

Effects of Hair Dressing Salon Wastewater on the Physicochemical and Bacteriological Properties of Soil in Awka Metropolis, Nigeria

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ABSTRACT

Wastewater refers to a combination of one or more domestic effluents or liquid effluents from commercial establishments. The ubiquity of microorganisms makes it easier for them to thrive in different habitats of which wastewater from hair dressing salon is not exempted. This work was aimed at determining the effects of hair dressing salon wastewater on the physicochemical and bacteriological properties of soil in Awka, Nigeria. Twenty different hair salon wastewater samples and pristine soil samples were collected from different locations in Awka metropolis. The soil was treated with the different wastewater samples daily for 7 days and analysed for its physicochemical and bacteriological properties. From the result, it was observed that the wastewater has a pH range of 6.20 to 9.60, conductivity range of 49.90 to 532.90, percentage total solid range of 0.19 to 9.32, percentage total carbon range of 0.06 to 1.90, percentage nitrogen of 0.41 to 0.85, chemical oxygen demand range of 97 to 197, biological oxygen demand range of 40.70 to 75.80, total phosphate range of 10 to 34 and total potassium range of 0.01 to 1.41. It was also observed that there was a significant decrease in all the physicochemical parameters of the soil upon treatment with the wastewater. The mean heterotrophic bacterial count of the wastewater was between 1.8 x 10^5 and 5.6 x 10^5 while that of pristine soil was 7.7 x 10^6 and wastewater treated soil was 2.1 x 10^4 to 7.2 x 10^4 . The significant decrease in the microbial population on the wastewater treated soil could be attributed to the presence of many chemicals like chlorine in the wastewater which has adverse effect on the soil microbiota. The findings from this study proved that salon effluents can be a potential public and environmental health hazard. There is need for proper treatment of wastewater before disposal into the soil.

INTRODUCTION

Wastewater is defined as a combination of one or more domestic effluent consisting of black water like excreta, urine and feacal sludge as well as and gray water from kitchen and bathing wastewater. It is also the water from commercial establishments and institutions like hospitals; industrial effluent, storm and urban runoff. Wastewater also include agricultural, horticultural and aquaculture effluent, either dissolved or suspended (Corcoran *et al.,* 2010). In addition, water contaminated by either chemical or biological agent is often unfit for drinking and other uses (Rathore *et al.*, 2014). Effluents from industries and its environments can alter the physical, chemical and biological nature of the soil which the water passes before and after entering the rivers (Fakayode, 2005).

The ubiquity of microorganisms makes it easier for them to thrive in different habitats of which wastewater from hair dressing salon is not exempted. Wastewater from domestic, industrial sector and even farms increase on the daily basis due to increase in population (Patil *et al*., 2014). Therefore, effluent and sludge from municipal sewage treatment plants, and that of water supplies to plants do contain micro and macro nutrients and heavy metals in high quantity which also lead to contamination of soil, thereby posing threat to microorganisms which are of benefits to agriculture as a result of land pollution. This surface pollution comes from both solid and liquid waste disposal practices, spills, agricultural practices and percolation of surface pollutants through unsaturated soil (Patil *et al*., 2014).

Pollution from wastewater is presently the greatest risk to the sustainable use of ground and surface water. Discharged wastewater may contain toxic substances, health-compromising pathogens, and/or chemical substances, which may cause adverse environmental impacts such as decrease in biodiversity, changes in

species composition and terrestrial and aquatic habitats and impaired use of contaminated drinking water and recreational waters. Domestic wastewater is generated from residential sources, such as sinks, toilets, laundry, and bathing while industrial wastewater is released by commercial enterprises and manufacturing processes. In general, wastewater is characterized based on its organic contents, specific contaminants and physical characteristics. Surface waters are the main repository of domestic and industrial wastewater disposal. The stagnating pools of wastewater on the roads and in the open gutters often provide habitat for several viruses and bacteria, as well as breeding grounds for mosquitoes (Mustapha and Getso, 2014).

The fashion industry (i.e. female beauty fashion) in Nigeria and most parts of African has come with its waste and effluent which are mostly discharged into the environment. Female beautification is a social change introduced to Africans as European body and hair are used to enhance beauty. Contemporary female body fashion or body care is undertaken through regular and intensive use of some chemical substances e.g. oils, lotions, sprays, dyes, shampoos and creams collectively called cosmetics. The mass adoption of beauty fashion treatments has led to widespread establishment of hair/body care shops popularly known as Beauty salon for fashion services in urban and rural settlements in Nigeria (Ajuzie and Osaghae, 2011).

Salons offer a variety of services such as hair treatment and styling, make-up application, manicure and other cosmetic services. Water is essential to every part of the services provided by beauty hair salons and as a result, copious amount of wastewater is generated. The produced wastewater contains a concoction of various chemicals such as acids, alkalis, dyes, relaxers, bleaches, and other organic and inorganic compounds. Nkansah *et al.* (2018) reported that some of the products in the beauty industry are unregulated and could be sources of carcinogens and volatile organic compounds (VOCs) such as ammonium thioglycolate, guanidine carbonate, lithium hydroxide and calcium hydroxide.

In Nigeria, fashion shop effluents are released directly into the environment without any treatment. This could be harmful to the environment, as the effluent contains chemicals which could alter soil and water physical and chemical properties (Bowers *et al*., 2002). Ajuzie and Osaghae (2011) reported increased chemical and biological oxygen demands in hair dressing salon effluent contaminated soil**.** Furthermore, toxicological evaluation of surfactants and detergents which are major ingredients of cosmetics by USEPA (1997) and Environmental Canada (1999) hinted that they cause endocrine disorders in fish and wildlife. Chude and Ekpo (2010) also reported that hair dressing salon effluent had acute toxic effect on fingerlings of both *Oreochromis niloticus* and *Clarias gariepinus*. Therefore, this study was aimed at determining the effects of hair dressing salon wastewater on the physicochemical and bacteriological properties of the soil in Awka Metropolis, Nigeria.

Aim and Objectives

The aim of this research work was to determine the effects of hair dressing salon wastewater on the physicochemical and bacteriological properties of the soil in Awka Metropolis, Nigeria.

Specific Objectives

The specific objectives were to:

- i. determine the physicochemical properties of hair salon wastewater, pristine soil and waste-water treated soil.
- ii. determine the bacterial load of hair salon wastewater, pristine soil and waste-water treated soil.
- iii. characterize the bacterial isolates associated with hair salon wastewater, pristine soil and waste-water treated soil.

MATERIALS AND METHOD

Materials

Autoclave, Incubator, weighing balance, Bunsen burner, Wire loop, Hot air Oven, Cotton wool, Spatula,

Aluminium foil, Microscope, Beakers, Conical flask, Slides, Cover slip, Petri dishes, Reagent bottles, Test tube and Calibrated measuring cylinder.

Nutrient agar, Methyl red, Kovac' reagent, Potassium hydroxide (KOH), Hydrogen peroxide (H₂O₂), Oxidase reagent and Peptone water.

Study Area

The study was conducted in Awka, Awka South L.G.A which is one of the Local Government in Anambra State. Awka occupies a total area of 198 square kilometres and has a median temperature of about 27° C. The estimated population of Awka was 242,800 inhabitants with the area mostly populated by members of the Igbo ethnic group according to National Population Commission of Nigeria and National Bureau of Statistics (2017).

Awka is the capital city of Anambra state in Nigeria, located in the southern part of the country, 42 kilometres east of Onitsha, 65 kilometres southwest of Enugu and 120 kilometres north of Owerri. It is famous for its cultural events, traditional markets and fairs attracting the attention of numerous foreign guests. There are lots of hotels and restaurant in the city. It is home to many government offices, such as Anambra state Governors lodge etc. The latitude of Awka, Nigeria is 6.210528, and the longitude is 7.072277. it has a GPS coordinate of 6° 12'37.9008" N and 7° 4' 20.1972" E. In Awka, the wet season is warm, oppressive and overcast while the dry season is hot, muggy and partly cloudy. Over the course of the year, the temperature typically varies from 66 $\mathrm{^{\circ}F}$ to 88 $\mathrm{^{\circ}F}$ and rarely below 59 $\mathrm{^{\circ}F}$ or above 91 $\mathrm{^{\circ}F}$.

Sample collection

Twenty (20) different Samples of hair salon wastewater were collected from 20 selected shops in different locations in Awka metropolis. The wastewater was collected directly from the salon discharge buckets immediately after the hair-washing procedures. Sterile sample bottles were used to collect the effluent samples and packed in cooler containing ice bags and transported to the Microbiology laboratory of Nnamdi Azikiwe University, Awka Metropolis for further analysis. 500g of soil sample was also collected from a farm in Nnamdi Azikiwe University, Awka, using a sterile polyethylene bag at a depth of 10cm using a sterile soil auger. 25g of the soil was treated with 25ml of the different wastewater daily for one month prior to analysis.

Physicochemical analysis

The physical and chemical composition of the salon wastewater, contaminated and pristine soil samples were determined using standard procedure. Samples were analyzed for pH, electrical conductivity (EC), total solids (TS), biological oxygen demand (BOD) and chemical oxygen demand (COD) as described by APHA (2012) while total carbon, total potassium, total nitrogen (TN) and total phosphorous (TP) were determined as described by Okalebi *et al.* (2018). These experiments were done in triplicates.

pH

The pH of the soil and wastewater samples were determined using the method of Ma *et al.* (2020). The electrode of the pH meter was calibrated as per the manufacturer's instructions. Two buffers that span the pH range of the sampled soils were used. Five grams (5g) of each of the sieved soil samples were weighed and 10ml of deionized water was added and stirred to mix well. It was allowed to stand for 30 minutes to equilibrate with atmospheric CO_2 after which it was stirred again, then the pH was read to nearest 0.1 pH unit using pH meter.

For the waste water, 5ml of waste water was stirred to mix well, then allowed to stand for 30minutes to equilibrate with the atmospheric CO_2 after which it was stirred again, then a pH was read nearest to 0.1 pH using pH meter.

Electrical conductivity

The measurements of electrical conductivity of the soil and wastewater samples were carried out according to

the standard method described by Rahmanian *et al.* (2015). Five grams (5g) of each of the sieved soil samples were weighed and 10ml of deionized water was added and stirred to mix well. It was allowed to stand for 30 minutes to equilibrate with atmospheric $CO₂$ after which it was stirred again. The Myron L multipara meter was calibrated using standard solutions of KCl and NaCl .The sample cup and the pH/ORP sensor were rinsed three times with the sample to be analyzed. The sample was then placed in the sample cup and sensor of the kit. The conductivity was measured by pressing the parameter button on the kit and the reading on the screen was recorded after 30 secs.

For the wastewater samples, 5ml of the samples were stirred to mix well. It was allowed to stand for 30 minutes to equilibrate with atmospheric $CO₂$ after which it was stirred again. The Myron L multipara meter was calibrated using standard solutions of KCl and NaCl. The sample cup and the pH/ORP sensor were rinsed three times with the sample to be analyzed. The sample was then placed in the sample cup and sensor of the kit. The conductivity was measured by pressing the parameter button on the kit and the reading on the screen was recorded after 30 secs.

Determination of total nitrogen

The total nitrogen content of the wastewater and soil sample was determined using the microkjeldahl method of AOAC (1999). One gram (1g) of the sample was weighed and transferred into the kjeldahl digestion flask followed by the addition of 3g of a mixture of sodium sulphate and copper sulphate pentahydrate in the ratio 10:1 as catalyst. Four anti-bumping chips was added to prevent sticking of the mixture to the flask during digestion and also to enhance boiling. The kjeldahl flask content was digested with 25ml concentrated H₂SO₄. The flask was inclined and heated gently at first until frothing ceased, then heated strongly with shakings, at intervals, to wash down charred particles from sides of the flask. Heating was continued until the mixture become clear and free from brown or black colour. This was allowed to cool and the content of the flask made up to 100ml using distilled water. 20ml of this diluted digest was placed in the distillation flask. 50ml of 2% boric acid solution was measured into a conical flask, and two drops of screened methyl red indicator was added into the conical flask. The conical flask and its content were placed on the receiver, so that the end of the delivery tube dips just below the level of the acid. Few pieces of granulated zinc and anti-bumping granules was added to the distillation flask and about 40ml of 40% NaOH solution was run into the flask to make the liquid in the flask alkaline. The content was boiled vigorously until the content of the flask bumps. The distillate was titrated with 0.1N HCl to a purple-coloured end point (Vml).

Calculation

Nitrogen (%) =
$$
\frac{1.4 \times \text{Time Volume x total volume of digest}}{1000 \times weight of Sample x Aliquot distilled} \times 100
$$

For the wastewater samples, the same procedure was repeated, using 1ml of the various wastewater samples.

Determination of organic carbon

The Organic carbon content of the soil and wastewater samples were determined using the method of Bisutti *et al.* (2004). One gram (1g) of the soil and one millilitre (1ml) were digested using Hydrogen peroxide and Nitric Acid by heating the samples in a mixture of the two acids until frotting cease. This was allowed to stay for 24 hours and then filtered. The samples were further washed with deionized water and dried. Percentage loss in mass of the soil and wastewater were calculated as organic carbon.

Biochemical oxygen demand (BOD)

Biochemical oxygen demand (BOD) of the soil and wastewater samples were determined using azide modification of Winkler's method as reported by Ma *et al*. (2020). BOD bottle was prepared and 10ml of the waste water was diluted with 290ml of deionized-distilled water and incubated at 20°C for 5 days in the dark. Accordingly, 10g of the soil samples were dissolved in 290ml of deionized-distilled water and incubated at 20°C for 5 days in the dark. After five days, incubated BOD bottle was mixed with 2ml of orthophosphoric

acid. This was shaken gently and titrated with sodium thiosulphate to the end point where there was change in colour. The titre value represents dissolve oxygen on day five. BOD was then calculated as the difference between dissolve oxygen on day one and that on day five.

Determination of chemical oxygen demand (COD)

Determination of chemical oxygen demand (COD) was done using the method of Ma *et al*. (2020). The wastewater sample (10 ml) was taken in a reflux flask and diluted with 40ml deionized-distilled water, and 10mL of potassium dichromate solution with 1 g mercuric sulphate was thoroughly mixed. Accordingly, 10g of the soil samples were dissolved in 40ml of deionized-distilled water and 10mL of potassium dichromate solution with 1 g mercuric sulphate was thoroughly mixed. Antibumping beads were added to control boiling of the solution. To this, 10mL of concentrated sulphuric acid containing silver sulphate was added through the open end of the condenser carefully and mixed by swirling motion. The reflux apparatus was operated for 1 hour and allowed to cool. The flask was removed, and its content was diluted to 150mL with distilled water. To the resulting solution, three drops of the ferroin indicator were added. This sample was titrated with standard ferrous ammonium sulphate to an end point where blue-green colour just changed to reddish-brown. Chemical oxygen demand (COD) of the blank sample was then calculated.

Potassium and Phosphorous Determination

This was determined using the method of APHA (1992). Two grams (2g) of each soil sample was weighed into a digestion flask and 20ml of acid mixture (650ml conc. HNO₃; 80ml perchloric acid; 20ml conc. H₂SO₄) was added. Similarly, 2ml of each wastewater was also weighed into a digestion flask and 20ml of acid mixture (650ml conc. HNO₃; 80ml perchloric acid; 20ml conc. H₂SO₄) was also added. The flask was heated using a heating mantle until a clear digest was obtained. The digest was diluted with metal-free distilled water to the100ml mark and used for the analysis using Chemetrics V-3000 Automated Multi-Analyte Photometer.

Total Solids

According to the modified method of AOAC (1990) the total solids content of the wastewater and soil samples were determined: 3g of soil samples and 3ml of wastewater sample respectively were weighed into a dry clean flat-bottomed aluminium dish, heated on a steam bath for 10 to 15 minutes. The dish was further put in an oven at 70°C overnight, and then cooled in a desiccator and weighed quickly. Heating and weighing were repeated until the difference between the two consecutive weighing was less than 0.1mg. The total solids content was determined from the following equation:

Total solids (
$$
\%
$$
) = $\frac{W_1}{W_0} \times 10$

Where:

 $W1$ = Weight of sample after drying, W0= Weight of sample before drying

Media Preparation

All media were prepared according to manufacturers' instruction.

Nutrient agar

Exactly 14g of nutrient agar powder was weighed and poured into 500ml distilled water in a conical flask. The mixture was swirled to obtain a homogenous mixture and thereafter sealed with aluminium foil and sterilized by autoclaving for 15mins at $121\textdegree C$ and cooled at $45\textdegree C$ and aseptically poured into Petri dishes and allowed to gel.

Inoculation of medium

Ten-fold serial dilution was done with the soil before and after treatment with salon wastewater. The procedure

was also repeated for the wastewater samples. The sample was inoculated using the spread plate method. 0.1ml of the samples were inoculated on sterile nutrient agar plates and incubated at 37° C for 24 hours for bacteria growth as described by Cheesbrough (2010).

Total heterotrophic count

The determination of the total heterotrophic bacterial count was carried out using colony-counting method as described by Cheese brough and calculated using the formula below;

$$
THC = \frac{n}{v \times d} (cfu/ml)
$$

Where $n =$ mean no of colonies

 $V =$ volume of sample inoculated

 $D =$ dilution factor.

Characterization and identification of the bacterial isolates

Bacteria with different cultural characteristics on nutrient agar plate were sub-cultured severally using the same nutrient agar plate to obtain pure culture. Distinct colonies were picked from each plate using a sterile wire loop and streaked on sterile nutrient agar plate. This was incubated for 24 hours at a temperature of 35° C after which the plate was preserved in agar slant for further biochemical tests and identification of specific bacteria.

The bacterial isolates were identified based on their cultural, biochemical properties and microscopic appearance as described by Cheesbrough (2010).

Gram's staining

An evenly spread smear of the isolate was made on a clean, dry slide using a sterile normal saline and the smears was allowed to air- dry in a safe place. The smear was fixed by passing three times over Bunsen flame and stained by the Gram technique as follows;

The smear was covered with crystal violet stain for 60 seconds. After that, the stain was rapidly washed off with clean water. All the water was tipped out, and then Lugol's iodine was added for 60 seconds. The iodine was washed off with clean water. Then, the smear was decolorized rapidly (10 seconds) with acetone – alcohol and washed immediately with clean water. After that, the smear was covered with neutral red stain (safranin) for 60 seconds. The stain was washed off with clean water. The back of the slide was wiped clean, and the slide was placed in a draining rack for the smear to air- dry. The smear was examined microscopically, with the oil immersion objective (x100) to look for bacteria and cells. The condenser iris was opened fully when using the oil immersion lens.

Indole test

Five ml of peptone water was incubated for 24 hours at 37°C in test tubes. The isolates were grown into the peptone water and allowed to stay for 24 hours. After, 5 drops of Kovac's reagent were added separately on each test tube and swirled gently for 5 minutes. Positive reactions were indicated by the development of a red colour in the reagent layer above while in the negative reaction (result) the indole reagent retained its yellow colour.

Methyl red test

The isolates were grown in 5 ml of MR both (glucose – phosphate peptone water) and incubated for 24 hours at 37°C. Thereafter, 3 drops of methyl red were added into each test tube. A reddish colour on the addition of indicator signified a positive result while a yellowish colour denoted negative result.

Voges – Proskauer test

Isolates were grown in 5 ml of Peptone water and glucose, respectively. This was incubated for 24 hours at 37°C then 5 drops of potassium hydroxide (KOH) were added. The tubes were shaken at intervals to ensure maximum aeration after 5 minutes. The development of red colour within 30s and 60s indicated a Voges-Proskauer positive test while no red colour showed a VP negative result.

Oxidase test

A piece of filter paper was wetted with a few drops of 1% oxidase reagent solution. A colony of the isolate was obtained with a sterile wire loop and smeared on the wetted portion of the filter paper. The development of an intense purple colour within 30 seconds indicated a negative test.

Catalase test

This test is used to differentiate those bacteria that produce the enzyme catalase, such as staphylococci, from non- catalase producing bacteria such as streptococci. About 2-3 ml of 3% hydrogen peroxide was poured into a test tube, by mean of a sterile wooden stick, a good growth of the test organism was removed and immersed in the hydrogen peroxide solution. Immediate bubbling in the solution indicates positive test.

Coagulase test

This test is used to differentiate *Staphylococcus aureus* (*S. aureus)* which produce the enzyme coagulase, from *S. epidermidis* and *S. saprophyticus* which do not produce coagulase. A drop of physiological saline was placed on a slide. A colony of the test organism was emulsified in the drop to make a thick suspension. A drop of plasma was added to the suspension and gently mixed. Clumping of the organisms within 10 seconds indicate positive test.

Sugar Fermentation test

This test was performed in a sugar broth medium to test an organism's ability to ferment sugars as well as its ability to produce gas and H_2S . The medium contains pH indicator bromothymol blue which converts to the yellow color in the acidic pH indicating sugar fermentation. The sugars were glucose, maltose, lactose, fructose and sucrose.

A suspension of the tested isolate was prepared using a sterile normal saline. Then each sugar broth (containing an inverted Durham tube inside a test tube) was inoculated separately with the prepared suspension using a sterile wire loop. The tubes were incubated at 35ºC for 24 hours. A change in the color to red indicating fermentation of the certain sugar found in that tube while bubbles in the Durham tubes indicate gas production.

Motility test

The bacterium was stabbed into a sterilized semi solid Nutrient agar contained in a sterile test tube, using a sterile inoculating needle. It was incubated for 24h at 37°C and observed for diffused lines of turbidity emerging from the original line of inoculation. The test was used to differentiate motile organisms from nonmotile ones.

Citrate utilization test

This test was carried out in test tube slants of Simmon's citrate agar. A 0.1 ml aliquot of each test organism was then inoculated by spreading on the Simmon's citrate slant and incubated at 37°C for 48h. Colour change from green to blue indicated that the organism was able to utilize citrate.

Urease test

The Urease test was carried out by streaking the isolate on urease agar, a change in colour from amber to pink

showed the production of the enzyme urease that hydrolyses urea into ammonia and carbon dioxide.

Data analysis

The data obtained was subjected to one-way ANOVA using Statistical Package for Social Sciences (SPSS) version 21 for windows evaluation. P-values < 0.05 was considered significant, indicating whether the wastewater composition will have a negative effect on the soil physicochemical and bacteriological properties.

RESULTS

The results of the analysis were presented using tables and charts.

Table 1 depicts the physicochemical parameters of the wastewater samples. The pH ranges from 6.20 to 9.60. The conductivity ranges from 49.90 to 532.90 μ s. The percentage total solid ranges from 0.19 to 9.32. The percentage total carbon ranges from 0.06 to 1.90. The highest percentage nitrogen was 0.85% while the lowest was 0.41%. The chemical oxygen demand ranges from 97.00 to 197.00 ppm while the biological oxygen demand ranges from 40.70 to 75.80 ppm. The total phosphate was between 10.00 and 34.00 ppm while the total potassium ranges from 0.10 to 1.41 ppm.

Table 1: Physicochemical parameters of the wastewater samples

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Table 2 shows the physicochemical parameters of the wastewater-treated soil samples. The pH ranges from 6.90 to 9.90. The conductivity ranges from 165.70 to 270.60 µs. The percentage total solids ranges from 80.00 to 99.40%. The percentage total carbon ranges from 0.70 to 2.90. The highest percentage nitrogen was 0.77% while the lowest was 0.41%. The chemical oxygen demand biological oxygen demand recorded zero values. The total phosphate was between 79.00 and 130.00 ppm while the total potassium ranges from 0.09 to 0.18 ppm.

Table 2: Physicochemical parameters of the wastewater-treated soil samples

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Table 3 shows the physicochemical parameters of the pristine soil sample. The pH was 10.3. The conductivity gave a value of 421.63 µs. The percentage total solids was 99.80%. The percentage total carbon wass 3.50. The highest percentage nitrogen was 0.88%. The chemical oxygen demand and biological oxygen demand recorded zero values. The total phosphate was 141.00 ppm while the total potassium gave a value of 0.20 ppm.

Table 3: Physicochemical parameters of the pristine soil samples

Table 4 shows the total heterotrophic bacterial count of the samples. The count of the salon wastewater ranges from 1.8 x 10^5 to 5.6 x 10^5 with sample from Udoka Estate having the lowest count and sample from Government house having the highest count. The bacterial count of wastewater-treated soil ranges from 2.1 x $10⁴$ (Isuaniocha) to 7.2 x $10⁴$ (Government house). The no of heterotrophic bacteria pristine soil sample was 7.6×10^6 .

Table 4: Mean heterotrophic bacterial count of the samples

Table 5 shows the biochemical characteristics of the isolated bacteria from the wastewater samples. The organisms exhibited varying characterizing to the different biochemical tests. The identified organisms are Staphylococcus aureus, Escherichia coli, Bacillus spp, Pseudomonas aeruginosa, Klebsiella spp, Micrococcus spp and Streptococcus spp.

Table 5: Biochemical characteristics of the organisms isolated from wastewater samples

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Table 6 shows the biochemical characteristics of the isolated bacteria from treated soil samples. The organisms exhibited varying characteristics to the different biochemical tests. The identified organisms are Staphylococcus aureus, Escherichia coli, Bacillus spp, Pseudomonas aeruginosa and Micrococcus spp.

Table 6: Biochemical Characteristics of the organisms isolated from treated soil samples

Key: $+=$ Positive

 $=$ Negative

 $A+$ = Positive with Acid production only

 $AG = Positive with Acid and Gas production$

 $Lac = Lactose$

 $Glc = Glucose$

 $Mal = Maltose$

 $Frc = Fructose$

 $Suc = Success$

$Gal = Galactic$

 M ann = Mannitol

 $Dev = Dextrose$

Table 7 shows the biochemical characteristics of the isolated bacteria from pristine soil. The organisms exhibited varying characterizing to the different biochemical tests. The identified organisms are Staphylococcus aureus, Escherichia coli, Bacillus spp, Pseudomonas aeruginosa, Klebsiella spp, Micrococcus spp and Streptococcus spp.

Table 7: Biochemical Characteristics of the Isolated organisms

$Glc = Glucose$ **Key:** $+$ = Positive $=$ Negative $Mal = Maltose$ $A+$ = Positive with Acid production only $Lac = Lactose$ $AG = Positive with Acid and Gas production$ $Frc = Fructose$ $Suc = Success$ $Gal = Galactic$ M ann = Mannitol $Dev = Dextrose$

Figure 1 shows the frequency of occurrence of the bacteria isolates in the three different samples. S. aureus was more prevalent in treated soil than in pristine soil and wastewater. Bacillus spp occurred most in the pristine soil while klebsiella spp occurred most in the waste water samples.

Figure 1: Frequency of occurrence of bacterial isolates in the samples

DISCUSSION, CONCLUSION AND RECOMMENDATION

Discussion

The effects of salon wastewater on the bacteriological and physicochemical parameters of soil were studied. The physicochemical analysis of shows the pH of the salon wastewater to be within the range of 6.20 ± 0.00 to 9.60 \pm 0.30 (Table 1). The results showed that the pH values of most of the analyzed samples was alkaline. The pH levels exhibited a highly significant difference $(P < 0.05)$. According to the classification by Metcalf and Eddy (2003), the pH levels were in the high category (Appendix 1). This could be attributed to the presence of chemicals like sodium hydroxide in hair relaxers and dyes used in hair conditioners (Dias, 2015). The composition of effluent varies from town to town depending on the type of public facilities, households, and industrial waste discharging into the environment, and this could be an essential contributory factor to the observed differences in pH. The pH values were however within the World Health Organization (WHO) and Federal Environmental Protection Agency (FEPA) acceptable limits of $6.0 - 9.0$ for drinking water and waste water discharge into the surrounding (WHO, 2004; FEPA, 1991). There was a notable decrease in the pH after treatment with salon wastewater (Table 2) while the pristine soil was strongly alkaline in [Table 3]

The conductivity of the salon wastewater ranged from $45.90 \pm 0.0.5$ to 511.90 ± 1.00 with a highly significant difference ($P < 0.05$), while that of treated soil ranged from 165.70 to 270.60 μ s. The high level of conductivity in the effluent could be ascribed to the high levels of dissolved ions in the wastewater (Levlin, 2010). All the mean recorded values were within the WHO permissible limits of 1000 and 1500 μS/m for wastewater (WHO, 2004). The highest and lowest total solid were measured at Tempsite and Isiagu with values of 99.40 ± 0.05 and 80.00 \pm 1.00 mgL⁻¹, respectively. All the mean recorded TS values where within WHO acceptable limits of 2000 mg/L, for effluents to be discharged into the environment (WHO, 2004). The TS values in the present study were higher than those found by Igbinosa and Okoh (2009). Also lower TS values of 2.210 – 2.655 mg/L was reported by Akan *et al*. (2008).

A highly significant difference ($P < 0.05$) of COD and BOD was observed with values ranging from 97.00 \pm 0.00 - 197.00 \pm 1.00 and 40.70 \pm 0.20 - 80.00 \pm 2.00 respectively. COD is a measure of the amount of oxygen required to break down both inorganic and organic particles in water system (Akan *et al*., 2008). High concentrations of COD in water systems may lead to drastic oxygen depletion (Fatoki *et al*., 2003). The levels of COD were in the low range according to Metcalf and Eddy (2003) wastewater classification (Appendix 1). According to Henze (2008), the composition of wastewater may change with time on a given location as a result of the variations in the amounts of substances being discharged. The mean COD levels were within the permissible limits of 250 mg/L recommended by the WHO for wastewater (WHO, 2004). A high COD value suggests more waste products or pollutants presence in the effluent such as sodium, dimethylphthalates and bis (2-ethylhexyl) and ammonium nitrogen. Since COD indirectly measures the amount of organic compound

present in water. It therefore means the water was heavily polluted. BOD is a measure of the concentration of biodegradable substances in the wastewater. Thus, BOD concentration was in the low category. The mean BOD concentrations was above the acceptable limit of 50 mg/L recommended by the WHO (2004). The BOD/COD ratios in the wastewater have a significant impact on the functioning and selection of wastewater treatment processes (Henze, 2008).

The nitrate levels showed a highly significant difference. Nitrate concentration in this study ranged between 0.41 ± 0.01 and 0.85 ± 0.05 mg/L and were the WHO permissible limit of 45 mg/L for wastewater. Phosphate levels in the wastewater varied from 10.00 ± 2.00 to 31.00 ± 2.00 mg/L. The nitrate level decreased upon treatment with the waste water. The phosphorus levels in this study were comparable to those reported by Ogunfowokan *et al*. (2005) and Akan *et al*. (2008). The phosphorus levels recorded in this study were above the WHO acceptable limit of 5 mg/L. The high phosphorus levels could be attributed to phosphate containing shampoos and conditioners used in these salons as well as organic and inorganic compounds present in dissolved and particulate forms (Mandiracioglu *et al*., 2006). Phosphate levels can also come from many diverse sources, such as agriculture, aquaculture, septic tanks, urban wastewater, urban storm water runoff, industry, and fossil fuel combustion (Paerl, 2006). These elevated levels of phosphorus, if not removed before discharge, could cause eutrophication of surface water bodies.

The mean viable count of salon wastewater ranged from 1.8 x 10^5 at Udoka Estate to 5.6 x 10^5 at Government house (Table 4). The mean viable count of pristine soil sample was 2.1×10^5 while wastewater-treated soil ranged from 2.1 x 10^4 at Isuaniocha to 7.2 x 10^4 at Government house (table 4). The high microbial counts recorded for the samples from most of the study areas could be attributed to the poor sanitary conditions of the hair dressing salons around that environment. The decrease in soil bacterial population could be attributed to the presence of chemicals in the wastewater which could be bactericidal to the soil flora. The low occurrence of the microbial content in the wastewater was as a result of the use of chlorine-treated pipe-borne water in the salons. Chlorine is bactericidal to enteric bacteria and therefore could account for a reduction in the microbe population.

In this study, 7 different bacterial organisms were isolated from hair dressing salon wastewater samples in Awka metropolis (Table 5). Pristine soil also had 7 different bacterial organisms (Table 6) while wastewatertreated soil had only 5 different bacterial organisms (Table 7). The organisms were characterized for the morphological parameters and biochemical tests were also conducted for their identification. The result showed that bacterial isolates identified in waste-water treated soil samples were *Staphylococcus aureus, E. coli, Bacillus* spp.*, Micrococcus* spp. and *Pseudomonas* spp. with the percentage occurrence of 7.00, 4.50%, 7.50%, 6.00% and 3.00% respectively (Figure 1). The untreated soil had *Staphylococcus aureus* (4.50%)*, Klebsiella* spp. (4.50%)*, E. coli* (6.00%)*, Bacillus* spp. (7.50%)*, Micrococcus* spp. (6.00%)*, Pseudomonas aeruginosa* (4.50%) and *Streptococcus* spp (3.00%). The salon waste water also had *Staphylococcus aureus* (6.00%), *Klebsiella* spp. (7.50%), *E. coli* (6.00%), *Bacillus* spp. (4.50%), *Micrococcus* spp. (3.00%), *Pseudomonas aeruginosa* (4.50%) and *Streptococcus* spp. (4.50%) (Figure 1).

Bacillus spp*.* has the highest percentage occurrence in both the pristine and treated soil while *Klebsiella* spp had the highest percentage occurrence in the salon wastewater. This present study agreed with the study of Udochukwu *et al*. (2021) who reported and proposed extensive microbial diversity including specie richness and evenness per gram of soil for contaminated and uncontaminated soil. *Klebsiella* spp, and *Streptococcus* spp. were absent in the treated soil. The absence of *Klebsiella* spp. in the treated soil can be attributed to the high chlorine content of salon waste water as chlorine is bactericidal to enteric bacteria (Ajuzie and Osaghae, 2011). The occurrence of *P. aeruginosa and S. aureus*, which were isolated from all the samples was not surprising since *P. aeruginosa* is a particularly adaptable organism found in various habitats (Auanya *et al*., 2016a).

Staphylococcus aureus was isolated from all the salon waste water sampled as was also seen in the work of Enemor *et al.* (2013). *Staphylococcus aureus* is among the important bacteria found to cause diseases such as boils, scalded skin syndrome, carbuncles and foliculitis in humans. Literatures have reported and proposed extensive microbial diversity including species of *Staphylococcus aureus* on human hair (Tharmila *et al*., 2012). *Staphylococcus aureus* is a normal flora of man and can also act as a potential source of infection

(Amna and Fozia, 2012). This result was found to be consistent with that reported by Ajuzie and Osaghae (2011) in Benin City.

Salmonella was not found in any of the water samples. This is in line with the findings of Mariam *et al*. (2020). However, *Escherichia coli* was isolated from all the samples. Ebah *et al*. (2020) also isolated *E. coli* in their findings. Inference can be drawn that there was some little faecal contamination of the process water used for hair dressing since *Escherichia coli* is a member of the family *Enterobacteriaceae* as well as an indicator organism of faecal contamination. Their low occurrence could be due to the antimicrobial content of some of the agents used in washing hair like shampoo, relaxers, conditioners and many others.

Conclusion

From the study, it was observed that following the treatment of the soil with the different salon wastewaters, there was a general decrease in the physicochemical and bacteriological parameters of the wastewater treated soil. This study shows that the use of wastewater effluent has significant negative effect on soil microbiota and soil physicochemical properties. Also, from the results, the mean levels of some of the physicochemical parameters were above the WHO regulatory limits for discharged wastewater. Therefore, an attempt should be made to treat salon effluents before disposal into salon premises. The findings of this study proved that salon effluents can be a potential public and environmental health hazard. Considering the lack of information on monitoring wastewater quality for the salon effluent in Nigeria, this study provides a basis for policy considerations to ensure public and environmental health protection.

Recommendations and contribution to knowledge

The indiscriminate disposal of untreated waste-water from the hair dressing salon into the environment should be discouraged by individuals and the government.

Mechanisms for proper treatment and disposal of salon waste-water should be developed for commercial hair dressers so that the waste-water from such commercial ventures are treated and properly disposed.

Agencies in the public health sector to sensitize and organize lectures, training workshops and seminars for the operators and workers of hair-dressing and beauty salons in the communities.

There should be routine inspection of hair dressing salons within a given locality by public health officers to check for hygienic standard and level of sanitation.

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