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Keywords: soil, bacteria, polyaromatic hydrocarbon, toxicity, hair dressing salon effluent.

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A 25 liter Jerri can with a height of about 30cm was filled with soil and polluted with the hair dressing salon effluent for 30 days. After 30 days of pollution, the top soil was collected from the height of 0-5cm, middle soil 12-17cm and the sub soil 25-30cm. The total heterotrophic bacterial count was determined, the acute and chronic toxicity of the effluent on bacterial was determined and the soil was analyzed for the presence of polyaromatic hydrocarbon using gas chromatography with mass spectrometry. The polyaromaic hydrocarbon (PAHs) detected in the test soil sample were 45.02ng/g biphenyl, 28.23ng/g Benzo[a] pyrene, 12.05ng/g Anthracene, 23.00ng/g and 5.07ng/g Phenanthrene. Only 2.01ng/g of biphenyl was detected in the control garden soil, while counts from the contaminated soil ranged from 1.0 x10² ± 1.10 to 4.0 x 10² ± 0.11. The counts from the control soil sample ranges from 2.0 x10³ ± 0.20 to 8.2 x10³ ± 0.20. The control soil sample had higher value compared to the test soil samples.

The following isolate were identified. Serretia sp., Klebsiella sp., Escherichia coli, Pseudomonas sp., Staphylococcus sp. Pseudomonas sp. and Staphylococcus sp had the highest percentage occurrence. The acute and chronic toxicity test showed a decline in bacterial count which could have occurred as a result of the presence of PAHs from Salon effluent. The findings from this research indicate that there is a constant release of PAHs into the soil which poses serious threat to the survival of soil bacteria and other soil biological sentinels.

Keywords: soil, bacteria, polyaromatic hydrocarbon, toxicity, hair dressing salon effluent.

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I. INTRODUCTION

Kogi state lokoja precisely battles with waste management, its efficient treatment as well as discharge. This is a major problem as Nigeria is counted among the developing countries which do not channel much attention towards efficient waste water management. Wastewater refers to any water that has been adversely affected in quality by anthropogenic influence. It comprises liquid waste discharged by domestic residences, commercial properties, small scale industries and aquifer treatment institutions. In general, waste water is characterized based on its bulk or organic contents, physical characteristics and specific contaminants (Damelle, 1995; Griffiths and Philippot, 2012). Efforts have been made towards curbing the menace of pollution around the world, particularly by the United Nations Environmental Programme. There have been many international conferences to this effect, such as the Rio de Janeiro conference of 1992 (Oyesola, 1998; OECD, 2004; Odokuma and Olewi, 2003). In many parts of the world, human activities still have negative impact on the environment. Some of the consequences of manmade pollution are transmission of disease by water-borne pathogens, eutrophication of natural water bodies, accumulation of topic or recalcitrant chemicals in the soil, destabilization of the ecological balance and negative effect on human health (Chikere and Okpokwasili, 2004).

The continuous trend toward the formulation of new beauty tips and manufacture of novel hair

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products to satisfy the demands of the growing populace could lead to some pollution problems.

Today's salons offer a wide range of services from hair styling and skin treatments to tanning, manicure and make up application. In providing these services, waste is generated. In most cases, this waste goes into the sanitary sewer system, where it can have a negative impact on the environment (Bowers *et al.,* 2002). A typical example of what happens, is logging of contaminated water in the soil. In this situation, oxygen becomes less available as electron acceptor, results in the reduction of available nitrate into gaseous nitrogen which has negative effects. Leaching into ground water is a major concern, because of the recalcitrant nature of some contaminants (Lapygina *et al.*, 2002; Toetora *et al.*, 1997). Different methods of waste treatment have been developed for reasons of public health and conservation which results in the destruction of pathogens and the mineralization of the organic components of sewage prior to discharge. Anaerobic wastewater treatment using granular sludge reactor is one of such methods (Lin, 2001). However, in Nigeria like in many developing countries, the discharge of untreated waste into the environment is still a problem, despite the establishment of Federal Environment Protection Agency (FEPA) since 1998. Other considerations for treatment can be the removal of toxic organic pollutants and heavy metal altering the physical conditions of the water (e.g. pH, electrical conductivity, etc), removing sediment loads or biochemical oxygen demand (BOD).

The aim of this study is to examine the extent of contamination in untreated wastewater of five different hair salons in lokoja, Kogi State, Nigeria and the impact on soil and soil biological sentinels. Soil pollution causes decrease in soil fertility, alteration of soil structure, disturbance of the balance between flora and fauna residing in the soil, contamination of the crops, and contamination of groundwater, constituting a threat for living organisms (Udochukwu *et al.* 2014)

II. MATERIALS AND METHODS

The study area for this research was victory road, Ganaja Village, Lokoja, Kogi State. The soil sample was collected from Ganaja village, Lokoja, Kogi State. A 25 liter Jerri can with a height of about 30cm was filled with soil. The Jerri can was cut open at the top and bottom. The salon effluent was collected in with another Jerri can from different salons in Ganaja village everyday for 30days and poured into the Jerri can containing the soil. After 30 days of pollution, the top soil was collected from the height of 0-5cm, middle soil (12-17cm) and the sub soil (25-30cm). A non polluted garden soil sample was collected which served as the control soil sample was collected.

The four soil samples were taken to the labouratory to air dry for two and were sieved. The soil samples (1gram) each were weighed on the analytically weighing balance in the laboratory. A total viable heterotrophic bacterial count was determined using pour plate technique. The bacterial isolates were identified using the various biochemical test (Burkhard *et al.*, 2001).

2.1 Preparation of Hair Dressing Saloon Effluent Concentration for Toxicity Test

For the determination of the median lethal concentration (LC_{50}), hair dressing saloon effluent concentrations of 100, 200, 300, 400 and 500 ml/l will be formulated by adding (100, 200, 300, 400 and 500 g) in 1000 ml of Winograsky medium respectively (Ibiene and Okpokwasili, 2011). For the median effective concentration, the following hair dressing saloon effluent concentrations (20, 40, 60, 80 and 100 ml/l.) will be formulated by adding (20, 40, 60, 80 and 100 g) in 1000 ml of Winograsky medium respectively.

A control experiment consisting of Winograsky medium only will be set up (Ibiene and Okpokwasili, 2011).

2.2 Soil Bacteria Acute and chronic Toxicity Test

The acute toxicity test will be carried out by determining the median effective concentration (EC_{50}) with these effluent concentrations (20, 40, 60, 80 and 100 ml/l). Also, the chronic toxicity

will be carried out by determining the median lethal concentration (LC_{50}) with these effluent concentrations (100, 200, 300, 400 and 500 ml/l). The winograsky medium which will be fortified by several milliliters of hair dressing saloon effluent (100, 200, 300, 400 and 500 ml/l) and (20, 40, 60, 80 and 100 ml/l) respectively.

They will be inoculated with ten milliliters of bacteria standard inoculum. They will be allowed to stand for an hour for growth. 1 ml of the suspension thereafter will be plated from mineral salt media composted with different volumes of hair dressing saloon effluent on a non-hair dressing saloon effluent composted winograsky agar plates. This will be carried out for the all concentrations and repeated for 2, 3 and 4 h interval (Okpokwasili and Odokuma, 1996). The colony forming units for each plate will be calculated and used to determination acute toxicity (EC_{50}) of the various hair dressing saloon effluent composted mineral salt media. The chronic toxicity of the effluents on soil bacteria will be determined by calculating the lethal concentration (LC_{50}) using probit analysis. All results will be subjected to the analysis of variance (ANOVA) (Ferrara *et al.,* 2006).

2.3 Instrumentation and Conditions

Hewlett Packard HP 5890 series II Gas chromatograph equipped with an Agilent 7683B injector (Agilent Technologies Santa Clara, CA, USA), A 30 m, 0.25 mm i.d. HP-5MS capillary column (Hewlett – Packard, Palo Alto, CA, USA) coated with 5% phenyl-methylsiloxane (film thickness 0.25 m) and an Agilent 5975 mass selective detector (MSD) will be used to separate and quantify the BPA compounds. The samples will be injected in the split less mode at an injection temperature of 300°C. The transfer line and ion source temperature will be 280°C and 200°C. The column temperature will be initially held at 40°C for 1min, raised to 120°C at the rate of 25°C/min, then to 160°C at the rate of 10°C/min and finally to 300°C at 5°C/min, held at final temperature for 15 min. Detector temperature will be kept at 280°C. Helium will be used as a carries gas at a constant flow rate of ml/min. Mass spectrometry will be acquired using the electron ionization (EI) and selective ion monitoring (SIM) mode. A PerkinElmer Gas Chromatograph model Autosystem XL, with Flame Ionization Detector will be used for identification of BPA, phthalate, organotin, alkyl phenol and other cosmetic chemicals by comparison between the retention times of the BPA sample peak and the standard compound.

The quantification carried out done by the internal normalization method. An Elite-5 fused silica capillary column (30 m x 0.25 mm i.d. crossbond 5% diphenyl – 95% dimethyl polysiloxane, 0.25 µm film thickness) will be used for the GC separation using the following oven temperature program: 150°C (5 min hold) heating to 250°C at 3°C/min and heating to 300°C at 10°C/min (5 min hold). The injector temperature will be 250°C. The injection volume will be 1.0 µL (n=3) in the split mode (1:50) (Burkhard *et al.*, 2001).

III. RESULTS

Fig. 1: Top Soil Sample

Fig. 2: Mid Soil Sample

Fig. 3: Sub Soil Sample

Table 3.1: Heterotrophic bacteria count for the effluent-enriched composting soil and the control garden soil. The garden soil sample had higher counts compared to the test salon effluent-enriched composting soil which was as result of the inhibition of bacteria by the PAHs present in the test soil sample

S/N	Bacterial Isolates	Frequency (%)
1	*Pseudomonas* spp.	27.27
2	*Staphylococcus* spp.	27.27
3	*Escherichia coli*	18.18
4	*Serretia* spp.	18.18
5	*Klebsiella* spp.	9.09

Table 3.2: Frequency of Bacterial Isolates

Time (Days)	Garden soil sample	Effluent composted soil
1-3	$7.8 \times 10^3 \pm 0.10$	$4.0 \times 10^2 \pm 0.11$
4	$7.5 \times 10^3 \pm 0.11$	$3.0 \times 10^2 \pm 2.10$
5	$8.2 \times 10^3 \pm 0.20$	$1.0 \times 10^2 \pm 1.10$
6	$6.8 \times 10^3 \pm 0.40$	No growth
7	$2.0 \times 10^3 \pm 0.20$	No growth

Table 3.3: Individual PAHs Detected in the Soil Samples and Their Concentration, Biphenyl, Benzo[a]pyrene, Anthracene and Phenanthrene Were Detected in the Plastic-Enriched Composting Soil While Only Biphenyl Was Detected in the Garden Soil Sample

PAHs	Effluent composted soil	Garden soil sample
Biphenyl	45.02	2.01
benzo[a]pyrene	12.05	NR
Anthracene	28.23	NR
phenanthrene	5.07	NR
Naphthalene	<LOD	NR
Flourene	<LOD	NR
Coronene	NR	NR
Total (ng/g)	90.37	2.01

Table 3.4: Bacterial Toxicity Analysis Showing the Values for the Median Effective Concentration (EC50) and the Median Lethal Concentration (LC50) Which Was Carried Out With the Salon-Enriched Composting Soil Sample at Different Time Intervals

Incubation time	EC_{50} for acute toxicity	LC_{50} for chronic toxicity
1h	123.13	13.39
2h	111.31	15.94
3h	81.72	23.93
4h	52.00	25.04

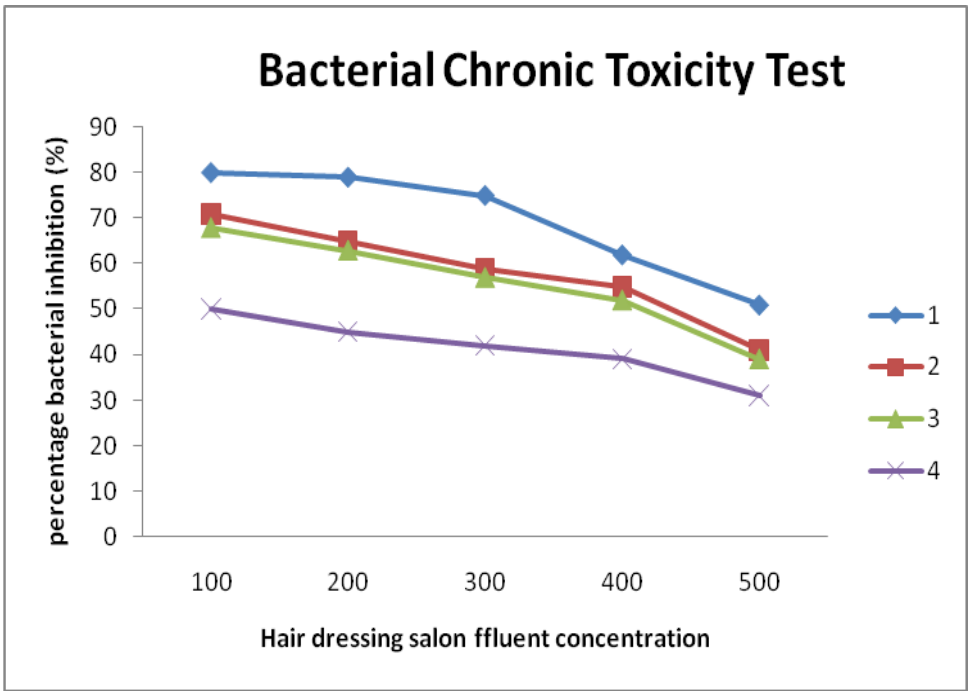

Fig. 3.3: A Growth Curve Showing Bacterial Inhibition at High Salon Effluent Concentration From 1 to 4 Hours

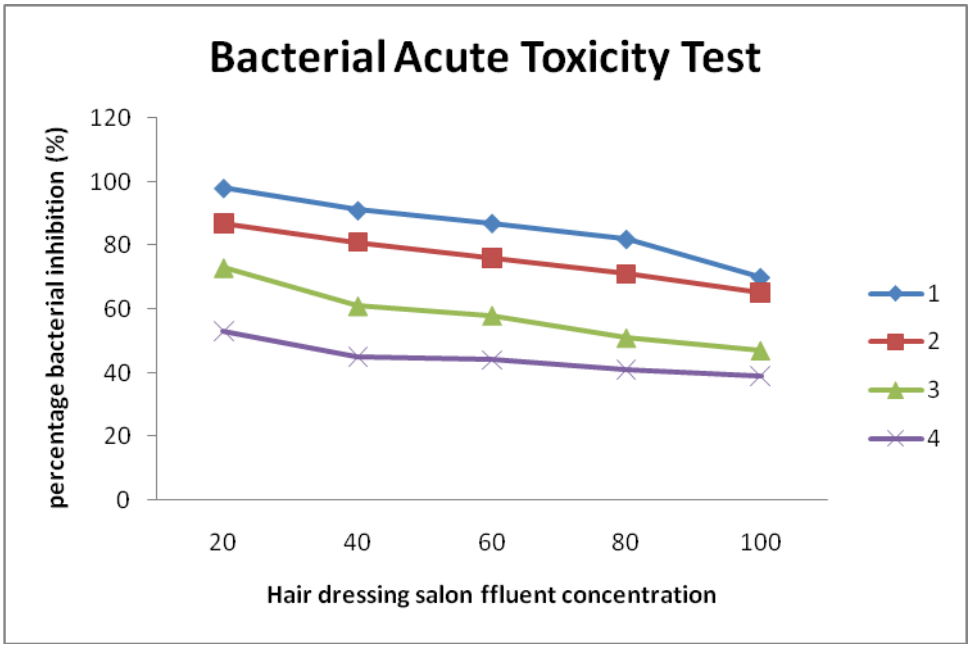

Fig. 3.4: Percentage Bacterial Inhibition at Low Salon Effluent Concentration From 1 to 4 Hours

IV. DISCUSSION

The results from this study showed that at the end of the pollution, there was a change in the colour of the soil sample from the top soil, mid soil to the sub soil which was as a result of the chemicals present in the salon effluent (Plate 1, 2 and 3).

This study revealed some of the degradation by-products of different PAHs the soil. Most of these compounds are in the degradation pathway of lots of PAHs like Biphenyl. The gas chromatography revealed the presence of PAHs in the Salon effluent composting soil. The concentration of the PAHs present in the soil were 45.02ng/g biphenyl, 28.23ng/g Benzo[a]pyrene, 12.05ng/g Anthracene, 23.00ng/g and 5.07ng/g Phenanthrene. Naphthalene and Flourene were below the limits of detection. Coronene was not recovered in the Salon effluent composting soil sample. Only 2.01ng/g of biphenyl was detected in the control garden soil, other PAHs were not recovered (Table 3.3). The total concentration of PAHs in the Salon effluent composted soil was 90.5ng/g and 2.01ng/g for the control garden soil sample. The presence of polyaromatic hydrocarbon, if not properly controlled, can affect the soil fertility and soil fauna which is has been established by (Lokke and Rasmussen, 1983).

These PAHs elicit toxic effects on the soil and soil biological sentinels. Atuanya *et al.* (2016) had earlier revealed that autotrophic transformation by nitrifying bacteria which enhances soil fertility may be hindered in an ecosystem polluted with high concentration of PAHs as nitrification processes will be altered.

PAHs have been shown to have acute effects on heterotrophic bacteria. The results showed that the bacterial counts from the control garden soil sample were higher than the test Salon effluent soil where bacterial growth was inhibited. The Salon effluent composted soil had counts ranging from 1.0 x 10^2±1.10 to 4.0 x 10^2±0.11cfu/g while the control soil sample had counts ranging from 2.0 x 10^3±0.20 to 7.8 x10^3±0.10cfu/g (Table 3.1).

This was as a result of the presence of acidic degradation PAHs in the soil which inhibited the growth of bacteria. Dalgaar *et al.* (2003) observed that pollution on soil can affect the growth of microorganisms in the soil. The degradation test was carried out; growth was observed in the mineral salt media which showed that some components of the PAHs were being degraded by bacteria. The result obtained in this study is in line with the previous study of (Wick *et al.*, 2010; Atuanya *et al.*, 2011). *Pseudomonas* sp. and

Staphylococcus sp had the highest percentage occurrence (Table 3:2).

The acute and chronic toxicity effect of the Salon effluent composted soil was conducted since growth is a function of enzyme activity and its measurement has been used as an indicator of pollution (Wilson *et al.,* 2001; Witter *et al.,* 20000). A decline in bacterial count was observed, and which could have occurred as a result of the presence of PAHs from Salon effluent which must have caused a toxic effect on the organism as earlier reported by Okpokwasili and Odokuma. (1997) who assessed the ecotoxicological impact of petroleum refinery oily sludge. The results of toxicity studies showed that the toxicity of Salon effluent composted soil on soil bacteria depended on the contact time and effluent concentration which corroborated Ibiene and Okpokwasili (2011) who assessed the toxicity of different insecticide concentrations on *Nitrobacter* sp. The EC_{50} values increased with increase in exposure time (Table 3.4) while the LC_{50} values decreased with increased exposure time. This shows that at low Salon effluent composted soil concentrations the bacteria were able to adapt and oxidize nitrite which increased with time (Figure 3.4). Also at higher Salon effluent composted soil concentration, the bacterial growth and metabolism were retarded even up to a hundred percent (Figure 3.3) resulting to decreasing LC_{50} values which is as a result of the inhibition of enzyme activities by the PAHs in the Salon effluent composting soil (Dokaniakis *et al.,* 2005; Atuanya *et al.,* 2012).

The comparison of LC_{50} and EC_{50} values of the test system showed that the LC_{50} values were lower than the EC_{50} values which suggest that LC_{50} was the best criterion for assessing response of the bacteria to toxicity. The results obtained from this study further suggest that autotrophic transformation may be hindered in an ecosystem polluted with these PAHs as bacterial growth and other microbial activities will be hindered (Ibiene and Okpokwasili, 2011).

Table 3: Shows the cultural, morphological and biochemical characteristics of bacteria isolate. The following isolate were identified. *Serretia* sp., *Klebsiella* sp., *Escherichia coli, Pseudomonas* sp., *Staphylococcus* sp. A total of 5 bacteria were isolated from the soil samples. *Pseudomonas* sp. and *Serretia* spp. had the highest percentage occurrence followed by *Klebsiella* sp. and *Escherichia coli*, this study agrees with the study of (Baath, 1989; Udochukwu *et al*., 2018; 2021).

The low occurrence of the Klebsiella can be attributed to the high Chlorine content of salon waste water as Chlorine is bactericidal to enteric bacteria (Ajuzie and Osaghae 2011; Auanya *et al*., 2016a).

The physiochemical parameters of waste water sample; the physiochemical analysis shows the pH to be 6.82. The result indicates that the pH value varies from weakly acidic. This could be attributed to the presence of chemicals like sodium hydroxide in hair relaxers and dyes used in hair conditioners (Donohue *et al.,* 2013). The pH value is 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 (Ferna´ndez, *et al.,* 2006; Fierer and Lennon, 2011; Fred, 2002; WHO, 2004; Auanya *et al.,* 2016b).

V. CONCLUSION

From the analysis of the impact of discharge from salon waste water on soil, it was discovered that salon waste water seeps into the top soil, the mid soil and the sub soil. This could affect the normal flora of the soil, and from the study, there was low occurrence of *Klebsiella,* and this could be attributed to high Chlorine content of salon waste water as Chlorine is bactericidal to enteric bacteria. Other industrial activities in the study area could have contributed and influenced the low occurrence of the organisms present in the soil. From the result of the test, it reveals that there was significant relationship between salon waste water parameters and soil microbes.

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