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Diversity of Soil Cyanobacteria in Relation to Dominant Wild Plants and Edaphic Factors at Western Saudi Arabia

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Authors' contributions

This work was carried out in collaboration between all authors. Authors YMAS, AAI, AAK and EFA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of *the manuscript. Authors YMAS, AAI and EFA managed the analyses and literature searches of the study. All authors read and approved the final manuscript.*

Article Information

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ABSTRACT

The study aims at evaluating cyanobacterial diversity along altitudinal gradient with respect to various edaphic factors at western Saudi Arabia. Thirty-one cyanobacteria species belonging to 17 genera were isolated and identified along the different sites of the study area. *Nostoc and Spirulina* had the highest number of species in the study area (four species each), followed by *Chroococcus* and *Oscillatoria* (two species each). The number of colonies had positive correlation with organic matter and phosphates. The application of the two-way indicator species analysis to the data set of

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the percentage presence of 31 cyanobacteria species in 40 stands resulted in agglomerating of 6 groups (communities) at $4th$ level of classification. The application of the detrended correspondence analysis (DCA) indicates reasonable segregation between these groups .The application of CCA on the cyanobacteria communities and environmental variables indicated that some cyanobacteria species are correlated positively with total number of associated plants, TSS and phosphates such as *Woella saccata* Wolle, *Chroococcus minor* Lemm*, Chroococcus majore* Lemm, *Microcystis areuginosa,* Smith *Anabaena spiroides* Lemm and *Nostoc muscorum* Agard, while others are negatively correlated with organic matter, chlorides, pH, EC, clay and silt such as *Oscillatoria limosa* Bory*, Synchococcus sp., Spirulina major* Kutz and *Lyngbya borgertii* Lemm. It is worthy to mentioned that, the soil samples dominated by *Commicarpus sinaicus* Meikle, *Verbesina encelioides* (Cav.) Benth. &Hook. f. ex A. Gray*, Argemone ochroleuca* Sweet*, Haloxylon salicornicum* (Moq.) Boiss.*, Acacia tortolis* (Forssk.) Galasso & Banfi and *Morettia parviflora* Boiss*.* had no cyanobacteria species.

Keywords: Wild plants; Cyanobacteria; edaphic factors; multivariate analysis; altitudes.

1. INTRODUCTION

Altitude is an important factor in habitat diversity because it presents changes in the availability of resources, such as heat and water [1,2]. Many researchers have explored altitudinal biodiversity patterns of plants and clarified that altitude has a role in regulating species richness patterns [3,4, 5,6]. The western region of Saudi Arabia is rich in vegetation when compared to the central and eastern region. The northwestern mountains are rugged and floristically poorer than the southwestern mountains, with affinities to the Mediterranean and North African floristic regions. However, the entire southwestern region is the richest in terms of species diversity and contains the highest concentration of endemism, despite the fact that these high altitude areas are heavily populated with human settlements dating to ancient times [7].

Many plant species including medicinal plants are able to produce and release bioactive compounds i.e. secondary metabolites into the environment and are capable of suppressing the growth of other plants. Such chemicals include tannins, phenolic acids, lignins, alkaloids, flavonoids, coumarins and terpenoids. They are present in all plant tissues including leaves, stems, roots, rhizomes, flowers, fruits and seeds, and even in pollen grains [8].

Cyanobacteria are often considered as the first prokaryotic microorganisms to colonies bare areas of rock and soil. Adaptations, such as ultraviolet absorbing sheath pigments, increase their fitness in the relatively exposed land environment. Many species are capable of living in soil and other terrestrial habitats, where they play significant role in ecosystem functioning and nutrient cycling [9]. The prominent habitats of

cyanobacteria are limnic and marine environments. They flourish in water that is salty, brackish or fresh, in cold and hot springs, and in environments where no other microalgae can exist [10,11]. A number of freshwater species are also able to withstand relatively high concentrations of sodium chloride and tolerate saline environments [12]. Freshwater localities with diverse trophic states are prominent habitats for cyanobacteria. Numerous species characteristically inhabit, and can occasionally dominate, both near-surface epilimnic and deep, euphotic, hypolimnic waters of lakes [13]. Others colonise surfaces by attaching to rocks or sediments, sometimes forming mats that may tear loose and float to the surface. Cyanobacteria have an impressive ability to colonise in fertile substrates such as volcanic ash, desert sand and rocks [14]. They are extraordinary excavators, boring hollows into limestone and special types of sandstone [15].

Another remarkable feature is their ability to survive extremely high and low temperatures. Cyanobacteria are inhabitants of hot springs [16], mountain streams [17], Arctic and Antarctic lakes [18] and snow and ice [19,20]. The cyanobacteria also include species that run through the entire range of water types, from polysaprobic zones to katharobic waters [21]. Enrichment of microorganisms in the rhizosophere is mainly due to the availability of some compounds released from the root. Numerous works have been published which deal with the effect of seed plants on soil fungi and bacteria. Very little studies, concerned with the inhibitory or stimulatory effects of seed plants on soil algae were demonstrated [22,23,24]. It has been pointed out that diversity and numbers of algae can be increased or decreased as a result of farming practices. Rhizosophere effects on soil algae vary with the species of vascular plant [24]. Positive rhizosphere effects have been noted for diatoms with *Artemesia* Linn and *Lasiagrostis* Link plants and for diatoms and blue-green algae with root of *Hibiscus esculantus* . Fawzy et al. [25], reported a negative effect of plant rhizosphere on algae. No attempts of soil Cyanobacteria have been conduct around wild plants at Western Saudi Arabia, especially in Taif area. Thus, the aims of the present study to investigate the role of different spatial and temporal scales in the variation of species composition and functional groups of Cyanobacteria and Wild plants. The different between Cyanobacterial assemblages and their functional groups in relation to environmental factors from the soils were studied.

2. MATERIALS AND METHODS

2.1 Study Area, Sampling Sites and Soil Analysis

Saudi Arabia land area of approximately 2,150,000 km² and extends from 16° 22' - 32° $14'$ N and 34º 29'-55º 40` E (Fig. 1). Taif is situated in the central foothills of the western mountains at an altitude of an approximately 2500 m above sea level. Bisha is located at south western part with an altitude of approximately 610 m above sea level. In general Taif climate is considered dry climate because the rainfall is less than 10 inch and the mean temperature ranges from 15.5°C in winter to 28.5°C in summer, while Bisha has a desert climate, where the mean temperature ranges from 28 °C in winter to 36°C in summer and the greatest amount of

precipitation occurs in April, with an average of 8 mm.

Forty stands surveyed in the study area(size 100X100m) to represent the prevailing variations in altitudes and vegetation during spring (March-May 2017). Seventeen of them were selected at Bisha (12) and Alsail (5) with altitudes ranges between <1500 m.a.s.l, twelve ones were selected at Hada (7) and Wahatt (5) with altitudes ranges between 1500 - 2000 m.a.s.l and eleven ones at shafa site with altitudes more than 2000 m.a.s.l (Table 1; Fig. 1). The first and second dominant plant species was listed in each stand.

Soil samples were collected at three random points from each site (composite samples) at a depth of 0 - 50 cm as a profile. The mechanical analysis of soil texture was carried out using hydrometer method. The electrical conductivity (EC) and pH for each sample determined as a 2:5 (w/v) dilution in deionised water. Soil analyses including total dissolved salts (TDS; g L^{-1}) and total bicarbonate (HCO₃), and chlorides (CI; g 100 g $^{-1}$ DW) were analyzed by titration by using AgCl. Major cations such as sodium (Na), potassium (K), calcium (Ca), and magnesium (g 100 q^{-1} DW) were determined by flame photometer (Jenway, PFP-7), according to the methods of Williams and Twine [26]. Phosphate was determined as described by Murphy and Riley [27]. Finally, nitrate was spectrophotometrically determined by chromotropic acid (1, 8-dihydroxynaphthalene-3, 6-disulphonic acid disodium salt) in concentrated sulfuric acid [28].

Fig. 1. Distribution of 40 stands along study area (red point) by using their coordinates (GPS)

Site	Stand No.	Latitudes (N)	Longitude (E)	Altitude (m)
	1	21.15085	40.23722	2313
	\overline{c}	21.15083	40.23835	2322
	3	21.14093	40.26475	2069
	4	21.10615	40.33471	2075
Shafa	5	21.10615	40.33471	2075
	6	21.11139	40.32981	2093
	7	21.12108	40.32021	2107
	8	21.12739	40.30928	2133
	9	21.13115	40.30467	2148
	10	21.15378	40.26634	2061
	11	21.14938	40.24406	2291
	$\overline{12}$	21.38997	40.34615	1766
	13	21.38783	40.34812	1766
	14	21.38490	40.34523	1763
Hada	15	21.38432	40.34410	1761
	16	21.37738	40.34276	1775
	17	21.36633	40.34149	1706
	18	21.35603	40.34515	1820
	19	20.12440	42.62877	1152
	20	20.11562	42.63089	1144
	21	20.15278	42.60614	1140
	22	20.15474	42.59858	1146
	23	20.17964	42.63998	1128
Bisha	24	20.30380	42.68302	1105
	25	20.19876	42.65363	1122
	26	19.85170	42.61546	1219
	27	19.91957	42.61074	1196
	28	19.94342	42.61649	1187
	29	20.01062	42.47854	1195
	30	19.99253	42.42082	1215
	31	21.21265	40.53893	1609
	32	21.21967	40.55899	1620
Wahatt	33	21.26203	40.55278	1563
	34	21.23424	40.53042	1579
	35	21.21362	40.51605	1603
	36	21.43473	40.49570	1512
Alsail	37	21.6673	40.44169	1143
	38	21.65186	40.39629	1121
	39	21.63541	40.41523	1198
	40	21.63544	40.41525	1198

Table 1. Description of the selected sites with their coordinates (GPS)

2.2 Isolation, Cultivation and Identification of Cyanobacteria

Rippka and Herdman [29] modified medium was used for isolation of Cyanobacteria and the 15 gL^{-1} of agar was used for preparation of solidified media. The cultivation and isolation of the Cyanobacteria that might be persisting in the form of spores, hormogonia, akinetes or any other perennating stages were carried out using the moist plate method. Five replicate Petridishes (9 cm diameter) were inoculated each with 1 ml of the soil extract (1:5) using dilution plate methods and 20 ml of the molten medium (45°C) was added. Petri-dishes were incubated

at $30 \pm 1^{\circ}$ C at $16/8$ h light-dark cycle with a light intensity of 3000 to 4000 Lux. Count of colonies was made after 25-30 days of inoculation, the number of colonies was proportioned to dry weight soil.

Cyanobacterial species was examined by means of a binocular microscope (Zeiss, with camera M35 W) and identified according to the following references: Bourrelly [30,31]. Tell, [32]; Waterbury [33] and Issa et al. [24]. Cyanobacteria sampling will be semi quantitative to determine the dominance or rareness of each species.

2.3 Algal Biomass

The technique of analysis is based on the distinction between phaeophytin-a and chlorophyll-a have absorption beaks in the same regions of the spectrum. The air dried algal crusts of a limited area (obtained with a sharp scalpel using 1.25 cm X 1.25 cm cover glass) was homogenized in 10 mL of 90% acetone and extracted in the dark at 5C for 24 hours. The extract was centrifugated for 5 minutes, then solvent and its component were decanted to a spectrophotometric tube and read at 665 nm and 750 nm before and after acidifying with one drop of HCl conc., for chlorophyll-a estimation and according to algal biomass, the following equations were applied [24].

Chlorophyll-a (mg / m²) =
\n26.37 (665 *b* - 750 *b*) X (665 *a* - 750 *a*) xV\n
$$
A
$$

Where: 665a = optical density reading at 665 nm after acidification, 665b = optical density reading at 665 nm before acidification, 750a = optical density reading at 750 nm after acidification, 750b = optical density reading at 750 nm before acidification, $V =$ volume in liters of extracting solution, $A =$ Area of the sample.

2.4 Data Analysis

Two-way indicator species analysis (TWINSPAN), for classification, and detrended correspondence analysis (DCA), for ordination, were applied to the presence percentage of 31 cyanobacterial species in 40 stands to recognize the Cyanobacteria communities in the study area [34,35,36,37]. Species turnover (beta-diversity) is calculated as a ratio between the total number of species recorded in a certain site and its alpha diversity [38]. Relative evenness or equitability (Shannon-Weaver index) of the importance value of species was expressed as $\hat{H} = -\sum^{s} P_i$ (Log P_i), where S is the total number of species and P*ⁱ* is the relative importance value (relative cover) of the species. The relative concentration of dominance is the second group of heterogeneity indices and expressed by Simpson's index: C = Σ^s (P_i)², where S is the total number of species and \overrightarrow{P}_i is the relative importance value (relative cover) of species. More details about these indices are available in Pielou [39] and Magurran [40]. In order to detect correlations between Cyanobacteria parameters and environmental

data, canonical correspondence analysis (CCA) according to Ter Break and Smilauer [41] was conducted with species recorded in stands and soil variables using the second matrix [42]. Means, standard deviations (SD) and one-way analysis of Variance (ANOVA) were calculated for the means of the soil samples in relation to sites (altitudes) to assess the heterogeneity of samples around their means. These techniques were according to SPSS software [43].

3. RESULTS AND DISCUSSION

Thirty-one cyanobacteria species were isolated along the different sites of the study area belong to 17 genera (Table 2). *Nostoc and Spirulina* had the highest contribution in the study area (four species each), followed by *Chroococcus* and *Oscillatoria* (two species each). Regarding to sites*,* Alsail site was dominated by *Nostoc linckia* Bornet*;* Bisha site was dominated by *Scytonema archangelii* Born*;* Whahatt site are doiminated by *Anabaena circinalis* Rabh*, Merismopedia glauca* Ehrenb and *Synchococcus* sp*.;* Hada site are dominated by *Phormidium* sp*.,* while Shafa site are dominated by *Aphanizomenon flosaquae* Ralfs*.* On the other hand, Shafa site was characterized by high diversity in both cyanobacteria and plant species (Table 3). It had the highest value of total number of cyanobacteria (29 spp = 93.6% of the total species), species richness or α -diversity (6.22 ± 2.86 sp/stand) with total mean of 5.31 ± 2.22 sp/stand, species turnover or β-diversity (4.66) with total mean of 5.84, Shannon index (H) (1.71) \pm 0.54) and Equitability (0.50 \pm 0.16), but the lower of Simpson index (C) (0.07 \pm 0.03). Large parts of the Middle Eastern countries are arid and semiarid regions, and thus characterized by limited higher plant cover. These areas are often densely covered by communities of cyanobacteria, green algae, fungi, lichens, and mosses, which form typical biological soil crusts. The information available on the dominant organisms in biological soil crusts are cyanobacteria. Identification of these organisms is very difficult, as they have different shapes under different growth conditions. In addition, when isolated and cultured, they undergo dramatic morphological, and perhaps physiological, modifications. With these limitations in mind, the recorded cyanobacteria for this area [44] include *Nostoc muscorum* Ag., *Microcoleus chtonoplastes* (Mert.) Zanard., *M. vaginatus* (Vauch.) Gom., *Oscillatoria* sp., and *Botrydium granulatum* (L.) Grev.

Table 3. Some diversity indices calculated for the study sites (Mean ± SD)

Diversity indices	Sites					Total
	Alsial	Bisha	Wahatt	Hada	Shafa	
Total Species	14	25	20	18	29	31
Species richness (α-diversity)	4.50 ± 1.29	5.00 ± 1.94	5.17 ± 2.23	5.17 ± 2.32	6.22 ± 2.86	5.31 ± 2.22
Species turnover $(\beta$ -diversity)	3.11	5.00	3.87	3.48	4.66	5.84
Shannon index (H)	1.47 ± 0.30	1.54 ± 0.41	1.54 ± 0.53	1.54 ± 0.54	1.71 ± 0.54	$1.58 + 0.46$
Simpson index (C)	0.09 ± 0.02	$0.08 + 0.02$	$0.08 + 0.03$	0.08 ± 0.03	0.07 ± 0.03	0.08 ± 0.02
Equitability	0.43 ± 0.09	0.45 ± 0.12	0.45 ± 0.15	0.45 ± 0.16	0.50 ± 0.16	0.46 ± 0.13
Plant species richness	15.33 ± 5.13	$3.36 + 1.96$	19.50±3.83	31.86±10.65	$37.27 + 13.15$	21.92+16.28

Table 4. Total colonies (X 100/gm soil), number of genera, number of cyanobacteria and plant species and Algal biomass (Kg/Hectare) in sampled stands

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Table 5. Means±SD of some soil characteristics collected from represented stands in the study sites

: P≤ 0.05, **: P≤ 0.01, *: P ≤ 0.001*

	Cyanobacteria sp.	Plant sp.	Colonies	Genera	Biomass
Sand	-0.230	-0.338	-0.110	-0.165	-0.130
Silt	0.192	-0.051	0.265	0.142	0.341
Clay	0.121	0.756	-0.244	0.078	-0.336
pH	-0.074	-0.338	0.169	-0.038	0.217
EC	0.031	-0.238	-0.189	0.030	-0.115
TSS	0.002	0.056	-0.167	-0.017	-0.157
OM	-0.086	-0.426	0.355	-0.065	0.448
СI	0.091	-0.243	0.148	0.118	0.181
NO ₃	-0.095	0.244	-0.267	-0.080	$-.318$
HCO ₃	-0.090	-0.250	-0.227	-0.123	-0.168
PO ₃	-0.061	0.037	0.358	-0.082	0.343
Сa	-0.016	-0.290	0.047	-0.061	0.010
Mg	-0.258	-0.517	0.255	-0.207	0.204
Na	-0.036	-0.230	-0.017	-0.066	-0.007
K.	0.119	-0.409	0.174	0.124	0.223
No. of algal		0.122	-0.022	0.967	0.024
species.					
No. of plant spp.			-0.283	0.053	-0.434
Colonies				0.024	0.837
Genera					0.061

Table 6. Pearson's correlation coefficients (r) between cyanobacteria community and soil variables

: P≤ 0.05, **: P≤ 0.01, *: P≤ 0.001*

The plant species, *Juniperus procera* Hochst. ex Endl.*, Ephedra alata* Decne*, Acacia gerrardii* Benth*., Ziziphus spina-chrita* (L.) Desf.*. Calotropis procera* (Aiton) W.T.Aiton and *Aerva javanica* (Burm.f.) Shult. had the highest diversity of Cyanobacteria, while some other species*, Commicarpus sinaicus* Meikle, *Verbesina encelioides* (Cav.) Benth. &Hook. f. ex A. Gray*, Argemone ochraleuca* Sweet*, Haloxylon salicornicum* (Moq.) Boiss.*, Acacia tortolis* (Forssk.) Galasso & Banfi and *Morettia parviflora* Boiss*.* had no cyanobacteria species (Table 4)*.* These results agree with that of Harborne [45] that indicated that the secondary compounds of *Verbesina encelioides* (Cav.) Benth. &Hook. f. ex A. Gray inhibit the germination and growth of other plants and as chemical defense against herbivory. On the other hand, aqueous extract of *Haloxylon salicornicum* (Moq.) Boiss. significantly inhibited the germination, growth and vigor of seedlings of some crops such as *Vigna aconitifolia* (Jacq.) Marechal [46]. Moreover, Noumi and Chateb [47] showed that water extracts of different parts of *Acacia tortilis* (Forssk.) Galasso & Banfi inhibits the germination and the radicel length of receptor plant. These results were also in conformity with those reported that the presence of alkaloids and phenolic compounds in the leaves of *Acacia tortilis* (Forssk.) Galasso & Banfi species, which implies that it, has the potential to inhibit seed germination [48,49,50,51].

The application of the two-way indicator species analysis (TWINSPAN) to the data set of the presence percentage of 31 cyanobacteria species in 40 stands, resulted in agglomerating of 6 groups (communities) at 4^{th} level of classification (Fig. 2A). The application of the detrended correspondence analysis (DCA) indicates reasonable segregation between these groups (Fig. 2B). The first group (community I) dominated by *Merismopedia glauca* Ehrenb*, Microcystis flos-aquae* Smith and *Woella saccata* Wolle*;* community II are dominated by *Merismopedia glauca* Ehrenb*, Microcystis flosaquae* Smith and *Nostoc muscorum* Agard*;* community III are dominated by *Anabaena circinalis* Rabh*, Nostoc linckia* Bornet and *Nostoc muscorum* Agard; community IV are dominated by *Nostoc* sp*, Lyngbya contorta* Lemm*, Spirulina major* Kutz and *Tolypothrix* sp*;* community V are dominated by *Chroococcus minor* Lemm and *Oscillatoria formosa* Bory*;* while community VI are dominated by *Chroococcus minor* Lemm *and Spirulina subsalsa* Nag. It is likely that many new species exist in desert soils, and problems associated with delimitation of cryptic species need to be the subject of future studies. As noted in previous studies, species of *Microcoleus , Nostoc* and *Schizothrix* are the most prevalent cyanobacteria in both arid and semiarid lands [52,53] and are possibly the species most important in developing the appearance of the soil surface. In most cases,

the dominant genera of filamentous
cyanobacteria in hot desert soils are cyanobacteria in hot desert soils are *Microcoleus, Phormidium, Plectonema*, *Schizothrix, Nostoc, Tolypothrix* and *Scytonema* [53].

The physico-chemical factors such as pH, temperature, dissolved oxygen; nutrient contents etc. play a very important role on the growth of the cyanobacteria in different habitats [23,54]. Generally, the sand, silt, clay, organic matter, phosphates, calcium, magnesium and potassium are differed significantly between soils of different sites (Table 5). The application of CCA on the cyanobacteria communities and environmental variables indicated that some cyanobacteria species are correlated positively with total number of associated plants, TSS and phosphates such as *Woella saccata* Wolle*, Chroococcus minor* Lemm*, Chroococcus majore* Lemm*, Microcystis areuginosa* Smith*, Anabaena. Spiroides* Lemm and *Nostoc muscorum* Agard*,* while others are negatively correlated with organic matter, chlorides, pH, EC,

Fig. 2A. Dendrogram of the 6 groups or communities (I - VI) derived after the application of TWINSPAN classification technique. B: Cluster centroids of the 6 groups along the axes 1 and 2 of DECORANA

Fig. 3. CCA Biplot ordination of the cyanobacterial species with environmental variables

clay and silt such as *Oscillatoria Limosa* Bory*, Synchococcus .*sp*, Spirulina major* Kutz and *Lyngbya borgertii* Lemm*.* Some other species such as *Nostoc commune* Vaucher*, Oscillatoria nigra* Vaucher*, Anabaena circinalis* Rabh*, Phormidium* sp and *Nostoc linckia* Bornet are correlated with sand, bicarbonates, nitrates, Na, Ca, Mg and K (Fig. 3). On the other hand, the correlation analysis between soil and community variables indicated a highly negative correlation of plant species richness with sand, pH, organic matter, Mg and K (r =-0.338, -0.338, -0.426, - 0.517 and -0.409; P < 0.01, respectively), but highly positive correlation with clay $(r = 0.756, P)$ < 0.001). The algal biomass had highly negative correlation with clay, nitrates and plant species richness (r = -0.336, -.318 and -0.434; P< 0.01, respectively), but highly positive correlation with silt, organic matter, phosphates and number of colonies (r = 0.341, 0.448, 0.343 and 0.837; P< 0,01, respectively). The number of colonies had positive correlation with organic matter and phosphates ($r = 0.355$ and 0.358, $P < 0.01$ respectively) (Table 6). Berry et al. [55] showed negative correlation between cyanobacteria and nitrate and phosphate contents of organically polluted freshwater body. Cyanobacteria usually prefer neutral to alkaline pH for their optimum growth. Venkataraman [56] observed that diversity of cyanobacteria species were high at temperature 30 0°C to 34 0°C before bloom formation. The species of *Microcystis* were found to be dominant m the lakes of Eastern Algeria and Elphmstone at warm temperature. Jarousha et al. [57] reported that total alkalinity of the Ramgarh Lake of Jaipur ranged between 192 0 and 347 0 mgl⁻¹ with an average value of 274 0 $mgl⁻¹$ and maximum value was during monsoon season. Issa et al. [54] reported that the abundance of cyanobacteria mainly depends upon the favorable contents of oxidisable organic matter of industrial effluents.

4. CONCLUSION

In conclusions, the high altitude at Taif city were richness by wild plant as well as numerous species of cyanobacteria associated to their roots. Some cyanobacteria species positively correlated with total number of associated plants,

TSS and phosphates such as *Woella saccata, Wolle Chroococcus minor* Lemm*, Chroococcus majore* Lemm*, Microcystis areuginosa*, Smith *Anabaena. Spiroides* Lemm and *Nostoc muscorum* Agard that may be useful in many purposes, in agriculture, medicines and industrial that needs more researches.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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