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Isolation and Identification of Bacteria from Soil Contaminated With Textile Dye And Tannery Effluents In Sokoto Metropolis

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Abstract

Soil contamination by textile and tannery effluents is a great concern to the environment, Complex effluents having a number of dyes accumulate in the soil there by polluting the environments and loss of microbial species in the soil. Samples were collected and the physicochemical analyses was determined. *The pH was basic with 8.0 and 10.1 from tannery and dyeing site respectively. The organic carbon was very low with 0.76% and 0.06%, Nitrogen (0.067% and 0,095%), phosphorus 0.8mg/kg, and 1.62mg/kg), calcium (1.35mol/kg in both the two samples. Magnesium recorded 0.30mol/kg and 1.65mol/kg, potassium 0.85 mol/kg1.65mol/kg , Sodium 3.13mol/kg and 30.0 mol/kg in tannery and dyeing site respectively. The sand, silt and clay recorded (78.8% and 90.6%)(7.3% and 5.3%), (13.9% and 4.1%). The heavy metal content of the soil sample were determined the concentration of chromium was 0.0258 and 0.0043 from tannery and dyeing site respectively, while Nickel was almost unavailable in the site with -1.0700 and -1.0756. Lead recorded a very low concentration with -0.9164 and -7803. Thirteen bacterial species were isolated and identified based on morphology and biochemical charachteristics from the samples, tannery soil with frequency occurrences of 23.07% for Bacillus laterosporus, 15.38% for Bacillus subtilis and Bacillus megaterium while Bacillus firmus, Bacillus cereus, proteus vulgaris, proteus mirabilis and clostridium had 7.69% each. Fifteen species were isolated from the dyeing soil sample with Bacillus subtilis, Bacillus megaterium, and Pseudomonas aeruginosa with the highest frequency of occurrences with13.33% each. Burkholderia cepacia, P. florescense, Bacillus laterosporus,Bacillus amyloliquifeciens, Bacillus brevis and Proteus vulgaris had 6.66% each, all these were determine with the use of Microgen identification kit, molecular analysis was done on two bacterial isolates and were confirmed to be Alishwanella solinqauinati and Bacillus subtilis. These indicate that the species isolated can resist high amount of toxic chemicals and can withstand any harsh environment and hence their ability to serve as a potential biosorbents for bioremediation of the affected environments at a cheap cost.*

INTRODUCTION

Microorganisms are ubiquitous participants in global biogeochemical cycles, and their ability to degrade or transform environmental pollutants that are otherwise persistent in the environment has sparked an interest in the mechanisms underlying these natural processes. Xenobiotics are chemically synthesized organic compounds that exhibit abnormal structural characteristics most of which do not occur in nature (Fetzner 2002).They can also be refer to as compounds that are foreign to a living organism, or any substance in high concentration can aso be regarded as xenobiotics(Bonjoko 2014). Xenobiotics include fungicides, pesticides, herbicides, insecticides, nematocides. Most of which are substituted hydrocarbons, phenyl carbonates, and similar compounds.

Metagenomics is a term that describes both field of a scientific research and a set of techniques that enables the culture independent analysis of a microbial community in any environment.(Sileator et al,. 2008). Studies have revealed that natural ecosystem is the best for exploiting these novel molecules and microorganisms; this can be explained in the vast richness of soil and other microbial niches (Schmeisser *et al.*, 2007). Despite the obvious importance of these organisms, very little is known of their diversity and ecological functions (Singh *et al.*, 2008).

Textile effluents also possess great threat to environment and human health. Complex effluents having a number of dyes accumulate

in the soil there by polluting the environments These effluents may contain a variety of chemicals that are used in the tanning process such as sodium sulphate, chromium sulphate, and non-ionic wetting agents and may accumulate in the immediate environments of the tannery. The high sulphide content of tannery effluents apart from being toxic to humans, may also pose serious odour problems when discharge into the environment. Chromium content of the effluent may pose great danger to humans in as much as it is toxic to humans from a level as low as 0.1mg/l (Tudunwada *et al.*, 2007). Also when the effluents are not properly managed, many pathogenic microorganisms in the effluents may predispose the inhabitants to serious health hazards (Ogbonna *et al.*, 2004). It may also deplete the dissolved oxygen of water bodies thereby affecting the aquatic ecosystems. Therefore, the aim of this study is to isolate and identify bacteria from soil contaminated with textile dye and tannery effluents .

MATERIALS AND METHODS

Sampling Sites

The dyeing and tanning sites are located within the Sokoto north metropolitan city, in Sokoto state Nigeria.

Sample Collection

Soil samples were collected aseptically at the surface of the sites with a soil auger in a sterile polythene bag and was taken straight to the laboratory for further analysis.

Physico Chemical Analyses and Heavy Metal Determination of the Soil Samples

Physico-chemical parameters of the soil sample was determined such as pH, Nitrogen, Potassium, Sand, Silt, Clay Organic carbon, Organic matter, Moisture content and Cation exchange. Heavy metals concentration of the soil samples were also determined.

Physicochemical Analysis of the Soil Samples

pH value

A fifty (50ml) beaker was filled with 30g of soil sample and deionised water,it was stir to obtain a soil slurry and was cover with watch glass. The solution was allowed to stand for 1h to stabilize.. Electrode of the pH meter (Starter 2100 B328559026) was immersed, and then the mixture was stirred for a few minutes.The suspension was allowed to stand for further 15 minutes and was allowed to stabilize for reading and the pH was recorded.(GTM,2015).**3.2**

Electrical conductivity (EC)

One hundred (100g) of the soil sample was crushed with hammer-mills. Suspension of water:soil (1:5) was prepared by weighing 10g and loss of microbial species in the soil, of an air dried soil (< 2mm) into a bottle, 50 ml of deionised water was added, it was shaked for 1hr with a mechanical shaker at 15 rpm to dissolve the soluble salts the conductivity meter was calibrated according to the manufacturers instructions using the KCL reference solution to obtain a constant cell solution, the cell was then rinsed thoroughly with the soil suspension, the conductivity cell was rinsed with the soil suspension and refilled and the value indicated on the conductivity meter was recorded in µs/cm.(Godson *et al.*,2002).

Moisture content

An air tight container was dried and cooled in a desiccator and weighed. One gram (1g) of the soil sample was placed in the container and the weight recorded, the sample was dried in air-circulation oven at 105°C to a constant weight. The sample and the container were cooled again in a desicator and weighed. The difference in weight after drying was used to calculate the moisture content, (Banerjee, 2004).

$$\text{Moisture (\%)} = \frac{\text{Loss in weight on drying (g)} \times 100}{\text{Initial sample weight (g)}}$$

Organic matter

The soil sample was ground and sieved with the use of 0.2mm sieve. One (1g) of the sieved soil sample was weighed in duplicates and transferred into 250ml Erlenmeyer flasks. Ten (10ml) of potassium dichromate solution and 20ml of concentrated sulphuric acid were added and the content of the flasks was shaked gently until properly mixed. One hundred milliliters of distilled water was added and allowed to stand for 30 minutes. This was followed by adding 4 drops of phenol red (indicator) and titrating against 0.5N ferrous sulphate solution. The percentage carbon was calculated according to the formula (Osuji and Adesiyan, 2005).

$$\text{\% Organic Carbon} = \frac{(M_e K_2C_2O_7 - M_e FeSO_4) \times 100}{\text{(wetsoil(g)- dry soil(g)}}$$

Where: Me = Mole equivalent.

Nitrogen content

The Macro-Kjeldahl method was used. Five (5) grams of soil sample was weighed into a dry 500 ml Macro-Kjeldahl flask and 20 ml of distilled water was added. The content was swirled for two minutes and allowed to stand for 30 minutes. One tablet of mercury catalyst and 10 g of K_2SO_4 were added and 30 ml of conc. H_2SO_4 was added through an automated pipette. The content of the flask was heated gently in the digestion stand for 30 minutes. After cooling, 100 ml of distilled water was added and

transferred into another clean Macro-Kjeldahl (750 ml) apparatus and the sand residue washed four times with 50 ml of distilled water. All the washings were transferred into the same flask. After that, 50 ml H_3BO_3 indicator solution was added into 500 ml Erlenmeyer flask, which was placed under the condenser of distillation apparatus and 150 ml of 10 N NaOH was added. This was followed by distillation and the condenser remained cooled (30^0C) by allowing sufficient cold water to flow and also to minimize frothing. Nitrogen was determined in the distillate by titrating with 0.01 N standards H_2SO_4. The color changed at the end point from green to pink. Percentage nitrogen was calculated using the formula: (Yaomans and Brenner, 1991)

%Nitrogen = (N× 0.014×VD×10) / (A× Wt. of sample) ×100

Where:

N = Normality of acid. VD = Volume of the digest

A = Aliquot of digest.Wt=Weight of the sample

Phosphorus

Two (2g) of soil and one teaspoon of carbon black were added to 40 ml of Bray extracting solution in 125 ml Erlenmeyer flask. The flask was shaken for 30 minutes and filtered through Whatman No. 2 filter paper. ½ teaspoon of carbon black was added to obtain a clear filtrate, thereafter 2 ml of the clear supernatant was dispensed in a 20 ml test tube and 5 ml of distilled water plus 2 ml of ammonium molybdate $(NH_4)2M_OO4$) were added. The contents were mixed properly and 1ml of dilute stannous chloride ($SnCl_2.2H_2O$) solution was added and mixed again. After 5 minutes, the absorbance in a spectrophotometer (Optima SP 300) at 660 nm wavelength was measured and the percentage phosphorus was calculated in accordance with the methods of Uriyo and Singh (1974):

P (mg/Kg) = (Reading × 0.61× dilution factor)
(Atomic weight of phosphorus).

Determination of Heavy Metals Content of the Soil Samples

The heavy metal content of the soil sample was determined by weighing one 1(g) of the sample into a 50 ml crucible, and then 10 ml of concentrated HNO_3 was then added. The sample was heated on a hot plate until the solution became semi-dry. This was followed by the addition of 10 ml of concentrated HNO_3.The solution was kept on a hot plate for 1h to allow the formation of a clear suspension, which was then cooled and subsequently filtered through Whatman No. 2 filter paper. The solution was then transferred to a 50 ml volumetric flask and 10ml distilled water was added to the mark and was analyzed using AA6300 ASC (Varian) spectrophotometer, and the concentrations of the analyte was calculated from calibration curves and conversions where made to obtain the final concentrations in mg/kg units (Koko and Jimoh, 2013).

Isolation of Bacteria Species Tolerant To the Contaminated Soil Samples.

The soil samples were homogeneously mixed and then sieved with the use of 2.0 mm sieve to remove unwanted soil debris. One gram (1g) of the soil was weighed into test tube containing 9 ml of sterile distilled water, and agitated for a minute. Serial dilution of the soil was made up to10^{-5} dilutions and 10^{-4} was used. Aliquot of 0.1ml of the prepared dilution was aseptically transferred onto the surface of solidified Nutrient agar for the isolation of bacteria. It was spread well with the use of a sterile bent glass rod. Plates were prepared in duplicates and incubated at 37°C for 24 h and were examined for bacterial growth. Different colonies observed were then purified by repeated streaking for each distinct colony on nutrient agar until pure colony was obtained. The purified bacterial isolates were transferred on sterile nutrient agar slants and stored for identification. Isolates were identified using the identification scheme provided in Bergy's manual of determinative Bacteriology (1997)

Characterization and Identification Of Bacterial Isolates

Isolates were macroscopically characterized and identified by microscopic, gram reaction and biochemical (indole, coagulase, citrate utilization, sugar fermentation, methyl red and Vorques Prokauer) characteristics. Microgen Kit was used to identify the isolates to species level.

Molecular Identification of Bacteria

The genomic DNA of bacteria were extracted by the DNA isolation kits according to Aiya (2020). PCR reaction was carried out in 200µl reaction containing template DNA, primers, dNTPs and Taq polymerase. The reaction was cycled 35 times at 94°C for 30sec, 58°c for 30 seconds and 72°C for 1 minute 30 seconds, followed by final extension at 72°C for 10 minutes. The PCR products were analyzed on 1% agarose gel in 1Xtbe BUFFER, run at 100v for 45minutes. Gel was stained with ethidium bromide and photograped (Pace, 1997). The sequence was aligned with corresponding sequences of 16srDNA from the database using BLAST from the website http://www.ncbi.nlm.gov/blast (Altschul *et al.*, 1997). Multiple alignments were generated by the CLUSTAL w program and phylogenetic tree were constructed by

neighbor-joining algorithm using MEGA6 software (Pace, 1997).

RESULTS

Table1. Physicochemical Analyses of the Soil Samples

Physicochemical parameters	Majema	Marina
pH	8.0	10.1
EC µs/cm	1629(µs/cm)	4.20(µs/cm)
Organic carbon	0.76%	0.06%
Organic matter	1.31%	0.10%
Nitrogen	0.067%	0.095 %
Phosphorus	0.81mol/kg	1.62 (mol/kg)
Calcium	1.35 (mol/kg)	1.35 (mol/kg)
Magnesium	0.30 (mol/kg)	1.65(mol/kg)
Potassium	0.85 (mol/kg)	0.67 (mol/kg)
Sodium	3.13 (mol/kg)	30.0 (mol/kg)
Cation exchange	12.6 (mol/kg)	17.2 (mol/kg)
Sand	78.8 (%)	90.6 (%)
Silt	7.3 (%)	5.3 (%)
Clay	13.9 (%)	4.1 (%)

Table 2. Heavy Metal Concentration of the Soil Samples

Heavy metals	Majema	Marina
Chromium	0.0258 (mol/kg)	0.0043 (mol/kg)
Nickel	-1.0700 (mol/kg)	-1.0756 (mol/kg)
Lead	-0.9164 (mol/kg)	-7303 (mol/kg)
Copper	0.1260 (mol/kg)	0.0751 (mol/kg)
Cadmium	0.0084 (mol/kg)	0.0145 (mol/kg)

Table 3 Biochemical characteristics of the soil sample

Sample ID	Grm rxn	Shape	Coa	Cat	Glu	Suc	Lac	H_2S	Gas	Citr	Urs	Mr	Vp	Mot	Bac
MJM1	+	Rod	+	+	+	+	-	-	-	+	-	-	+	+	*B.subtilis*
MJM2	+	Rod	+	+	+	+	-	-	-	+	-	-	+	+	*B.subtilis*
MJM3	+	Cocci	+	+	+	+	+	-	-	-	+	+	-	-	*S.aureus*
MRN4	-	Rod	-	+	-	-	-	-	+	+	-	-	-	+	*P.aeriginosa*
MRN1	+	Rod	+	+	+	+	-	-	-	+	-	-	+	+	*B.subtilis*
MRN2	+	Rod	+	+	+	+	-	-	-	+	-	-	+	+	*B.subtilis*
MRN3	+	Rod	-	-	+	-	-	+	-	+	-	+	-	+	*Clostridium*
MRN4	+	Rod	-	-	+	-	-	+	-	+	-	+	-	+	*Clostridium*

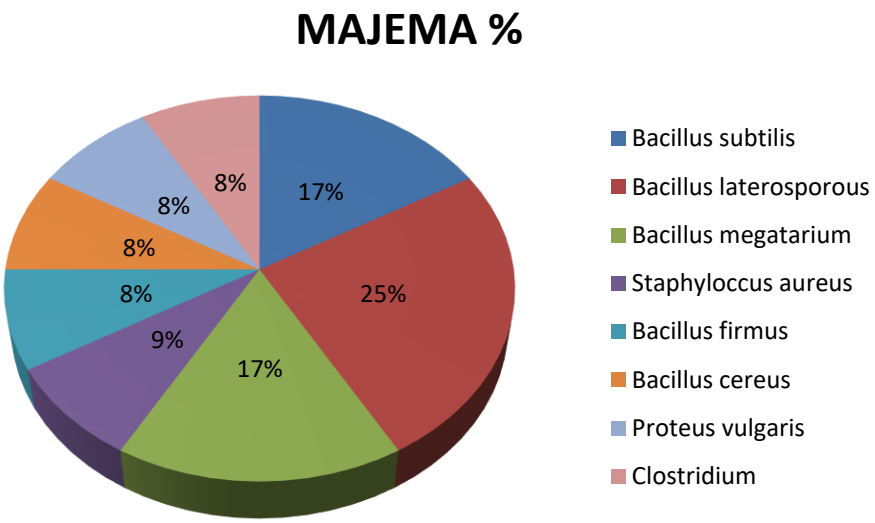

Figure 1: Distribution of bacteria species in Majema.

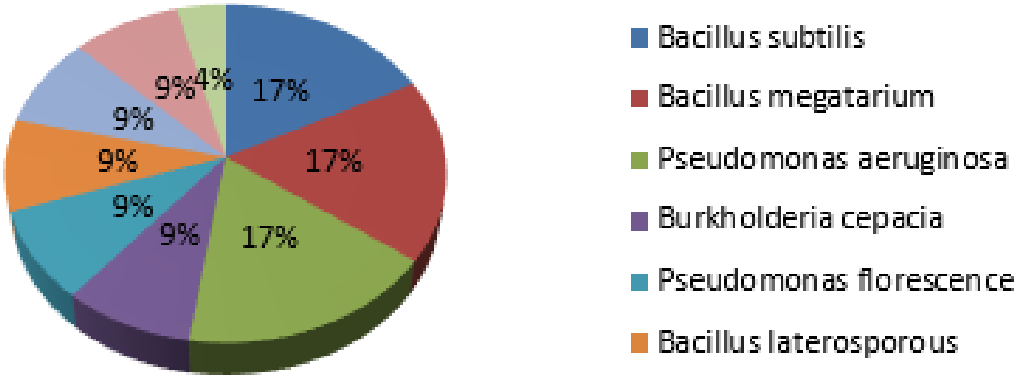

Figure 2: Distribution of bacteria species in Marina

Agarose Gel Image Showing 16S PCR amplification of Metagenomic DNA from Some selected isolates.

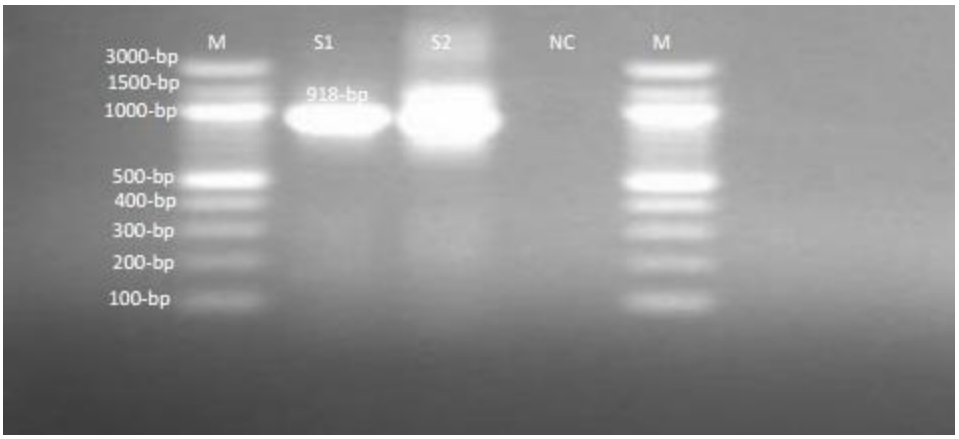

Figure : 3 Amplified fragment of 16SrRNA gene of bacterial isolates from the soil at 918bp. Lane M (Molecular maker of 100bp;Lane (Nuclease free water as Negative Control; Lane S1 Marina and S2 Majema(Bacterial isolates).

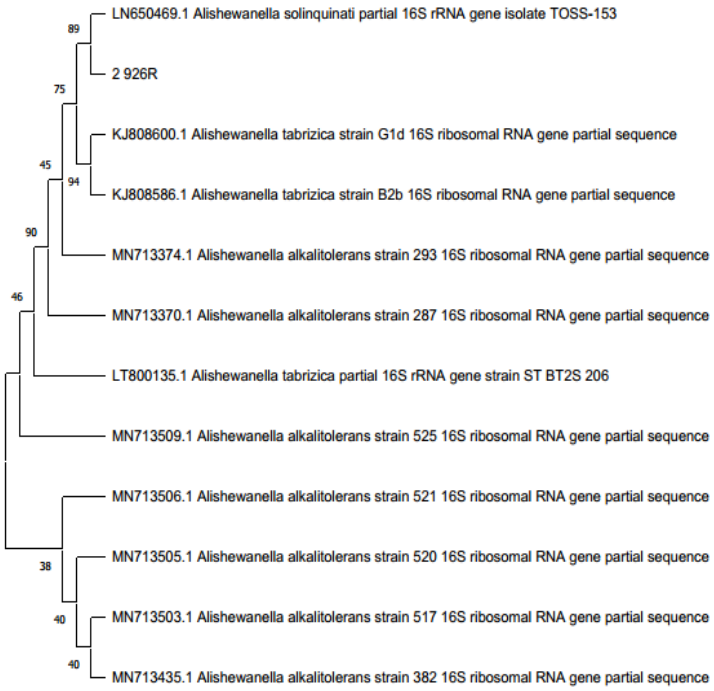

Figure 4. Phylogenetic tree showing *Alishwanella solinqui*

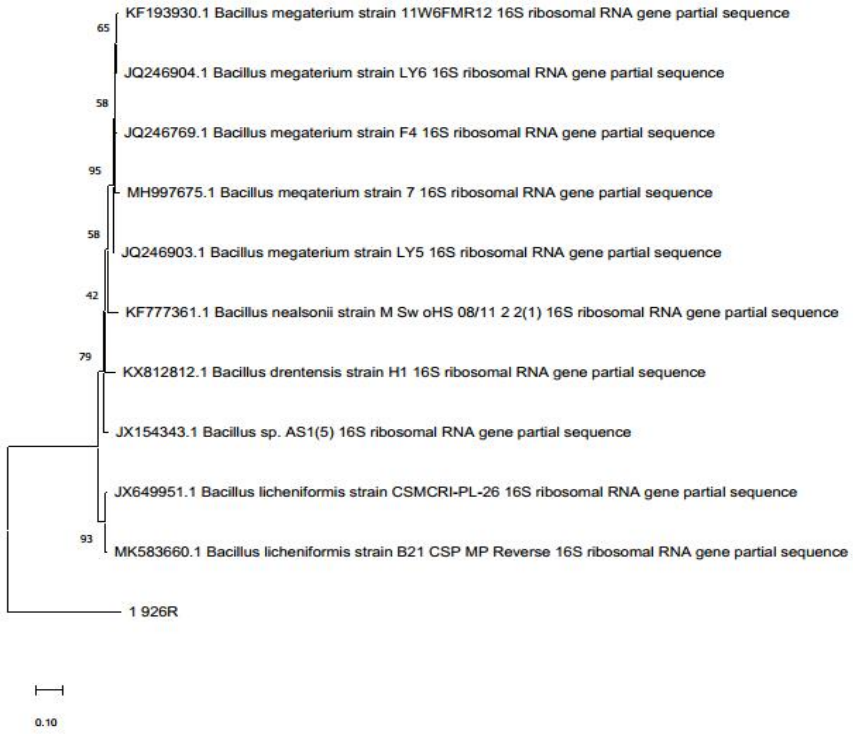

Figure 4 Phylogenetic tree showing evolutionary relationship among the bacteria species identified

Figure 5: phylogenetic tree showing evolutionary relationship of *Alishwanella specie strain*

DISCUSSION

Table 1; Shows the result of physico-chemical parameters of the two soil samples. pH value is an important parameter of soil, which has a great impact on chemistry reaction of soil, it plays an important role in complexation, redox-coordination, mineralization reactions and a leading role in precipitation, and desorption of soils. The values recorded shows that the two soil samples are basic in nature with 8.0 and 10.1 from majema and marina respectively; acidic soil decreases the availability of plant nutrients and increases the availability of some heavy toxic metals in the soil. This work disagrees with the findings of Hauwa *et al.,* (2019). The values recorded for calcium content (1.35 and 1.35 respectively) agrees with the report of Kiran (2013), but disagree with report of Hauwa *et al.,* (2019). Sand, Silt and clay content recorded very high in this study with (78.8,90.6), (7.3, and 5.3) and (13.9 and 4.1) respectively. The high value recorded could be due to the toxic heavy metals present in the soil as a result of chemicals use in dying and tannin activities, thereby decreasing the soil nutrients and contaminating the ground water and nearby well and boreholes water use by the communities around for consumption and other house chores.

Table 2; Shows the result of heavy metals content of the soil samples with high copper content in tanning soil 0.1260 and 0.751 in majema and marina soil while lead and Nickel where almost not available in the two soil samples with -0.9164,-7303 and -1.0700 and -1.0756 respectively. The values recorded disagree with the report of Hauwa *et al.,* (2019) and Majjiya *et al.*, (2015). Chromium and Copper recorded a high value in all the samples with 0.0258, 0.0043 and 0.0084 and 0.0145 respectively. This could be due to the toxic acid use in dying and tanning like acetic acid, hydrochloric acid, sulphuric acid formic acid and hydrogen peroxide etc.

Table 3 shows the result of biochemical characterization of bacterial isolates obtained from the samples. A total of twenty eight bacterial species where isolated. A total of thirteen bacteria species were isolated from Majema with *Bacillus laterosporus* having the highest percentage occurrence of 23.07%, followed by *Bacillus subtilis, Bacillus megaterium*, with 15.38% each the least recorded was by *S.aureus, Bacillus cereus, Proteus vulgaris, Proteus mirabilis, and clostridium* specie with 7.69% each.

Fifteen bacteria species was also isolated from marina Soil the highest occurrence was by *clostridium* specie with 20%, followed by *Bacillus subtilis, Bacillus Bacillus megaterium,, Pseudomonas aeruginosa* with 13.33% and the least recorded was by *Burkholderia cepacia, Pseudomonas flouresecense, Bacillus laterosporus, Bacillus amyloliquifeciens, Bacillus brevis,and Proteus vulgaris* with 6.66% each.. The highest percentage recorded by *Bacillus* could be attributed to their ability to resist harsh environmental conditions and heavy metal contamination in soils. *Bacilli* species are known to produce spores that enable them withstand environmental harshness. This agrees with Lugauskas *et al.* (2005), who found *Bacillus* species as the most

abundant bacteria in the soils contaminated The presence and abundance of *Bacillus subtilis* identified in this study may not be surprising because apart from their ability to produce spores they are also indigenous to soil environment and are known to persist in such environments (Atlas and Bartha, 2007). This finding is also in agreement with Kafilzadeh *et al.*, (2012) who reported *Bacillus* species among the organisms that resist lead in their findings.

The molecular identification of the two bacterial isolates reveals the identity of two bacteria namely *Allishewanella solinquinati sp and Bacillus Subtilis. Allishewanella solinquinati sp* was once isolated from a contaminated textile soil. This agrees with the work of Kolekar *et al.*, (2013) and Gunti (2020).

CONCLUSION

Twenty eight bacteria were repeatedly isolated from the soil sample. Only two isolates were molecularly identified as *Allishewanella solinquinati sp* which is not found frequently in the soil environment *and Bacillus Subtilis* that is found in abundance in the soil and entire ecosystem.

REFERENCES

Atlas RM, And Bartha R. (2007). Fundamentals And Applications. Addison Wesley Longman, Inc. Menlo Park, California, Microbial Ecology 237-238.

Benerjee, Dipak., K. I (2004). Speciation Of Metals In Sewage Sludge And Sludge Amended Soils. Water Air And Soils Pollution 152:1 219-232. **[Crossref]**

Bonjoko B 2014. Environmental Pharmacoogy An Overviewin; Pharmacology And Therapeutics. INTECH PP 133-178. **[Crossref]**

Esteve Nenez A Ramos JL (2001). Biological Degradation Of 2, 4,6-Trinitrotoluene. Microbiol Molecular Biology Review. (65):335-352. **[Crossref]**

Fetzner S. 2002 Biodegradation Of Xenobiotics. In Encyclopedia Of Life Support Systems(Eolss) Publishers, Developed Under The Auspices Of The Unesco. Biotechnology Editted By Doelle Ans Da Silva. Eolss Oxfor, U.K P32.

Godson, R. E. E. Ana and M.K.C Sridhar, (2002) Soil quality near a chemical fertilizer industry at Port Harcourt. Nigeria. AJEAM/RAGEE,4 50-57

Hauwa H, C.M.Z. Whong, S.A Ado., Abdulmuminu A. Nuhu. 2019. Optimization Of Culture Condition For Biosorption Of Lead Using Pseudomonas Aeruginosa Isolated From Gold Mining Site Of Anka Zamfara State. Umyu Journal Of Microbiology 5(1) Pp 43-48. **[Crossref]**

Koko, I, and Jimoh, WLO. (2013). Determination of Heavy Metals in Soil from Dump Site of Tenneries and Farmlands in Challawa Industrial Estate Kano, Nigeria. Bayero Journal of Pure and Applied Sciences 6:2.**[Crossref]**

Lugaskas A, Levinskaite L, Peciulte D, Repeckiene J, Motuzas A, Vaisvalavicius R And Prosycevas I. (2005). Effect Of Copper, Zinc And Lead Acetates On Microorganisms In Soil. Ekologija 1: (6)1-69.

Majiya M Hassan, Abdulmimun A Nuhu, M.S. Sallau, Hussaini M. Majiya And AK Mohammed. Assessment Of The Heavy Metals Pollution In Water And Soil Samples Of Artisanal Gold Mining Region In Anka Local Government Area, Zamfara State (Msc Research Work).

Ogbonna DN, Soker TC, And Benson GI, (2004). Studies On Bacterial Population And Inorganic Content Of Waste Dumpsites In Okrika Local Governments Area Of Rivers State, Nigeria. Nigerian Journal Of Environmental Science 2:(1) 6-11.

Osuji, LC., and Adesiyan,S.O (2005). Extractable Hydrocarbons, Nickel and Vanadium Contaminants Of Ogbodo-Isokpo Oil Spills Polluted Soils In Niger-Delta, Nigeria. .Environmental Monitoring Assessement 110:129-139. **[Crossref]**

Schloss PD. & Handelsman J.(2005). Metagenomics For Studying Unculturable Microorganisms: Cutting The Gordianknot.Genome Biology 6:29. **[Crossref]**

Schmeisser Christel, Helen Steel, And Wolfgang R. Street. (2007) Metagenomics, Biotechnology With Non Culturable Microorganisms. Applied Microbiology And Biotechnology 75:(5) 955-962. **[Crossref]**

Sileator RD, Shortall C, And Hill C. 2008 METAGENOMICS. Letter Appl. Microbiol. 47.5.361-6. **[Crossref]**

Tudunwada IY, Essiet EU, And Mohammed SG. (2007).The Effects Of Tannery Sludge On Heavy Metals Concentration In Cereals On Small- Holder Farms In Kano, Nigeria.Notulae Botanicae Horti Agrobotanici Clui- Napoca 35:(2) Pp 55-60

Uriyo, A.P. and Singh, B.R. (1974). Practical Soil Chemistry Manual. Department of Soil Science and Agricultural Chemistry, Faculty of Agriculture and Forestry University of Dar Es Salam-Morogoro..