by the unique microbial communities in soils [2]. Biodiversity, as this assemblage of life forms is referred to have now been acknowledged as the foundation for sustainable livelihood and food security. Researchers are also aware of the immense potentials of the various life forms especially, in the context of recent advances made in science and technology. Biological diversity refers to the variety and variability among living organisms and the ecological complexes in which they occur. Indirect benefits of biological diversity include nutrient trapping, maintaining water cycles, soil protection, absorption and breakdown of pollutants etc. Paradoxically, despite the acknowledged commercial value of microorganisms our knowledge of their diversity and many of their key roles in sustaining global life support systems is extraordinarily meager [3]. With respect to microbial diversity, microorganisms have been used in a variety of ways to serve the mankind for sustainable development [4]. The driving forces of microbial (bacterial, viral, viroid, filamentous fungal, yeast, micro-algal, and protozoan) diversity include the genetic constitution of these organisms, the environment in which they are found, and ecological interactions with other components of the biosphere.

The root system of higher plants is always associated with vast populations of metabolically active microorganisms in addition to an inanimate environment composed of organic and inorganic substances. The living roots of the plants are known to influence the rhizosphere microflora quantitatively and qualitatively. Investigations by earlier workers have shown that the rhizosphere microorganisms are important in soil plant relationships. The population of microorganism increases in the presence of plant roots only [5].

Starkey [6] showed that the microflora of the rhizosphere differs both quantitatively and qualitatively from that in the soil beyond the influence of the root. The quantity and quality of the microflora in the plant rhizosphere depend largely upon the physiological conditions of the plant [7; 8]. Katznelson [5] made a comparison of the microbial counts per gram of firmly adhering soil (R) with the counts per gram of soil away from the roots (S) and the ratio between R and S (R:S) was suggested as standard for comparing the rhizosphere effect in different plants growing in different soils.

The region of North Eastern part of Karnataka covering Gulbarga and Bidar districts have unique agricultural ecosystems with black and red soils respectively. These soils are categorized as vortisol type and are known for the crop productivity because of their physico chemical and nutritional attributes. Although, a lot of work has been carried out on the nitrogen fixing and phosphate solubilising bacteria of these soils, integrated approach considering total bacterial communities prevailing in the rhizosphere of crops is very meager. Therefore, it is aimed to understand the structural and functional bacterial communities from the rhizosphere and non rhizosphere black and red soils of chickpea and sorghum.

2. MATERIALS AND METHODS

Selection of fields and collection of soil samples

Rhizosphere and non-rhizosphere soil samples of both chickpea and sorghum from the selected field stations in Gulbarga and Bidar districts were collected as per the standard procedure by Jackson [9]. Plants (8 to 10) at the tender age were carefully uprooted from each field station. The soil adhered to root system of all the collected plants pooled together and about 500 g of soil was collected in a polythene bag as rhizosphere soil samples of that particular plant. Similarly, the soil samples nearer to the location of uprooted plants but away from the influence of the root systems were also collected and pooled. 500 g of the pooled samples was considered as non rhizosphere soil sample of that particular plant. Soil adhered to the root system were collected at three different length of the root system to obtain surface, sub surface and deep soils. Surface, subsurface and deep soils were collected between three regions vertically, at the depth of 01 to 05 cm, 06 to 10 cm and 11 to 15 cm respectively. The soil on the surface level was considered as surface soil. At the similar levels, non-rhizosphere soil samples were also collected.

Isolation and enumeration of Rhizobia

Rhizobia were isolated from different rhizosphere and non rhizosphere soil samples by following serial ten fold soil dilution plate count technique as described by Vincent [10]. Yeast extract mannitol agar (Yeast Extract - 1 g; Mannitol - 10 g; Dipotassium Phosphate - 0.5 g; Magnesium Phosphate - 0.2 g; Sodium Chloride - 0.1 g; pH - 6.8 ± 0.2; Water – 1 L and Agar - 20 g;) along with congo red as an indicator was employed as specific medium for the isolation of rhizobioum. Population of rhizobia was estimated by standard formula [10] using average colony count, dilution factor and volume of the inoculum.

Isolation and enumeration of Azotobacter

Azotobacter from all the collected different rhizosphere and non rhizosphere soil samples were isolated on Jensen's medium (Sucrose - 20 g; Dipotassium Phosphate - 1 g; Magnesium Sulphate - 0.5 g; Sodium Chloride - 0.5 g; Ferrous Sulphate - 0.1 g; Sodium Molybdate - 0.005 g; Calcium Carbonate - 2 g; Water - 1 L and Agar - 20 g) by following the standard serial dilution plate count technique [11]. The colonies showing the standard features were accounted for the enumerations of Azotobacter per gram of the soil. The calculation was carried out as mentioned earlier.

Isolation and enumeration of phosphate solubilising bacteria

Phosphate solubilising bacteria from the collected rhizosphere and non rhizosphere soil samples were isolated and enumerated on Pikvoskay's medium (Tricalcicum Phosphate - 10.0 g; Ammonium Sulphate - 5.0 g; Potassium Chloride - 0.5 g; Magnesium Sulphate - 0.2 g; Manganese Sulphate - 0.1 g; Ferrous Sulphate - Trace; Yeast Extract - 0.5 g; Water – 1 L and Agar - 20 g) by following the standard serial dilution plate culture method Pikovskay's [12].

3. RESULTS AND DISCUSSION

There are two schools of though regarding the genesis of the associated red and black soils. One school of thought attributed their genesis to the differences in the topography. Wherever the soil is red it is usually close to the hills and overlies a thin layer of decomposed granite and highly kaloinised feldspar. With increase in the distance from the hills black soil of increasing thickness occurs and this is found overlying a thicker layer of decomposed and kalonised material. Very few have dealt with the close association of only red and black soils as distinct entities with clear lines of demarcation, under almost similar [13]. Another school of thought attributed the genesis of these complex soils to the differences in the mineralogical make up of the parent materials. Ramaiah and Raghavendrachar [14] reported that although black and red soils in the Madras Presidency (India) are derived from granite and gneiss and occur side by side, the two types are associated with rocks containing minerals of different chemical composition.

Rhizobium population of chickpea rhizosphere and non rhizosphere black soils are as presented in Figure 1. Bhangargi, Pattan, Dandhoti, and Kirangi locations have recorded maximum population of Rhizobia in chickpea rhizosphere black soils followed by Gobbur, Doranhalli, Malkhed and Ainoli locations. The lowest population was recorded in Yadgir and Rangampet locations. In the chickpea non rhizosphere black soils, the maximum population of Rhizobia was recorded in Pattan, Kirangi and Bhangargi locations followed by Dandhoti, Gobbur, Ainoli and Malkhed locations. The less population of Rhizobia in chickpea non rhizosphere black soils was reported in Doranhalli, Rangampet and Yadgir locations. The maximum population of Rhizobia was determined from subsurface soils followed by surface and deep region of chickpea non rhizosphere black soils. A survey conducted by Rupela and Saxena [15] on chickpea root nodulation in different growing regions of the country revealed poor to adequate nodulation. In general, red soils of Rayalseema region (Andhra Pradesh) contained fewer nodules as against very good nodulation in medium black soils of Dharwad and Raichur districts of Karnataka and Parbhani and Latur districts of Maharashtra. Poor nodulation was also observed in coastal plains of Saurashtra while medium black calcareous soils of Junagadh, Rajkot and Amreli districts supported adequate nodulation. However, ineffective nodules exceeded the number of effective nodules. Out of eighty Rhizobium cultures isolated from different parts of the country, only five were found to be effective.

Phosphate solubilising bacterial population of chickpea rhizosphere and non rhizosphere soils are as shown in Figure 2. Sub surface soils of both rhizosphere and non rhizosphere chickpea black soils have shown maximum population followed by deep and surface soils. In both rhizosphere and non rhizosphere soils, Kirangi, Pattan and Bhangargi have shown maximum population of phosphate solubilising bacteria. Dandhoti, and Gobbur have recorded moderate population. All the remaining locations have shown the lowest population. Sundara Rao and Sinha [16] isolated a number of phosphate solubilisers free wheat rhizosphere by Corithead method and colonies with clear zone on Pikovskaya's medium were purified by repeated plating. Subba Rao and Bajpai [17] and Chhonkar and Subba Rao [18] isolated a number of fungi from legume nodule surfaces. These results give a way to expect possible beneficial interactions between PO_4 solubilisers and N_2 fixing Rhizobium. Bhurat and Sen [19] isolated PO_4 solubilisers from leaf surfaces of pea, barley and gram by incubating discs of leaves on solid medium with tricalcium phosphate.

Rhizobium population in chickpea rhizosphere and non rhizosphere red soils are as presented in Figure 3. In both rhizosphere and non rhizosphere regions, sub surface soils have shown maximum populations followed by surface and deep soils. Hudgi, Basavakalyan, Mudabi and Kallur locations have given more population of Rhizobia followed by remaining locations of chickpea rhizosphere red soils. However, Hudagi, Basavakalyan and Mudabi soils have shown maximum population in chickpea non rhizosphere red soil followed by the remaining locations.

Phosphate solubilising bacterial population in rhizosphere and non rhizosphere red soils of chickpea are as presented in Figure 4. In the chickpea rhizosphere and non rhizosphere red soils, sub surface soils have recorded higher population count followed by deep and surface soils. Mudabi, Hudagi and Basavakalyan have shown maximum population of phosphate solubilising bacteria. The least population was recorded in Bidar soils, where as the remaining locations have shown the moderate population.

Not much information is available on the occurrence and activities of specific groups of microorganisms, in general, and of azotobacteris in particular, in the rhizosphere of important crop plants, especially in the black and red soils of north eastern part of Karnataka. The present study reveals high variations among the nitrogen fixing and phosphate solubilising bacterial population in the rhizosphere and non-rhizosphere soils, of both black and red from Gulbarga and Bidar districts respectively. In addition to the soil physico chemical and nutritional properties, the root exudates of chickpea may be mainly responsible for the significant variations among the bacterial populations. Figure 5 reveals Azotobacter population of sorghum rhizosphere and non rhizosphere black soils. In both rhizosphere and non rhizosphere black soils, population of Azotobacter was more in sub surface soils followed by surface and deep soils. In sorghum rhizosphere black soils Pattan, Kirangi, Bhangargi and Dandhoti locations have shown highest population of Azotobacter followed by Malkhed, Gobbur, Ainoli and Rangampet. The least population was established in Yadgir and Doranhalli soils. In sorghum non rhizosphere black soils Kirangi, Pattan, Bhangargi have recorded maximum population of Azotobacter followed by Dandhoti, Gobbur and Malkhed soils. The lowest population was recorded in Rangampet followed by Doranhalli, Ainoli and Yadgir soils.

Phosphate solubilising bacterial population in sorghum rhizosphere and non rhizosphere soils are shown in Figure 6. In rhizosphere and non rhizosphere black soils of sorghum sub surface soils have shown maximum population followed by deep and surface soils. Pattan, Kirangi and Bhangargi have recorded the maximum population followed by Dandhoti and Gobbur. All the remaining locations have recorded the least population of phosphate solubilising bacteria in both rhizosphere and non rhizosphere black soils of sorghum.

Azotobacter population of sorghum rhizosphere and non rhizosphere red soils are presented in Figure 7. In sorghum rhizosphere red soil Basavakalyan, Mudabi and Hudagi have shown maximum population of Azotobacter followed by Koudihal, Humanabad and Chitaguppa locations. Kallur, Khanapur and Bidar locations have recorded the low population of Azotobacter. In the non rhizosphere red soils of sorghum Mudabi, Basavakalyan and Hudagi have recorded maximum population of Azotobacter. Humanabad, Koudihal, Chitaguppa, Kallur and Khanapur locations have recorded moderate population count of Azotobacter followed by Bidar location with the lowest population count.

Phosphate solubilising bacterial population of sorghum in rhizosphere and non rhizosphere red soils are shown in Figure 8. In the sorghum rhizosphere and non rhizosphere red soils also, sub surface soils have shown higher population of phosphate solubilising bacteria than deep and surface soils. Mudabi, Basavakalyan and Hudagi locations have given maximum population of phosphate solubilising bacteria in both rhizosphere and non rhizosphere red soils of sorghum. Koudihal and Chitaguppa locations have given moderate count. Bidar location has shown the lowest population count of phosphate solubilising bacteria in both rhizosphere and non rhizosphere red soils of sorghum.

Evarsince Hiltner [20] brought out the significance of rhizosphere effect in plants. Several investigators have probed into the inter relationships that exists between plant roots and rhizosphere microorganism. The rhizosphere effect has been, in general, studied along two broad lines, one concerning the influence of plant on the microorganisms and the other of rhizosphere microorganisms on the plant. The latter is concerned with the study of role of rhizosphere microorganisms on the growth and health of the plant. However, it has been proved beyond doubt that the microbial mantle around the root exerts a profound effect, positive or negative on plant growth. Among many beneficial soil bacteria, Rhizobia, Azotobacters and Phosphate solubilisers are important, particularly, in the rhizosphere of crop plants. In the present study, the influence of root exudates on the rhizosphere microorganisms is very high resulting into the more population of Azotobacter in the rhizosphere, when compared to the non rhizosphere, especially in black soils. However, the influence of root exudates seems to be high on the phosphate solubilising bacteria in red soil when compared to black soil. It clearly reveals that, not only soil properties but also the host crop plays a vital role in regulating the total bacterial population in the rhizosphere.

4. CONCLUSIONS

The significance of rhizosphere effect in plants is well established. Several investigators have probed into the interrelationships that exist between plant roots and rhizosphere microorganisms. The rhizosphere effect has been, in general, studied along two broad lines. One concerning the influencing of plant on microorganisms and the other of rhizosphere microorganisms on the plant. However, it has been proved beyond doubt that, the microbial mantle around the root exerts a profound effect, positive or negative on plant growth. Among many beneficial soil bacteria, Rhizobia Azotobacter and Phosphate solubilising bacteria are important, particularly, in the rhizosphere of crop plants. Rhizobia, Azotobacter and Phosphate solubilising bacterial population were also determined from the rhizosphere and non-rhizosphere soils of chickpea and sorghum in both black and red soils. Naturally, because of the several root exudates, the population of these bacteria was predominantly observed in the rhizosphere soils than non-rhizosphere soils, in both the crops and soils.

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REFERENCES

[1] Samuel L. Tisdale, Werner L. Nelson, James D. Beaton: Soil fertility and fertilizers. Macmillian Publishing Company, New York 1985, 60-65.

[2] Jennifer L. K., Lee, A. B., Miranda H., Peter M., John N. K., Hung Lee, Jack T. T: Methods of studying soil microbial diversity. J. Microbiol. Meth 2004. **58:** 169-188.

[3] Colin Campbell: Soil biodiversity. Information and Advisory Note. Scottish Natural Heritage 2002. No. 151.

[4] Colwell: Microbial Biodiversity and Biotechnology, Chapter 19, In Biodiversity II: Understanding and Protecting our Biological Resources, Eds: Marjorie, L., Reaka, K., Don, E. Wilson and Edward O, Wilson. Joseph Henry Press, Washington, D.C. 1997

[5] Katznelson H: The rhizosphere effect of mangoles on certain groups of soil microorganisms. Soil Sci 1946. **62:** 343-354.

[6] Starkey, R. L.: Some influences of higher plants upon the microorganisms in the soil. II. Influence of stage of plant growth upon the abundance of organisms. *Soil. Sci.* **27:** 355-374.

[7] Lochhead A. G., Timonin M. I., West P. M.: The microflora of the rhizospheric in relation to resistance of plants to soil born pathogens. Sci. Agri 1940. **20:** 414-418.

[8] Timonin M. I.: The interaction of higher plants and soil microorganisms. I. Microbiol population of seedlings of certain cultivated plants 1940.

[9] Jackson M L: Soil chemical analysis, Prentice Hall of India Pvt. Ltd., New Delhi 1973.

[10] Vincent J. M.: A Manual for the Practical Study of Root Nodule Bacteria, IBP Handbook, No. 15 Blackwell Scientific Publications, Oxford 1970. p. 164.

[11] Rangaswami G. and Bagyaraj D.J.: Methods of studying microorganisms. 1993.

[12] Pikovskaya R. I.: Mobilization of Phosphate in Soil in Connection with the Vital Activities of some Microbial species. Microbiologia 1948. **17:** 362-370.

[13] Pal, D. K., Deshpande S. B.: Genesis of clay minerals in a red and black soil complex of southern India. Clay Research 1987. **6:** 6-13.

[14] Ramaiah P. V., Raghavendrachar C. S.: The origin of black soils in the Madras Presidency. Proc. Soc. Biol. Chem., India 1936, **1:** 9.

[15] Rupela O. P., Saxena M. C.: Nodulation and nitrogen fixation. In: The chickpea (Eds. M. C. Saxena and K. B. Singh). CAB International Wallingford 1987, pp. 191-206.

[16] Sundara Rao W. V. B., Sinha M. K.: Phosphate Dissolving microorganisms in the Soil and Rhizosphere. Indian J. Agric. Sci 1963. **33:** 272-278.

[17] Subba Rao N. S., Bajpai P. D.: Fungi on the surface of legume root nodules and phosphate solubilisation. Experimentia 1965, **21:** 386-387.

[18] Chhonkar P K, Subba Rao N S: Phosphate solubilisation by fungi associated with legume root nodules. Con. J. Microbial 1967. **13:** 749-752.

[19] Bhurat M.C., Sen, A.: Phosphate solubilizing bacteria in phyllosphere of winer crops. Indian Journal of Microbiology 1968. 8 (21): 255-256.

[20] Evarsince Hiltner, R: Uber neurere Erfahkrengen und problema and dem Gabiet der Bodenobackeriologie and unterbesoderer. Berucksichtigune der Grundungung and Brache, Arb. Dert. Land Wirstschges, Osterrieich 1904, **98:** 59-78.

Fig. 1: Diversity of rhizobia in chickpea rhizosphere and non rhizosphere black soil

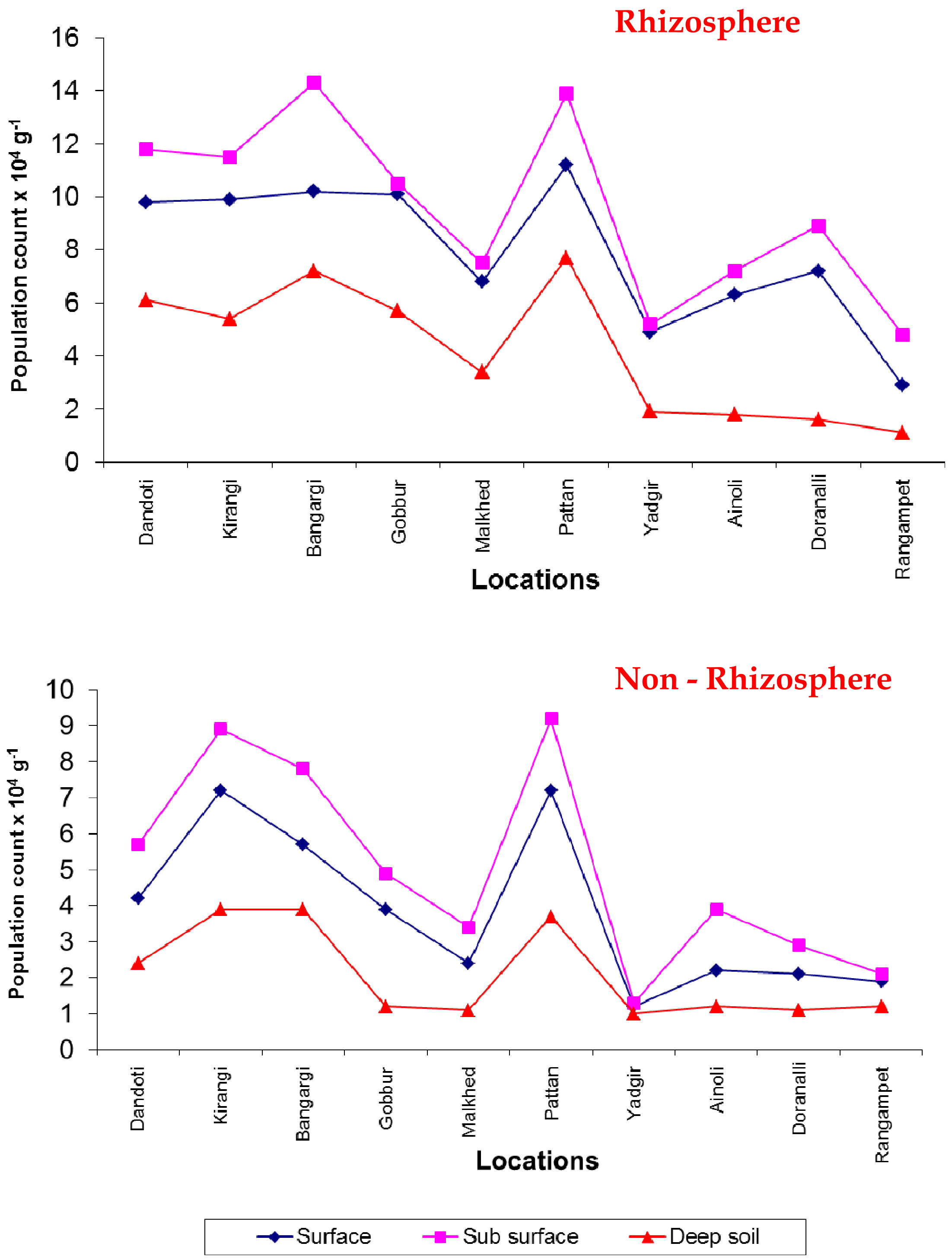


Fig. 2: Diversity of phosphate solubilizing bacteria in chick pea rhizosphere and non rhizosphere black soil

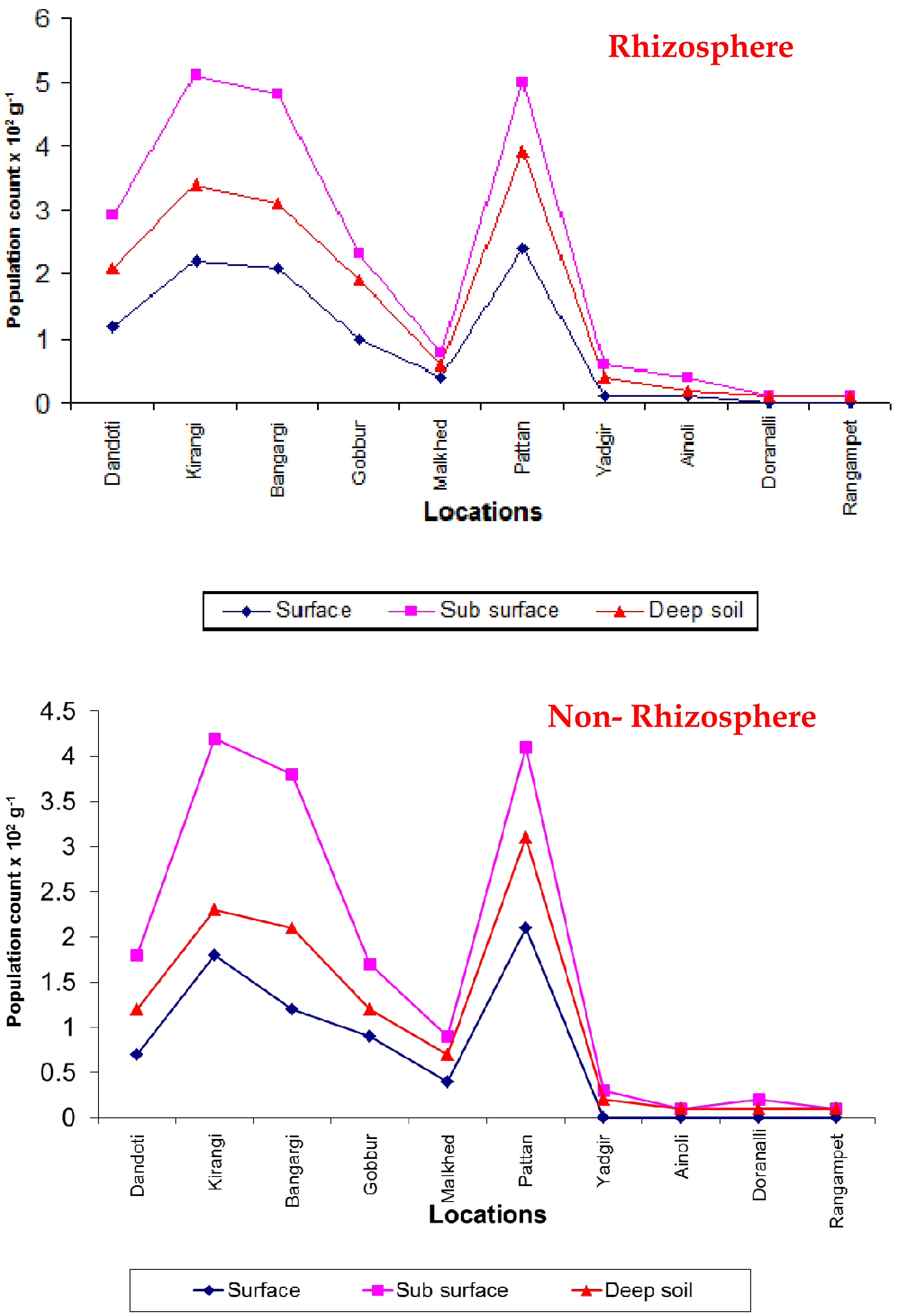


Fig. 3: Diversity of rhizobia in chickpea rhizosphere and non rhizosphere red soil

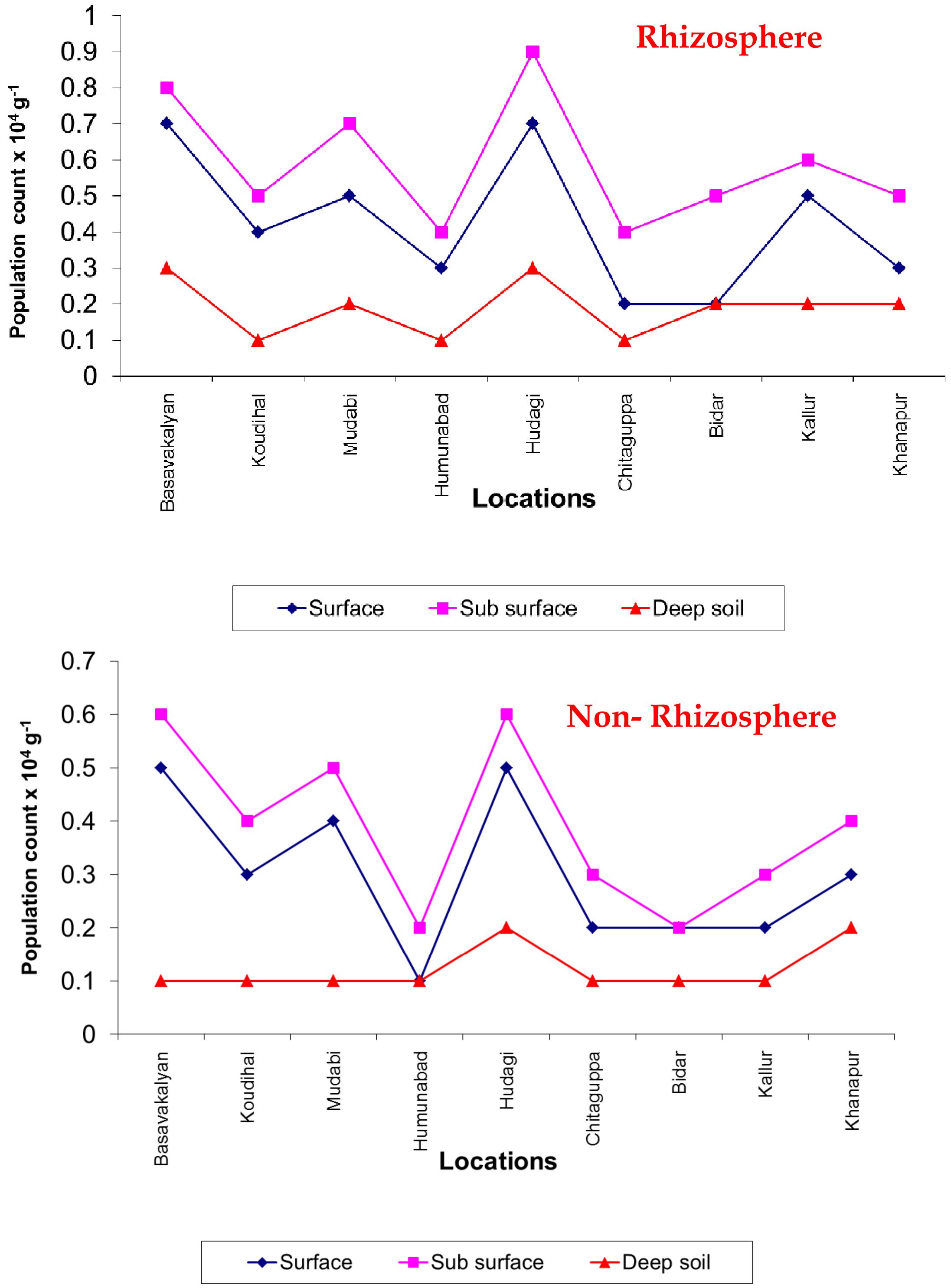
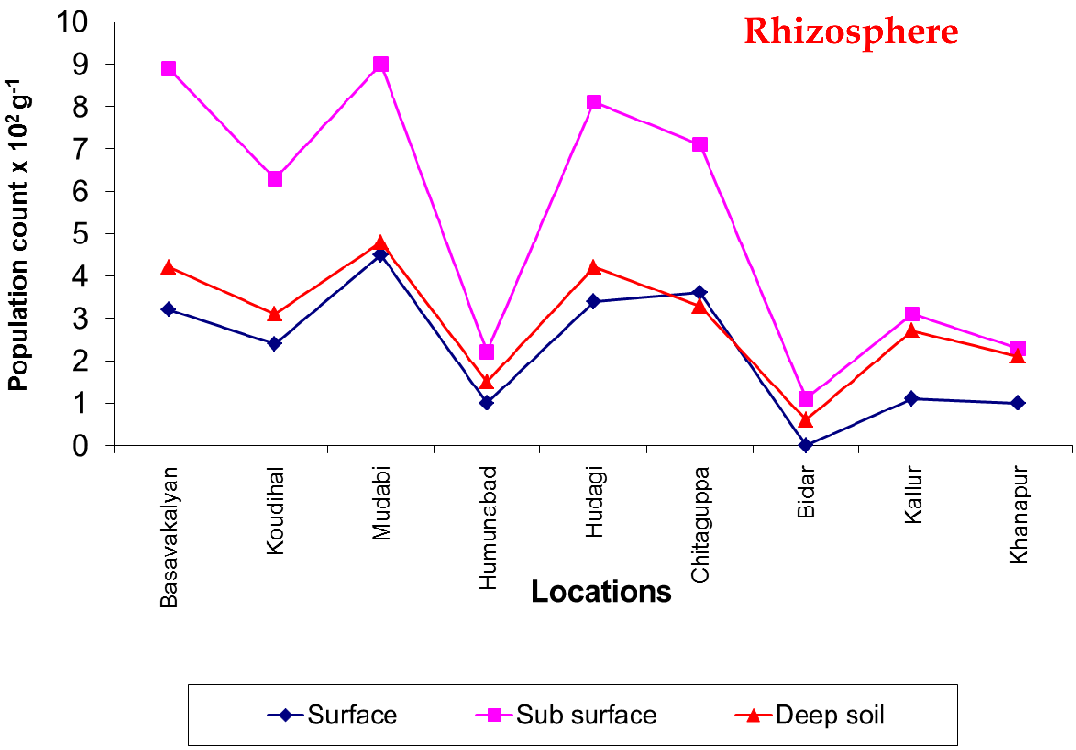


Fig. 4: Diversity of phosphate solubilising bacteria in chick pea rhizosphere and non rhizosphere red soil



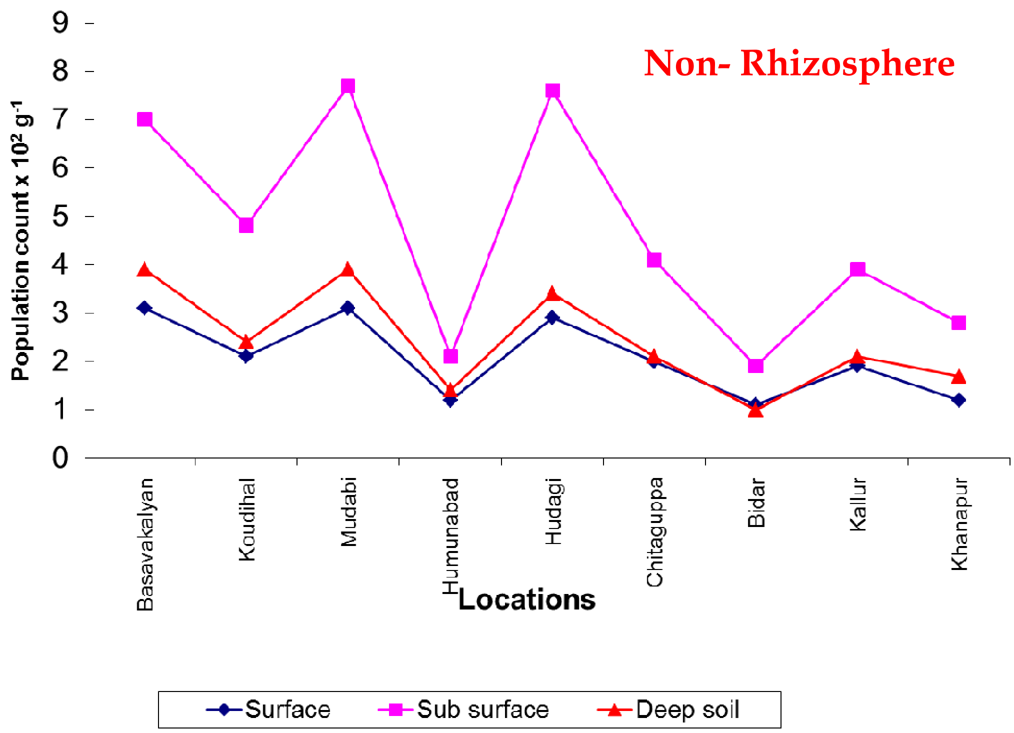
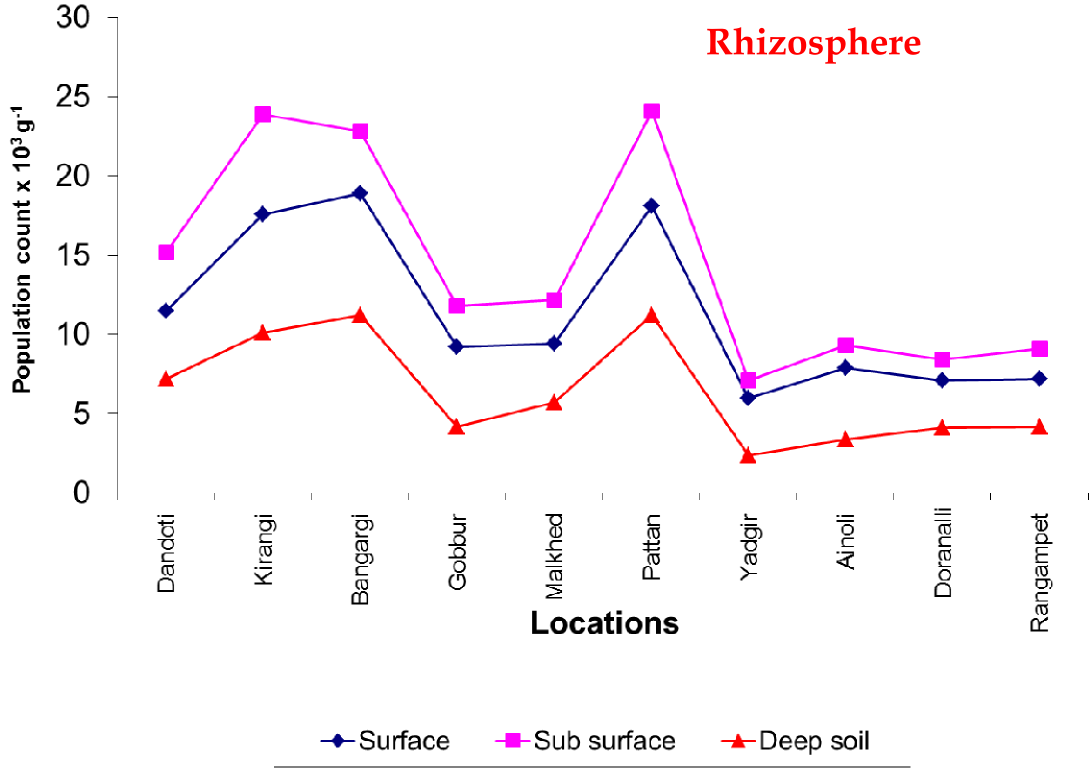


Fig. 5: Diversity of azotobacter in sorghum rhizosphere and non rhizosphere black soil



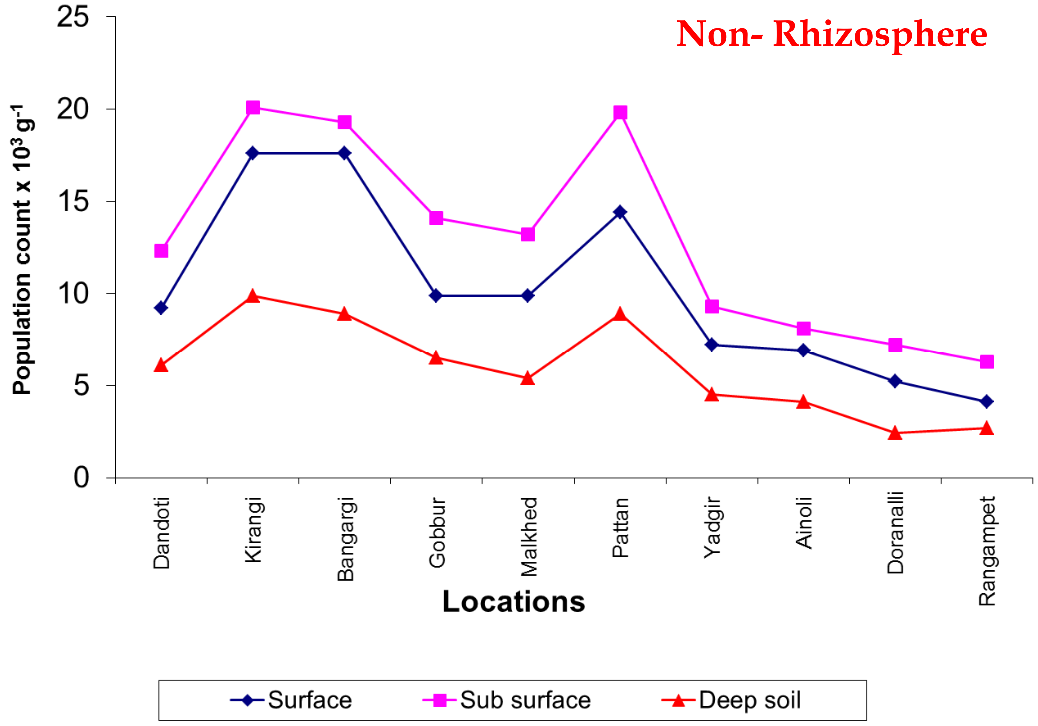
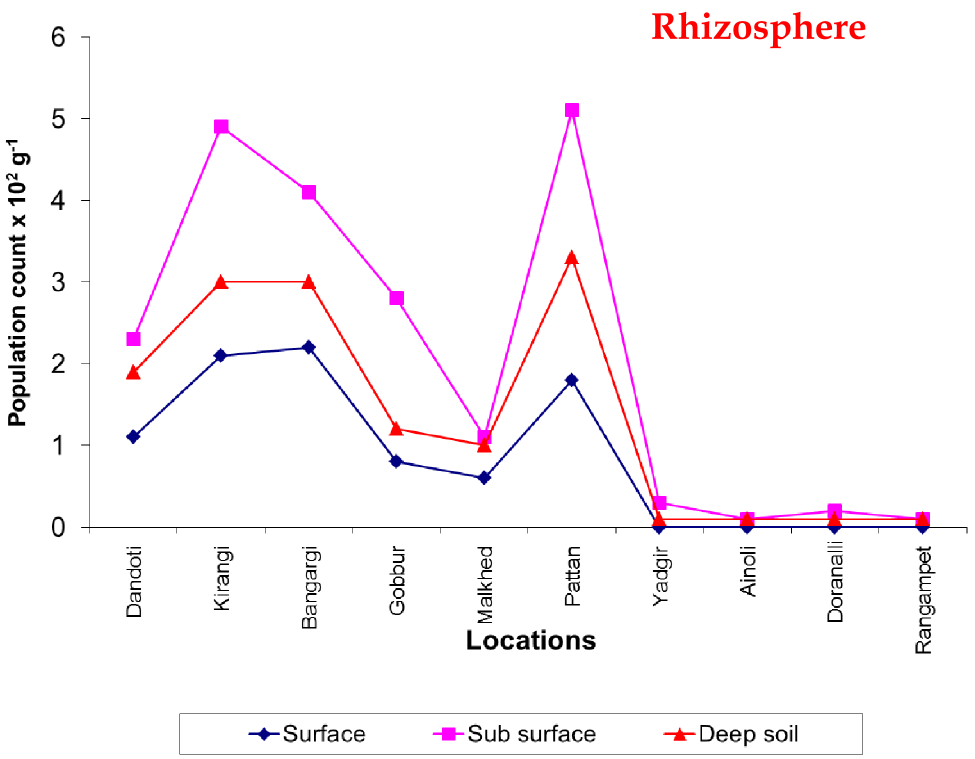


Fig. 6: Diversity of phosphate solubilising bacteria in sorghum rhizosphere and non rhizosphere black soil



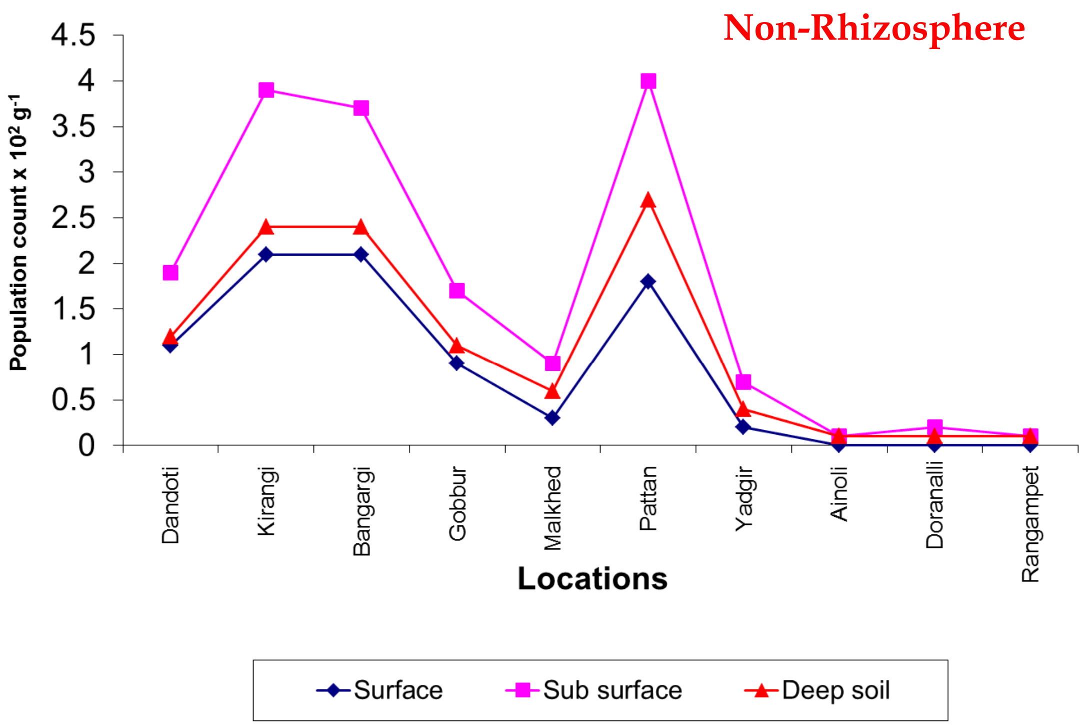
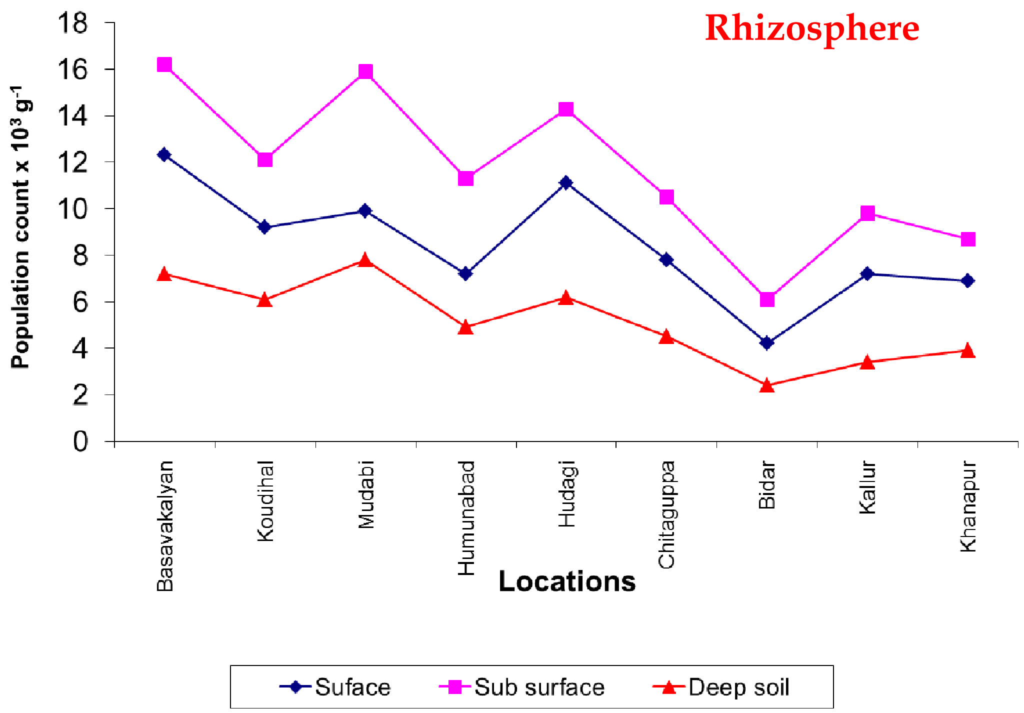


Fig. 7: Diversity of azotobacter in sorghum rhizosphere and non rhizosphere red soil



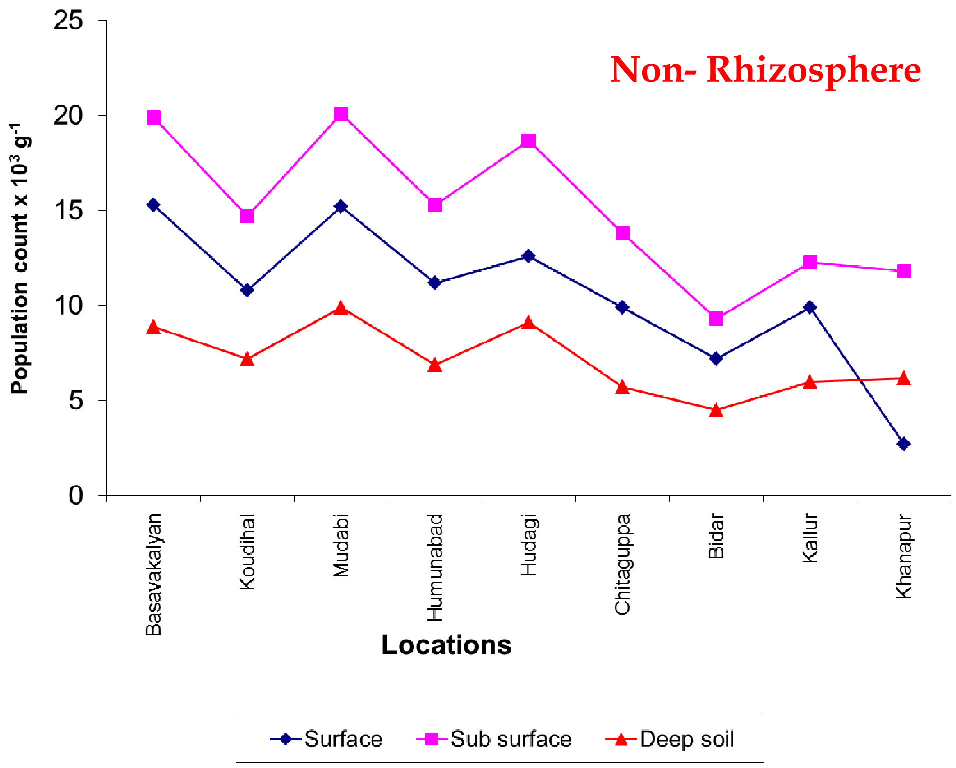


Fig. 8: Diversity of phosphate solubilising bacteria in sorghum rhizosphere and non rhizosphere red soil