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RESEARCH ARTICLE

BACTERIAL POPULATION STUDY IN OIL-CONTAMINATED AND UNCONTAMINATED SOILS

Noor Faizul Hadry Nordin[1*], Tg Shilatul Hirwany Tg Mahassan[2], Ahmed Jalal Khan Chowdhury[2]

[1]International Institute for Halal Research and Training (INHART), International Islamic University Malaysia (IIUM), Jalan Gombak, 53100 Kuala Lumpur, Malaysia
[2]Kulliyyah of Science, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, Bandar Indera Mahkota, Kuantan, Pahang, Malaysia
****Corresponding Author Email: faizul@iium.edu.my***

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ABSTRACT

Soil provides a vital habitat primarily for bacteria to continue the process of biogeochemical cycle. The remarkable metabolic diversity and capacity of the soil bacteria is increasingly being harnessed for the benefit of humankind. One of the beneficial spin-offs from the understanding of the metabolism of soil microbes is the development of bioremediation for contaminated soils. Investigation on bacterial diversity was conducted on several types of samples for both contaminated and uncontaminated soils. The soil texture test determined that the soil samples obtained were sandy, silt and clay, respectively. The microbial population were enumerated using colony counting while bacteria characterisation and identification were performed using Gram staining, morphological and biochemical analysis. This study indicated that microbial population is higher in hydrocarbon-contaminated soils compared to uncontaminated soils. On the other hands, the bacterial diversity is lower in contaminated soils compared to uncontaminated soils. The study also portrays that Gram-positive bacteria, *Staphylococcus* spp was dominant in untreated soils while Gram negative bacteria, *Pseudomonas* spp was dominant genus in hydrocarbon contaminated soil.

KEYWORDS

Hydrocarbonoclastic bacteria, Bacterial population, Biodegradation

1. INTRODUCTION

Soil bacteria are the major decomposer in most terrestrial ecosystems and are the basis for a complex food chain in soils. These microbes have several functions and purposes such us improving soil structure and releasing nutrient from organic matter during decomposition [1]. Furthermore, soil microbes able to break down toxins and pollutants and avoid those chemicals from entering into the groundwater [2]. Without microorganisms, the Earth surface would be piled high with dead organic matter.

The remarkable metabolic diversity and capacity of the soil microflora is increasingly being harnessed and put to good use by human. A most beneficial development from understanding of the metabolism of soil microbes has been the expansion of methods for the bioremediation of soils contaminated with hazardous wastes or spilled petroleum products [3].

Various methods were used to characterise hydrocarbon-degrading bacterial population in soil. Soil biological investigations such as measurement of respiration, enzyme activities and microbial counts can give information about the presence of viable microorganisms thus indicates the impact of environmental stresses such as hydrocarbon contamination.

There is limited information about the prevalence and geographical distribution of various hydrocarbon-degrading bacterial population in soils. The aim of this study was to investigate the bacterial population changes in soil towards hydrocarbon contamination.

2. EXPERIMENTAL METHOD

2.1 Soil samples

Different types of soil samples were collected from various places in Langkawi Island (Mount Mat Chincang [MC], Tuba Island [PT] and Kilim river [SK]) and Kuantan, Pahang (Teluk Chempedak [TC], Taman Gelora [TG] and Jabor Landfill [JL]). Soil were collected using sterilised shovel and transferred to sterilised plastic bag and kept in 4C for further analysis.

2.2 Physical properties of soil

Soil characteristics were determined by physical properties analyses. Several properties such as soil colour were soil texture were determined based on FAO training manual [4]. The colour of individual horizons was defined when the soil was moistening while the soil texture was analysed using three simple tests; the bottle test, Mud-Ball test and Shaking test.

2.3 Preparation of hydrocarbon-contaminated soil

An amount of 100g of each soil was mixed with filter-sterilised used motor oil in a beaker. The oil-contaminated soil was stirred until the mixture homogenised. The oil-contaminated soils were left for one week for the hydrocarbon degrading bacteria to flourish.

2.4 Isolation and enumeration of bacteria

An amount of 10g of soil sample both contaminated and uncontaminated was mixed with 20mL of sterilised distilled water in 50mL Falcon tube and vortexed to make it homogenised. The mixtures were incubated for 30 minutes at 37C in incubator with 200rpm shaker. Following the incubation, the mixtures were centrifuged at 5000rpm for 20 minutes. 10 ml of the supernatant was pipetted into a fresh 15 mL Falcon tube and serial dilution was performed. Subsequently, 100ul of diluted solution was spread on Tryptic Soy Agar (TSA) plate and incubated at 37C for 18-24hours. The bacterial enumeration was conducted by counting the colony number on TSA plate after the incubation and the population density was described as CFU/100mL.

2.5 Bacterial Identification

Bacterial identification was initiated using Gram staining to differentiate between Gram negative and Gram-positive bacteria. The isolated bacteria were further tested based on biochemical analysis with API identification system. Two type of API strips were used namely API 20E (bioMerieux, Inc.) for Enterobacteriaceae and other non-fastidious Gram negative bacteria and API Staph (bioMerieux, Inc.) for Staphylococci and Micrococci of Gram-positive. The procedures for API 20E and API Staph were according to manufacturer protocol.

3. RESULTS AND DISCUSSION

3.1 Physical properties of soil

Physical properties such as soil colour, proportions and texture of the soil were classified according to the simple method adapted from FAO training module. Depending on their texture, structure and consistency, various kinds of soils hold more or less water and air, which indirectly determine microbial population in that particular soil. The bottle-test gives a general idea of the proportions of sand, silt and clay in the soil samples. The percentage of approximate proportions of soils resulted from the bottle test was referred to USDA textural classes of soils (Figure 1). Soil obtained from Kilim River and Tuba Island of Langkawi were categorised as clay with major proportion of clay is 87% with fine texture. Even though soil samples from Teluk Chempedak and Taman Gelora beach were categorised as sandy, the Taman Gelora beach indicate moderately coarse texture with light brown colour compared to Teluk Chempedak which is coarser in texture with yellow colour soil. Jabor Landfill and Mount Mat Chingcang soils showed colour ranged from reddish to dark brown with medium to moderately fine texture of silt. The overall physical properties of soil samples were simplified as Table 1.

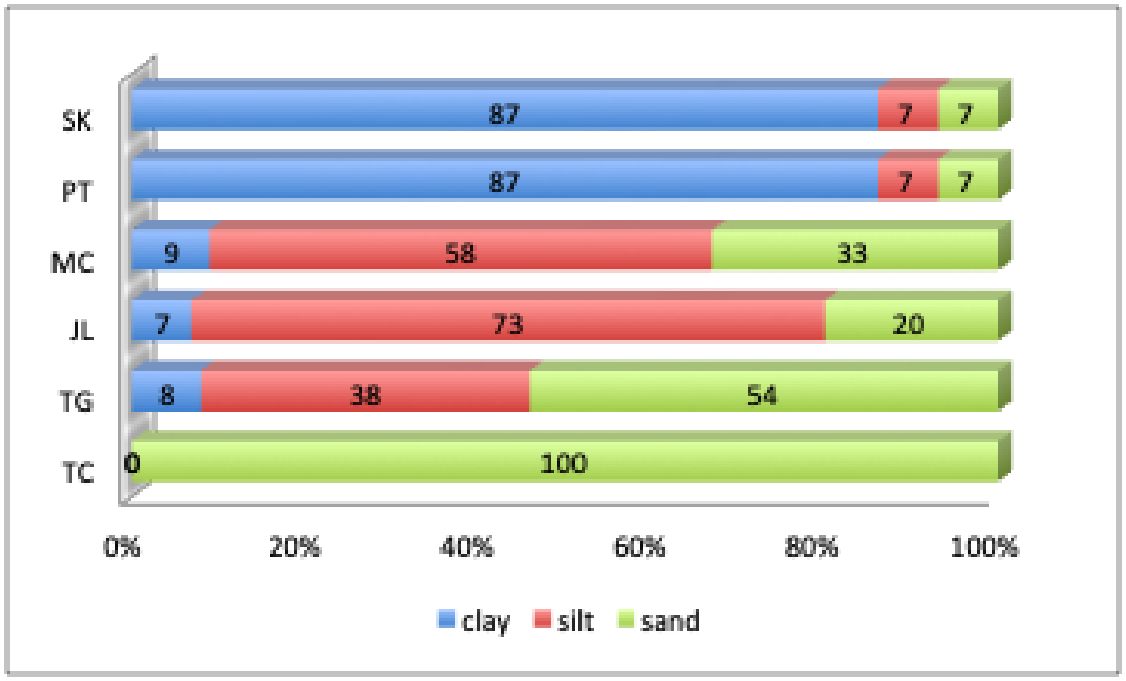

Figure 1: The percentage of sand, silt and clay proportions in the soil samples (SK= Kilim River; PT= Tuba Island; MC= Mount Mat Chincang; JL=Jabor Landfill; TG= Taman Gelora beach; TC= Teluk Chempedak beach)

Table 1: Physical properties of soil samples

Sample	Soil colour	Soil texture		
		Bottle test	Mud-ball test	Shaking test
TC	Yellow	Sand	Coarse texture	Sand
TG	Light Brown	Sandy Loam	Moderately coarse texture	Sand
JL	Reddish Brown	Silty Loam	Medium texture	Silt
MC	Dark Brown	Silty Loam	Moderately fine texture	Silt
PT	Dark Grey	Clay	Fine texture	Clay
SK	Dark Grey	Clay	Fine texture	Clay

3.2 Bacterial enumeration

Bacterial enumeration using TSA plate counting was expressed as CFU/mL to indicate bacterial population for every millilitre of supernatant mixture of soil and water. The result showed that oil-contaminated soil generally has higher bacterial population compared to non-contaminated soil. Soil with oil contamination provides indigenous bacteria that able to utilise the special carbon with selective advantages to growth and proliferate faster over non-hydrocarbon utiliser (Figure 2). The finding is in agreement with previous research conducted by Oluwafemi and coworkers [5]. However, the changes of bacterial population will be governed by the type and texture of the soil samples. Sand with less organic material may have lower bacterial population at initial condition (8.30 x 10^4 CFU/mL). However, due to the contamination, higher availability of carbon in the sand matrix with higher aeration due to its porosity, the bacterial population increased significantly in oil-contaminated (1.94 x 10^5 CFU/mL). Similar situation was indicated in silt where bacterial population is higher in oil-contaminated soil (9.1 x 10^4 CFU/mL) compared to uncontaminated soil (4.6 x 10^4 CFU/mL), even though it was not as high as in sand. However, the opposite situation was indicated in clay where the bacterial population was decrease toward exposure to oil. Non-contaminated clay which rich in organic material support significant degree of bacterial population (1.61 x 10^5 CFU/mL), however, the exposure to hydrocarbon contaminant may allow only selected bacteria to flourish. Furthermore, due to lower permeability of clay, the penetration of oil through them is not remarkable. Also, clay form double diffused layer around it which does not allow penetration of oil through it as oil tends to float around its surfaced reduced the bioavailability of motor oil for microbial utilisation [6]. Additionally, the fine texture of clay may clog with oily characteristics of motor oil which lead to less oxygen within soil matrix thus bacterial population decreased (8.4 x 10^4 CFU/mL). (Figure 3)

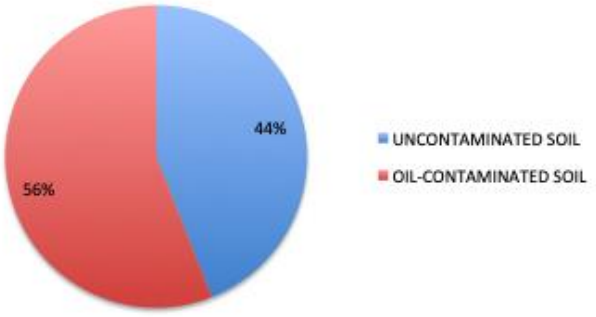

Figure 2: Percentage of total bacterial population in oil-contaminated and uncontaminated soils.

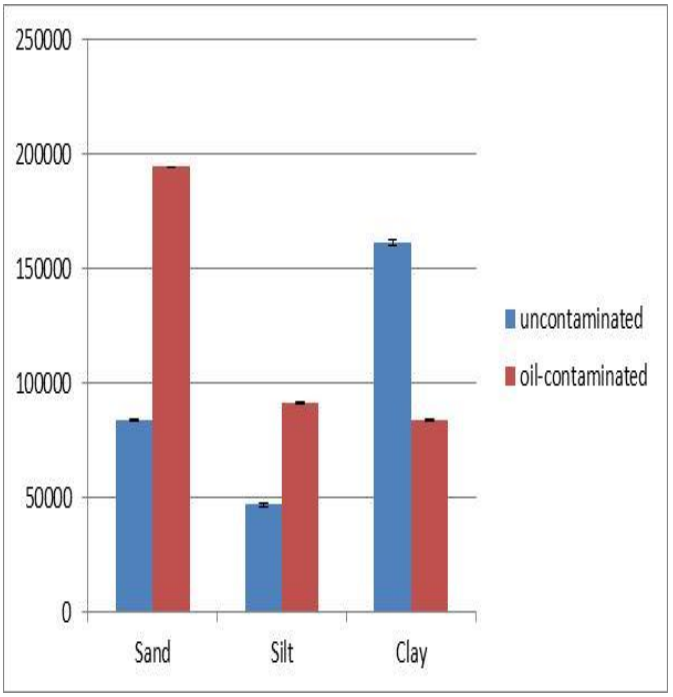

Figure 3: Average bacterial population (CFU/mL) in oil-contaminated and uncontaminated sand, silt and clay soils

3.3 Bacterial Identification

API 20 E and API Staph identification kit (bioMerieux Inc) were used in identification of Gram-negative and Gram –positive bacteria, respectively.

The results were referred to API database, which provide a list of possible species, comment indicating the reliability of the identification and a full biochemical profile of the microorganisms. The species obtained from this analysis were listed in Table 2. The diversity in all uncontaminated soils was higher than oil-contaminated soils. The adaptation of bacterial population towards specific carbon flourished only bacteria that able to utilise hydrocarbon as carbon and energy sources. Genus *Pseudomonas* is dominant in both contaminated and uncontaminated soil even though the present of *Pseudomonas* in contaminated clay was not indicated. Many reports indicated the ability of *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* in degrading hydrocarbons including diesel oil and other PAHs such as anthracene and phenanthrene [7,8]. Gram-positive *Staphylococcus* was also one of hydrocarbon degraders that were vastly studied especially for *Staphylococcus aureus* [9].

Table 2: API microbial identification system for uncontaminated and oil-contaminated soils.

Soil type	Sample	Uncontaminated soil	Oil-contaminated soil
Sand	TC	*Pseudomonas aeruginosa* *Pseudomonas fluorescens/putida* *Erwinia spp* *Chryseomonas luteola*	*Pseudomonas aeruginosa* *Pseudomonas fluorescens/putida*
	TG	*Pseudomonas fluorescens/putida* *Staphylococcus hominis* *Micrococcus spp* *Burkholderia cepacia*	*Micrococcus spp* *Pseudomonas fluorescens/putida*

Silt	JL	*Acinetobacter baumanii* *Staphylococcus hominis* *Staphylococcus sciuri* *Staphylococcus xylosus* *Poteus mirabilis*	*Pseudomonas fluorescens/putida* *Chromobacterium violaceum*
	MC	*Pseudomonas fluorescens/putida* *Staphylococcus xylosus* *Staphylococcus hominis* *Staphylococcus cohnii spp cohnii* *Staphylococcus aureus*	*Staphylococcus lentus* *Staphylococcus auricularis*
Clay	SK	*Pseudomonas aeruginosa* *Staphylococcus auricularis* *Burkholderia cepacia*	*Chromobacterium violaceum* *Staphylococcus auricularis*
	PT	*Staphylococcus haemolyticus* *Pseudomonas aeruginosa*	*Staphylococcus auricularis*

4. CONCLUSION

Six different soil samples obtained from different locations were used for investigation of bacterial population with- and without exposure of hydrocarbon contaminants. The investigation indicated bacterial population changes in soil samples when hydrocarbon was introduced. Generally, the bacterial population was increased, and diversity was reduced when exposed to hydrocarbon contaminants. This might due to proliferation of selected strain of bacteria that able to utilise hydrocarbon thus avoid competition in carbon source from other non-hydrocarbonoclastic bacteria. This study revealed that several bacterial strains especially from genus *Pseudomonas* and *Staphylococcus* have potential in hydrocarbon degradation for different type of soils. The utilisation of these bacteria for hydrocarbon bioremediation needs to consider the type of soils to be treated besides other physico-chemical factors.

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