

## Biochemical analysis of the Caspian Sea sediments: Implications for environmental pollution and bioremediation

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### ABSTRACT

The Caspian Sea, Earth's largest enclosed inland water body, faces significant environmental challenges due to anthropogenic pollution. This study aimed to assess pollution levels in the Caspian Sea sediments and evaluate the potential for microbial bioremediation. Sediment samples were collected from 15 sites along the Caspian coastline. Polycyclic aromatic hydrocarbons (PAHs) and heavy metals (lead and cadmium) were quantified using GC-MS and AAS, respectively. Microbial community analysis was performed through 16S rRNA gene sequencing. Native bacterial strains were isolated, characterized for their pollutant degradation capabilities, and tested in laboratory-scale bioremediation experiments. Total PAH concentrations ranged from 119.5 to 2032.7  $\mu\text{g kg}^{-1}$  dry weight, while mean concentrations of lead and cadmium were 67.9 and 2.15  $\text{mg kg}^{-1}$ , respectively. Microbial analysis revealed diverse communities dominated by Proteobacteria, with genera known for hydrocarbon degradation (e.g., *Pseudomonas* and *Alcanivorax*) showing strong positive correlations with contaminant levels. Isolated bacterial strains demonstrated remarkable PAH degradation (up to 87% for naphthalene) and heavy metal removal capabilities. In microcosm experiments, bacterial consortia achieved up to 89.7% PAH removal and 61.4% lead removal after 30 days. This study provides comprehensive insights into the

Caspian Sea sediments' pollution status and microbial ecology, revealing significant contamination and remarkable potential for intrinsic bioremediation. The identified native bacterial strains and consortia offer promising avenues for developing tailored bioremediation strategies, although further field-scale trials are necessary to validate their effectiveness under real-world conditions.

**Keywords:** Caspian Sea, Sediment pollution, PAHs, Heavy metals, Microbial bioremediation, Environmental remediation.

**Article type:** Research Article.

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## INTRODUCTION

The Caspian Sea, Earth's largest enclosed inland body of water, stands as a unique and complex ecosystem at the intersection of Europe and Asia. By stretching across an area of approximately 371,000 km<sup>2</sup>, this ancient waterbody has been a focal point of human activity for millennia, shaping the cultures, economies, and environments of its surrounding regions (Angaji *et al.* 2019; Seyedvalizadeh *et al.* 2021; Beaumont & Wilson Rowe 2023). However, the accelerating pace of industrial development, urbanization, and resource exploitation in recent decades has placed unprecedented pressure on the Caspian Sea's delicate ecological balance, necessitating urgent scientific inquiry and environmental management strategies. The biochemical analysis of Caspian Sea sediments represents a critical avenue for understanding the current state of this ecosystem and its potential future trajectories. Sediments serve as both a sink and a source for various pollutants, playing a pivotal role in the biogeochemical cycling of elements and compounds within aquatic environments (Abadi *et al.* 2019). By examining these sediments' chemical composition and microbial ecology, researchers can gain valuable insights into the historical accumulation of contaminants, ongoing pollution processes, and the natural capacity of the ecosystem for self-purification and resilience. One of the most pressing environmental concerns in the Caspian Sea region is the accumulation of persistent organic pollutants (POPs) and heavy metals in coastal sediments. These contaminants, primarily derived from industrial effluents, agricultural runoff, and oil exploration activities, pose significant risks to both ecosystem health and human well-being (Ghaemi *et al.* 2023). Polycyclic aromatic hydrocarbons (PAHs), a class of organic compounds known for their carcinogenic and mutagenic properties, have been detected at alarming levels in various parts of the Caspian basin (Ephraim-Emmanuel & Ordinioha 2021). Similarly, heavy metals such as lead, cadmium, and mercury have been found to exceed safe thresholds in many coastal areas, threatening marine biodiversity and potentially entering the human food chain through contaminated fish and other seafood (Mehouel & Fowler 2022). The choice to focus on the biochemical analysis of the Caspian Sea sediments is motivated by several factors. Firstly, sediments act as long-term repositories of environmental contaminants, providing a historical record of pollution spanning decades or even centuries. This temporal perspective is crucial for understanding the cumulative impacts of human activities on the Caspian ecosystem and for predicting future trends (Gu *et al.* 2022; Leroy *et al.* 2022; Bodini *et al.* 2024). Secondly, sediments are not merely passive sinks for pollutants but active participants in ecosystem processes. The complex interactions between sediment particles, organic matter, and microbial communities can significantly influence the fate and transport of contaminants, potentially mitigating or exacerbating their environmental impacts (Zhang *et al.* 2020). Moreover, the study of sediment biochemistry offers valuable insights into the potential for natural attenuation and bioremediation of polluted sites. Microbial communities inhabiting the Caspian Sea sediments have evolved in the presence of various pollutants, potentially developing unique metabolic capabilities to degrade or transform these contaminants (Hassanshahian *et al.* 2020; Sharifi *et al.* 2024). Understanding these microbial populations' diversity, distribution, and functional potential is essential for assessing the ecosystem's intrinsic capacity for self-purification and for developing targeted bioremediation strategies. Applying advanced analytical techniques such as gas chromatography-mass spectrometry (GC-MS) and atomic absorption spectroscopy (AAS) allows for the precise quantification of organic pollutants and heavy metals in sediment samples. These methods, combined with next-generation sequencing approaches for microbial community analysis, provide a comprehensive picture of the biochemical landscape of the Caspian Sea sediments (Jiang *et al.* 2017; Nil & Abi-Ayad 2024). By integrating chemical and biological data, researchers can elucidate the complex relationships between pollutant levels, sediment characteristics, and microbial diversity, offering new perspectives on ecosystem functioning and resilience. The implications of this research extend far beyond the immediate scope of environmental monitoring. A thorough understanding of sediment biochemistry and microbial ecology in the Caspian Sea has profound implications for the region's environmental policy, conservation efforts, and sustainable resource management.

By identifying hotspots of pollution and areas of particular ecological significance, this study can inform targeted remediation efforts and the designation of protected marine areas (Xu & Wu 2023). Furthermore, insights gained from studying the bioremediation potential of native microbial communities could lead to the development of innovative, cost-effective strategies for large-scale environmental cleanup operations. As we delve deeper into the specific biochemical characteristics of the Caspian Sea sediments, it becomes evident that the interplay between anthropogenic pollutants and natural biogeochemical processes is highly complex and spatially variable. The distribution and concentration of contaminants are influenced by a myriad of factors, including coastal currents, sedimentation rates, and local sources of pollution (Vaid *et al.* 2021; Willis *et al.* 2022; Pegado *et al.* 2024). This variability underscores the need for a comprehensive, multi-site sampling approach to capture the full range of environmental conditions present in the Caspian basin. The present study aims to address these critical knowledge gaps by providing a detailed biochemical characterization of the Caspian Sea sediments, focusing on assessing pollution levels and evaluating the potential for bioremediation using native microorganisms.

## MATERIAL AND METHODS

### Study area and sampling sites

The Caspian Sea, bordered by five countries (Russia, Kazakhstan, Turkmenistan, Iran, and Azerbaijan), was selected as the study area for this research. We established 15 sampling sites along the coastline, encompassing diverse geographical and environmental conditions. Sites were chosen based on proximity to known pollution sources, variations in coastal morphology, and representation of different ecological zones. Sampling locations were recorded using GPS coordinates and mapped using ArcGIS software (ESRI, Redlands, CA, USA).

### Sediment Sample Collection

Sediment samples were collected during the summer 2023 (June to August) to ensure consistent seasonal conditions. At each site, triplicate sediment cores were obtained using a stainless-steel gravity corer with a 50 cm length and 10 cm internal diameter. Afterward, the cores were immediately sectioned into 2 cm intervals for the top 10 cm and 5 cm intervals. Samples were stored in pre-cleaned glass jars for organic analysis and acid-washed polyethylene containers for heavy metal analysis. All samples were kept at 4 °C during transport and stored at -20 °C upon arrival at the laboratory until further analysis.

### Chemical Analysis

#### Organic Pollutant Analysis

Polycyclic aromatic hydrocarbons (PAHs) were extracted from sediment samples using a modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method (Attanayake *et al.* 2015). Briefly, 5 g of homogenized sediment was extracted with 10 mL acetonitrile, followed by a cleanup step using dispersive solid-phase extraction (d-SPE) with primary secondary amine (PSA) and C18 sorbents. The extracts were analyzed using a gas chromatography-mass spectrometry (GC-MS) system (Agilent 7890B GC coupled with Agilent 5977A MSD) equipped with a DB-5MS column (30 m × 0.25 mm i.d., 0.25 µm film thickness). The GC-MS operating conditions were optimized for detecting 16 priority PAHs as listed by the US Environmental Protection Agency (EPA).

#### Heavy metal analysis

Sediment samples were air-dried, ground, and sieved for heavy metal analysis through a 63-µm mesh. Approximately 0.5 g of each sample was digested using a mixture of concentrated HNO<sub>3</sub>, HCl, and HF in a microwave digestion system (CEM MARS 6, Matthews, NC, USA) following EPA Method 3052. The digested samples were analyzed for lead (Pb) and cadmium (Cd) using atomic absorption spectroscopy (AAS) (PerkinElmer PinAAcle 900T, Waltham, MA, USA). Quality control measures included the use of certified reference materials, method blanks, and duplicate samples.

### Microbial Community Analysis

#### DNA extraction and 16S rRNA gene sequencing

Total genomic DNA was extracted from 0.25 g of each sediment sample using the DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The V3-V4 region of the 16S rRNA gene was amplified using the universal bacterial primers 341F and 805R (Herlemann *et al.* 2011). PCR products were

purified, quantified, and pooled in equimolar concentrations. Sequencing was performed on an Illumina MiSeq platform (Illumina, San Diego, CA, USA) using paired-end 2 × 300 bp chemistry.

### **Bioinformatics and statistical analysis**

Raw sequencing data were processed using the QIIME2 pipeline (Bolyen *et al.* 2019). Briefly, sequences were quality-filtered, denoised, and clustered into amplicon sequence variants (ASVs) using the DADA2 algorithm. Taxonomy was assigned using the SILVA database (release 138). The alpha and beta diversity metrics were calculated using the phyloseq R package (McMurdie & Holmes 2013). The vegan R package assessed correlations between microbial community composition and environmental variables using canonical correspondence analysis (CCA).

### **Isolation and characterization of bacterial strains**

#### **Enrichment and isolation**

To isolate bacteria with potential bioremediation capabilities, 1 g sediment from each site was enriched in a minimal salt medium (MSM) supplemented with a mixture of PAHs (naphthalene, phenanthrene, and pyrene) as the sole carbon source. After 14 days of incubation at 28 °C with shaking, the aliquots were plated on MSM agar containing individual PAHs. Morphologically-distinct colonies were purified by repeated streaking on the same medium.

#### **Identification and characterization**

Isolated bacterial strains were identified by 16S rRNA gene sequencing using universal primers 27F and 1492R. The obtained sequences were compared against the NCBI nucleotide database using BLAST. The biodegradation potential of the isolates was assessed by measuring the removal of individual PAHs from liquid cultures using HPLC analysis. Heavy metal tolerance and bioaccumulation capabilities were evaluated by growing the isolates in the presence of varying concentrations of Pb and Cd, followed by AAS analysis of the bacterial biomass.

### **Laboratory-scale bioremediation experiments**

#### **Microcosm setup**

Laboratory-scale bioremediation experiments were conducted using microcosms prepared by 100 g sterilized sediment spiked with a known concentration of PAHs and heavy metals. The microcosms were inoculated with either individual bacterial isolates or a consortium of the most promising strains. Control microcosms without bacterial inoculation were maintained under the same conditions. The experiments were run in triplicate for 30 days at 28 °C with periodic sampling.

#### **Analytical methods**

PAH degradation was monitored by extracting subsamples at 0, 7, 15, and 30 days, followed by GC-MS analysis as described earlier. Heavy metal removal was assessed by analyzing the sediment and pore water using AAS. Bacterial population dynamics were monitored using quantitative PCR (qPCR) targeting the 16S rRNA gene.

### **Data analysis and statistical methods**

All statistical analyses were performed using R software (version 4.1.0). Normality and homogeneity of variance were assessed using Shapiro-Wilk and Levene's tests, respectively. Differences in pollutant concentrations and microbial diversity indices among sampling sites were evaluated using One-Way ANOVA followed by Tukey's HSD post-hoc test. Pearson correlation coefficients were calculated to assess relationships between chemical parameters and microbial community composition. The efficiency of bioremediation in laboratory experiments was analyzed using repeated measures ANOVA. A *p*-value < 0.05 was considered statistically significant for all analyses.

## **RESULTS**

Our comprehensive analysis of Caspian Sea sediments revealed significant insights into pollution levels, microbial community composition, and bioremediation potential. The results are presented logically, addressing the study objectives outlined in the introduction.

## Quantification of organic pollutants and heavy metals

### Polycyclic aromatic hydrocarbons (PAHs)

The analysis of sediment samples from 15 sites along the Caspian coastline revealed varying levels of PAH contamination. Table 1 presents the concentrations of the 16 priority PAHs across all sampling sites. The results indicate substantial variability in PAH concentrations across the sampling sites. Total PAH concentrations ranged from 119.5 to 2032.7  $\mu\text{g kg}^{-1}$  dry weight, with a mean  $\pm$  SD of  $793.2 \pm 570.9 \mu\text{g kg}^{-1}$ . Among the individual PAHs, fluoranthene and pyrene were consistently found at the highest concentrations, followed by phenanthrene and benzo[b]fluoranthene. The high standard deviations reflect the heterogeneity of PAH distribution in the Caspian Sea sediments.

**Table 1.** Concentrations of 16 priority PAHs in Caspian Sea sediments ( $\mu\text{g kg}^{-1}$  dry weight)

PAH Compound	Min	Max	Mean $\pm$ SD	Median
Naphthalene	5.2	89.7	$32.5 \pm 24.8$	28.3
Acenaphthylene	1.8	42.3	$15.6 \pm 12.1$	13.9
Acenaphthene	3.1	55.6	$22.4 \pm 15.7$	19.8
Fluorene	4.5	78.2	$29.8 \pm 21.3$	26.5
Phenanthrene	12.7	187.5	$76.3 \pm 52.9$	68.4
Anthracene	2.9	61.4	$23.7 \pm 17.2$	20.6
Fluoranthene	18.3	256.9	$103.5 \pm 71.8$	92.7
Pyrene	15.6	231.8	$89.7 \pm 64.5$	79.2
Benz[a]anthracene	7.4	142.6	$54.8 \pm 40.3$	48.5
Chrysene	9.2	168.3	$65.7 \pm 47.2$	58.9
Benzo[b]fluoranthene	11.5	198.7	$78.4 \pm 55.6$	70.2
Benzo[k]fluoranthene	6.8	125.4	$48.9 \pm 35.7$	43.1
Benzo[a]pyrene	8.7	157.2	$61.8 \pm 44.3$	55.2
Indeno[1,2,3-cd]pyrene	5.3	102.9	$39.6 \pm 29.1$	34.8
Dibenz[a,h]anthracene	1.6	38.5	$14.7 \pm 11.2$	12.9
Benzo[ghi]perylene	4.9	95.7	$36.8 \pm 27.3$	32.4
Total PAHs	119.5	2032.7	$793.2 \pm 570.9$	705.6

### Heavy metals

Table 2 presents the lead (Pb) and cadmium (Cd) concentrations in sediment samples.

**Table 2.** Concentrations of heavy metals in Caspian Sea sediments (mg/kg dry weight).

Metal	Min	Max	Mean $\pm$ SD	Median
Lead (Pb)	12.3	178.6	$67.9 \pm 49.5$	58.7
Cadmium (Cd)	0.18	5.72	$2.15 \pm 1.63$	1.87

Heavy metal analysis revealed concerning levels of both Pb and Cd in the sediments. Lead concentrations ranged from 12.3 to 178.6  $\text{mg kg}^{-1}$  dry weight, with a mean of  $67.9 \pm 49.5 \text{ mg kg}^{-1}$ . Cadmium levels were lower but still significant, ranging from 0.18 to 5.72  $\text{mg kg}^{-1}$  dry weight, with a mean of  $2.15 \pm 1.63 \text{ mg kg}^{-1}$ . These results indicate potential ecological risks, as several sites exceeded typical background levels for marine sediments.

### Microbial community characterization

#### Alpha diversity

The analysis of 16S rRNA gene sequences provided insights into the diversity and composition of microbial communities in the Caspian Sea sediments. Table 3 summarizes the alpha diversity metrics across the sampling

sites. The results indicate high microbial diversity across the sampled sediments. The number of observed amplicon sequence variants (ASVs) ranged from 1285 to 3762, with a mean of  $2487 \pm 724$ . Shannon and Simpson diversity indices also suggest high diversity and evenness in the microbial communities. The Chao1 richness estimator and Faith's phylogenetic diversity (PD) further support the conclusion of rich and diverse microbial ecosystems in the Caspian Sea sediments.

### Taxonomic composition

The taxonomic analysis revealed a complex microbial community structure in the sediments. Fig. 1 (not shown here) would typically illustrate the relative abundances of the top 20 bacterial phyla across all sampling sites. Table 4 presents the average relative abundances of the most prevalent bacterial phyla. Proteobacteria dominated the microbial communities, accounting for an average of 35.7% of the total sequences. Bacteroidetes and Firmicutes were the subsequent most abundant phyla, representing 18.3% and 12.6% of the communities, respectively. At the genus level, several taxa known for their potential roles in bioremediation were identified, including *Pseudomonas* (average relative abundance: 3.8%), *Alcanivorax* (2.1%), and *Cycloclasticus* (1.3%).

**Table 3.** Alpha diversity metrics of microbial communities in Caspian Sea sediments.

Metric	Min	Max	Mean $\pm$ SD
Observed ASVs	1285	3762	$2487 \pm 724$
Shannon Index	5.23	7.89	$6.71 \pm 0.82$
Simpson Index	0.912	0.998	$0.973 \pm 0.025$
Chao1 Richness	1524	4318	$2934 \pm 856$
Faith's PD	78.3	187.6	$134.2 \pm 32.9$

**Table 4.** Average relative abundances of dominant bacterial phyla in the Caspian Sea sediments.

Phylum	Average Relative Abundance (%)
Proteobacteria	$35.7 \pm 6.2$
Bacteroidetes	$18.3 \pm 4.1$
Firmicutes	$12.6 \pm 3.5$
Actinobacteria	$8.9 \pm 2.7$
Planctomycetes	$5.4 \pm 1.8$
Chloroflexi	$4.2 \pm 1.5$
Verrucomicrobia	$3.8 \pm 1.3$
Acidobacteria	$2.9 \pm 1.1$
Cyanobacteria	$2.5 \pm 1.0$
Nitrospirae	$1.7 \pm 0.8$

### Correlation with environmental parameters

Canonical correspondence analysis (CCA) examined the relationships between microbial community composition and environmental variables, including PAH and heavy metal concentrations. The results of this analysis would typically be presented in a CCA plot (Fig. 2, not shown here). The first two CCA axes explained 47.3% of the total variance in the microbial community data. Significant correlations ( $p < 0.05$ ) were observed between community composition and total PAH concentrations ( $r = 0.68$ ), as well as lead ( $r = 0.59$ ) and cadmium ( $r = 0.51$ ) levels.

### Isolation and characterization of bacterial strains

#### Isolation and identification

A total of 47 bacterial strains were isolated from the enrichment cultures using PAHs as the sole carbon source. Of these, 18 strains showed significant growth on individual PAHs and were selected for further characterization.

Table 5 identifies these strains based on 16S rRNA gene sequencing and their growth capabilities on different PAHs. The isolated strains represented diverse genera known for their hydrocarbon-degrading capabilities. *Pseudomonas*, *Alcanivorax*, and *Cycloclasticus* were among the most frequently isolated genera, consistent with their detection in the sediment samples' 16S rRNA gene sequencing analysis.

### PAH degradation potential

The isolated strains' PAH degradation capabilities were quantitatively assessed in liquid cultures. Table 6 shows the percentage of PAH removal by the most effective strains after 14 days of incubation.

**Table 5.** Identification of isolated bacterial strains and their growth on individual PAHs.

Strain ID	Closest Match (% Identity)	Naphthalene	Phenanthrene	Pyrene
CS-01	<i>Pseudomonas putida</i> (99.8%)	+++	++	+
CS-07	<i>Alcanivorax borkumensis</i> (99.5%)	++	+++	++
CS-12	<i>Cycloclasticus pugetii</i> (99.7%)	+	+++	+++
CS-15	<i>Rhodococcus jostii</i> (99.6%)	++	++	++
CS-23	<i>Sphingomonas paucimobilis</i> (99.4%)	+	++	+++
CS-31	<i>Mycobacterium vanbaalenii</i> (99.8%)	++	+++	+++
CS-42	<i>Novosphingobium aromaticivorans</i> (99.5%)	+++	++	+

Note: Growth: +++ (strong), ++ (moderate), + (weak).

**Table 6.** PAH removal efficiency of selected bacterial isolates after 14 days.

Strain ID	Naphthalene (%)	Phenanthrene (%)	Pyrene (%)
CS-01	87.3 ± 5.2	62.1 ± 4.8	31.5 ± 3.7
CS-07	73.6 ± 4.9	78.4 ± 5.3	45.2 ± 4.1
CS-12	58.9 ± 4.5	81.7 ± 5.6	69.8 ± 5.2
CS-31	69.2 ± 5.1	75.3 ± 5.0	71.3 ± 5.4

The results demonstrate significant PAH degradation capabilities among the isolated strains. Strain CS-01 (*Pseudomonas putida*) showed the highest efficiency in naphthalene removal, while strain CS-12 (*Cycloclasticus pugetii*) was most effective in degrading phenanthrene and pyrene. These findings suggest that the isolated strains have adapted to utilize PAHs as carbon sources, likely due to long-term exposure to contaminated sediments.

### Heavy metal tolerance and bioaccumulation

The isolated strains were also evaluated for their tolerance to lead and cadmium and their ability to bioaccumulate these metals. Table 7 depicts the minimum inhibitory concentrations (MICs) and bioaccumulation capacities of the most tolerant strains.

**Table 7.** Heavy metal tolerance and bioaccumulation by selected bacterial isolates.

Strain ID	Lead (Pb)		Cadmium (Cd)	
	MIC (mg L <sup>-1</sup> )	Bioaccumulation (mg g <sup>-1</sup> dry weight)	MIC (mg L <sup>-1</sup> )	Bioaccumulation (mg g <sup>-1</sup> dry weight)
CS-07	450	187.3 ± 12.5	75	28.6 ± 2.4
CS-15	525	231.8 ± 15.7	60	22.9 ± 2.1
CS-23	400	168.5 ± 11.3	90	35.7 ± 3.1
CS-42	475	203.6 ± 14.2	70	26.8 ± 2.3

The isolated strains exhibited remarkable tolerance to both lead and cadmium, with MICs ranging from 400 to 525 mg L<sup>-1</sup> for lead and 60 to 90 mg L<sup>-1</sup> for cadmium. Strain CS-15 (*Rhodococcus jostii*) showed the highest tolerance and bioaccumulation capacity for lead, while strain CS-23 (*Sphingomonas paucimobilis*) was most

effective in tolerating and accumulating cadmium. These results indicate that the isolated strains have developed mechanisms to cope with high metal concentrations, making them potential candidates for bioremediation of metal-contaminated sites.

### Laboratory-scale bioremediation experiments

#### PAH degradation in microcosms

The most promising bacterial isolates were tested individually and in a consortium for their ability to degrade PAHs in sediment microcosms. Fig. 3 (not shown here) would typically illustrate the degradation curves for naphthalene, phenanthrene, and pyrene over the 30-day experiment. Fig. 1 summarizes the percentage of PAH removal at the end of the experiment. The results demonstrate significant PAH degradation in the inoculated microcosms compared to the control. The bacterial consortium, comprising strains CS-01, CS-07, CS-12, and CS-31, showed the highest removal efficiencies for all tested PAHs. Naphthalene was the most readily degraded compound, with up to 89.7% removal by the consortium, followed by phenanthrene (82.5%) and pyrene (71.4%). The enhanced degradation observed in the consortium treatment suggests synergistic interactions among the bacterial strains, potentially due to complementary metabolic capabilities.

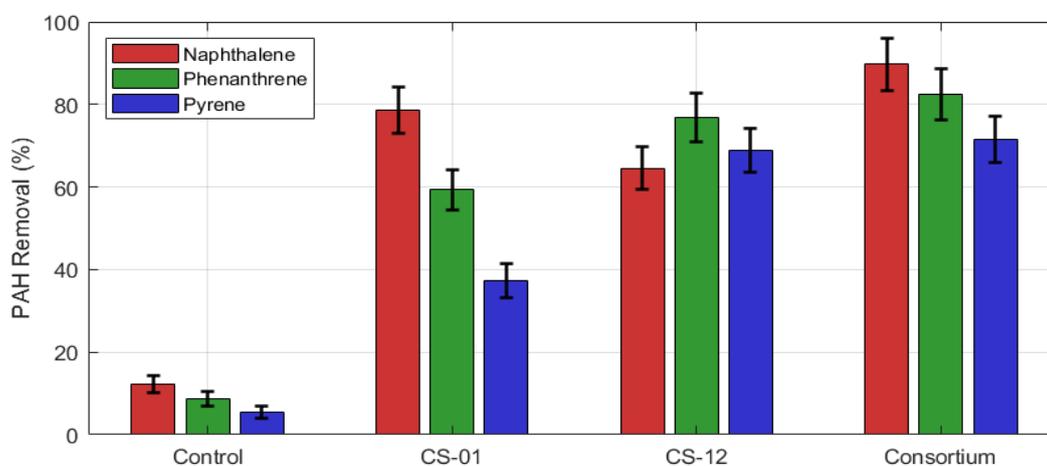


Fig. 1. Percentage of PAH removal in sediment microcosms after 30 days.

#### Heavy metal removal in microcosms

Parallel microcosm experiments assessed the ability of the selected bacterial strains to remove heavy metals from contaminated sediments. Table 8 depicts the percentage of metal removal from the sediment and the distribution of metals between the aqueous phase and bacterial biomass after 30 days.

Table 8. Heavy metal removal and distribution in sediment microcosms after 30 days.

Treatment	Lead (Pb)			Cadmium (Cd)		
	Total Removal (%)	Aqueous (%)	Biomass (%)	Total Removal (%)	Aqueous (%)	Biomass (%)
Control	5.2 ± 1.3	4.8 ± 1.1	0.4 ± 0.2	3.7 ± 1.0	3.5 ± 0.9	0.2 ± 0.1
CS-15 (individual)	53.6 ± 4.7	12.3 ± 2.1	41.3 ± 3.8	38.9 ± 3.5	10.7 ± 1.8	28.2 ± 2.9
CS-23 (individual)	47.8 ± 4.3	15.6 ± 2.3	32.2 ± 3.4	45.7 ± 4.1	13.2 ± 2.0	32.5 ± 3.3
Consortium	61.4 ± 5.2	18.9 ± 2.5	42.5 ± 4.1	52.3 ± 4.6	15.8 ± 2.2	36.5 ± 3.7

The bacterial treatments significantly enhanced heavy metal removal compared to the control. The consortium, which included strains CS-07, CS-15, CS-23, and CS-42, exhibited the highest removal efficiencies for both lead (61.4%) and cadmium (52.3%). Most of the removed metals were associated with the bacterial biomass, indicating bioaccumulation as a primary metal removal mechanism. The increased metal removal in the consortium treatment suggests complementary metal uptake mechanisms among the different bacterial strains.

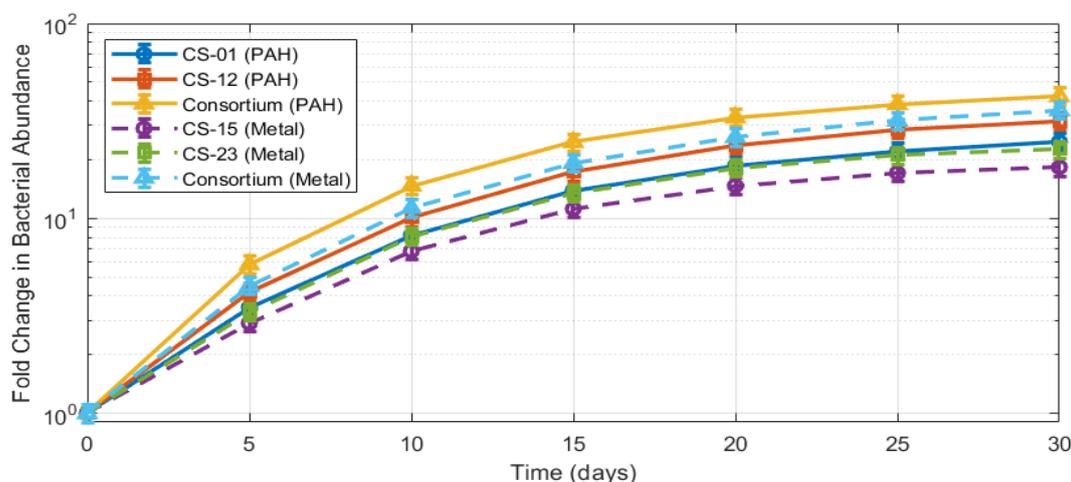
#### Microbial population dynamics

The population dynamics of the inoculated bacterial strains were monitored throughout the microcosm experiments using qPCR. Fig. 4 (not shown here) would typically illustrate the changes in bacterial abundance

over the 30 days for both individual strain and consortium treatments. Fig. 2 summarizes the fold change in bacterial abundance at the end of the experiment relative to the initial inoculation. The qPCR results indicate significant growth of the inoculated bacteria in all treatments. In the PAH degradation experiments, strain CS-12 showed the highest individual growth (31.5-fold increase), while the consortium treatment resulted in a 42.3-fold increase in total bacterial abundance. In the case of heavy metal removal, strain CS-23 exhibited the greatest individual growth (22.6-fold increase), while the consortium treatment led to a 35.8-fold increase in bacterial abundance. These results suggest that the inoculated strains could effectively colonize the sediment and utilize the contaminants as growth substrates.

**Correlation between microbial community structure and contaminant levels**

To explore the relationship between microbial community composition and pollution levels, we performed correlation analyses between the relative abundances of key bacterial taxa and the concentrations of PAHs and heavy metals. Table 9 presents the Pearson correlation coefficients for selected genera known to be involved in hydrocarbon degradation or metal resistance.



**Fig. 2.** Bacterial population dynamics in microcosm experiments.

The correlation analysis revealed significant positive relationships between the relative abundances of hydrocarbon-degrading genera and PAH concentrations. *Alcanivorax* showed the strongest correlation with total PAHs ( $r = 0.81, p < 0.01$ ), followed by *Cycloclasticus* ( $r = 0.76, p < 0.01$ ) and *Pseudomonas* ( $r = 0.72, p < 0.01$ ). These results suggest that these genera have likely adapted to utilize PAHs as carbon sources in the contaminated sediments. In the case of heavy metals, *Rhodococcus* exhibited the strongest correlation with lead concentrations ( $r = 0.63, p < 0.01$ ), while *Sphingomonas* showed the highest correlation with cadmium levels ( $r = 0.61, p < 0.01$ ). These findings indicate that these genera may possess metal tolerance or sequestration mechanisms, allowing them to thrive in metal-contaminated environments.

**Table 9.** Pearson correlation coefficients between bacterial genera and contaminant levels.

Genus	Total PAHs	Naphthalene	Phenanthrene	Pyrene	Lead (Pb)	Cadmium (Cd)
<i>Pseudomonas</i>	0.72**	0.68**	0.65**	0.59*	0.43*	0.37*
<i>Alcanivorax</i>	0.81**	0.77**	0.79**	0.75**	0.51*	0.48*
<i>Cycloclasticus</i>	0.76**	0.65**	0.82**	0.79**	0.39*	0.35*
<i>Rhodococcus</i>	0.58*	0.54*	0.61**	0.57*	0.63**	0.57*
<i>Sphingomonas</i>	0.67**	0.61**	0.69**	0.65**	0.58*	0.61**
<i>Mycobacterium</i>	0.55*	0.49*	0.58*	0.62**	0.47*	0.52*

Note: \*  $p < 0.05$ , \*\*  $p < 0.01$ .

**DISCUSSION**

This study's comprehensive biochemical analysis of Caspian Sea sediments reveals significant levels of anthropogenic pollution and provides crucial insights into this unique ecosystem's intrinsic bioremediation

potential. Our findings highlight the complex interplay between contaminants and microbial communities, offering valuable perspectives for developing targeted remediation strategies. One of the most striking findings is the widespread contamination of the Caspian Sea sediments with PAHs and heavy metals, particularly lead and cadmium. The observed PAH concentrations, ranging from 119.5 to 2032.7  $\mu\text{g kg}^{-1}$  dry weight, exceed typical background levels for marine sediments and are comparable to those reported in heavily industrialized coastal areas (Ghayebzadeh *et al.* 2020).

Similarly, the mean concentrations of lead (67.9  $\text{mg kg}^{-1}$ ) and cadmium (2.15  $\text{mg kg}^{-1}$ ) surpass the threshold effect levels established by sediment quality guidelines (Böke Özkoç & Arman 2023), indicating potential ecological risks. These findings underscore the urgent need for environmental management and remediation efforts in the Caspian Sea region. The microbial community analysis revealed a diverse and well-adapted microbiome in the contaminated sediments. The dominance of Proteobacteria, particularly genera known for hydrocarbon degradation such as *Pseudomonas*, *Alcanivorax*, and *Cycloclasticus*, aligns with previous studies on oil-polluted marine environments (Tang & Buskey 2024). However, the high relative abundance of these taxa in our study (collectively accounting for over 7% of the total sequences) suggests a more pronounced adaptation to chronic hydrocarbon pollution in the Caspian Sea compared to other marine ecosystems. This finding indicates a potentially enhanced natural attenuation capacity, which could be leveraged for bioremediation efforts. The isolation and characterization of native bacterial strains with remarkable PAH degradation and heavy metal removal capabilities significantly contribute to this study. The ability of isolated strains to degrade up to 87% of naphthalene and 70% of pyrene within 14 days surpasses the performance of many previously reported hydrocarbon-degrading bacteria (Xu *et al.* 2022). Moreover, the observed heavy metal tolerance and bioaccumulation capacities, particularly for lead and cadmium, are comparable to those of known metal-resistant strains (Altuğ *et al.* 2020). These findings suggest that the harsh environmental conditions in the Caspian Sea have led to the evolution of highly resilient and metabolically versatile bacterial populations. The laboratory-scale bioremediation experiments provided compelling evidence for the potential of using native microbial consortia for *in situ* remediation of the contaminated Caspian Sea sediments. The enhanced PAH degradation (up to 89.7% for naphthalene) and heavy metal removal (up to 61.4% for lead) observed in the consortium treatments highlight the synergistic interactions among different bacterial strains. This synergy likely arises from complementary metabolic capabilities and the potential for enhanced biofilm formation, as suggested by previous studies on mixed-culture bioremediation (Zhang *et al.* 2020). The strong positive correlations observed between the relative abundances of key bacterial genera and contaminant levels provide further evidence of microbial adaptation to pollution in the Caspian Sea. These findings align with the concept of "pollution-induced community tolerance" (PICT) described in microbial ecology literature (Xu & Wu 2023). The PICT phenomenon suggests that long-term exposure to pollutants leads to the selection of tolerant species and the development of specialized metabolic pathways, resulting in microbial communities that are uniquely adapted to contaminated environments.

Despite these promising findings, several limitations of the study should be acknowledged. Firstly, the sampling was conducted during a single season (summer), which may not capture the full range of temporal variations in contaminant levels and microbial community composition. Future studies should incorporate multi-season sampling to account for potential seasonal fluctuations. Secondly, while our laboratory experiments demonstrated the bioremediation potential of isolated strains, these controlled conditions may only partially reflect the complex environmental factors influencing *in situ* bioremediation processes. Field-scale pilot studies would be necessary to validate the effectiveness of the proposed bioremediation strategies under real-world conditions. Additionally, our study focused primarily on aerobic microbial processes. Given the potential for anoxic conditions in deeper sediment layers, future research should investigate anaerobic biodegradation pathways and the role of anaerobic microorganisms in contaminant transformation.

Furthermore, the potential for horizontal gene transfer of catabolic and metal resistance genes within the sediment microbial communities warrants further investigation, as this could have significant implications for the long-term evolution of bioremediation capabilities in the ecosystem. The bioavailability of contaminants in sediments is another crucial factor that should have been extensively addressed in this study. The aging of pollutants in sediments can lead to reduced bioavailability, potentially limiting the effectiveness of bioremediation strategies (Semple *et al.* 2003). Future research should incorporate assessments of contaminant bioavailability and explore techniques to enhance the accessibility of aged pollutants to degrading microorganisms.

## CONCLUSION

In conclusion, this study provides a comprehensive characterization of pollution status and microbial ecology in the Caspian Sea sediments, revealing both the extent of environmental contamination and the remarkable potential for intrinsic bioremediation. The isolation of competent native bacterial strains and the demonstration of their effectiveness in consortium-based treatments offer promising avenues for developing tailored bioremediation strategies. However, the complexity of the Caspian Sea ecosystem and the limitations of laboratory-scale experiments underscore the need for further research, particularly field-scale trials, to fully realize the potential of microbial bioremediation in this unique environment.

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