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Isolation of Bacteria from Spent Engine Oil Polluted Site in Bali and Its Environs Taraba State, Nigeria

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ABSTRACT

Isolation and identification of microorganisms associated with engine oil contaminated soil was carried out. Five different mechanic workshops within Bali and its environs Taraba State were selected and soil samples were collected from each site. Twenty-five grams (25g) of contaminated soil was added to conical flask containing 250 mL of distilled water. The media was prepared by dissolving 3.5g of nutrient agar powder in 125ml of distilled water. The mixture was autoclave at 1210C for 15minute allow to and cool but not solidify. The sterile nutrient agar was aseptically pure plate, 25ml each of the five different petri dish and allowed to solidify. A wire loop was used to transfer amount of spent engine oil polluted soil (stock solution) and inoculated by striking the solidified upper surface layer of the agar plate. The plate was invertically incubated for 24hrs at 370C Singly colonies growing on nutrient agar plate was transferred using wire loop and sub-culture on to freshly prepared culture media (certified agar). The pure culture- plate was inoculated for 24hrs at 370C.The pure culture isolate was identified macroscopically, microscopically and biochemically using standard protocols .Result: The bacterial species isolated were Pseudomonas aeruginosa. Whereas Gazabu has the highest count of the species and Garba Chede has the least.

INTRODUCTION

Waste engine oil is a brown to black oil removed from automobiles when oil is changed it could also be defined as a thick mineral liquid applied to a machine or engine so as to reduce friction between the moving parts of the machine (Shahida *et al.*, 2015). Used engine oil as the name implies represent oil that has undergone destructive changes in the property when subjected to oxygen, combustion gases, and high temperature. The said oil also undergoes viscosity changes as well as additive depletion and oxidation (Mark *et al.*, 2018). The disposal of spent engine oil (SEO) into gutters, water drains, open plots and farms is a common practice in Nigeria especially by motor mechanics. This indiscriminate disposal of spent engine oil adversely affect plants, microbes and aquatic lives (Nwoko *et al.*, 2015; Adenipekun *et al.*, 2018) because of the large amount of hydrocarbons and highly toxic polycyclic aromatic hydrocarbons contained in the oil (Wang *et al.*, 2016; Vwioko and Fashemi, 2015). Heavy metals such as vanadium, lead, aluminium, nickel and iron which are found in large quantities in used engine oil may be retained in soil, in form of oxides, hydroxides, carbonates, exchangeable cation and/or bound to organic matters in the soil (Ying *et al.*, 2017). These heavy metals may lead to build up of essential organic (carbon, phosphorous, calcium, magnesium) and non-essential (magnesium, lead, zinc, iron, cobalt, copper) elements in soil which are eventually translocated into plant tissues (Vwioko *et al.*, 2016). Although heavy metals in low concentration are essential micronutrients for plants, but at high concentrations, they may cause metabolic disorder and growth inhibition for most of the plant species (Yadav, 2018). According to Nwadinigwe and Onwumere (2016), contamination of soil arising from oil spills affect the growth of plants and causes tremendous negative impacts on food productivity (Onwurah *et al.*, 2007). Microbial degradation is the major mechanism for the elimination of used petroleum products from the environment. Soils contain very large numbers of microorganisms which can include a number of hydrocarbons utilizing bacteria and fungi. Hydrocarbon or oil biodegradation as a process makes use of natural microbial biodegradative activities and this often employs the enzymatic capabilities of indigenous hydrocarbon-degrading microbial populations and modifying environmental factors (Atlas, 2019). One major requirement for oil biodegradation is the presence of microorganisms with the appropriate metabolic capabilities. Soil contaminated by used lubricating oil is rapidly increasing due to the global increase in the usage of petroleum products. However, presence of different types of automobiles and machinery results in an increase in the usages of lubricating oil (Ameen *et al.*, 2018). Hydrocarbon contamination of the soil especially by Polyclic Aromatic Hydrocarbons (PAHs) attracts public attention because many PAHs are toxic, mutagenic and carcinogenic. Prolonged exposure to high oil concentration may cause the development of liver or kidney diseases, possible damage to the bone marrow and an increased risk of cancer (Olukunle and Boboye, 2016).

MATERIALS AND METHOD

Sample Collection The Soil samples were taken from 5 different mechanic workshops that had highly contaminated with spilled used engine oil in Bali Local

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Government and its environs which include Bali1, Bali2, Garba Chede ,Maihula and Gazabu. A sterile hand gloves and trowel were used to collect the soil sample at a depth 5-8 cm into clean labelled plastic bags and conveyed to Science laboratory Technology Department of Federal Polytechnic, Bali, Taraba State. 25g from of each sample were collected into the conical flask containing 250 ml of distilled water.

Preparation of Nutrient Agar

The media was prepared by dissolving 3.5g of nutrient agar powder in 125ml of distilled water. The mixture was autoclave at 1210C for 15minute allow to cool but not solidify.

Inoculation of the Sample

The steriled nutrient agar were aseptically pure plate, 25ml each of the five different petri dish and allowed to solidify. Wire loop was used to transfer amount of spent engine oil polluted soil (stock solution) and inoculated by striking the solidified upper surface layer of the agar plate. The plate was invertically incubated for 24hrs at 370C.

Obtaining Pure Culture

Singly colonies growing on nutrient agar plate was transferred using wire loop and sub-culture on to freshly prepared culture media(certified agar).The pure culture-plate was inoculated for 24hrs at 370C.The pure culture isolate was identified macroscopically,microscopically and biochemically using standard protocols as stated in Owuama (2013).

RESULTS AND DISCUSSIONS

The table below shows the characterization and identification of bacteria isolates from spent engine oil polluted sites

Table 1: The characterization and identification of bacteria isolates from spent engine oil polluted sites.

Characterestic	Bali1	Bali12	Garba Chede	Maihula	Gazabu
Culture media	25ml steriled nutrient agar plate	25ml steriled nutrient agar plate	25ml steriled nutrient agar plate	25ml steriled nutrient agar plate	25ml steriled nutrient agar plate
Colour	Creamy	Creamy	Creamy	Creamy	Creamy
Shape	Large and irregular	Large and irregular	Large and irregular	Large and irregular	Large and irregular
Morphological					
Cell type	Rod	Rod	Rod	Rod	Rod
Cell arrangement	Single	Single	Single	Single	Single
Gram reaction	-ve	-ve	-ve	-ve	-ve
Biochemical					
Catalase	+ve	+ve	+ve	+ve	+ve
Probable organism	*Pseudomonas aeruginasa*	*Pseudomonas aeruginasa*	*Pseudomonas aeruginasa*	*Pseudomonas aeruginasa*	*Pseudomonas aeruginasa*

Note: +ve = Postive, -ve = Negative

DISCUSSIONS

The results revealed that, Pseudomonas sp. was the best degraders of the used engine oil for certain period of incubation. The superiority of Pseudomonas species over Bacillus sp. in hydrocarbon degradation has been well established by many researchers (FA. and Adamu. 2022; Tirmizhi *et al.*, 2022). The bacterial species isolated were Pseudomonas aeruginosa. Whereas Gazabu has the highest count of the species and Garba Chede has the least. 13 species isolated and screened 5 bacterial isolates for their ability to utilize spent engine oil. The authors found that Pseudomonas putrefacience CR33 (68%) had the best degradation potential compared with other isolates, while Bacillus coagulans CR31 (45%) had the least potential. (15) screened 19 isolates for their potential to utilize 0.5% 2T spent engine oil and discovered that Pseudomonas sp. GD18 was the best candidate based on its increasing optical density (OD600) in M9 broth. Nevertheless, Bacillus sp. has been used with great success in the biodegradation of used engine oil. The extent of degradation is determined by hydrocarbon concentration and usually, microbes degrade at lower concentrations rapidly while th higher concentration was reported to be lethal for many soil microorganisms(Abioye *et al.*, 2012). Therefore, the higher degradation observed in used engine oil concentration in this study might be attributed to its low toxicity.

Muniz *et al.*, (2004); Adesodun and Mbagwu (2008); Adeniyi and Owoade (2010); Abdullah *et al.* (2011) as expected, the microbial population of the polluted soils is generally lower than that of the control soil samples. This may be due to a shift in the ecological balance of the biota in favour of metal-tolerant strains (Jiang *et al.*, 2008); or may result from the sparse population of plants in the polluted soils, which deprives the soil of the organic matter inputs necessary to support the growth of the microbial population (Kuperman and Carreiro, 1997). It is expected that microbial processes will play

an important role in the restoration of these and other similar polluted ecosystems.

CONCLUSION

The search for efficiently used engine oil-degrading bacteria has been on the increase. Therefore, in this study the isolate tested, Pseudomonas sp. was the best degrader of spent engine oil. The results revealed that Pseudomonas sp. was able to degrade higher percentage of the used engine oil.

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