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Microbiological analysis of cigarette butts and cigarette butt fibers on a tourist beach in Cartagena, Colombia

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Cigarette butts (CBs) discarded on tourist beaches represent an emerging waste concern in marine-coastal ecosystems due to their persistence, low degradability, and toxicity. This study investigated CBs, cigarette butt fibers (CBFs), and beach sand in Cartagena, Colombia, as potential substrates for microbial growth. Samples were collected over five months from different beach usage zones (active, rest, and service) and analyzed for bacterial isolation, morphological and biochemical characterization, 16 S rRNA gene sequencing, and the presence of total coliforms *and Escherichia coli*. The results revealed the presence of both Gram-positive and Gram-negative bacteria in sand, CBs, and CBFs. Notably, *Virgibacillus pantothenticus* and *Virgibacillus dokdonensis* were identified and documented for the first time in Colombia. These findings contribute to understanding the sanitary and environmental quality of tourist beach sand and highlight potential risks to human health. Moreover, the identification of *V. dokdonensis* in cigarette butts discarded on beach sand is particularly relevant, as previous research suggests that certain bacterial taxa may have bioremediation potential for heavy metals.

Keywords Microorganisms, Cigarette butts, Cigarette butts fibers, Sand, Bacteria, Beach

CBs are waste commonly found on tourist beaches and are composed of cellulose acetate fibers. These are persistent plastic wastes in the environment that break down into various types of microplastic fibers (MPs)[1]. MPs refer to plastic particles that are less than 5 mm in size. These particles are either created in micro-sizes and released into the environment (primary MPs) or result from the breakdown of larger plastics due to environmental factors (secondary MPs), thus producing pollution in coastal marine areas[2–4]. MPs can be quickly colonized by a microbial biofilm, giving rise to distinct communities known as the plastisphere, where potential pathogens may also be present[5]. The survival capacity of these potential pathogens during transitions between different environments increases their persistence and the risk of spreading in the environment. For instance, *Escherichia coli*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa* were able to survive and persist on polyethylene or glass particles, whether they remained in a single environmental matrix or moved between different environmental matrices. Furthermore, it was found that the concentrations of these bacteria were higher on the microplastic particles compared to glass[6].

The use of tourist beaches for recreational activities in water and sand provides opportunities for human exposure to this microorganism-colonized plastic waste, with the potential for pathogen transfer and subsequent risks to public health[7]. Plastic microbeads on beaches near urban areas have shown higher concentrations of potential pathogens in the plastisphere. These urban sites are located near large wastewater treatment plants (i.e., which also receive hospital effluents), and this is likely a significant source for plastics being colonized by pathogens. The potential for pathogens to persist in the plastisphere and the subsequent transport and dispersion of microplastics across the landscape increase the possibility of human exposure routes and environmental transfer pathways[5].

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Previous studies, such as the analysis conducted on the beaches of the Madeira Archipelago, Portugal, explored the relationship between the presence of microorganisms and characteristics such as sand granulometry and chemical composition. These parameters did not prove to be decisive for the presence of microorganisms[8]. However, it is necessary to investigate whether factors such as sand granulometry and temperature play a critical role in the decomposition processes of residues, such as CBs transforming into CBFs and, subsequently, into fiber-type microplastics. Data from the beaches of Tenerife, Canary Islands, indicated that 85.7% of the analyzed fragments and 57.1% of the pellets showed the presence of intestinal enterococci[9]. Furthermore, research has indicated that *E. coli* populations in the environment can be influenced by environmental factors that affect their long-term viability[10].

In this study, the potential for microbial proliferation in cellulose acetate, a key component of cigarette butt filters (CBFs), and discarded CBFs across different beach use zones (active, rest, and service) in Cartagena, Colombia, was investigated for the first time. Notably, *Virgibacillus dokdonensis* and *Virgibacillus pantothenticus* were identified in the CBF samples collected. Total coliforms were detected in CBs from the rest and service zones, as well as in CBFs from the rest zone, where higher *E. coli* concentrations were also observed.

Principal Component Analysis (PCA) revealed that the first principal component, accounting for 44.18% of the variance, was primarily influenced by variables such as *E. coli* in the sand, CBs, and CBFs, as well as total coliforms in the sand, CBs, and CBFs. The second component, contributing 28.32% of the variance, was predominantly influenced by total coliforms in CBs, *E. coli* in CBFs, and sand temperature. The PCA results showed that active and service zones clustered closely within replicates but remained distinct from each other, highlighting patterns that warrant further investigation. These findings open avenues for future research to better understand human behavior in different beach use zones and assess the potential health risks associated with CBs presence due to direct user exposure.

Materials and methods

Study beach and monitoring campaigns

The study area is Bocagrande Beach (Cartagena, Colombia), located at coordinates 75° 33' 42.0" W 10° 28' 56.7" N (Fig. 1). This urban tourist beach was selected for this study due to previous research monitoring environmental quality on tourist beaches, specifically assessing solid waste in the sand as part of the Ibero-American Network of Beach Certification (PROPLAYAS) project. Between June 2021 and May 2022, high abundances of cigarette butts (0.5–1.0 CBs/m²) and cigarette butt fibers (0.1–0.2 CBFs/m²) were documented at the study site[11].

The sampling area spanned a 500-meter section of the beach, with monitoring campaigns carried out over five field surveys between August and December 2022, encompassing both rainy and dry seasons. CBs and CBFs samples were collected to assess their densities in the sand throughout the study period. The collected materials were stored in reusable glass jars and transported to the laboratory, where they were counted separately as individual units of cigarette butts and fibers, categorized by each sampling zone. Meanwhile, duplicate composite samples of CBs, CBFs, and sand were gathered for microbiological analysis. Sampling was performed in three zones, classified according to areas identified based on the activities conducted in each, considering the recreational and tourist nature of the beaches under investigation. The following considerations were applied to the project. (A) Active area: along the coastline, around the waterline; intended for sports and recreational activities such as walking, running, beach soccer, and beach volleyball. This area allows access to the bathing zone. (B) Rest area: designated for the relaxation and rest of visitors, equipped with umbrellas, chairs, and other services. (C) Service area: occupies the innermost part of the beach, where tourist and support services like bars, restaurants, and souvenir shops are located[11,12]. Zoning was considered to identify potential differences in the concentrations of microorganisms found in cigarette butts, butt filters, and sand, and their potential direct contact with beach users, as well as various factors associated with environmental parameters such as sand temperature and the presence of organic material, among others.

Methodology for Microbiological analysis

For each study zone and monitoring campaign, composite samples (CBs, CBFs and sand) were collected in duplicate. The samples were stored in sterile Ziplock bags to avoid cross-contamination and were transported refrigerated, maintaining the cold chain, for subsequent analysis in the laboratory.

The methodology used for bacterial identification consisted of three stages: i. Isolation of bacteria, for which modified LB broth and agar with seawater were used. Seawater is filtered with 0.22 µm filter paper and then autoclaved to prepare LURIA BROTH (Luria Bertani LB); 20 g of LB medium should be weighed for one liter of sterile filtered seawater, LB medium contains 5 g of NaCl with a concentration of 3.5% w/v, 5 g of yeast extract 5.0 g and 10 g of casein peptone with a (pH 7.2 ± 0.2). The samples were incubated in Petri dishes containing sterilized modified agar Luria-Bertani with filtered and sterile seawater (LB NaCl) and incubated at 37 °C for 24 h under aerobic conditions. The isolates of the bacterial strains were incubated at 37 °C; at pH range of 7 ± 0.2, for 24 h, without agitation and under aerobic conditions in Broth and LB NaCl Agar (modified with sea water); according to the indications of Acevedo-Barrios et al. [13–16]. Growth and morphology were observed with an optical microscope (Olympus BX41). Strains were scraped onto glass slides and stained for identification. The analyzed characteristics of the isolates included colony morphology, staining, spores if any and cell shape. Gram staining was used for microscopic description, according to Koneman et al. (2006)[17]. An antifungal (Fluconazole: 500 g of Fluconazole in 1000 mL) was used to inhibit fungal growth, as the scope of the study was limited to bacteria.

ii. Morphological and biochemical characterization of the isolated strains, conducted through Gram staining, catalase and oxidase tests, and BBL Crystal; iii. Two representative isolates of Gram-positive bacteria were selected from the isolated samples, one from the active zone (QRY_UTB 39 – 8) and the other from the service

Fig. 1. Location of monitoring transects on Bocagrande Beach, Cartagena. (CO). Reference: Esri, Maxar, Earthstar Geographics & GIS user community. Basemap: Imagery. Figure created in ArcMap 10.8.2. https://www.esri.com/en-us/home (accessed [20 Dec 2024]).

area (QRY_UTB 64 – 6). The samples were sent for analysis to the CorpoGen laboratory, a private, non-profit research center conducting scientific research and technological development in Colombia since 1995 (Table 1).

For phylogenetic analysis based on the 16 S rRNA gene sequence for the *Virgibacillus* genus, the 16 S rRNA records were searched in the RefSeq-NCBI database. The sequences were aligned using the Mafft program (v7.487) before being utilized by IQtree (v1.6.12) to construct a maximum likelihood tree, using the best

Sample	Host	size of the amplicon	Primer name	Oligonucleotide sequence (5´–3´)	Reference
QRY_UTB 39 – 8	CBFs active zone	1538 pb	337 F	GACTCCTACGGGAGGCWGCAG	(Park et al., 2021)[18]
			518 F	CCAGCAGCCGCGGTAATACG	
			800R	TACCAGGGTATCTAATCC	
			1100R	AGGGTTGCGCTCGTTG	(Sikolenko & Valentovich, 2022)[19]
QRY_UTB 64 – 6	CBFs service zone	1500 pb	337 F	GACTCCTACGGGAGGCWGCAG	(Park et al., 2021)[18]
			518 F	CCAGCAGCCGCGGTAATACG	
			800R	TACCAGGGTATCTAATCC	
			1100R	AGGGTTGCGCTCGTTG	(Sikolenko & Valentovich, 2022)[19]

Table 1. Primer sequence used for PCR.

substitution model (GTR + F + I + G4) and running a bootstrap analysis with 1000 replicates to assess branch support. The resulting tree was visualized using the iTOL web tool (v6).

To determine the presence of Total coliforms and *E. coli*, ten grams of sand were diluted in 90 ml of sterile distilled water. After the filtration process under vacuum conditions, 20–30 ml of sterile distilled water was added to rinse the sample salts. Once the membrane was removed from the filtration system using sterile forceps, it was transferred to containers with Chromocult medium, avoiding the formation of air gaps between the membrane and the medium. The containers with the membranes were incubated for 22–24 h at 35 ± 0.5 °C in an inverted position. After the incubation period, the Colony Forming Units (CFU) of the coliform group were counted, considering the macroscopic characteristics of the group in the culture medium used. Red colonies (Total coliforms) and blue colonies (*E. coli*) were reported in CFU/100 ml of the sample[20]. The same procedure was used to process the samples of CBs and CBFs.

Statistical analysis

The statistical analysis involved a cluster analysis to determine the clustering patterns of the elements analyzed across the different beach zones and to assess the behavior of microorganism concentrations in these areas. The paired group algorithm was applied, utilizing Euclidean distance as the similarity index (Supplementary Table 1). Euclidean distance measures the separation between two datasets in a multidimensional space, with smaller values indicating higher similarity and larger values indicating greater dissimilarity.

Additionally, a principal component analysis (PCA) was performed to understand the relationships between samples based on the usage zone. This test utilizes a dataset consisting of multiple observations described by independent or dependent variables without presupposing their relationships (Supplementary Table 2). The primary objective of PCA was to reduce the dimensionality of the dataset while preserving as much variability as possible, thereby enabling its use for classification or regression tasks and simplifying decision-making processes[21].

All statistical analyses and visualizations were carried out using the software GraphPad Prism 8.0 (GraphPad Software, Boston, Massachusetts, USA, www.graphpad.com) and R version 4.3.1, employing the Rcmdr and ggplot2 packages (R Core Team, 2021).

Results and discussion

Abundance and density of CBs and CBFs on the study beach

During the study period, the abundance and density of CBs and CBFs were assessed on Bocagrande Beach (Cartagena, Colombia). The results shown in Table 2 indicated an average density of 0.53 CBs/m² and 0.15 CBFs/m², with maximum values of 0.95 CBs/m² and 0.29 CBFs/m² and minimum values of 0.11 CBs/m² and 0.03 CBFs/m². It is common for the highest densities of both CBs and CBFs to be found in the service area, which could be associated with the commercial activity of restaurants and food and beverage sales.

Isolation and identification of gram-positive bacteria

The objective of the present study was to establish the potential for microbial growth in discarded CBs and CBFs on the study beach and their interaction with specific sand conditions, such as moisture, temperature, salinity, and variations in organic matter, may provide favorable or unfavorable conditions for bacterial growth. Analysis of the isolated strains through Gram staining revealed that 93% of the bacteria were Gram-positive, while 7% were Gram-negative. Morphological characterization indicated variability ranging from long bacilli in clusters and non-sporulating diplobacilli to short bacilli, both sporulating and non-sporulating.

Two bacterial samples isolates were sent to CorpoGen, a private, non-profit research center that has been conducting scientific research and technological development in Colombia since 1995. Molecular identification was conducted on two isolated Gram-positive bacterial strains, named QRY_UTB 39 – 8 and QRY_UTB 64 – 6 (Table 3).

For the first strain QRY_UTB 39 – 8, the results of the taxonomic analysis of the assembled 1538 bp problem sequence against the NCBI ref_seq database indicate a 99% identity over 97% of its length with ribosomal 16 S gene sequences from the species *Virgibacillus dokdonensis*, *Virgibacillus pantothenticus*, and *Virgibacillus chiguensis*. The phylogenetic distance tree generated by the leBIBI tool classifies the sample as belonging to the species *Virgibacillus dokdonensis* (Fig. 2). *Virgibacillus* are Gram-positive, moderately elongated, or slightly

Date	Zone	CBs (units)	CBFs (units)	CBs/m^2	CBFs/m^2
August 2022	Active	420	63	0.84	0.126
	Rest	125	15.5	0.250	0.031
	Service	416	133	0.831	0.266
September 2022	Active	369	93.5	0.738	0.187
	Rest	253	82.5	0.505	0.165
	Service	385	106.5	0.769	0.213
October 2022	Active	205	135	0.410	0.270
	Rest	280	64	0.560	0.128
	Service	475	147	0.950	0.294
November 2022	Active	208	61	0.416	0.122
	Rest	99	19	0.198	0.038
	Service	57	26	0.114	0.052
December 2022	Active	473	95	0.946	0.190
	Rest	101	28	0.202	0.056
	Service	75	36	0.150	0.072

Table 2. Density of CBs and CBFs on the study beach.

Sample/codes		QRY_UTB 39 – 8	QRY_UTB 64 – 6
Length of the assembled sequence		1538 pb	1500 pb
Sequencing reference Results NCBI	Microorganism	*Virgibacillus dokdonensis*	*Virgibacillus pantothenticus*
		Virgibacillus pantothenticus	*Virgibacillus dokdonensis*
		Virgibacillus chiguensis	*Virgibacillus chiguensis*
	% identity	99	98
	% coverage	97	98
leBIBI Results		*Virgibacillus dokdonensis*	*Virgibacillus pantothenticus*
Conclusion	genus	*Virgibacillus*	*Virgibacillus*
	species	*dokdonensis*	*Species probability Virgibacillus pantothenticus.*
GenBank accession number (NCBI)		PV105771	PV105772

Table 3. Molecular identification of bacterial isolates.

halophilic bacteria mostly isolated from the environment or fermented marine products[22]. *Virgibacillus* species have been identified in the literature, isolated from Salt Lake sediments, traditionally salt-fermented marine products, a permafrost core collected in the high Canadian Arctic, a solar salt marsh, soil, seawater, field soil, dairy products, wastewater washwater produced during wastewater processing[23].

Given the results found in different databases and in accordance with the origin of the sample, it can be concluded that it is most likely that the sample belongs to the species ***Virgibacillus dokdonensis.*** *T*he significance of this result lies in the fact that it is the first time, to the best of our knowledge, that these isolates have been reported in Cartagena, Colombia. *Virgibacillus dokdonensis* has been described in studies with limited focus on the mineralization of Fe^{3+} ions; however, *V. dokdonensis* can be employed to enhance the mineralization process of Ca^{2+} and Fe^{3+} ions in hypersaline oilfield wastewater (NaCl: 50 g/L)[24].

Recent research on marine organisms has explored and discovered various important bioactive properties of polysaccharides, which are essential for developing high-value biomaterials[25]. Bacterial exopolysaccharides (EPS) are high-molecular-weight organic biomacromolecules secreted in the natural environment by the host organism, involved in defense, symbiosis, phagocytosis, signaling processes, nutrient entrapment-driven cell development, and desiccation prevention[26]. Halophilic bacteria are considered novel sources of EPS for diverse biological activities. In a study conducted in the saltern region in Kumta, on the Arabian Sea Coast of India, EPS was produced by *Virgibacillus dokdonensis* VITP14 and extracted using ethanol. The maximum EPS production (17.3 g/L) was observed after 96 h of fermentation[27]. Such in-depth studies on these bacterial isolates are crucial and deserve further research endeavors from CBs and CBFs present inside marine habitats.

The second bacterial isolated strain QRY_UTB 64 – 6, as determined by the RDP classifier, was identified as a sequence from a microorganism belonging to the genus *Virgibacillus*. The phylogenetic tree (Fig. 3) constructed from the thirty closest cultivable microorganism sequences available in the NCBI RefSeq_RNA database shows that the analyzed sequence clusters with sequences from the genus *Virgibacillus sp.*

It is concluded that the sample likely belongs to the species ***Virgibacillus pantothenticus***. This genus is polyphyletic, as indicated by the 16 S rRNA gene sequence analysis, and thus does not form a well-defined group. Further in-depth phylogenetic revision of the genus is still needed to assign taxonomic categories confidently. A DNA-DNA hybridization analysis may be helpful in resolving the species level.

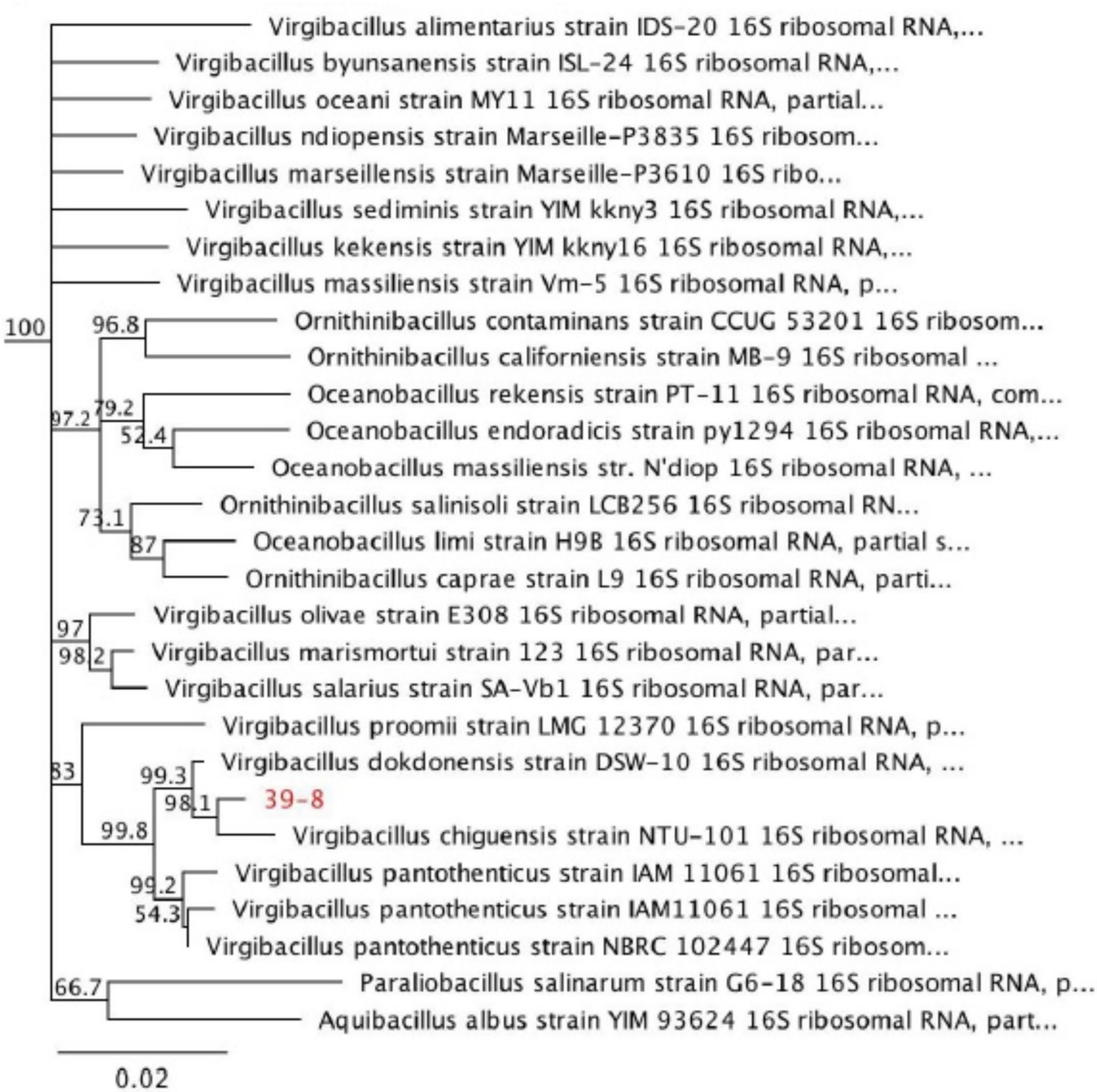

Fig. 2. Section of the phylogenetic distance tree generated by the leBIBI tool. The species names included in the figure are written in italics.

Virgibacillus sp. VITP14 has been reported in the literature as a Gram-positive bacterium belonging to the *Bacilli* subclass of phylum *Firmicutes* (*Bacillota*). The study conducted in Kumta, Arabian Sea Coast of India, describes them as halophilic and halotolerant bacteria that inhabit high ionic strength habitats such as seawater, salt lakes, brines, saltpans, saline soils, and salty foods, where they have to cope with osmotic stress[28]. *Virgibacillus* strains were isolated and reported in the study conducted in the National Library of Slovakia's repository, where they were isolated from brown-yellow stains on parchment and paper documents. Firmicutes strains belonging to the *Virgibacillus* genus have been associated with stains on parchment caused by copper oxidation, and these strains have shown a high adaptation and copper sorption capacity[29]. Similarly, bacterial strains were isolated from the southeast coast of India from the mangrove rhizosphere and identified as phylum Firmicutes, genus *Virgibacillus*, and species *V. dokdonensis*[30].

Virgibacillus pantothenticus, formerly known as *Bacillus pantothenticus*, was first described, isolated from a marine sponge, with the potential to inhibit the adhesion of fouling bacteria in natural environments[31]. Furthermore, the accelerating effects of microorganisms, including *Virgibacillus pantothenticus*, were reported in the biodeterioration of stone monuments under air pollution and low-temperature climatic conditions in Turkey[32].

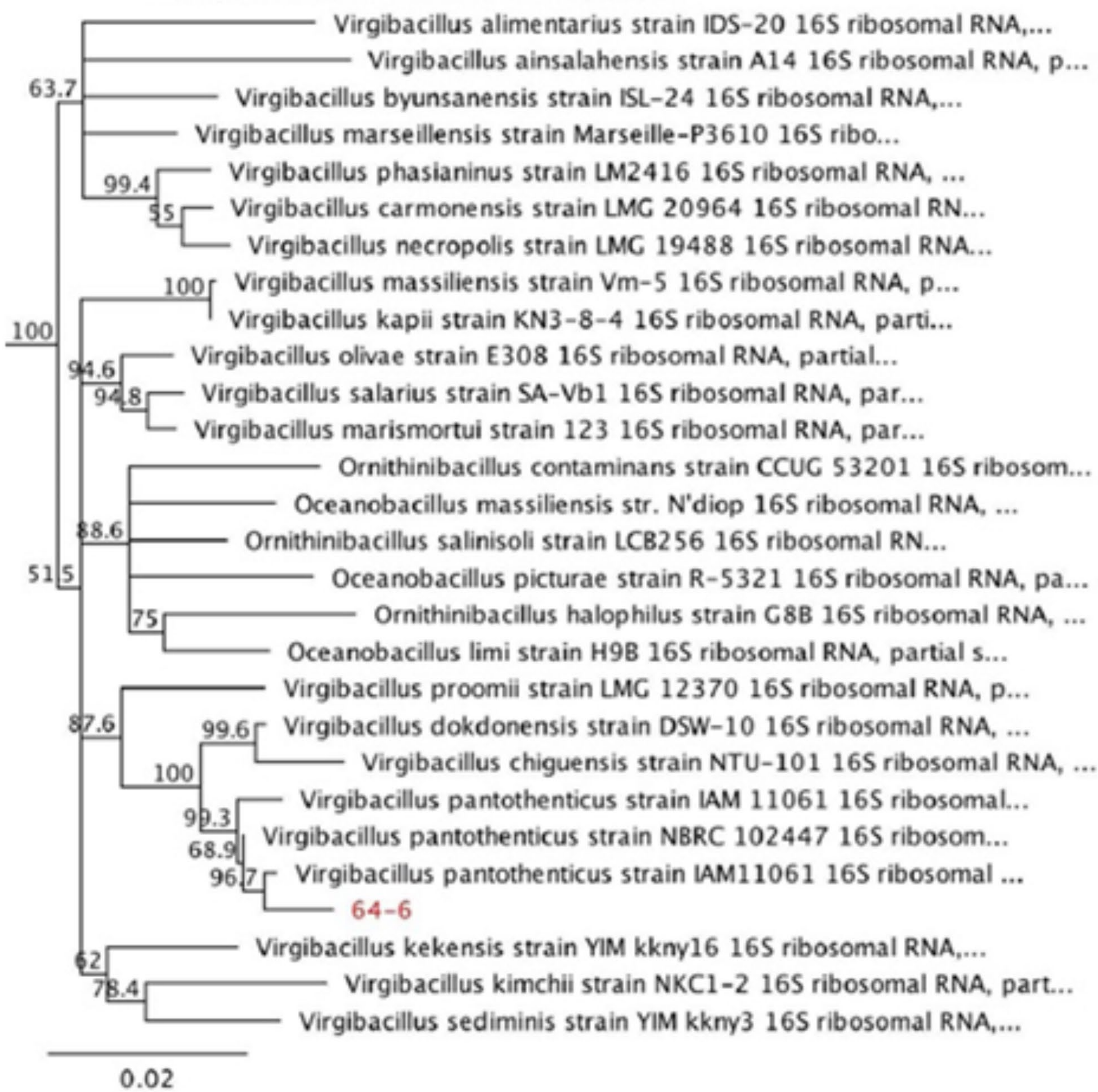

Fig. 3. Phylogenetic Tree of the Second Gram-Positive Sample. The species names included in the figure are written in italics.

Analysis and distribution of Gram-negative bacteria according to the usage zone

To evaluate the colonization and growth capacity of Gram-negative bacteria, specifically Total coliforms and *E. coli*, on residues such as CBs and CBFs discarded on the sand, and to establish their relationship with the potential exposure risk for beach users based on contact durations in different usage zones, a cluster analysis was conducted (Fig. 4).

In Fig. 4, the formation of four major groups of elements was identified. The first group consists of CBs in the active zone and CBFs in the service area. The second group is formed by CBs in the resting and service zones. The third group is composed of sand samples and CBFs in the active area, while the fourth group consists solely of CBFs collected in the resting zone. This latter group was characterized by having the highest concentration of bacteria, especially Total coliforms. Additionally, this same group of microorganisms exhibited the highest values in the CBFs located in the active zone, as well as in the sand from the three zones analyzed (resting, service, and active). *E. coli* bacteria were found in the rest zone in CBFs and were absent in the rest of the elements and zones of the beach.

There are no reports on indicators of the proliferation of these microorganisms in cigarette butts and fibers, which constitutes a contribution to research knowledge. It is crucial to continue with additional research to delve

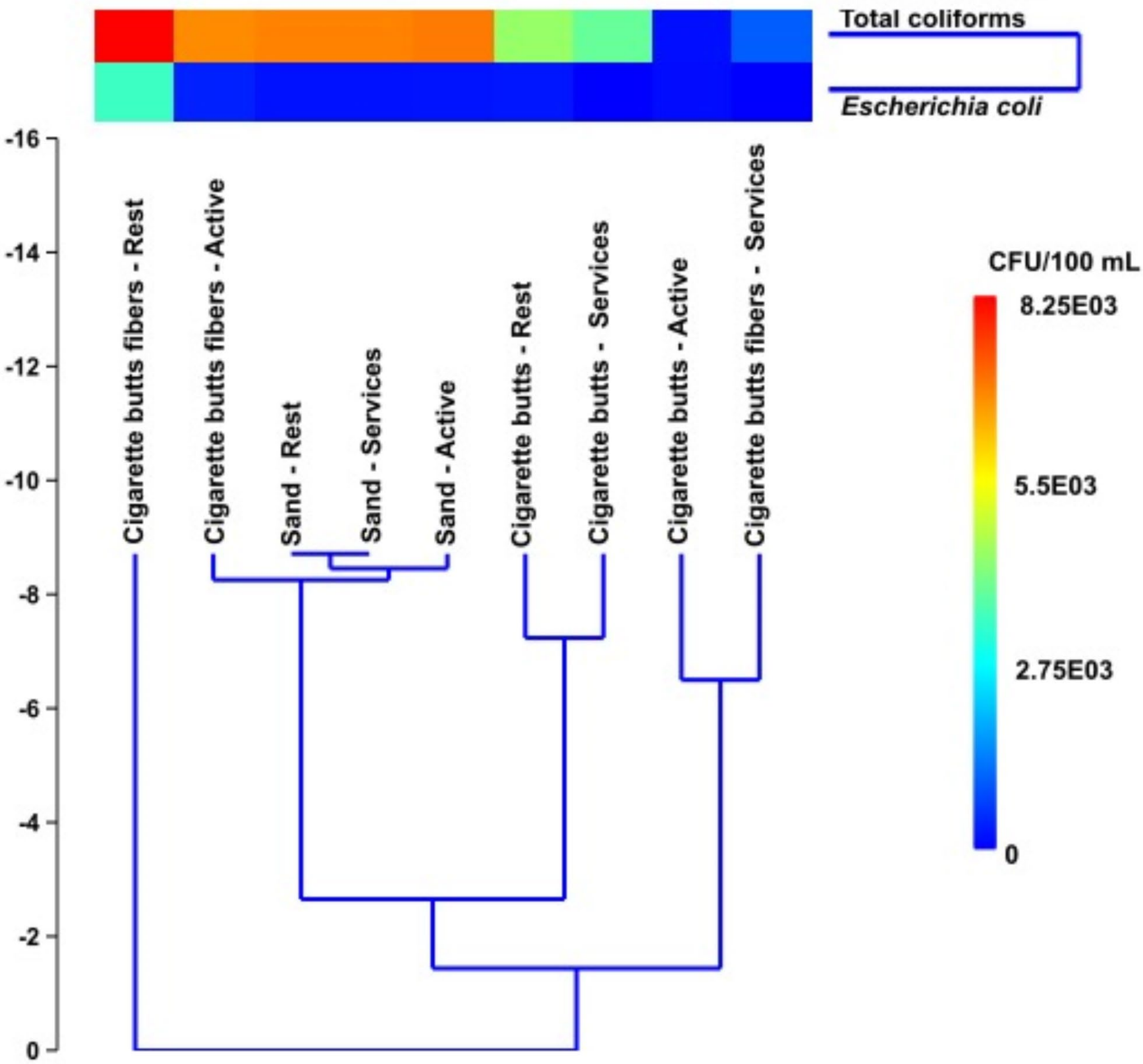

Fig. 4. Cluster analysis for identification of microorganism behavior.

deeper into assessing the potential public health risk associated with microbial growth in this specific type of waste.

Beach sand provides a suitable habitat for microorganisms such as Enterococci and *E. coli*; therefore, microbiological monitoring of beach sand is of vital importance to assess the safety of beaches for public use. Studies on the quality and monitoring of beach sand are gaining greater significance due to public concern about potential health risks associated with beaches[33].

To examine the relationship between environmental parameters on the beach, such as sand temperature (T_Sand), sand granulometry (Sand), and organic matter content (OM), with the presence of Total coliform microorganisms colonizing CBs, CBFs, and sand (TC_CB, TC_CBF, TC_Sand), as well as *E. coli* identified in CBs, CBFs, and sand (*E_coli*_CB, *E_coli*_CBF, *E_coli*_Sand), and their distribution across active, resting, and service zones, a principal component analysis (PCA) was performed, as shown in Fig. 5.

The Principal Component Analysis (PCA) revealed that the first two components account for 72.5% of the data variability. The first component, with a weight of 44.18%, was characterized by a higher weight of variables such as *E. coli* in Sand, *E. coli* in CB, *E. coli* in CBF, Total coliforms in Sand, Total coliforms in CBF, and Total coliforms in CB. Similarly, in the second component, with a weight of 28.32%, the variables with the highest weighting were Total coliforms in CB, *E. coli* in CBF, and Sand Temperature.

In addition to the considerations, the representation of the PCA shown in Fig. 5 allows for the identification of a clear spatial pattern regarding the distribution of higher values for some variables in two beach zones. The active area stands out for having a higher weighting of the variables Total coliforms CBF, Total coliforms in Sand,

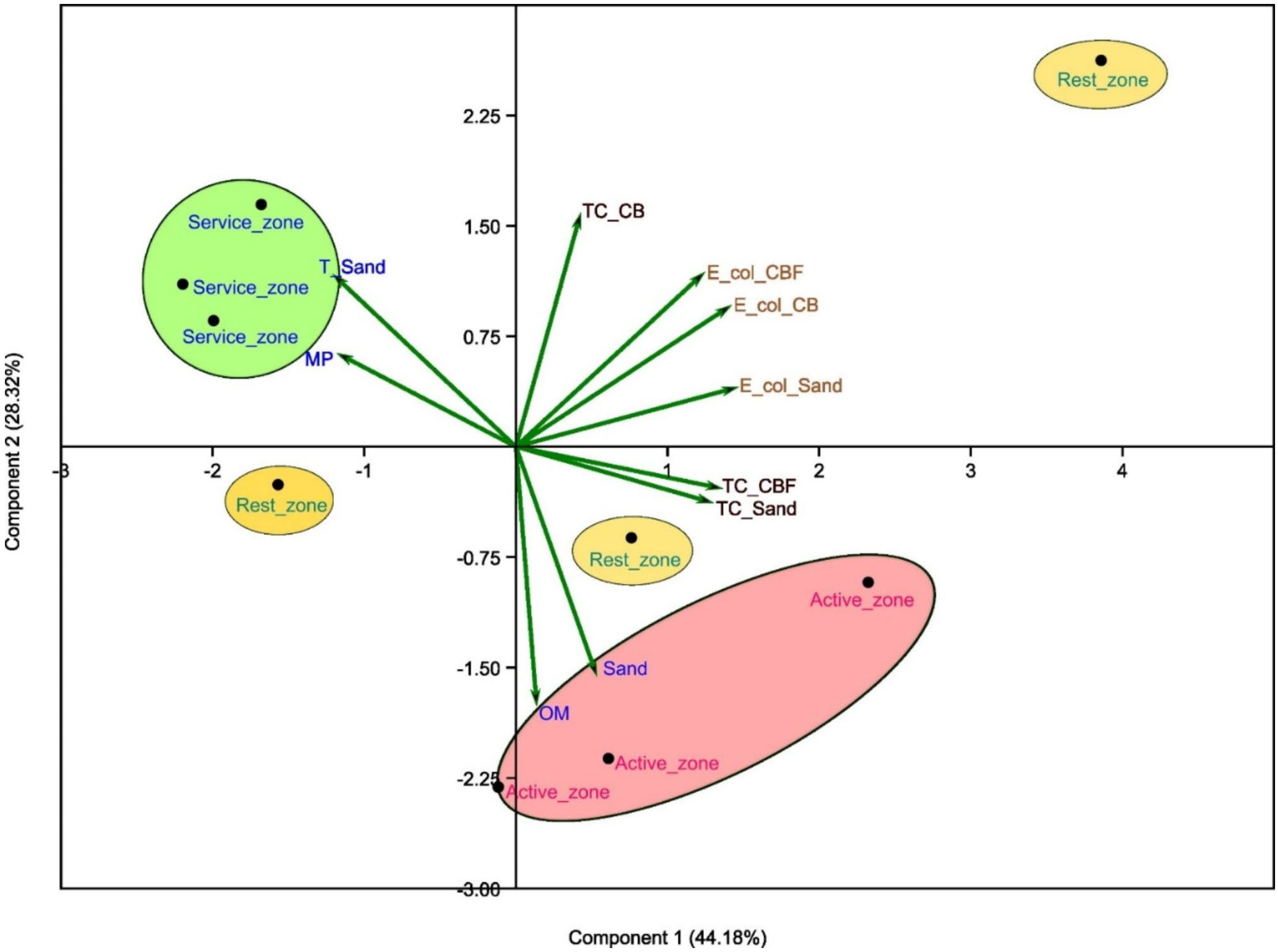

Fig. 5. Principal Component Analysis of the variables analyzed in the different usage zones of Bocagrande Beach.

Sand gradation (higher percentage of particles sized 150 microns), and Organic Matter. Meanwhile, the service area was notable for having a higher weighting of the variables Temperature and Microplastics. Regarding the rest area, it did not show a clear association with the analyzed variables.

As indicators of fecal matter are concentrated in beach sand, fecal pathogens may also be accumulating, indicating that contact with sand carries health risks related to beach use. The study developed by Bonilla et al. shows that bacteria were more concentrated in dry sand than in wet sand; however, the epidemiological study conducted in that study indicated that despite high pathogen levels in dry sand, exposure risks were decreased, which was attributed to the lack of contact with water[34]. The present study shows a decrease in isolation in the service area, which is the dry zone, but this is attributed more to the high temperatures present in the area. The survival rates of *E. coli* in coastal environments are influenced by various environmental factors, with the temperature being the most prominent, while other factors such as pH, salinity, and sunlight intensity also play a significant role[35]. Beach sand, biofilms, and water present both advantages and unique challenges in terms of the introduction, growth, and persistence of pathogens. These dynamics are further complicated by the continuous exchange between sand and water habitats[36].

Cigarette butts, composed of cellulose acetate (CA), a synthetic polymer derived from cellulose, produced through an acetylation process that includes the addition of acetic anhydride and acetic acid, as well as the incorporation of plasticizers such as polyethylene glycol[37]. CA are a highly abundant litter on beaches, and their decomposition is attributed to the presence of a significant percentage of fiber-type microplastics. It is crucial to note that the global production of cellulose acetate intended for cigarette filter manufacturing reaches approximately 640,000 tons. Assuming that 60% of these filters are discarded in various aquatic environments, including coastal areas, we are directly and continuously introducing around 0.3 million tons of potential microplastics from this source[1].

During the decomposition process from CBs to CBFs, numerous environmental factors associated with the structural characteristics of the filters influence the degradation rate. These environmental factors include temperature, humidity, pH, sunlight exposure, and the availability of oxygen, nutrients, and colonizing microorganisms[38]. In the decomposition process, various microorganisms present in the environment can colonize cellulose acetate with a high degree of substitution, serving as a prerequisite for subsequent biodegradation.

Environmental parameters are a crucial factor associated with the decomposition status of discarded cigarette butts (CBs) and cigarette filters (CBFs) in the sand, as well as with the absorption of metals and the growth of microorganisms. The results of the sand temperature analysis in the study area reveal that the service area exhibited the highest temperatures, with a mean of 50.4 ºC, followed by the rest area, with an average temperature of 47.8 ºC. This could potentially explain the low presence of *E. coli* in CB and CBF in these two zones.

The sand environment could potentially provide a protective shield to *E. coli* and other bacteria by mitigating the impacts of radiation. It has been observed that *E. coli* levels are higher in the morning and on cloudy days than in the afternoon or sunny days[39]. Sunlight exposure, rather than UV radiation alone, correlates with the inactivation of *E. coli*. Temperature affects the persistence and replication of *E. coli* in the sand. In cooler temperatures, *E. coli* may persist for longer periods on beach sand. Furthermore, the survival of *E. coli* and Enterococci significantly decreases at sand temperatures above 50 °C. Ambient temperatures ranging from 23 to 32 °C have been observed to favor the maximum level of replication[39]. This aligns with the results obtained in the present study, where sand temperatures in the service area exceed 50 °C, and ambient temperatures fluctuate between 33 and 35 °C, leading to a decrease in microbial growth in the area.

Conclusions

The present study analyzed the interaction of cigarette butts (CBs) and cigarette butts fibers (CBFs) waste discarded on the sandy beach of Bocagrande (Cartagena, Colombia). Variable densities ranged from 0.11 to 0.95 CBs/m^2 and 0.03 to 0.29 CBFs/m^2. This data is of interest when considering the composition of cellulose acetate plasticized in CB, highlighting the relationship between the high persistence and low degradability of these wastes in the beach sand.

Regarding the growth and isolation of microorganisms, Gram-positive bacteria (93%) and Gram-negative bacteria (7%) were reported. Molecular analysis of Gram-positive bacteria allowed for identifying bacteria belonging to the species *Virgibacillus pantothenticus* and *Virgibacillus dokdonensis*, which are documented for the first time in a tourist beach in Cartagena, Colombia, in this study. Isolating bacteria from the genus Virgibacillus is significant because some species within this genus are known for their potential in bioremediation, a process where microorganisms are used to break down environmental pollutants. Virgibacillus species are particularly notable for their ability to withstand extreme environmental conditions, including high salinity, temperature, and pH, which are typical in coastal ecosystems.

Additionally, total coliforms and *E. coli* were found in samples of sand, CBs, and CBFs, with a higher incidence in the active and rest zones. A considerable decrease in microbiological growth was observed in the service area, possibly associated with the high sand temperatures, averaging 51.0 ºC. The PCA results demonstrate how active and service zones cluster closely within replicates but remain distinct from each other. This pattern raises questions for future research aimed at understanding human behavior in different beach use zones.

The main challenges that persist in the study of cigarette butts as pollutants in coastal ecosystems have been identified. Additionally, the ability of these residues to serve as substrates for bacterial proliferation highlights the complexity of this issue. Furthermore, new challenges are proposed that represent current knowledge gaps, such as the detection of viruses and the adaptation or growth of fungi on these residues, encouraging further research in this area. Finally, the results obtained in this study emphasize the importance of developing strategies and public policies aimed at reducing or banning cigarette consumption on beaches to mitigate the negative impacts of these residues on coastal ecosystems and protect public health.

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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C.D-M.: Conceptualization, Methodology, Investigation, Writing - Original Draft, Project administration. J.M-B.: Writing - Review & Editing. C.M.B.: Conceptualization, Writing - Review & Editing. R.A-B.: Investigation and Conceptualization. L.G.: Data Curation, Writing - Review & Editing.

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The authors declare no competing interests.

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