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MICROBIOLOGICAL AND PHYSICOCHEMICAL ASSESSMENT OF TANNERY POLLUTED SOIL INOCULATED WITH PURE AND MIXED FUNGI SLURRY

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ABSTRACT
The biological remediation capacity of tannery waste polluted soil by individual Aspergillus aculeatus (MH634499.1) isolated from unpolluted soil, Aspergillus niger (MH780917.1) isolated from tannery waste polluted soil and Co-slurry of both fungi was analyzed. From the polluted soil, microbial and physicochemical parameters were determined before amendments and amended tannery waste polluted soil was monitored for two weeks interval up to 12 weeks; Nutrient agar (NA) was used for total heterotrophic bacteria count (THBC), Sabouraud dextrose agar (SDA) for total heterotrophic fungi counts (THFC), minimal salt agar (MSA) for total tannery waste utilizing bacteria counts (TtWUBC) and total tannery waste utilizing fungi counts (TtWUFC) while pH, Phosphorus, Nitrogen, Organic Carbon, potassium, sodium, Calcium, Magnesium, Cation Exchange Capacity and Soil texture as Physicochemical. Twelve (12) conical flasks each with 1000ml sabouraud dextrose broth and colony of each fungi were incubated in a rotary shaker at 300C for 7days. These were used as slurry for bioremediation. The soil polluted with tannery waste 500g each were amended with fungi slurry containing Spore cells 105 per gram of soil in the designed treatments, T1, T2, T3 and T4. The pollution intensity of the tannery waste polluted soil before amendment include THBC (2.5×106 CFU/g), TtWUBC (1.5×106 CFU/g), THFC (1.5×105 CFU/g) and TtWUFC (8×103 CFU/g) microbiologically while pH 7.5, organic carbon (4.7%), Nitrogen (0.22%), Phosphorus (1.25mg/kg), Calcium (3.75mg/kg), Magnesium (3.55mg/kg), sodium (20mg/kg), potassium (12.1mg/kg), CEC (47.3mg/kg) were physicochemical properties. The mean range of the amended tannery waste polluted soil had pH 7.5-8.8 with organic carbon reduced from 4.7-1.1%, Nitrogen (0.2-0.09mg/kg), phosphorus (1.25-0.69mg/kg), calcium (3.75-2.02mg/kg), magnesium (3.55-0.12mg/kg), potassium (12.1-3.6mg/kg) and sodium (20-6.9mg/kg). In the microbial changes, THBC, TtWUBC, THFC and TtWUFC were significantly different ($P<0.05$) in all the treatment sets which takes the form T3>T2>T1>T4, T4<T1<T2<T3, T2>T3>T1>T4, and T3>T2>T1>T4 respectively. Meanwhile, Co-slurry indicated greater remediation potential in the polluted soil than individual fungi slurry.

Keywords: tannery waste pollution, soil, microbial, organic carbon, treatment

INTRODUCTION

Limited research information has been available on the use of fungi to solve environmental problems, although they have strong morphology and varied metabolic strength due to which they are specially suited for bio-restoration of land (Deshmukh *et al*., 2016). Fungi are eukaryotes, heterotrophs that feed by ingestion after digestion (Taddei and Gasser, 2012). They do not produce their own food; they always depend on available and already processed nutrients from other organisms. The organisms uses enzyme to break down nutrients. Some fungi grow rapidly while others grow slowly, depending on the growth conditions. They characteristically form long hyphae, branched to give a mycelium (Bridge and Spooner, 2001). They have the ability to spread their mycelium covering large surface area.

The strategic process of treating animal skins into desired leather products have been accomplished in a particular area of land referred as tannery. During treatment process in tannery, residual substances including wash and falls of the remains of substances used (NaCl, $NaSO_4$, Cr_2SO_4 and many other biocides) are found in higher concentration in the soil (Junior *et al*., 2006). Tanneries are always moistened because the process use up huge amount of water and produces about two third of the consumed water as effluents or waste water (Chowdhury *et al*., 2013). The effluents induce heavy pollution load as a result of massive containment of synthetic compounds, variety of organic and inorganic matters, Sulphate compound and sodium chloride, toxic metals, various tanning agents that can either be use directly or indirectly by microbes and mass decayed suspended matter (Akan *et al*., 2007).

Many precise scientific technical processes available for use to limit or vanish the substances that their concentration may passes the permissible limit accumulating soil. Bioremediation is ecologically amicable which depends on natural machinery to

lower the pollutants quantity by either removing or petrifying pollutants towards a safe status (Azubuike *et al*., 2016). The technique have been a good alternative to common traditional clean-up technologies in the field, and has been used in a number of areas like Europe with effective level of achievement (Vidali, 2001). This study was designed to determine bioremediation effectivity on tannery polluted soil of *Aspergillus aculeatus, Aspergillus niger* and combined activity of both isolates (in their slurry form).

MATERIALS AND METHODS

Study Area

The area of study was Unguwar Rogo, Rijiyar Dorowa and Tudun wada located within the metropolitan of sokoto state, Nigeria (Fig. 1). The seats of the caliphate, sokoto state have some certain areas that carryout tanning process at each and every day. While carrying out skin treatments, extensive water volume, wastewater or effluents has been discharged to the surrounding thereby polluting the soil.

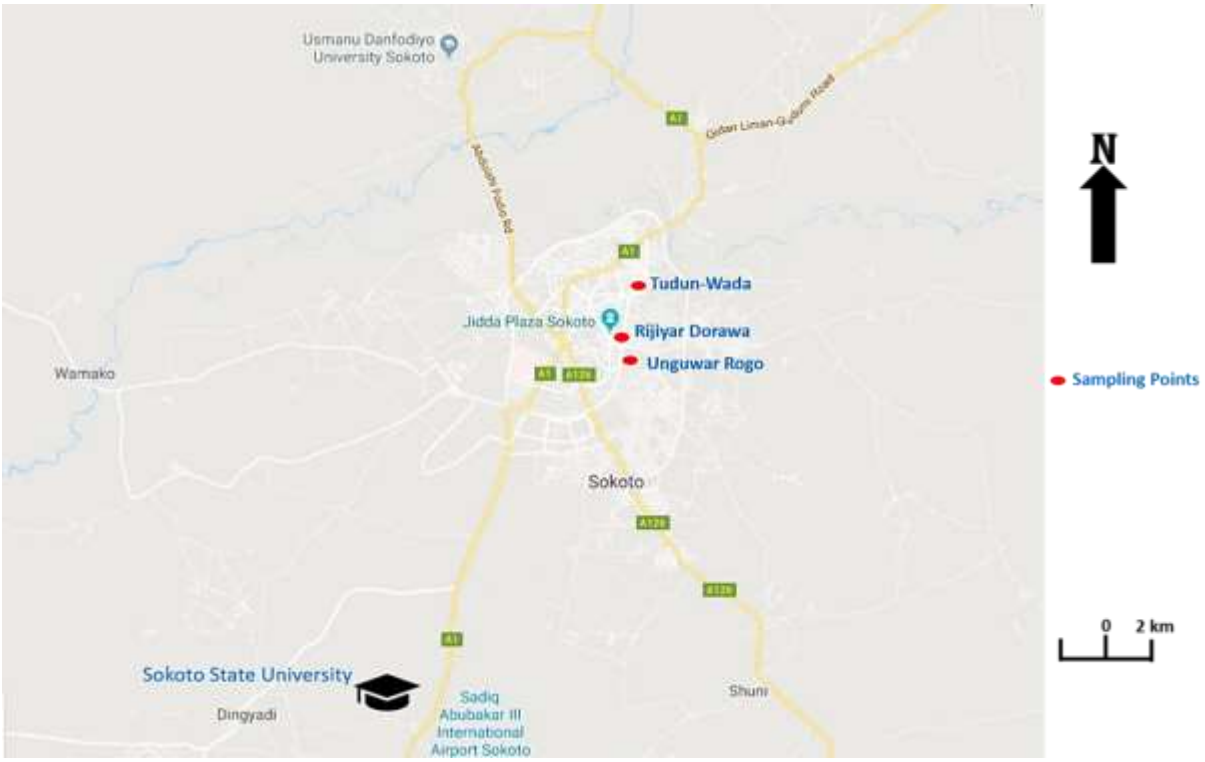

Fig. 1: Sampling Point and Their Position in Sokoto

Collection of Samples

Clean bags of polyethylene were used during collection of tannery polluted soil. Samples were collected randomly from a spot in the study area from a depth of 14cm below ground and were immediately transported to the laboratory. Soils from different sites were mixed together in order to get composite sample (Khan *et al*., 2007). The physicochemical and microbiological properties of the soil samples were determined.

Determination of Microbiological Properties of Tannery Waste Polluted Soil before Amendments.

Tannery waste polluted soil sample was serially diluted and spread inoculated on agar medium; Sabouraud Dextrose Agar (SDA) for total heterotrophic fungi count (THFC) added with Streptomycin (1mg/100ml) to inhibit the growth of bacteria. Nutrient agar for total heterotrophic bacteria count (THBC) added with Fluconazole (1mg/100ml) to inhibit fungal growth (Ameh and Kawo, 2017). Minimal salt agar (MSA) mixed with tannery effluents (1% per 100ml water); added with Streptomycin (1mg/100ml) for total tannery waste utilizing fungi counts (TtWUFC) and that added with Fluconazole (1mg/100ml) for total tannery waste utilizing bacteria counts (TtWUBC). The plates (for fungi) were incubated at 25^0C for 5days while plates (for bacteria) were incubated at 37^0C for 24hours. Microbial colonies which developed on the agar plates were counted and reported as colony forming units per gram of soil (CFU/g) (Obiakalaije *et al*., 2015).

Determination of Physicochemical Properties of Tannery Waste Polluted Soil

The soil physicochemical properties were determined by adopting the procedure described by Sadiq and Malami (2009) and Rabah and Ibrahim (2010).

pH Determination

This was obtained by the use of pH meter (model 8314, Hanna HI, U. K.). Twenty mililitres (20ml) of distilled water was

poured in a beaker and stirred together with 20g of sample (soil). The mixture was allowed to settle for 30minutes. The pH meter electrode was then put into the mixture after buffer solution was used to standardize the pH meter. Readings of pH were taken.

Phosphorus Determination

Phosphorus was determined by Vanado-molybdo phosphoric acid colometric method. In acidic condition, ammonium molybdate produces molybdo-phosphoric acid. Spectrophotometer was used which measured the color intensity of the mixture.

Nitrogen Determination Macro-kjeldahl method for nitrogen determination were adopted for this research. Two grams (2g) of soil sample was transferred to kjeldahl flask followed by addition of kjeldahl catalyst (K_2SO_4-HgO) and 10ml of concentrated H_2SO_4 was added to the mixture. This was heated for one hour and allowed to stand until the mixture became clear. This was followed by addition of 50ml of distilled water to the flask containing the mixture. The digest was transferred into another clean kjeldahl flask and 20ml of 40% NaOH was added. The flask was mounted on the distillation apparatus. Twenty millilitres (20ml) of boric acid (H_3BO_3) indicator was added into a conical flask and placed under the condenser thereafter distillation process commenced. Forty millilitres (40ml) of the distillate was collected and the distillate was titrated against 0.01M H_2SO_4 from green to pink color endpoint. Titre value was recorded.

$$\%N = \frac{14\times M\times V+Vx}{\text{Weight of sample (mg)}\times vx}\times 100$$

where M= Actual molarity of acid

V= Titre volume of H_2SO_4 used

Vx= Aliquot volume distilled

Organic Carbon Determination

Walkey-Black method procedure for the organic carbon determination and was adopted for this research. Soil sample (1g) was placed in 250ml conical flask followed by addition of 10ml 1N K_2 Cr_2 O7 solution and 10ml of Concentrated H_2SO_4. The mixture contained in the flask was whirled and allowed to stand for 30minutes. One hundred millilitres (100ml) of deionized water was added into the flask and a drop of barium diphenylamine indicator was added. The mixture was titrated against 0.5M ferrous sulfate solution to dark green color endpoint. Titre value was recorded.

$$\text{Org. C (\%) is given by} = \frac{\text{Black titre (B)}-\text{sample titre (T)}\times 0.003\times 0.5\times 1.33}{\text{weight of the sample (x)}}$$

Potassium and Sodium Determination

Flame photometer method was used. The photometer was arranged according to the manufacturer's instruction. The instrument was set to zero reading using distilled water and was set to 100% transmittance by feed with 10ppm potassium and sodium solution. The standard solution and soil extract was regulated. From the standard curve the value of K (potassium) and Na (sodium) present in the soil as milliequivalent per 100g was determined.

Calcium and Magnesium Determination

Ethylenediaminetetra-acetic acid (EDTA) method was used. Soil extract (1ml) was introduced into the conical flask and 19ml of distilled water was added to the flask. For calcium determination, 1ml 10% NaOH was added to the mixture. A tip of murexid indicator was added to the mixture and titrated against 0.01M EDTA from pink to purple endpoint. Titre value was recorded.

For magnesium determination, 5ml buffer solution and 2-drops of ericochrome black T indicator was added to the mixture titrated against 0.01ml EDTA from purple to blue endpoint. The titre value was recorded.

Determination of Cation Exchange Capacity (CEC)

Kjeldahl distillation method was used. The soil extract was put into kjeldahl flask together with 50ml distilled water. Twenty millilitres (20ml) of 40% NaOH was added to the mixture. The flask was mounted on the distillation apparatus and 20ml boric acid indicator was added into the conical flask by placing under the condenser and distillation commenced. Forty millilitre (40ml) of the distillate was collected and filtrated against 0.1M HCl from green to pink color endpoint. The titre value was recorded.

Determination of Soil Texture (Sand, Clay and Silt)

Hydrometer method of soil analysis was used. Fifty one grams (51g) of soil sample was placed into 1000ml capacity measuring cylinder containing 500ml of water and 50ml of 5% calgon solution was added to the mixture and stirred for 15minutes. Soil hydrometer was inserted and water was added to 1000ml mark. The hydrometer was removed. The cylinder was covered, inverted severally and then placed on a flat surface. Hydrometer was inserted into the mixture again. First reading (H_1) was taken after 40seconds. Thermometer was inserted and first temperature reading (T_1) was taken. The mixture was allowed to stand again for 3hours. The second hydrometer (H_2) and second temperature (T_2) readings were taken.

The formulae used to determine the values are as follows:

$$\text{Sand} = \frac{51-(H_1-2)0.3\,(T_1-20)}{51}\times 100$$

$$\text{Clay} = \frac{51-(H_2-2)\,0.3(T_2-20)}{51}\times 100$$

$$\text{Silt} = 100-(\%\text{Sand}+\%\text{Clay})$$

Soil texture was determined by the use of textural triangle.

Preparation of Fungal Slurry

Fungal slurry was prepared by picking 7days old colony culture of each isolate and inoculated in sterile prepared saboraud dextrose broth (SDB) contained in 500ml Erlenmeyer flask.

These were incubated in a rotary shaker (150rpm) at 30^0C for 7days (Asgher *et al*., 2012).

Inoculation of Tannery Waste Polluted Soil with Fungi Slurry

The experimental design adopted was completely randomized design (CRD). The soil sample was dispensed in 500g of four (4) sets of 8containers. Bioremediation evaluations were carried out for 3 months. Each set was inoculated with 200ml fungal slurry (Akpomie and Ejechi, 2016) for a concentration of 10^5 spore cells per gram of soil for each isolate carrying each treatment (Olivier *et al*., 2004). The first set-up consisted of slurry of *Aspergillus aculeatus* (T1). The 2nd set contained slurry of *Aspergillus niger* (T2) while the 3rd contained co-culture of both fungi (T3). Control (T4) was the 4th set containing no test organism. Each treatment was in duplicates. These were incubated aerobically at room temperature (25±2˚C) in the laboratory for 84days.

Monitoring of Remediated Tannery Waste Polluted Soil

The amended tannery waste polluted soil was sampled and monitored for microbiological and physicochemical properties (for two week interval up to 12 weeks).

Microbiological Parameters

i) Enumeration of Total Aerobic Heterotrophic Bacteria

Sub-sample of soil (25g) in 225ml clean sterile water was used and serially diluted. The dilution 10^{-6} (0.1ml) was spread plated (in duplicates) on sterile nutrient agar (NA) containing Streptomycin (1mg/100ml, Ameh and Kawo, 2017) for the enumeration of aerobic THB (Obiakalaije *et al*., 2015). The plates were incubated at 37˚C for 18-48hours (Salami and Atoyebi, 2017). The colonies were counted and reported as CFU/g (colony forming units per gram) of soil sample.

ii) Enumeration of Tannery Waste Utilizing Bacteria

The bacteria used tannery waste and its constituents, TtWUB were enumerated on minimal salt agar (MSA) containing (g/1000ml): K_2HPO_4, 6.8; $NaHPO_4$. $2H_2O$, 7.8; NH_4Cl, 2.0; $MgSO_4$, 0.2; $Ca(NO_3)_2.4H_2O$, 0.05, and agar, 20g (Thakur and Srivastava, 2006), tannery effluent, 1% in 1000ml of the medium (Abioye *et al*., 2009) amended with fluconazole (1mg per 100ml, Ameh and Kawo, 2017) as fungal growth inhibitor. The plates were incubated at 37˚C for 48hours. The colonies were counted and reported as colony forming units per gram (CFU/g) of soil.

iii) Enumeration of Total Heterotrophic Fungi

Soil sample (1g) was diluted serially to 10^{-6}. Aliquot (0.1ml) of 10^{-4} dilution was

inoculated (in duplicates) onto plates of Sabouraud dextrose agar (SDA) containing Streptomycin antibiotics (1mg/100ml, Ameh and Kawo, 2017) and spread plated using sterile bent glass rod (Obiakalaije *et al*., 2015). The plates were incubated at 30˚C for 5-7days. Colonies were counted and reported as colony forming units per gram (CFU/g) of soil.

iv) Enumeration of Tannery Waste Utilizing Fungi

Minimal salt agar (MSA) containing tannery effluents (1%) was used and amended with streptomycin (1mg per 100ml, Ameh and Kawo, 2017) to suppress bacterial growth. Aliquot (0.1ml) of 10^{-4} dilution was spread plated, incubated at 30˚C for 5-7days. Colonies were counted and reported as colony forming units per gram (CFU/g) of soil (Ameh and Kawo, 2017).

Determination of Physicochemical Properties of the Amended Soil

Parameters including pH, Organic carbon, total nitrogen, Phosphorus, Potassium, sodium, Calcium, Magnesium and Cation exchange Capacity were determined using procedures outlined previously in the present study.

Statistical Analysis of Data

GenStat Seventeenth edition PL 25.1 statistical software was used for the analysis. Comparison of means between treatment sets carrying slurry of fungi and control sets without inoculum was used. Analysis of variance (ANOVA) was adopted and determined where there were mean differences. Level of significance was at $P \leq 0.05$. Results were presented in graphical lines carrying error bars with standard deviation.

RESULTS

Microbiological and Physicochemical Characteristics of Tannery Waste Polluted Soil Before Amendment with Fungal Slurry

Microbial Properties

The microbiological characteristics of soil polluted with tannery waste before slurry amendments are presented in Table 1. The total heterotrophic bacterial count (THBC) were 2.5×10^6 CFU/g and tannery waste utilizing bacterial counts were 1.5×10^6 CFU/g. The total heterotrophic fungal count were 1.5×10^5 CFU/g and 8×10^3(CFU/g) for total tannery waste utilizing fungal count.

Physicochemical Properties

The values for physicochemical characteristics of the tannery waste polluted soil before amendments were 7.5, 4.7%, 0.22%, 1.25mg/kg, 3.75 mg/kg, 3.55 mg/kg, 12.1 mg/kg, 20.0 mg/kg, 47.3 mg/kg for pH, Organic carbon, nitrogen, phosphorus, calcium, magnesium, potassium, sodium and cation exchange capacity (CEC) respectively, while the soil texture was determined as sandy loam with 72.5%, 11.7% and 15.7% for sand, clay and silt respectively (Table 1).

Bioremediation of Tannery Waste Polluted Soil

The microbiological and physicochemical changes in the amended soil were monitored time after time up to twelve (12) weeks.

Physicochemical Changes in Tannery Waste Polluted Soil Amended with Fungal Slurry

The changes in physical and chemical parameters of tannery waste polluted soil amended with fungal slurry are presented in Table 2. The polluted soil amended with *Aspergillus aculeatus* slurry from unpolluted soil (T1) had pH 7.3-8.85 while polluted soil carrying *Aspergillus niger* slurry isolated from that same polluted soil (T2) had pH 7.3-8.7 pH. Contaminated soil amended with fungal consortium T3 (*Aspergillus aculeatus* and

Aspergillus niger), had pH 7.2-8.8 while the unamended, control soil had pH 7.0-7.1 (Table 2). The pH changed in all the treatments and at week 8, 10 and 12 were significantly difference. The organic carbon was 1.4-4.6%, 1.6-4.7%, 1.1-4.7% for T1, T2 and T3 respectively as compared to 3.67-4.27% for T4. The low nitrogen contents remained almost same in all the treatments with 0.09-0.2mg/kg (T1), 0.09-0.2mg/kg (T2) and 0.09-0.2mg/kg (T3) as compared to 0.16-0.22mg/kg for T4. Phosphorus was 0.69-1.4mg/kg (T1), 0.7-1.4mg/kg (T2), 0.8-1.5mg/kg (T3) and 1.17-1.25mg/kg (T4). Calcium, magnesium, potassium and sodium were lower in the unamended soil (T4) than the amended soils and were 2.4-3.7, 0.15-3.5,9.4-12.1 and 8.81-20.5mg/kg (T1), 2.4-4.0, 0.12-3.5, 6.0-12.1 and 12.54-20.0mg/kg (T2), 2.02-4.0, 0.12-3.5, 3.6-12.1 and 6.9-20.0mg/kg (T3) respectively compared to their corresponding T4 (Table 2). Cation exchange capacity (CEC) was 25.2-75.2mg/kg (T1), 25.9-80.1mg/kg (T2), 25.9-80.1mg/kg (T3) against 46.7-47.75mg/kg (T4). The particle size of soil showed higher percentage of sand followed by silt and clay in all treatments. It orderly takes the form sand>silt>clay (Table 2).

Microbial Changes in Tannery Waste Polluted Soil Amended with Fungi Slurry

Throughout the experiments, there were changes in microbial counts in tannery waste polluted soil amended with fungi slurry. The results are presented in Figure 2 to 5. Aerobic heterotrophic bacterial counts in the soil are presented in Figure 2. The results revealed that the counts increased gradually in all the treatments from week 0 to week 6 and then decreased between week 6 and week 8 particularly for T1 (19±3.5 - 31.5±4.04×10^6), T2 (19.5±2.89 - 34.5±2.89×10^6) and T4 (19±2.31 - 25.5±1.73×10^6) cfu/g of soil. Counts in T3 (19.5±5.19 to 38.5±0.58×10^6 cfu/g of soil sample) increased sharply from week 8 to week 12 (Figure 2). Statistical analysis of the counts revealed that there were significant differences at P<0.05 among T3, T2, T1 and T4. Similarly, the counts observed at week 4 for T2 (34.5 ±2.88×10^6) cfu/g were more than all the other treatments (T1, T3 and T4) and that counts in (T3 and T1) remained approximately same during the entire study at week 4.

However, the counts at week 6 and 8, T2 was less, recorded same bacterial growth counts as T4 while T3 (31.5±5.19×10^6) cfu/g was higher than T1 (28.5±1.73×10^6) cfu/g at week 6 and T1 (28.5±0.57×10^6) cfu/g was higher than T3 (25.5±4.04×10^6) cfu/g at week 8. The bacterial growth counts increased again, T3 (30±1.15×10^6) cfu/g and was higher than T2 (23.5±0.57×10^6) cfu/g at week 10. Likewise at week 12, T3 (38.5±0.57×10^6) cfu/g bacteria counts increased and T4 were observed to have less bacterial counts. There were significant differences (P<0.05) among the treatments at week 10 and 12 and the results appeared in this order T3>T2>T1>T4 (Figure 2).

The counts of bacteria that were able to utilize tannery waste are presented in Figure 3. Contaminated soil containing slurry of fungi and unpolluted soil (T1) showed high number of bacteria (29±3.46×10^6 cfu/g) at week 2 while T2 and T3 recorded same bacterial counts (22.5±1.73×10^6 cfu/g each). However, at week 4, T3 maintained similar counts as the previous weeks while the counts in T2 and T1 decreased. There were no significant differences (P>0.05) among the treatments at weeks 2 and 4. Likewise at week 6, the counts T1 (25.5±2.88×10^6 cfu/g) increased while at week 10 T2 (25±1.15×10^6 cfu/g) had the highest bacterial counts. At week 12, the counts in all the treatments increased. There were significant differences (P<0.05) at week 12 among the treatments in the order T4<T1<T2<T3 (Figure 3).

The number of culturable fungi were determined during the remediation process and presented in figure 4. T3 recorded higher number of fungi (16±2.30×10^5 cfu/g) than T1 (14±2.30×10^5 cfu/g) and T2 (10.5±1.73×10^5 cfu/g) at week 2 while at week 4, T2 had the highest fungal counts (18.5±0.57×10^5 cfu/g) and hence, they are statistically significantly difference in the order T2>T3>T1>T4. However, at week 6, 8 and 10, fungal counts in T2 were not as high as in T1 and T3 and are 15±1.15×10^5, 16±2.30×10^5 and 16±3.53×10^5 cfu/g respectively as presented in figure 4. Counts in T3 increased gradually up to week 12. Similarly, counts in T2 increased at week 12, hence there were significant differences (P<0.05) among the treatments in the order T3>T2>T1>T4 (Figure 4).

The counts of fungi capable utilizing tannery waste was presented in Figure 5. From the results, T2 and T3 maintained similar fungal counts (9±2.30×10^5 cfu/g each) at week 2 and hence there were no significant differences at P>0.05 among the treatments. The counts in all treatments with the exception of the control (T4) increased gradually to week 6 (Figure 5). There were significant differences (P<0.05) among the treatments at week 4 and 6. At week 8, the counts in all treatments decreased while that of T3 increased slightly and lasted till the end of the experiments at week 12 (Figure 5). Fungal counts in T1 and T2 increased between week 10 and 12. There were significant differences (P<0.05) among the treatments at week 12. Throughout the experiments, T4 had tannery waste utilizing fungal counts lower than their corresponding treatments (Figure 5).

Table 1: Microbiological and Physicochemical Characteristics of Tannery Waste Polluted Soil

Parameters	Values
Total heterotrophic bacteria (CFU/g)	2.5×10^6
Tannery waste utilizing bacteria (CFU/g)	1.5×10^6
Total heterotrophic fungi (CFU/g)	1.5×10^5
Tannery waste utilizing fungi (CFU/g)	8.0×10^3
pH	7.5
Organic Carbon (%)	4.71
Nitrogen (%)	0.22
Phosphorus (mg/kg)	1.25
Calcium (mg/kg)	3.75
Magnesium (mg/kg)	3.55
Potassium (mg/kg)	12.1
Sodium (mg/kg)	20.0
Cation Exchange Capacity (mg/kg)	47.3
Sand (%)	72.5
Clay (%)	11.7
Silt (%)	15.7
Soil texture	Sandy loam

CFU/g: colony forming unit per gram, mg/kg: milligram per kilogram

Table 2: Changes in Physicochemical Parameters of Tannery Waste Polluted Soil Amended with Fungal Slurry over a Period of 12 Weeks

Parameters	Range Values Obtained			
	Treatment(s)			
	T1	T2	T3	T4
pH	7.3–8.85	7.3–8.7	7.2–8.8	7.0–7.1
Org.Carbon (%)	1.4–4.6	1.6–4.7	1.1–4.7	3.67–4.27
Nitrogen (mg/kg)	0.09–0.2	0.09–0.2	0.09–0.2	0.16–0.22
Phosphorus(mg/kg)	0.69–1.4	0.7–1.4	0.8–1.5	1.17–1.25
Calcium (mg/kg)	2.4–3.7	2.4–4.0	2.02–4.0	3.2–3.75
Magnesium(mg/kg)	0.15–3.5	0.12–3.5	0.12–3.5	3.2–3.54
Potassium (mg/kg)	9.4–12.1	6.0–12.1	3.6–12.1	11.94–12.1
Sodium (mg/kg)	8.81–20.5	12.54–20.0	6.9–20.0	19.54–20.51
CEC (mg/kg)	25.2–75.2	25.9–80.1	25.9–80.1	46.7–47.75
%sand	72.5–80.4	72.5–81.4	72.5–81.4	72.5–81.4
%silt	9.8–17.1	9.8–13.3	9.8–13.3	11.1–17.1
%Clay	8.4–15.6	8.4–15.7	8.4–15.7	5.5–15.7

The values (ranges) were determined from mean of duplicate samples
T1: treatment with *Aspergillus aculeatus* from unpolluted soil
T2: treatment with *Aspergillus niger* from tannery waste polluted soil
T3: treatment with fungi co-culture (*A. aculeatus* and *A. niger*)
T4: treatment without the test organism (control)

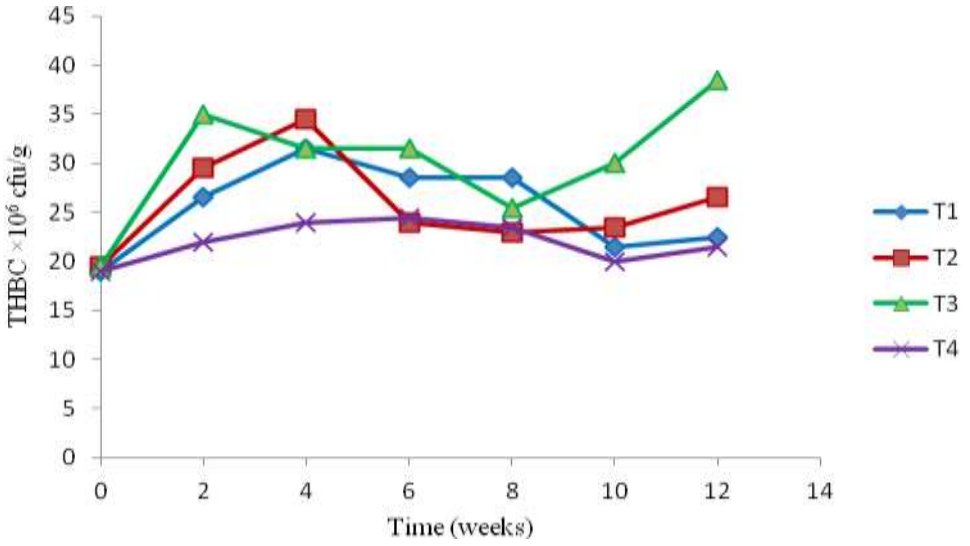

Figure 2: Changes in Total Heterotrophic Bacterial Counts in Tannery Waste Polluted Soil Amended with Fungal Slurry

T1: treatment with fungi *A. aculeatus* from unpolluted soil
T2: treatment with fungi *A. niger* from tannery waste polluted soil
T3: treatment with fungi co-culture (*A. aculeatus* and *A. niger*)
T4: treatment without the test organism (control)

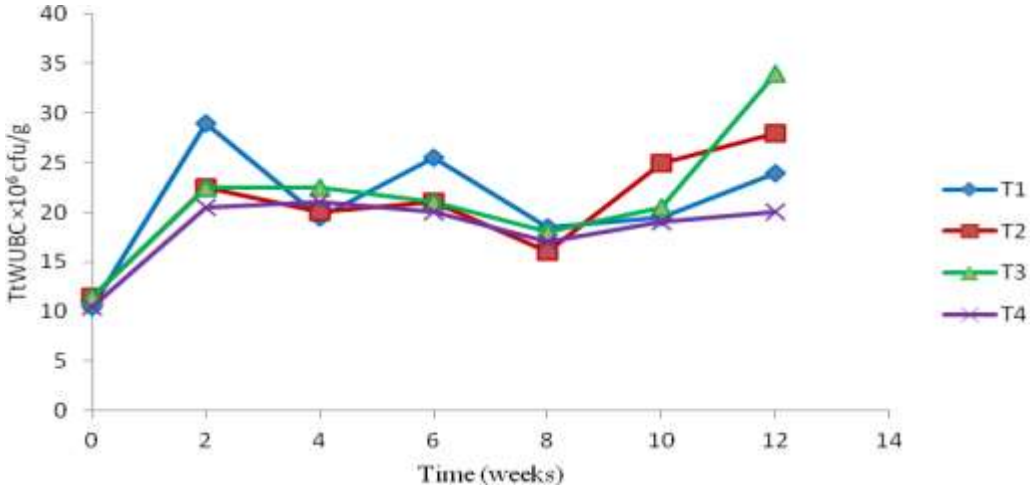

Figure 3: Changes in Tannery Waste Utilizing Bacterial Counts in Tannery Waste Polluted Soil Amended with Fungal Slurry

T1: treatment with fungi *A. aculeatus* from unpolluted soil
T2: treatment with fungi *A. niger* from tannery waste polluted soil
T3: treatment with fungi co-culture (*A. aculeatus* and *A. niger*)
T4: treatment without the test organism (control)

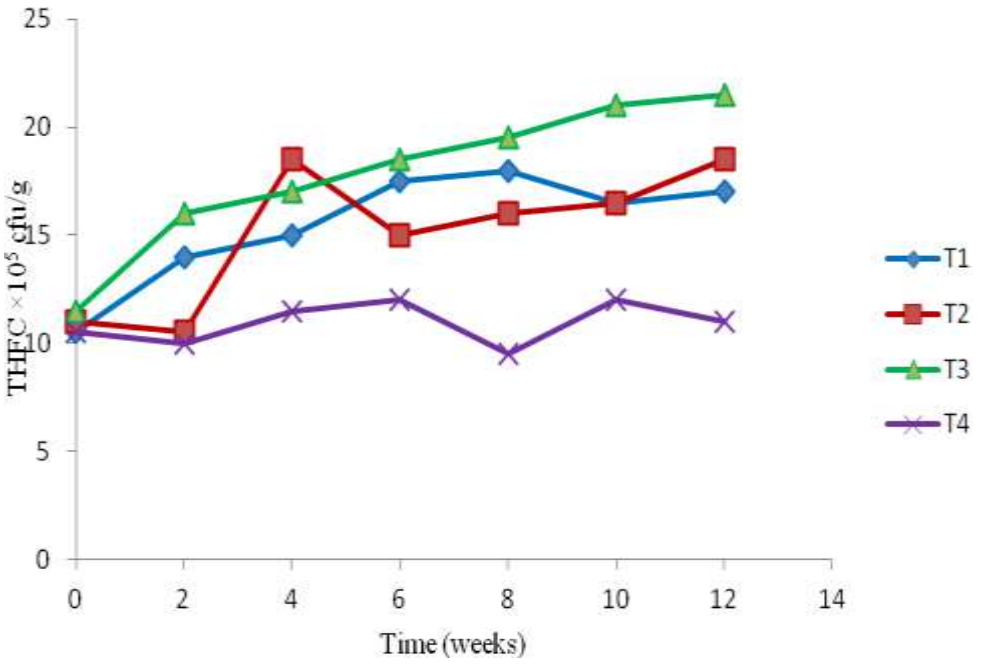

Figure 4: Changes in Total Heterotrophic Fungal Counts in Tannery Waste Polluted Soil Amended with Fungal Slurry

T1: treatment with fungi *A. aculeatus* from unpolluted soil
T2: treatment with fungi *A. niger* from tannery waste polluted soil
T3: treatment with fungi co-culture (*A. aculeatus* and *A. niger*)
T4: treatment without the test organism (control)

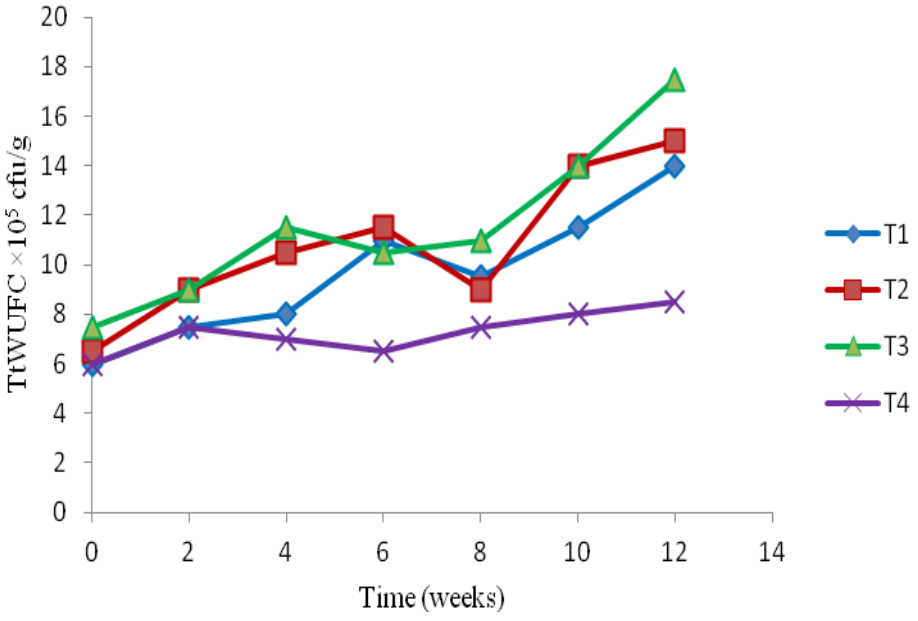

Figure 5: Changes in Total Tannery Waste Utilizing Fungal Counts in Tannery Waste Polluted Soil Amended with Fungal Slurry

T1: treatment with fungi *A. aculeatus* from unpolluted soil
T2: treatment with fungi *A. niger* from tannery waste polluted soil
T3: treatment with fungi co-culture (*A. aculeatus* and *A. niger*)
T4: treatment without the test organism (control)

DISCUSSION

In the initial microbial enumeration, both total heterotrophic bacterial and fungal counts were much higher than their corresponding total tannery waste utilizing microbial counts because of toxicity posed by wastewater that hindered the growth of many viable microbes. These counts were higher than those reported by Rabah and Ibrahim (2010), Sharaf and Alharbi, (2013), Umar *et al*. (2017). The differences in results could be due to environmental conditions and organic matter contents which may favor their proliferation (Ameh and Kawo, 2017). The pH reported in this study agrees with that obtained by Rabah and Ibrahim (2010) and Umar *et al*. (2017). The pH 7.5 is within the value that favors most microbial growth (Tortora *et al*., 2010). The nitrogen content was low (0.22%) in the contaminated soil before amendments. This agrees with the report by Ezeigbo *et al*. (2013) that nitrogen level decreased with increased pollution which could account for the limited content of nitrogen utilizing microbes in the contaminated soil. The organic carbon (4.71%) appreciated under investigation. Organic carbon may as well influence organic matter content (Amos-Tautua *et al*., 2014). Presence of organic carbon in the soil vicinity may be due to co-utilization of natural barks of trees and grasses. Phosphorus was low (1.25mg/kg) in the soil. This result is not in agreement with the report of Rabah and Ibrahim (2010) that in all the sampling sites (sokoto tannery site), phosphorus was not detected. However, phosphorus content was within the level set by Federal ministry of environment. The food and Agriculture Organization, FAO (1976) reported calcium content of >4.0mg/kg as the value contained in the soil and calcium (3.75mg/kg) was above the limit set by the Federal Ministry of Environment (Nigeria). Exchangeable Na, K and Mg were also present in greater amounts, with sodium (Na) having a very high content of 20.0mg/kg. This may be attributed to the use of sodium sulphate by tanners. Ezeigbo *et al*. (2013) reported that Na and K increased with decreased pollution. Another reason could be that it is derived from substances during preservation of skin (Tariq *et al*., 2009). Mohan and Saranya Devi (2015) reported that in the tannery, sodium chloride (NaCl) has been used to preserve skins from microbial deterioration and the excess salt have been removed prior to skin processing in the tannery.

In soil amended with fungi slurry, the change in pH over time may be as a result of microbial activities that favor the growth of other displaced microbes to re-thrive again. This is because ordinary soil environments are basically neutral with range of pH 5 to 9 for most microbial growth (Krulwich and Guffanti, 1989; Dubey and Maheshwari, 2013). Organic carbon trend in all the treatments decreased and may be because active microbes utilized it as their energy source (Khantoon *et al*., 2017). Besides, energy production, available microbes may use organic carbon for building of cellular material or production of other molecular nutrients and elements for cellular growth (Dubey and Maheshwari, 2013). The nitrogen levels remained almost the same in all treatments though, Nabavinia *et al*. (2015) reported that wastes from tannery are good sources of nitrogen. The low content of nitrogen may have been due to the fact that nitrogen content remains limited in the soil and no matter what amount found present, it is controlled by microbial activities. However, Ca, K and Na were significantly different as their levels were reduced in all treatments sets. Geoffrey (2010) explained the trends by which fungi accumulate and translocate nutrients via mycelium or concentrate in the fruiting bodies. Rosenstock *et al*. (2016) reported uptake of nutrients by many fungi including *Aspergillus* sp. Treatment with combined fungal slurry indicated much higher activity than other corresponding treatments which managed these nutrients effectively.

Microbial growth is very sensitive to changes in conditions like pH, nutrient availability, atmospheric temperature and others (Rousk and Baath, 2011). One of the methods of studying growth in soil is by successive increase in the number of colony of fungi or bacteria usually during condition of growth after amending or augmenting inhabitant fauna (Christensen *et al*., 2007). In the total heterotrophic bacterial counts, the setup containing co-slurry of fungi (T3) had increased counts as against control (T4) at week 2. This can be attributed to the synergistic adaptation and action in the tannery waste by these strains that stimulated the other inactive bacteria to proliferate to the maximum population at week 2. Another possible explanation may be the co-slurry (T3) had allowed bacterial cells to exploit condition that could involve log phase (Rolfe *et al*., 2011). Similarly, soil containing slurry of innate fungi (T2) had singly favored bacterial counts increased at week 4 as against control (T4) while T3 was low. Decrease or increase of microbial counts in the environment relies on adaptation of available microbes for their growth and multiplication with no incoming hindrances. Low microbial population was probably due to less active bacteria to interact with contaminant (Piakong and Zaida, 2018). Progressively co-slurry (T3) at week 10 and 12 was much effective and increased above all other treatments (T1 and T2) as against T4. So also T2 actively responded from its declined position at week 6 and 8 and improved at 10 and 12. In the tannery waste utilizing bacterial counts, slurry of fungi of unpolluted soil (T1) in the tannery waste polluted soil was much effective at 2 and 6 week compared to other treatments sets as against the control (T4) which was low, and at week 10 (T2) increased compared to other treatments set and hence T3 improved effectively at week 12. In this case, bacteria (in T1) require little time to adapt and improved the soil habitat and with time subsequently the counts droped. The bacteria may be disturbed by the contaminants toxicity over time or competition by microbes to use available substrate drops the less effective bacteria among them (Tortora *et al*., 2010). Bacteria that can uses tannery waste (in T2 and T3) took longer duration to grow in the contaminated soil. They may be affected by atmospheric condition or preparing for adaptation (Stuart, 2010). Organic matter may absorb the pollutants in soils contaminated for quite

longer time often less available for microbial utilization (Olivier *et al*., 2004). This do not agree with the report by Tripathi *et al*. (2011) that stated that microbes require substrate for growth during amendments process because they do not obtain energy from transformation reaction.

In this study part, total heterotrophic fungal count, the counts by (T1) had increased and greater above (T2), though (T3) was higher more than all other treatments as against control (T4) at week 2. The counts of total fungi by (T3) had successively increased from week 6 to 12 greater than all other treatments. The mean counts by T2 were previously dominant at week 4 only as against control. The use of individual fungi as enrichment for bioremediation strategy involves metabolism of only a narrow range of substances whereas fungal consortium with comprehensive enzymatic dimensions are indispensable to break down substances into smaller parts for use by other indigenous microbes (Piakong and Zaida, 2018).

In tannery waste utilizing fungal counts, it was observed that, there are few number of tannery waste utilizing fungal counts in the experiments. Its worthy to know that increase or decline and fluctuation of colony counts in all the treatment set are explained due to the fact that the tannery waste may be effected on bacteria/fungi and the period for adaptability or the competition among the indigenous microbes in the soil (Carquira *et al*., 2011).

CONCLUSION

Tannery polluted soil remediation by individual inherent fungi, *A. niger* perform greater than foreign fungi *A. aculeatus* that was isolated from unpolluted soil but their co-slurry have been shown to be active in restoring soil missed properties.

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