

Bacterial morphotypes as biodiversity indicators in a scarcely explored ecosystem in Santiago de Cali, Colombia

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Abstract

Bacteria are considered as the most diverse microorganisms on earth. However, bacterial diversity is unknown in some ecosystems, such as leachate treatment plants. In the present study, we characterized culturable bacteria isolated from six lagoons and one pure leachate to assess whether bacterial morphotypes can be used as biodiversity indicators. The sampling site was a leachate treatment plant located in Santiago de Cali. Overall, 311 bacterial morphotypes were collected from six lagoons, and a pure landfill leachate, and were identified using colony characters. Four biodiversity indexes related to species richness and abundance as well as to the estimation of the number of species present were calculated. The results show a great diversity of bacterial morphotypes in all samples. Further, the highest estimates of biodiversity were observed at lagoon 6 physicochemically treated, and the lowest estimates were observed at lagoons 5 and 8. The leachate generated in the sampled lagoons, which were composed of different elements, allows for the colonization of diverse bacteria. Moreover, these bacteria were resistant to the treatment plant processes, thereby explaining the highest diversity observed at the lagoon 6. Our results showed that bacterial morphotypes can be used as biodiversity indicators in ecosystems.

Introduction

Colombia is considered a vastly diverse country with a great diversity of flora and fauna¹⁻³. Mountainous ecosystems and tropical forests extensively contribute to the biodiversity of the country, where approximately 58,312 species have been recorded without considering the enormous diversity of microorganisms⁴. Currently, a list of 100 bacterial species registered in Colombia was published⁵. Bacteria are organisms that belong to the domain Bacteria and have been found in different environments⁶. In Colombia, studies in bacterial isolates from soil⁷ air⁸, and water⁹ have been performed. However, the presence of bacteria and their diversity in some areas remain unknown. An example of these ecosystems is the leachate treatment plant located in the Antiguo Vertedero de Navarro (old Navarro landfill), a place of environmental importance because it was a landfill for >40 years. Since 2014, the plant has been in operation to collect and conduct leachates to the treatment system and subsequently manage leachates through physicochemical treatments¹⁰.

Biodiversity is a measure of variation between different organisms and is classified into three levels: genes, species, and ecosystems^{11,12}. There are mathematical operations for the estimation of alpha, gamma, and beta biodiversity^{13,14}. Alpha diversity quantifies species richness in a community and beta diversity estimates the degree of species differentiation at a certain geographic site. Further, gamma diversity is the richness of species in a community integrating the landscape¹⁵.

In ecology, the most used indexes are Simpson (D) and Shannon–Wiener (H')¹⁶, which have been applied in biodiversity studies in different organisms such as plants^{17,18}, mosquitoes^{19,20}, and fungi isolated from soil^{21,22}. The D index reflects the heterogeneity of the sample considering two factors, the number of species and their relative abundance²³. This index has been commonly used in the analysis of the species richness of bacteria of clinical and environmental interest that have been identified up to genus and/or species level using molecular techniques²⁴⁻²⁶. In Aquixtla, Mexico, the diversity of Enterobacteria present in soil and in tomatoes was evaluated in three greenhouses that used a fertigation system. Three indexes were estimated: the H' index indicated differences in the microbiota, the D index showed that few species are predominant in this bacterial community, and the Chao index (SChao1) showed a greater abundance of *Enterobacter cloacae* in tomatoes from the three greenhouses²⁷. In another study, the structure of a cultivable heterotrophic bacterial community was evaluated in samples obtained from three sampling sites of the Almendares River in La Habana, Cuba. The D and H' indexes showed significant differences in the bacterial diversity at the three sampling sites²⁸. In Colombia, 1,060 microbial morphotypes (bacteria and fungi) were isolated from Los Nevados National Natural Park; the morphotypes were macroscopically and microscopically characterized as well as analyzed using 16S rDNA sequencing⁷. In the mentioned studies, bacteria were evaluated as estimates of biodiversity and were identified at the genus and/or species level. However, to date, to the best of our knowledge, no studies on the use of bacterial morphotypes as biodiversity indicators when bacteria were only macroscopically and microscopically characterized have been conducted. Therefore, in the present study, four biodiversity indexes were evaluated to determine whether the bacterial morphotypes isolated from a leachate treatment plant can be useful as biodiversity indicators. This study contributes to the knowledge of microorganism biodiversity in a scarcely explored ecosystem that currently benefits the city of Santiago de Cali, Department of Valle del Cauca, and the country itself.

Methods

Study site and morphotypes determination

The Antiguo Vertedero de Navarro is located in the southwest of Colombia in the city of Santiago de Cali, Department of Valle del Cauca (Figure 1). Sampling was performed from March to October 2019 using a methodology described for conducting rapid biodiversity inventories⁵⁵. The plant was visited three times and each lagoon was divided into quadrants (3–6) depending on the structure and access. Moreover, samples were collected from a pure leachate located diagonally to the leachate treatment plant. Leachate samples were collected in sterile bottles and transported to the microbiology laboratory of the Universidad Santiago de Cali. Serial dilutions from 10⁻³ to 10⁻⁶ were prepared using nutritive agar as the culture medium to identify the largest possible number of bacterial morphotypes. Bacteria were plated in triplicates and grown for 48–72 h at room temperature⁵⁶. Bacterial morphotypes were identified using taxonomic characteristics such as color, form, size, elevation, surface, margin, and texture⁵⁷. Consecutive numbers were assigned to each morphotype, and Gram staining and spore staining were performed, last in gram-positive bacilli only.

The results were recorded in an Excel 2016 file. Additionally, a photographic record for each of the morphotypes was obtained. Morphotypes were stored in 1.5-mL tubes with nutrient broth and skim milk agar at -20°C .

Biodiversity analysis

Four indexes were used to estimate the alpha biodiversity from bacterial morphotypes. The Simpson index of diversity or dominance, calculated as $D = \sum p_i^2$ (1), evaluates the probability that two individual samples collected at random belong to the same species⁵⁸. In the present study, this corresponds to the probability that two bacterial morphotypes collected at random have the same morphotype. The Shannon index of species richness, calculated as $H' = -\sum p_i \ln p_i$ (2), assumes that the individual samples are randomly selected and are all represented in the sample⁵⁸. Therefore, the number of morphotypes present in a sample (species richness) and the relative number of individuals of each morphotype (abundance) are considered. The Pielou's species evenness index, calculated as $J = \frac{H}{\ln(S)}$ (3), estimates the proportion of the observed diversity in relation to the expected maximum⁵⁸. In the present study, we evaluated whether the morphotypes in the samples are equally abundant or whether some morphotypes are present in a greater abundance than the others. The non-parametric index, calculated as $Chao1 = S + \frac{a^2}{2b}$ (4), estimates the number of species in the sample based on the number of species represented by one individual and the number of those represented by two individuals⁵⁹. This index was applied to estimate the number of morphotypes that should be present in the different lagoons. In the equations, S represents the number of bacterial morphotypes present in a sample, the proportional abundance of the morphotypes divided by the total number of morphotypes, a is the number of species represented by one individual, and b is the number of species represented by two individuals.

Results

First item

Overall, 765 isolates were obtained. After careful examination, 311 bacterial morphotypes showing differences in form, elevation, margin, and color were identified (Figure 2, Table 1). Some of these morphotypes were reported in an educational booklet addressed to the university community of the Universidad Santiago de Cali²⁹. Only 3 of the 311 morphotypes were present in the 6 sampled lagoons and the pure leachate. These morphotypes were assigned the numbers 34, 38, and 41. The morphotypes 38 and 41 were collected at all 3 samplings (March, August, and October), whereas morphotype 34 was collected in the August and October samplings. Typically, a high number of morphotypes was observed, ranging from 8 to 19 in March 14 to 63 in August, and 16 to 55 in October (Figure 3). Gram staining showed that most morphotypes were gram-positive [76.2% (236/311)], whereas a lesser extent of gram-negative morphotypes [4.8% (16/311)] and gram-variable morphotypes [19% (58/311)] were observed (Figure 3, Table 1). Bacilli was the predominant microscopic form (186/311). Additionally, 92.5% (172/188) of the gram-positive bacillary morphotypes produced spores, whereas 7.5% (14/188) did not produce spores.

Second item

The diversity indexes obtained according to lagoon and sampling date are presented in Table 2. A Simpson index (D) close to 0 indicates a greater diversity in the sample. Lagoon 6 presented the highest estimation of diversity in the global analysis, particularly in the second and third samplings (Table 2). Shannon index (H') values <2 represent low diversity, whereas values ≥ 3 represent a high diversity in the sample. In the first sampling occasion, all the studied lagoons showed low diversity, whereas in the second sampling occasion, lagoons 5 and 6 presented high diversity. In the third sampling, lagoons 4, 5, and 8 showed low diversity, and in the global analysis lagoons 5 and 8 showed low diversity. Moreover, lagoon 6 had the highest diversity according to the Shannon index (H') (Table 2). A Pielou's index (J) close to 0 represents little evenness in the sample, and a value of 1 represents total evenness. At the first sampling, all the results showed little evenness. At the second sampling, lagoon 6 and the pure leachate presented high evenness, indicating that the abundance of the different bacterial morphotypes present in these lagoons is evenly distributed. At the third sampling as well as in the global analysis, lagoon 6 showed the largest evenness. However, lagoons 5 and 8 presented low evenness at the third sampling and in the global analysis (Table 2). For the Chao1 index, we hypothesized the presence of numerous bacterial morphotypes. However, the estimation of numerous bacterial morphotypes was not possible in the first sampling from lagoons 5 and 7 and the third sampling from lagoon 4. The Chao1 index is based on the morphotypes represented by one individual and the morphotypes represented by exactly two individuals. In the present study, the equation could not be applied because values of 0 were found. According to this index, it was estimated that, in the first, second, and third sampling occasions, lagoon 3, lagoon 8, and pure leachate would have the highest amount of morphotypes, respectively. In the global analysis, lagoon 7 was estimated to present the highest number of bacterial morphotypes (Table 2). Cumulatively, these results suggest that lagoons 3, 7, and 8 and the pure leachate provided the highest number of bacterial morphotypes in the study site.

Discussion

Bacteria are the most abundant microorganisms in the world and their diversity has not yet been completely elucidated³⁰⁻³⁴. In the environmental field, bacteria have been useful in the analysis of water quality^{35,36}, in the evaluation of soil contamination^{37,38}, and as the indicators of food quality^{39,40}. Regarding biodiversity, studies on the diversity of bacterial communities in treatment plants have identified bacterial genera and/or species^{41,42}. In addition, in a recent report, bacterial morphotypes were used as biodiversity indicators in moorland ecosystems in Colombia. However, in that study, the

morphotypes were identified using conventional bacterial taxonomic characteristics and 16S rDNA sequences⁷. In the present study, bacterial morphotypes only macroscopically and microscopically identified were evaluated. Although bacteria were not identified at the genus and/or species level, our results demonstrate that bacterial morphotypes can be used as biodiversity indicators. The inference is based on the fact that the bacterial morphotypes identified in the lagoons and pure leachate are characterized based on different taxonomic characteristics, rendering them different from each other. Morphotypes 34, 38, and 41 were identified as dominant morphotypes because they were found in all samplings. However, this represents a minimal percentage because each lagoon and the pure leachate contain unique morphotypes, which are representatives of their own biodiversity. In addition, our results allowed the evaluation of two indexes related to species richness (Simpson and Shannon), one index related to the evenness of the bacterial morphotype distribution (Pielou's), and the Chao1 index, an estimator of the number of species.

The Simpson index showed that there is high diversity among bacterial morphotypes in the six sampled lagoons and the pure leachate, showing that these microorganisms are present throughout the treatment plant contributing to its operation. Reportedly, a high bacterial diversity indicates that the bacterial community is in constant interaction defining inter- and intra-specific relationships for the functioning of the ecosystem^{43,44}. In the sampled lagoons and the pure leachate, we consider the role of bacteria to be crucial for the operation of the treatment plant.

The global analysis results of the Shannon species richness index suggested that lagoon 6 is the most diverse, i.e., the one presenting the largest number of morphotypes. This is important because this lagoon is supplied with the waste that the leachate treatment plant cannot process. In addition, evaporation occurs in this lagoon, and the lagoon is subjected to physicochemical treatments that bacteria could use as a nutrient source. Bacteria present in lagoons 3, 5, 7, and 8 (lagoons for the collection and conduction of leachate to the plant) may be resistant to physicochemical treatments, resulting in a higher diversity in lagoon 6. Further, these results suggested that the resident bacteria were able to degrade pollutants that were not degraded by the previous chemical treatment^{45,46}. Most of the isolated morphotypes were gram-positive spore-forming bacilli. This is in contrast to previous studies performed in this type of ecosystems where gram-negative bacteria were the main representatives⁴⁷⁻⁴⁹. Spore-forming bacteria may be advantageous compared with non-spore-forming ones, considering that under adverse conditions, they can form spores to protect themselves until the appropriate conditions for growth and development are present^{50,51}.

According to the results of the global analysis of the Pielou's evenness index, all bacterial morphotypes were evenly distributed in the studied lagoons, except for lagoons 5 and 8. Although the Simpson index indicated a diverse bacterial population, the bacterial morphotypes were not evenly distributed in these lagoons. Consequently, these lagoons presented the lowest values in terms of abundance and evenness of morphotypes. These results are important because they demonstrated that bacteria were not evenly distributed in an environmental matrix³⁴. Besides the low diversity of bacterial morphotypes in lagoons 5 and 8, these results indicated that some of the morphotypes were present in a higher proportion than the others, suggesting that these lagoons, which collect leachate and are not subjected to any treatment, present optimal conditions for the development of certain bacteria that has the ability to produce metabolic waste that limits the growth of other bacteria or that limit the nutrients from their environment when optimally developed, thereby hindering further bacterial colonization⁵².

The bacterial morphotype compilation described in the present study contributes to the knowledge of bacterial diversity in – scarcely explored ecosystems, such as the leachate treatment plant located in the Antiguo Vertedero de Navarro. Some studies have proposed the identification of bacterial phylotypes and determination of their abundance and ecological attributes^{34,53,54}. In the present study, we proposed the use of bacterial morphotypes as biodiversity indicators because a wide morphotype diversity was observed in the six studied lagoons and the pure leachate. Moreover, the correct operation of the plant is probably attributed to the resident bacteria, which could be spore-forming extremophilic bacteria. Furthermore, because these morphotypes may represent bacterial species having the potential of degrading different compounds, further studies for the identification of genus and/or species as well as degradation studies are warranted. Finally, the results of this study and use of bacterial morphotypes as biodiversity indicators may constitute a valuable input for biodiversity, taxonomic, and systematics studies.

Declarations

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Tables

Table 1. Characteristics of bacterial morphotypes obtained from the landfill leachate treatment plant

Morphological characteristics	Isolates	Photography	
Form	Circular	144	
	Evase	7	
	Filamentous	6	
	Puffiform	13	
	Indeterminate	43	
	Irregular	22	
	Lobate	6	
	Paucifidens	37	
	Rhizoid	27	
	Oval	1	
Urchinate	5		
Margin	Entire	51	
	Erode	3	
	Filamentous	21	
	Indeterminate	35	
	Irregular	40	
	Lobate	17	
	Rounded	81	
	Urchinate	63	
Elevation	Convex	114	
	Flat	147	
	Hilly	18	
	Flat in the middle	5	
	Indeterminate	6	
	Urchinate	21	
Texture	Creamy	233	
	Coarse	16	
	Filamentous	1	
	Indeterminate	8	
	Stocheraceous	51	
Color	Binate	36	
	Colorless	15	
	Indeterminate	26	
	Orange	46	
	Pink	13	
	Red	2	
	White	133	
Yellow	40		
Surface	Smooth	221	
	Rough	99	
Gram classification	Gram-negative	15	
	Gram-positive	237	
	Gram-variable	59	

Table 2. Diversity indexes applied to bacterial morphotypes

Lagoon	First sampling (March)				Second sampling (August)				Third sampling (October)				Global analysis			
	D	H'	J	SChao1	D	H'	J	SChao1	D	H'	J	SChao1	D	H'	J	SChao1
L3	0.77	0.73	0.25	147	0.16	2.26	0.86	39	0.15	2.45	0.63	72	0.15	2.6	0.63	72
L4	0.56	1.48	0.53	100	0.28	2.2	0.64	55	0.29	1.91	0.61	NA	0.15	2.69	0.64	NA
L5	0.92	0.28	0.13	NA	0.12	3.05	0.80	130	0.39	1.28	0.46	34	0.31	1.68	0.41	34
L6	0.49	1.35	0.50	31	0.05	3.04	0.97	48	0.11	2.76	0.86	32	0.10	3.21	0.79	32
L7	0.46	1.44	0.56	NA	0.22	2.75	0.66	153	0.21	2.16	0.57	84	0.12	2.94	0.63	84
L8	0.93	0.23	0.10	18	0.09	2.87	0.87	745	0.39	1.52	0.45	54	0.25	1.97	0.48	54
PL	0.58	1.16	0.44	74	0.06	2.9	0.95	202	0.15	2.45	0.60	128	0.13	2.57	0.59	128

D = Simpson index, H' = Shannon index, J = Pielou's index, SChao1 = Chao1 index, NA= not available

Figures

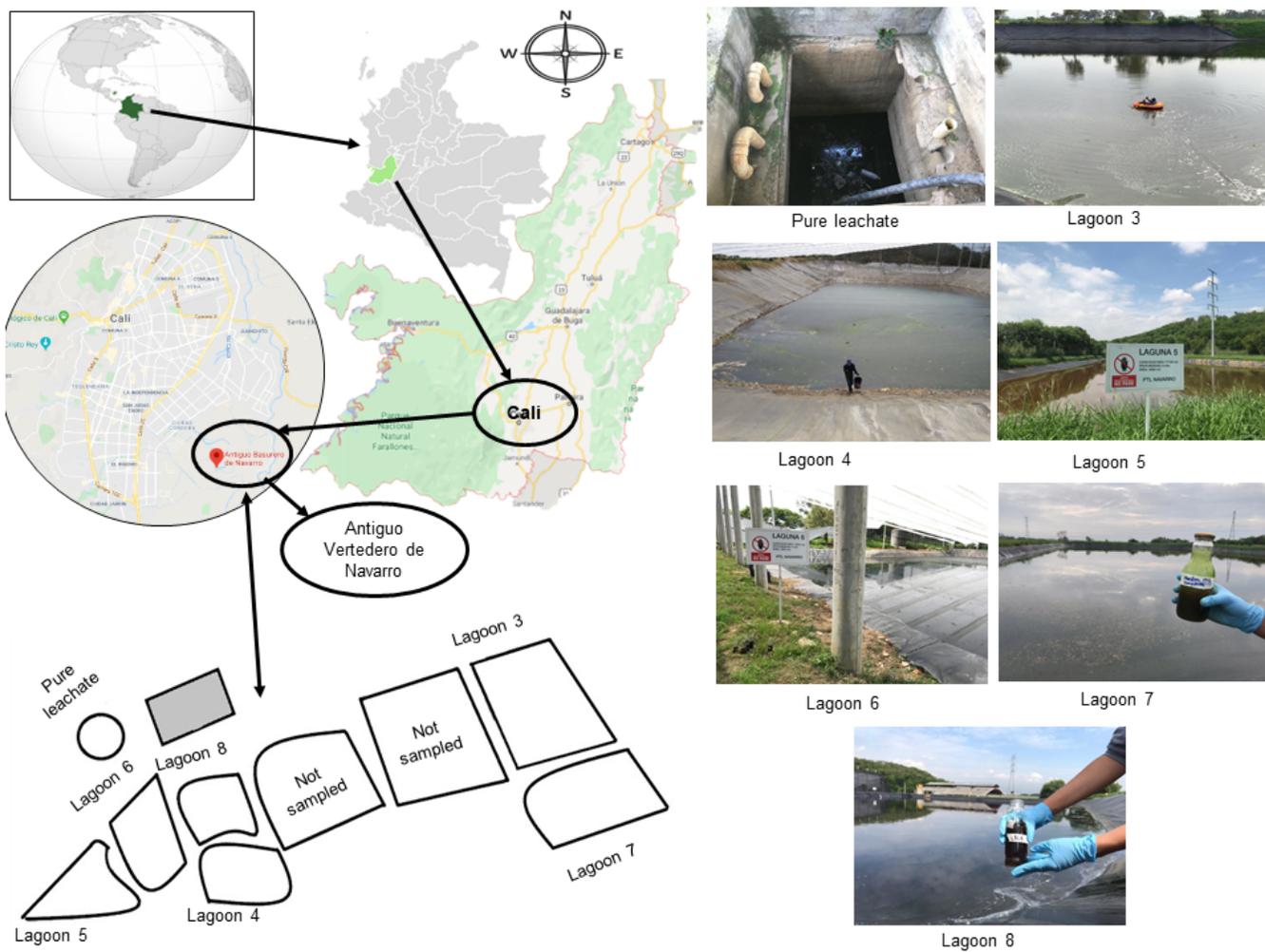


Figure 1

Landfill leachate treatment plant located at Valle del Cauca, Colombia Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

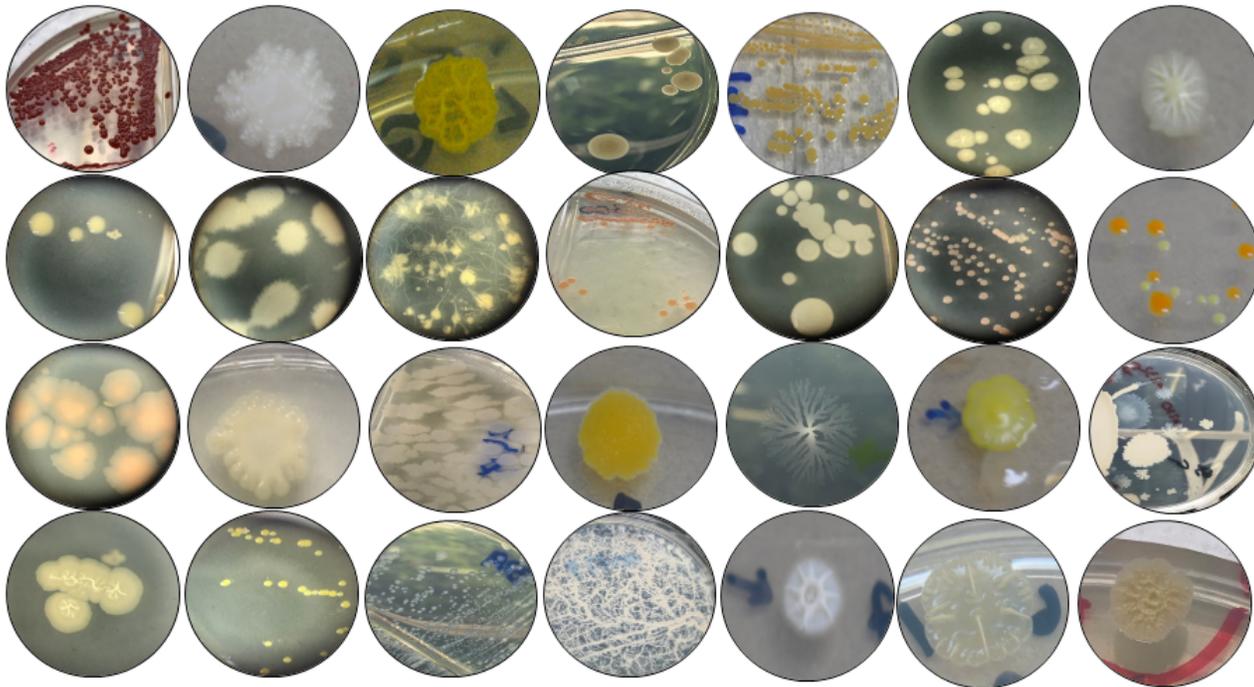


Figure 2

Photography of some bacterial morphotypes obtained from the landfill leachate treatment plant

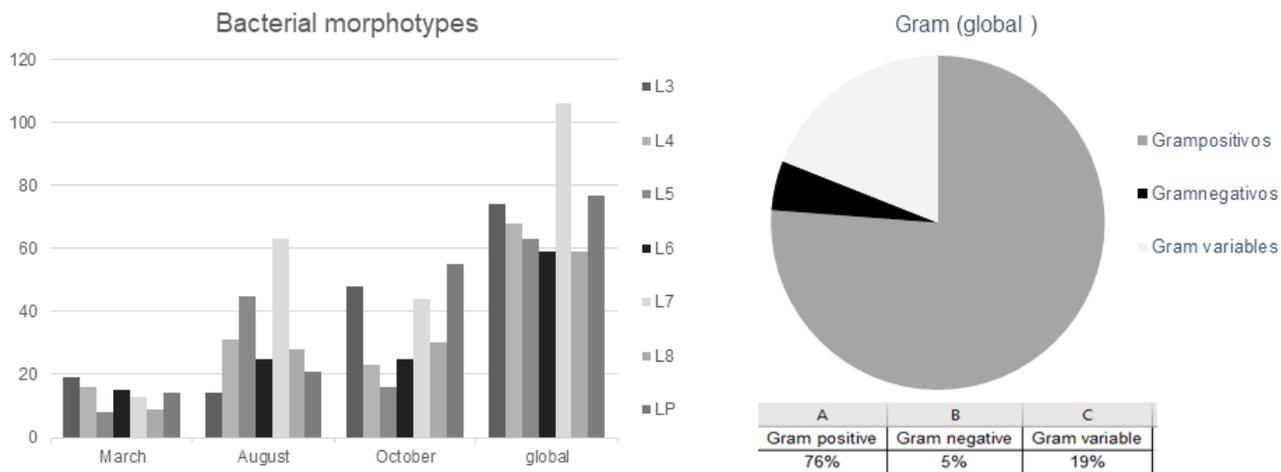


Figure 3

Number of bacterial morphotypes collected in the three samplings conducted at the studied lagoons and pure leachate and bacterial differentiation based on Gram staining