Diversity of Nitrogen Fixing and Phosphate Solubilizing Bacteria in Black and Red Soils

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Abstract

In recent years scientists have recognized the high potential productivity of different kinds of soils in the country. However, a gap in the current knowledge is the precise assessment of not only soil properties but also microbial diversity of the soils. In this direction, an attempt was made in the present study to understand physico chemical properties, nutritional attributes and potential bacterial spectrum of soils of north eastern part of Karnataka, especially black and red soils of Gulbarga and Bidar districts. Neutral to alkaline condition is an important attribute of the black soil, when compared to acidic nature of the red soil. The analysis of black soils in general reveals more organic matter and nitrogen content. However, red soils were less in organic matter and nitrogen content, but were rich in phosphorus content. Black soil reveals the more content of molybudum, sulphur and zinc, but red soils were rich in copper, iron and manganese. Among nitrogen fixing bacteria, rhizobium and azotobacter were recorded to be more in black soils. The red soils reported to harbor more number of phosphates solublising bacteria.

KEYWORDS: Diversity, Nitrogen fixing, Phosphate solubilizing, Black soil and Red soil

INTRODUCTION

Microorganisms constitute a huge and almost unexplained reservoir of resources likely to provide innovative applications. It has been estimated that only 1-6% of all microorganisms on earth have been studied [1]. A large proportion of these unknown species is thought to reside in the soil. Microbial diversity is the key to human survival and economic wellbeing and provides a huge reservoir of resources, which we can explored for the human welfare. The biological diversity of the Indian subcontinent is one of the richest in the world owing to its vast geographical area, varied topology and climate [2]. Biodiversity is the variety of life on Earth at all its levels, from genes to ecosystems, and the ecological and evolutionary processes that sustain it. There are three main categories of biodiversity which are genetic diversity, taxonomic diversity and functional diversity. Genetic diversity is the diversity of genes within a species as well as between the group to which it belongs. Taxonomic diversity is identifying the number of different taxa at the species level and functional diversity is to recognize the variety of roles of any given organism in the ecosystem. The diversity of soil microorganisms is more extensive than any other environment in the world when all living forms are considered [3].

The soil is a habitat for a vast, complex and interactive community of soil microorganisms whose activities largely determine the chemical and physical properties of the soil. In a fertile soil, the soil biota may have a biomass exceeding 20 tons ha^{-1}, with life forms ranging from microscopic bacteria to the largest of earthworms [4]. The role of soil microorganisms in the development and maintenance of soil structure,

nutrient cycling and their various interactions with plant roots is known. Even if it were possible to sample the full diversity of soil organisms at a given location the data would be of limited value since, many soil microorganisms, are opportunistic with the capacity for rapid population increase to exploit temporary availability of favorable substrates [5]. The full extent of diversity of all groups of soil inhabiting organism has not been and could not be determined for any one given location (Manish Kapoor and Rakesh Kumar, 2004). Prospects for the management of the soil biota to promote sustainable productivity are illustrated by describing the agricultural practices on the composition of soil organism community. Management technologies that conserve the biodiversity of communities may provide the greatest benefits for the long term sustainability of the soil resource. The importance of soil organisms in soil formation and in the maintenance of soil fertility has long acknowledged but, support for scientific study has, until recently, been slight. Maintenance of biodiversity is a major factor contributing to nutrient turnover and control of harmful organisms in soils. Technological options for the sustainable management of soil and water resources thus need to be those that maintain or enhance high populations and taxonomic diversity of the soil biota. At the same time, there is a need to concentrate more research on the complex communities of soil organisms to understand better significance of their extreme diversity, the functional significance of individual groups and the interactions between groups, so that, they may be modulated and managed without compromising their sustainability [6].

The region of North Eastern part of Karnataka especially, the districts of Gulbarga and Bidar have unique agricultural ecosystems with black and red soils respectively. Although, a lot of work has been carried out on the nitrogen fixing and phosphate solubilizing bacteria of these soils, integrated approach considering total bacterial communities and soil attributes is very meager. Therefore, it is aimed to understand the structural and functional microbial communities of black and red soils.

MATERIALS AND METHODS

Selection of fields and collection of soil samples

A survey was made during March, April and May 2012 in the northern part of Karnataka covering Bidar and Gulbarga districts with an aim to establish different field stations [7] for the collection of suitable black and red soil samples for the required study.

The black and red soil samples (about 500 gm) were collected from all the selected field stations during off season (April and May) as per the standard procedure prescribed by Skinner [8] and Jackson [7]. Surface, subsurface and deep soils were collected between three regions vertically, at the depth of 01 to 10 cm, 11 to 20 cm and 21 to 30 cm respectively.

A handful of soil samples were collected from seven to eight different spots of each selected field station. The samples collected from the different spots were pooled on the spot and mixed thoroughly. The representative soil sample of about 500 gm from the heap of each field station was collected in sterile polythene bag and brought to the laboratory indicating the sample with a code [7]. The coded samples were cleaned to remove stones debris and air dried and stored at 40^{o}C for further studies.

Analysis of soil

All the collected black and red soils samples were subjected for the analysis of certain physico-chemical and nutritional components as per the standard procedures. The analysis of soil samples was carried out at District soil testing Centre, Department

of Agriculture, Kotnoor, Gulbarga and Prestine Laboratory, Bangalore. The analysis of various components of soils in brief is as follows.

Soil moisture: Soil moisture was determined after collection of soil samples by adopting gravimetric method Jackson [7] and expressed in percentage.

Soil pH: The pH of the soil water suspension (1:2.5) was determined using a pH meter with combined glass electrode Jackson [7].

Electrical conductivity: The electrical conductivity was determined in 1:2.5 soil extract using conductivity bridge Jackson [7] and expressed as dSm^{-1}.

Organic carbon: The organic carbon of the collected soils was determined by Walkley and Blacks wet oxidation method Jackson [7] and same was expressed in percentage.

Soil nutrients

Total Nitrogen: The total nitrogen content of the soil was determined by Micro-Kjeldhal's method Jackson [7]. One gram of soil was transferred into a clean and dried Kjeldhal's digestion tube. A pinch of catalyst digestion mixture containing potassium sulphate, copper sulphate, selenium (100:10:1), two crystals of sodium sulphate and 5 ml of 4% salicylic acid in H_2SO_4 were added to the tubes and kept for digestion. The digestion was continued until the digest became clear and the sample appeared green in colour. After sufficient cooling the contents of the tubes were distilled in Kjeldahl's distillation set by adding excess of 40% NaOH. The ammonia released was collected in 20 ml of 2% boric acid solution containing mixed indicator of bromo cresol green and methyl red and back titrated with 0.05 N H_2SO_4. From the value of acid consumed the percentage of nitrogen in the sample was determined and calculated as Kg per hectare [9].

Available Phosphorus: The soil was extracted with Bray's No. 1 extractant containing ammonium fluoride. The concentration of phosphorus in the extract was determined by chlorostannous reduced phosphomolybdic blue colour method as described by Jackson [7]. The intensity of blue colour was read by Spectrophotometer (ELICO – Cl. 24) at 600 nm and calculated as Kg/ha [10].

Available Potash: Available potash in the collected soil samples was determined from a suitable aliquot, obtained by 1.0 N neutral ammonium acetate. The potash content in the filtrate was determined by Flame Photometer (Model – AIMIL), using 'K' filter as described by Jackson [7] and calculated as Kg/ha.

Micronutrients

Iron, manganese, zinc and copper are extracted from the soil with DTPA (Diethylne triamine penta acetic acid) extractant. The concentration of these elements in solution was determined by using atomic absorption spectrophotometer [11] and expressed in ppm.

Available sulphur: Available sulphur was estimated by Turbidimetric method [12] and expressed in ppm.

Available molybdenum: Available molybdenum was estimated using Grieg's reagent by colorimetric method [13] and expressed in ppm.

Isolation and enumeration of Rhizobia

Rhizobia were isolated from different soil samples by following serial ten fold soil dilution plate count technique as described by Vincent [14]. 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 (Vincent, 1970) using average colony count, dilution factor and volume of the inoculum.

Isolation and enumeration of Azotobacter

Azotobacter from all the collected different 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 [15]. 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 solubilizing bacteria

Phosphate solubilizing bacteria from the collected 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 [16].

RESULTS AND DISCUSSION

The black and red soils are the most extensive soil groups in India next to alluvial soils. The red and black soils representing two broad soils groups observed in India often occur side by side under apparently, the same climatic and geological conditions and the mode of formation of these soil types has been long debated issue. The red and black complex soils are most common in the Deccan Plateau of India. In India these red and black complex soils are commonly reported in Andhra Pradesh, Madhya Pradesh, Tamil Nadu and Karnataka. In Karnataka these soils are predominant in the northern districts.

Physico chemical properties of black soil are as mentioned in Table–1. The maximum moisture content was recorded in the soils of Bhangargi (22.80%) and Kiranagi (19.28%). The least moisture was recorded in the soils of Doranhalli (8.40%) and Yadgir (8.60%). Moderate moisture content was recorded in the remaining soils (9.20 to 16.28%). Soils of Dhandoti, Malkhed, Pattan and Ainoli showed more pH (8.0 to 8.7). Remaining soils of Kirangi, Bhangargi, Gobbur, Yadgir, Doranhalli and Rangampet have showed the pH from 7.2 to 7.9. The electrical conductivity determined in all the soil samples was in the range of 0.15 millimoles/cm (Dandoti soil) to 1.22 millimoles/cm (Pattan soil). The highest organic carbon was recorded in soils of Kirangi (0.86%) and Bhangargi (0.84%). The least organic carbon content was recorded in soils of Doranhalli (0.36%) and Rangampet (0.39%). The physicochemical properties of red soil are shown in Table–2. The maximum moisture content was observed in soils of Basavakalyan (16.24%) and Chitaguppa (15.62%). The least moisture content was observed in Khanapur (7.9%) and Humnabad (7.9%). The range of moisture content from the remaining soil samples observed was from 9.50% (Hudagi soil) to 15.62% (Chitaguppa soil). The soils of Basavakalyan, Mudabi, Hudagi, Chitaguppa and Bidar have shown the pH in the range 6.0 to 6.3 and the remaining soil have shown the pH ranging from5.3 to 5.8. The electrical conductivity of all the soils observed was in the range of 1.08 millimoles/cm (Chitaguppa soil) to 1.90 millimoles/cm (Basavakalyan soil). The organic matter determined from all the sample was in the range of 0.12%

(Humunabad soil) to 0.18% (Basavakalyan, Hudagi and Kallur soils). Soil samples of Gulbarga district are classified as vertisol type of soils, which are medium black to black in colour, clay-to-clay loam in texture having maximum water holding capacity.

Nutritional properties of black and red soils are presented in Table–2. Nitrogen (N), Phosphorous (P) and Potash (K) were observed as macronutrients and copper (Cu), Iron (Fe), Manganese (Mn), Molybedon (Mo), Sulphur (S) and Zinc (Zn) were recorded as micronutrients. Maximum amount of macronutrients were recorded in the soils of Kirangi and Bhangargi followed by Pattan, Dandhoti and Gobbur. Remaining soils have shown moderate amount of macronutrients. The amount of micronutrients are varied from soil to soil. The nitrogen content was more in Mudabi (130.6 Kg/h) followed by phosphorus in Hudagi (20.2 Kg/h) and content of potash in Basvakalyan (190 Kg/h) red soils. Highly varied contents were observed among all micronutrients from soil to soil. The productivity of a land is dependent on the availability of nutrients of the major essential plant nutrients; phosphorus occupies a prime place after nitrogen and plays an important role in plant metabolism reflecting on the crop yields. The cycle of phosphorus in soils shows a high percentage of Pi to be locked up in water insoluble forms and unavailable for plant uptake. Phosphorus is one of the major essential plant nutrients which plays an important role in obtaining higher crop yields. In a developing country like India, the supply of P to crops in the form of different phosphatic fertilizers is of great importance as the soils are low in available P content and also due to the problems of P fixation.

Population of Rhizobia estimated from black and red soil are recorded in Figure 1 and 2 respectively. Maximum population of Rhizobia were recorded in Pattan, Kirangi and Bhangargi black soils followed by Dandoti, Gobbur and Doranhalli. The lowest population was recorded in Malkhed, Yadgiri, Ainoli and Rangampet black soils. Subsurface black soils in all the locations have shown maximum population followed by deep soil and surface soils. In red soils also, sub surface soils have shown maximum population of Rhizobia in comparison to deep soil and surface soils. Among the different locations of red soils, the maximum populations of Rhizobia were recorded in Mudabi, Basavakalyan, Hudgi and Chitaguppa red soils. The remaining locations have shown the low population of Rhizobia.

Survival of Rhizobia is greater in heavier or organic soils due to the interaction of the Rhizobia with clay or other charged particles Danso and Alexander,[17]. Venkateshwarlu *et al.* [18] observed a positive and significant correlation between the phosphate solubilizing microbes and organic matter content in the soil. While working with the distribution of Rhizobium at soil depths running from 3 to 120 cm, Kumar Rao and Dart [19] observed variation in the rhizobial population with the depth even in the same field. The reduction was more pronounced below100 cm, in soil profile. Surveys of the research stations and farmer fields by Toomsan *et al.* [20] indicated 3.9 x 10^2 to 1.8 x 10^6, 10 to 3.8 x 10^3 and 10 to 5.4 x 10^4 Rhizobia per gram of soil samples from Hisar, Gwalior and ICRISAT respectively. Similarly Rupela *et al.* [21] observed decline in rhizobial population with soil depth. There was highest population of Rhizobia (about 10^4/gm soil) in the top 30 cm of the profile and lowest but still present (10^2 to 10^3/gm soil) at 90 to 120 cm depth.

The populations of Azotobacter estimated in black and red soils are as presented in Figure 3 and 4 respectively. Pattan, Kirangi and Bhangargi were the prime locations indicating the maximum population of Azotobacter in black soils. Dandhoti, Gobbur, Malkhed, Yadgir and Ainoli were the locations indicating moderate population followed

by Doranhalli and Rangampet locations, indicating low population count. Subsurface soils have shown higher population of Azotobacter followed by deep soils and surface soils. In red soils also, sub surface region has shown higher population of Azotobacter followed by deep and surface soils. Basavakalyan, Mudabi, Hudagi and Humanabad locations have shown maximum population of Azotobacter followed by Kouadihal, Chitaguppa, Khanapur and Kallur red soils. The least population of Azotobacter was recorded in Bidar soil. In all, surface soil has shown maximum population of Azotobacter followed by deep and surface soil.

Studies on quantitative occurrence of *Azotobacter* spp., in Indian soils have been reported. In the four major soils types of Karnataka state, viz., red soils of Hebbal, black soils of Dharwar, alluvial soils of T. Narasipur and lateritic soils of Sirsi, the population of Azotobacter ranged from 0 to 11.4 x 10^2/g of moisture free soil. The alluvial soil of T. Narasipur with a pH of 7.5 recorded maximum number. In all the soils of Karnataka and Andhra Pradesh examined, surface soils were found to have more of Azotobacter and their number decreased with increase in depth [22]. Acidity of the soil was the most important factor affecting the Azotobacter population [23]. In an investigation carried out to study the relationship of some soil properties with Azotobacter population Channal *et al.* [24] observed that Azotobacter requires almost neutral to slightly alkaline pH. The highest population 84 x 10^2 g^{-1} was recorded in forest soils of Prabhunagar where it had the maximum amount of organic carbon 2.5 percent. The lowest population of 6 x 10^2 g^{-1} was found in acids soils of Sirsi with 0.94 percent organic carbon. The trend indicated, increase in organic carbon in soil favoured Azotobacter proliferation.

Phosphate solubilizing bacterial population of black and red soils is presented in Figure 5 and 6 respectively. In both black and red soils, deep soils have shown more population count followed by sub surface and surface soils. In black soils, Bhangargi, Kirangi and Pattan locations have shown maximum population count followed by Dandhoti and Gobbur. Remaining all locations has shown least count of phosphate solubilizing bacteria. In red soils, Hudagi, Basavakalyan and Mudabi have shown maximum followed by Koudihal and Chitaguppa soils. The lowest population was recorded in Kallur, Khanapur, Bidar and Humanabad soils.

Raj [25] found that the population of PS bacteria was 3 x 10^5, 5.86 x 10^5, 0.43 x 10^5 and 0.11 x 10^5 cfu/g of soil in red sandy loam, medium black clay, laterite and alkali soil types respectively. Similarly, the number of phosphate solubilizing bacteria from 42 soil horizons of 18 profiles at different locations of Himachal Pradesh indicated considerable inter horizon and inter profile variations in their number as demonstrated by Gupta *et al.*[26]. The population of these organisms was higher in A than in B horizons and in majority of the soils it constituted less than 10% of the total bacteria. Organic carbon content of the soils markedly influenced their population size, while pH in the range of 5.5 – 8.0 had no significant role. Higher values of organic carbon in A horizons (1.31 to 1.70%) showed a positive influence on the number of phosphate dissolving bacteria as compared to the B horizons which had lower contents of organic carbon (0.58% to 1.05%).

The present study reveals that, the population of Rhizobium and Azotobacter was high in black soil, when compared to red soil. However, the population of phosphate solubilizing bacteria was more in red soil than black soil. Generally, the metabolic activities of a particular group of bacteria will be more in the soils, where there is a scarcity of that particular nutrient. But, in the present investigation, nitrogen fixing bacteria are more in the black soil and phosphate solubilizing bacteria are more

in the red soil. It is obvious to note that, the quantum of these available nutrients, are not sufficient enough to meet the requirement of these bacteria. Hence, even with the present level of nutrients, the present soils are poor in their fertility. It clearly reveals that, these potential groups of bacteria are the index of soil fertility. Further, studies on the prevalence of bacteria at different depths of the soil, indicated that population increased with soil depth, but only up to the subsurface level, and at the deeper level, population decreased. Several ecological features, such as soil moisture, pH and temperature are known to contribute for these variations.

CONCLUSIONS

The present study indicated considerable variations in physico chemical and nutritional properties of black and red soils. Black soils were found to be neutral to alkaline in nature with more amount of organic carbon and nitrogen. The red soils were nearly acidic in nature and were poor in organic matter and nitrogen content, but were rich in phosphorus content. Micronutrients are also known to play a vital role in soil fertility and crop productivity. Copper, iron, manganese, molybdenum, sulphur and zinc were the micronutrients assessed in both black and red soils. Copper, iron and manganese were found to be more in red soils when compared to the black soils. Molybdenum, sulphur and zinc were found to be more in black soils than red soils. The different parent materials from which the black and red soils have been originated, believed to be responsible for these high variations in the micronutrients. The present study reveals that, black soil harbors a high population density of nitrogen fixing bacteria, both Rhizobia and Azotobacter. However, red soils although indicated a less population count of Rhizobia and Azotobacter, shown relatively more population density of phosphate solubilizing bacteria. Further, it was observed that, irrespective of type of soils and bacteria, sub surface level of the soils has shown a higher population count, followed by surface and deeper levels.

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Table–1: Physicochemical properties of black and red soils

Sl. No.	Location	Properties of black soil			
		Moisture (%)	pH	EC*	OC ** (%)
1.	Dandoti	9.20	8.0	0.15	0.48
2.	Kirangi	19.28	7.8	0.16	0.86
3.	Bhangargi	22.80	7.2	0.24	0.84
4.	Gobbur	16.00	7.4	0.28	0.69
5.	Malkhed	16.28	8.4	0.52	0.49
6.	Pattan	12.24	8.7	1.22	0.54
7.	Yadgir	8.60	7.2	0.34	0.48
8.	Ainoli	15.80	8.6	0.51	0.63
9.	Doranhalli	8.40	7.9	1.15	0.36
10.	Rangampet	9.60	7.6	0.24	0.39
Properties of red soil					
11.	Basavakalyan	16.24	6.1	1.90	0.18
12.	Koudihal	9.24	5.8	1.12	0.14
13.	Mudabi	11.12	6.1	1.20	0.13
14.	Humunabad	7.90	5.7	1.96	0.12
15.	Hudagi	9.50	6.3	1.34	0.18
16.	Chitaguppa	15.62	6.0	1.08	0.16

17.	Bidar	12.24	6.1	1.09	0.15
18.	Kallur	9.76	5.3	1.76	0.18
19.	Khanapur	7.9	5.8	1.78	0.13

** OC – Organic Carbon; * EC – Electrical Conductivity (millimoles/cm)

Table–2: Nutritional Properties of black and red soils

Sl. No.	Location	Properties of black soil								
		Macro nutrients (Kg/h)			Micro nutrients (ppm)					
		N	P	K	Cu	Fe	Mn	Mo	S	Zn
1.	Dandoti	150.2	14.6	280	3.1	4.5	10.6	0.9	13.2	1.2
2.	Kirangi	160.5	15.4	360	3.2	4.9	11.21	0.8	13.0	1.4
3.	Bhangargi	160.8	14.2	340	3.4	4.5	12.5	0.8	13.5	1.4
4.	Gobbur	150.2	13.2	300	2.9	5.0	16.4	0.5	12.1	0.9
5.	Malkhed	140.8	11.5	240	2.4	5.4	15.9	0.6	11.2	0.5
6.	Pattan	150.9	14.1	320	3.0	4.6	13.2	0.7	13.0	1.2
7.	Yadgir	140.2	11.8	210	1.9	4.9	12.8	0.6	11.8	0.5
8.	Ainoli	150.4	12.4	210	1.8	5.2	16.8	0.6	12.0	0.6
9.	Doranhalli	150.8	12.6	260	2.1	4.8	14.2	0.5	11.5	0.8
10.	Rangampet	140.2	11.4	210	1.5	5.1	17.8	0.5	11.2	0.5
Properties of red soil										
11.	Basavakalyan	125.2	18.9	190	5.4	7.8	14.8	0.5	8.5	0.2
12.	Koudihal	130.21	18.2	170	6.2	6.9	17.0	0.5	8.9	0.2
13.	Mudabi	130.6	19.1	160	4.8	8.1	15.1	0.5	7.8	0.2
14.	Humunabad	120.2	16.2	110	3.4	3.9	7.8	0.4	8.0	0.1
15.	Hudagi	130.2	20.2	160	5.2	4.5	15.2	0.6	9.6	0.3
16.	Chitaguppa	125.6	18.5	170	4.9	7.2	14.6	0.5	9.2	0.2
17.	Bidar	120.2	17.8	120	4.1	6.5	8.6	0.4	8.7	0.1
18.	Kallur	110.2	17.1	130	3.9	5.6	5.9	0.3	8.9	0.2
19.	Khanapur	120.8	19.1	170	4.2	5.7	12.1	0.4	9.1	0.1

Fig. 1: Diversity of rhizobia in black soils

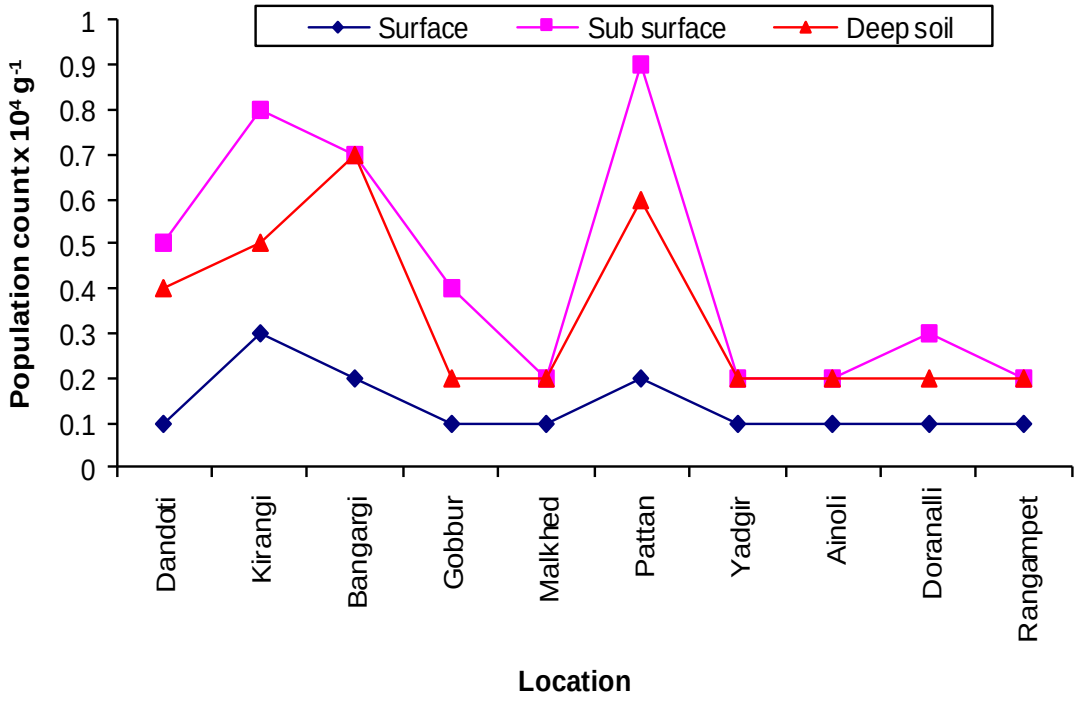

Fig. 2: Diversity of rhizobia in red soils

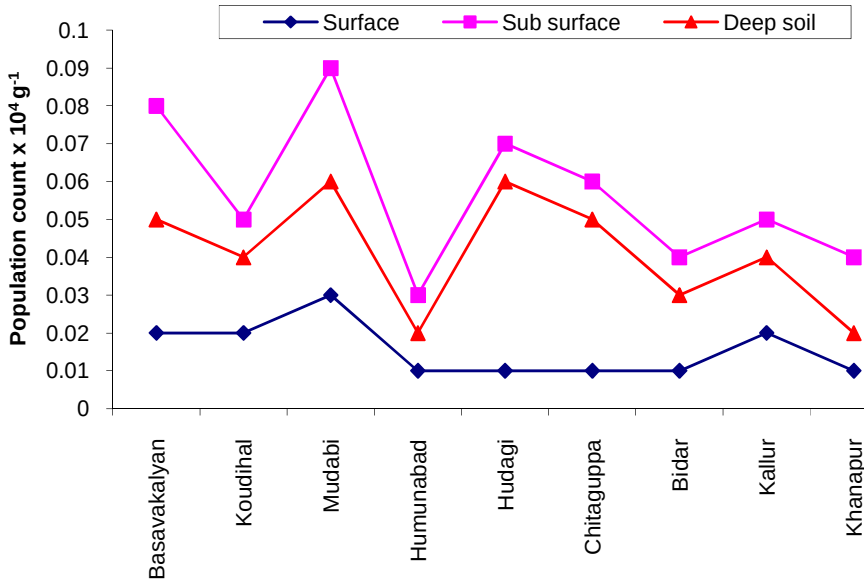

Fig. 3: Diversity of azotobacter in black soils

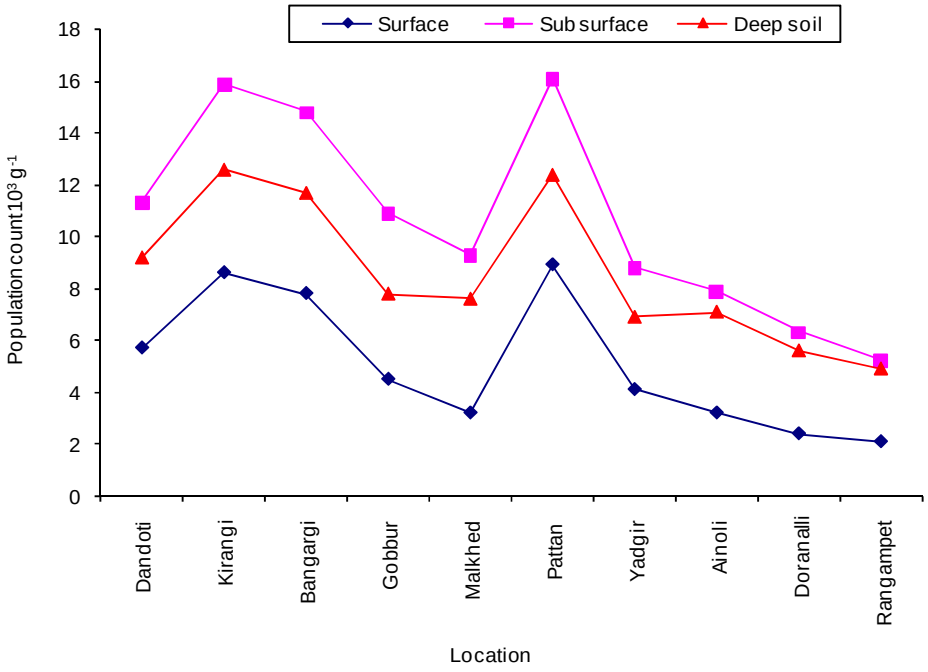

Fig. 4: Diversity of azotobacter in red soils

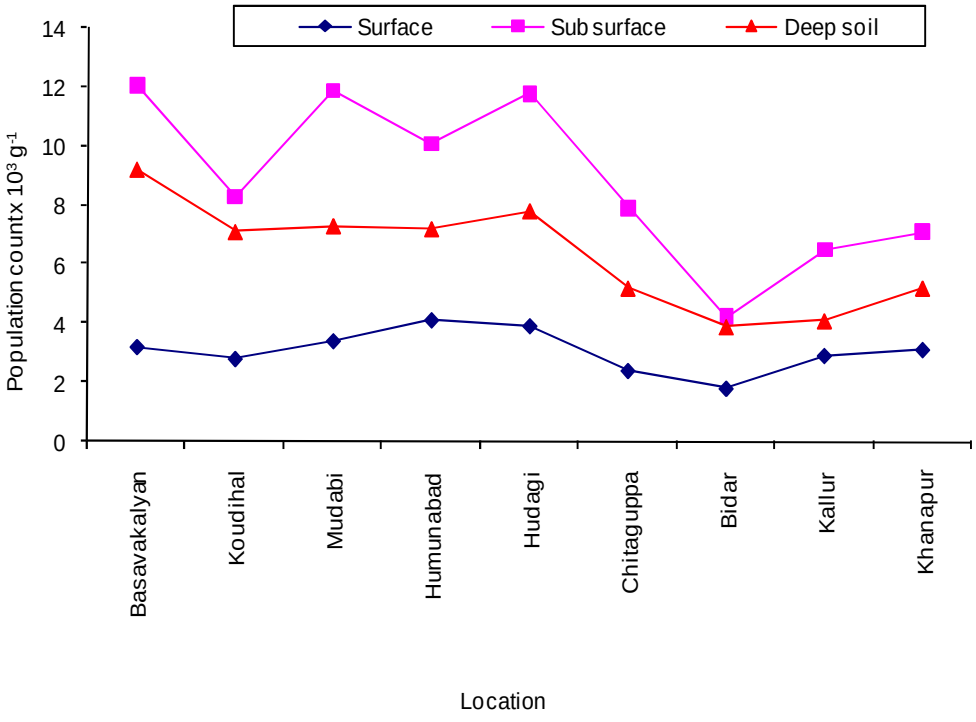

Fig. 5: Diversity of phosphate solubilizing bacteria in black soils

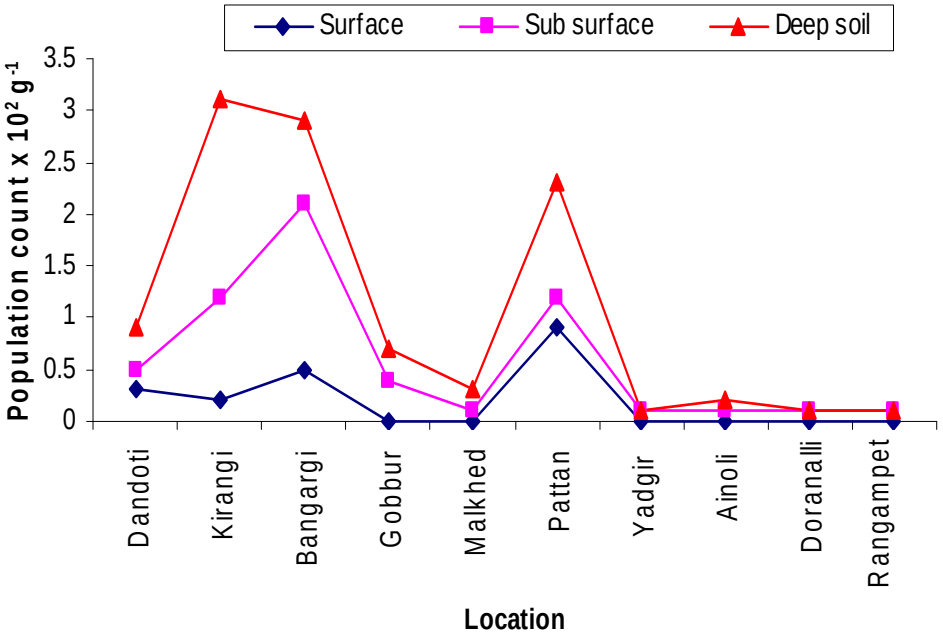

Fig. 6: Diversity of phosphate solubilizing bacteria in red soils