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Impact of Treated Palm Oil Mill Effluent (POME) on Soil Microflora

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ABSTRACT

This work on the Effects of Treated Palm oil mill effluents (POME) on soil microflora was carried out using standard microbiological and biochemical methods. Activated carbon used for the treatment of POME were generated corn cob, coconut shell and a combination of the two materials. Palm oil mill effluents were obtained from Umulolo community in Okigwe Local Government Area of Imo State. Top soil (10-15cm) and sub soil (of 15-30cm) samples were obtained from Federal University of Technology, Owerri farms. The Activated carbon were used separately and in combined forms to treat POME samples and the resultant wastewater, by watering the top and sub soil samples for four (4) weeks. The microbial flora of the watered soil samples was assessed using pour plate method. The results obtained revealed that the high microbial load of the untreated POME (TVC: 3.28 x 10^7 cfu/ml) reduced after the treatment with the activated carbon from agrowastes (Coconut shell TVC: 5.7 x 10^6 cfu/ml, Corn cob TVC: 1.03 x 10^7 cfu/ml and Cocunut shell + Corn cob TVC: 2.04 x 10^7 cfu/ml). There was a general increase in the microbial load of the top and sub soil samples watered with the treated POME when compared with the top and sub soil watered with the raw POME. The bacterial isolates obtained in this study were; *Kebsiella pneumoniae, Bacillus subtilis, Staphlococcus aureus, Corynebacterium glaucum, Bacillus cereus, Pseudomonas aeruginosa, Streptococcus faecalis and Escherichia coli.* The fungal isolates obtained were *Cladosporium herbarum*, *Aspergillus niger*, *Penicillum chrysogenum*, *Rhizopus stolonifera* and *Candida albicans*. From the results obtained in this study, it can be inferred that POME treatment with activated carbon removed the colloidal properties (usually one of the characteristics of POME), reduced the Biochemical oxygen demand (BOD), the chemical oxygen demand (COD) thus conditioning the soil for more microbial growth.

Introduction

The palm oil mill industry is one of the most prevalent industries in Nigeria, especially in the southern part where palm trees (*Elaeis guinensis*) are found both in the plantations and in the wild. Both the traditional and the mechanized palm oil mills release the wastes associated with palm oil processing in large amounts into the environment, with little or no treatment (Wu et al., 2010). A lot of previous research has reiterated the negative consequences of the indiscriminate disposal of wastes associated with the processing of palm oil into land and water bodies. Palm oil mill effluents (POME) is a thick, brownish sludge with pollution indicators such as high biological oxygen demand (BOD), chemical oxygen demand (COD), high amounts of solids and oil & grease, but at the same time is highly rich in nutrients such as phosphorus, sodium and magnesium (Okereke and Ginikanwa, 2020). During palm oil processing, the extraction of oil from the ripe palm fruits (during sterilization and clarification) requires large quantity of water leading to palm oil mill effluents (POME) generation (Soleimaninanadegani and Soheila, 2014). Averagely, for every 1 tonne of crude palm oil produced, about 5-7 tonnes of water ends up as POME (Ahmad *et al.* 2003).

Certain parameters of palm oil mill effluent (POME) affect the soil microbial flora, which in turn affects the fertility of the soil. POME contains several nutrients including light (nitrogen, potassium, magnesium, phosphorous, sodium) and heavy nutrients/metals (zinc, copper, cadmium, chromium, iron etc.) (Awotoye *et al.* 2011; Ohimain *et al.* 2012.). In addition, it contains high pollution indicators including oil and grease, chemical oxygen demand (COD) and biological oxygen demand (BOD). POME contains diverse groups of microorganisms including lipolytic bacteria, methanogens, hydrocarbon degrading bacteria and fungi (Ohimain *et al.* 2012; Ohimain and Izah 2014).

Palm oil as a lipid derives its distinctive properties from the hydrocarbon nature of a major portion of the structure. Studies have also shown that for organisms to degrade palm oil, they must be able to produce lipase (Okechalu *et al.* 2011).

Soil constitutes the most important medium for the survival, growth and multiplication of microorganisms. Microflora of soil is an integral part of soil organic matter (Jacoby *et al.* 2017). The microorganisms present in the soil affect its structure and fertility. Soil microorganisms are classified into bacteria, actinomycetes, fungi, algae and protozoa. Each of these groups have their specific functions in soils (Mendes *et al.* 2013). Bacteria constitutes the most abundant group of microorganisms in soil, while the fungal population is made up of a heterogeneous group of organisms. The population of these microorganisms is highest

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in the rhizosphere than that of other zones due to the promotion of growth promoting substances by plants. Soil microorganisms break down a variety of organic materials and use some of the products of the breakdown to generate and synthesize humus (a dark, coloured amorphous substance), polysaccharides, non-humic substances and humin (Yan and Quin 2015). These materials impact on the physical, chemical and biochemical properties of the soil in many ways. Autotrophic bacteria are found in higher numbers in soil when compared to heterotrophic bacteria because autotrophs derive energy by making their own food through oxidation, rather than feeding on plants or other species. These autotrophic bacteria play an important role in nitrogen fixation, for example *pseudomonas*. In well aerated soils, bacteria and fungi dominate, with bacteria accounting for almost all the biological and chemical changes that occur in soil with little or no oxygen. The abundance of microbial population in soils vary, depending on the condition of the soil being reviewed, such as soil pH and other environmental parameters. The microbial activity of the soil is also influenced by chemical fertilizers, organic amendments and other planting practices such as application of plant protection chemicals. All these could affect the quantity and quality of soil microflora (Harleen and Anshu, 2018).

Therefore, while enjoying the highly profitable commodity palm oil, a challenge exists to convert POME to an environmentally friendly waste, through the adoption of simple but efficient treatment methods and disposal techniques. Recently, agricultural wastes have proven to be low-cost alternatives for the treatment of effluents containing heavy metals (for e.g. POME) through the adsorption process. Many studies on the treatment of effluents bearing heavy metals have revealed adsorption to be a highly effective technique for the removal of heavy metals from the waste streams and activated carbon has been widely used (Obi *et al.* 2016). Cheap agricultural wastes such as sugarcane bagasse, rice husk, coconut husk, coconut shell, saw dust, palm kernel shell, walnut shells, jujube seeds, neem bark, corn cob etc. have been investigated by various researchers as raw materials that can be used to produce activated carbon. These materials are abundant in nature, highly renewable, have high mechanical strength, low ash contents and are also easily available. This makes them good sources of raw materials for activated carbon (Kini *et al.* 2015).

Activated carbon, also known as activated charcoal or activated coal is a common term for carbon materials made up of charcoal. It is a type of processed carbon that has small and low-volume pores that increase the surface area available for chelating chemical reactions and adsorption (Perrich, 2018). Activated carbon is a highly adsorptive medium that has a complex structure composed of carbon atoms (Amin and Alazba, 2017). During the adsorption process, the molecules will be trapped in the internal pores of the carbon structures by Van der Waals Forces or other bonds of attraction and then accumulate into a solid surface (Singh, 2018). Activated carbon is widely used in the treatment of drinking water because it can remove a lot of compounds including heavy metals. Also in waste water treatment, activated carbon plays a very important role in that it is used to remove organic and some inorganic substances.

Corn (*Zea mays*) is one of the largest agricultural commodities in many parts of the world. The use of corn as a food ingredient and most times as a staple diet has led to an increase in corn production, which leaves the cobs to waste without any form of utilization. Therefore to increase the economic value of the plant, corn cobs can be used as basic materials for activated carbon, to serve as bio-adsorbents through an activation process. (Magfiroh *et al.* 2018). Also, according to Farma *et al*. (2018), the structural component of corn cobs i.e. cellulose (41%), hemicellulose (36%), lignin (6%) and low ash content indicates that corn cobs have high potentials and can be used effectively as raw materials for activated carbon production.

On the other hand, the abundant supply of coconut shells makes it another good source of material for activated carbon. They are cheap to acquire and requires no extra amount for procurement. Also, apart from generating an amorphous form of carbon that can absorb a lot of gases, vapours and colloidal solids, coconut shell activated carbons also have high density, high purity and are also dust-free. This makes them very good bio-adsorbents.

As supported by many researchers, the soil has always been a very effective purification medium, its microflora having a great capacity to receive and decompose waste matter, yielding nutrients in the process (Lyakndue *et al.* 2017). However, if the content of the soil pollutants exceeds the soil purifying limit, the efficiency of the soil microbial activity will be reduced considerably. The discharge of POME (untreated and treated) has a direct impact on the minerals, organic matter and microbial community of the soil (Nagaraju *et al.* 2009).

In recent years, the proliferation of traditional palm oil milling centres in several parts of Nigeria and also the continued disposal of the wastes associated with the milling of palm oil even by the mechanised centres has led to an increase in POME generation and subsequently, increased pollution in the environment e.g. nearby farmlands. These reasons have made it justifiable to investigate the impact of treated palm oil mil effluents on soil microflora, in a bid to enhance the biodegrading properties of the soil.

The objective of this study was to assess the effects of raw and treated POME on soil microflora.

Collection of Samples

Soil Sample

Soil samples of 0-15cm (top soil) and 15-30cm (sub soil) were collected from 9 locations in a zigzag manner from an agricultural soil at FUTO farms, Federal University of Technology, Owerri, using a soil auger and spade. The collected top soil samples from the 9 locations were thoroughly mixed on the spot in order to obtain a composite sample, and same was done for the sub soil. Each of the top and sub soil samples were weighed (10kg) and placed into 2 different black polythene bags and transported to the laboratory for air drying (Lyakndue *et al.* 2017)

Palm oil mill effluent (POME) samples

Palm oil mill effluent (POME) samples were collected from a local palm oil mill in Umulolo community, Okigwe Local Government Area, Imo State, Nigeria. The sample were collected in sterile plastic 10 litre containers and transported the same day to the Laboratory for analyses and treatment.

Treatment of POME samples

The palm oil mill effluents (POME) samples were treated using activated carbon from corn cob and coconut shell, individually and then in combination. The agrowastes (coconut shell and corn cob) used for the treatment of the palm oil mill effluents were sourced from Relief market,

Owerri, Imo State. The method used was Batch Adsorption. (Abugu *et al*. 2015)

Pollution of soil samples

Pollution of the dried soil was achieved by employing standard methods (Lyakndue *et al.* 2017). Each of top and sub soil (100g) were weighed out and added into 5 labelled containers (5 for top soil and 5 for sub soil). Measured 40ml of the raw and treated POME were added into the containers and the soil was mixed thoroughly to ensure homogeneity. The polluted and unpolluted (control) top and sub soil were left on the work bench under normal environmental conditions for four (4) weeks.

Microbial Analysis

10-fold Serial Dilution in 4 steps

Serial dilution is done to reduce the microbial load to a countable number. The test tubes for serial dilution were sterilized in the oven (dry heat), at 160C for 1hour. After sterilization, the test tubes were carefully covered with cotton wool and 9ml of sterile distilled water dispensed into each test tube, using a syringe. The syringes for inoculation were appropriately labelled.

One millilitre (1ml) of raw POME was added to one of the test tubes containing 9ml of sterile water, making it up to 10ml. Then, 1ml was pipetted from first tube and added to the next tube, up until the last (4^{th}) tube. From the 4^{th} tube, 1ml was taken out and discarded, then about 3ml was taken from that last tube using a syringe. About 4 drops each (approximately 0.1ml) were inoculated into the agar plates.

Inoculation

After sterilization in the oven, the petri dishes were laid out and appropriately labelled. The agar was allowed to cool before pouring into the petri dishes. Chloramphenicol capsules were added to the dishes containing SDA to inhibit bacterial growth, at a standard measurement of 0.5g/l of media. A spirit lamp was lighted in the laboratory to ensure aseptic conditions. After the foil covering the media was removed, the rims of the beakers were flamed to avoid contamination. Spread plate method was used to inoculate the samples into the solidified media after serial dilution. The petri dishes were placed in the incubator at 37 C for 24hrs. After about 24hours, colonies were visible in the nutrient agar plates.

Subculturing

Appropriate measurements of Nutrient Agar (NA) and Saboraud Dextrose Agar (SDA) were weighed out. The media was prepared and sterilised. Upon cooling, they were poured on Bejou bottles in agar slants and a pure culture of the organisms were inoculated in the bottles. At the end of inoculation, the organisms were placed in the incubator at 37C for 24hours.

Biochemical Tests

Motility Test using Half-Strength Nutrient Agar (NA)

The test tubes were marked and sterilized using oven dry heat (at 160° C for 1hour). After this, the prepared media was sterilized and allowed to cool before pouring into the test tubes. The organisms were inoculated before the marked line. After inoculation, the test tubes were incubated at 37°C for 24hours.

Citrate Utilization Test using Simon Citrate Agar (SCA)

The prepared media was poured in slants and allowed to solidify. The slant culture was inoculated using stab inoculation technique, the butt was stabbed and the slope was streaked. After inoculation, the test tubes were incubated at 37°C for 24 hours.

Sugar fermentation Test using Triple Sugar Iron Agar (TSI)

The prepared media was poured in slants and allowed to solidify. The slant culture was inoculated using stab inoculation technique. The butt was stabbed while the slope was streaked. After inoculation, the test tubes were incubated at 37°C for 24 hours.

Indole Test using Peptone Water (PW)

The prepared media was sterilised and allowed to cool before poured into the labelled test tubes. After inoculation, the test tubes were incubated at 37°C for 24 hours. At the end of 24 hours, Kovac's reagent was introduced into the test tubes.

Catalase Test

Hydrogen peroxide (4ml) was added to each of the sterilized test tubes. The wooden sticks for inoculation were sterilised by wiping with 99% ethanol. Using the sticks, the organisms were collected from the pure culture and inoculated into the test tubes containing hydrogen peroxide. The test tubes were allowed to sit for a while. Active bubbles in the test tubes indicated a positive result.

Oxidase Test

Peptone water broth was used from the Indole Test (before the addition of the Kovac's reagent but 24hrs after inoculation). Oxidase strips were impregnated with oxidase reagent (this was done by pouring the oxidase reagent on the strips and drying in the oven at 60°C minimum temperature for 1hour). The oxidase strips were placed in a petri dish, and the peptone water broth containing the organisms were poured on the strips. The petri dish was allowed to sit for a while. A purple colour change indicated a positive result.

Gram Staining

The slides were washed with water and ethanol to wash off the stains and grease. They were appropriately labelled. After labelling, the slides were once again wiped with ethanol. Using a syringe, a drop of water was dropped on each slide. Prior to this, an ethanol lamp was lighted in the laboratory for sterility. Using a sterilised wire loop (sterilised by flaming), the organisms were collected from the pure culture and smeared on the slides. After smearing, the slides were fixed by allowing to air-dry. Using a stop-watch, Crystal violet dye (60 secs), Lugol's Iodine (60secs), Ethanol (10secs) and Safaranine (secondary stain, 2mins) were used to flood the slides in that order.

At the time of expiration of each dye, the slides were flooded with water before flooding with the next dye. Afterwards, the slides were fixed by allowing to air-dry.

Spore Staining Test

The clean slides were appropriately labelled and placed over hot water steam. Malachite green stain was flooded on the slides for 5mins. If the stain dried up before 5mins was up, the slides were flooded again with the stain. At the end of 5mins, the slides were flooded with water and flooded with safaranine dye for 2mins, after which they were flooded again with water. Afterwards, the slides were fixed by allowing to air-dry.

Coagulase Test

Blood specimen (2ml) was allowed to separate and the serum collected. 0.1ml of the serum were dropped on each of the slides and the organisms were inoculated using a flamed wire loop. Clumps on the surface of the slides indicated a positive result while smooth surfaces indicated a negative result.

Lactophenol cotton blue stain (Fungi)

The slides were washed with water and wiped with ethanol. Lactophenol stain was dropped on the slides. The isolates were teased out and placed on the slides using an inoculating needle, and the slides were viewed under the microscope.

Characterization and Identification of Isolates

Various methods were used to characterize and identify the isolates (Antai *et al*., 2014; Mill 2017). The test results for bacteria were evaluated using characteristics presented in Bergey's Manual of Determinative Bacteriology as used by Lyakndue *et al.* (2017).

Representative colonies of fungal isolates were characterized and identified based on their cultural and morphological features. The characteristics were identified through staining techniques, with the use of lactophenol cotton blue.

Results

The results of the total microbial load of the untreated Top and Sub-soil samples as well as the raw palm oil mill effluent (POME) as presented in **Table 1** which show that the untreated Top Soil had total viable count (TVC) of $4.01x10^7$cfu/g while the subsoil had TVC of $3.90x10^7$cfu/g and the raw POME had TVC of $3.28x10^7$cfu/g. The pome treated with the activated carbons from agrowastes showed a reduction in the microbial load when compared with the raw POME. The Coconut shell treated POME (PCS) showed the highest reduction in the microbial load ($5.7x10^6$cfulg) while the combination of the Coconut shell and corn cob (PC+S) showed the least reduction ($2.04x10^7$cfu/g). According to Pritha and Karpagam (2019), the more solid nature of the coconut shell may have absorbed more microorganisms by impingement. The same authors reported that the antimicrobial properties of coconut shell may have also contributed to these reductions. Total coliform count (TCC), was obtained only with the POME treated with corn cob and coconut shell ($1.0x10^5$cfu/g) among the three agro waste-treated POME samples. The POME treated with the combination of corn cob and coconut shell also showed the highest total staphylococcal counts (TSC) and total fungal count (TFC) of $3.0x10^6$cfu/g and $2.03x10^7$cfu/g respectively. The total viable count of the untreated subsoil and topsoil showed the highest value of $3.90x1o^7$cfu/g and $4.01x10^7$cfu/g and also showed considerable staphylococcal and fungal counts. However, there was a reduction in the microbial load when watered with the raw palm oil mill effluent. Such reduction revealed the adverse effect of the palm oil mill effluent on the soil microbiota.

Morphological and Biochemical characterization of Bacterial Isolates

The results of the morphological and biochemical characterization of the bacterial isolates are presented in Table 2. During this study, a total of 13 colonies were primarily selected from different culture media. Among them, eight (8) representative isolates were randomly selected for detailed studies towards identification based on colony morphology, microscopic observation, physiological and biochemical characteristics. Colonies of the isolates were found to be different in form, elevation, margin, surface, color and optical characteristics. Among the Gram positive bacterial isolates, three (3) were rod shaped while two (2) were cocci. On the other hand, the gram negative bacteria were short rods and non-spore forming. Among the tested isolates, four (4) were catalase positive, while four (4) were catalase negative. Five (5) of the tested isolates were able to utilize citrate as the only carbon source. Of all the isolates, only one (1) showed positive results for motility test and could produce the enzyme oxidase. Only two (2) isolates formed complex reddish rings with Kovac's reagent and therefore tested positive for Indole Test. Only four (4) organisms produced gas and none produced H_2S in test for sugar fermentation. Only one (1) organism tested positive for Coagulase test.

Table 3 showed the percentage occurrence of the bacterial isolates from the samples. *Klebsiella pneumoniae* had the highest occurrence (21.31%), while *Bacillus cereus* showed the least (8.84%).

Table 4 shows the results of the fungal isolates obtained from the samples using Lactophenol cotton blue stain. The molds showed both branched and unbranched hyphae when viewed under the microscope. Three out of the branched hyphae had spores bearing sterigmata. One isolate was unbranched with terminal spores and one isolate was identified as budded yeast cells.

Table 5 shows the results of the percentage occurrence of the fungal isolates. *Penicillum chyrysogenum* had the highest (36.36%), while *Aspergillus niger* had the least (0.09%).

Table 1. Total microbial load of treated, untreated pome and soil samples.

Sample	TVC (cfu/g)	TCC (cfu/g)	TSC (cfu/g)	TPC (cfu/g)	TFC (cfu/g)
TS CONTROL	$4.01x10^{7\,a}$	$1.20x10^{6\,a}$	$1.80x10^{6\,a}$	$1.30x10^{6\,a}$	$1.00x10^{6\,b}$
SS CONTROL	$3.90x10^7$	$1.00\ x10^6$	$8.00x10^5$	$1.00x10^6$	4.00x10
RP	$3.28\ x\ 10^7$	$1.00\ x\ 10^5$	$3.70\ x\ 10^6$	-	$6.80\ x\ 10^6$
PCC	$1.03\ x\ 10^7$	-	$1.10\ x\ 10^6$	-	$5.00\ x\ 10^6$
PCS	$5.70\ x\ 10^6$	-	$2.50\ x\ 10^6$	-	$9.00\ x\ 10^6$
PCC+CS	$2.04\ x\ 10^7$	$1.00\ x\ 10^5$	$3.00\ x\ 10^6$	-	$2.03\ x\ 10^7$
TrP	$2.80\ x10^6$	$7.00\ x\ 10^5$	$2.00\ x\ 10^5$	-	$1.20\ x\ 10^7$
SrP	$6.90\ x\ 10^6$	$7.00x10^5$	$1.20x10^6$	-	$1.10x10^6$
TCC	$2.70x10^6$	$1.00x10^5$	$7.00x10^5$	$1.30x10^6$	$1.00x10^5$
SCC	$4.50x10^6$	$2.00x10^5$	$9.00x10^5$	$1.70x10^6$	$5.00x10^5$
TCS	$5.10x10^6$	-	$1.90x10^6$		$2.00x10^6$
SCS	$2.67x10^7$	$8.00x10^5$	$8.00x10^5$	$1.00x10^6$	$1.21x10^7$
TCC+CS	$7.00x10^6$	$3.00x10^5$	$9.00x10^5$	$8.00x10^5$	$1.90x10^5$
SCC+CS	$1.16x10^7$	$1.00x10^5$	$1.00x10^5$	$1.10x10^6$	$9.00x10^5$

TVC = Total viable count, TCC = Total coliform count, TSC = Total staphylococcal count, TPC = Total pseudomonas count, TFC = Total fungal count, RP = Raw POME, C= corn cob, CS= coconut shell, CC= corn cob, CS= coconut shell, TrP= top soil polluted with raw POME, SrP= sub soil polluted with raw POME, TCC= top soil watered with CC treated POME, SCC= sub soil watered with CC treated POME, TCS== top soil treated watered with CS treated POME, SCS=sub soil watered with CS treated POME, TCC+CS= top soil watered with CC+CS treated POME, SCC+CS= sub soil watered with CC+CS treated POME.

Table 2. Morphological and biochemical characteristics of bacterial isolates.

Media	Morphological characteristics	Gram reaction	Oxidase Test	Mot Test	Indole Test	Spore G@5test	CataTest	Citrate Test	Coagulase Test	Sugar ferm. TestS B G H_2 S	Possible bacteria
M.S.A	Golden yellow raised non mucoid colonies	Gram positive cocci	_	_	_	_ ND	+	_	+	R Y + _	*Staphylococcus aureus*
N.A	Milkish flat non mucoid colonies with rough edges	Gram positive rod	_	_	_	+ ND	+	+	_	R Y _ _	*Bacillus subtilis.*
C.A	Bright greenish pigmented colonies	Gram negative rod	+	+	+	_ ND	_	+	_	R Y_ _	*Pseudomonas aeruginosa*
N.A	Milkish flat non mucoid rhizoid-like colonies	Gram positive rod	_	_	_	+ ND	+	+	_	R Y _ _	*Bacillus cereus*
M.A	Pinkish raised mucoid colonies	Gram negative rod	_	_	+	_ ND	_	+	_	Y Y + _	*Escherichia coli*
N.A	Milkish raised needled-pointed non mucoid colonies	Gram positiverod	_	_	_	_ ND	+	_	_	R R + _	*Corynebacterium glaucum*
N.A	milkish raised extremely mucoid colonies	Gram negative rod	_	_	_	_ +	_	+	_	R Y $+^2$ _	*Klebsiella pneumoniae*
N.A	Milkish raised non mucoid colonies growing in chains	Gram positive cocci in chains	_	_	_	_ ND	_	_	_	R R _ _	*Streptococcus faecalis*

Key: M.S.A = Mannitol salt agar, M.A = Macconkey agar, N.A=Nutrient agar., C.A=Centrimide agar; + = positive, -=negative, S=slope coloration, B=butt colouration, G=gas production, H_2S=Hydrogen sulphide production, Y=Yellowish coloration (acidic), R=Reddish pinkish coloration (alkaline production); G@5 = Growth at 5^0C.

Table 3. Percentage occurrence of the bacterial isolates.

Isolates	Samples	No. of Samples	Occurrence	percentage occurrence %
Klebsiella pneumoniae	PCC+CS, RP, PCC, TCS, PCS, TCC, SCC, SrP, SCS, SCC+CS, TrP, UTS, USS	14	13	21.31
Bacillus subtilis	PCC+CS, RP, PCC, TCS, SCC, SCS, SCC+CS, TCC+CS, TrP	14	9	14.75
Staphylococcus alreus	PCC+CS, PCC, TCS, TCC, SCC, SrP, SCS, SCC+CS, TCC+CS, TrP	14	10	16.39
Corynebacterium glaucum	RP, TCS, PCS, SrP, TrP, USS	14	6	9.84
Bacillus cereus	RP, PCC, SCS, TCC+CS, UTS, USS	14	6	8.84
Pseudomonas aeruginusa	TCC, SCC, SCS, TCC+CS, UTS, USS	14	6	9.84
Streptococcus fecaelis	SCC+CS, UTS, USS	14	3	4.92
Escherichia coli	SCS, TrP, SCC+CS, SrP, RP, PCC+CS, TCC+CS, TCC	14	8	13.11
TOTAL				61.100

RP = Raw POME, CC= corn cob, CS= coconut shell, CC= corn cob, CS= coconut shell, TrP= top soil polluted with raw POME, SrP= sub soil polluted with raw POME, TCC= top soil watered with CC treated POME, SCC= sub soil watered with CC treated POME, TCS== top soil treated watered with CS treated POME, SCS=sub soil watered with CS treated POME, TCC+CS= top soil watered with CC+CS treated POME, SCC+CS= sub soil watered with CC+CS treated POME.

Table 4. Identification of Fungal Isolates.

Macroscopic characteristics On SDA	Microscopic appearance	Possible Fungi
Whitish broom like cottony colony with yellowish green centre	Septate hyphae with conidia bearing sterigmata	*Aspergillus niger*
Whitish broom like centre with bluish green centre	septate hyphae with spores	*Penicillium sp*
Whitish broom-like cottony colonies	Non septate hyphae with terminal spores	*Rhizopus stolonifera*
Creamy raised non - mucoid colonies	Budded yeast cells	*Candida albicans*
Whitish broom like cottony colonies with whitish elevated centre that later turned brown	Septate hyphae with erect and conidiophores and conidia	*Cladosporium herbarum*

Table 5. Percentage Occurrence of the Fungal Isolates.

Isolates	Samples	No. of Samples	Occurrence	percentage occurrence %
Cladosporium herbarum	SCS, TrP, PCC, TCC+CS, PCS, TCS	14	6	27.27
Aspergillums niger	SCS, SrP	14	2	0.09
Penicillium chrysogenum	SCS, SCC, TrP, PCC+CS, SCC+CS, TCC+CS, TCC, TCS	14	8	3636
Rhizopus stolonifera	SCC, SCC+CS	14	2	9.09
Candida albicans	PCC+CS, PCC, PCS, RP	14	4	18.19
Total			22	100

Discussions

From Table 1, soil irrigation with CC treated POME at the topsoil and CC+CS treated POME at the subsoil would be a cost-effective method for controlling total coliforms threats to surface and subsurface water bodies. The decomposition of POME by soil microbes could have induced O_2-depletion in the surface soil, thereby inhibiting aerobic microbial activity (Nwoko and Ogunyemi, 2010). It is generally believed that the toxicity effects of POME can be attributed to the presence of phenols and other organic acids which are responsible for their phytotoxic effects and antibacterial activity (Pascual *et al*. 2007). In a study carried out by Ezeokoye *et al.* (2018), the total culturable hydrocarbon utilizing fungal count in the polluted soil pre remediation, during remediation, after remediation and the control were $1.51 \times 10^4 \pm 0.03$cfu/g, $1.77 \times 10^5 \pm 0.02$ cfu/g, $6.01 \times 10^3 \pm 0.06$ cfu/g and $8.15 \times 10^4 \pm 0.13$cfu/g, respectively. The difference in counts could be due to pH and organic matter content which could aid the proliferation of microorganisms (Ameh and Kawo, 2017).

In a microbiological analysis of palm oil mill effluent from Nigeria Institute for Oil Palm Research (NIFOR), Osaro (2002) reported that the genera *Pseudomonas* sp, *Bacillus* sp, *Penicillium* sp and *Aspergillus* sp were predominant. Eze *et al*. (2014) also reported bacterial isolates to be *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella* sp., *Staphylococcus aureus*, *Micrococcus* sp., *Bacillus* sp., *Citrobacter* sp. And *Streptococcus* sp. which are similar to the bacteria isolates recorded in this study. The breakdown of petroleum hydrocarbon by fungi particularly of the genera *Aspergillus, Mucor, Penicillium* and *Fusarium* has been reported by several authors (Obire *et al*. 2008; Ibiene *et al*. 2011). *Aspergillus* species in particular are reported to be good producers of cellulase, the enzyme responsible for the breakdown of cellulose in petroleum products (Wong *et al*. 2008). There was higher population of bacteria compared to fungi populations in this study. Similarly, Gomez *et al*. (2014) reported significantly lower F: B ratio in soil samples amended with fast pyrolysis activated carbon after 12 months of incubation. It is notable that the F: B ratio of activated carbon amended soil depends on its C:N ratio, as a result of activated carbon application (Brewer *et al*. 2011; Farrell *et al*. 2013; Muhammad *et al*. 2014) or its native C:N ratio status (Rousk *et al*. 2013). However, the results were in agreement with Hu *et al*. (2014) who found 12%, 30% and 37% higher bacterial diversity and 17%, 40% and 23% lower fungal diversity in forest-litter-activated carbon amended loamy soil. The variations in the range of microbial populations are an indication of several reasons such as nutrient, minerals, temperature, oxygen level, acidity, volume of wastewater (Okereke *et al.* 2007), concentration of oil and grease and sugars in the POME.

In general, activated carbon increases the soil microbial community and biomass (Zhang *et al.* 2014; Demisie and Zhang, 2015; Xu *et al*. 2016) mainly by providing favourable habitat and labile C needed by the microbes. Also, activated carbon pores serve as a habitat (Pietikainen *et al*. 2000; Warnock *et al*. 2007; Quilliam *et al*. 2013; Jaafar *et al*. 2014) and refuge to soil microorganisms such as bacteria (size range from 0.3 to 3 mm), fungi (2–80 mm), and protozoa (7–30 mm), which protect them from predatory soil microarthropods (Warnock *et al*. 2007). However, activated carbon may also negatively affect microbial biomass, especially when applied at high concentrations (Zhang *et al.* 2014; Demisie and Zhang, 2015), though this negative effect varies with soil properties. This was not reported in the present study as the activated carbon was not directly applied on soil but on the POME samples used to water the soil samples. It is important to note that volatile compounds (such as benzene) found in activated carbon also have the potential to decrease soil microbial biomass (Girvan *et al.* 2005; Dempster *et al.* 2012). Increase in microbial biomass leads to an increase in microbial activities e.g. soil respiration (Kolb *et al.* 2009; Smith *et al.* 2010; Xu *et al.* 2016) and therefore could lead to an increase in soil fertility. This is in line with the results reported in this study as there was a general increase in the TVC, TCC, TSC and TPC (7.0×10^6, 3.0×10^5, 9.0×10^5 and 8.0^5 cfu/g) respectively of top soil watered with CC + CS treated POME. Total fungal count

(TFC) also showed highest values (9.0 x 10^5 cfu/g) in sub soil sample watered with CC+CS treated POME. Therefore, POME treatment with activated carbon derived from a combination of corn cob and coconut shell agrowastes can be used in conditioning the soil for more microbial growth, thereby improving soil fertility.

Conclusion

This work has clearly shown that freshly prepared palm oil mill effluent (POME) reduces soil microbial load possibly due to some physiochemical composition of the POME such as high Biochemical Oxygen Demand (BOD). However, treatment of POME with agrowastes such as corn cob and coconut shell reduces the microbial load of the raw POME and also improves the biochemical parameters of POME and applying this treated waste increases the microbial load of the receiving subsoil and topsoil when compared with the soil polluted with the raw POME. The soil samples watered with the CC + CS treated POME also showed higher microbial diversity - higher total coliform count, total viable count, total staphylococcal count, total fungi count and total pseudomonas count and so POME treatment with activated carbon from a combination of corn cob and coconut shells is a good option for its treatment before discharge.

Recommendation

Based on the findings of this work, the researcher recommends that POME be treated with cheap and readily available agrowastes such as corn cob and coconut shell before depositing them on arable soil to avoid the reduction of soil microbial diversity.

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