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# Metagenomics profiling for assessing microbial diversity in both active and closed landfills

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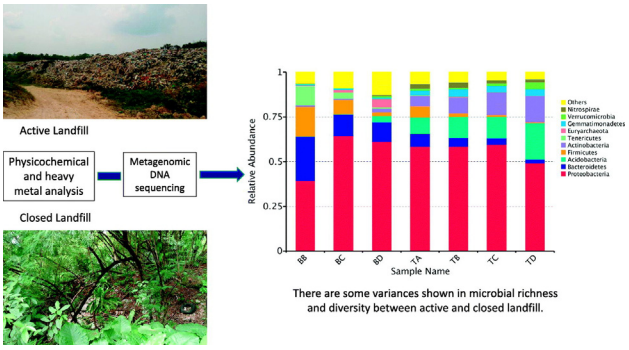
## HIGHLIGHTS

- The biosphere and lithosphere of landfill soils are not fully explored and their microorganism complexities remain unknown.
- The high-throughput sequencing analysis showed the difference in bacteria composition between active and closed landfill.
- Physicochemical conditions and heavy metal content of the soil samples indirectly effects the microorganism composition.

## GRAPHICAL ABSTRACT

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## ABSTRACT

The municipal landfill is an example of human-made environment that harbours some complex diversity of microorganism communities. To evaluate this complexity, the structures of bacterial communities in active (operational) and closed (non-operational) landfills in Malaysia were analysed with culture independent metagenomics approaches. Several points of soil samples were collected from 0 to 20 cm depth and were subjected to physicochemical test, such as temperature, pH, and moisture content. In addition, the heavy metal contamination was determined by using ICPMS. The bacterial enumeration was examined on nutrient agar (NA) plates aerobically at 30 °C. The soil DNA was extracted, purified and amplified prior to sequence the 16S rRNA gene for statistical and bioinformatics analyses. As a result, the average of bacteria for the closed landfill was higher compared to that for the active landfill at $9.16 \times 10^7$ and $1.50 \times 10^7$, respectively. The higher bacterial OTUs sequenced was also recorded in closed landfills compared to active landfill i.e. 6625 and 4552 OTUs respectively. The data from both landfills showed that the predominant phyla belonged to Proteobacteria (55.7%). On average, Bacteroidetes was the second highest phylum followed by Firmicutes for the active landfill. While the phyla for communities in closed landfill were dominated by phyla from Acidobacteria and Actinobacteria. There was also Euryarchaeota (Archaea) which became a minor phylum that was detected in active landfill, but almost completely absent in closed landfill. As such, the composition of bacterial communities suggests some variances between the bacterial communities found in active and closed landfills. Thus, this study offers new clues pertaining to bacterial diversity pattern between the varied types of landfills studied.



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## 1. Introduction

Municipal solid waste (MSW) landfills have turned into a habitual spot to dump solid wastes in many countries, including Malaysia. As such, the case of leachate has emerged as a primary concern that has yet to be addressed due to this landfills waste disposal practice (Banar et al., 2006; Oller et al., 2011) for the increasing rate of deleterious soil and groundwater pollutions, as a consequence of discharged leachate, is rather alarming (Han et al., 2016; Heberer, 2002). Furthermore, leachate that consists of unusual amount of contaminants like heavy metals, organic matter, as well as chlorinated organic and inorganic salts (Dao et al., 2016; Slack et al., 2005) that are hazardous to the surrounding environment, are also affecting the public health. In addition, this contamination is slow in its degradation process and its harmful residue can last for more than three decades (Perez-Leblic et al., 2012).

The local microbial communities, especially in leachate and leachate soil, transform most pollutants and organic elements into less toxic compounds (Staley et al., 2015). Some microorganisms that have displayed the potential to degrade pollutants are fungi, protozoa, and bacteria (Fang et al., 2014). Hence, this study of microbial communities in contaminated landfills reflects the level of contamination, whereby this precise knowledge can be applied as a measurement to predict and to monitor their rates of natural degradation (Jain et al., 2005; Tavares et al., 2016). Although several studies have tapped into the basic microbial reaction at a lab scale, along with a pilot study concerning landfill bioreactor (Sang et al., 2008); the aspects of structural and functional in microorganism communities in the actual landfill have yet to be discovered.

In general, researches concerning bacteria in landfills have looked into the application of conventional methods, such as culture dependent and culture independent techniques. The latter method of genetic molecular tools, for instance, denaturing gradient gel electrophoresis (DGGE) (Nayak et al., 2009), fluorescence in-situ hybridization (Burrell et al., 2004), and PCR cloning (Huang et al., 2005), have been employed to characterize microbial communities without undergoing the cultivation process. Hence, the fundamental findings of these studies are in line with the notion that landfills do contain high complex microbial communities, such as Proteobacteria, Firmicutes and Bacteroidetes as the dominating phyla and archaeal population observed includes the methanogenic species (Kochling et al., 2015). Nevertheless, these valuations have remained incomplete and fail to reflect the complete picture of the community structure, primarily due to limitation in methodologies.

This study aimed to investigate bacteria communities through the use of novel and high-throughput sequencing approaches that offer more readable sequences for analyses, thus enabling a more complete picture pertaining to landfill microbial communities. Two non-sanitary landfills represent operational and non-operational landfill soil samples were used for HiSeq-based 16S rRNA gene sequence analysis in order to carry out an in-depth genetic survey, as well as to gain better taxonomic resolution. The physicochemical test and ICPMS were also conducted to quantify the relative level of heavy metal in the soil samples. This, in turn, enhances one's comprehension concerning microbial communities that involve bioremediation in landfills and also for further application to practice better MSW disposal.

## 2. Materials and methods

### 2.1. Site and soil sampling

Two types of non-sanitary landfills had been selected for this study; the active landfill situated at Bukit Beruntung (BBL) and the closed landfill located at Taman Beringin (TBL), all found at Selangor, Malaysia (Fig. 1). These landfills have and had served as domestic and industrial waste dumping sites; known as MSW landfill.

Table 1 presents the general condition of both landfills. Soil samples with a depth of 0–20 cm were collected from several selected points at each landfill from areas contaminated with leachate by using a one-piece auger in adherence to 2014 ASTME – 1197 standard guideline in performing terrestrial soil-core microcosm test (Sprocati et al., 2012). As for the Taman Beringin landfill site, four various sampling points were opted, while three for Bukit Beruntung landfill, as described in Table 2. Besides, for each point, several small sub-points were gathered and mixed well to obtain the final homogenised soil. After that, some portion of the composite soil samples were kept in a sampling bag with ice pack for transportation purpose before stored at 4 °C and −20 °C for further analysis.

### 2.2. Soil analysis and physicochemical determination

The sample soil suspensions were prepared by mixing 1 g of sleeved soil sample with 2.5 ml sterile distilled water (1:2.5 ratio) before measuring its pH by dipping it in PB-11 pH probe (Sartorius, USA). The moisture content was determined based on the dry mass of the sample soil that had been oven dried at 105 °C for overnight.

In addition, the composition of heavy metals concentration had been analysed via USEPA 3050B method by using the Agilent 7500 Series Inductively Coupled Plasma-Mass Spectrometry (ICP-MS ChemStation G1834B) (Agilent Technologies, Japan).

### 2.3. Enumeration and isolation of bacteria populations

As for isolation of bacteria, 1 g of soil sample was transferred into a tube that contained 9 ml of saline water (0.3 salt water w/v) and was homogenised via vortex followed by serial dilution to produce the next dilution factor solutions. Next, 100 μl of sample diluted soil was pipetted on agar media and spread using a hockey stick. Three types of media were used in this research, i.e. nutrient agar (NA), MacConkey agar (MCA), and mannitol salt agar (MSA), which were prepared based on the instructions provided by the manufacturer. After that, all inoculated plates were incubated at 30 °C for two days. The growth of bacteria colony was observed daily and the colony forming unit (CFU) was determined. The colony with different morphology was collected and purified on the fresh NA plate prior to maintain in the slant agar and stored at 4 °C for future use.

### 2.4. DNA extraction and purification

The DNA of landfill soil samples was extracted directly by using the Powersoil® DNA Isolation Kit by adhering to the instructions given by the manufacturer (MO BIO, USA). The purity of the harvested DNA was measured by using Nanodrops 2000 UV–Vis spectrophotometer (Thermo Scientific, USA) and followed by 1% (v/v) agarose gel electrophoresis.

### 2.5. The 16S rRNA amplicon Illumina sequencing soil bacteria

The recovered DNA samples were further analysed for sequencing at Novogene Bioinformatic Technology Co., Ltd (Beijing, China). The 16S protocol was designed to amplify prokaryotes (bacteria and archaea) using paired-end 16S community sequencing on the Illumina platform. The Polymerase Chain Reaction (PCR) amplification was conducted using primers with barcode 515F (GTGCCAGCMGCCGCGGTAA) and 806R (GGACTACHVGGGTWTCTAAT) which target the V4 region of the 16S rRNA. The 30 μl PCR reaction mixture contained 15 μl of Phusion® High Fidelity PCR Master Mix (New England Biolabs): 0.2 μM of forward and reverse primers, and about 10 ng templates DNA. The PCR was performed using standard procedure: initial denaturation at 98 °C for 1 min followed by 30 cycles of denaturation (98 °C for 10 s), annealing (50 °C for 30 s), and elongation (72 °C for 60 s) with a final extension at 72 °C for 5 min. The PCR products were mixed with 1× loading buffer