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# 16S rRNA Metabarcoding Inference Reveals Adaptive Bacterial Communities and Predicted Copper-Lead Resistance in Tanjung Emas Harbor Sediments

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**Abstract:** Bacteria employ diverse mechanisms to cope with environmental stress, yet how sediment-associated microbial communities adjust to contamination—particularly heavy metals—in tropical port ecosystems remains insufficiently characterized. This study characterized the bacterial community profile based on amplicon sequencing and predicted the functional potential for heavy-metal resistance in surface sediments from Tanjung Emas Port, Semarang (TEPS), an area chronically exposed to wastewater pollutants and metal contamination from intensive anthropogenic activities. Amplicon-based metabarcoding targeting the 16S rRNA V3–V4 region was applied. Sequence processing using Cutadapt and DADA2 yielded 38,742–42,064 high-quality Amplicon Sequence Variants (ASVs) per sample. Functional potentials were inferred using PICRUSt2, and downstream analyses were conducted in RStudio. The community was dominated by the phylum Pseudomonadota (31–39%), class Gammaproteobacteria (27–35%), order Steroidobacterales (8–10%), family Woeseiaceae (8–10%), and genus *Woeseia* (13–16%), reflecting adaptation to organic-rich, pollutant-impacted conditions including Cu and Pb contamination. Alpha and beta-diversity analyses revealed significant differences among sampling locations ($p<0.05$). Predictive functional profiling indicated a high potential for putative heavy-metal resistance genes (HMRGs) associated with Cu and Pb resistance, highlighting the ecological resilience and bioremediation potential of the indigenous bacterial community.

**Keywords:** bacteria; sediment; Tanjung Emas Port; metabarcoding; HMRGs.



## 1. Introduction

The tropical waters of Tanjung Emas Port, Semarang (TEPS), constitute a highly dynamic yet vulnerable transition zone where freshwater from three river mouths mixes with seawater and receives runoff from intensive anthropogenic activities. Recent assessments by Purnomo *et al.* reported that dissolved heavy-metal concentrations in TEPS waters reach 1 mg/L for Pb and 0.01 mg/L for Cu [1]. Sediment compartments harbor even higher burdens, with Pb at 15.51 mg/kg and Cu at 19.84 mg/kg [2], leading to significant bioaccumulation in sentinel species such as green mussels [3]. While these chemical signatures establish a clear

baseline of contamination, there is a critical need to evaluate the concurrent biological response of the sedimentary microbiome. This study therefore synchronizes biological sampling with documented chemical gradients to determine how persistent metal pressure shapes the extant microbial landscape.

Sediment-associated bacterial communities play central roles in biogeochemical cycling and in the biotransformation of xenobiotic compounds. Their phylogenetic complexity and diversity underpin ecosystem productivity and post-disturbance recovery [4]. Environmental variables—such as organic matter content and heavy metal loads—act as ecological filters that shape community structure and metabolic function. Given that contaminated conditions often enrich opportunistic taxa and metal-resistance determinants [5], accurate monitoring requires 16S rRNA amplicon-based metabarcoding, which remains a robust tool for high-resolution taxonomic profiling [6]. Functional potential within the microbial community is inferred through PICRUSt2, a reference-based bioinformatic framework that generates predictive metabolic profiles [7]. This approach provides a comprehensive overview of the community's genetic repertoire, offering critical insights into the potential pathways involved in metal resistance and detoxification under environmental pressure. Ultimately, such locally grounded datasets are essential for sustainable coastal monitoring and management.

This research delineates the taxonomic diversity and community structure of sedimentary bacteria at TEPS under Pb and Cu exposure, while simultaneously inferring the potential functional repertoire through predictive profiling. Specifically, the analysis targets genes and metabolic pathways associated with resistance and detoxification mechanisms to resolve how these communities adapt to heavy-metal stress. By distinguishing between observed taxonomic shifts and predicted functional capacities, this study establishes a nuanced scientific basis for formulating effective bioremediation strategies. These anticipated outcomes serve to strengthen environmental quality management and safeguard public health in the adjacent coastal communities.

## 2. Materials and Methods

### *2.1. Sampling.*

This study was conducted in the coastal waters of Tanjung Emas Port, Semarang City, Central Java, Indonesia. Surface sediments were collected from three distinct stations (Figure 1). At each station, surface sediment (0–20 cm depth) was sampled using a three-point composite design with an Ekman grab, yielding approximately 0.4 kg per station. The sampling depth of 0–20 cm was selected due to the highly dynamic nature of the study site, which is subject to frequent maintenance dredging by port authorities. Such anthropogenic physical disturbances lead to the homogenization of the upper sediment layers and the resuspension of deeper materials [8]. These conditions prevent the establishment of stable, stratified redox gradients typically found in undisturbed environments [9], thus justifying the use of a broader surface integration to characterize the impacted substrate. Samples were transferred into sterile sample bottles and stored at −20°C until further analysis.

Additionally, heavy metal concentrations (Cu and Pb) were measured using Atomic Absorption Spectrophotometry (AAS). The sediment samples for these heavy metal analyses were collected concurrently with the samples used in the present study. However, the detailed

measurement results and findings have been previously published in the Jurnal Ilmu Lingkungan (https://ejournal.undip.ac.id/index.php/ilmulingkungan/article/view/72778) [1].

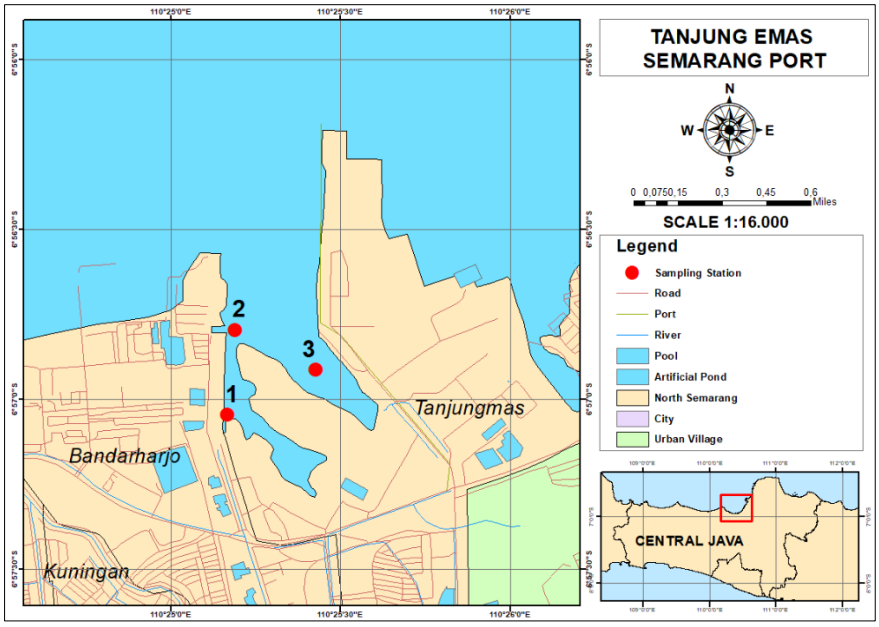


**Figure 1.** Sampling locations and coordinates in the coastal waters of Tanjung Emas Port (TEPS), Semarang, Central Java, Indonesia.

*2.2. DNA extraction.*

Genomic DNA was extracted following the protocols described by Kachiprath *et al.* and Tatti *et al.* [10,11]. For each composite sample per site, three biological replicates were processed (n = 3). The lysis buffer contained 5% cetyltrimethylammonium bromide (CTAB), 20% sodium dodecyl sulfate (SDS), and proteinase K at 20 mg $mL^{-1}$. Extracted DNA was resuspended in DNase/RNase-free water to a final volume of 50 µL. DNA concentration and purity were assessed using a NanoDrop 2000 spectrophotometer and the Qubit dsDNA HS assay, while DNA integrity was evaluated via electrophoresis on a 1% agarose gel. Following extraction, the DNA templates were maintained at −20 °C and subsequently outsourced to PT Genetika Science Indonesia (https://ptgenetika.com) for comprehensive quality assessment, library construction, and high-throughput sequencing (HTS).

*2.3. Library construction and 16S amplicon sequencing.*

The 16S rRNA V3–V4 region was amplified using primers 341F (5′-CCTAYGGGRBGCASCAG-3′) and 806R (5′-GGACTACNNGGGTATCTAAT-3′) carrying universal Illumina adapters [12]. PCR reactions were performed with KOD Multi & Epi (KME-101, Toyobo, Japan) DNA polymerase. The thermal cycling conditions consisted of an initial denaturation at 94°C for 2 min, followed by 30 cycles of denaturation at 98°C for 10 sec, annealing at 55°C for 10 sec, and extension at 68°C for 15 sec, with a final extension at 68°C for 2 min. The expected ~470-bp amplicon was confirmed via 15 TBE agarose gel electrophoresis, and the final PCR products were used directly for library preparation. Libraries were sequenced on an Illumina MiSeq platform to generate paired-end raw reads.

*2.4. Analysis and workflow of 16S amplicon sequencing.*

The resulting raw reads were adapter- and primer-trimmed using Cutadapt, followed by processing via the DADA2 pipeline for error modeling, quality filtering, and chimera removal.

Specifically, reads were truncated to 240 bp for the forward reads and 200 bp for the reverse reads. Filtering parameters were strictly enforced, allowing a maximum of two expected errors per read (maxEE = 2). Chimeric sequences were identified and removed utilizing the default 'consensus' method in DADA2, and the remaining cleaned reads were grouped into high-resolution amplicon sequence variants (ASVs). Taxonomic classification of these ASVs was performed against the SILVA reference database (v138.20), applying a minimum bootstrap confidence threshold of 80%. Marker-based functional profiles were inferred using PICRUSt2 and then mapped to the Kyoto Encyclopedia of Genes and Genomes (KEGG) Orthology (KO) and the BacMet database [13]. These functional profiles are predictive estimates inferred from 16S rRNA gene sequences and do not constitute a direct representation of actual in situ metabolic activities [14]. Downstream analysis and visualizations were executed in RStudio (R version 4.2.3) (https://www.R-project.org/).

*2.5. Data analysis.*

Prior to community analyses, the ASV table was rarefied to a uniform minimum library size of approximately 25,000 reads per sample to mitigate potential biases introduced by uneven sequencing depths. Rarefaction curves were subsequently generated to verify that the sequencing effort reached an asymptote, indicating adequate capture of taxonomic diversity across all samples. Community analyses comprised the evaluation of alpha diversity (Observed ASVs, Chao1, Shannon, and Simpson indices) and beta diversity. Significant differences in alpha diversity were assessed using Analysis of Variance (ANOVA), followed by Tukey's Honestly Significant Difference (HSD) post-hoc tests with a 95% family-wise confidence level to account for multiple comparisons. Variations in community composition (beta diversity) among locations were statistically tested using Permutational Multivariate Analysis of Variance (PERMANOVA) with 999 permutations, implemented via the adonis2 function in the vegan package. All statistical analyses and visualizations were performed in RStudio (R version 4.2.3) using appropriate R packages (vegan, fossil, and multcompView).

## 3. Results and Discussion

16S amplicon sequencing analyses yielded a total of 361,810 valid reads and 8,022 ASVs, comprising sequences that successfully passed all quality control, merging, and denoising filters. Across samples, the number of reads ranged from 38,742 to 42,064, corresponding to high read proportions of 38.80–42.11%. The number of ASVs per sample spanned 870–920, directly reflecting among-sample heterogeneity in bacterial community structure. Good's coverage was consistently high across all samples (99.87–99.92%), indicating that the vast majority of sedimentary bacterial diversity was captured by the sequencing effort.

*3.1. Sediment bacterial community profile.*

The sedimentary bacterial community at TEPS (Figure 2) was dominated by the phylum Pseudomonadota (~30%), a common constituent of coastal ecosystems owing to its adaptability and capacity to utilize diverse organic substrates [15,16]. Thermodesulfobacteria (17–21%), a phylum comprising sulfur-reducing bacteria (SRB), was also highly abundant. At the class level, Gammaproteobacteria (27–35%) were prominent as pollutant-tolerant degraders of organic matter [17], accompanied by Desulfobacteria and Desulfobulbia (≈8–12%

and 8–9%, respectively), which are sulfate-reducing taxa with potential capabilities in heavy metal detoxification [18,19]. Several additional classes were consistently detected at notable abundances across all samples, including Bacteroidia (9–12%), Anaerolineae (6–7%), Alphaproteobacteria (4–6%), Planctomycetes (5–8%), Verrucomicrobia (3–5%), and Thermoanaerobaculia (4–5%).

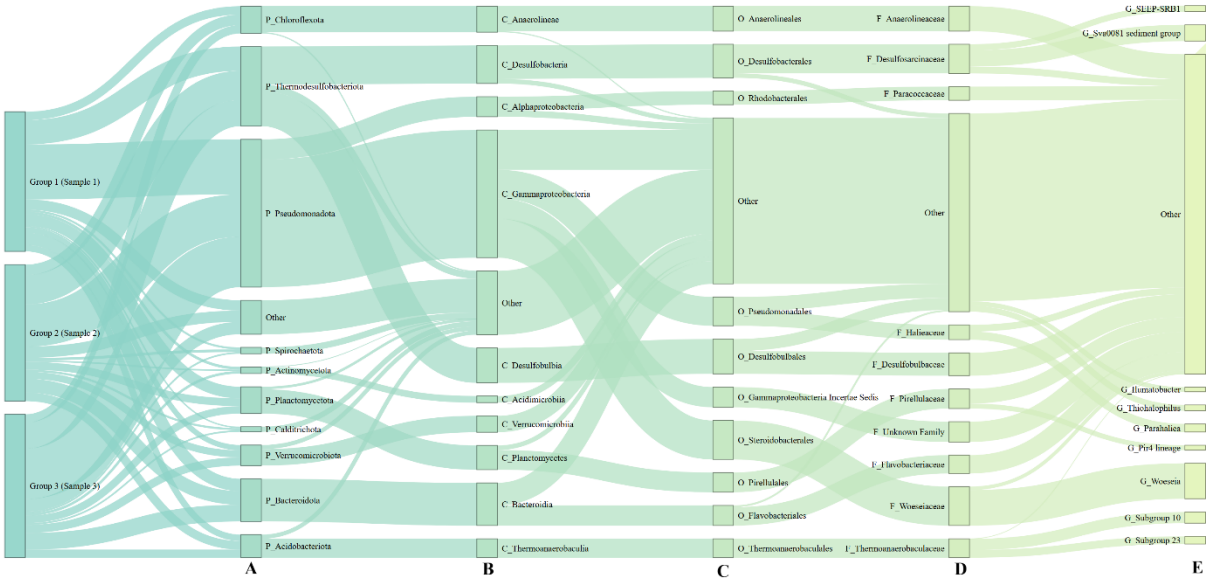


**Figure 2.** Relative abundance of the top 10 taxa for each sampling group/station: **(A)** Phylum; **(B)** Class; **(C)** Order; **(D)** Family; **(E)** Genus.

At the order level, Desulfobulbales and Desulfobacterales predominated. These taxa belong to the SRB group [20] and possess the potential to immobilize heavy metals via sulfide formation [18]. Pseudomonadales, Steroidobacterales, and Flavobacteriales were also detected; these orders can decompose complex organic substrates and may further contribute to metal bioremediation [20,21]. Additionally, Anaerolineales are known to possess heavy-metal resistance, potentially mediated by cell wall functional groups and adsorptive extracellular polymeric substances (EPS) [22]. At the family level, Woeseiaceae, Desulfobulbaceae, and Desulfosarcinaceae were dominant. Members of Woeseiaceae are well adapted to contaminated environments and participate in the transformation and immobilization of heavy metals [23], whereas Anaerolineaceae support hydrocarbon degradation and anaerobic carbon flux [24,25].

At the genus level, *Woeseia* emerged as the most dominant taxon (13–16% relative abundance), which is notable, as previous studies have characterized it as highly adaptive to organic-rich and heavy-metal-polluted marine environments, with documented tolerance mechanisms for Cu and Pb [23,26]. Alongside *Woeseia*, consistently detected genera such as *Parahaliea* and *Ilumatobacter* have been reported to contribute to heavy metal detoxification through chelation and enzyme-mediated pathways [27]. Beyond these primary groups, the community includes known sulfate-reducing bacteria (SRB) such as *Desulfobacter*, *Desulfurivibrio*, *Desulfatiglans*, and *Sulfurovum*. The presence of these taxa suggests a latent biological potential for sulfate-to-sulfide transformation—a process widely documented to precipitate insoluble metal sulfides (e.g., CuS and PbS) and restrict heavy metal bioavailability [28,29]. Furthermore, diverse taxa like *Sedimenticola* and *Vibrio* are recognized in prior research for their bioaccumulation capabilities [15,30], while *Acinetobacter*, *Marinobacter*, *Methylobacterium*, *Thauera*, and *Pseudoalteromonas* are frequently associated with metal

resistance via the production of extracellular polymeric substances (EPS), biosurfactants, and *copABCD* genes [31-33]. Collectively, based on their established ecological profiles, this diverse microbial architecture implies that the sediment community possesses a strong intrinsic potential for natural bioremediation, which may help buffer the coastal ecosystem against anthropogenic Cu and Pb stress.

*3.2. Diversity analyses.*

The α-diversity analysis of nine sediment samples from TEPS revealed a remarkably high bacterial diversity (Figure 3). According to ANOVA results ($p < 0.05$), bacterial α-diversity differed significantly among sampling stations. Tukey's HSD post-hoc test ($p < 0.05$) further indicated that the bacterial microbiome composition at Station 1 was significantly distinct from that of Stations 2 and 3, whereas no significant difference was observed between Stations 2 and 3. This pattern likely reflects spatial variation in pollutant exposure and hydrodynamic conditions. Station 1, located near the Kali Baru estuary, is more directly influenced by urban effluent transported via river discharge, while Stations 2 and 3 are situated closer to the open sea and areas of intense shipping activity.

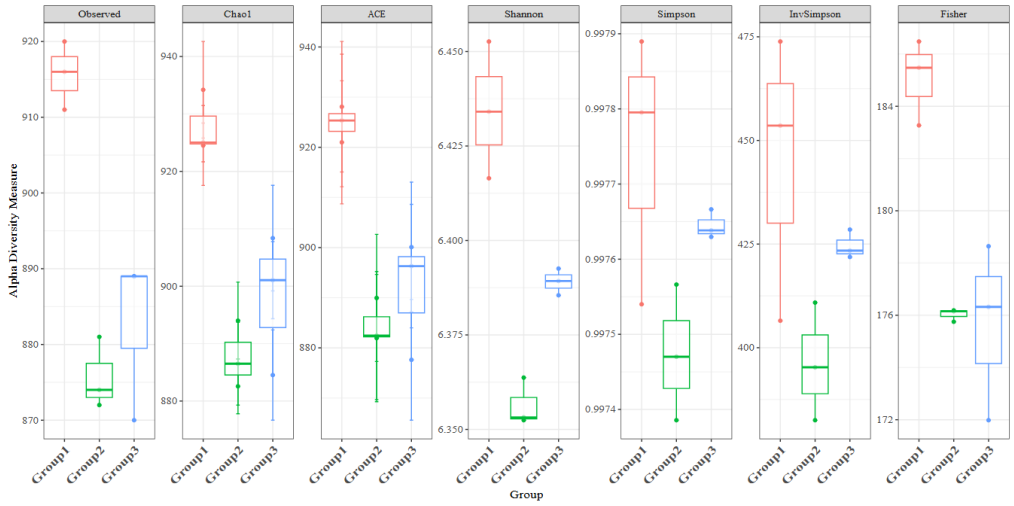


**Figure 3**. Bar plots comparing α-diversity among the three sampling stations: Group 1 (Station 1), Group 2 (Station 2), and Group 3 (Station 3).

PERMANOVA revealed significant differences in microbial community composition among location groups ($p < 0.05$). The model attributed approximately 53% of the variance to between-group differences (PERMANOVA $R^2 \approx 0.53$). This effect was supported by the homogeneity of multivariate dispersions (beta-dispersion) test, which was non-significant ($p > 0.05$), indicating comparable within-group dispersion across locations. Together, these results show that the PERMANOVA signal reflects genuine shifts in community structure among locations rather than artifacts of unequal dispersion. This interpretation is consistent with the finding that when PERMANOVA is significant, but the beta-dispersion test is not, the group or location effect on community structure is considered robust and not driven by differences in spread among groups [34].

Weighted UniFrac–based PCoA (Figure 4) resolved three clearly separated sample clusters across both PCoA1–PCoA2 and PCoA1–PCoA3 projections. The first coordinate (PCoA1) explained 48.37% of the total variance, with PCoA2 and PCoA3 accounting for an additional 21.01% and 6.34%, respectively. The lack of overlap among clusters indicates consistent differences in community structure among groups, corroborating the

PERMANOVA results. These findings align with those of Wang *et al*., who demonstrated that Weighted UniFrac PCoA effectively discriminates microbial community composition across sites or sample groups, with distinct cluster separation reflecting meaningful compositional divergence [29]. Moreover, the use of Weighted UniFrac enriches β-diversity interpretation by incorporating both phylogenetic relatedness and taxon abundance [35,36].

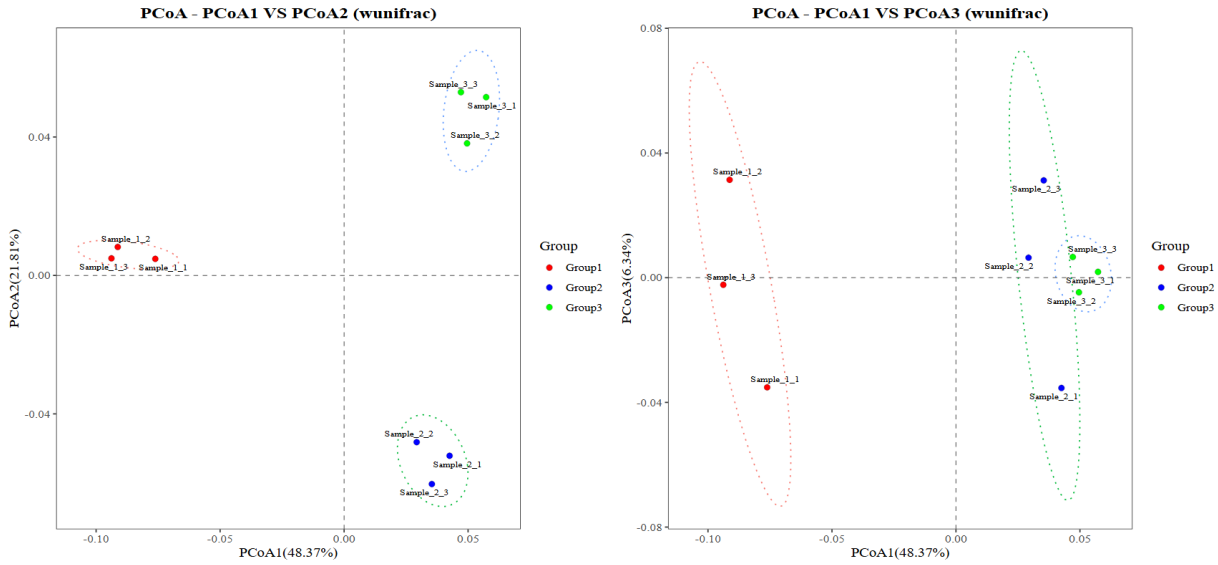


**Figure 4.** Principal coordinates analysis (PCoA) based on Weighted UniFrac distances for the three sample groups: Group 1 (Station 1), Group 2 (Station 2), and Group 3 (Station 3).

*3.3. Presence of unique (site-specific) taxa.*

Each sampling location exhibited a distinct microbiome structure (Figure 5). Group 1 harbored ~27.80% of bacterial taxa that were unique—i.e., not detected in Groups 2 or 3. Groups 2 and 3 contained ~21.07% and ~20.61% unique taxa, respectively. Importantly, these percentages were derived from the normalized dataset, ensuring that the observed variations reflect true ecological divergence rather than artifacts of unequal sequencing effort (Figure 6). Pairwise overlaps comprised ~5.16% (Groups 1 and 2), ~3.18% (Groups 1 and 3), and ~7.80% (Groups 2 and 3). Taxa shared across all three locations accounted for ~14.39%, indicating the presence of a broadly distributed core community.

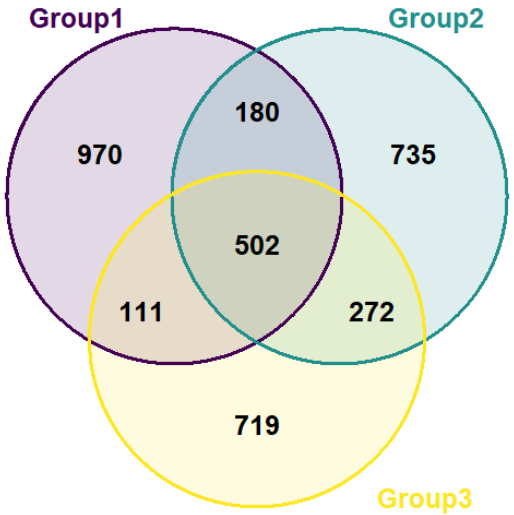


**Figure 5.** Venn diagram illustrating the distribution of shared and unique bacterial taxa among the three sampling stations. Numerical values are calculated based on ASV counts.

The occurrence of site-specific taxa suggests environmental filtering and geographic isolation shaping local assemblages [37]. Heterogeneity in source materials, variation in pH, the availability of N and P, and environmental pressures such as temperature extremes and spatial isolation can drive microbiome turnover and the emergence of unique species or higher taxa [38]. Beyond structuring, unique taxa may diversify community functional potential and hold promise for biotechnological applications [38,39]. Collectively, the presence of unique taxa not only reflects the complexity of microbial community structure but also underscores their role in sustaining overall ecosystem stability and functional resilience [37,40].

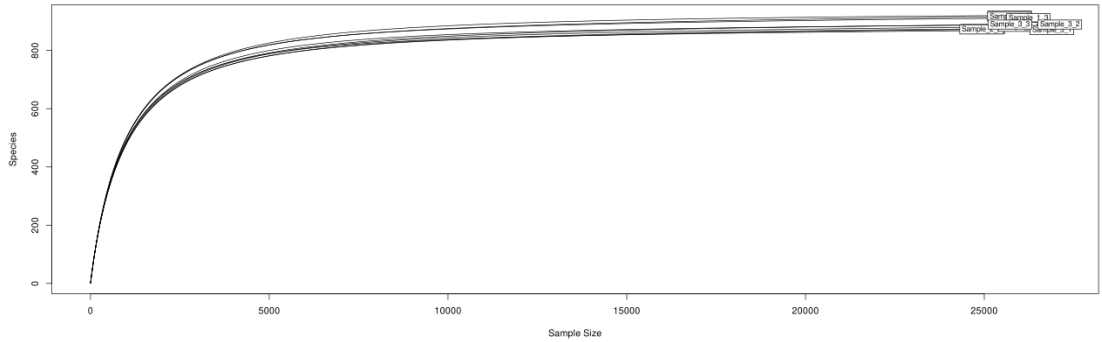


**Figure 6.** Rarefaction curves illustrating the observed species richness across all samples. The plot confirms that sequencing coverage was adequate across all sample groups, as evidenced by the curves reaching a clear plateau.

*3.4. Functional gene prediction.*

PICRUSt2 was applied to the taxonomic profiles to infer functional metabolic pathway predictions using the Kyoto Encyclopedia of Genes and Genomes Orthology (KO) and Enzyme Commission (EC) number databases [41]. The reliability of these functional inferences was supported by an average Nearest Sequenced Taxon Index (NSTI) of 0.19, a value characteristic of complex environmental sediment microbiomes that indicates an acceptable level of reference genome coverage [42].

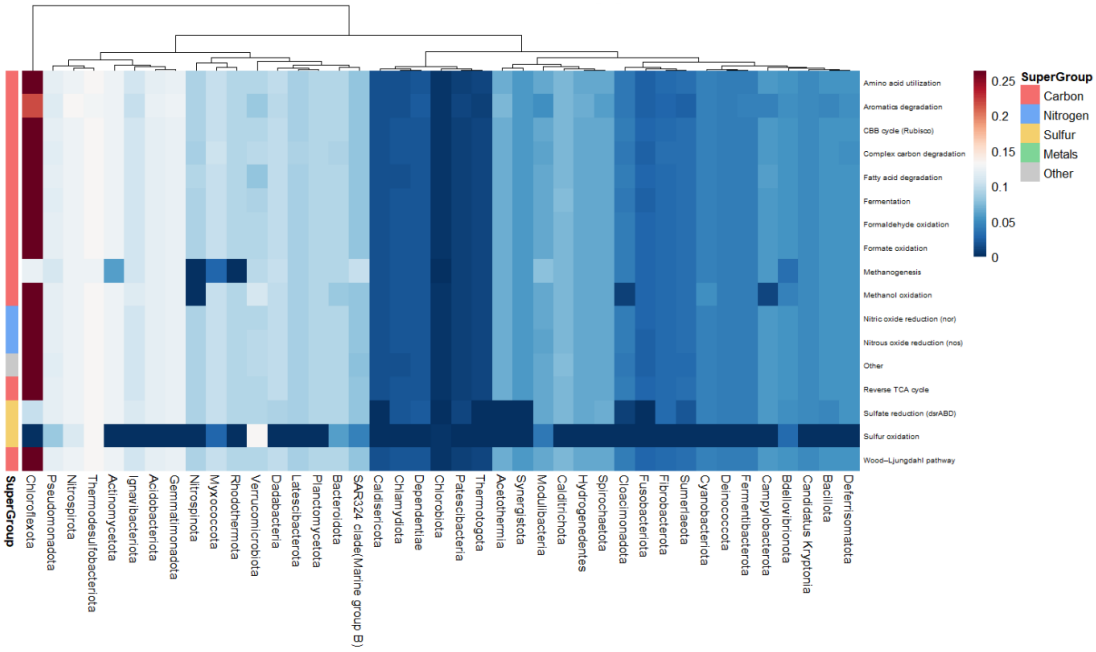


**Figure 7.** Heatmap of predicted functional metabolic pathways differing across detected phyla.

The analysis predicted 5,917 genes, 1,991 enzymes, and 394 functional metabolic pathways, which were subsequently grouped into major metabolic categories (Figure 7). The breadth of predicted pathways in TEPS sediment communities underscores substantial environmental pressures and the ecological complexity of the Semarang coastal system. As shown in Figure 7, functional pathways were diverse and varied in relative abundance. The aerobic respiration I (cytochrome c) pathway dominated across all sediment bacterial groups. Aerobic respiration is a principal energy-generating process in bacteria, producing ATP by oxidizing organic substrates with oxygen as the terminal electron acceptor [43]. Ecologically, aerobically respiring communities can strongly influence nutrient and carbon cycling and biofilm formation [44].

In addition, the presence of glycolysis, homolactic fermentation, and N10-formyl-tetrahydrofolate biosynthesis indicates baseline energetic capacities in these polluted coastal sediments. Detection of the superpathway of chorismate metabolism, together with 4-hydroxyphenylacetate degradation, suggests enhanced microbial potential to transform aromatic compounds typically associated with industrial and domestic effluents [45,46]. Collectively, these pathways indicate exposure to complex organic pollutants and xenobiotics in TEPS waters [47], suggesting adaptive selection within the resident microbiota to support detoxification and resilience under contaminant stress.

### *3.5. Functional gene predictions related to Cu and Pb resistance.*

Based on PICRUSt2 predictions mapped to the KO and the BacMet database, we inferred the presence of HMRGs associated with Cu (Figure 8) and Pb (Figure 9) in TEPS sediment microbiomes.

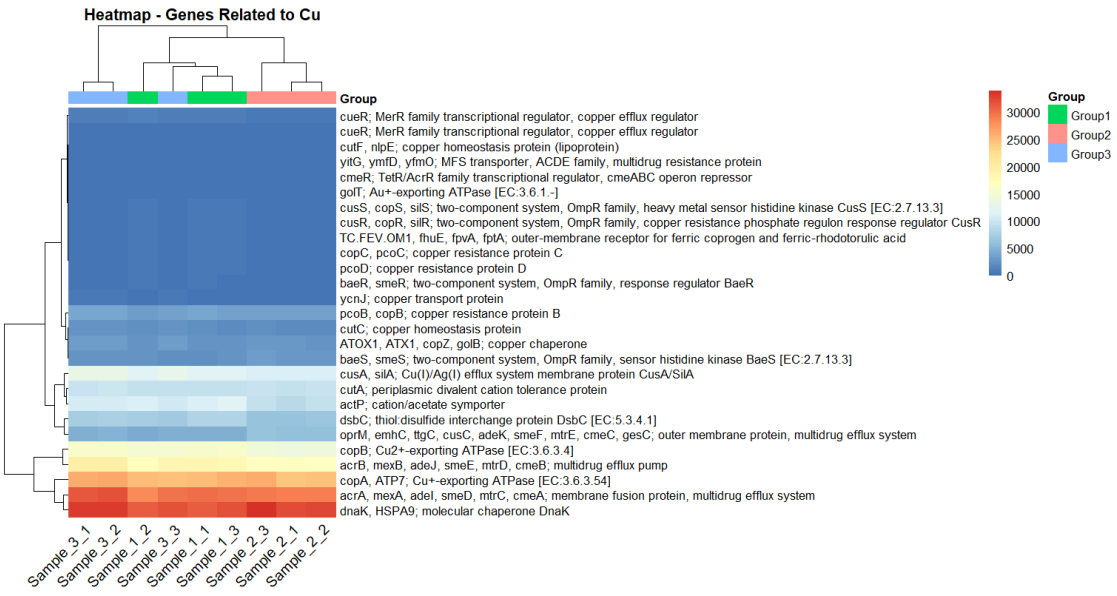


**Figure 8.** Pheatmap of predicted functional genes associated with copper (Cu) resistance.

The predicted Cu-resistance repertoire encompassed functions spanning enzymes, transcriptional regulators, efflux systems, metal-binding proteins, membrane proteins, chaperones, porins, membrane transporters, and outer-membrane lipoproteins. Inferred regulatory genes included *baeR*, *baeS*, *copR*, *copS*, *cueR*, *cusR*, and *cusS* [13], which comprise bacterial two-component and MerR-family systems that sense excess metal and orchestrate an appropriate Cu-resistance response. Several Cu-resistance genes encoding detoxification and

homeostatic enzymes were also inferred—*actP*, *copA*, *copB*, *golT*, *pcoA*, *pcoB*, and *dsbC* [13]. Notably, *copA* and *copB* encode P-type ATPases that export $Cu^{+}/Cu^{2+}$ from the cytoplasm to reduce intracellular toxicity arising from Cu overload [48,49]. Pb-related HMRGs inferred from BacMet comparisons (Figure 9) comprised three key functional genes: *cadC*, *zntA*, and *zraS*. Among these, *cadC* and *zraS* encode regulatory proteins, whereas *zntA* encodes a cation-transporting P-type ATPase implicated in Pb efflux [13]. However, it must be noted that the presence of these specific genes was inferred solely from 16S rRNA gene amplicon data and remains to be experimentally validated by quantitative PCR or shotgun metagenomic sequencing.

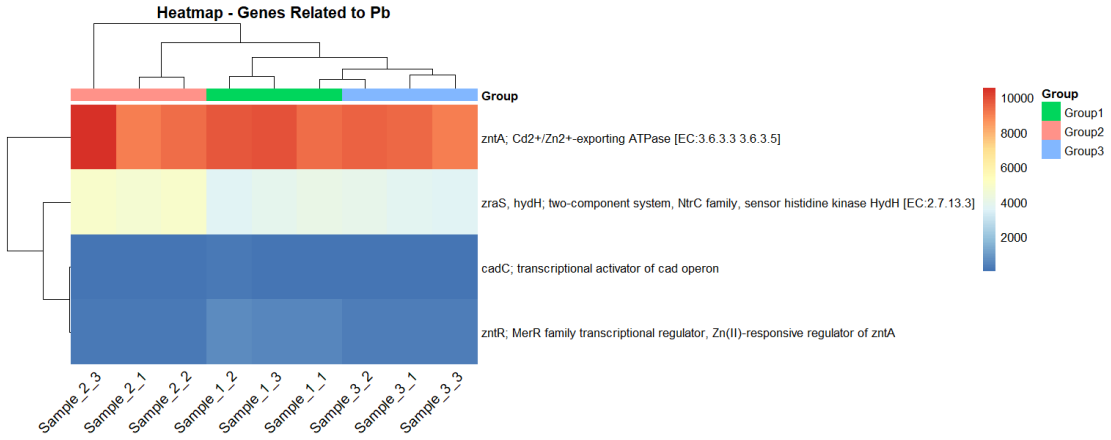


**Figure 9.** Pheatmap of predicted functional genes associated with lead (Pb) resistance.

The predicted presence of HMRGs associated with copper (Cu) and lead (Pb) resistance in TEPS sediment microbiomes suggests a potential for bioremediation [48]. Theoretically, these genes underpin sustainable, environmentally friendly strategies for mitigating Cu and Pb contamination. Microbial communities harboring such determinants are hypothesized to tolerate, detoxify, and attenuate heavy-metal pollutants through multiple mechanisms, including efflux, sequestration, chelation, enzymatic transformation, and redox-mediated immobilization [50]. While their inferred genetic profiles suggest an adaptive capacity to metal-impacted environments, further physiological and isolation studies are required to confirm their actual remediation efficacy before establishing these taxa as viable bioagents for targeted, site-appropriate heavy-metal remediation.

### *3.6. Strategies for remediating copper and lead contamination.*

Bioremediation of heavy metals can be explored and hypothesized using modern sequencing approaches. Leveraging 16S amplicon sequencing via Next-Generation Sequencing (NGS) enables comprehensive, cultivation-independent profiling of microbial communities, including non-culturable bacteria [51]. Consistent with our findings, the detected genera—*Acinetobacter*, *Desulfobacter*, *Desulfurivibrio*, *Marinobacter*, *Methylobacterium*, *Pseudoalteromonas*, and *Thauera*—are inferred to be potential candidates as bioagents for heavy-metal remediation. Furthermore, computational tools applied to an amplicon-based microbial profile facilitate the inference of potential metal-resistance determinants, including efflux systems, metal-binding proteins, and EPS-mediated sequestration [51]. In this study (Figures 8 and 9), predictive functional profiling identified 31 HMRGs. However, while the predicted presence of these HMRGs indicates a theoretical potential for heavy-metal resistance, direct experimental validation is required to confirm their actual bioremediation capacity. Specifically, dedicated functional assays, isolation experiments, and validation via qPCR or

whole-metagenome sequencing are necessary to strengthen and verify these computational predictions. Thus, 16S amplicon sequencing primarily resolves bacterial community composition while generating hypotheses regarding functional pathways for future targeted applications.

Further, integrating multi-omics approaches is essential to build a holistic view of microbial mechanisms and interactions, thereby streamlining the screening and optimization of high-performance microbial consortia for remediating diverse metals [52,53]. Synergizing amplicon-based microbial profiles with true shotgun metagenomics, transcriptomics, and in vitro functional assays can accelerate the rational design of consortia, enable real-time bioreactor monitoring, and inform more precise remediation strategies [54]. Collectively, integrating sequence-guided hypotheses with multidisciplinary experimental validation offers adaptive, effective, and sustainable solutions for restoring aquatic environments impacted by heavy-metal contamination.

## 4. Conclusions

The sedimentary bacterial community at Tanjung Emas Port, Semarang, exhibited pronounced dominance by the phylum Pseudomonadota, class Gammaproteobacteria, order Steroidobacterales, family Woeseiaceae, and the genus *Woeseia*. This taxonomic profile is consistent with those of microbiomes frequently associated with organic-rich, pollutant-impacted environments, including those contaminated with Cu and Pb. Furthermore, α- and β-diversity analyses revealed significant spatial heterogeneity in community structure across the sampled sites. Crucially, predictive functional inference suggested the putative presence of HMRGs associated with Cu and Pb. While these findings point toward a potential genetic basis for community-level metal tolerance, this adaptive capacity remains theoretical without targeted experimental validation. Therefore, the hypothetical utility of these sedimentary communities for heavy-metal bioremediation applications provides a promising framework that necessitates future empirical studies to confirm their actual functional activity.

## Author Contributions

Conceptualization, E.P.; formal analysis, H.P.K. and A.B.; writing—original draft preparation, E.P.; writing—review and editing, H.P.K. and A.B.; visualization, E.P.; validation, A.T.L. and C.S.; supervision, A.T.L. and C.S. All authors have read and agreed to the published version of the manuscript.

## Institutional Review Board Statement

Not applicable.

## Informed Consent Statement

Not applicable.

## Data Availability Statement

All data produced or examined in this study are provided within this published article and its supplementary materials. Any additional datasets or raw data used and/or analyzed in the present study can be obtained from the corresponding author upon reasonable request.

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## Conflicts of Interest

The authors declare that they have no competing interests.

## Abbreviations

The following abbreviations are used in this manuscript:

| Abbreviation | Definition |
|---|---|
| TEPS | Tanjung Emas Port, Semarang |
| AAS | Atomic Absorption Spectrophotometry |
| CTAB | Cetyltrimethylammonium Bromide |
| SDS | Sodium Dodecyl Sulfate |
| HTS | High-Throughput Sequencing |
| TBE | Tris-Borate-EDTA |
| NGS | Next Genome Sequencing |
| ASV | Amplicon Sequence Variants |
| EPS | Extracellular Polymeric Substances |
| ANOVA | Analysis of Variance |
| HSD | Honestly Significant Difference |
| PERMANOVA | Permutational Multivariate Analysis of Variance |
| KO | Kyoto Encyclopedia of Genes and Genomes Orthology |
| EC | Enzyme Comission |
| NSTI | Nearest Sequenced Taxon Index |
| HMRGs | Heavy-Metal Resistance Genes |

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