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Defunct gold mine tailings are natural reservoir for unique bacterial communities revealed by high-throughput sequencing analysis

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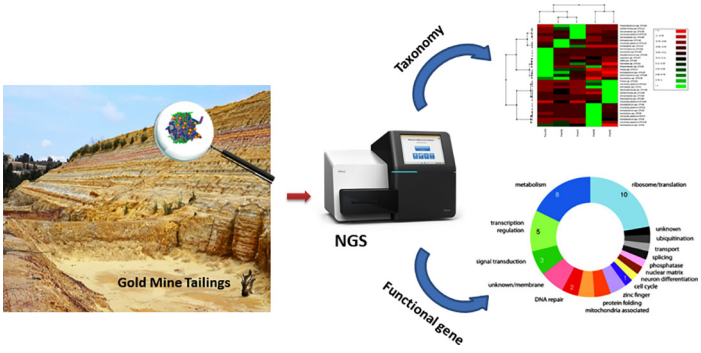
HIGHLIGHTS

- This study provides fundamental knowledge of bacterial diversity in gold mine tailings.
- Phylum *Actinobacteria* was the most dominant, followed by others.
- Highlights the vulnerability and resilience of native microorganisms to environmental disturbances in their ecological niche
- The study highlights the functional gene reservoir and metabolic processes of microorganisms native to gold mine tailings.

GRAPHICAL ABSTRACT



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ABSTRACT

Mine tailing dumps are arguably one of the leading sources of environmental degradation with often both public health and ecologically consequences. The present study investigated the concentration of heavy metals in gold mine tailings, and used high throughput sequencing techniques to determine the microbial community diversity of these tailings using 16S rRNA gene based amplicon sequence analysis. The concentration of detected metals and metalloids followed the order Si > Al > Fe > K > Ca > Mg. The 16S rRNA gene based sequence analysis resulted in a total of 273,398 reads across the five samples, represented among 7 major phyla, 41 classes, 77 orders, 142 families and 247 major genera. Phylum *Actinobacteria* was the most dominant, followed by *Proteobacteria*, *Firmicutes*, *Chloroflexi*, *Cyanobacteria*, *Bacteroidetes*, *Acidobacteria* and *Planctomycetes*. Redundancy analysis (RDA) and pairwise correlation analysis positively correlated the distribution of *Alphaproteobacteria* and *Gammaproteobacteria* to Al and K; *Actinobacteria* to Cr and *Chloroflexi* to Si. Negative correlations were observed in the distribution of *Bacteroidetes* with respect to As concentrations, *Actinobacteria* to Al, and *Alphaproteobacteria and Gammaproteobacteria* to high As and Te content of the soils. Predictive functional analysis showed the presence of putative biosynthetic and degradative pathways across the five sample sites. The study concludes that mine tailing sites harbour diverse and unique microbial assemblages with potentially biotechnologically important genes for biosynthesis and biodegradation.

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1. Introduction

Metal mining has contributed immensely to industrial and infrastructural development among all countries in each continent (Keshri et al., 2015), howbeit with consequences such as the accumulation of waste mine tailing dumps from abandoned mines across the globe (Liu et al., 2014). According to Huang et al. (2012), tailings are residual wastes from processing ores that are rich in metals and metalloids (e.g. Al, As, Au, Cr, Cu, Ni, Pb, Zn, U), salts and unwanted gangue minerals (e.g. silicates, carbonates, oxides/hydroxides, sulphides, etc.). At inception, these residues of fine particle sizes are pumped in slurry form into purposely built facilities and deposited as sediments across hundreds to thousands of hectares of landscape at each mine, eventually forming the "tailing mounds" that have become a characteristic feature of either active, disused or abandoned mines. In some countries like South Africa, gold mining dates back to more than a century ago, creating a mine tailing footprint covering >13 km^2 of land (Rosner and van Schalkwyk, 2000). These dumps are arguably one of the leading sources of environmental degradation (de Andrade Lima et al., 2008), of which the most commonly reported cause is the generation of acid mine drainage (AMD) by the large percentage of sulphide minerals like pyrite (Rosner and van Schalkwyk, 2000), and also the introduction into the environment of toxic heavy metal species (Wang et al., 2011). Additionally, mine tailings are also a source of radionuclides which, together with heavy metal species, pose potentially detrimental effects on human and environmental health (Qing et al., 2007). Ecologically, heavy metals are known to affect soil microbial diversity and species distribution, favouring the growth of selective dominant species (Sheoran et al., 2010), limiting microbial reproduction (Wei et al., 2009) and reducing the various ecological role of microbes that are relevant to the ecosystem, like carbon and nitrogen metabolism (Qing et al., 2007).

Regardless of the type of ore being mined, mine tailings are characterised by sub-optimal concentrations of organic or micronutrients for plant growth, and generally support only minimal microbial populations (Pepper et al., 2012). Left alone, mine tailing become unsightly mounds of bare soil which constitute a health risk through the spread of vast amounts of contaminated soil via aeolian and water erosion (Callender, 2014). Facilitated reclamation of tailings is therefore required to allow for faster subsequent revegetation and ecosystem stability. Among methods that can be used for reclamation, mechanical methods tend to be prohibitively costly (Wang et al., 2011), as opposed to bioremediation strategies using microorganisms indigenous to heavy metal contaminated soils which have been proven to be efficient and less costly (Wei et al., 2009). For such microbial assemblages to be effectively used in bioremediation, there is need to both ascertain the extent of heavy metal contamination of the soils and also to determine the diversity of microbial communities inhabiting those soils (Teng et al., 2017). With the advancements in high-throughput metagenomic sequence studies, it is now feasible to characterise the ecological role played by uncultured microbial taxa, and to elucidate their ecological significance in acidified or extreme environments (Sajjad et al., 2018). The present investigation was aimed at quantitatively determining the concentration of heavy metals of gold mine tailings, and to use high throughput sequencing techniques to provide an insight into the microbial community diversity of these tailings using 16S rRNA gene based metagenomic analysis.

2. Methods and materials

2.1. Description of study site

Soil samples were collected from a defunct gold mine tailing mound, Durban Deep Gold Mine, in June 2017 across five transects (Point A 26°10′52.4″S 27°52′39.5″E, Point B 26°10′55.0″S 27°52′42.2″E, Point C 26°10′59.2″S 27°52′44.5″E, Point D 26°11′05.3″S 27°52′47.1″E, and Point E 26°11′01.6″S 27°52′34.7″E) (Fig. 1). During its operational days, the mine was also nicknamed the Grand Old Lady in mining circles, although operations at this mine formerly ceased in 2001. The site is located close to the Central Business District hub of Roodepoort, which is surrounded by industrial, residential and recreational facilities. Aeolian erosion of the exposed tailings is a notable problem especially during windy seasons, putting the lives of people in surrounding communities in increased risks of airborne heavy metal toxicities.

The soil samples were collected at a depth of 5 to 10 cm range after removing the surface soil (0 to 5 cm). The collected soil samples were then placed in the cooler box at 4 °C and then transported to the laboratory at the University of South Africa (UNISA Florida Campus, Johannesburg, South Africa) immediately for microbial and metal content analysis.

2.2. Soil metal concentration analysis

For each sample, about 1 g soil sample duplicates (of 2 No) were separately weighed into beakers followed by digestion with 15 ml of HCl and 5 ml of HNO_3 (aquaregia) in a 3:1 ratio at 110 °C until all soil particles were completely dissolved. Another 15 ml of aquaregia was added to the mixture after which the samples were evaporated by cooling to room temperature. This was followed by 1 M HNO_3 being added to the mixture and the extract was filtered through acid washed Whatman no. 42 filter paper. The filtrate was collected to quantify the metal concentrations using inductively coupled plasma-optical emission spectrometer (ICP-OES, Perkin Elmer). The samples were subjected to further analysis for various trace metals with dilutions where necessary. A calibration blank and an independent calibration verification standard were analyzed together with all samples to confirm the calibration status of the ICP-OES. The method set-up protocols included a minimum of four calibration standards covering a range of concentrations and analytes, internal quality control (QC) samples, blanks, replicates and reference materials. QC samples were read after calibration standards, periodically during sample analysis and at the end of the analysis. To ensure that the process was running correctly, the results of the QC samples were checked if they are within a pre-determined control limit before any samples were tested.

2.3. Extraction of genomic DNA and 16S rRNA based amplicon sequencing

About 10 g of each soil sample was first sieved (2 mm mesh size) to remove large debris and then added into 50 ml of phosphate buffered saline (PBS) buffer (pH 7.4). The mixture was agitated in a shaking incubator (50 rpm, 25 °C) for 1 h to dislodge bacterial cells from their adhesion to soil particles after which the supernatant was obtained by filtering the mixture through a Whatman filter paper. About 400 μl of the filtrate was used to extract genomic DNA using a Faecal/Soil Total DNA extraction kit (ZYMO RESEARCH, Irvin, USA) according to the manufacturer's protocol. The obtained genomic DNA was then amplified by polymerase chain reaction (PCR) using the universal bacterial primers, 27F (5′-AGAGTTTGA TCMTGGC-3′) and 518R (5′-GTATTACCGCGGCTGCTGG-3′) targeting the conserved bacterial 16S rRNA gene as described by Ramganesh et al. (2018). PCR amplicons were purified using a DNA Clean & Concentrator Kit (ZYMO RESEARCH, Irvin, USA). The purified PCR products were then sequenced by paired end sequencing chemistry along with its multiplex sample identifiers on the Illumina MiSeq Platform by Inqaba Biotechnology (Pretoria, South Africa). The sequence datasets were submitted to the NCBI Sequence Read Archive (SRA) library under the accession number: SRP151301.

2.4. Sequence data analysis

The obtained raw sequence (Fastq) datasets were scrutinized for PCR artefacts and low-quality reads (reads with >50% bases having a