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Spacial distribution of heavy metal (cadmium, iron, lead, aluminum) and community structure of bacteria from Sendangbiru Beach based on environmental DNA 16S rDNA

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ABSTRACT

Heavy metal pollution in coastal waters has emerged as a significant environmental concern, particularly near river estuaries and urban areas, due to uncontrolled industrial, mining, and domestic activities. This study aimed to assess the spatial distribution of heavy metals (cadmium, iron, lead, and aluminum) and the corresponding bacterial community structures at Sendangbiru Beach, Malang, Indonesia. The methods employed included heavy metal concentration analysis using atomic absorption spectrophotometry (AAS) and evaluation of bacterial community structures through environmental DNA (eDNA) metabarcoding. Results revealed that iron (Fe) exhibited the highest concentrations, suggesting significant anthropogenic and natural inputs. Cadmium (Cd) and lead (Pb) showed lower but consistently distributed levels, indicating chronic pollution from human activities. Metagenomic profiling identified diverse bacterial genera, notably *Psychrobacter, Neptunomonas*, and *Pseudomonas*, known for their resistance to heavy metal contamination. High microbial diversity indices at specific sites, such as Waru-Waru and Watu Pecah, indicated ecological resilience and adaptability in response to environmental stressors. These findings highlight the effectiveness of integrating eDNA metabarcoding with geospatial analyses as a valuable tool for monitoring ecological health and guiding sustainable coastal ecosystem management.

Keyword: heavy metals, environmental DNA, metabarcoding, microbial diversity, pollution.

INTRODUCTION

Sendangbiru Beach is one of Indonesia's ecologically and economically significant coastal ecosystems, serving as a major hub for fisheries and maritime activities. However, increasing anthropogenic pressures such as industrial effluents, ship fuel residues, and agricultural runoff have resulted in the accumulation of heavy metals, particularly Cd, Pb, and Fe, in the aquatic environment (Sharma et al., 2025; Bertucci et al., 2022). These pollutants pose substantial ecological risks, including disruptions to ecosystem equilibrium, reduction in biodiversity, and bioaccumulation in marine food webs, ultimately threatening human health (Kim et al., 2024). Therefore, sensitive and efficient monitoring methods are essential to assess pollution impact in marine environments, with environmental DNA (eDNA) emerging as a promising biomonitoring tool.

eDNA has been widely recognized as a revolutionary approach for biodiversity assessment and ecosystem health evaluation. This technique enables species detection without direct sampling, instead relying on genetic material released into the environment through excretion, cell shedding, or tissue decomposition (Takahashi et al., 2023; Wang et al., 2022). eDNA-based monitoring has been successfully applied in various ecological studies, including the detection of rare species, identification of invasive organisms, and analysis of microbial community structures in aquatic ecosystems (Macher et al., 2023). Compared to conventional monitoring techniques, eDNA provides higher sensitivity, broader spatial and temporal coverage, and rapid detection of ecological changes (Zhou et al., 2025).

The relationship between heavy metal pollution and microbial communities has become a critical focus in ecotoxicological research. Heavy metal exposure often leads to shifts in microbial community composition, favoring metal-resistant species while suppressing sensitive ones (Bertucci et al., 2022; Sharma et al., 2025). Recent advancements in eDNA metabarcoding have enabled the identification of microbial community responses to heavy metal contamination, including genetic markers associated with metal resistance (Kim et al., 2024). This molecular-based approach offers valuable insights into microbial ecological functions and biogeochemical processes within polluted marine ecosystems.

The integration of eDNA technology with heavy metal characterization holds significant potential for environmental monitoring and conservation. Combining eDNA analyses with geospatial modeling can facilitate pollution mapping and provide early warning indicators for ecosystem degradation (Takahashi et al., 2023; Wang et al., 2022). Given the increasing recognition of eDNA-based biomonitoring, this study aims to investigate the spatial distribution of heavy metals and microbial community structures at Sendangbiru Beach using eDNA as an innovative environmental assessment tool. The findings will contribute to a deeper understanding of the ecological impacts of heavy metal pollution and support sustainable coastal management efforts.

MATERIAL AND METHODS

Sample collection

Water and sediment samples were collected from ten sampling sites representing different levels of anthropogenic activities at Sendangbiru Beach. Water samples were obtained from a depth of 0.5 to 2 meters using a Niskin bottle sampler and were subsequently filtered through a 0.45 µm nylon membrane to capture environmental DNA (eDNA) (Takahashi et al. 2023). Each sample was transported to the laboratory under cold-chain conditions to prevent DNA degradation and contamination (Macher et al. 2023).

The sampling sites included various ecological and anthropogenic influence zones (Figure 1): Watu Meja, Waru-Waru, Harbor area, Watu Pecah, 3 Warna.

Heavy metal analysis

Heavy metal concentrations of Cd, Pb, Fe, and Al in both water and sediment samples were analyzed using atomic absorption spectrophotometry (AAS) and inductively coupled plasma mass spectrometry (ICP-MS) (Sharma et al. 2025; Bertucci et al. 2022). The accuracy and precision of measurements were ensured through the use of certified reference materials and procedural blanks, minimizing the risk of contamination and ensuring data reliability (Kim et al. 2024).

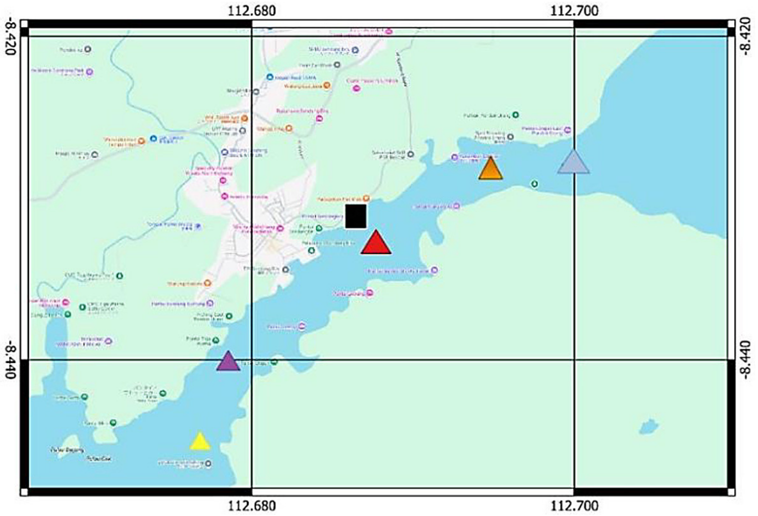

Figure 1. Sampling site at Sendangbiru Beach, Malang, Indonesia: blue triangle – Watu Meja, orange triangle – Waru-Waru, red triangle – Harbor area, yellow triangle – Watu Pecah, purple triangle – 3 Warna

DNA extraction and sequencing

DNA extraction from water samples was performed using the DNeasy PowerWater Kit (Qiagen), which is optimized for recovering environmental DNA while minimizing inhibitors present in aquatic samples (Macher et al. 2023). The extracted DNA was quantified using a NanoDrop spectrophotometer, and DNA integrity was assessed through 1.5% agarose gel electrophoresis with GelRed staining (Kim et al. 2024).

Subsequent Polymerase Chain Reaction (PCR) amplification targeted the 16S rRNA gene V3–V4 region. This study used forward primer 341F (5'-CCT ACG GGN GGC WGC AG-3') and reserve primer 785R (5'-GAC TAC HVG GGT ATC TAA TCC-3') from the Illumina kit (Illumina, USA), which is widely used for characterizing bacterial community diversity in aquatic environments (Takahashi et al. 2023). The PCR conditions consisted of an initial denaturation step at 95 °C for 30 seconds, followed by 35 cycles of denaturation (95 °C, 30 seconds), annealing (55 °C, 30 seconds), and extension (72 °C, 45 seconds) (Macher et al. 2023). Amplicon sequencing was performed on the Illumina MiSeq platform, generating high-throughput sequencing data for microbial community analysis (Kim et al. 2024).

Diversity index analysis

Microbial diversity at each sampling site was assessed using two widely applied ecological metrics: the Shannon diversity index (H') and the Simpson diversity index (D). These indices were calculated based on the taxonomic data obtained from 16S rRNA gene sequencing results.

The Shannon diversity index was used to estimate species richness and evenness, and is calculated using the formula:

$$H' = -\sum_{i=1}^{s} pi\, Ln(pi) \tag{1}$$

where: H' – Shannon diversity index, S – total number of species (or operational taxonomic units, OTUs), p_1 – proportion of the *i-th* species relative to the total number of sequences.

A higher H' value indicates greater community diversity, reflecting both a larger number of taxa and a more even distribution of individuals among those taxa (Takahashi et al. 2023). The Simpson diversity index (D) quantifies the probability that two individuals randomly selected from a sample belong to the same species. The index is calculated using the formula:

$$D = 1 - \sum_{i=1}^{s} pi^2 \tag{2}$$

where: D – Simpson diversity index, p_1 – proportion of the *i-th* species

The value of D ranges between 0 and 1, where values closer to 1 indicate higher diversity and lower dominance of individual species (Macher et al. 2023).

Bioinformatics and statistical analysis

The raw sequencing reads were processed using QIIME2 and DADA2 for quality filtering, chimera removal, and operational taxonomic unit (OTU) clustering at a 97% sequence similarity threshold (Macher et al. 2023). Taxonomic classification was performed using the SILVA rRNA database and NCBI GenBank reference sequences (Wang et al. 2022).

RESULTS AND DISCUSSION

Heavy metal distribution

This study provides insights into the spatial distribution of heavy metals and the associated bacterial community structure at Sendangbiru Beach, Malang, Indonesia (Figure 2). The analysis revealed that Fe presented the highest concentration across all sampling locations, notably around 0.024 mg/L, suggesting Fe as the predominant heavy metal contaminant. This high concentration could originate from natural sediment erosion, boat engine residues, or industrial effluents, aligning with findings from recent studies indicating similar anthropogenic and natural sources contributing to elevated Fe levels in marine environments (Bertucci et al., 2022; Sharma et al., 2025). High concentrations of Fe can lead to increased turbidity, impacting photosynthesis in marine phytoplankton, and subsequently affecting the entire food web structure (Kim et al., 2024).

In contrast, concentrations of Cd and Pb were consistently lower yet uniformly distributed across

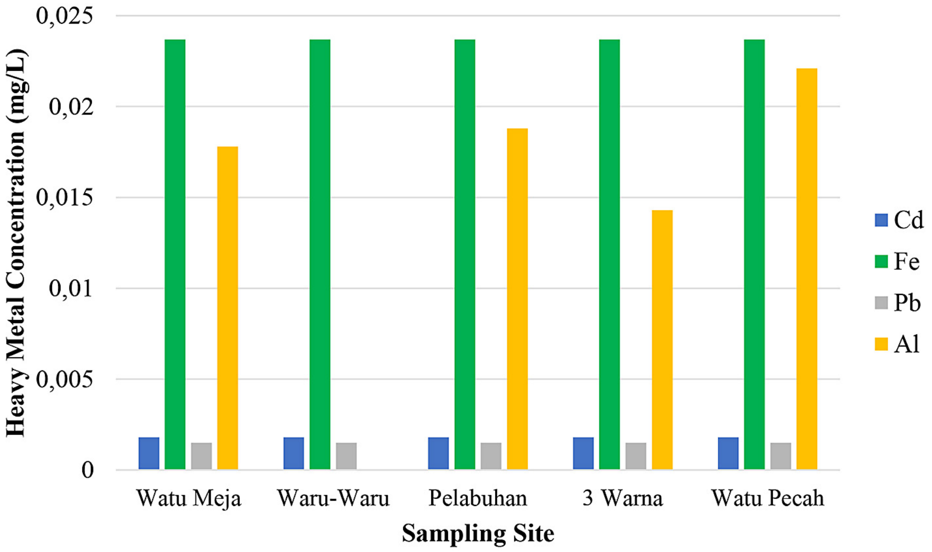

Figure 2. Heavy metal (Cd, Fe, Pb and Al) concentration among sampling sites at Sendangbiru Beach, Malang, Indonesia

all locations. This distribution pattern suggests chronic pollution potentially linked to historical fuel combustion, industrial discharges, or agricultural runoff, corroborating other recent research that highlighted the persistent presence of these metals due to ongoing anthropogenic activities (Kim et al., 2024; Sharma et al., 2025). Both Cd and Pb are highly toxic, even at low concentrations, with the potential to bioaccumulate in marine organisms, causing chronic toxicological effects such as reproductive impairment, immune suppression, and developmental anomalies in aquatic species (Bertucci et al., 2022; Sharma et al., 2025).

Al concentrations varied significantly among sites, reaching peak values at Pelabuhan (Harbor) and Watu Pecah. Such localized variability in Al concentration likely indicates distinct, point-source pollution possibly associated with marine traffic, ship maintenance activities, or localized industrial discharges, supporting the notion that Al pollution tends to show spatially discrete patterns due to its specific anthropogenic sources (Bertucci et al., 2022; Zhou et al., 2025). Elevated levels of Al can result in gill damage and impaired respiratory functions in fish and other aquatic organisms, ultimately reducing their survival rates and reproductive success (Sharma et al., 2025).

Metagenomic analysis

Microbial community analyses using eDNA revealed distinct bacterial genera dominating at different locations (Figure 3). At Watu Meja, the dominant genera were *Psychrobacter* and *Candidatus Actinomarina*, while Waru-Waru was characterized by *Neptunomonas* and *Pseudomonas* dominance. *Jannaschia* was dominant in the Pelabuhan area, whereas *Psychrobacter*, *Pseudomonas, Candidatus Actinomarina*, and *Prochlorococcus* prevailed at 3 Warna. *Neptunomonas, Poseidonibacter, Neptuniibacter*, and *Prochlorococcus* were dominant at Watu Pecah. These microbial patterns align with recent studies, which suggest that microbial communities are sensitive indicators of heavy metal contamination, with shifts favoring metal-resistant taxa under elevated heavy metal exposure (Kim et al., 2024; Macher et al., 2023; Sharma et al., 2025).

The genus *Psychrobacter* is often associated with cold, saline environments and has been documented to exhibit resistance to heavy metals such as Cd and Pb, indicating its potential as a bioindicator for heavy metal contamination (Kim et al., 2024). Similarly, the presence of *Pseudomonas*, a genus well-known for its metabolic versatility and resilience in polluted environments, likely reflects anthropogenic stressors from fuel combustion and industrial discharges (Sharma et al., 2025). *Neptunomonas*, another dominant genus identified at multiple sites, is known for its capabilities in hydrocarbon degradation and tolerance to heavy metal pollutants, underscoring the direct influence of human maritime activities (Bertucci et al., 2022).

Prochlorococcus, while predominantly recognized for its ecological role in primary production,

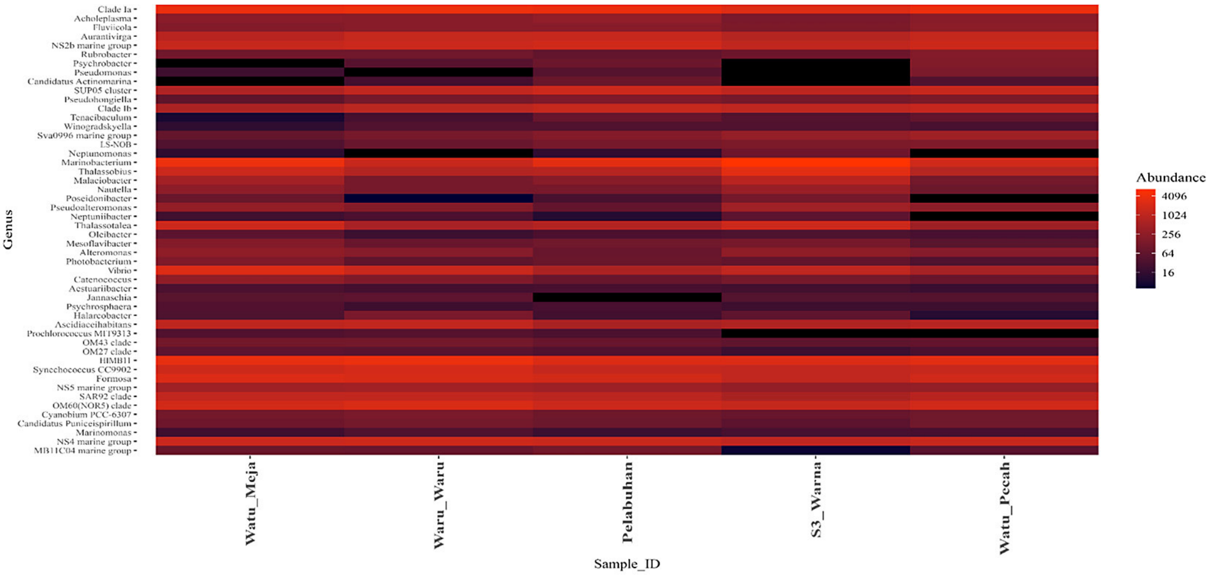

Figure 3. Heatmap of bacterial abundance from Sendangbiru Beach, Malang, Indonesia

can be sensitive to heavy metal stress, and its presence in varying abundance may indicate a threshold of pollution tolerance in different areas. *Jannaschia* and *Poseidonibacter* are commonly found in marine sediments and waters impacted by organic and inorganic contaminants, further emphasizing the direct impact of local human activities, such as harbor operations and maintenance, on microbial community dynamics (Zhou et al., 2025).

Diversity analysis

The results of this study indicated that diversity indices across all sampling locations were not significantly different (Figure 4). The Shannon and Simpson diversity indices were consistent across the locations, with the highest values observed at Waru-Waru (Shannon index = 4.689; Simpson index = 0.982), followed closely by Watu Pecah (Shannon index = 4.682; Simpson index = 0.982). These high values fall into the category of very high diversity, reflecting robust ecosystem health and significant heterogeneity in microbial communities at both sites.

High microbial diversity, as observed at Waru-Waru and Watu Pecah, is typically associated with stable and healthy aquatic ecosystems. The presence of diverse microbial taxa suggests these ecosystems possess resilience against environmental stressors such as heavy metal pollution (Sharma et al., 2025). Additionally, microbial diversity is crucial for maintaining ecological functions, including nutrient cycling, organic matter decomposition, and supporting food webs (Abrar et al., 2025). Therefore, monitoring microbial community composition through eDNA metabarcoding, as applied in this study, provides valuable insights into the ecological integrity and resilience of marine ecosystems (Takahashi et al., 2023; Macher et al., 2023).

Furthermore, previous studies emphasize that heavy metal contamination, notably Cd, Pb, Fe, and Al, can profoundly alter microbial community structure by favoring metal-resistant taxa while reducing overall diversity (Bertucci et al., 2022; Kim et al., 2024). The high microbial diversity indices at Waru-Waru and Watu Pecah indicate either relatively low levels of contamination or the presence of highly adaptable microbial communities capable of tolerating metal pollutants. Consequently, the maintenance of microbial diversity at these sites may reflect the ecosystem's ability to mitigate adverse impacts of environmental stressors through inherent microbial community adaptability and functional redundancy (Abrar et al., 2025; Sharma et al., 2025). Continuous monitoring using sensitive techniques such as eDNA-based metabarcoding is essential to track temporal changes in microbial diversity, which serve as early indicators of ecosystem degradation or recovery (Zhou et al., 2025). The integration of eDNA technology with geospatial mapping can also enhance the precision of pollution impact assessments and improve conservation strategies (Takahashi et al., 2023).

The Venn diagram analysis across sampling locations (Figure 5) indicated considerable overlap in bacterial community characteristics.

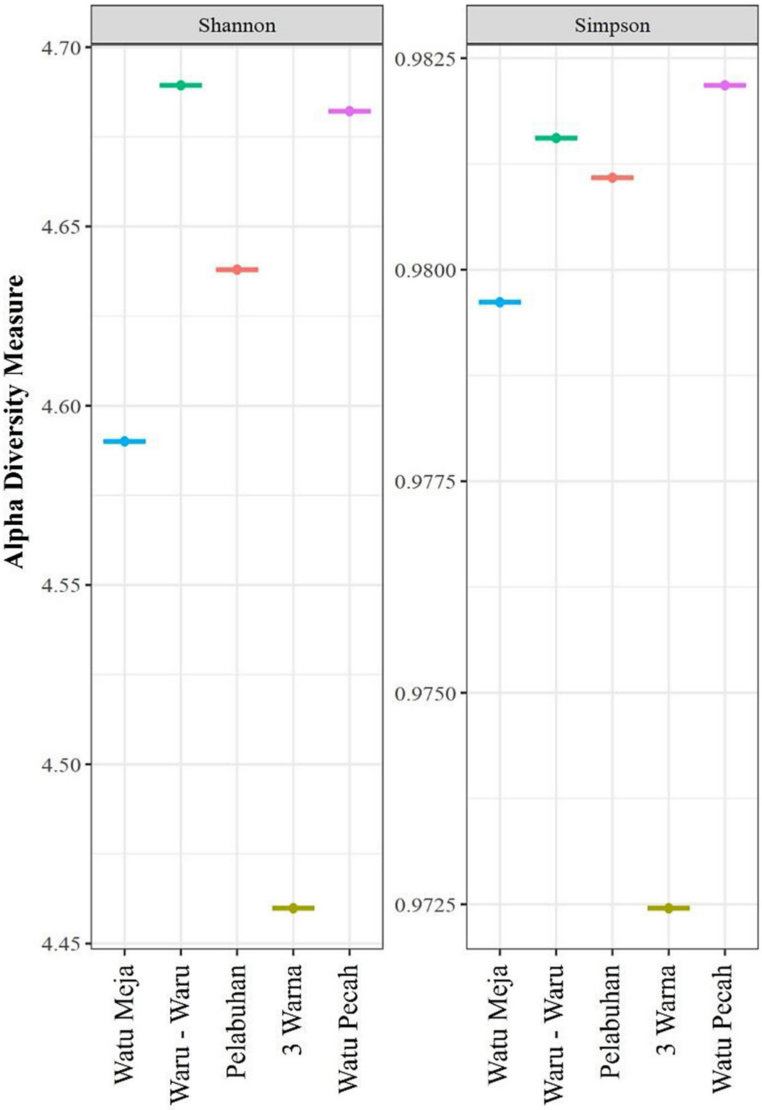

Figure 4. Alpha diversity of bacteria from Sendangbiru Beach, Malang, Indonesia; left – Shanon index, right – Simpson index

A notable finding was the universal presence of *Vibrio brasiliensis* at all sampling sites, indicating a widespread distribution and ecological adaptability of this species within the studied coastal ecosystem. Such distribution patterns often reflect the intrinsic ability of certain bacterial taxa to thrive under varied environmental conditions, including those influenced by anthropogenic activities and heavy metal contamination (Kim et al., 2024; Sharma et al., 2025).

In contrast, certain bacterial families were distinctively site-specific, which can be associated with differences in environmental conditions, particularly varying heavy metal concentrations. The bacterial families Halieaceae, Cryomorphaceae, Salinisphaeraceae, Wohlfahrtiimonadaceae, Flavobacteriaceae, Rhodobacteraceae, Shewanellaceae, and Rubrobacteriaceae were characteristic of Watu Meja and Watu Pecah locations. Such community distinctions suggest specialized niche adaptation likely driven by environmental pressures including contamination and nutrient availability (Bertucci et al., 2022).

At Waru-Waru, distinctive bacterial families such as Francisellaceae, Nannocystaceae, Kiloniellaceae, Cyclobacteriaceae, Bacteriovoracaceae, Lachnospiraceae, Flavobacteriaceae, and Rubrobacteriaceae were observed. The presence of diverse bacterial taxa including both pathogenic and environmentally beneficial genera could indicate a complex interplay between pollutant-driven selection and natural

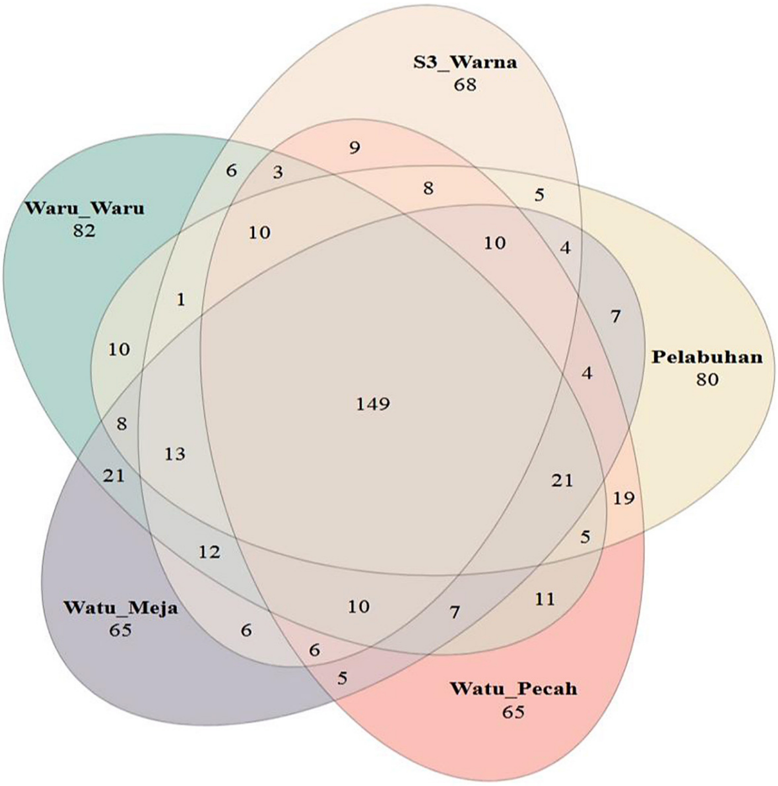

Figure 5. Venn diagram of bacterial species from Sendangbiru Beach, Malang, Indonesia

ecological succession (Macher et al., 2023). Particularly, families such as Flavobacteriaceae are known for their metabolic versatility, which can aid their survival and dominance in metal-stressed environments (Sharma et al., 2025).

The bacterial families that characterized the harbor area (Pelabuhan) notably included Cryomorphaceae, Caulobacteraceae, Trueperaceae, Flavobacteriaceae, and Endozoicomonadaceae. Harbors are often exposed to significant anthropogenic pressures including heavy metals from shipping and industrial discharges. Previous studies have indicated that bacterial communities in harbor environments often include specialized taxa capable of tolerating and metabolizing pollutants (Kim et al., 2024; Zhou et al., 2025).

At the 3 Warna location, families Flavobacteriaceae, Nitrosomonadaceae, Oceanospirillaceae, Rubritaleaceae, Lentimicrobiaceae, and Thiotrichaceae were predominant. The occurrence of Nitrosomonadaceae, recognized for their role in nitrogen cycling, emphasizes the potential functional roles of bacterial communities in maintaining ecosystem stability and health in moderately impacted marine environments (Mohsin Abrar et al., 2025). The site-specific microbial diversity observed across Sendangbiru Beach emphasizes that microbial community structure and function can serve as effective indicators of ecosystem health, and that eDNA-based methodologies offer a sensitive approach for detecting subtle yet ecologically significant shifts resulting from environmental contamination and human activities (Takahashi et al., 2023; Wang et al., 2022).

CONCLUSIONS

The present study investigated the spatial distribution of heavy metals (Cd, Fe, Pb, Al) and associated bacterial community structures at Sendangbiru Beach, Malang, Indonesia, employing eDNA metabarcoding as a sensitive biomonitoring approach. Results highlighted Fe as the predominant heavy metal contaminant across all sampling sites, while Cd and Pb exhibited lower yet consistent distributions indicative of chronic anthropogenic pollution. Distinct microbial communities were identified at different locations, dominated by genera such as *Psychrobacter, Neptunomonas*, and *Pseudomonas*, all known for their heavy metal resistance. Diversity analyses revealed high microbial diversity indices, particularly at Waru-Waru and Watu Pecah sites, suggesting ecosystem resilience and adaptability despite ongoing anthropogenic pressures. The integration of eDNA technology with geospatial analyses provided robust insights into pollution dynamics, demonstrating the method's efficacy for ecological assessment and sustainable management of coastal environments.

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