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Isolation and characterization of microbial diversity in phenanthrene-degrading consortia in a pollution zone in Ciudad del Carmen, Mexico

Aislamiento y caracterización de la diversidad microbiana de consorcios degradadores de fenantreno de una zona contaminada en Ciudad del Carmen, México

K. García-Uitz[1]*, J.C. Cruz[2], M.G. León-Pech[1], I. Moreno-Andrade[3], G. Giácoman-Vallejos[4], C. Ponce-Caballero[4]

[1]*CONAHCYT-Tecnológico Nacional de México/ I. T. de Chetumal, Chetumal, Quintana Roo. 77013, México.*
[2]*Tecnológico Nacional de México/ I.T. de Chetumal, Chetumal, Quintana Roo. 77013, México.*
[3]*Laboratory for Research on Advanced Processes for Water Treatment, Instituto de Ingeniería, Unidad Académica Juriquilla, Universidad Nacional Autónoma de México, Blvd. Juriquilla 3001, Querétaro. 76230, México.*
[4]*Universidad Autónoma de Yucatán. Facultad de Ingeniería. Av. Industrias No-Contaminantes x Periférico Norte. Tablaje Catastral 12685, Mérida, Yucatán. 97111, México.*

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Abstract

Human health is adversely affected by polycyclic aromatic hydrocarbons. In fossil fuels, phenanthrene is a simple polycyclic aromatic hydrocarbon with elevated carcinogenicity, teratogenicity, mutagenicity, and ecotoxicity. Several bacterial species have been isolated from soil and sediment samples to assess their ability to utilize phenanthrene. The aim of this study was to isolate and characterize microbial diversity in phenanthrene-degrading consortia. Four aerobic bacterial consortia were isolated from both seawater and sediments from Ciudad del Carmen, Mexico. Synthetic seawater was enriched with phenanthrene at a concentration of 100 mg L^{-1}, serving as the growth medium to assess the pollutant's degradation potential. Bacterial richness was gauged using the 16S rRNA gene. The biodegradation data revealed a range of 58% to 75% phenanthrene degradation within a span of 7 days. The specific microbial growth rate (μ) ranged from 0.015 to 0.031 h^{-1}, and degradation rates (DR) ranged from 0.732 to 1.94 (mg L^{-1}) / h. The most predominant phyla observed were *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Cyanobacteria*, and *Firmicutes*. Within the *Proteobacteria* phylum, the dominant classes were *Gammaproteobacteria* and *Alphaproteobacteria*. The primary operational taxonomic units (OTUs) were identified as *Alcalinovorax venustensis* and *Halomonas* sp.

Keywords: consortium, pyrosequencing, *Proteobacteria*, phenanthrene-degrading.

Resumen

La salud humana se ve afectada negativamente por los hidrocarburos aromáticos policíclicos. En los combustibles fósiles, el fenantreno es un hidrocarburo aromático policíclico simple con elevada carcinogenicidad, teratogenicidad, mutagenicidad y ecotoxicidad. Varias especies bacterianas se han aislado de muestras de suelo y sedimento para evaluar su capacidad para utilizar el fenantreno. El objetivo de este estudio fue aislar y caracterizar la diversidad microbiana en consorcios degradadores de fenantreno. Se aislaron cuatro consorcios bacterianos aeróbicos tanto de agua de mar como de sedimentos de Ciudad del Carmen, México. Se enriqueció el agua de mar sintética con fenantreno a una concentración de 100 mg L^{-1}, sirviendo como medio de crecimiento para evaluar el potencial de degradación del contaminante. Los datos de biodegradación revelaron un rango de degradación de fenantreno del 58% al 75% en un período de 7 días. La riqueza bacteriana se evaluó utilizando el gen 16S rRNA. La tasa de crecimiento microbiano específico (μ) varió de 0.015 a 0.031 h^{-1}, y las tasas de degradación (DR) oscilaron entre 0.732 y 1.94 (mg L^{-1}) / h. Los phyla más predominantes observados fueron *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Cyanobacteria* y *Firmicutes*. Dentro del phylum *Proteobacteria*, las clases dominantes fueron *Gammaproteobacteria* y *Alphaproteobacteria*. Las principales unidades taxonómicas operativas (OTUs) se identificaron como *Alcalinovorax venustensis* y *Halomonas* sp.

Palabras clave: consorcio, pirosecuenciación, *Proteobacteria*, degradación fenantreno.

*Corresponding author. *E-mail*: karla.gu@chetumal.tecnm.mx;
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1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) stand out among environmental pollutants for their disruptive impact on ecosystems, primarily due to their persistence and accumulation along the trophic chain (Eze, 2021). These compounds are particularly noteworthy due to their prolonged existence resulting from natural combustion processes and their substantial accumulation in marine environments (Eze, 2021b). As a prevalent contaminant in soil and water, PAHs have garnered significant attention due to their detrimental effects on human health (Zeng *et al*., 2010; Kuppusamy *et al*., 2016; Zhao *et al*., 2017).

Phenanthrene, a simple form of PAHs, is a vital constituent of incomplete organic matter combustion and serves as a principal component within fossil fuels (Juhasz and Naidu, 2000). Its molecular structure comprises three fused benzene rings and two distinct regions, referred to as bay and K, contributing to its heightened reactivity both chemically and biologically. The concerning aspect of phenanthrene lies in its elevated carcinogenicity, teratogenicity, mutagenicity, and eco-toxicity (Gu *et al*., 2021).

Numerous consortia and individual bacterial species have been isolated from soil and sediment samples, aiming to assess their capacity to utilize phenanthrene as a source of carbon and energy (Janbandhu and Fulekar 2011; Govarthanan *et al*., 2020; Martin *et al*., 2012; Patel *et al*., 2012). These microorganisms include *Rhodocyclaceae*, *Sphingomonadaceae*, *Actinobacteria*, *Sphingobacterium* sp, *Halomonas* sp, and *Pseudoxanthomonas* sp (Janbandhu and Fulekar 2011; Govarthanan *et al*., 2020; Martin *et al*., 2012; Patel *et al*., 2012).

While some experimental studies have employed pure bacterial cultures for phenanthrene degradation, it is important to acknowledge that such models do not accurately represent microbial behavior in their natural habitats (Sauret *et al*., 2014; Ghazali *et al*., 2004). Effective bioremediation necessitates the use of microbial consortia, leveraging the cooperative metabolic capabilities of various microbial populations to enhance the conversion of hazardous compounds into less toxic or non-toxic elements (Sauret *et al*., 2014; Ghazali *et al*., 2004; Zakaria *et al*., 2020; Loreto-Muñoz *et al*., 2024).

Advancements in microbial ecology have introduced culture-independent community analysis methods, revolutionizing the identification of non-cultured and previously unknown microbes (Laas *et al*., 2014). Molecular techniques, centered around the analysis of phylogenetically informative genes like the 16S rRNA gene, offer a more comprehensive understanding of the diverse microbial communities present in different marine environments (Andersson *et al*., 2009; Gilbert *et al*., 2009; Kirchman *et al*., 2010). These techniques prove invaluable in the analysis of seawater communities and phenanthrene degradation.

Given its molecular weight, phenanthrene is present in both water and sediments, contributing to pollution in Ciudad del Carmen, Mexico. This city is a hub for significant economic activities, notably hydrocarbon exploration and production, underscoring the importance of identifying phenanthrene-degrading bacteria. Therefore, this study aimed to explore the biodegradation of phenanthrene and describe the microbial diversity of the bacterial consortia sourced from sediments and seawater in Ciudad del Carmen, Mexico.

2 Materials and methods

2.1 Sampling

Seawater and sediment samples for consortium bacteria isolation were collected at 0-15 and 0-5 cm depth, respectively. Four different collecting sites were selected in Playa Norte, Ciudad del Carmen, Campeche, Mexico, and the stations identified by the following: S-1 (N 18°39´54.3 - WO 91°50´56.3); SW-1 (N 18°39´00.7 - WO 91°50´42); SW-2 (N 18°39´06.4 - WO 91°50´12) and S-2 (N 18°39´13.7 - WO 91°50´40)). Samples were contained in amber sterilized glass bottles and stored at 4 °C until used.

2.2 Isolation of microbial consortia

Consortia were adapted to 100% artificial seawater (AS). The AS consisted of the following composition per liter (according to Lyman and Fleming, 1940): 24.5 g of NaCl; 11.1 g of $MgCl_2{\cdot}6H_2O$; 4.10 g Na_2SO_4; 1.54 g of $CaCl_2$; 0.7 g of KCl; 0.2 g of $NaHCO_3$; 0.1 g of KBr; 0.03 g of H_3BO_3; 0.02 g of $SrCl_2{\cdot}6H_2O$; 3 mg of NaF. Was adjusted with HCl and NaOH solutions to a pH of 7.5. All the media and solutions were sterilized at 121°C for 15 min.

The initial growth medium was prepared with 250 mL of artificial seawater contaminated with 50 ppm of phenanthrene, and either one gr of soil or 30 mL of seawater was added. They were then incubated at 30°C and 150 rpm for seven days. After this period, a 20 mL inoculum was taken, repeating this process 3 times (Figure 1).

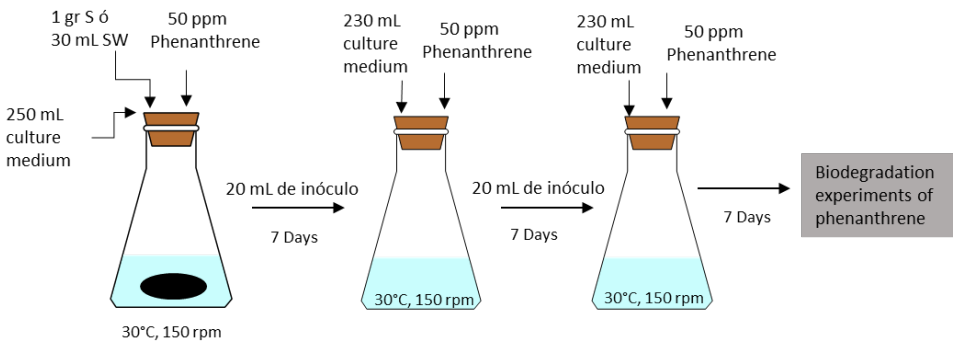

Figure 1. Culture of Microbial Consortia.

2.3 *Biodegradation experiments of phenanthrene*

Consortia bacteria degradation capability of phenanthrene degrading was performed on the micro-scale. Phenanthrene solution was prepared: 100 ppm of phenanthrene was aggregated to 50 mL of acetone. And 14.5 mL of AS was added after the acetone was vaporized. 0.5ml of the solution was inoculated to each microbial consortium.

The experiment involved three repetitions for every combination of microorganisms used. Additionally, a control group was included. Each set of treatments underwent incubation on a shaker at 150 rotations per minute for seven days at ambient temperature. Samples were collected from the flasks at intervals of 1, 3, 5, and 7 days. Following that, the remaining phenanthrene was extracted and measured.

Concurrently, the growth kinetics of each microbial consortium were assessed by employing microbial plate counts (CFU mL^{-1}) on tryptic soy agar (TSA). To prepare the bacterial dilutions, 1 mL of the medium was mixed with 9 mL of sterile saline solution. These dilutions, along with all inoculated plates, were then incubated at ambient temperature for three days. The microbial growth was subsequently evaluated.

2.4 *Analytic method*

The quantitative analysis of phenanthrene was conducted using a GC-MS system (TRACE GC ULTRA-ITQ) equipped with a TG-SQC capillary column (30 m length, 0.25 mm I.D., and 0.25 μm film thickness). Injector, detector, and transfer line temperatures were set at 250°C, 270°C, and 270°C, respectively. The oven ramp conditions included an initial temperature of 50°C for 1 minute, followed by a temperature increase of 15°C per minute until reaching 225°C, then ramping up to 300°C at a rate of 30°C per minute, which was maintained for 1 minute. Injection was performed in splitless mode with a volume of 1 μL, using helium as the carrier gas at a flow rate of 1 mL per minute.

The recovery of phenanthrene was executed following method 610 of dichloromethane (US-EPA, 1986) with the following modifications:

1. Three separations were performed between the organic solvent and the aqueous phase.

2. Three organic extracts were combined in a 30 mL vial for GC-MS analysis.

3. Linearity of the method was determined (100-1000ng mL^{-1}) with a correlation coefficient consistently > 0.999 for phenanthrene concentration. The recovery efficiency was assessed under extraction conditions by varying the concentration of phenanthrene, which yielded a value of > 92.2 +2.86%. 9.28 ng mL^{-1} was determined to be the detection limit of the method.

Quality control measures were implemented, and the method was validated prior to analysis.

2.5 *Microbial growth*

The specific growth rate (μ) of the culture, degradation rates (DR), and doubling time (td) were calculated utilizing experimental data on microbial growth of initial phenanthrene and residual concentrations. These calculations were performed using the Monod equation:

$$\mu = \frac{\ln X/Xo}{t} \tag{1}$$

$$DR = \frac{C(0) - C(t)}{t} \tag{2}$$

$$t_d = \frac{\ln 2}{\mu} \tag{3}$$

Where C presents the concentration of phenanthrene, and X indicates the cell density. The unit of DR, μ and t_d are (mg L^{-1}) / h, h^{-1} and h, respectively.

2.6 Statistical analyses

The mean and standard deviation values for phenanthrene were calculated from three separate sample extractions. To evaluate the statistical significance of the differences observed among the consortia, a parametric one-way ANOVA test was conducted, and the results are reported as $p < 0.05$. Descriptive statistics were generated using Statgraphics Centurion XV (StatPoint, Inc).

2.7 DNA extraction

To identify the microbial consortium, the genomic DNA of bacteria was cultivated in artificial water for a duration of five days. Subsequently, it was isolated utilizing the method reported by Rojas-Herrera *et al*., (2008). The purified total DNA underwent quantification using a spectrophotometer (NanoDrop Technologies Inc) at wavelengths of 260 and 280 nm. The integrity of the total DNA was confirmed by running samples on 1.2% denaturing agarose gels.

2.8 Processing of the 16S rRNA gene data

The bacterial community composition of the isolated consortia was assessed through 16S rRNA gene sequencing, employing 454 pyrosequencing. Once sequencing had completed, the data analysis process consisted of the quality checking and reads denoising stage and the diversity analysis stage.

2.9 Quality checking and denoising

The quality trimming and clustering stages were conducted using the algorithm implemented in USEARCH (Edgar RC, 2010). Chimera detection was performed utilizing the de novo method integrated into UCHIIME (Edgar *et al*., 2011). Denoising checking was carried out using the Research and Testing denoiser. In the FASTA formatted sequence and quality files, sequences failing to meet the specified quality control criteria were removed. These criteria included failed sequence reads, sequences with low-quality tags, and sequences that did not reach at least half the expected amplicon length or 250 bp in length, whichever was shorter. Sequences that passed the quality control screening were then consolidated into a single FASTA formatted sequence and quality file.

2.10 Microbial diversity analysis

2.10.1 Taxonomic identification

During the diversity analysis stage, each sample underwent an analysis pipeline to ascertain taxonomic information for every read, enabling the determination of microbial diversity within the sample. To identify the identity of each remaining sequence, the sequences were initially sorted in descending order by length in the FASTA formatted file. Subsequently, these sequences were clustered into operational taxonomic unit (OTU) clusters with 100% identity (0% divergence) employing the algorithm integrated into USEARCH (Edgar RC, 2010). Reference sequences were chosen from the SILVA ribosomal RNA database (Pruesse *et al*., 2007).

2.10.2 Data analysis

Results were analyzed using the publicly available SEED MG-RAST program (Metagenome Rapid Annotation using Subsystem Technology) (Meyer *et al*., 2008). Data analysis was performed, Richness, α-Diversity, Shannon and Chao index were estimated. Non-metric multidimensional scaling plots were generated based on Bray-Curtis dissimilarities. Dissimilarities were calculated.

3 Results and discussion

3.1 Degradation of isolated consortia

The sampling area exhibits a pronounced industrial expansion, encompassing industrial and fishing ports, as well as offshore crude exploitation. From the sediment, two consortia were derived (S-1, S-2), and likewise, two were obtained from seawater (SW-1, SW-2) (Figure 2).

Each consortium underwent assessment for phenanthrene degradation at a concentration of 100 mg L^{-1} in synthetic seawater. Following seven days of treatment, the degradation percentages were observed as follows: consortium S-2 demonstrated a degradation of 58% ± 5.9, while consortium S-1 exhibited the most significant degradation at 75% ± 4.9 among all isolated consortia. For the remaining consortia, phenanthrene degradation percentages were measured as 60% ± 6.48 for SW-1 and 62% ± 5.1 for SW-2 (Figure 3).

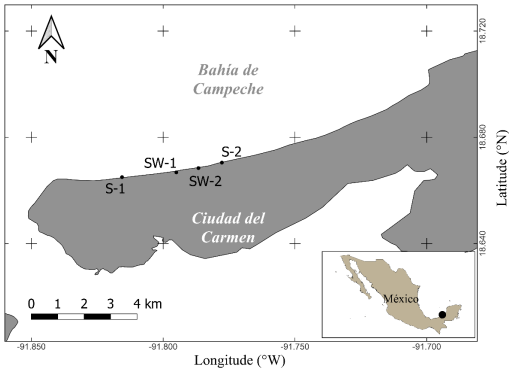

Figure 2. Map of the sampling area location in the Ciudad del Carmen, Mexico.

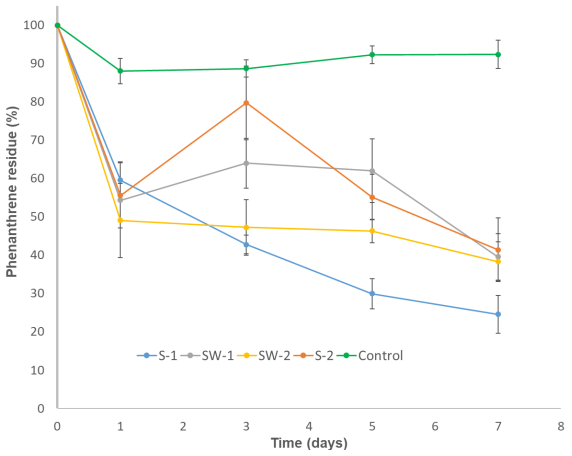

Figure 3. Degradation of phenanthrene by consortia.

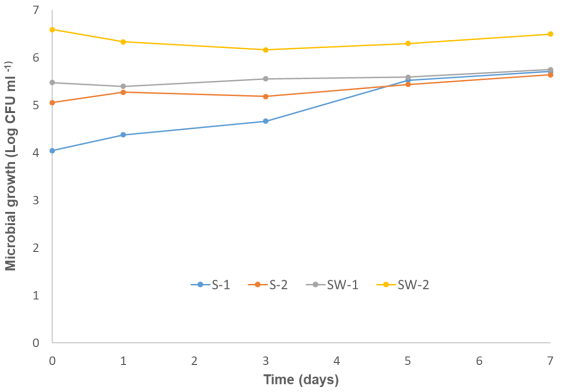

Figure 4. Microbial growth in various consortia (Log CFU mL^{-1}).

In the absence of inoculum, the control sample showed an 11% reduction in phenanthrene. This decrease can be attributed to substrate loss through volatilization or chemical degradation (Cerniglia, 1992).

Various consortia have been isolated and utilized for phenanthrene degradation in a mineral medium. Janbandhu and Fulekar (2011) reported a 56.9% degradation of phenanthrene over 14 days of treatment, while Pedetta *et al.*, (2013) observed complete mineralization of 150 mg/L within three days. Tam *et al.* (2002) indicated around 40% phenanthrene degradation in six days of treatment by consortia, starting from an initial concentration of 50 mg/L in seawater. These outcomes suggest that the metabolites generated during phenanthrene degradation by different consortium members could aid in the comprehensive elimination of contaminants, acting as intermediate carbon sources. Phenanthrene biodegradation is influenced by several environmental factors, including salinity (higher salt percentage > 20% leads to reduced degradation), initial phenanthrene concentrations, and the addition of carbon and nutrients (Fortunato *et al.*, 2012; Lozupone and Knight, 2007; Wu *et al.*, 2006).

3.2 *Microbial growth*

The microbial-specific growth rates (μ) were computed for all consortia and are presented in Table 1. In this study, the microbial growth rates across different consortia exhibited variation. For consortium S-1, the growth rate was calculated as 0.031 h^{-1} (75% degradation), while for S-2, it was 0.018 h^{-1} (58% degradation). In comparison, the growth rates for consortia SW-1 and SW-2 were 0.018 h^{-1} and 0.015 h^{-1}, corresponding to degradation percentages of 60% and 62%, respectively (Figure 4).

Table 1.- Specific growth rate (μ), degradation rate (DR) and doubling time (t_d) of isolated consortia.

Consortium isolated	μ (h^{-1})	t_d (h)	DR (mg L^{-1}) h^{-1}
S-1	0.031	22.35	1.94
S-2	0.018	38.5	1.85
SW-1	0.018	38.5	1.9
SW-2	0.015	46.2	0.73

Based on degradation times (td) and degradation rates, it becomes apparent that consortium S-1 exhibited the highest phenanthrene consumption rate per CFU. Moreover, it achieved a faster degradation rate compared to the other three consortia, where the substrate consumption exhibited similarity.

Table 1 results underscore that consortium S-1 demonstrated effective assimilation of phenanthrene, positioning it as the most proficient degrader among the consortia.

Throughout this study, diverse specific growth rate (μ) values were derived from the various consortia, pointing towards their capacity to utilize phenanthrene as the primary carbon source. These values can be juxtaposed with findings from other investigations. For instance, *Rhodotorula glutini* attained a μ of 0.028 h^{-1} after 15 days of initial inoculation with a concentration of 200 mg L^{-1} (Romero *et al.*, 1998). In another study, Tian *et al.*, (2002) employed *Pseudomonas mendocina* and achieved a μ of 0.33, signifying 95% degradation after 48 hours of inoculation. Elevated μ values indicate that these consortia could indeed exhibit efficiency in phenanthrene degradation.

3.3 *Microbial diversity in consortia isolated*

DNA quantification ranged from 42.6 to 54.9 $\mu g\ \mu L^{-1}$, and sample purity was confirmed by 260/280 nm ratios ranging from 1.64 to 1.72.

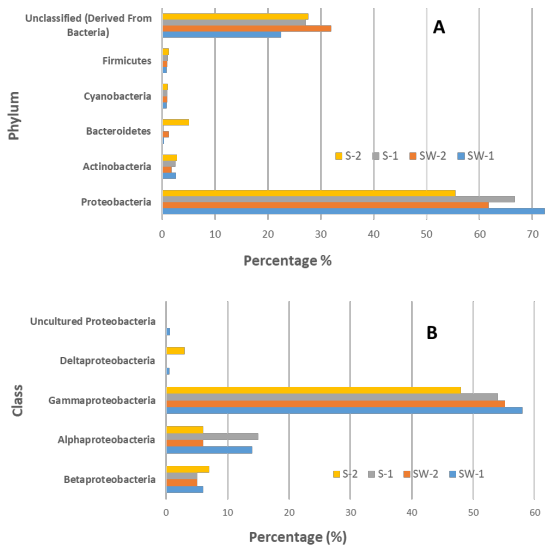

Figure 5. Taxonomic assignment of 16S rRNA gene sequences retrieved from sediment and seawater consortia (S-1, S-2, SW-1, and SW-2), respectively, classified by (A) phylum and (B) class within the phylum *Proteobacteria*.

The pyrosequencing analysis yielded a total of 19,805 bacterial 16S rRNA sequences from the consortia (Table 1), which were then utilized for community analyses. The 16S rRNA gene sequences determined in this study were submitted to NCBI GenBank database (http://www.ncbi.nlm.nih.gov/genbank/index.html), under number BioProject PRJNA251719, accession numbers SAMN02840637, SAMN02840638, SAMN02839426, and SAMN02840640. Within the microbial community, five primary phyla were identified across the four isolated consortia (Figure 5A). The most prevalent among these was the *Proteobacteria* phylum, ranging from 55.5 to 72.3%. Additionally, *Actinobacteria*, *Bacteroidetes*, *Cyanobacteria*, and *Firmicutes* phyla were present in percentages below 5.1. Notably, a substantial portion remained unclassified (derived from bacteria), constituting 22.5 to 32% of the community, lacking a specific bacterial phylum assignment in the MG-RAST database.

At the class level within the *Proteobacteria* phylum, *Gammaproteobacteria* emerged as the dominant group, representing 48 to 58%. It was followed by *Alphaproteobacteria* (6 to 15%), *Beta Proteobacteria* (5 to 7%), and *Deltaproteobacteria*, *uncultured Proteobacteria*, accounting for less than 3% (Figure 5B). *Gammaproteobacteria* is often related to n-alkane degrading genes, as demonstrated in previous studies (Eze, 2021), and the dominance of *Alphaproteobacteria* in bacteria communities suggests that these genera are tolerant of petroleum hydrocarbon concentrations and have the potential to degrade organic contaminants (Eze, 2021, Garrido-Sanz *et al*., 2019; Liu *et al*., 2014; Shao and Wang 2013; van Beilen *et al*., 1994, 2001; Aguilar-Vilchis *et al*., 2023).

Laas *et al*., (2014) reported the isolation of a consortium where the community primarily consisted of *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, and *Cyanobacteria*. However, differences at the class level were observed; the main classes identified included *Alphaproteobacteria* (28.8%), *Epsilonbacteria* (25.9%), *Actinobacteria* (14.6%), *Gammaproteobacteria* (3.3%), *Betaproteobacteria* (5.1%), *Flavobacteria* (3.2%), among others. These distinctions likely stemmed from the various depths (5 - 40cm) sampled for isolation.

Table 2. Predominant species in the different consortia isolated (from seawater SW-1 and SW-2, from sediments S-1 and S-2).

Species	Percentage %			
Alcalinovorax venustensis	63.33	26.52	40.44	32
Halomonas	0.56	46.86	20.66	27.62
Bradyhizobiaceae	4.72	0.23	2.75	0.8
Bosea	3.67	0	2.33	0.47
Rhodospirillaceae	13.58	1.12	12.37	5
Agrobacterium	0.49	3.42	0.87	2.52
Clostridia	0.15	0	0	0
Pseudomonas	4.45	4.64	7.29	7.34
Alcaninovorax sp.	0.91	0.57	0.64	0.12
Bacillaceae	0.15	0	0	0
Rihizobium	0.12	0.2	0.23	0
Mesorhizobium	0.21	0	0	2.74
Clostridiales	0.12	0.1	0.17	0.16
Pseudomonas stutzeri	0.46	0.65	0.67	1.07
Acidovorax	0.165	0.26	0.17	0.18
% TOTAL	94.68	92.85	91.37	85.623

Table 3. Richness estimators to predict the number of species in the different consortia from seawater (SW-1 and SW-2) and sediments (S-1 and S-2), observed OTUs, α- Diversity, Shannon and Chao index.

Consortia	Observed OTU´s	α- Diversity	Shannon Index	Chao index
SW-1	106	5.954	1.51	279
SW-2	101	8.153	1.66	255
S-1	129	9.709	2.17	260.13
S-2	153	13.318	2.46	324.42

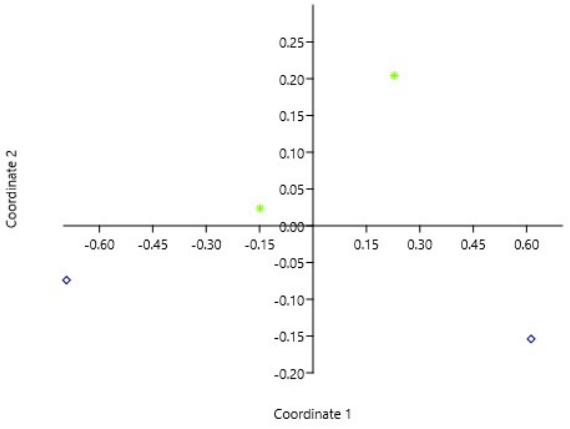

Figure 6.- Analysis non-metric multidimensional scaling (♦ sediment diversity; ♦ seawater diversity).

Notably, depth stands as a vital parameter in accessing specific bacteria. Gomes *et al*., (2013) conducted sampling at a depth like this study (5 cm) and identified a predominance of the *Proteobacteria* phylum (> 50%) and *Gammaproteobacteria* class (30%). The species-level analysis is presented in Table S2, indicating that *Alcalinovorax venustensis* was the most abundant species in consortia S-1 and SW-1, constituting 63 and 40.44%, respectively. Similarly, *Halomonas* was prevalent in consortia SW-2 and S-2, accounting for 46.86 and 27.62%, respectively. However, in consortium SW-1, these species were present in percentages below 1%.

Variance analysis (ANOVA) at the species level revealed not significant difference among the four consortia, yielding a p-value of 0.1685 upon comparing individual consortia, a significant difference emerged only between SW-2 and S-2 consortia, with a p-value of 0.0092. Nonetheless, Non-metric multidimensional scaling revealed slight differences between the sediments and water samples of the polluted soil and water samples (Figure 6).

Consortium S-2 exhibited the highest count at 13,318, while the less diverse consortium SW-1 contained 5,954 species. Based on reference sequences, diversity was assessed with a 5% dissimilarity threshold. Richness and diversity estimators confirmed varying species counts across consortia (Table 3). α Diversity, Shannon, and Chao index indicated Consortium S-1 and S-2 consortium were the most diverse (13.318, 2.46, and 324.42 respectively). In contrast, SW-1 and SW-2 consortium were the least diverse (Table 3).

These results underscore how the richness and diversity of the community differ concerning the percentage of phenanthrene degradation, reflecting the community's enrichment due to acclimatization to the contaminated medium (Wang *et al*., 2021). However, sediment consortiums were found to be the most diverse and, greater phenanthrene consumers in general.

Notably, *Alcalinovorax venustensis* exhibited growth in the presence of NaCl concentrations of 3-10%. Research by Fernández-Martínez *et al*., (2003) and Schneiker *et al*., (2006) identified communities of the *Alcanivorax* genus and closely related *Proteobacteria*, suggesting these microorganisms could serve as bioindicators for water contaminated with varying levels of polycyclic aromatic hydrocarbons. This phylum has been found in marine and coastal ecosystems around the world, including the Mediterranean Sea, the Pacific Ocean, the seas near Japan and China, and the Arctic Sea (Harayama *et al*., 2004; Kasai *et al*., 2001; Yakimov *et al*., 2005).

Conclusions

This study represents one of the initial endeavors to explore microbial diversity in seawater amid the presence of polycyclic aromatic hydrocarbons like phenanthrene in Ciudad del Carmen, Mexico a city where hydrocarbon exploration and production is an important economic activity. Four phenanthrene-degrading consortia were established, exhibiting degradation percentages of 58, 60, 62 and 75%. The corresponding degradation rates for S-2, SW-1, SW-2, and S-1 were measured at 0.014, 0.010, 0.008, and 0.025, respectively. Consortium diversity was subsequently assessed, revealing a predominance of the *Proteobacteria* phylum (55 - 72.3%). The class *Gammaproteobacteria* was the most prevalent, constituting 48 - 58%, with species such as *Halomonas* sp and *Alcalinovorax venustensis* emerged as the most abundant, associated with the degradation of n-alkanes. A further study of *Alcalinovorax*

venustensis in the presence of NaCl suggests that these microorganisms may serve as bioindicators for water contaminated with polycyclic aromatic hydrocarbons. This research revealed that the community's enrichment is due to acclimatizing to the contaminated medium, and sediment consortiums consume the most phenanthrene and are the most diverse.

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