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Polyaromatic Hydrocarbons Levels and Bacterial Load on Soil after Consistent Disposal of Untreated Hairdressing Saloon Effluent in Lokoja, Kogi State, Nigeria

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ABSTRACT: This study estimated polyaromatic hydrocarbons levels and bacterial load on soil after thirty (30) days consistent disposal of untreated hairdressing saloon effluent in Lokoja, Kogi State, Nigeria using standard methods. After 30 days of pollution, the topsoil was collected from the height of 0-5cm, the midsoil (12-17cm) and the subsoil (25-30cm). A non-polluted soil sample was also collected which served as the control for this experiment. The total heterotrophic bacteria count was determined. Toxicity analysis was carried out to determine the effect of the effluent on soil bacteria. The soil samples were also analyzed for the presence of polyaromatic hydrocarbon using gas chromatography with mass spectrometry. The polyaromatic 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. 2.01ng/g of Biphenyl was detected in the control garden soil. Bacteria counts from the contaminated soil range from $1.0 \times 10^2 \pm 1.10$ to $4.0 \times 10^2 \pm 0.11$. The counts from the control soil sample range from $2.0 \times 10^3 \pm 0.20$ to $8.2 \times 10^3 \pm 0.20$. The control soil sample had a higher value compared to the test soil samples. Bacteria species isolated from these soil samples were: *Serretia* sp., *Klebsiella* sp., *Escherichia coli, Pseudomonas* sp., and Staphylococcus sp. with *Pseudomonas* sp. and *Staphylococcus* sp. showing increased percentage occurrence. The acute and chronic toxicity test showed a decline in the bacterial count, which could have occurred due to the presence of PAHs from Saloon effluent. It was observed that a constant release of PAHs into the soil poses serious threat to the survival of soil bacteria, and can alter the various beneficial roles these bacteria play in the soil ecosystem.

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Kogi State is one of the states in Nigeria which battles with waste management, its efficient treatment as well as discharge. It is a significant problem as Nigeria is counted among the developing countries which do not channel much attention towards efficient wastewater 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, wastewater 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 worldwide, particularly by the United Nations Environmental Programme (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 artificial pollution are the transmission of disease by water-borne pathogens, eutrophication of natural water bodies, accumulation of toxic and recalcitrant chemicals in the soil, destabilization of the ecological

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balance, and adverse effect on human health (Chikere and Okpokwasili, 2004).

The continuous trend toward the formulation of new beauty tips and the manufacture of novel hair products to satisfy the demands of the growing populace could lead to some pollution problems. Today's saloons 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 harm the environment (Bowers *et al.,* 2002). A typical example of what happens is logging of contaminated water in the soil. Leaching into groundwater is a significant 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 of public health and conservation which results in the destruction of pathogens and the mineralization of the organic components of sewage before to discharge. Anaerobic wastewater treatment using a granular sludge reactor is one such method (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 the Federal Environment Protection Agency (FEPA) in 1998. Other treatment considerations can be the removal of toxic organic pollutants and heavy metals, altering the physical conditions of the water (e.g., pH, electrical conductivity, etc.), removing sediment loads, or biochemical oxygen demand (BOD). The objective of this study was to estimate of polyaromatic hydrocarbons levels and bacterial load on soil after thirty (30) days of consistent disposal of untreated hairdressing saloon effluent in Lokoja, Kogi State, Nigeria

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 Jerrican with a height of about 30cm was filled with soil. The Jerrican was cut open at the top and bottom.The saloon effluent was collected in with another Jerrican from different saloons in Ganaja village everyday for 30days and poured into the Jerrican containing the soil. After 30 days of pollution, the topsoil was collected from the height of 0-5cm, the midsoil (12-17cm) and the subsoil (25-30cm). A non-polluted garden soil sample was also collected, which served as the control soil sample for the experiment. The four soil samples were taken to the laboratory to air dry for two days and were sieved. The soil samples (1gram) each was weighed on the analytically weighing balance in the laboratory. A total viable heterotrophic bacterial count was determined using the pour plate technique. The bacterial isolates were identified using various biochemical test (Burkhard *et al*., 2001). The soil samples were analysed for the presence of PAHs using a Hewlett Packard HP 5890 series II Gas chromatograph with Mass spectrometry (Udochukwu *et al*. 2014).

Preparation of Hairdressing saloon effluent concentration for Toxicity Test: For the determination of the median lethal concentration (LC_{50}), hairdressing saloon effluent concentrations of 100, 200, 300, 400, and 500 ml/l was formulated by adding (100, 200, 300, 400 and 500 g) to 1000 ml of Winograsky medium respectively (Ibiene and Okpokwasili, 2011). For the median effective concentration, the following concentrations (20, 40, 60, 80 and 100 ml/l.) of the effluent was formulated by adding (20, 40, 60, 80, and 100 g) to 1000 ml of Winograsky medium respectively. A control experiment consisting of Winograsky medium only was set up (Ibiene and Okpokwasili, 2011).

Soil Bacteria Acute and chronic Toxicity Test: The acute toxicity test was 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 was 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 was fortified with several milliliters of hairdressing saloon effluent (100, 200, 300, 400, and 500 ml/l) and (20, 40, 60, 80, and 100 ml/l), respectively. They were inoculated with ten milliliters of bacteria standard inoculums and allowed to stand for an hour for growth. 1 ml of the suspension after an hour was inoculated from the mineral salt media composted with different volumes of hairdressing saloon effluent on a non-hairdressing saloon effluent composted Winograsky agar plates. It was repeated for 2, 3, and 4 h intervals (Okpokwasili and Odokuma, 1996). The colony-forming units for each plate was calculated and used to determine the acute toxicity (EC_{50}) of the various hairdressing saloon effluent composted mineral salt media. The chronic toxicity of the effluents on soil bacteria was determined by calculating the lethal concentration (LC_{50}) using Probit software. All results were subjected to the analysis of variance (ANOVA) (Ferrara *et al.*, 2006; Udochukwu *et al*., 2022).

RESULTS AND DISCUSSION

The results from this study showed that at the end of the pollution, there was a change in the color of the soil sample from the topsoil, midsoil to the subsoil, which was a result of the chemicals present in the saloon effluent (Plate 1, 2 and 3). This study revealed some of the degradation by-products of different PAHs in the test soil sample. Most of these compounds are in the degradation pathway of many PAHs like Biphenyl. The gas chromatography revealed the presence of PAHs in the Saloon 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 Fluorene were below the limits of detection. Coronene was not recovered in the Saloon effluent composting soil sample.

Plate. 1: Topsoil sample

Plate. 2: Midsoil sample

Only 2.01ng/g of Biphenyl was detected in the control garden soil (Table 3). The total concentration of PAHs in the Saloon effluent composted soil was 90.5ng/g and 2.01ng/g for the control garden soil sample. The presence of polyaromatic hydrocarbon, if not adequately controlled, can affect the soil fertility and soil fauna, which 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, was hindered in an ecosystem polluted with high concentration of PAHs as nitrification processes were altered.

Plate. 3: Subsoil sample

The acute and chronic toxicity effect of the Saloon effluent composted soil was conducted since growth is a function of enzyme activity and its measurement has been used as an indicator for pollution (Wilson *et al.,* 2001; Witter *et al.,* 20000). A decline in bacterial count was observed, which could have occurred due to the presence of PAHs from Saloon effluent. This must have elicited toxic effects on the organisms, as earlier reported by Okpokwasili and Odokuma. (1997), who assessed the ecotoxicological impact of petroleum refinery oily sludge. The toxicity studies showed that the toxicity of Saloon 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 decreased with an increase in exposure time (Table 4), while the LC_{50} values increased with increased exposure time. It shows that at low Saloon effluent composted soil concentrations, the bacteria were able to adapt and oxidize nitrite (Fig. 2). Also, at higher Saloon effluent composted soil concentration, the bacterial growth and metabolism were retarded (Fig. 1), resulting in increasing LC_{50} values, which is a result of the inhibition of enzyme activities by the PAHs in the Saloon 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 suggests that LC_{50} was the best criterion for assessing the response of the bacteria to toxicity. The results obtained from this study further indicate that autotrophic transformation may be impeded in an ecosystem polluted with these

PAHs as bacterial growth and other microbial activities will be hindered (Ibiene and Okpokwasili, 2011).

Table 1: Heterotrophic bacteria count for both soil samples

Time (Days)	Control Garden soil sample	Effluent-composted soil sample
1-3	7.8 x10^3±0.10	4.0 x 10^2±0.11
4	7.5 x 10^3±0.11	3.0 x 10^2±2.10
5	8.2 x 10^3±0.20	1.0 x 10^2±1.10
6	6.8 x 10^3±0.40	No growth
7	2.0 x 10^3±0.20	No growth

Table 2: Frequency of Bacterial Isolates

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: Individual PAHs seen detected in both soil samples

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

Key: *LOD: Limit of Detection; R: Not Recovered*

Table 4: EC_{50} and LC_{50} values

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

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 compared to the test Saloon effluent soil, where bacterial growth was inhibited. The Saloon 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 1). This was due to the presence of acidic degradation by products of PAHs in the soil, which inhibited the growth of bacteria. Dalgaar *et al.* (2003) observed that pollution on soil could affect the growth of microorganisms in the soil. *Pseudomonas* sp. and *Staphylococcus* sp. had the highest percentage occurrence (Table 2). The result obtained in this study is in line with the previous studies (Wick *et al.*, 2010; Atuanya *et al.*, 2011).

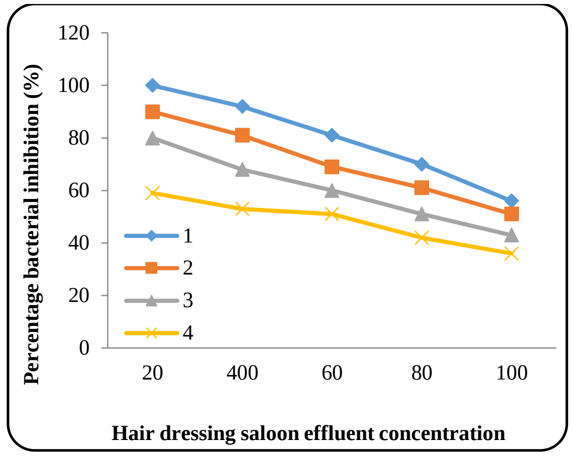

Fig. 1: A growth curve showing bacterial inhibition at high saloon effluent concentration from 1 to 4 hours

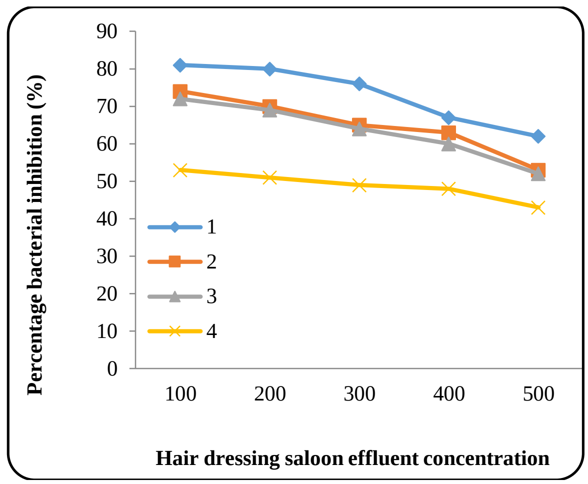

Fig. 2: percentage bacterial inhibition at low saloon effluent concentration from 1 to 4 hours

The following isolates were identified. *Serratia* sp., *Klebsiella* sp., *Escherichia coli*, *Pseudomonas* sp., *Staphylococcus* sp. A total of five (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 analysis agrees with (Baath, 1989; Udochukwu *et al.*, 2018; Udochukwu *et al.*, 2021). The low occurrence of Klebsiella can be attributed to the high Chlorine content of saloon wastewater, as Chlorine is bactericidal to enteric bacteria (Ajuzie and Osaghae, 2011; Atuanya *et al.*, 2016a).The physiochemical analysis of the wastewater sample had a pH of 6.82. The result indicates that the pH value varies from weakly acidic, which could be attributed to 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 wastewater discharge into the surrounding (Ferna´ndez, *et al.,* 2006; Fierer and Lennon, 2011; Fred, 2002; WHO, 2004; Atuanya *et al.,* 2016b).

Conclusion: From the analysis of the impact of discharge from saloon wastewater on the soil, which revealed that saloon wastewater seeps into the topsoil, the midsoil, and the subsoil. This could affect the normal flora of the soil. From the study, there was a low occurrence of Klebsiella, which could be attributed to the high Chlorine content of saloon wastewater as Chlorine is bactericidal to enteric bacteria. Other industrial activities in the study area could have influenced the low occurrence of the organisms present in the soil. The results from the test reveal that a continuous release of untreated hair dressing effluents had significant impact on microbial growth.

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