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Metagenomics Profiling of Microbial Community in Soil and Leachate Sample Isolated from a Landfill in Selangor, Malaysia

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ABSTRACT

A landfill is a site for disposal of waste material by burying it in the ground. Understanding the impact of landfill ecosystems and studying the bioremediation methods require an understanding of their microbial diversity and functional ability. However, little is known about the microbial communities that found in landfill sites, especially in tropical areas. In order to further our knowledge of water quality and environmental sustainability, this study aims to outline the metabolic pathways and microbial community structure in soil and leachate samples from Jeram landfill. Sample of soil and leachate were carefully collected at the selected sites within the Jeram Landfill in Selangor, Malaysia. Metagenomic DNA was extracted and Illumina shotgun sequencing was performed for taxonomic and functional analysis. Taxonomic profiling showed that bacteria dominated in both soil (55.31%) and leachate (30.98%), whereas archaea were significantly more common in leachate (8.91%) than in soil (1.74%). Leachate contained a high percentage of unidentified sequences (59.60%), indicating the presence of a new or unknown microorganisms. The most prevalent species in the soil sample were *Bacteroides graminisolvens*, *Methanosarcina barkeri*, and *Klebsiella pneumoniae*. Methanogens like *Methanoculleus bourgensis and Methanosarcina mazei*, as well as *Arcobacter* species known for their roles in the nitrogen cycling, was dominant in leachate samples. KEGG pathway functional analysis revealed that metabolic pathway, biosynthesis of secondary metabolites, and carbon metabolism were the most common in both soil and leachate samples. However, leachate showed a significantly greater methane metabolism thus indicating the active anaerobic breakdown processes. These findings highlight how important microbial communities are to the breakdown of the organic matter, nitrogen cycling, and methane production which have a significant impact on the stability of landfills and the likelihood of water. This study enhances our knowledge of waste degrading processes and the implications for environmental sustainability and water resource

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management by providing insight into the metabolic processes and microbial diversity found in landfill sites. The findings provide an outline for further research on landfill impact assessment, bioremediation, and pollution control.

1. Introduction

1.1 Background Research

Across the world, landfill are one of the most common places to dispose of waste, especially in urbanization. They are made to bury waste to keep it out of the environment and avoiding contamination [1]. However, microbial communities are essential to the breakdown of organic matter, nutrient cycling, and production of methane in landfill, which are complex ecosystems [2,3]. Understanding the environmental impact of landfill ecosystems and developing practical bioremediation methods require a knowledge of the microbial diversity and functional capabilities that present in these environments. Landfill in tropical areas are subjected to unique environmental factors, like high humidity and temperatures, which might affect microbial activity and the community composition. Research on the microorganisms in tropical waste is limited, although it is global significance of landfill [4].

1.2 Literature Review

In the landfill ecosystem, microbial diversity is essential for the decomposition of organic matter, biogeochemical cycling, and the breakdown of pollutants. The environmental concerns include soil pollutions, greenhouse gas emissions, and leachate contamination that are caused by the landfills. Soil and groundwater are at the a risk from leachate, a toxic liquid that forms when water seeps through the waste and contain organic contaminants, ammonia, and heavy metals. Furthermore, methane (CH_4) and carbon dioxide (CO_2), two greenhouse gases that can contribute to climate change that produced during anaerobic decomposition in the landfills [5,6]. The landfill release a volatile organic compounds (VOCs), which can impact air quality and have an impact on the nearby ecosystems [7]. Even while methane capture, leachate treatment and waste-to-energy conversion method are used in the modern landfills, their effectiveness is frequently restricted, especially in the areas with insufficient waste management infrastructure [8,9].

Tropical landfills encounter unique potential and challenges because of their different climate, which include the high humidity, high temperature, and heavy rains. These factors have a major impact on the landfill environmental effects, waste decomposition rates, and microbial activity [10]. High rainfall may contribute to the leachate formation, raising the risk of soil and groundwater contamination where the tropical landfill leachate often contains a higher concentrations of organic contaminants, ammonia, and heavy metals [11-13]. Tropical climate has a higher temperatures and moisture contents that enable the microbial activity which speed up the decomposition of organic waste and increase the production of methane [12].

In the landfill, microbial communities consisting of bacteria, fungi, and archaea are important for the breakdown of the waste and the cycling of nutrients [8,14]. Methanogenic archaea, such as, *Methanosarcina* and *Methanobacterium*, can convert organic matter into methane, while anaerobic bacteria such as *Clostridium* and *Bacteroidetes*, can breakdown complex chemical substances [15,16]. *Methanosarcina barkeri* and *Methanosaeta concilii* are two microbial groups that are more common in the tropical landfills because of their ability to resist high temperatures and moisture levels, while anaerobic bacteria such as *Clostridium thermocellum* and *Bacteroides vulgatus* are essential for the breakdown of the complex organic matter in the tropical environments [17-19]. The environmental factors like waste type, moisture content, and oxygen availability affect the microbial composition [20]. Bacteria such as *Pseudomonas* and *Bacillus* contribute to the breakdown of the organic matter in the aerobic zones, where facultative and strictly anaerobic bacteria dominant in the anaerobic zone, thus promote the fermentation and methanogenesis [21,22]. Nutrient-recycling bacteria like *Bacteroides* and *Klebsiella* are frequently dominate soil microbial communities, while nitrogen-cycling bacteria including *Arcobacte*r and methane-producing archaea such as *Methanoculleus* are found in the landfill leachate [23-25]. Increases in the microbial population in the landfill is related to less environmental impact and better waste breakdown efficiency [20].

Microbial population in the landfill are important to the stability and ecological impact of the landfill ecosystems because they provide an important roles in the waste digestion, nutrient cycling, and the production of gas. Their ability is to break down complex organic substances into simpler components is one of their main roles [15]. This process is started by bacteria, which hydrolyse and ferment organic waste to produce smaller molecules including fatty acid and sugars. Following their utilization, methanogenic archaea use an anaerobic digestion to transform these intermediates into methane (CH4) and carbon dioxide (CO2) [26]. The dual function of the microbial activity in the landfill is shown by this process, which not only improves the waste breakdown but also increases the greenhouse gas emissions. The rapid anaerobic breakdown of the organic waste in the tropical landfills often occurs in the presence of the volatile organic compounds (VOCs), including the benzene, toluene, ethylbenzene, and xylene (BTEX) [27]. Since these compounds are more volatile at a high temperatures, there is a greater impact on the environment and higher emission rates.

In tropical climates with a higher microbial activity, bioremediation techniques are being studied more and more as sustainable ways to manage leachate and landfill emissions. Using the specific strains of *Bacillus* and *Pseudomonas* for the bioaugmentation has been shown to enhance the breakdown of organic contaminants in the landfill leachate [28,29]. The use of plants to absorb and detoxify pollutants is known as phytoremediation, and it work especially well in the tropical areas because of the high microbial activity in the rhizosphere and the quick development of the plants. Comparing the tropical landfills to temperate landfills, the leachate frequently has higher levels of heavy metals, ammonia, and volatile fatty acids (VFAs). The excessive of VFA levels can prevent methanogenesis, which can result in the buildup of the intermediate products and possible harmful conditions for the microbial diversity [30,31].

Researchers can analyse the genetic information from environmental samples using the metagenomic, a powerful approach for analyzing microbial communities at landfill sites without the requirement for culturing. Researchers are able to identify both the culturable and unculturable microbes using metagenomic, which reveals a wide variety of previously unidentified species and genes by sequencing the entire genome of microbial community [8]. The microbial communities and their functional genes can be identified through the high-throughput sequencing technologies like Illumina shotgun sequencing which also provide the roles that microorganisms play in waste breakdown, nutrient cycling, and production of methane [8]. The adaptation of the microbial populations to various environmental factors such as differences in the temperature, moisture content level, and waste composition, can be better understand using the metagenomic analysis [32]. Metagenomics has been helpful in identifying the metabolic pathways and functional genes in the landfill ecosystems that are involved in the breakdown of organic waste, the cycling of nutrients, and the production of methane. For example, research has shown the important of microbial contributor in anaerobic digestion, including fermentative bacteria and methanogenis archaea, and their functions in producing carbon dioxide and methane from the organic waste [8,33]. Furthermore, metagenomics has shown that a significant percentage of waste sample contain undiscovered sequences, thus indicating that the present of new microbes with special metabolic abilities. By understanding these functional functions, microbial-based method to improve the landfill sustainability and reduce the ecological concerns can be implemented.

Although a lot of research has been done on the landfill microbial communities, most of it has been done in the temperate environments. The microbial diversity and the metabolic processes in the tropical landfills, especially in Malaysia, are poorly understand. Few studies have been examined how the environmental conditions, such as high humidity and temperatures changes, affect microbial communities of functional pathways and composition. To close this gap, the microbial diversity and functional capacities of the soil and leachate from the Jeram landfill are being profiled using the metagenomic sequencing. Understanding the functions of the microorganisms in the organic matter decomposition, nitrogen cycling, and methane metabolism can help us better understand the landfill microbial diversity in the tropical climate. The result will help in the developing of the microbial-based method to reduce environmental effects and enhance landfill sustainability.

2. Methodology

2.1 Sample Collection

Sample of soil and leachate had been collected at the Jeram Landfill (coordinate). These landfills are found in Selangor, Malaysia, a tropical area with high humidity and temperature. In order to minimize the cross contamination, soil samples were collected between 0 – 15cm below the surface. Multiple subsamples were collected from different sites within same sampling location and homogenized to form a composite sample. To detect a potential contamination, field blanks and a negative controls had been used. All samples were immediately kept in sterile sampling bottles, packed in ice boxes, and delivered to the laboratory for analysis within 4 hours of collection. Samples were then kept for further examination at -20 °C to avoid bacteria degradation. The same steps were taken again to obtain leachate samples.

2.2 DNA Extraction and Sequencing

The DNA of landfill soil samples and leachate was extracted using the DNeasy PowerSoil Kit, following the manufacturer’s procedure with adjustment to improve yield and purity. DNA integrity was evaluated using three quality control (QC) procedures which is the gel electrophoresis to detect the DNA fragmentation, Qubit fluorometry to measure the DNA concentration, and Nanodrop spectrophotometry (A260/280) for determining the purity. After passing QC, the DNA samples were purified using the magnetic bead-based purification for removing tannin. The DNA samples then undergo mechanical shearing method using a Covaris instrument to ensure that all of the sample length are within 300 and 350 bp, as determined by the bioanalyzer. Only samples with a greater than 70% recovery and at least 1 µg of DNA per 50 µl volume were further processed [34]. According to the microfluidic cell chip’s final data processing, each DNA fragment was given a unique barcode of an index adapter sequence to identify and categorise each read. Qubit and Bioanalyzer are used to assess the quality and concentration of the library in order to ensure that the fragment size is appropriate for sequencing. Illumina NovaSeq was used for shotgun sequencing on the generated libraries, generate the paired-end reads. FASTQ files containing nucleotide sequences and the quality scores for each read are created from the raw sequencing data [34].

2.3 Data Processing and Metagenomics Analysis

The raw sequencing data in the FASTQ files went through a preprocessing and quality. SolexaQA++ is used for sequencing the data quality control and enhances data dependability through read trimming and filtering, data correction, and tile-based assessment. Bowtie2 was used to remove PhiX control DNA and prevent the contamination [35]. The remaining reads were trimmed and filtered to ensure consistent in length and quality. FastQC was used to determine the sequence quality parameters thus ensuring that only high-quality reads are kept. The metagenome is rebuilt using the SPAdes v3.15.5 with the metaSPAdes mode after the clean reads have undergone de novo assembly [35]. Assembly quality was assessed using QUAST v5.2.0, which took into consideration the total assembly size, N50 value, and number of contigs. The taxonomic classification was conducted with Kraken2 v2.1.3, the abundance was estimated with Bracken.

2.4 Gene Prediction

After the assembled contigs were examined for the gene prediction analysis using the Prodigal (v2.6.3; -p meta -c -m), to detect the possible coding seqeunce (CDS) and open reading frames (ORFs) in assembled contigs. After CDS has been predicted, a gene set clustering approach is used to the group similar genes based on sequence homolog and functional features, taking into considering the functional diversity present in the microbial community.

2.5 Functional Annotation

Multiple database were used to accomplish the functional annotations. DIAMOND v2.1.9 was used to align peptide sequences to SwissProt and Refseq protein database using the following parameters, -max_target_seqs 1 -mmax_hsps 1 -evalue 1e-5. Functional classification was carried out with eggnog-mapper v2.1.12, which used the eggNOG 5.0 database to generate functional categories. InterProScan was also used to discover protein domains and motifs, which provided a more insight into the gene function. The gene were assigned KEGG Orthology (KO) identifiers which allow them to be mapped the metabolic pathways in the KEGG pathway [36]. Using orthology-based inference, eggNOG-mapper v2.1.12 was able to accomplish this by connecting sequences to KEGG pathways. This multi-database method enabled a thorough knowledge of the functional of annotated genes in the biogeochemical cycles.

2.6 Statistical Analysis

The microbiological diversity in the landfill soil and leachate samples was assessed using the alpha-diversity indices. The Shannon, Berger-Parker, Simpson, Inverse Simpson, and Fisher indices were among the several indices used to measure the diversity of microbial communities. The Shannon index is to measure the species, considering both richness and evenness, while the Simpson Index is to estimate the probability that two randomly selected individuals belong to the same species. Furthermore, Inverse Simpson Index is to provide measure of diversity that account for dominant species. The Berger-Parker Index is to assess species dominance by measuring the proportion of the most abundance species, whereas the Fisher index is to estimate species richness based on the log-series distribution. The species richness, dominance patterns, and evenness of the microbiomes linked to landfill are revealed by these indexes [37]. Potential variations in microbial community structure were identified by statically comparing diversity metrics between soil and leachate samples.

3. Results

3.1 Raw Data Assessment

Table 1 shows the significant amount of raw data that generated through high-throughput sequencing from soil and leachate samples that had been collected from the Jeram landfill. According to the sequencing data, both samples types have read size of 150.00bp. For the soil sample, 64,764,536 reads were collected thus producing 9,714,680,400 bases. By comparison, leachate sample generated 7,414,535,400 bases, yielding 49,430,236 reads. The soil sample's higher sequencing depth than leachate sample indicates likely a more varied microbial population or a higher extraction efficiency of DNA. It also possible that the differences in the microbial biomass, with soil possibly sustaining a richer microbial community that leachate, might be the reason for the observed contrast in sequencing reads [38].

Table 1
Sequencing read statistics of raw data

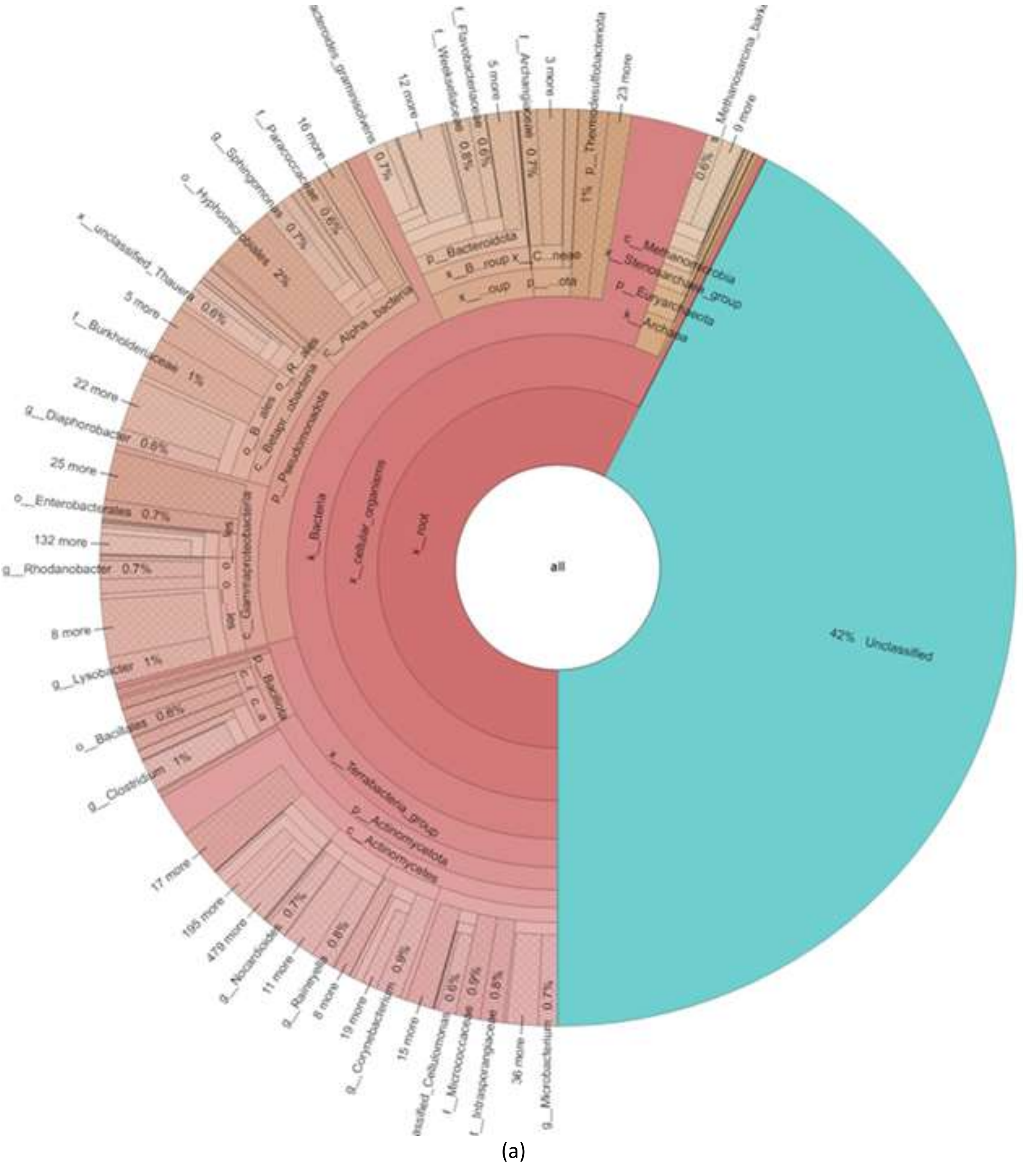
Sample ID	Read size (bp)	Total reads	Total bases
Jeram soil	150.00	64,764,536	9,714,680,400
Jeram leachate	150.00	49,430,236	7,414,535,400

Both dataset offer enough sequencing coverage for subsequent taxonomic and functional research, despite the differences between them. Although these differences, both datasets offer significant sequencing coverage for future functional and taxonomic research. The higher of sequencing depth in the soil samples highlight the complexities and variety of microbial populations in terrestrial ecosystems, which play an important role in the organic matter breakdown and nutrient cycling. These processes are critical for keeping soil healthy and minimising the contaminants from leaking into groundwater [39]. On the other hand, the lower sequencing depth in the leachate samples could be indicate that harsh environmental circumstances, such as high organic matter and low oxygen availability, which can limit diversity in microbes and favour specialized species that are well-adapted to such environments [40]. It has significant consequences for water quality, as landfill leachate can contaminate neighbouring bodies of water, threatening aquatic ecosystems and humans. As stated by Anand *et al.*, [41], the microbial composition of landfills influences how the pollutants break down and the possibility of leachate contamination in the water bodies nearby.

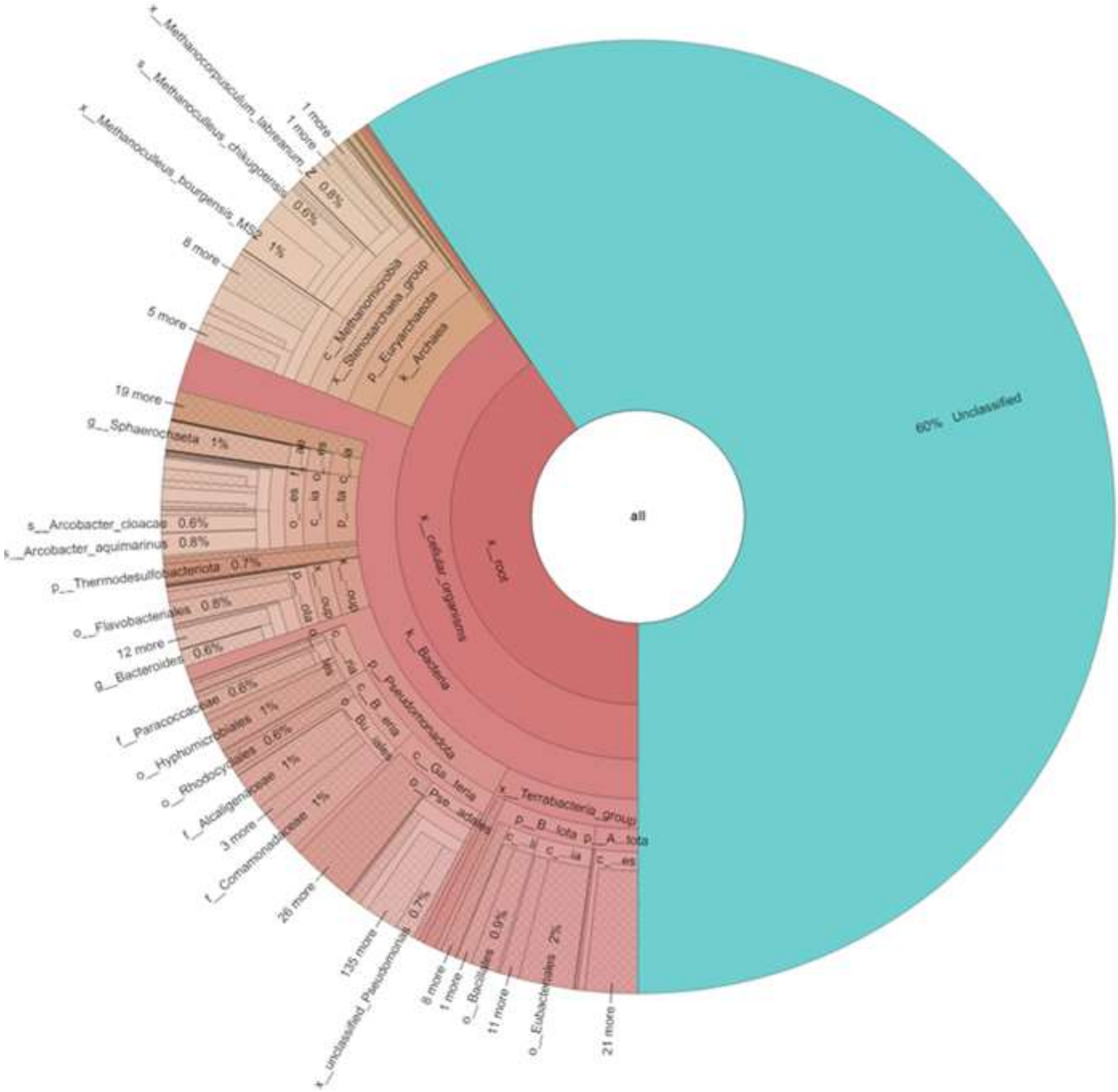
3.2 Taxonomy Profiling

Based on Figure 1, Kraken2 database was used to analyse the microbial diversity of the Jeram soil and leachate samples thus display the different taxonomic composition. Bacteria dominated for both samples, followed by viruses, eukaryote, and archaea. However, there were significant variances in proportions between the two environments. Table 2 provides a summary of the taxonomic composition of both samples. Bacteria made up of 55.31% of the classified sequences in the Jeram soil, but only 30.98% of the sequence in the Jeram leachate. The difference might be result from the stable conditions in soil, which produce a rich and varied bacterial community [42]. On the other hand, the high concentrations of organic contaminants, heavy metals, and anaerobic environments that found in the landfill leachate may restrict bacterial development and favour particular microbial species that are suited to harsh conditions [43].

Meanwhile, archaea were common in the leachate (8.91%), with methanogenic species like *Methanosarcina mazei* and *Methanoculleus bourgensis* contributing to a significantly to the production of methane [44,45]. As state by Sauk and Hug [46], the leachate offers an ideal habitat for anaerobic archaea, especially those that breakdown organic matter in an oxygen-deficient environment. Furthermore, the percentage of unclassified sequences in Jeram leachate was much higher (59.60%) compared to Jeram soil (42.47%), indicating that there were more unknown or novel microbial species in the leachate sample. This might be as a result of the unique and diverse microbial communities found in the leachate, which have received fewer studies than the soil ecosystems. The presence of previously unidentified bacteria adapted to harsh landfill conditions may be indicated by the greater the percentage of the unclassified sequence in the leachate [47].



(a)



(b)

Fig. 1. Krona plot of profiled taxonomy against Kraken2 database for (a) Jeram landfill soil sample and Krona plot of profiled taxonomy against Kraken2 database for (b) Jeram landfill leachate sample

Table 2
Taxonomic composition of Jeram soil and leachate sample

Domain	Jeram soil (%)	Jeram leachate (%)
Bacteria	55.31%	30.98%
Eukaryota	0.17%	0.20%
Archaea	1.74%	8.91%
Viruses	0.03%	0.08%
Others	0.28%	0.23%
Unclassified	42.47%	59.60%

These result show that the variations in the composition of the microbial communities between the soil and leachate, which could be affected by the environmental factors such as pollution levels, organic load, and oxygen availability [48]. Bacteria dominance in the soil samples indicates their essential role in the organic matter degradation and nutrient cycling, which both are required to maintain soil health and prevent contaminants from leaking into groundwater [49]. Meanwhile, archaea are more abundant in the leachate samples, thus highlight their relevance in the production of methane under anaerobic factors, which has substantial consequences for the landfill greenhouse gas emissions [50]. The substantial percentage of the unclassified sequences in the leachate indicates the presence of unique species of bacteria with a potential biotechnological uses, such as heavy metal and organic pollutant bioremediation in the damaged water system. Understanding the activities of the microbes in the landfill ecosystem, especially their roles in the organic matter breakdown, pollutant decomposition, and greenhouses gas emissions, which require an awareness of these variations. Deeper understanding of these metabolic capacities of the microbial diversity and possible application in the waste management and landfill bioremediation can be obtained through the additional functional study [51].

3.3 Top 10 Most Abundance Species Name

Table 3 illustrate the impact of the environmental on the microbial diversity through studying the top 10 most abundant microbial species in Jeram soil and leachate. This analysis shows a significant variations in the microbial composition. The detection of *Bacteroides graminisolvens* and *Cellulomonas* sp. NTE-D12 in the soil samples indicates their importance in the breaking down of complex organic compounds, which is critical for the recycling of nutrients and the soil fertility [52,53]. This process can minimise the leaching of the organic compound into the groundwater, thus protecting the water quality. However, methanogenic archaea that produce methane and thrive in anaerobic environments, such a *Methanoculleus bourgensis*, *Methanosarcina mazei*, and *Methanocorpusculum labreanum*, predominate in the leachate sample [16,54]. The abundance of methanogenic archaea in the leachate highlight their role in the production of methane, as en essential function in the landfill ecosystems with a substantial consequences for the greenhouse gas emissions. The detection of *Acrobacter* taxa in the leachate shows that they are adapted to the harsh environments, making them an ideal options for the bioremediation of polluted water systems [55]. These results show that soil has a metabolically adaptable and ecologically varied microbial population while leachate contains organisms that suited to harsh and nutrient-rich environments.

Table 3
Top 10 most abundant microbial species in Jeram soil and leachate

Jeram soil	Jeram leachate
Bacteroides graminisolvens	*Methanoculleus bourgensis*
Methanosarcina barkeri	*Methanosarcina barkeri*
Cellulomonas sp.NTE-D12	*Methanosarcina mazei*
Thauera sp.GDN1	*Methanocorpusculum labreanum*
Klebsiella pneumoniae	*Arcobacter aquimarinus*
Diaphorobacter sp.JS3051	*Aliarcobacter skirrowii*
Propioniciclava sp.MC1595	*Aliarcobacter cryaerophilus*
Rhodanobacter thiooxydans	*Methanoculleus chikugoensis*
Archangium violaceum	*Arcobacter cloacae*
Pseudoxanthomonas mexicana	*Neopusillimonas aromaticivorans*

Environmental factors like oxygen availability, nutritional content, and pollution levels are the main causes of the observed variations in the microbial diversity between soil and leachate. A variety of bacteria involved in the breakdown of organic matter, recycling of nutrients, and stability of ecosystems that are supported by the stable and aerated habitat that soil environments provide [49]. On the other hand, low oxygen levels, a high organic matter content, and possibly harmful contaminants characterize the landfill leachate. Therefore, organisms that are highly resistant to the environmental stress are selected for the harsh chemical environment of leachate [12]. These findings highlight the significance of microbiological observation in the landfill management, especially in determining a possible water pollution risks that caused by leachate movement into groundwater or nearby aquatic habitats.

3.4 KEGG Annotation

The study of the top 20 KEGG pathway in the leachate and soil samples, as shown in Figure 2 and 3, provide a significant insight about the environmental adaptations and metabolic processes of the microbial populations. Common pathways between the two samples, including “Metabolic pathways”, “Biosynthesis of secondary metabolites”, and “Microbial metabolism in diverse environments”, indicate essential metabolic functions that are important to the microbial survival and function in a variety of environments. However, the differences in the environment between soil and leachate are highlighted by the relative abundance and particular pathways that found in each samples.

The finding of ‘carbon metabolism” and “oxidative phosphorylation” pathways in the soil samples indicate the significance of energy production and nutrient cycling in the terrestrial environments [56,57]. These activities contribute to soil health by reducing the leaching of the contaminants into groundwater, hence maintaining the water quality. The presence of pathways like “Purine metabolism” and “Glycolysis/Gluconeogenesis” highlight the significance of nucleotide synthesis and energy metabolism in the soil microorganisms. These pathways demonstrate that soil microorganisms rely on the producing essential compound for survival and breakdown organic matter [56,58]. However, The dominance of “ABC transporters” and “Aminoacyl-tRNA biosynthesis” in the leachate samples indicate that the bacteria in these environments concentrate in the nutrients uptake and protein synthesis, mostly likely due to the high organic matter and resource availability [59,60]. This aligns with the leachate environments that is frequently abundant in nutrients and dissolved organic matter, requiring a quick protein synthesis and effective transport routes for microbial developments.

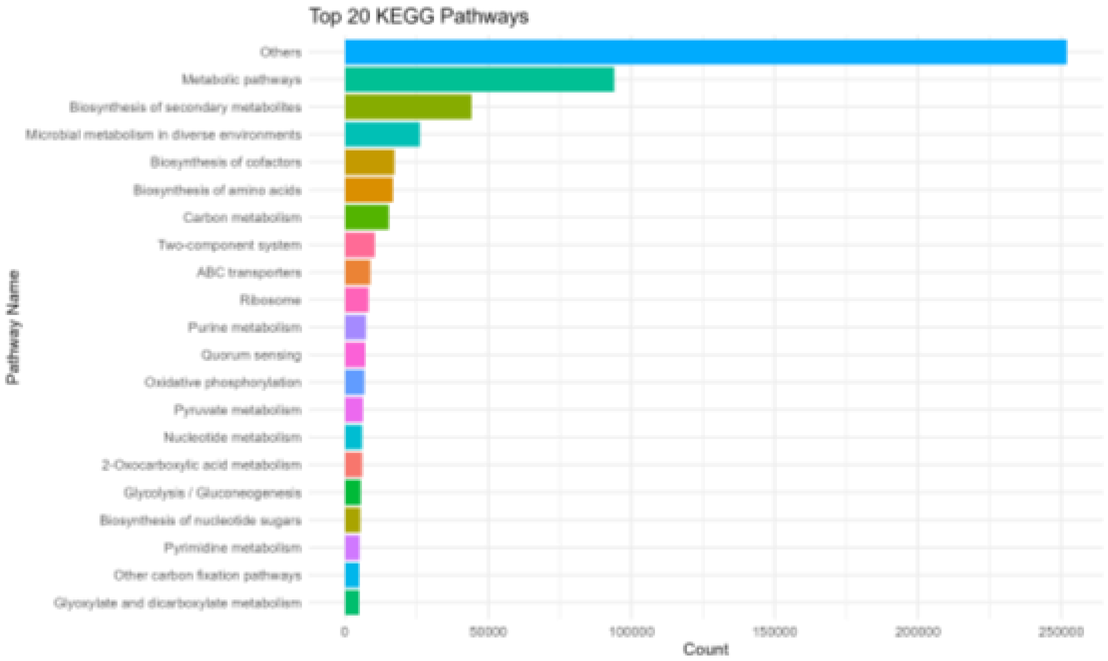


Fig. 2. Top 20 KEGG pathways identified in the soil samples

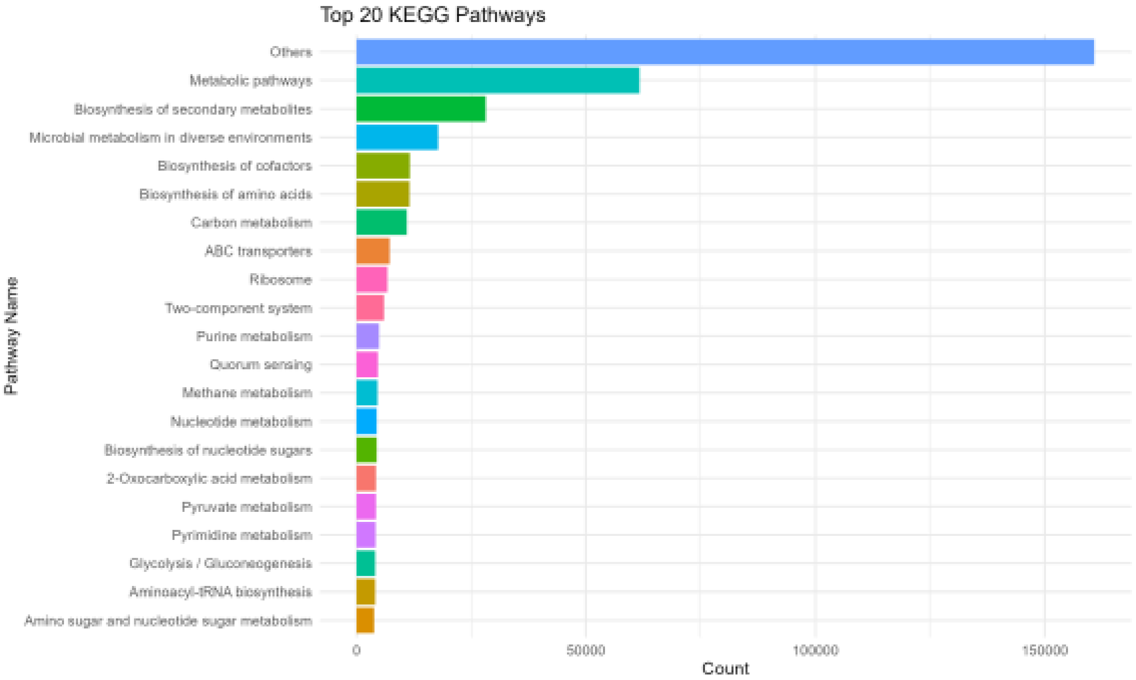


Fig. 3. Top 20 KEGG pathways identified in the leachate samples

In general, the variation between the leachate and soil samples highlight how the microbial populations can adapt to their particular environments. Whereas leachate bacteria concentrate on the nutrient intake and quick development, soil microorganisms that prioritize energy metabolism and nutrient cycling. The complex interaction between microbial diversity and environmental factors is in the environment of water and soil ecosystems is highlighted by these variations, which is also show how the variety of metabolic methods used by the microbial population in response to changing environmental conditions [61]. These findings provide a useful knowledge into the metabolic strategies used by the microbial communities in various landfill sites, which can be used to improve waste degradation and reduce the environmental effects on the water systems.

3.5 Diversity Index Analysis in Microbial Community

The Figure 4 visualizes the Berger-Parker, Fisher, Inverse Simpson, Simpson and Shannon, which they measure species diversity within each sample group which is soil and leachate. The Berger-Parker index represents the proportion of the most abundant species which is the higher values indicate lower evenness. Leachate samples (red) show a higher Berger-Parker index value thus indicates that a few dominant species are more prevalent in these environments. Soil samples (blue) has lower index values, showing that the microbial population in soil is more evenly distributed, with no single species being overwhelmingly dominant [62]. In the Fisher index, soil samples (blue) consistently show higher fisher alpha-diversity compared to leachate (red). The variability in diversity is also more prominent in soil samples. This suggest that soil samples generally support a more diverse microbial community compared to leachate [63].

Furthermore, Inverse Simpson Diversity Index is a metric that emphasized both species richness and evenness in the microbial communities across different sample. Leachate sample (red) has very low Inverse Simpson index values indicates that they are dominated by only a few microbial species. In soil samples (blue) have a significantly higher values, suggesting that the soil communities are more diverse with species being more evenly distributed [64]. Moreover, Simpson diversity index, which measure species dominance in the microbial population across different samples. Soil samples (blue) have very high Simpson index values (~1) indicating that they are more dominated by a few highly abundant species. Leachate sample (red) have lower Simpson index values, suggesting greater species diversity in leachate environments [62]. Shannon diversity index, which measures species diversity within each sample group. Soil samples (blue) generally have higher Shannon diversity than leachate samples (red). This suggest microbial diversity greater in soil, while leachate sample have lower diversity. Soil sample (blue) have consistently higher microbial diversity than leachate sample (red) [62].

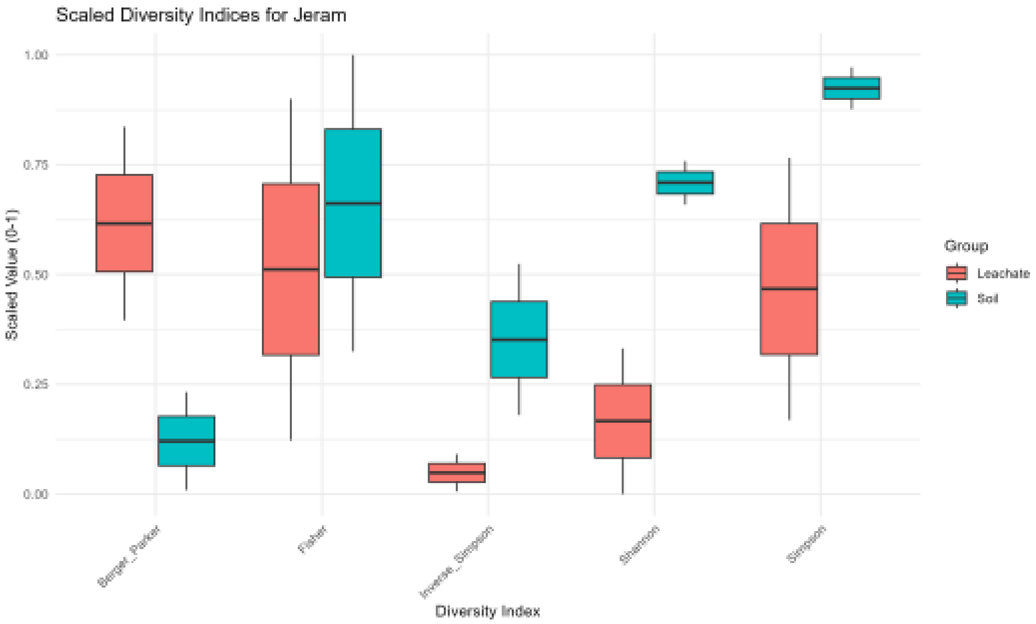


Fig. 4. Boxplot of alpha-diversity indices

Alpha diversity study using indices like Shannon, Simpson, and Inverse Simpson, revealed a significant variations in the microbial diversity between soil and leachate. The increased of microbial diversity in soil samples indicates a more robust and resilient ecosystem capable of supporting a wide range of metabolic processes required for organic matter decomposition and nutrient cycling. As state by Wydro *et al*., [38], microbial diversity in soil is important for nutrient cycling, ecosystem stability, and environmental sustainability, an efficient waste and water management techniques are necessary to reduce the risk of contamination. In contrast, the lower diversity and higher species richness in the leachate samples may be due to the harsh environmental circumstances that favour specialized organisms that can adapted to high organic matter and low oxygen availability [65]. These findings highlight the need of to maintain microbial variety within the landfill ecosystems to improve waste degrading efficiency and minimise environmental consequences on the water systems.

4. Future Research

Although this study reveals a valuable knowledge into the diversity of microbes and metabolic pathway in the landfill ecosystem, some areas need a further study to enhance our awareness of their contribution to water quality and the sustainability of the environment. Future research should concentrate on long-term monitoring of microbial populations in the landfills to better understand how they change as time passes as they adapt to the environmental change such as waste composition, moisture, and temperature [66]. This will provide an insight into the resilience and adaption of microbial populations, which are important to sustainable landfill management. Climate change significantly impacts the diversity of microbes and the function in the waste ecosystems. The rising in temperatures and changing patterns of precipitation could affect the microbial activity, influencing waste breakdown effectiveness and greenhouse gas emissions [67]. Understanding these processes will help in the developing adaptive measure for reducing the landfill environmental impact. Future research should look into how microbe data can be utilised and applied to the landfill management processes to improve waste breakdown and reduce the impact on the environment [68]. For example, increasing the metabolic activity of certain microbial populations could improve the methane absorption capacity and lower the greenhouse gas emissions. Furthermore, microbial-based techniques can be developed to reduce the leachate pollution in the nearby water supplies. The use of modern omics technologies, including metatranscriptomics and metabolomics, might reveal more information about the functional roles of the microorganisms in the waste ecosystems [69]. These methods can show the active metabolic pathways and the gene expression pattern of microbe, thus providing a more complete picture of their relationship to waste degradation and environmental sustainability.

5. Conclusion

This study highlight the unique microbial communities that found in soil and leachate samples, providing substantial understanding into the microbial diversity and functional capacities within the Jeram Landfill. The results show that, according to the specific environmental requirements of each habitat, bacteria are prevalent in the soil, whereas archaea and unclassified sequences common in the leachate. *Bacteroides graminisolvens*, *Methanosarcina barkeri*, and *Cellulomonas* sp. NTE-D12 are the most abundant species in Jeram soil, while *Methanoculleus bourgensis*, *Methanosarcina mazei*, and *Methanocorpusculum labreanum* are the most prevalent in the leachate sample. The metabolic pathways that have been found, like the metabolism of carbon and the production of methane has highlight the important roles that microbial population plays in the breakdown of organic matter, the cycling of nutrients, and the emission of greenhouse gases. This study provides light on the diversity of microbes and the functional potential of the landfill soil and leachate, which has impact on the environmental management and the water quality monitoring. The discovery of important microbial taxa and metabolic processes involved in the organic matter breakdown, production of methane, and nitrogen cycle indicates the importance of improved of landfill waste treatment procedures. Furthermore, the discovery of the novel and unclassified microbes in the leachate emphasized the importance of continued investigation into the landfill-associated microbes, especially in terms of pollution management and sustainable waste management techniques. Furthermore, documenting the microbial communities and abundance can help use to acknowledge these microbes as a part of the natural heritage. Understanding how they contribute to ecology offers an information about the cycling of nutrients, decomposition, and overall sustainability of the ecosystem. This understanding can also help the conservation efforts by emphasizing the significance of the microbial diversity in ensuring environmental health and adaptability. In addition to showing the adaptability of microorganisms life in the harsh environments, their ecological roles that provides significant biotechnological potential for environmental remediation, such as the bioremediation of the heavy metal pollution in the landfills. Sustainable environmental management and ecosystem conservation may profit in the future from the preservation and research of these microbial populations.

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