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MYCOREMEDIATION OF POLYAROMATIC HYDROCARBON CONTAMINATED ANOXIC ECOSYSTEM BY *TRICHODERMA* SPECIES IN SYNERGY WITH *BACILLUS* SPECIES

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ABSTRACT

Fungi-base remediation is a cheap, effective and ecofriendly procedure for the cleanup of crude oil contaminated ecosystem. The study investigated the reduction of lag in crude oil degradation by Trichoderma in synergy with Bacillus in biofilm mode Sediment samples were collected from Bodo Ogoni River State using random stratified method.at depth 0 – 15 cm and 15 – 30 cm. The experimental setup consists of consortium of 1 % and 50 % crude oil (bonny light) with Trichoderma inoculum alone and/or Trichoderma and Bacillus inoculum for sixty (60) days. Microbiological test, Biochemical test, Physiochemical test (pH, conductivity) salt (nitrate, phosphate, sulphate) metal (lead, nickel, chromium, vanadium, iron) and total petroleum hydrocarbon and polyaromatic hydrocarbon using Gas chromatography flame ionization detector. Trichoderma koningopsis strain (MT111912.1): accession no (ON564694) and Bacillus velezensis strain (ON287164.1) accession no. (ON584354) were molecularly identified. 1 % crude oil Trichoderma only setup recorded 33.3 % reduction of PAHs while Trichoderma and Bacillus 1 % crude oil setup recorded 78.8 %. The 50 % crude oil Trichoderma only setup recorded 57.5 % reduction of PAHs while Trichoderma and Bacillus 50 % crude oil setup recorded 90.8 %. For TPH 1 % crude oil Trichoderma only setup recorded 29.9 % reduction while Trichoderma and Bacillus recorded 71.9 %. The 50 % crude oil Trichoderma only setup recorded 49.2 % reduction of while Trichoderma and Bacillus 50 % crude oil setup recorded 85.8 %. The study revealed that Trichoderma species can degrade polyaromatic hydrocarbon and total petroleum hydrocarbon faster in synergy with Bacillus.

Keywords:*Trichoderma, Bacillus,* Mycoremediation, contamination, Polyaromatic hydrocarbon

INTRODUCTION

In the Niger Delta, especially Rivers State, environmental degradation brought on by crude oil spills is a significant problem (Tamunoiyowuna *et al*., 2016). Given the danger it causes to both the environment and people, particularly polycyclic aromatic hydrocarbons, hydrocarbon contamination is a major concern. Exploration for crude oil hastens the introduction of unintentional oil spills, drilling mud, leaks, and other petroleum byproducts into the environment. Since contaminated soils have an impact on various ecosystem components, there has been increased focus on the concurrent generation

of large amounts of toxic and persistent petroleum hydrocarbon pollutants like benzene, oil, phenols, toluene, arsenic, grease, and ethyl benzene (BTEX) and polyaromatic hydrocarbons (PAHs) (Okoye *et al*., 2019).

Crude oil spills cause ecological and toxicological impacts on plants, the disruption of the natural state of the oilfield, and harm to the ecosystem by reducing the aeration and water permeability of the soil by filling the pores. Among many additional effects of crude oil pollution, carcinogenic and mutagenic crude oil chemicals may result in fatal alterations in genetic material (Jia *et al.,* 2017). Crude oil spills also lower the quality of the air, water, and soil, wasting non-renewable resources (Tamunoiyowuna et al., 2016).

Due to the use of ex situ treatments, many traditional physical and chemical cleanup techniques (soil cleansing, chemical reduction or oxidation of pollutants, and cremation) are expensive. In addition to the financial costs, they frequently result in secondary pollution issues due to the transportation of contaminants and chemical reagents (Fatima *et al*., 2015). This has raised concerns about natural remediation using living organisms, such as plants, and microbial methods, which have been shown to not endanger life and have other negative effects.

Mycoremediation is the process of utilizing fungi to break down organic substances. Several distinct traits of filamentous fungal species include the production of oxidative enzymes, organic acids, chelators, and extracellular enzymes. Numerous studies haveshown that most filamentous fungal species are good hydrocarbon degraders, (Vanishree, 2014). Trichoderma is one of the fungal species that may clean up pollution in the soil since it is petroleum-resistant. Pratibha *et al,* 2012) demonstrated that bioremediation efforts using Trichoderma spp. is a potent technique. Trichoderma is a genus of filamentous fungi that inhabits soil and bears teleomorphs. It is a member of the Hypocreales order of the class Ascomycota. Trichoderma is a genetically diversified genus that includes several strains with important agricultural and industrial properties.

Surprisingly, microbial remediation is regarded as one of the most promising solutions for the reasonably priced restoration of places contaminated by crude oil. Due to genetic alterations, the native microbial communities exposed to petroleum hydrocarbons develop a tolerance and show increased rates of biodegradation (Azubuike *et al.,* 2020). Given the intricate network of waterways and the magnitude of the areas affected by a spill, inadequate accessibility to contaminated sites presents a significant problem for the clean-up of these sites.

Due to their suitable metabolic abilities and the availability of degradative enzymes, bacterial communities have long been recognized as one of the most active agents in the degradation of petroleum. When different bacterial species work together, the growth factors, metabolic processes, and enzymes that speed up the breakdown of complicated hydrocarbon combinations perform better. According to research by Sowani *et al.* (2019), bacteria are essential for biogeochemical cycling and the degradation of contaminants derived from petroleum.

Numerous studies show that the majority of *Bacillus* species are efficient hydrocarbon degraders. According to reports, some bacterial species can remove pollutants from soil since they are resistant to petroleum (Vanishre, 2014). It offers a potent tool for connecting bioremediation efforts. The study looked into how Trichoderma and Bacillus working together in a biofilm mode could shorten the time it took for crude oil to degrade, its objective includes (1) to isolate and identify Trichoderma and Bacillus species molecularly (2) to evaluate the remediation rate at which Trichoderma sp. will remediate crude oil polluted ecosystem (3) to evaluate the remediation rate at which Trichoderma sp. and Bacillus sp. will remediate crude oil polluted ecosystem

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MATERIAL AND METHODS

Sample collection

A total of ten(10) crude oil polluted soil samples were collected with sterile soil auger at the depth of 0-15cm and 15-30cm at different point (A with a latitude of 04°36'429" N/ longitude of007°15'643" E; point B, with latitude of 04° 38'0.439" N/ longitude of 007°642" E ; point C, with a latitude 04°36'430" N/ longitude of 007°15'642" E, point D with a latitude of 04°39'834" N/ longitude of 007°15'684" E ; point E, with latitude of 04°36' 421" N/ longitude of 007°15'642" E) using a Randomized Stratified Method. The soil samples were taken with a sterile polythene bag and were transported to the laboratory, stored at 4^oC for physiochemical and microbiological analysis.

Physiochemical analysis

After the collection of the soil samples, a portion of the soil sample A (0-15cm), (15-30cm) was taken with the aid of sterile spatula, it was put in a sterile universal container, labeled properly and taken to the laboratory to check for physiochemical parameters such as pH, Electric conductivity, salts (Nitrate, phosphate and sulphate), heavy metals (Pb, Ni, Cr, V, Fe), TPH (total petroleum hydrocarbon) and PAH (polycyclic aromatic hydrocarbon). Association of analytical chemists (AOAC), 2012 method was adopted.

Enumeration of total heterotrophic bacteria Count

A 10-fold serial dilution was carried out to achieve this adopted Ogbonna *et al*, 2020 method. One gram of each soil sample was weighed out and dispensed into a beaker containing 10ml of normal saline. Using a sterile micro pipette, 1ml of the sample was pipette into the first test tube containing 9ml of normal saline to give a 10^{-1} dilution, it was shaken and 1ml was taken from it to the next test tube containing another 9ml of normal saline to give a 10^{-2} dilution, it was diluted serially, up to 10^{-5}. This procedure was used for the other samples. The test tubes were covered with cotton wool. Exactly 0.1ml aliquot of the dilutions starting from 10^{-3} and 10^{-4} was inoculated into Petri dishes containing Nutrient Agar (NA) in triplicate and spread with a sterile spreader. The plates were incubated for 18-24hours.

Enumeration of Total Hydrocarbon Utilizing Bacteria

The hydrocarbon utilizing bacteria of the soil sample were enumerated in triplicates on the Bushnell Hass media, using the spread plate method as descripted by Mulet *et al*., (2018) Vapour phase transfer was used was used to introduce crude oil. Serial dilution of each sample was carried out by suspending 1gof the sample into normal saline, which was diluted serially into 5 test tubes containing 9ml of normal saline. Exactly 0.1 ml of the appropriate dilution was inoculated by spread plate method onto the duplicated agar plates. A sterile filter paper (WhatmanNo.1) saturated with crude oil was placed inside the cover of the Petri dish closed, inverted and incubated at 28^oC for 5-8days. The filter paper saturated with crude oil served as a sole source of carbon. The plates were counted after 7 days of incubation. The percentage hydrocarbon utilized within the heterotrophic bacterial population were determined.

Microbial colonies inside the culture plates were purified by sub-culturing into nutrient agar, identification of the pure isolate was doneinvolved biochemical tests(triple sugar iron agar, Simmon's citrate agar, motility test, oxidase test, indole test, Methyl red and Voges-Proskauer test, Sugar fermentation/acid gas production) and microscopy and stocked in Nutrient agar slants in bijou bottles.

Enumeration of Total Fungi (TF) Count

A tenfold serial dilution was carried out to achieve this. One (1) gram of each soil sample was weighed out and dispensed into a beaker containing 10ml of normal saline; it was rocked thoroughly for proper mixing. With the aid of a sterile micro pipette, 1ml of the sample was pipette into the first test tube containing 9ml of normal saline which represent 10^{-1} dilution, The content of the test tube shaken

and 1ml was taken from it to the next test tube containing another 9ml of normal saline which represent10^{-2} dilution, Stepwise dilution continued up to 10^{-5}. The same procedure was used for the other samples. The test tubes were covered with cotton wool. 0.1ml aliquot of the dilutions starting from 10^{-3} and 10^{-4} were noculated into Petri dishes containing potato dextrose agar (PDA) in triplicate and spread with sterile bent glass rod. The plates were incubated at 27^{o}C for 3-7 days(Ogbonna et al., 2020).

Enumeration of total culturable hydrocarbon utilizing fungi

Vapour phase transfer method was used to isolate hydrocarbon utilizing fungi. The vapour phase transfer method includes the addition of crude oil, it is suitable for hydrocarbon utilizers. The culturable hydrocarbon utilizing fungi of the soil samples were enumerated in triplicates on the Bushnell Haas media during the spread plate method. Serial dilution of each sample was carried out by suspending 1gof the sample into 10ml of normal saline which was diluted serially into 5 test tubes containing 9ml of normal saline. Aliquot of 0.1ml of the appropriate dilution 10^{-3} and 10^{-4} was inoculated in triplicates by spread plate method respectively.A sterile filter paper saturated with crude oil was place inside the cover (lid) of the Petri dish with the aid of a sterile forcep immediately, the plates were closed, inverted and incubated at 28^{o}C for 3-8 days. The filter paper saturated with crude oil seated as a solesource of carbon and energy (Ogbonna et al., 2020).

Microscopic identification of fungal isolates

To properly identify the microscopic features of the fungi pure cultures, a drop of lactophenol cotton blue was placed on a clean sterile glass slide, with a drop of normal saline placed on the same glass slide. Using a sterile wire hoop, the fungal colony was picked and placed on the glass slide.It was covered with a sterile cover slip and was examined under the microscope with a x40 objective lenses (magnification). Microscopic examination and morphological characteristics were noted. The method of Ogbonna et al., 2020 was adopted with slight modification.

Molecular Identification of Fungal/ Bacterial Isolate

Extraction was done using a Zymo Quick DNA fungal/bacterial extraction kit adopting Sarkar *et al*., (2017). The ultra-pure DNA was then stored at -20^{o}C for downstream reaction. DNA quality and purity were checked using Nanodrop2000c spectrophotometer (Thermo fisher scientific Inc. Wilmington, Delaware, USA). Purity is measured as a ratio of ultra violet (Uv) light absorbance at 260nm to that of 280nm.Gel electrophoresis was performed using 1.5% agarose gel. Primer squence for fungi identification (ITS4): TCCTCC GCTTATTGATATGS and (ITS5): GGAAGTAAAAGTCGTAACAAGG, while for bacteria GAAATTGAAAGACGG CTTCGGCTGTCACTTATGGATGGACCC GCGTCGCATTAGCTAGTTGGTGAGGTA ACGGCTCACCA and AGGCAACGATG CGTAGCCGACCTGAGAGGGTGATCGG CCACACTGGGACTGAGACACGGCCCA GACTCCTACGGGAGGCA

Microbiological sample set-up for evaluation of crude oil degradation

This set-up was done using the mixture of Bushnell Haas broth and crude oil inoculated with the organism isolateadopting APHA, 2017 with slight modifications. A total of 5 sterile conical flasks were used for different concentrations of fungi.The concentration were; 1%, 10%, 25% and 50% thecontent of the fifth flask is the control. Exactly 2.9g Bushnell Haas broth was autoclaved at 121^{o}C for 15mins at 15psi, allowed to cool and was suspended into the 5 sterile conical flasks. Ten millimeter (10ml) of peptone water was suspended in a test tube, with the help of a sterile wire loop, an inoculum of the sub-cultured plate (FF11) was taken and inoculated into the test tube, the content of the test tube was stirred with the wire loop and covered with a cotton wool, it was stored at room temperature for 18-24 of hrs. Exactly 1ml of

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the organism was inoculated into the different conical flask and covered properly.The procedure was repeated using bacteria isolate.

Preparation of Media for Optical Density and Microbial Count.

Four (4)Petri dishes for fungi and 1 Petri dish for the control were used adopting APHA, 2017 with slight modificationsExactly 3.27g of Bushnell Haas broth was dissolved in 1000ml of distilled water, 15g of agar was added for solidification.Using 2ml of sterile syringe, 2 ml each was taken from the various concentration of the setup, exactly 1 ml for optical density and 1ml for the serial dilution. Exactly1ml of each concentration was mixed properly into the sterilized Bushnell Haas Agar before taken the reading for the optical density. 1% and 50% of the concentration bacteria was taken to the laboratory for physiochemical analysis (total petroleum hydrocarbon and polycyclic aromatic hydrocarbon only) on the zero (o) day. The procedure was repeated using bacteria isolate.

This set-up was carried out and allowed to last for 2months. On the last day of monitoring, the samples were taken to the laboratory for TPH and PAH analysis before the setup was terminated.

RESULTS AND DISCUSSION

Table 1: Physiochemical analysis of the crude oil polluted soil sample

Parameters	Soil sample A(0-15cm)	Soil sample C(0-15cm)	Soil sample A(15-30cm)	Soil sample C(15-30cm)
pH	6.58	6.72	6.17	6.28
E.C(µS/cm)	1947.13	1420.00	1310.62	951.34
Nitrate (mg/kg)	36.84	28.78	21.73	17.05
Phosphate (mg/kg)	0.762	0.946	0.391	0.573
Sulphate (mg/kg)	240.19	210.36	213.85	180.11
Pb (mg/kg)	3.84572	4.01371	4.92103	6.21549
Ni (mg/kg)	0.42178	1.06287	1.41724	3.13708
Cr (mg/kg)	11.12134	9.47341	14.83036	13.52831
V (mg/kg)	0.01181	0.02913	0.08574	0.19470
Fe (mg/kg)	9.81275	8.34086	7.95245	7.03624

Table 2: Biochemical Characteristics of Bacterial Isolates

Isolate code	Glucose	Lactose	Sucrose	Citrate	Indole	Catalase	MR	VP	Slant	Butt	H2S	Gas	Oxidase	Gram Stain	Probable genera
St1	+	-	-	-	-	+	-	-	A	B	-	-	-	+VE	*Bacillus* spp
St2	+	-	-	-	-	+	-	+	A	B	-	-	+	+VE	*Bacillus* spp
St3	-	-	-	-	-	+	-	-	A	B	-	-	+	-VE	*Alcaligenesspp*
St4	+	-	-	+	-	+	-	-	A	B	-	-	+	-VE	*Pseudomonas* spp
St5	-	-	-	+	-	+	-	-	A	B	-	-	+	-VE	*Bacillus* spp
St6	+	-	-	+	-	+	-	-	A	B	-	-	-	-VE	*Klebsiella*spp

St7	-	-	-	-	-	+	-	-	A	B	-	-	-	-VE	*Alcaligenes*spp
St8	+	-	-	+	-	+	-	-	A	B	-	-	-	+VE	*Staphylococcus* spp
St9	+	-	-	-	-	+	-	-	A	B	-	-	-	+VE	*Bacillus* spp
St10	-	-	-	-	-	+	-	-	A	B	-	-	-	-VE	*Acinetobacter*spp
St11	-	-	-	-	-	+	-		A	B	-	-	-	-VE	*Acinetobacter*spp
St12	+	-	-	+	-	+	+	+	B	A	+	+	-	+VE	*Staphylococcus* spp
St13	+	-	+	+		+	+		B	A	+	+	-	-VE	*Acinetobacter*spp
St14	+	-	-	-		+	+	+	B	A	+	+	-	+VE	*Staphylococcus* spp

Key: MR= Methyl red, VP = Voges-Proskauer, +ve = growth positive, -ve + growth negative, A= acid, B = Base

Table 3: Morphological Characteristics of the Fungal Isolates

Isolate Code	Macroscopic characterization	Microscopic characterization	Probable organisms
FF1	White dry mycelia with black spores and a white reverse	Conidiophores are protrusions from septate a and hyaline hyphae. The conidia head is septate and biseriate. Conidia	*Aspergillus niger*
FF2	White dry raised mycelia and white reserve	Branched hyphae septate , presence of spores	*Mucor spp*
FF3	Yellowish-green mycelia and dark yellow reserve	Conidiophores are hyaline and coarsely roughened. Conidia shape is globose to sub globose	*Aspergillus flavus*
FF4	White dry mycelia and white reverse	Branched hyphae septate , presence of spores	*Mucor spp*
FF5	White fluffy mycelia. The reverse is brown and white zonal	Presence of thick wall hyphae, unbranched, non-septate bearing conidia	*Fusarium spp*
FF7	White cottony dry mycelia. The reverse is brown	Presence of hyaline branched hyphae (scattered), no conidia seen	*Rhizopus nigricans*
FF11	Green raised aerial mycelia, white cotton powdery mycelia with bubbles, white flat margin and a white reverse.	Presence of spore, branched thin hyphae bearing conidia, septate	*Trichoderma spp*

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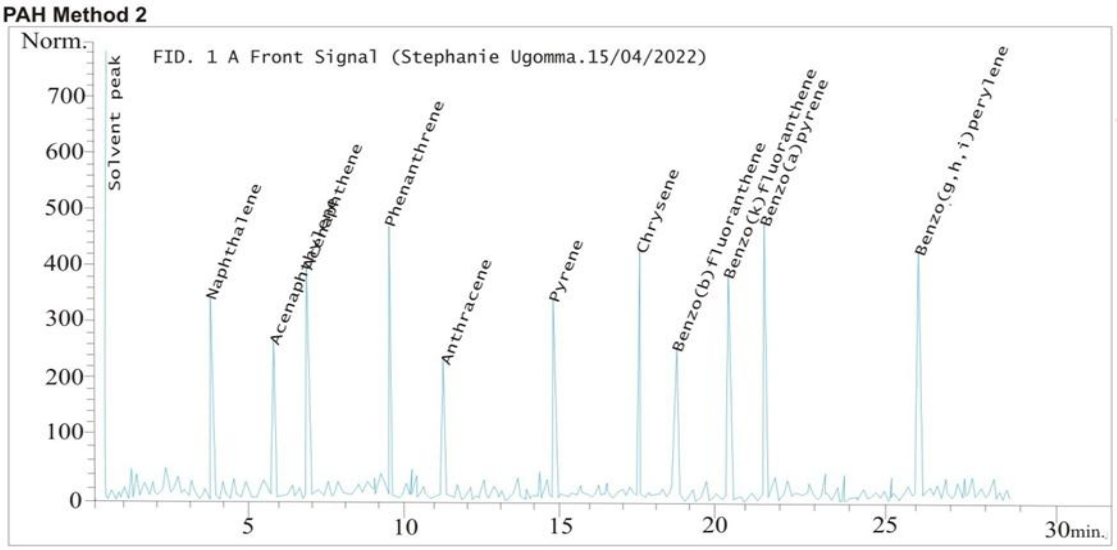

Fig 1: Polycyclic aromatic hydrocarbon (PAH) set-up (1%) Bacillus spp. day 0

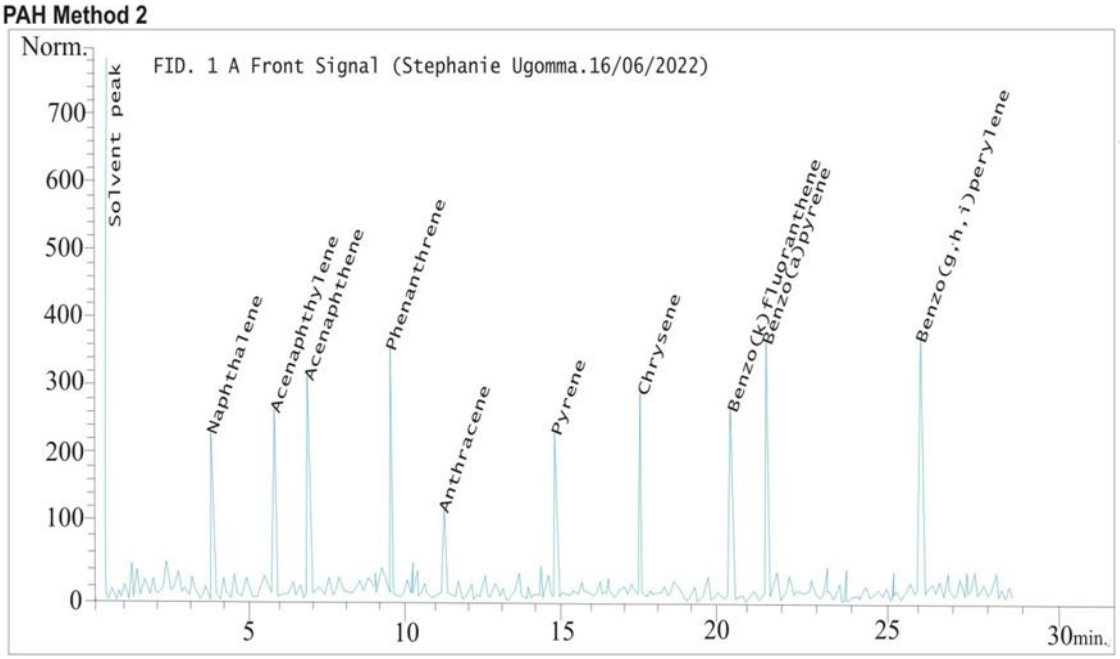

Fig 2: Polycyclic aromatic hydrocarbon (PAH) set-up(1%) with *Bacillus* spp day 60

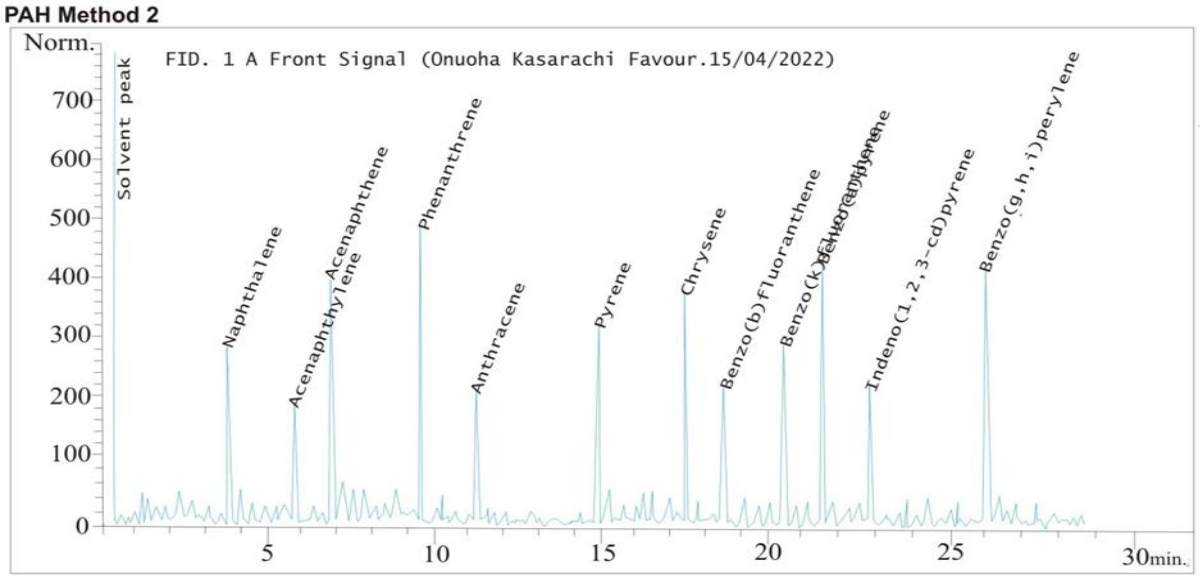

Fig 3: PAH of the microbiological crude oil set-up 1% amended with *Trichoderma* spp. day 0

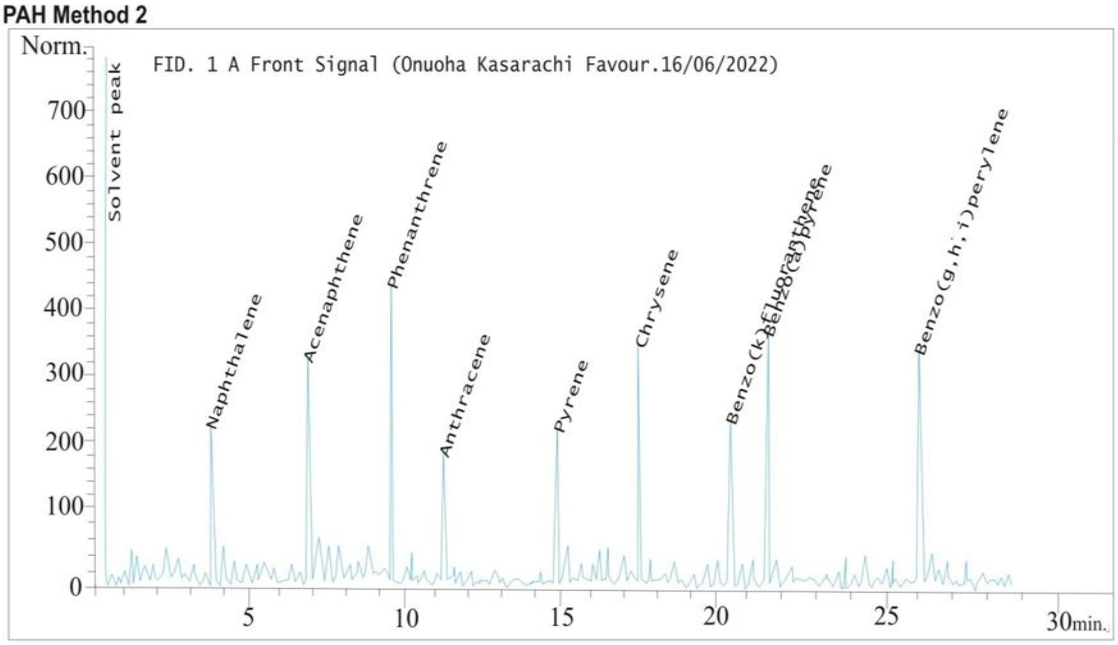

Fig 4: PAH of the microbiological crude oil set-up 1% amended with *Trichoderma* spp. day 60

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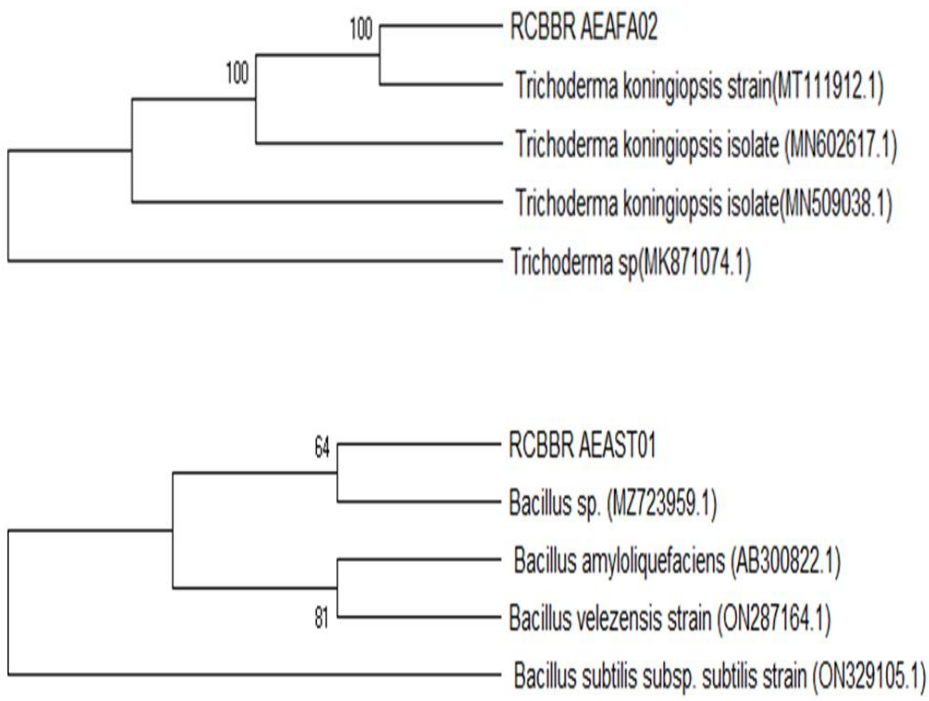

Fig 5: Phylogenic tree of *Trichodermakoningopsis*strain (MT111912.1) and *Bacillus velezensis* strain (ON287164.1)

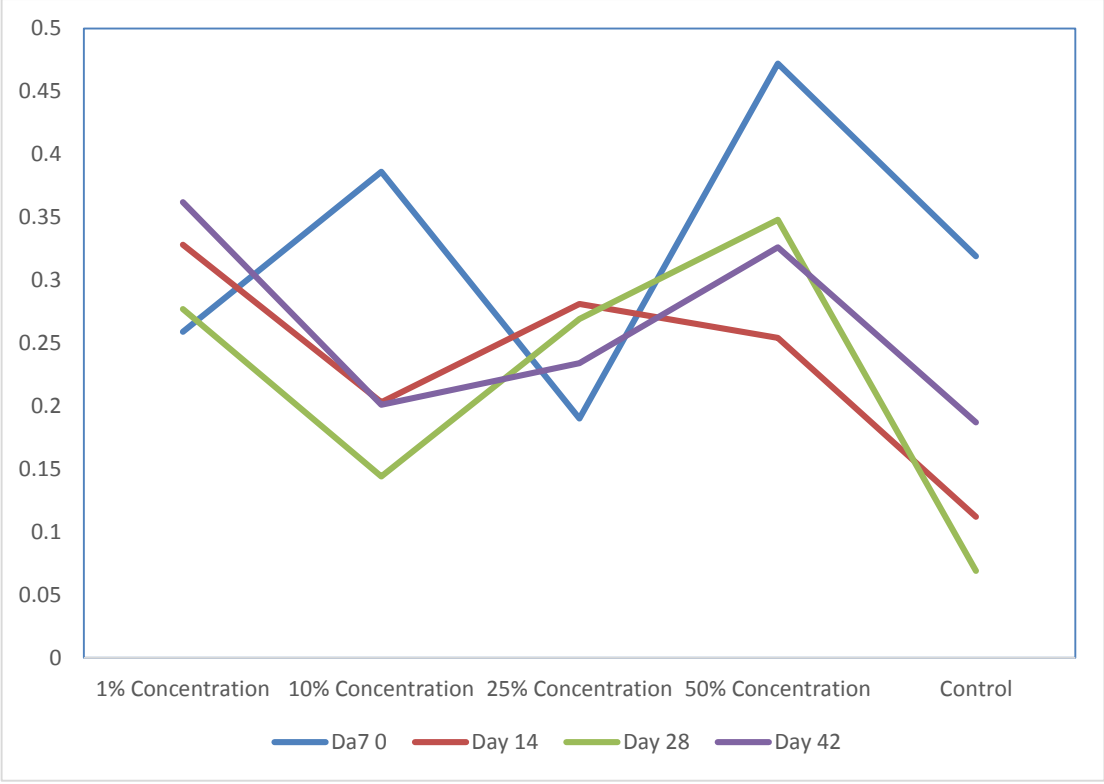

Fig. 5: Optical density of bacterial isolate (St1) at various concentration.

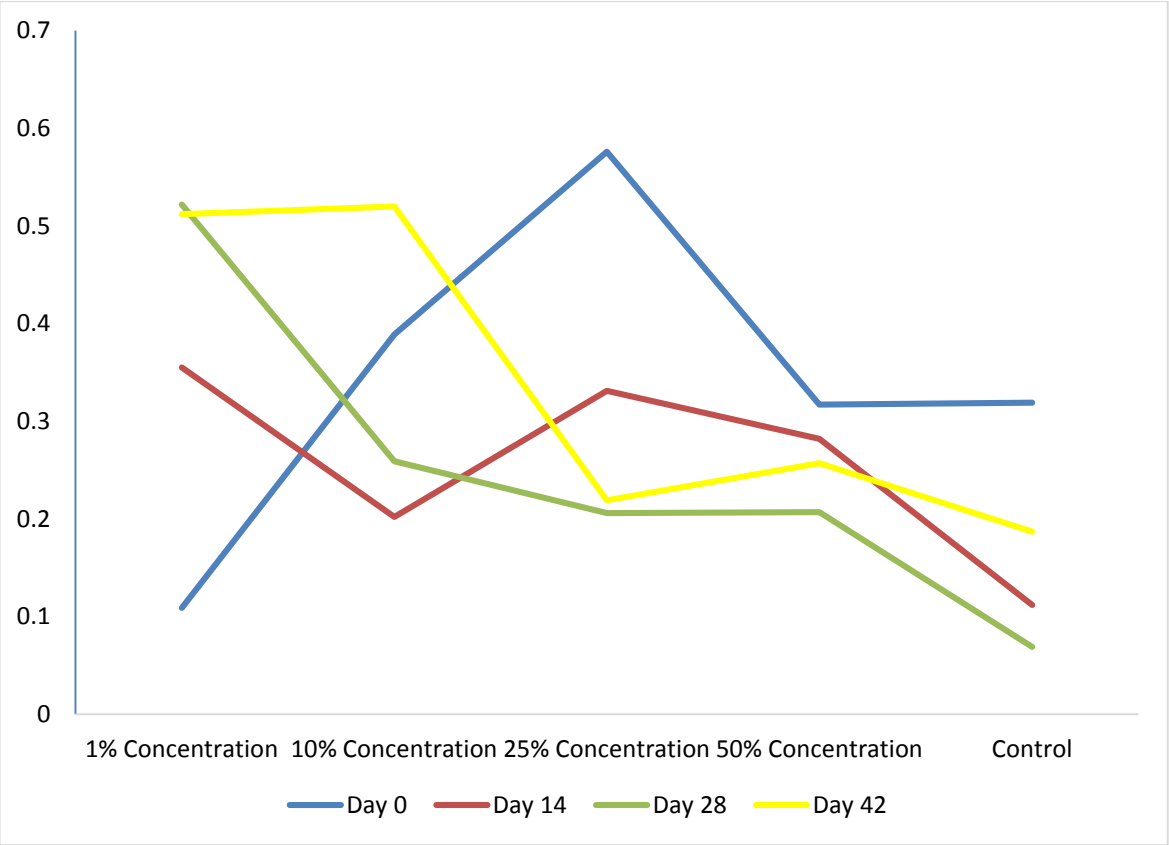

Fig. 6: Optical Density of Fungal Isolate at various Concentration

The soil sample collected from 0-15cm depth had higher pH, electrical conductivity, nitrate, sulphate, and phosphate levels than the soil sample collected from 15-30cm. The soil sample also had higher Pb, Ni, Cr, V, and Fe levels than the soil sample collected from 15-30cm. The total petroleum hydrocarbon and polycyclic aromatic hydrocarbon levels were higher in the 0-15cm depth soil sample than15-30cm depth soil sample. Crude oil contamination is the cause of the elevated pH level seen at 0–15 cm of depth, as shown in Table 1. As depth increased the pH roseas was the case at 15–30 cm of depth. Additionally, the lower electrical conductivity (574.10) seen at depths of 0–15 cm compared to 15–30 cm suggests that the organic matter in the soil has decreased at depths of 0–15 cm as a result of crude oil contamination, as indicated in Table 1. These results concur with the study by Jiang*et al*. (2014) on the effects of crude oil contamination on the physical and chemical characteristics of soil in China's Momoge Wetland.

This experiment revealed that the overall heterotrophic bacterial count was higher in the nutrient agar plates than the hydrocarbon-impregnated agar plates using bacterial count. This may be due to the presence of crude oil, which has a high concentration of TPH, PAH, and heavy metals, all of which are toxic tomicroorganisms.

The Total petroleum hydrocarbon (TPH) at 0-15cm and 15-30cm depth were 16089.0 mg/kg and 19685.3 mg/kg while Polycyclic aromatic hydrocarbon at 0-15cm and 15-30cm depth were 7747.5m mg/kg and 6694.6 mg/kg respectively.

The total petroleum hydrocarbon TPH concentration of the soil sample obtained from the crude oil polluted site at 0-15cm and 15-

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30cm depths ranged from 16089.0-19685.3mg/kg which is above the USEPA maximum possible limit of 30mg/kg(EPA, 2013). Azubuike *et al*.,(2020) reported a similar result that soil samples in Rivers State, Nigeria was contaminated with TPH concentration of 1534.7 and 1438.0 mg/kg at depth of 0.0-50cm and 50-100cm respectively which were similar to the values obtained in this study. These TPH compounds are generally carcinogenic and immuno-toxic in nature (EPA, 2013).

In this present study, both macroscopic and biochemical characteristic shows *Bacillus* spp(26.6 %)is the frequently occurring isolate followed by *Acinetobacter* spp, and *Staphyloccus* spp,(21.4 %) then *Alcaligens* spp (14.3 %),while *Pseudomonas* spp, and *Klebsiella spp*. being the least (7.2 %)(table 2) A similar genera were reported by Tremblag *et al*., (2017); Varjani and Gnansounou, (2017) and Xu *et al.,*(2017) from a related study. Organisms such as *Bacillus velezensis* was observed to be metal tolerant (able to withstand the harsh environment heavy metal of crude oil created) by being able to withstandsuch conditions. (Varjani and Gnansounou, 2017; Sharma *et al*., 2015). Sharma *et al*., (2015) identified *Bacillus thuringiensis* PW-05from marine and *Bacillus* sp. SD-43 from steel industry waste posing inbuilt mechanisms for adapting to their environment. It was noted that this organism plays a vital role in remediation and clean-up of oil spillage by utilizing crude oil as its sole carbon source, low cost and environmentally friendly (Guerra *et al*., 2018) and spore former shielding them from the noxious effects of the hydrocarbon (Ogbonna *et al*., 2020).

When *Bacillus velezensis* was tested for its remedial ability using optical density analysis, it was proven to remediate and clean up oil spillage properly by having a heavy growth of the organism as well as *Trichoderma koninggiopsis*(Fig(s) 5 and 6).

Evaluation of the microscopic/macroscopic characteristics revealed the presence of *Aspergillus niger, Mucorsp, Aspergillus flavus, Fusarium* sp*, Aspergillus japonicas, Rhizopus nigricans, Trichoderma sp, Penicillum* sp**.** The frequency of occurrence of fungal isolates showed that a total of twenty-three (23) fungal isolates were gotten from the samplesthe frequency of occurrence of the isolatesis higher than four (4) isolates reported by Ogbonna *et al*., (2020).The possible reason could be as a result of the duration of simulation, in this case 21 days simulation was allowed in order to mimic a naturally polluted soil ((Varjani and Gnansounou, 2017) *Aspergillus* sp and *Fusarium sp* were the most frequent occurring isolates identified during the course of this study. This is followed by *Penicillium* sp, *Mucor* sp with *Rhizopus* sp and *Trichoderma* sp being the least as shown Table 3.

Ascomycetes have lots of advantages as a tool for remediating polluted ecosystems; These include: they do not require co-metabolic carbon sources to trigger the secretion of the extracellular enzymes, transformation of recalcitrant compound, (Zavarzina *et al*., 2010) synthesis of soil organic matter, metal detoxifying, ability to degrade organic contaminants (Coa *et a*l., 2018) and uptake and translocation of Ni, Zn and Cd (Arriagada *et al.,* 2019); and *Trichoderma* sp. belong to the phylum ascomycetes.

The result of the polycyclic aromatic hydrocarbons in the study site at the depths 0-15cm and 15-30cm ranged from 7747.5 - 6694.6 mg/kg respectively. The sum of the PAH concentration obtained in this study were higher than the recommended levels of 1mg/kg, 1.5mg/kg, and 5mg/kg imposed by soil cleanup guidelines from Denmark, Netherlands and Australia respectively. (EPA, 2013). The high pH of the soil sample indicates contamination of this study site with petroleum. Generally, mixtures of petroleum were known to cause carcinogenic Geno-toxic effect and are potential immunosuppressant (EPA, 2013).

The total petroleum hydrocarbon of the microbiological samples first day set-up from the concentration (1%FF and50%FF) have

several carbons which ranged from C8-C27 with the total that ranged from 5114.2-9467.1 mg/kg. The total petroleum hydrocarbon of the microbiological samples for the last day set-up from the concentration (1%FF and50%FF) have several carbons which ranged from C8-C27 while the total that ranged from 2598.4-6625.5 mg/kg

The polycyclic aromatic hydrocarbon of the microbiological samples first day set-up from the concentration (1%FF) had different groups of aromatic rings which include naphthalene, acenaphthylene, acenaphthene, phenanthrene, anthracene, pyrene, chrysene, benzo(b) fluoranthene, benzo(k)fluoranthene, indeno (1,2,3-cd) pyrene, benzo(g,h,i)perylene and (50%FF) has the same groups of aromatic ringswith (1%FF) exceptbenzo(b)fluoranthene with their sum total that ranged from 2257.4 - 3470.3 mg/kg.

The Polycyclic Aromatic Hydrocarbon of the microbiological samples for the last day set-up from the concentration (1%FF) has different groups of aromatic rings which includes; naphthalene, acenaphthene, phenanthrene, anthracene, pyrene, chrysene, benzo(k) fluoranthene, benzo(a)pyrene, benzo(g,h,i) perylene and (50%FF) has acenaphthene, phenanthrene, pyrene, chrysene, benzo(a) pyrene, benzo(g,h,i)perylene with their sum total that ranged from 960.8 - 2315.1 mg/kg.

The Polycyclic Aromatic Hydrocarbon of the microbiological samples first day set-up from the concentration (1%FF + ST) has different groups of aromatic rings which includes; naphthalene, acenaphthylene, acenaphthene, phenanthrene, anthracene, pyrene, chrysene, benzo(b)fluoranthene, benzo(k)pyrene, benzo (g,h,i)perylene and (50%FF + ST)has the same groups of aromatic rings with (1%FF) except chrysene with their sum total that ranged from 960.7 – 1580.6 mg/kg.

The Polycyclic Aromatic Hydrocarbon of the microbiological samples for the last day set-up from the concentration (1%FF + ST) has different groups of aromatic rings which includes; naphthalene, acenaphthene, phenanthrene, benzo(a)pyrene, benzo(g,h,i) perylene and (50%FF +ST) has naphthalene acenaphthene, phenanthrene, pyrene, benzo(b)fluoranthene benzo(a)pyrene, benzo (g,h,i)perylene with their sum total that ranged from 335.1 mg/kg – 871.8 mg/kg.

The study revealed that 1 % crude oil *Trichoderma* only setup recorded 33.3 % reduction of PAHs while *Trichoderm*a and *Bacillus* 1 % crude oil setup recorded 78.8 %. The 50 % crude oil *Trichoderma* only setup recorded 57.5 % reduction of PAHs while *Trichoderma* and *Bacillus* 50 % crude oil setup recorded 90.8 %. A workdone by Harman *et al* 2014; 2018 employed *T. atroviride and T. harzianum* on naphthalene, acenaphthen, fluorene, phenanthrene recorded similar result.

Phylogenetic tree of isolate GA1 base on neighbor-joining revealed fungus isolated and used for the experiment is *Trichoderma koninggiopsis*with accession ID number (ON564694.1)while the bacterial isolate*bacillus velezensis* with accession ID number (ON584354.1)

CONCLUSION

The fungus named *Trichoderma koningiopsis* with the accession number (ON564694.I)) was observed to readily degrade TPH and PAH, in the contaminated site. Further test referred as optical density was carried out also proved that *Trichoderma koningiopsis* could be applied for remediation of crude oil polluted site. Trichoderma-bases eco-friendly PAH-removal technology can be developed by mass-cultured PAHs tolerant *Trichoderma* isolated from crude oil contaminated site.

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