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Presence and variation of cyanobacteria related to the physical properties of soil on the coast of Oaxaca, México

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Abstract: The aim of the research was to determine the influence of particle size (sand, clay, silt) and the water holding capacity (WHC) of soils in coastal Oaxaca, Mexico on the presence of cyanobacteria. These bacteria are present in many different soil types and can function as a bio-indicator of soil quality and fertility. Soil samples were obtained at seven sites along the coast of Oaxaca. These soils are used for the production of maize, sorghum, and papaya crops and the use of agrochemicals has altered the biological activity in the soil and, potentially, the presence or absence of cyanobacteria. The presence or absence of the cyanobacteria genera, or P(r), in the soil at each collection site was then validated by using the confirmatory probability estimation with a radial classification model. In the soils studied, filamentous cyanobacteria predominated (78 %) over non-filamentous cyanobacteria. The greatest diversity of cyanobacteria was found in the soil with highest percentage of sand, medium water holding capacity and without agricultural activity. We consider that the main variables that control the presence of the cyanobacteria genera are the WHC, followed by sand, clay and silt constituents.

Key words: Agricultural soil, diazotrophic cyanobacteria, filamentous, genera composition, radial probability.

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Introduction

Cyanobacteria are photosynthetic prokaryotes and are one of the biological constituents of the soil (Whitton 2000). Cyanobacteria play an important role in the ecology of terrestrial ecosystems because they contribute to soil fertility due to the production of organic carbon (Kabirov & Gaisina 2009) and because they facilitate the processing of fixed atmospheric nitrogen (Pankratova 2006). Like other bacteria or plants, cyanobacteria need nutrients (e.g. minerals, nitrogen, phosphorus,

etc.) and optimal environmental conditions (e.g. salinity, electrical conductivity, dissolved oxygen, etc.) in order to survive in soil (Nayak & Prasanna 2007; Rejmánková & Komárková 2005; Whitton 2000; Yelemou *et al.* 2015).

Manchanda & Kaushik (2000) reported that in aridisol soil with high alkalinity (pH 8.9) and low salinity (CE 2.4 dS m⁻¹) (e.g. Rohtak, India), the number of cyanobacteria species was higher than in soil with high salinity (CE 21 dS m⁻¹) and same pH.

Thamizh & Silvakumar (2011) reported from a

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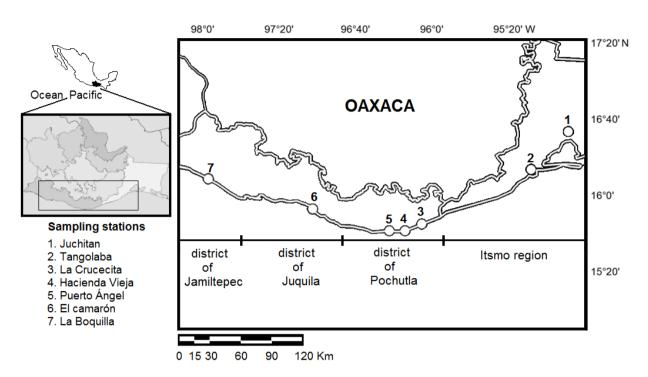


Fig. 1. Study area: coastal zone of Oaxaca, México. Sampling sites: (1) Juchitan, (2) Tagolaba, (3) La Crucecita, (4) Hacienda Vieja, (5) Puerto Ángel, (6) El Camaron, and (7) La Boquilla.

rice field in Tamilnadu, India, that cyanobacteria genera with heterocystous characteristics (e.g. Anabaena, Calothrix, Nostoc) were predominant over cyanobacteria genera that were not heterocystous (e.g. Lyngbya, Oscillatoria, Phormidium). Heterocysts are specialized nitrogen fixing cells formed during nitrogen starvation by some filamentous cyanobacteria genera, such as Nostoc, Cylindrospermum and Anabaena (Whitton 2000).

Some studies that consider the effects of soil particle size (e.g. sand (S), clay (C) and silt (L)) and water holding capacity (WHC) on the formation and establishment of cyanobacteria crusts have been reported previously (Bowker et al. 2010; Delgado-Baquerizo et al. 2010; Yu et al. 2012). Similar studies have not been done so far in Oaxaca, México, and for this reason we considered the evaluation of the presence or absence of cyanobacteria genera at seven sampling stations along the coast of Oaxaca. In order to explain the presence or absence of cyanobacteria genera, multiple correlations were used that included analysis of S, C, L and WHC.

Materials and methods

The climate in southern Oaxaca is warm and wet (Aw₂) and sub-wet (Aw₀) with average tempe-

ratures between 26 and 30 °C all year round. The rainy season is from June to October, with a rainfall range between 500 and 1500 mm per year. Along the coast of Oaxaca there are wide areas of sub-deciduous forest and deciduous forest, where mangrove proliferates (17,297 ha) (INEGI 2013). The predominant soil types are Regosol, Luvisol, while the Phaeozem soil type is least common (Garcia-Mendoza *et al.* 2004). The Regosol soil type is not very deep (from 10 to 30 cm); moreover, it is a geological constituent of beaches and mountain slopes. According to Garcia-Mendoza *et al.* (2004), the Regosol soil type has forestry, agricultural and livestock uses.

Soil samples were obtained at seven agricultural sites along the coast of Oaxaca in from the agricultural cycles of spring-summer and autumnwinter over the years 2009 - 2011 (Fig. 1). The sampling stations are identified according to the name of the community where they are located (Fig. 1). Two sampling stations were located in Juchitan (JU) and Tagolaba (TG) in the Istmo region, three in the district of San Pedro Pochutla, namely: La Crucecita (LC), Hacienda Vieja (HV) and Puerto Angel (PA). El Camarón (EC) was located in the district of Juquila, and La Boquilla (LB) in the district of Jamiltepec. Sampling stations had different land use: EC and TG, culti-

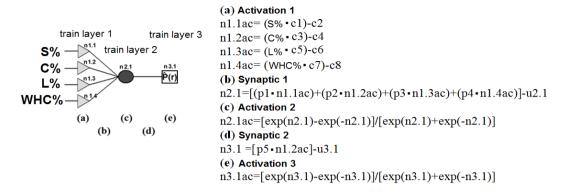


Fig. 2. Radial neuronal classification model to validate P(r). The internal layers consist of ni.j neurons that after the synaptic signal were activated (ac). Coefficients (cn), threshold (un), weights (pn). Sand (S), Clay (C), Silt (L), Water-holding capacity (WHC).

vated with maize (Zea mays L.) and sorghum (Sorgum vulgare L.); PA and LC, uncultivated soil and secondary vegetation; and finally, LB, JU and HV, newly cleared soil cultivated with maize (Zea mays L.).

At each sampling station a square of 1 ha was delimited. Samples were collected within each square following a zigzag pattern from the top right corner to the bottom right corner. At each zigzag point (20 in total) 50 g of soil were sampled at 5 cm depth from the surface and put inside sterile labeled glass vials with a capacity of 250 ml. In addition, between 1 and 2 kg of soil were sampled at 20 cm depth from the surface and put inside labeled plastic bags. All samples were shipped to the Laboratory of Environmental Biotechnology at the Universidad del Mar, Puerto Angel, Oaxaca, México. The first soil samples were used for bacteriological analysis and the second set of soil samples was used for the analysis of physical soil constituents.

For the integrated bacteriological analysis 10 g of soil from each sampling station was added to 5 ml of distilled water. The soil samples were then vacuum-filtered by using Whatman filters # 40 and four aliquots of 4 ml were taken from the supernatant and added to the BG-11 $_{0}$ culture medium (Rippka *et al.* 1979) (with strict sterility conditions). The samples were incubated for 21 days at 25 ± 1 °C with a photoperiod time of 12:12 and a pH constant at 7.5. Cyanobacteria were identified up to the genera level using phase contrast microscope (Olympus BX51) and the taxonomic keys of Geitler (1932) and Komarek & Anagnostidis (1999, 2005).

For the analysis of the soil physical properties, soil samples were dried at ambient temperature and sieved using square mesh of 2 mm. The soils

were then classified according to Gee & Bauder's method (1986) to obtain the soil classification according to soil particle size: S, C and L. This information was recorded in percentages; e.g., S %, C % and L %, respectively. At each sampling station percentage variation was analyzed by using a multiple correlation analysis, including the WHC % which was estimated as follows: triplicate 20 g field-moist soil samples were placed in a funnel and filter paper mounted on a suitable, preweighed collecting flask. The exact soil weight was recorded to the nearest 0.01 g. Distilled water in small portions of 100.0 ± 0.01 ml was added and allowed to stand overnight while covering the funnel with aluminum foil to prevent evaporation. The funnel of the neck of the flask was gently tapped to move water drops adhering to the flask. The collecting flask was weighed to the nearest 0.001 g. Duplicate blanks were run which included the funnel and filter paper without soil. WHC % = $[(100-Wp) + Wi]/dwt \times 100$; where, Wp was the weight of the percolated water in grams, Wi the initial amount of water in grams contained in the sample, and dwt the dry weight of the soil in grams (Forster 1995).

For the statistical analysis we assigned a value of 1 to identify the presence of the cyanobacteria genera in the S %, C % and L %. Conversely, we assigned a value of 0 to identity the absence of cyanobacteria genera in the S %, C % and L %. In both cases the presence and absence of cyanobacteria genera was called P(r). At each sampling station, P(r) values were validated by using a confirmatory probability estimator or $\hat{P}(r)$. In accordance with the recommendation of Haykin (1999), a Radial Neural Networks model was used to estimate P(r) values (Fig. 2).

The Radial Neural Networks model was

constructed with three train layers using the S %, C %, L %, and WHC % as entrance signs. A lineal model was used to activate the first train layer (a) (Fig. 2). Between this train layer and the second train layer (c), a lineal model was used as synaptic signal and, in order to activate the second train layer, a hyperbolic model was used (Fig. 2). Between the second train layer (activated) and the third train layer (e), a lineal model was used as synaptic signal and, in order to activate the third train layer, a hyperbolic model was used (Fig. 2). The third train layer was used in order to estimate P(r) values (Fig. 2).

Seven Radial Neural Networks models were established at each sampling stations (Fig. 2). Although weights (Wn), threshold (Tn) and coefficients (Cn) were estimated in each case, those values were not included here. Finally, $\hat{P}(r)$ values were converted to a logarithm scale (between 0 and 1) in order to compare them with $\hat{P}(r)$ values. $\hat{P}(r)$ values, S% and the total sum of C% and L% were used in order to infer the preference of cyanobacteria genera to inhabit specific soil constituents under WHC% variation effects. The minimum squares were used to optimize the Radial Neural Networks model and developed with the software Statistica® version 7.

Results

The cyanobacteria genera that were identified are given in Table 1. Overall, 45 % cyanobacteria genera were identified as heterocystous (Fischerella sp., Anabaena sp., Nostoc sp., Calothrix sp.), 33 % were identified without heterocystous characteristics (Phormidium sp., Lyngbya sp. and Oscillatoria sp.) and 22 % were unicellular (Microcystis sp. and Dermocarpa sp.).

At the sampling stations, sand was the main soil constituent (between 49 and 76 %) (Table 2). Clay and silt were recorded with low percentage variation that was never dominant: clay showed a variation interval between 3 and 31 %, while silt varied from 10 to 48 % (Table 2). WHC was less than 60 % in JU, TG, LC and PA; and greater than 60 % in HV, EC and LB. In PA, the water holding capacity was close to 60 % (Table 2).

Initially, the WHC % was inversely correlation with the S % (R = -0.76, P < 0.05) and positively correlated with the C % (R = -0.21, P > 0.05) and with L % (R = 0.50, P > 0.05). Due to statistical correlation of clay and lime on the WHC % resulted non-significant, we used the sum of C % and L % in order to generate a better statistical

correlation with the WHC %. Once done, the WHC % was inversely correlated with the S % (R = -0.99, P=0.00) and a direct correlation with the sum of C % and L % (R = 0.74, P=0.05). We observed that the WHC % increased from south to north, and additionally, when the sum of C % and L % was over 40 %, WHC % increased. Whereas when the sum of C % and L % was under 40 %, WHC % decreased (Fig. 3). In JU the aforementioned relation was not observed, but the S % was higher than the C + L %.

Table 1. Relation of the cyanobacteria genera identified at collection sites: Juchitan (JU), Tagolaba (TG), La Crucecita (LC), Hacienda Vieja (HV), Puerto Ángel (PA), El Camarón (EC) and La Boquilla (LB).

Sampling stations	Cyanobacteria genera
JU	Phormidium sp. 3, Dermocarpa sp. 2,
	Oscillatoria sp. 3
TG	Phormidium sp. 2
LC	Nostoc sp. 2, Anabaena sp. 2, Calothrix
	sp. 2, Oscillatoria sp. 2, Phormidium
	sp. 1,
HV	Lyngbya sp. 1, Nostoc sp. 3
PA	Fischerella sp. 1, Anabaena sp. 1, Nostoc
	sp. 1, Calothrix sp. 1, Dermocarpa sp. 1,
	Microcystis sp. 1, Oscillatoria sp. 1,
EC	Fischerella sp. 2
LB	Fischerella sp. 3

Almost all records of the presence or absence of cyanobacteria genera were validated in JU, TG, LC, HV, PA, EC and LB. New presence cases were found (Table 3): case a in HV and EC; case c in EC; case e at all sampling stations; case e in LC and HV; case e in HV and EC; case e in PA and EC. Conversely, case e had less presence in EC, but new presence in JU; and case e and e showed no change.

Anabaena and Lyngbya genera were recorded with low $\hat{P}(r)$ values in sand (Fig. 4) and clay/silt (Fig. 5). Lyngbya genera showed a reduction in their $\hat{P}(r)$ values when S % began to rise and, when clay/silt began to rise (from 45 %), their $\hat{P}(r)$ values increased. Calothrix and Nostoc genera were recorded with high $\hat{P}(r)$ values inside wide intervals of sand (from 47 to 77 %) (Fig. 4) and clay/silt (from 15 to 75 %) (Fig. 5). Dermocarpa and Oscillatoria genera increased their $\hat{P}(r)$ values when S % rise more than 57 % and 52 %, respectively (Fig. 4), and, between 15 and 55 % of clay/silt, their values decreased (Fig. 5). Fischerella

Table 2. Relation of the cyanobacteria genera present (1) and not present (0) in terms of P(r) in relation to the particle size at the collection sites: (a) Anabaena sp. 1 and Anabaena sp. 2, (b) Calothrix sp. 1 and Calothrix sp. 2, (c) Dermocarpa sp. 1 and Dermocarpa sp. 2, (d) Fischerella sp. 1, Fischerella sp. 2 and Fischerella sp. 3, (e) Lyngbya sp. 1, (f) Nostoc sp. 1, Nostoc sp. 2 and Nostoc sp. 3, (g) Microcystis sp. 1, (h) Oscillatoria sp. 1, Oscillatoria sp. 2 and Oscillatoria sp. 3, (i) Phormidium sp. 1, Phormidium sp. 2 and Phormidium sp. 3. Sand (S), clay (C), silt (L), water-holding capacity (WHC).

Ŷ(r)	a	b	c	d	е	f	g	h	i				WHC
JU	0	0	1	0	0	0	0	1	1	53	31	16	30
TG	0	0	0	0	0	0	0	0	1	76	14	10	27
LC	1	1	0	0	1	1	0	1	1	70	14	16	40
HV	0	0	0	1	1	1	0	0	0	64	12	24	65
PA	1	1	1	1	1	1	1	1	0	74	6	20	54
EC	0	0	0	1	0	0	0	0	0	76	9	14	65
LB	0	0	0	1	0	0	0	0	0	49	3	48	77

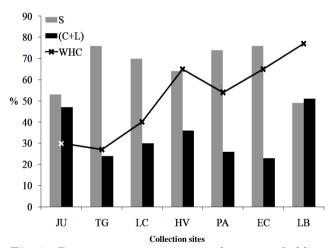


Fig. 3. Percentage variation of the water-holding capacity (WHC) and particle size: sand (S), clay plus silt (C + L); at the collection sites: Juchitan (JU), Tagolaba (TG), La Crucecita (LC), Hacienda Vieja (HV), Puerto Ángel (PA), El Camarón (EC) and La Boquilla (LB).

and *Microcystis* genera were not recorded in sand (Fig. 4), while in clay/silt intervals from 15 to 65 % in their P(r) values were constant (between 0.3 and 0.4) and decreased, respectively (Fig. 5). *Phormidium* genera showed an increase in their P(r) values when S % and clay/silt began to rise (from 69 % and 45 %, respectively) (Figs. 4 & 5).

Table 3. Relation of the cyanobacteria genera present (1) and not present (0) in terms of validated records or $\hat{P}(r)$ in relation to the particle size at the collection sites: (a) Anabaena sp. 1 and Anabaena sp. 2, (b) Calothrix sp. 1 and Calothrix sp. 2, (c) Dermocarpa sp. 1 and Dermocarpa sp. 2, (d) Fischerella sp. 1, Fischerella sp. 2 and Fischerella sp. 3, (e) Lyngbya sp. 1, (f) Nostoc sp. 1, Nostoc sp. 2 and Nostoc sp. 3, (g) Microcystis sp. 1, (h) Oscillatoria sp. 1, Oscillatoria sp. 2, and Oscillatoria sp. 3, (i) Phormidium sp. 1, Phormidium sp. 2 and Phormidium sp. 3.

P (r)	a	b	c	d	e	\mathbf{f}	g	h	i
JU	0	0	0	1	1	0	0	0	0
TG	0	0	0	0	1	0	0	0	1
LC	1	1	0	0	1	1	1	1	0
HV	1	0	0	0	1	1	1	1	0
PA	1	1	1	1	1	1	1	1	1
EC	1	0	1	0	1	0	0	1	1
LB	0	0	0	1	1	0	0	0	0

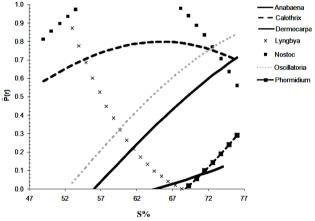


Fig. 4. Trend of the presence of cyanobacteria genera $\hat{P}(r)$ in relation to percentage of sand (S %) at the collection sites. (a) *Anabaena* sp., (b) *Calothrix* sp., (c) *Dermocarpa* sp., (d) *Fischerella* sp., (e) *Lyngbya* sp., (f) *Nostoc* sp., (g) *Microcystis* sp., (h) *Oscillatoria* sp., (i) *Phormidium* sp.

The variation in WHC influenced the presence of cyanobacteria (Table 2). When the WHC was under 30 %, the number of cyanobacteria genera decreased, similarly, when WHC was over 70 %, the number of cyanobacteria genera also decreased, whereas between 40 and 65 % of WHC, the number of cyanobacteria genera increased. The maximum number of cyanobacteria genera was found in PA at 54 % of WHC.

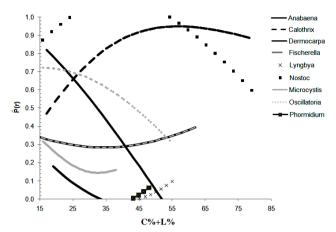


Fig. 5. Trend of the presence of cyanobacteria genera $\hat{P}(r)$ in relation to percentage of clay plus silt (C % + L %) at the collection sites. (a) *Anabaena* sp., (b) *Calothrix* sp., (c) *Dermocarpa* sp., (d) *Fischerella* sp., (e) *Lyngbya* sp., (f) *Nostoc* sp., (g) *Microcystis* sp., (h) *Oscillatoria* sp., (i) *Phormidium* sp.

Discussion

Nine genera of cyanobacteria were identified in the seven soils examined in Oaxaca. The filamentous cyanobacteria dominated (78 %), 45 % which presented heterocystous characteristics. These results are similar to those reported by Bharadwaj & Baruah (2013) and Nayak Prasanna (2007). The soils examined differed in granulometry, with the following pattern: S % > L % > C %. This influenced the establishment of cyanobacteria genera, which showed a preference for the sandy soils, especially the filamentous cyanobacteria that were heterocystous. The preference was confirmed by Manchanda & Kaushik (1997) and Potts (2000). They observed that cyanophytes in general survive adverse conditions where there are nutrient deficiencies and even long periods of desiccation. However, Tomaselli & Giovanetti (1993) have shown that the different genera of heterocystous cyanobacteria have different capacities to survive in clay soil and silt-loam soil in dry conditions. Falchini et al. (1996) have studied the role of cyanobacteria in the maintenance and improvement of the soil structure, they found that the primary aggregation of clays is the consequence of the interaction of exopolysaccharide (EPS) produced by Nostoc AfS49 and KaS35 strains and morphologic units from the fine fraction of the soil.

In the soils of Oaxaca the pattern of filamentous cyanobacteria composition with heterocystous and unicellular types was similar to the one

reported by Mansur & Shaaban (2010), Chun-Xiang et al. (2002), and Hahn & Kusserow (1998). According to the authors, the presence of nitrogenfixing cyanobacteria is characteristic of sandy soil that is poor in nutriments, but not for the unicellular cyanobacteria and the filamentous with heterocystous characteristics. Considering that most of the soils examined have had some agricultural activity, resulting in degradation and erosion, resulting in edaphic conditions similar to those of poor soil. This probably explains the similar and low cyanobacteria diversity in these soils as compared to the results of Thamizh & Sivakumar (2011) in soils dedicated to the cultivation of rice, where 35 species of cyanobacteria belonging to 12 genera were identified, out of which four were heterocystous and eight nonheterocystous. Similarly, Zancan et al. (2006) described 92 taxa of algae, 23 of these belonging to cyanophyceae; however, cyanobacteria were not abundant and showed little diversity or were even absent in soils cultivated with corn and fertilized with large amounts of inorganic fertilizers for many years. This inducing a periodic loss of algae species in general and of cyanobacteria in particular filamentous types, like Nostoc sp., Phormidium sp. and Pseudoanabaena sp. (Kuzyakhmetov 1998; Zancan et al. 2006).

The soil in PA and LC has the largest diversity of cyanobacteria (7 and 5, respectively). These soils, although they had a high content of sand and medium water retention has not had agricultural activity. On the other hand, the soils with more agricultural activity (TG, EC), despite presenting percentages of sand and clay similar to the ones in PA and LC, only had one filamentous genera of the heterocystous type (Fischerella). It is probable that the agricultural practices, including the application of pre-emergent herbicides and chemical fertilizers, especially the ones with nitrogen, and because of the high concentrations of ammonium, inhibited for establishment and growth nitrogen-fixing cyanobacteria, as stated by Zancan et al. (2006).

The cyanobacteria *Nostoc*, was only present in soils without agricultural activity and without a defined vegetal surface, thus presenting some similarities with the results reported by Dubovik *et al.* (2007) and Tiwari *et al.* (2005). They mentioned that *Nostoc* is found mainly in places without upper plants as land cover. They also proposed that the presence or absence of *Nostoc* can, therefore, be considered as an indicator of the pastoral load. Furthermore, *Nostoc* creates favo-

rable conditions for the growth of other representatives of the soil biota and microscopic algae. From the results presented by Malam *et al.* (2009), the *Nostoc* genera showed the biggest colonial increase during the humidification of the soil surface. In the present research, *Nostoc* had a probability of 0.5 for being found in soils with a WHC between 35 and 85 %; this is consistent which was reported by the same author.

In the case of Oscillatoria and Phormidium, Kuzyakhmetov (1998), states that they adapt well to unfavorable conditions, especially in soils with strong disturbance and with an absence of plants. However, in this research, Oscillatoria was present in places with a WHC between 30 and 54 % (LC. PA, JU), while Phormidium was present in places with a WHC of 27 to 30 % (TG and JU). The reason why cyanobacteria dominate the sandy soils is its ability to resist desiccation during long periods of time because of the production of mucilage that is used to store water. Besides, the capacity of cyanobacteria to retain water and to maintain a humid environment is also related to the physicalchemical properties of the soil, that is, the hydrophilic and hydrophobic molecules that liberate the cyanobacteria to the environment (Decho 1990; Kidron et al. 1999; Stainer & Cohen-Bazire 1977). The polysaccharides exuded to the medium are adhesive hydrophilic exopolymers that allow the cells to stay linked to other cells or to particles of the environment (Decho 1990), while the glycoprotein and lipo-polysaccharides that were found are hydrophobic molecules that increase the adhesive forces between the adjacent filaments (Kidron et al. 1999; Malam et al. 2001). Mazor et al. (1996) found that high concentrations of polysaccharides in sandy soils reduce the quantity of water that penetrates the soil. This suggests that the capacity for water retention is related to the hygroscopic properties of the polysaccharides, allowing cyanobacteria and other microorganisms to endure the osmotic stress from the water and from the matrix (De Winder et al. 1989) and its recuperation in the periods of dryness (Ernst et al. 1987). On the other hand, the mucilage not only improves the stability of the additives, protecting the surface from erosion (De Winder et al. 1989), but also provides a source of additional carbon that, along with the nitrogen fixes the heterocystous cyanobacteria, facilitates and improves the biological activity of the soil (Lange et al. 1994; Zaady et al. 1998).

Conclusions

In the studied soils from the coast of Oaxaca and through a probabilistic-statistical analysis filamentous cyanobacteria (78 %) with heterocystous characteristics were shown to dominate over non-filamentous cvanobacteria. Cvanobacteria are known for colonizing soil that is poor in nutriments and for enduring long desiccation and the cyanobacteria examined here show a selection of habitats according to particle size. We found that they express a preference for sandy soils with medium WHC and no agricultural activity. The Lyngbya genera presented the highest probability to establish P(r) in a soil with a WHC ranging from 27 to 65 %. Although Lyngbya were not found at all of the collection sites with the identification technique used, the probability analysis suggested that this type of cyanobacteria is more common. It is, therefore, necessary in the future to use molecular techniques to identify this species. In summary, we propose that the main variable controlling the presence of cyanobacteria in soils is WHC %, followed by S %, C % and L % constituents.

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