Bacterial Diversity of Discarded Face Masks from Landfills, Mangroves, and Beaches, in Java, Indonesia

Anna Rakhmawati[1*], Bernadetta Octavia[1], Siti Marwati[2]

[1]Department of Biology Education, Faculty of Mathematics and Natural Sciences, Universitas Negeri Yogyakarta, Jl. Colombo 1, Karangmalang, Yogyakarta, 55281, Indonesia Tel./Fax. +62-274-565411/+62-274-548203, email: anna_rakhmawati@uny.ac.id ;
[2]Department of Chemistry Education, Faculty of Mathematics and Natural Sciences, Universitas Negeri Yogyakarta, Jl. Colombo 1, Karangmalang, Yogyakarta, 55281, Indonesia

Abstract

As the number of Covid-19 cases has increased, the production and use of face masks have also increased accordingly. This widespread use of face masks generates millions of tons of mask waste. This study analyzed the bacterial community composition of discarded masks from landfills (Piyungan, Jatibarang, Burangkeng); mangroves (Wanatirta, Tirang, Teluk Naga); and beaches (Parangtritis, Marina, Tanjung Pasir) in Yogyakarta, Semarang, Bekasi, and Tangerang, Java, Indonesia using 16S rRNA sequencing. Analyzing all samples from landfills, mangroves, and beaches revealed that the Proteobacteria phylum is the predominant. In addition, Firmicutes was the second-highest phylum in the samples from landfills and mangroves. In the meantime, Actinobacteria and Cyanobacteria dominated the phyla found in samples from beaches. Analyses at the genus level revealed that Bacillus members predominated in samples of discarded face masks from landfills. In addition, the most prevalent genus found in samples from mangroves and beaches was Vibrio. According to the findings, the distribution of bacterial communities differed among the various regions. Dissimilar bacterial communities and gradient distributions were found among discarded face masks in landfills, mangroves, and beaches. It was the first examination of bacterial distribution in discarded disposable face masks from various locations.

Keywords: bacterial communities, discarded mask, Proteobacteria, 16S amplicon sequencing.

1. Introduction

During the COVID-19 pandemic, the face mask is one of the essential fundamental equipment to prevent the spread of the Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) from one human to another (Prata et al. 2020). The waste from disposable face masks could reach 1 million tons per month (Nghiem et al. 2021). In addition, between 0.15 and 0.39 million tons of plastic debris can accumulate annually in the world's oceans due to improper management of face masks in coastal regions (Chowdhury *et al*., 2021). Since the COVID-19 pandemic began, the widespread use of disposable face masks has become a critical ecological concern. Due to poor waste management practices and a lack of environmental consciousness, a substantial amount of untreated mask waste is dumped into the environment (Cordova *et al*., 2021). After a single use, face masks are discarded in dumpsites and landfills, although some are discarded in public places (Babaahmadi *et al*., 2021). Reports indicate that improper disposal of face masks has occurred globally. Various face masks have been discovered in urbanized areas (gardens, streets, parks), beaches, natural reserves, high mountains, and other aquatic environments (Gunasekaran *et al*., 2022).

Mishandling of face masks results in physical, physiological, and ecotoxicological harm to domestic and wild animals. Typically, face masks are composed of nonrenewable polymers derived from petroleum that are non-biodegradable and hazardous to the environment and public health. It has resulted in a great new type of pollution from improperly discarded masks, including heavy metals, volatile organic compounds (VOCs), and microfibers (Liu *et al*., 2022). Non-biodegradable face masks can cause significant problems for our ecosystem, such as microplastic pollution and increased transmission of antibiotic-resistant genes, if not properly disposed of (Pereira de Albuquerque et al. 2021). The disposable face masks are composed primarily of polypropylene (PP) and polyethylene (PE) with a high density and may also contain polyesters, polystyrene, polyurethane, and polyacrylonitrile. The slow degradation of the PP and PE fibers in face masks creates large reservoirs of microplastic pollutants that have acute and chronic effects on the physiology of aquatic organisms (Hasan *et al*., 2021; Patrício Silva *et al*., 2021; Spennemann, 2022).

In addition, the presence of discarded face masks has direct chemical effects on bacterial communities in the environment. Bioremediation of face mask-contaminated sites requires extensive knowledge of functional bacterial diversity and factors influencing bacterial functions. The role of indigenous bacteria in the biodegradation of discarded face masks is crucial. With increased discarded face mask contamination in landfills, mangroves, and beaches, the bacterial community may undergo significant changes. Due to natural selection resulting from contaminant pressure, the most dominant species in the locations are the most tolerant species (Panigrahi *et al*., 2018). Face masks discarded in the environment served as

a viable substrate for the growth of microbial biofilm and hydrocarbonoclastic microbes (Crisafi *et al.*, 2022). Various bacteria, biofilm-forming bacteria, bacterial consortia, and fungi can be utilized for the biodegradation of the polymers contained in face masks. Several variables affect the biodegradation process, including microorganism type, polymer type, physicochemical properties of polymers, and environmental conditions such as pH, temperature, and UV radiation (Oliveira *et al.*,2020).

Even though the microbiome is involved in the degradation of face masks, no analysis has been conducted to compare the bacterial composition of face masks discarded in different locations. Using a culture-independent method and 16S rRNA amplicon sequencing, the primary objective of this study was to identify the bacteria from discarded face masks in landfills, mangroves, and beaches in the western, central, and southern regions of Java, Yogyakarta, Indonesia. Although the degradation of polymers by bacteria has been extensively studied, little is known about the bacteria that degrade face masks. In the future, using face mask-degrading bacteria may offer an excellent eco-friendly face mask degradation process. Therefore, it is necessary to investigate the bacterial community in discarded face masks through the analysis of bacterial diversity, with the hope that the results of this study will improve the management of face mask waste. In this study, three landfills (Piyungan, Jatibarang, Burangkeng); three mangroves (Wanatirta, Tirang, Teluk Naga); and three beaches (Parangtritis, Marina, Tanjung Pasir) in Yogyakarta, Semarang, Bekasi, and Tangerang, Java,

Indonesia were chosen since these sites harbored a high abundance of discarded face masks and served as a hot spot for discovering bacterial communities for face mask degradation.

2. Materials and Methods

2.1. Discarded Face Mask Sampling

Between March and June 2022, discarded face mask samples were collected from landfills, mangroves, and beaches in Yogyakarta, Semarang, Bekasi, and Tangerang, Java, Indonesia. The discarded face mask at each location was collected with 50 mL sterile centrifuge tubes. On the island of Java, nine locations were chosen to study and represent diverse geographical conditions. The landfill site was chosen based on the size of the largest landfill in each province. The mangrove and beach were selected based on their proximity to Java Island's southern and northern tourist destinations. The sampling locations were Piyungan-Yogyakarta/A1, Jatibarang-Semarang/A2, Burangkeng-Bekasi/ A3 landfills; Wanatirta-Yogyakarta/B1, Tirang-Semarang/B2, Teluk Naga-Tangerang/B3 mangroves,and Parangtritis-Yogyakarta/C1, Marina-Semarang/C2, Tanjung pasir-Tangerang/C3 beaches as illustrated in Figure 1.
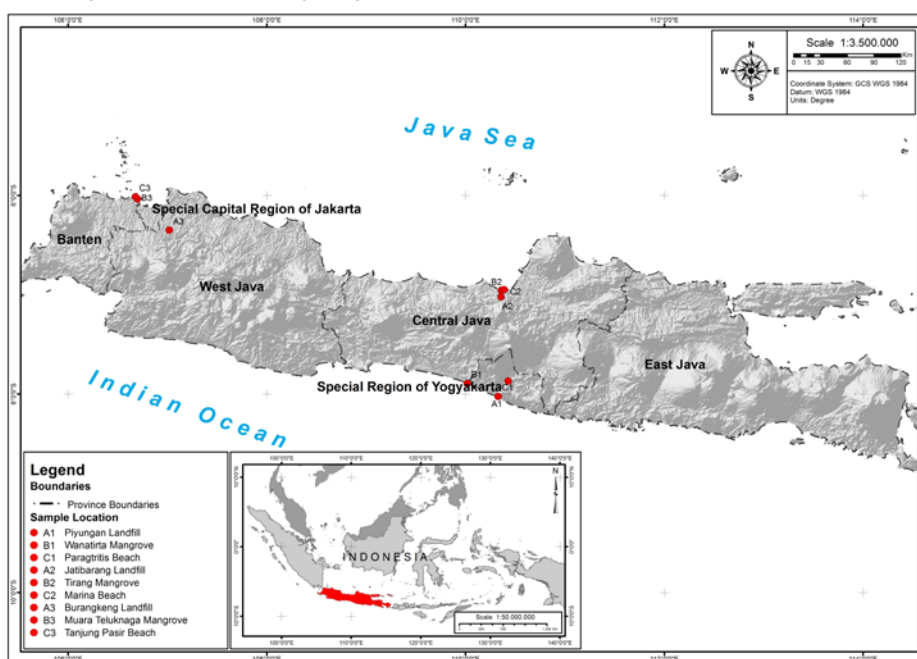

Figure 1. The location of discarded face masks sampling are landfills (A), mangroves (B), and beaches (C) in Java, Indonesia

2.2. Extraction, amplification, and sequencing of total DNA

All samples were sent for DNA extraction to PT Genetika Science Indonesia. Following the manufacturer's instructions, total DNA was extracted from each sample using a ZymoBIOMICSTM DNA Miniprep Kit (Takarina *et al.*, 2022). The bacterial community was analyzed using

Illumina HiSeq 2500 PE250 (Novogene, Tianjin, China). The replicated metagenomic DNA was pooled, and the hypervariable V3-V4 regions of the bacterial 16S rRNA gene were amplified using universal primers 341F (5'-CCTAYGGGRBGCASCAG-3') and (5'-GGACTACNNGGGTATCTAAT-3') primer pair (806R) to generate an amplicon library. All PCR reactions were performed with a Phusion High-Fidelity PCR master mix

(New England Biolabs). PCR amplicons were generated under the following conditions: 98 °C for 3 minutes, followed by 35 cycles of 98 °C for 45 s, 50 °C for 60 s, and 72 °C for 90 s, and final elongation at 72 °C for 10 minutes (Takahashi *et al.*, 2014). We combined 2% agarose gel electrophoresis with equal volumes of 1X loading buffer (containing SYBR green) and 2 μL PCR products. Samples with a bright DNA bands of 400–450 bp were chosen for further experiments. We were mixing PCR products with equal density ratios. Qiagen Gel Extraction Kit (Qiagen, Germany) was used to purify the mixed PCR products. Using the NEBNext® UltraTM DNA Library Prep Kit for Illumina, a sequencing library was generated, quantified using Qubit and QPCR, and analyzed using the Illumina platform. The library's quality was evaluated using a Qubit® 2.0 Fluorometer (Thermo Scientific) and an Agilent Bioanalyzer 2100 system. The library was sequenced on an Illumina platform to generate 250 bp paired-end reads (Zhang *et al.*, 2021).

2.3. Bioinformatics analysis

Based on the samples' unique barcodes, paired-end reads were assigned and truncated by removing the barcode and primer sequences. The FLASH software combined the data from each paired-end sequenced reading (Magoč and Salzberg, 2011). Using the QIIME (V1.7.0) software (Kuczynski *et al.*, 2011), high-quality, clean tags were obtained by performing quality filtering on the raw tags under specific filtering conditions (Bokulich *et al.*, 2013). Filtering the raw tags with the UCHIME algorithm produced high-quality tags, which were then clustered into operational taxonomical units (OTUs) using a cutoff percentage of bases with a quality score > 20 and an error rate of 0.01 (Q20). Edgar *et al.* (2011) compared the clean tags to databases (Gold database) using the UCHIME algorithm to detect chimera sequences, which were then eliminated to achieve effectiveness. Using the UPARSE program (Edgar, 2013), sequenced data (effective tags) were then analyzed. Sequences with a similarity of 97% were classified as belonging to the same OTU. Each OTU was then compared to the SILVA 132 database (https://www.arb-silva.de/) to identify species at each taxonomic rank (threshold: 0.8–1). Information regarding the abundance of OTUs was normalized using the standard sequence number corresponding to the sample with the fewest sequences. Based on these output normalized data, subsequent alpha, and beta diversity analyses were performed. Principal coordinate analysis (PCoA) was then used to calculate the Shannon-Weiner index (relative abundance) and its effect on data distribution based on the annotation results. The Shannon-Weiner relative abundance and index were calculated using R (R Core Team, 2019) and Minitab 19 (Minitab, 2021) for the PCoA (Sessa *et al.*, 2022).

3. Results and Discussion

The on-site collection of discarded face masks from landfills, mangroves, and beaches in three clusters (Yogyakarta, Semarang, and Tangerang-Bekasi) revealed the presence of different types of disposable face masks (duckbill, surgical, KF94, and KN95). During the collection of discarded face masks, it was discovered that they were mixed with other wastes in the landfill, sediments in the mangrove, and sand along the beach, as determined by a one-day sampling at each location. This study did not specify the residence time, mask type, quantity, manufacturer, or status of the physicochemical and biological processes. Illumina sequencing investigated the bacterial community structure in discarded face masks. With the advent of high-throughput amplicon sequencing methods focusing on the 16S rRNA gene, a wealth of data is now available for describing the composition and diversity of the microbiome in natural environments. All discarded face mask samples yielded 1,020,654 effective sequences clustered into 35,612 OTUs. Our sample classified 901,664; 900,711; 885,140; 864,393; 739,319; and 278,125 sequences at the phylum, class, order, family, genus, and species levels, respectively.

In addition, differences in the microbiome's diversity and abundance between samples were compared using Chao1, ACE, Shannon, and Simpson indices (Table 1) and rarefaction curves (Figure 3). Through clustering with 97% identity on the Effective Tags of all samples, the OTUs were determined and then identified to examine each sample's bacterial community composition. Table 1 displays the bacterial community's parametric and non-parametric diversity indices in the discarded face masks. Alpha diversity indices demonstrated the abundance (observed species, Chao1 indices) and diversity (Shannon and Simpson indices) of the microbiome within the samples. Alpha diversity indices showed the richness (observed species, Chao1 indices), and diversity (Shannon and Simpson indices) of the microbiome in the samples. The observed alpha diversity indices for discarded face masks from landfills were 3403 ± 387 (observed OTUs), 8.62 ± 0.82 (Shannon), 0.99 ± 0.01 (Simpson), 4018 ± 544 (chao1), and 4126 ± 2463 (ACE). The alpha diversity indices for discarded face masks from mangroves had mean values of 3794 ± 774 (observed OTUs), 7.96 ± 2.00 (Shannon), 0.94 ± 0.07 (Simpson), 4442 ± 999 (chao1), and 4522 ± 989 (ACE), whereas the alpha diversity indices for discarded face masks from beaches had mean values of 3271 ± 520 (observed OTUs), 7.83 ± 0.52 (Shannon), 0.97 ± 0.01 (Simpson), 3891 ± 816 (chao1), and 4013 ± 717 (ACE). These indices indicated that the species richness, evenness/abundance, number of different taxa, and phylogenetic distances of the discarded face masks varied significantly depending on the collection location. Teluk Naga Mangrove contained the highest microbial taxa (4269), and Parangtritis Beach contained the lowest number (2861). The average number of bacterial taxa across all nine samples was 3,489557, with mangrove, landfill, and beach samples having the highest, middle, and lowest numbers, respectively. The samples from Wanatirta Mangrove (B1) and Parangtritis Beach (C1) had the highest and lowest Chao1 non-parametric diversity indices, respectively. In addition, discarded face masks from the Wanatirta mangrove contained the highest bacterial diversity (Shannon's H index), while samples from the Tirang mangrove contained the least (B2).

Table 1. Normalized summary of sequence library including OTUs, diversity, and richness estimated at 97% level

Sample name	Observed species	Shannon	Simpson	Chao	ACE	Coverage
A1	3496	8.855	0.992	4467.486	4522.181	0.986
A2	3736	9.286	0.995	4174.874	4241.347	0.990
A3	2978	7.707	0.981	3412.896	3616.884	0.989
B1	4213	9.415	0.993	5115.607	5127.164	0.985
B2	2900	5.682	0.858	3294.709	3380.020	0.991
B3	4269	8.794	0.980	4918.264	5060.180	0.986
C1	2860	7.646	0.980	3286.100	3485.942	0.990
C2	3857	8.425	0.971	4819.719	4830.337	0.985
C3	3098	7.427	0.970	3568.520	3722.750	0.989

A1 (Piyungan), A2 (Jatibarang), A3 (Burangkeng) landfill; B1 (Wanatirta), B2 (Tirang), B3 (Teluk Naga) mangrove; C1(Parangtritis), C2 (Marina), C3 (Tanjung Pasir) beach

To further differentiate the three location groups, Figure 2.a displays the unique and shared OTUs, where 870 OTUs were unique to the landfill samples, 1367 OTUs were unique to the mangrove samples, and 695 OTUs were found exclusively in the beach samples out of the 3381 OTUs shared by the three location groups. In addition, based on Figure 2.b, the number of core OTUs obtained from a Venn diagram analysis was 989. It indicates that the 989 OTUs were shared by the discarded face mask in nine different locations and indicated a high degree of similarity in the bacterial community structure of all samples (Figure 2.b). This study revealed that several bacterial phyla populations in discarded face masks actively adapt to various environmental conditions.
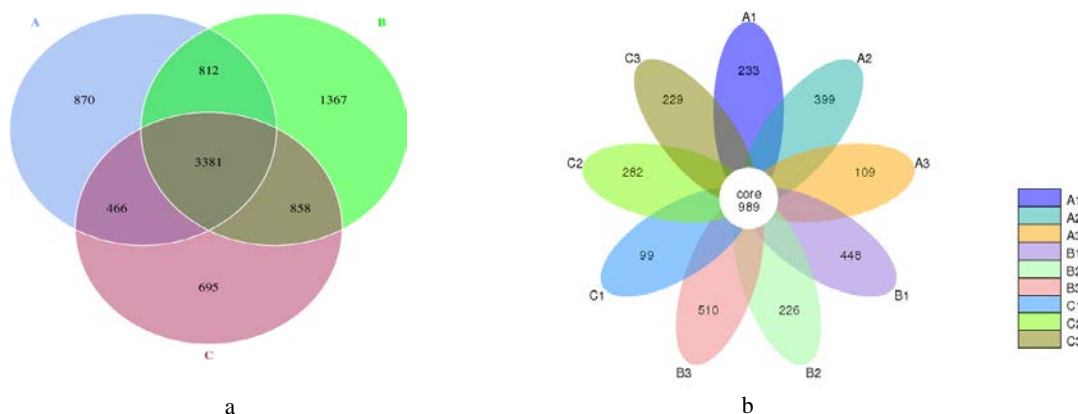

a

b

Figure 2. Venn diagram showing the amount of bacterial OTUs shared among the studied discarded face mask community from A, B and C (landfill, mangrove, and beach), respectively. A1 (Piyungan), A2 (Jatibarang), A3 (Burangkeng) landfill; B1 (Wanatirta), B2 (Tirang), B3 (Teluk Naga) mangrove; C1(Parangtritis), C2 (Marina), C3 (Tanjung Pasir) beach

Figure 3 depicts the rarefaction curves generated at a threshold of 1% to compare the diversity of microbial species between sites. Environmental factors significantly impacted the abundance of bacterial groups and OTUs, as well as the appearance of some bacteria. The composition of microbial habitats can influence bacterial diversity in various environments. Notably, microbial communities are dynamic, and the results presented here represent a particular sampling time point. As observed in this study, variations in microbial diversity at various taxonomic levels in discarded face masks were related to the surrounding environment in which they originated. Considering the sampling area as a significant determinant of community composition, we can predict that the bacterial communities associated with discarded face masks will rapidly adapt to shifting environments rather than remain stable over long distances. Figure 3.a depicts that the OTUs indices of microbial communities in discarded face masks from mangroves were more significant than those from landfills and beaches. It was caused by the unique physicochemical characteristics of mangrove soil, such as oxygen, pH, salinity, and chemical

compositions that were diverse for various microbial life forms and can therefore regulate the structure of mangrove microbial communities (Ceccon *et al*., 2019). The high organic carbon content of mangrove sediments results from the deposition of mangrove litter, root exudates, and phytoplankton debris. In addition, mangrove sediments support the development of taxonomically and functionally diverse microbial communities (Zhu *et al*., 2018). Changes in the physicochemical properties of mangrove soils facilitated the modification of functional microbial groups (Yang *et al*., 2022). Mangroves are transitional coastal ecosystems between terrestrial and marine environments that have been contaminated in recent decades (Cabral *et al*., 2018) and whose microbial ecosystem functions may be impacted (Cotta *et al*., 2019). Therefore, such a coastal region's sediment properties and seawater qualities result from natural and anthropogenic inputs and interactions. Physical properties, hydrological conditions, organic matter, and pollutants inputs influence microbial communities' biogeography (Su *et al*., 2018). Mangrove ecosystems are highly susceptible to the bioaccumulation of heavy metals and frequently act as metal absorbers in

coastal areas. In order to survive in environments with a high metal content, the microbial population in mangrove sediments develops various mechanisms to combat metal toxicity (Puthusseri *et al*., 2021).
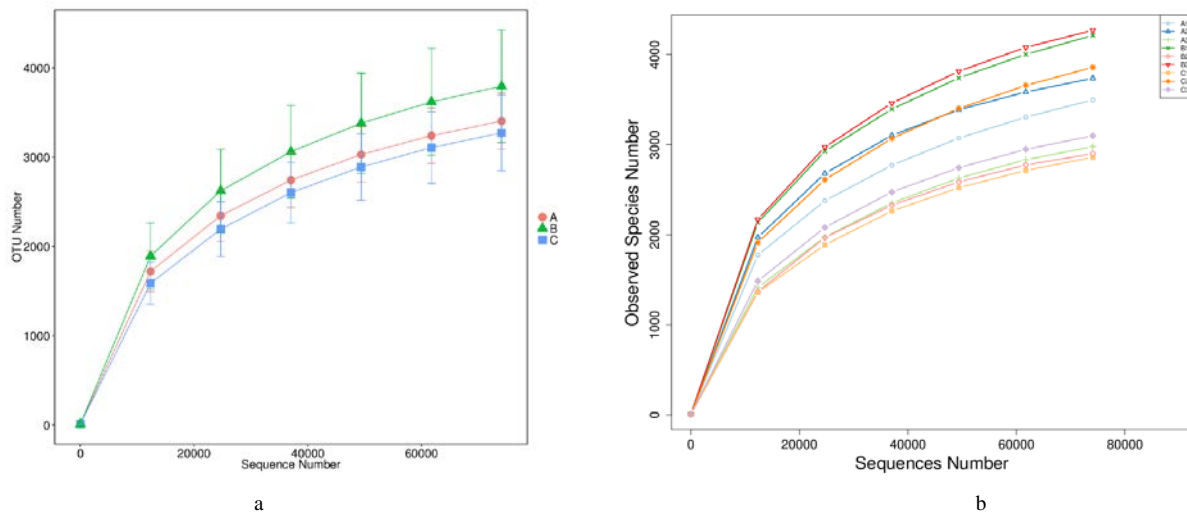

a

b

Figure 3. Rarefaction curves showing the observed number of operational taxonomic units (OTUs) at 1% dissimilarity for discarded face masks.

a. The group samples of landfills A (round-redline), mangroves B (triangle-greenline), and beaches C (square-blueline).

b. A1 (Piyungan), A2 (Jatibarang), A3 (Burangkeng) landfill; B1 (Wanatirta), B2 (Tirang), B3 (Teluk Naga) mangrove; C1(Parangtritis), C2 (Marina), C3 (Tanjung Pasir) beach.

The beta diversity pattern of discarded face mask samples was depicted on a PCoA plot utilizing unweighted UniFrac based on Bray-Curtis distances. The PCoA plot reveals area-specific clustering of samples from regions A, B, and C. 38.07% (PCo1) and 19.43% (PCo2) of the spatial variances of the bacterial community were explained by unweighted UniFrac distance (Figure 4). However, the three plots revealed dissimilarities in the structure of the bacterial communities originating from discarded face masks (B). This difference was primarily caused by the dominance of Proteobacteria in B1 and Firmicutes, which were significantly more prevalent in B3 than in B2 metagenomes. It was discovered that different bacterial communities were associated with different types of discarded face masks and collection locations. The effect of the face mask's surface on its colonization can be caused by hydrophobicity, degradability, electric charge, or roughness, or indirectly by the formation of a conditioning film on top of the particles. The bacterial communities are associated with the distinction between microplastics and natural particles, such as cellulose, particle-attached water fraction, and sediment (Oberbeckmann and Labrenz, 2020).
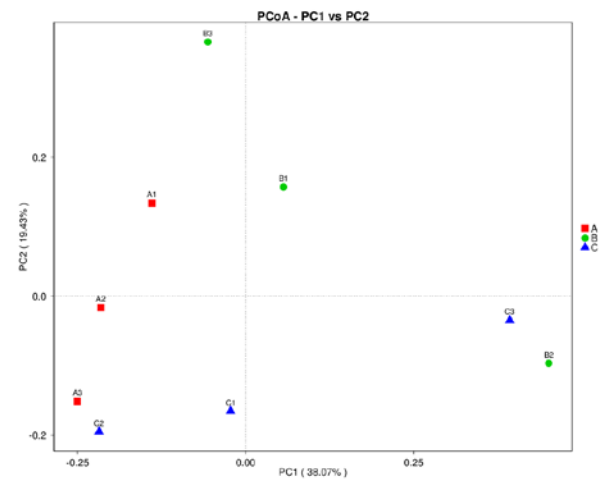

Figure 4. Beta diversity of the nine bacterial communities at studied landfills (A), mangroves (B), and C (beaches) sites. Principal coordinates analysis (PcoA) plots were constructed by applying the weighted Unifrac distance matrix on the CSS normalized OTU table data

Figure 5.a depicts Proteobacteria as the dominant phylum in all samples from landfills (33.32%), mangroves (52.22%), and beaches (53.63%). It suggests that Proteobacteria may be capable of degrading organic and inorganic materials in discarded face masks. The second dominant phylum in landfill samples was Actinobacteria (30.29%), followed by Firmicutes (17.25%). In contrast, the second dominant phylum in mangrove samples was Firmicutes (19.85%). In addition, the second dominant phylum in beach samples was Actinobacteria (12.62%), and the third was Cyanobacteria (11.52%). The natural environment influenced the presence of microorganisms in the face mask samples. According to previous studies, Proteobacteria is the dominant phylum in mangrove (Shah *et al*., 2022); tropical estuarine (Khandeparker *et al*., 2017); contaminated site (Kumar *et al*., 2020); and coastal waters (Li *et al*., 2021) environments. In addition, under various landfill conditions, Proteobacteria, Actinobacteria, and Firmicutes predominate because they can degrade complex substrates inaccessible to other microbial groups and grow with limited nutrients (Hu *et al*., 2022). Proteobacteria are the most diverse group due to their versatile metabolism and ability to survive in various environments. They are crucial in nutrient cycling and maintenance due to their metabolic flexibility (Chakraborty *et al*., 2021). Firmicutes can constitute up to 90% of the human gut microbiota (Quillaguamán *et al*., 2021), effectively indicating sewage contamination in mangroves. Proteobacteria and Firmicutes possess a diverse metabolic capacity for hydrocarbon degradation. Proteobacteria and Firmicutes can tolerate and survive in a contaminated environment due to their high prevalence (Muccee and Ejaz, 2020). They were present in all samples from landfills, mangroves, and beaches, but phylum composition varied by location (Figure 5.b). It appears that environmental factors had a more significant impact on these microbial communities' structure than geographic variation (Abuzahrah *et al*., 2022). Continuous discharge of wastes can alter the physical and biological properties of the backwaters, which can have a growing impact on the ecosystem's fauna and flora (Balasubramanian *et al*., 2021). Nathan *et al*. (2020) found that bacterial communities in mangrove environments varied significantly based on the composition and location of sediments, with each sampled mangrove ecosystem exhibiting unique environmental characteristics.

Figure 5.c depicts the diversity of bacteria at the genus level. Bacillus (5.96%), Nocardioides (3.00%), and Gardonia (2.91%) dominated the samples collected from landfills. Vibrio (19.41%), Weisella (5.49%), and Lactobacillus (3.8%) predominated in the mangrove samples. In contrast, Vibrio (8.34%), Coleofasciculus (5.22%), and Deinococcus (4.44%) dominated beach samples. There was also considerable variation in the occurrence (and persistence) of some of the microbiome's most abundant members. For instance, Bacillus was the most prevalent group in the discarded face masks from landfills (Figure 5.d), but its prevalence varied greatly between landfills. It has been reported that Bacillus can be isolated from soil (Massadeh and Mahmoud, 2019), compost (Xie *et al*., 2021), and face masks (Delanghe *et al*., 2021). In this study, we observed that Vibrio dominated mangrove and beach samples. Nevertheless, the genus Vibrio is predominantly endemic to marine aquatic environments, ranging from the deep sea to shallow aquatic environments (Junaid *et al*., 2022); tolerant of heavy metals isolated from mangrove sediments (De Fretes *et al*., 2019), and sewage-associated plastic wastes (Metcalf *et al*., 2022).

Bacterial communities are hypothesized to have significant functional contributions to face mask degradation. The biodegradability of bacteria has been repeatedly demonstrated. However, the current research focused on discovering new microorganisms that can degrade biodegradable and non-biodegradable face mask components. The contents of the face mask inhibited the production of bacterial enzymes. The interaction between microplastics and dissolved organic pollutants in the environment depends on the physicochemical properties of the organic compounds. Bacillus was detected in the metagenome of discarded face masks from landfills, as determined by NGS analysis results about one of the most abundant genera in our samples. Bacillus, which can bind heavy metals to the cell surfaces and provide bioremediation, was also responsible for degrading disposable face mask materials (Muzammil *et al*., 2021). Bacillus from various locations has been demonstrated to be an effective azo dye degrader (Guembri *et al*., 2021); plastic degrader *(Fibriarti et al*., 2021); and organometallic degrader (Tripathi *et al*., 2021). Figure 5.b illustrates the distribution of the predominant bacterial genera in samples collected from various locations, including landfills, mangroves, and beaches. Briefly, the results demonstrated that the composition of bacterial communities differed across regions. It has been demonstrated that differences in face mask types and compositions, disposal period, and environmental factors (salinity, pH, temperature, dissolved organic carbon loading, etc.) influence bacterial community composition and dominance during degradation processes.
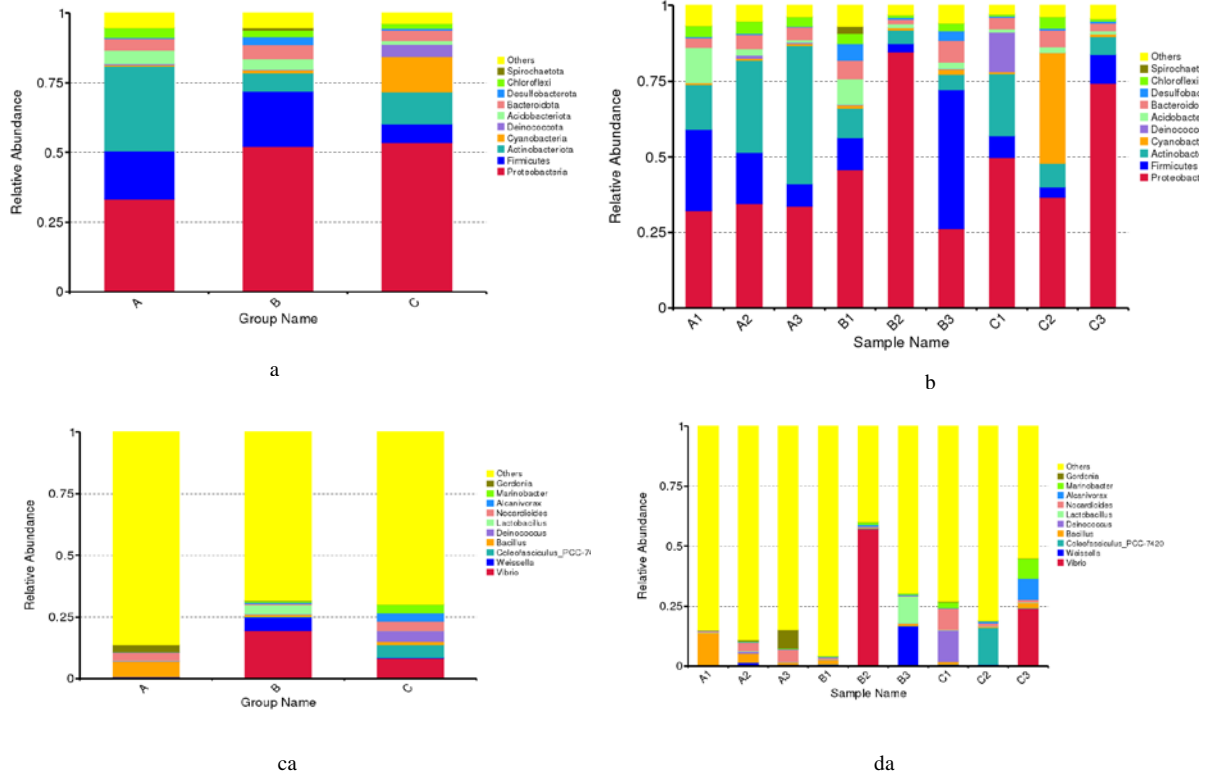
Figure 5. The composition of bacterial communities at the phylum (a,b) and genus (c,d) level.

The same taxa were marked by the same color.

Based on the abundance data of the top 35 genera of all samples, a heatmap was created to determine whether samples with similar processing were clustered or not and to observe the samples' similarities and differences (Figure 6). The bacterial communities on discarded face masks from landfills, mangroves, and beaches were distinct (Figure 6.a). Gemmatimonadota, Nitrospirota, and Eucaryarchaeota were only found in the Piyungan landfill sample, while Sumerlaota was only found in the Burangkeng landfill sample. Only the sample from

Wanatirta Mangrove contained the phyla Crenarchaeota, Desulfobacterota, Methylomirabilota, Spirochaetota, Entotheonellaeota, Elusimicrobiota, and Verrucomicrobiota. In contrast, Campilobacterota, Halobacterota, and Latescibacterota were discovered in the Teluk Naga Mangrove sample. Only face masks from Parangtritis Beach, Marina Beach, and Tanjung Pasir Beach contained the phyla Deinoccocota, Cyanobacteria, and Thermoplasmatota.
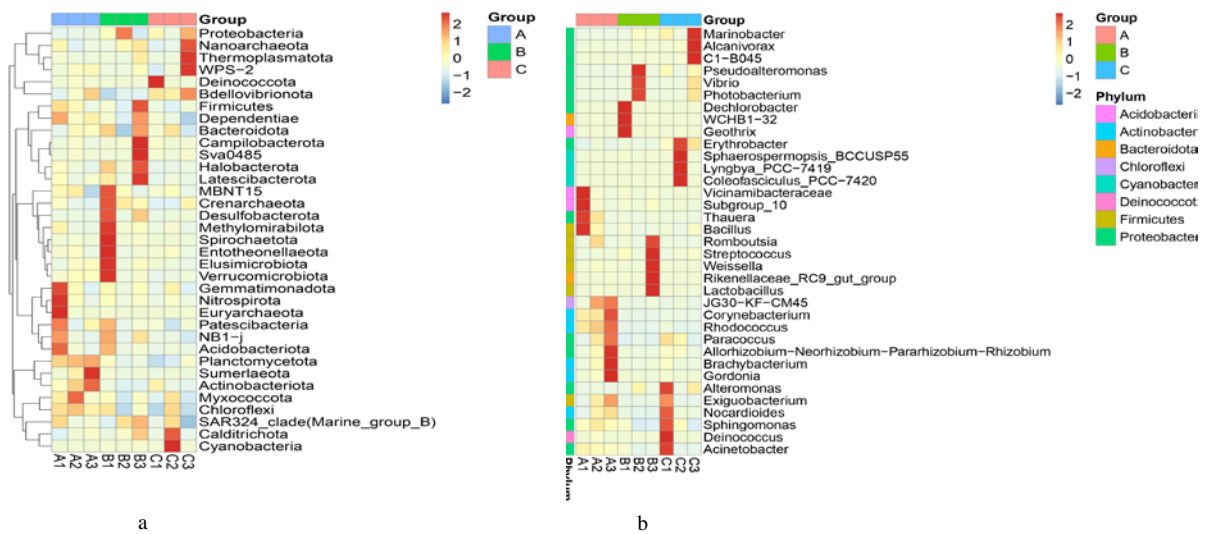

Figure 6. Heatmap displaying the differences in the relative abundances of the top 35 a) phylum and b) genus.

The colors indicate the relative abundance of taxa, ranging from blue (low) to red (high).

In particular, Vicinamibacteraceae, Thauera, and Bacillus were found in the Piyungan landfill. Rhizobium, Brachybacterium, and Gordonia were found in the Burangkeng landfill sample (Figure 6.b). Regarding the activity of the bacterial community, the abundances of the six genera were relatively higher in landfills. The disposable face masks' utilization or degradation efficiency was an essential factor to consider. It showed that the microbiome in landfill can potentially degrade pollutants. Vicinamibacteraceae has the dominant genus in contaminated soils (Yi *et al*., 2022) and textile dye wastewater (Patel *et al*., 2021). Thauera is the predominant genus in leachate landfills (Saleem et al. 2018) and contaminated sediments (Y. Chen *et al*., 2018); therefore, it can be used as a bioremediation for heavy metals (Yin *et al*., 2019). Gordonia is commonly found in plastic (Adamovsky *et al*., 2021); therefore, it has the potential for polymer degradation and removal (Bhandari *et al*., 2021).

In this study, face masks found in mangroves may have been the source of the genera Dechlorobacter, Geothrix (Wanatirta mangrove); Pseudoalteromonas, Vibrio, Photobacterium (Tirang mangrove); and Rombutsia, Streptococcus, Weisella, Lactobacillus (Teluk naga mangrove), respectively (Figure 6.b). Since Dechlorobacter was discovered in waste-activated sludge in a previous study (Chen *et al*., 2021), this genus could be utilized as an organic biodegradation agent (Wang *et al*., 2020). Geothrix is prevalent in drinking water treatment residues (Wang *et al*., 2021), geothermal areas (Massello and Donati, 2021), wastewater treatment plants (Valk *et al*., 2022), and contaminated soil (Peixoto *et al*., 2022). Pseudoalteromonas can be isolated from mangroves (Alzubaidy *et al*., 2016) that can degrade hydrocarbon (Bhattacharjee *et al*., 2020). Streptococcus can be found in sediment mangroves (Chithira *et al*., 2021) that can degrade plastic *(Dang et al*., 2018), hydrocarbon (Sarkar *et al*., 2020), heavy metal biosorption (Priyadarshanee and Das, 2021), and azo dyes degradation (Slama *et al*., 2021). Weisella can be isolated from municipal solid waste (Jurado *et al*., 2020).

Considering the bacterial groups assigned at the genera level, we observed that marine bacteria dominated the predominant genera found on beach-discarded face masks. Alteromonas, Exiguobacterium, Nocardioides, Sphingomonas, Deinococcus, and Acinetobacter predominated on Parangtritis beach. However, Erythrobacter, Sphaerospermopsis, Lyngbya, and Coleofasciculus were present at Marina Beach. Figure 6.b accounts for the genera Marinobacter and Alcanivorax on Tanjung Pasir beach. Xia *et al*., (2021) demonstrated unequivocally that Alteromonas can be isolated from coastal and ocean environments. Halophilic Exiguobacterium can be isolated from arsenic-rich thermal springs and estuarine sediments (Prieto-Barajas *et al*., 2018; Cui *et al*., 2021). Sphingomonas can be isolated from marine and mangrove sediments (Al Farraj *et al*., 2021; Guo *et al*., 2011). Known as freshwater bacteria (Medeiros *et al*., 2016), Deinococcus can be isolated from lakes (Chakraborty *et al*., 2021) and antarctic soils (Ramírez-Fernández *et al*., 2021). Acinetobacter dominates in polar habitats (Ali *et al*., 2020) and urban lakes (Quillaguamán *et al*., 2021). Erythrobacter can be isolated from a marine containing microplastic (Oberbeckmann and Labrenz, 2020) has saline stress tolerance (Benidire *et al*., 2020) and can be utilized as a hydrocarbon-related pollutant degrader in oceanic and coastal environments (Ramírez *et al*., 2020). Lyngbya, also called benthic marine cyanobacteria (Narayana *et al*., 2020), can be isolated from the estuarine mangrove ecosystem (Sengupta *et al*., 2020). In addition, Marinobacter has been identified in lake ecosystems (Moopantakath *et al*., 2020), the South Atlantic Ocean (Coutinho *et al*., 2021), plastisphere bacteria (Sumithra *et al*., 2020), and hydrocarbon-degradering organisms (Hidalgo *et al*., 2021). An increased abundance of Alcanivorax sequences in oil-contaminated sand (Panigrahi *et al*., 2018) forms biofilms in marine environments. It possesses the genomic capability to degrade multiple oil-derived hydrocarbons (Roager and Sonnenschein, 2019).

A cluster tree was constructed using clustering analysis to examine similarities between various samples. Using the weighted UniFrac, the differences in microbial communities based on the occurrence and abundance of OTUs were observed. At the phylum and OTU levels, eight areas except C2 clustered together (Figure 7.a), revealing a significantly more significant distinction. In the meantime, the unweighted UniFrac calculations identified inter-community variables by determining the occurrence of OTUs. In addition, Figure 7.b demonstrates that although the samples were collected from distinct locations, they contained similar bacterial communities. To further investigate the phylogenetic relationship between the genera, the top 100 genera were chosen, and an evolutionary tree was constructed from the aligned sequences of representative species. Figure 7.c also displays the relative abundance of each genus and its genus.
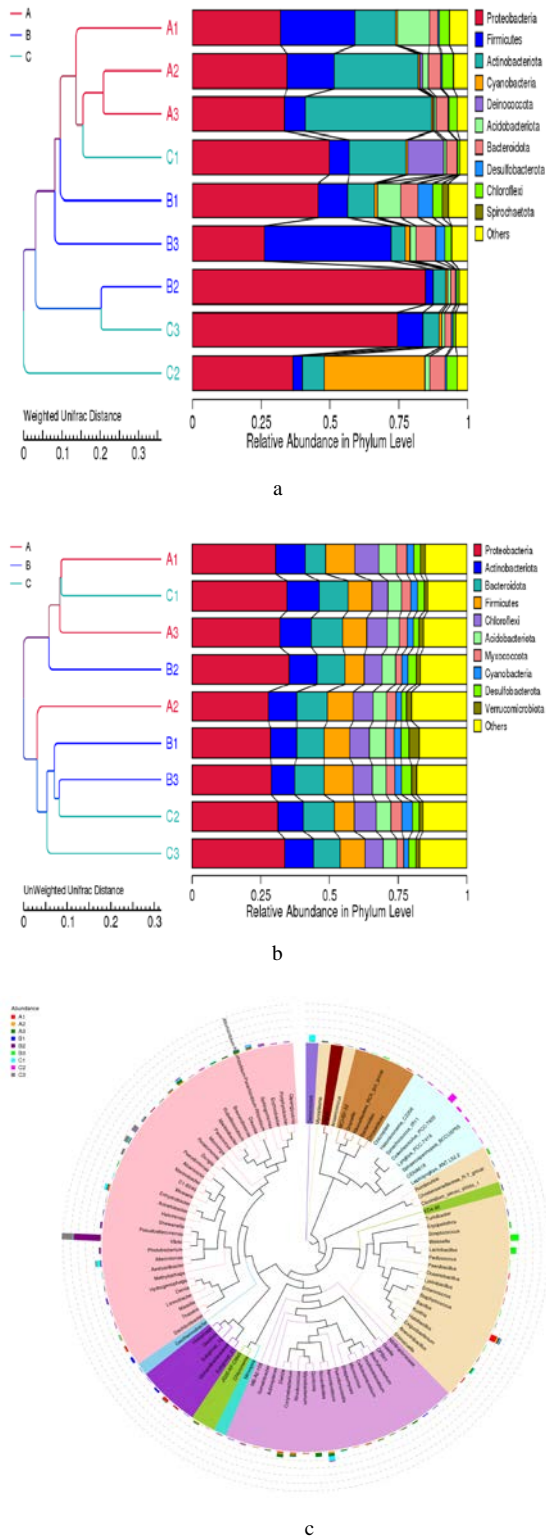
a

b

c

Figure 7. Hierarchical clustering of microbiomes from different sampling points locations. Distance matrices were created with (a) weighted and (b) unweighted UniFrac calculations. (c)16S rRNA gene-based phylogeny showing representatives of all microbial genera in discarded face masks.

In conclusion, 16S rRNA gene sequencing was used to investigate the microbial communities inhabiting discarded face masks from landfills, mangroves, and beaches on Java Island, Indonesia. The current study reported detailed investigation results regarding bacterial communities' abundance, composition, and diversity at various locations. The bacterial community composition of discarded face masks was dynamic and susceptible to many environmental factors. This study was an essential step toward elucidating the bacterial community's potential role in modulating face mask degradation. Therefore, future research will concentrate on identifying new bacterial taxa involved in the degradation of face masks, thereby advancing the current research on this topic.

Acknowledgements

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