

PAHs-contaminated water and sediment marine habitats shape microbial diversity and community structure in a eutrophic multi-stressed coastal system

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Abstract This study investigates the influence of Polycyclic Aromatic Hydrocarbons (PAHs) driven contamination on microbial diversity and community structure in seawater and sediment samples from five sites in the Inner Saronikos Gulf and Elefsis Bay, Greece, two marine coastal areas under intense anthropogenic pressure. PAHs were quantified in both seawater (dissolved and particulate phase) and sediments. The molecular profiles of the determined PAH compounds indicate mixed petrogenic and pyrolytic sources, with low molecular weight (LMW) PAHs dominating seawater and high molecular weight (HMW) ones prevailing in sediments. High-throughput sequencing revealed distinct microbial communities, highlighting the use of microbial taxa as contamination bioindicators. Canonical Correspondence Analysis (CCA) grouped the bacterial communities according to PAH sources. The results describe pollutant-driven microbial responses and highlight the ecological risks of chronic PAH contamination in the study area.

Keywords: Polycyclic aromatic hydrocarbons, pollution, metabarcoding, bacterioplankton, biodegradation

1. Introduction

PAHs are a group of persistent organic pollutants originating from combustion, industrial, and petroleum-related activities. They enter the marine realm through atmospheric deposition, oil spills, and continental runoffs, and tend to accumulate in sediments due to their hydrophobic properties (Simoneit 1984). Microbial communities, as first responders, can degrade PAHs through specific metabolic pathways or cooperative interactions and serve as bioindicators due to pollution-induced shifts in their community structure (Jokanović et al., 2021). The Saronikos Gulf and Elefsis Bay (Aegean Sea), adjacent to Athens and the port of Piraeus, are affected by multiple anthropogenic pressures, including industrial activities, shipping, and wastewater discharges, amongst others. This study investigates the links between PAH contamination and microbial communities in the study area by: (a) identifying PAH sources, concentrations, and spatial distribution; (b) characterizing microbial

diversity in seawater and sediments; and (c) assessing how PAHs and environmental variables shape microbial community structure.

2. Materials and Methods

Seawater and sediment samples were collected in June 2024 from five sites across the Saronikos Gulf and Elefsis Bay (Table 1). For PAH analysis, 2.5 L of surface and deep euphotic zone water were filtered *in-situ* using pre-combusted GF/F filters. Surface sediments were collected using an Ekman grab, and stored at −20 °C till further analyses. Instrumental analysis of PAHs was carried out by gas chromatography-mass spectrometry (GC-MS), quantifying 47 compounds (TPAH₄₇), including the 16 US-EPA priority parent PAHs and alkylated homologues of naphthalene, dibenzothiophene, phenanthrene, pyrene, and chrysene (Skylaki et al., 2025). DNA was extracted from filters and sediments using the NucleoSpin® Soil Kit. PCR amplification was performed using primers targeting the V3-V4 hypervariable region of the 16S rRNA gene, with metabarcoding sequencing conducted at a 300+300 bp paired-end Illumina MiSeq sequencer.

Table 1. Sampling sites across the study area.

Sampling site	Coordinates	Sampling Depth	Sampling point code
S43	37.5316N, 23.3520E	2 m	S43.2
		90 m	S43.B
		sediment	S43.sed
S7	37.5542N, 23.3545E	2 m	S7.2
		45 m	S7.45
		60 m	S7.B
KER	37.5655N, 23.3419E	sediment	S7.sed
		2 m	Ker.2
		sediment	Ker.sed
S1	38.1594N, 23.3327E	2 m	S1.2
		20 m	S1.B
		sediment	S1.sed
S2	37.5960N, 23.27180E	2 m	S2.2
		30 m	S2.B
		sediment	S2.sed

CCA was implemented to explore relationships between microbial diversity and PAH sources.

3. Results and Discussion

PAH concentrations in sediments ranged from 860 to 5451 ng g⁻¹, avg. 3027 ± 1816 ng g⁻¹. The molecular composition of the studied compounds suggested mixed petrogenic and pyrolytic origins, with LMW PAHs dominating the water column, while HMW compounds, due to their hydrophobicity, accumulating in sediments. Positive Matrix Factorization analysis identified four common PAH sources in both seawater and sediments, however their distributions varied. In seawater, contributions were evenly distributed among LMW fossil (27%), petrogenic (28%), low-temperature combustion (22%), and high-temperature combustion (24%), indicating labile inputs. In contrast, sediments were largely influenced by high-

temperature combustion (50%), characteristic of persistent HMW PAHs from sources like wood and coal (Skylaki et al., 2025), while the remaining sources (23%, 14%, 12%) indicated long-term accumulation and selective degradation. Metabarcoding analysis revealed distinct microbial communities in seawater and sediment habitats. Several detected Operational Taxonomic Units (OTUs) belonged to genera commonly associated with PAH-contaminated environments, including *Alcanivorax*, *Marinobacter*, *Mycobacterium*, *Nocardioides*, *Colwellia*, *Vibrio*, *Spirochaeta*, and *Microbulbifer*, though most appeared at low relative abundances. CCA showed that all four PAH sources significantly influenced microbial composition in the water column (Figure 1). These results suggest that specific bacterial taxa, employing biodegradation gene machinery or developing tolerance mechanisms (Genitsaris et al., 2025) can be used as bioindicators of pollution.

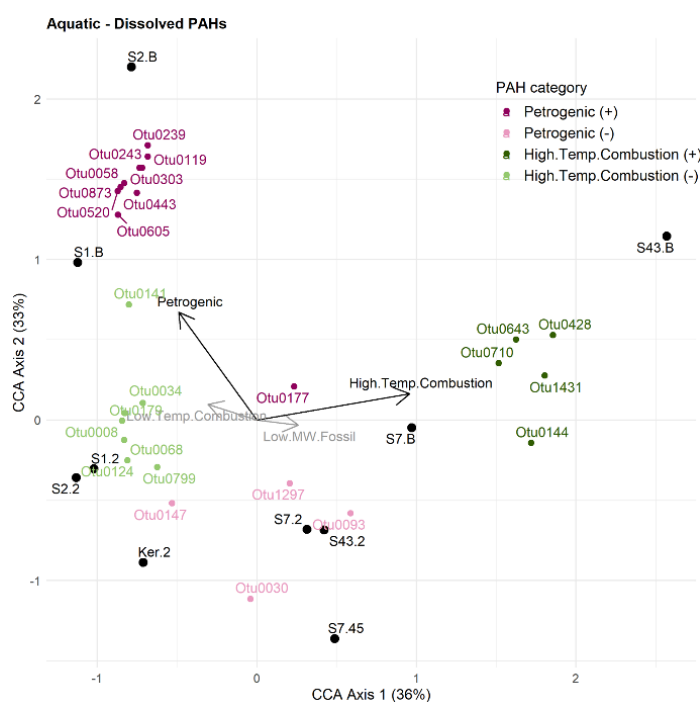


Figure 1. Biplot of PAHs sources and bacterial communities based on Canonical Correspondence Analysis (CCA).

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