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Determination of Total Petroleum Hydrocarbon Composition and Screening for Metallotolerant Bacteria from Polluted Effluent Discharge in Kaduna Metropolis, Nigeria

***Musa Nomsu[1]; Sambo Dearest[1]; Masha Glory[2] and Abdu Mohammed[1]**

[1]Department of Microbiology, Faculty of Pure and Applied Sciences, Kaduna State University, Nigeria
[2]Department of Microbiology, Faculty of Life Sciences, Bayero University, Kano-Nigeria
*Correspondence author: nomsumusa35@gmail.com

Abstract

The release of heavy metals into aquatic systems results in various environmental challenges. The combined presence of hydrocarbons and heavy metals within the environment also poses a health risk to humans, plants, and animals. This study aimed to screen for metallotolerant bacteria from a polluted effluent discharge in Kaduna metropolis, Nigeria. Physicochemical assessment, determination of total petroleum content, and heavy metal screening of the wastewater samples were conducted using standard protocols. Metallotolerant bacteria were screened and identified using standard methods. It was revealed from the result that, on the total petroleum hydrocarbons (TPHs), eicosane had the highest detectable similarity with percentage quality (99%), while oxasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl (50%) was the lowest. Lead had the highest concentration of 0.95±0.017 Mg/L, while zinc had the lowest value, 0.057±0.00 mg/L. The bacterial species identified were Escherichia coli, S. aureus, Klebsiella sp., Pseudomonas sp., and Bacillus species, respectively. Furthermore, the tolerance assay results showed that Pseudomonas species had the maximum tolerance for Cu (0.72 mg/mL), Bacillus species for Pb (0.79 mg/mL), Escherichia coli for Zn (0.76 mg/mL), and Staphylococcus aureus for Fe (0.92 mg/mL), respectively. Based on the maximum tolerance exhibited by these bacterial isolates to heavy metals, this study proved they are good candidates, especially isolates of (Staphylococcus aureus, Pseudomonas species, and Bacillus species) for use in bioremediation and bioaugmentation of heavy metals in polluted industrial sites.

Keywords: Hydrocarbon composition, Metallotolerant, Bacteria, Effluent discharge, Nigeria

INTRODUCTION

The rapid pace of industrialization, population growth, and unplanned urbanization have led to significant contamination of water and soil (Li *et al.,* 2018). Freshwater contamination can primarily be attributed to the release of untreated and toxic industrial waste, the dumping of industrial wastewater, and runoff from agricultural fields. It has been estimated that 70-80% of all problems in developing countries are traceable to water pollution, and this typically affects women and children (Li *et al.,* 2018). The toxic pollutants released into wastewaters can be harmful to aquatic organisms, which also makes regular waters unfit for consumption as water sources (Sahmoune, 2018). A substantial number of poisonous substances, including toxic metals, pharmaceuticals, pesticides, dyes, and surfactants, have polluted global water resources and are considered ecologically hazardous to humans and wildlife (Grace-Pavithra *et al.*, 2019; Saravanan *et al.*, 2019).

The release of heavy metals into aquatic systems may result in various physical, chemical, and biological processes (Guo *et al.*, 2018). These can be generally divided into two: the effects of heavy metals on the environment and the effects of the environment on heavy metals (Guo *et al.*, 2018). The first classification depends on the natural conditions; there may be adjustments in diversity, density, species composition of the population, and structure of the microbial community (Guo *et al.*, 2018). The nature and degree of change depend to a great extent on the concentration of heavy metal species in the water and the residues (Guo *et al.*, 2018). Hence, the physicochemical processes within the effluents and aquatic systems have a noteworthy, albeit indirect, impact on the biological responses (Zhao *et al.*, 2018). Heavy metals have been characterized by their inability to biodegrade, the possibility of their transformation into more toxic forms, and their ability to accumulate and remain within the tissues of living organisms, thus transferring through the food chain (Igiri *et al.*, 2018). Therefore, the presence of heavy metals in the environment must be within specific concentrations for certain metals, especially those that are essential but become toxic at high concentrations, such as cobalt, zinc, copper, nickel, and chromium (Gossuin and Vuong, 2018). As for non-essential metals, such as Lead, cadmium, mercury, and silver, they are very toxic even in low concentrations and have no significant metabolic role in the organisms (Jaishankar *et al.*, 2014; Gossuin and Vuong, 2018).

Hydrocarbons are defined as organic compounds made up primarily of carbon and hydrogen; however, they can also contain oxygen, nitrogen, and halogens (Huang *et al.*, 2018). Hydrocarbons can be gases, volatiles, oil vapour, and oil mist (Huang *et al.*, 2018). The sum of the various hydrocarbons yields the total hydrocarbon content, which is measured in milligrams per liter (Tariq *et al.*, 2016). High concentrations of hydrocarbons and heavy metals present in contaminated sites could pose a health risk to human, plant, and animal lives (Huang *et al.*, 2018). The study of bacteria from heavy metal-polluted wastewater and their bioremediation potential will enable the use of microorganisms for the treatment of industrial effluents instead of conventional methods that are unfriendly to the environment, less effective, have an extended processing time, and are expensive (Grace-Pavithra *et al.*, 2019). Bacteria play a vital role in the cost-effective and efficient removal of heavy metals from polluted wastewater. Bacteria are capable of absorbing pollutants and removing heavy metals from the environment; they are particularly well-suited for bioremediation due to their ease of manipulation (Grace-Pavithra *et al.*, 2019).

Microorganisms have evolved various mechanisms of metal resistance, and scientists have tried to exploit the genetic and metabolic basis of such mechanisms for the engineering of superior strains (Huang *et al.*, 2018). Scientists have explored this attribute to ascertain if tolerance to higher concentrations can subsequently aid in the biodegradation of such toxicants (Huang *et al.*, 2018). In a previous study, the specific metal uptake capacity and metal removal (%) of adapted cells were found to be higher than those of non-adapted cells at all concentrations tested (Zhao *et al.*, 2018). Although the authors could not elucidate the mechanism of adaptation, they suggested that constitutive synthesis of methalothionein, other copper-binding proteins, or changes in the genetic makeup could be the factors responsible for the adaptation (Huang *et al.*, 2018; Zhao *et al.*, 2018).

Bacteria, in particular, have developed mechanisms for tolerating and removing heavy metals from polluted wastewater, including the production of metabolites that aid in heavy metal bio-removal, which can be applied in the bioremediation of industrial effluents (Mathivanan *et al.*, 2021).

Insufficient/updated data on the total petroleum hydrocarbon composition, heavy metal levels, isolation, and identification of indigenous bacteria, as well as their tolerance to these heavy metals, and assessment of the bioremediation potential of the bacteria, necessitate this present study.

MATERIALS AND METHODS

Study Area and Sample Collection

The study area was the Panteka stream, located in Kaduna metropolis, along the Nnamdi Azikiwe Expressway bypass. The study area lies between latitudes 10 ° 33’ 39’’ to 10 ° 34’ 26’’ N and longitudes 7 ° 24’ 23’’ to 7 ° 25’ 17’’ E, with a total area of 3.21 km^2. A wastewater sample was collected aseptically in a sterile bottle. The lid of the bottle was removed, held by its base, and completely submerged below the surface of the wastewater. The bottle was filled by holding it upstream in the flowing water and in a sweeping motion to prevent any water that had come into contact with the hand from entering the bottle. The sample was transported to the Department of Microbiology Laboratory at Kaduna State University, Nigeria, for further analysis.

Determination of Total Petroleum Hydrocarbons (TPHs)

Effluent samples were analyzed for the presence of total petroleum hydrocarbons using gas chromatography coupled to mass spectrometry (GC-MS). Gas chromatography was performed using a Hewlett Packard HP5890-II (HP, Palo Alto, USA) machine. The MS-DOS workstation was used for both system control and data acquisition. For the extraction, approximately 5.0 mL of the effluent sample was used, thoroughly mixed with 150 mL of extraction solvent, and extracted over 4 hours (Olayinka and Alo, 2014). The extraction was performed in 1,1,2-trichloro-1,2,2-trifluoroethane. After the extraction, 0.3g of silica gel was added to adsorb the polar material, such as vegetable oils and animal fats. The USEPA Method 8440 regards all "oil and grease" materials that are not eliminated by silica gel adsorption as "petroleum hydrocarbons." The extracts were filtered through Whatman GF/C filters (UK) using a DINKO D-95 vacuum pump (Spain). Sodium sulphate was added to the samples during the extraction procedure and in the filtration process to eliminate residual water. The extracts thus obtained were analyzed by carrier gas GC-MS.

Determination of Heavy Metals in Effluent Discharge Samples

The concentrations of heavy metals in effluent samples were analyzed using a fast sequential atomic absorption spectrophotometer (Abdullah *et al.,* 2020). (Model AA240S, Varian Technologies, Florida, USA). The instrument's settings and operational conditions were set in accordance with the manufacturer's specifications by calibrating with an analytical-grade metal standard stock solution. The wet digestion method was used for the samples, in which 5.0 mL of the sample was placed in a 100 mL volumetric flask, and 4 mL of HNO3 was added. The solution was allowed to stand for a few hours, and then it was carefully heated over a water bath till the red fumes completely ceased coming out of the flask. The flask was allowed to cool to room temperature, and then 4 mL of perchloric acid was added. The flask was thereafter heated again over a water bath to evaporate, until only a small portion was filtered through Whatman filter paper. The volume was made up to 100 mL using distilled water (Mathivanan *et al.,* 2021).

Physiochemical Parameters and Determination of Heavy Metals in Waste Water Sample

The wastewater sample was processed to determine several physicochemical parameters of the wastewater, including total dissolved solids (TDS), total alkalinity (TA), total hardness (TH), pH, CO2, nitrate, and chloride, using standard methods as described by Thipeswamy *et al.* (2012).

Isolation and Characterization of Bacteria from Waste Water Sample

From the wastewater sample, 1.0 mL was serially diluted aseptically using sterile distilled water until a dilution of 10^{-6} was obtained. Aliquots of 0.1 mL from $10^{(-4)}$ dilutions were inoculated on nutrient agar in duplicate and incubated to isolate total culturable heterotrophic bacteria. The plates were incubated at 37 ˚C for 24 hours. The developed colonies on the plates were counted and expressed as colony-forming units per millimetre (CFU/mL) of wastewater. Distinct colonies were isolated and sub-cultured on selective and differential media. Identification of the isolates was achieved by adopting conventional methods of bacterial identification, including cultural and morphological features, microscopy, and biochemical characterization (such as Gram staining, catalase, citrate, methyl red, indole, motility, triple sugar iron, and Voges-Proskauer) as described in Bergey's Manual for Determinative Bacteriology.

Heavy Metal Tolerance Concentration Determination

Each isolated bacterium was inoculated into a nutrient broth containing different concentrations of heavy metals. The heavy metals were used in their salts namely: $ZnSO_4$, $FeSO_4$, $CuSO_4$, and $PbSO_4$. The concentrations of copper, Iron, zinc, and lead were 0.5, 1.0, 1.5, 2.0, and 2.5 mg/mL, respectively. The growth optical density was observed using a spectrophotometer at an absorbance of 600 nm, and nutrient broth containing the same amount of heavy metals was used as a blank. After 24 hours, 48 hours, 72 hours, 96 hours, and 120 hours (Pandit et al., 2013), the growth optical density was measured.

RESULTS

It was revealed that eicosane recorded the highest detectable similarity of the total petroleum hydrocarboncomposition with percentage quality (99%), followed by hexadecanoic acid methyl ester (98%), 11-octadecenoic acid methyl ester (97%), heptacosane (97%), hexacosane (96%), nonadecane (94%), 1-decanol, 2-hexyl (72%) octane-2-methyl (54%), and octadecamethyl-(50%).

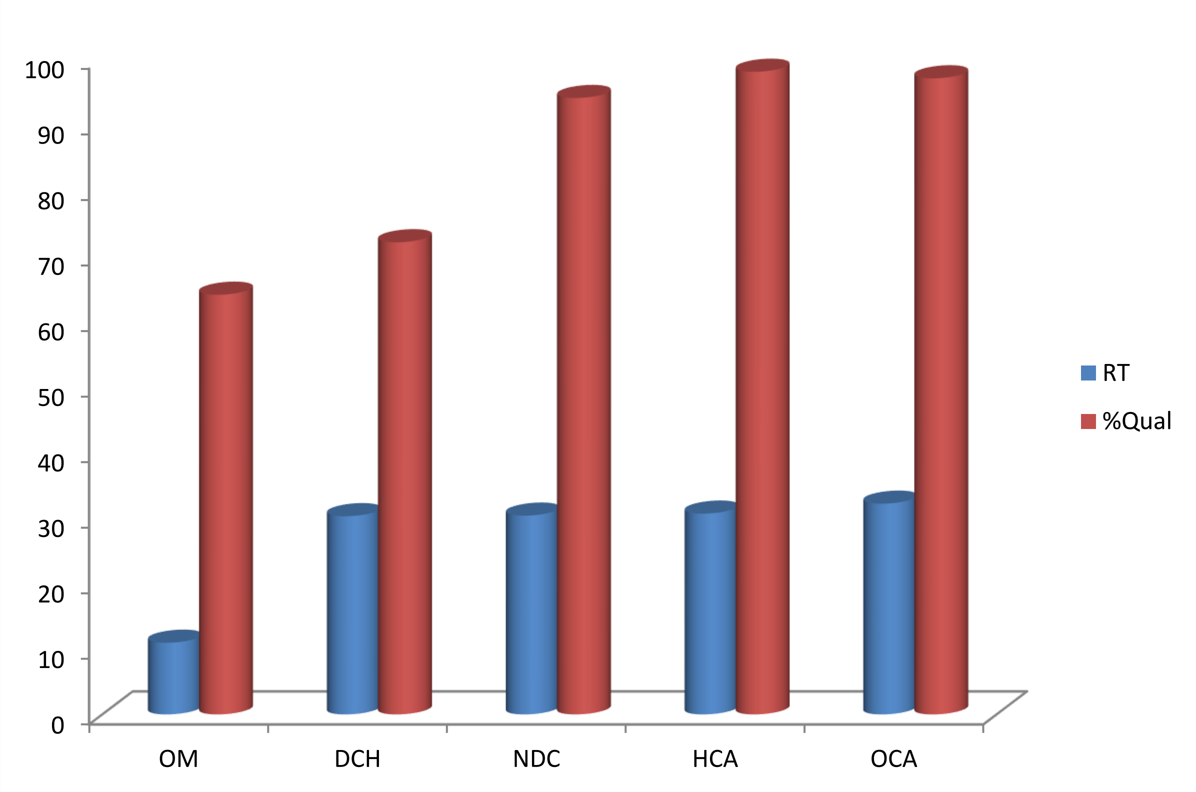

Fig. 1 above shows the total petroleum hydrocarbons indicating the retention time and percentage quality.
Key:RT = retention time; QUA = percentage quality; OM = octane 2-methyl; DCH = 1-decanol, 2-hexyl; NDC = nonadecane; HCA = hexadecanoic acid, methyl ester; OCA = 11-octadecenoic acid, methyl ester

Table 1: Physicochemical Properties of the Wastewater Sample

Parameter	Value (Mean ± SD)	WHO Standard
Total Dissolved Solids (mg/L)	99.7 ± 3.162	500
Nitrate (ppm)	0.62 ± 0.072	NS (Not Specified)
Total Alkalinity (mg/L)	245 ± 7.214	100
Total Hardness (mg/L)	0.28 ± 0.000	100
Chloride (mg/L)	56 ± 0.821	250
pH	7.32 ± 0.17	6.5-8.5
Carbon Dioxide (mg/L)	80 ± 0.086	50

Table 2: Heavy Metal Concentrations of Waste Water Samples

Parameter	Concentration (mg/L)	WHO Standard (mg/L)
Copper (Cu)	0.79 ± 0.00 **	2.00
Lead (Pb)	0.95 ± 0.017 *	0.05
Zinc (Zn)	0.06 ± 0.00 **	3.00
Iron (Fe)	0.32 ± 0.001 *	0.31

Key: values with a single asterisk are statistically significant, while values with double asterisks indicate a high statistical significance value at $p < 0.05$ level of significance using SPSS.

The physicochemical parameters of the wastewater, as shown in Table 1 below, reveal the values obtained for each parameter and the corresponding World Health Organization (WHO) standards. According to the table, the levels of total dissolved solids, nitrate, pH, chloride, and total hardness are within the standards set by the WHO, while carbon dioxide and total alkalinity exceed the WHO limits.

The analysis of heavy metals in the wastewater samples indicated that Lead (Pb) had the highest concentration, at 0.95 mg/L, followed by Iron (0.32 mg/L), zinc (0.06 mg/L), and copper (0.79 mg/L), respectively. It was noted that the concentrations of Lead (Pb) and Iron (Fe) in the wastewater discharge were far above the Standard set by the World Health Organization (WHO), as shown in Table 2 below.

Table 3 below shows the Gram reaction and biochemical characterization of the bacteria isolated from the wastewater sample. Isolates of *Escherichia coli*, *Klebsiella* species, and *Pseudomonas* species were Gram-negative (-) rod-shaped bacteria, while *Staphylococcus aureus* and *Bacillus* species were confirmed to be Gram-positive cocci and rod-shaped bacteria.

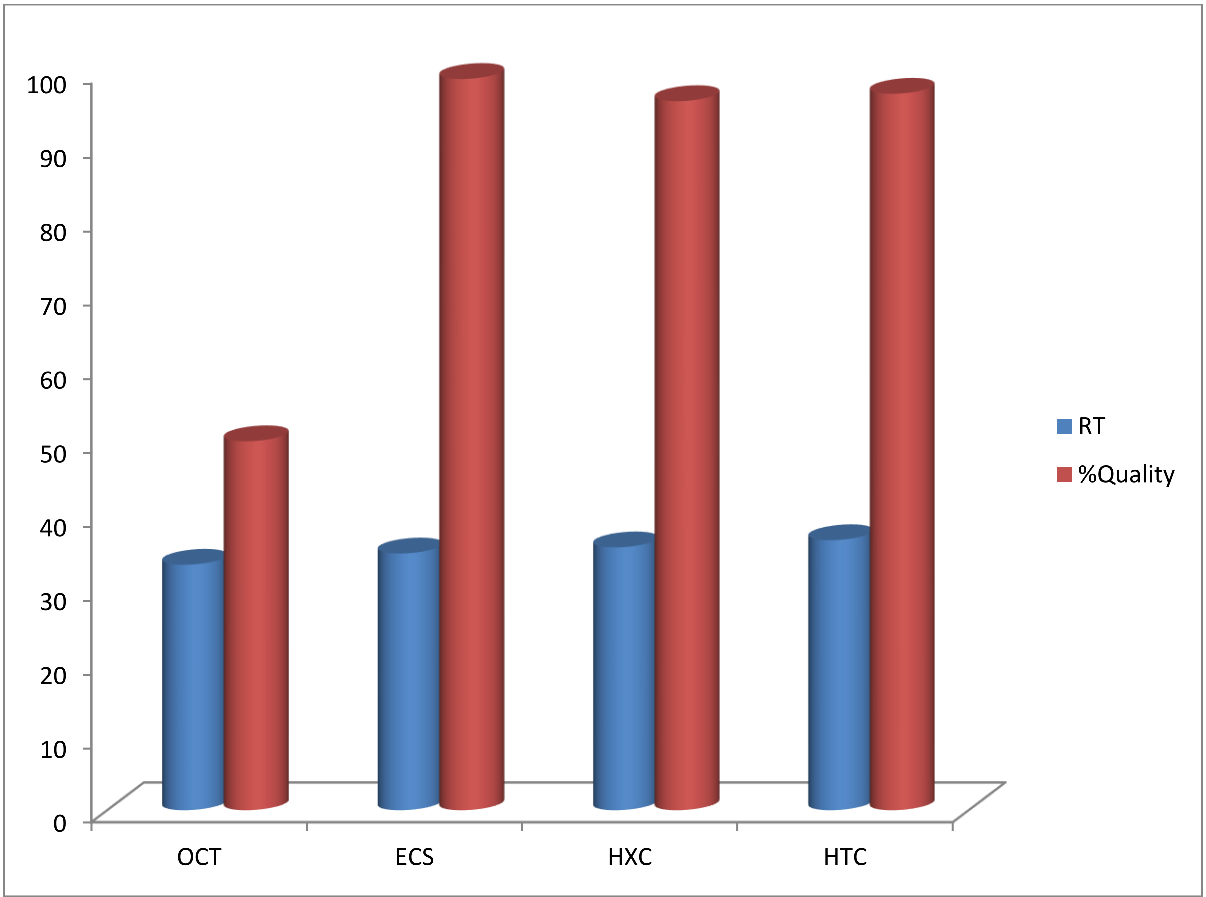

Fig. 2 above shows the total petroleum hydrocarbons indicating the retention time and percentage quality.
Key:RT = retention time; QUA = percentage quality; OCT = oxasiloxane; ECS = eicosane; HXC = hexacosane; HTC = heptacosane.

Table 3: Identification and Characterization of Bacteria Isolated from Waste Water Sample

Suspected Organism	GS	CAT	CT	MR	VP	IN	MT	TSI
Escherichia coli	-	+	-	+	-	+	+	A/AG
Klebsiella spp.	-	+	+	-	+	-	-	A/A
Pseudomonas spp.	-	+	+	-	-	-	+	A/A
Staphylococcus aureus	+	+	+	+	+	-	-	A/A
Bacillus spp.	+	+	+	-	+	-	+	A/A

Key: + (positive),- (negative), GS = Gram Stain, CAT= Catalase, CT= Citrate, MR= Methyl Red, VP= Voges Proskauer, IN= Indole, MT= Motility, TSI= Triple Sugar Iron, A/AG= Acid/Acid Gas (yellow/yellow butt), A/A= Acid/Acid (Red/Red butt).

Table 4 showed the percentage occurrences of the bacteria isolates from Panteka effluents. Thirty-two (32) bacterial isolates were isolated from the samples, and it was revealed that isolates of *S. aureus*had the highest occurrence 11/32 corresponding to (34.37%), *Klebsiella* species 8/32 (25.00%), *E. coli* and *Bacillus* species had each 5/32 (15.63%) respectively and *Pseudomonas* species having the lowest percentage 3/32 corresponding to (9.37%).

Table 4: Percentage Occurrence of Bacteria Isolates from Panteka Effluents

Bacteria	Frequency (n)	Percentage (%)
Escherichia coli	5	15.63
Klebsiella spp.	8	25.00
Pseudomonas spp.	3	9.37
Staphylococcus aureus	11	34.37
Bacillus spp.	5	15.63

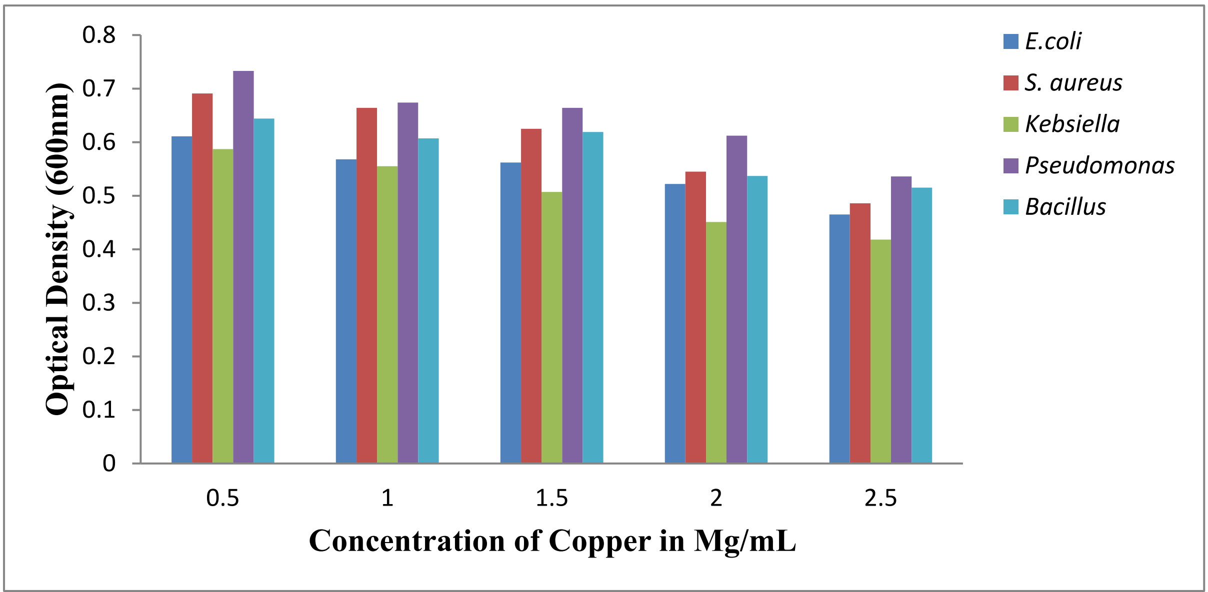

Figure 3: Effect of different copper concentration on the growth rate of bacteria isolates

For copper (Fig. 3), *Pseudomonas* species had maximum tolerance (0.72) while *Klebsiella* species had the minimum tolerance (0.48)

For Lead (Fig. 4), *the Bacillus species* had the maximum tolerance (0.79), while the *Klebsiella* species had the minimum tolerance (0.47). The bacterial isolates were able to tolerate 2.5 mg/mL of the heavy metal, as seen below.

This presents the optical density of bacteria growing in different concentrations of Zn. It was shown that *Escherichia coli* had the maximum tolerance (0.76), while *Klebsiella* species had the minimum tolerance (0.38). The bacterial isolates were able to tolerate 2.5 Mg/mL of the heavy metal (Fig. 5).

The figure below (Fig. 6) shows the optical density for Iron. It was found that Staphylococcus aureus had the maximum tolerance (0.92), while *Escherichia coli* had the minimum tolerance (0.53). The bacterial isolates were able to tolerate 2.5mg/ml of the heavy metal.

DISCUSSION

The total petroleum hydrocarbons found in this study were of significant concern to environmental health, as their occurrence and composition were shown to be extremely high (Figures 1 and 2), respectively. The presence of petroleum hydrocarbons in water bodies poses a great danger to the aesthetic nature of the river, creating an unpleasant odor and causing harm to both the micro- and macrofauna of such bodies. These petroleum products become attached to growing plants along the riverbank, subsequently transferring their effects to humans as they consume the irrigated farm produce and seafood (WHO, 2017).

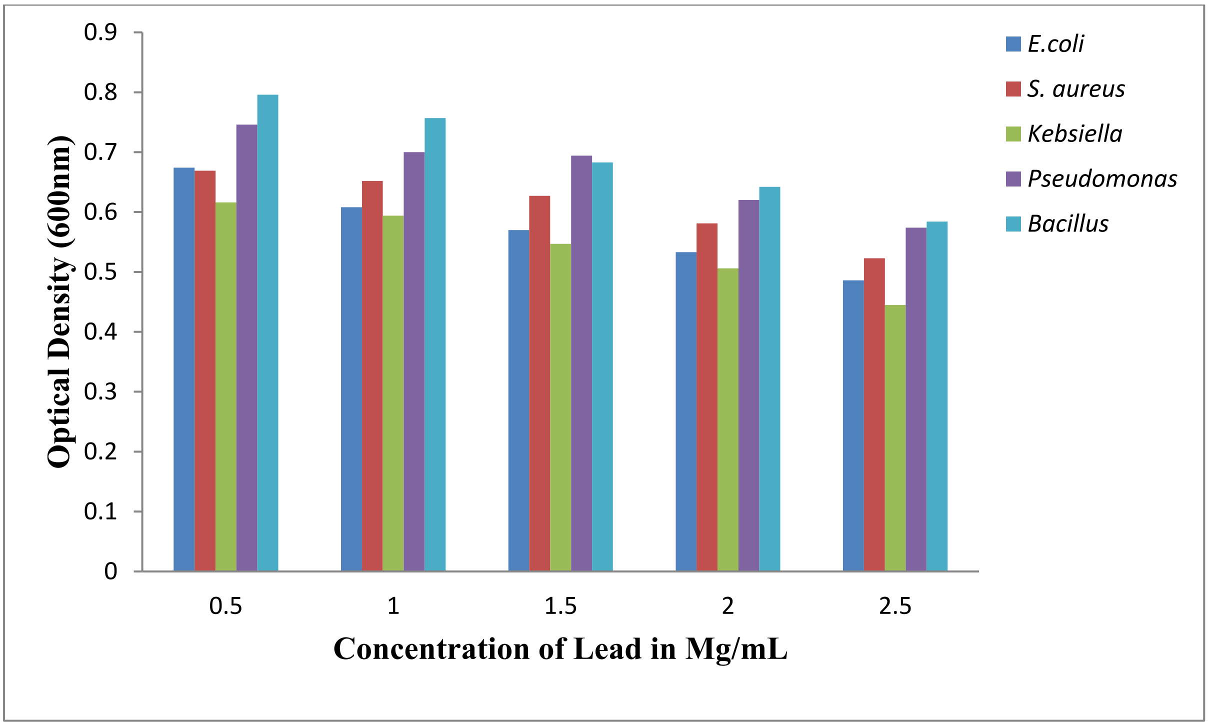

Figure 4: Effect of different lead concentration on the growth rate of bacteria isolates.

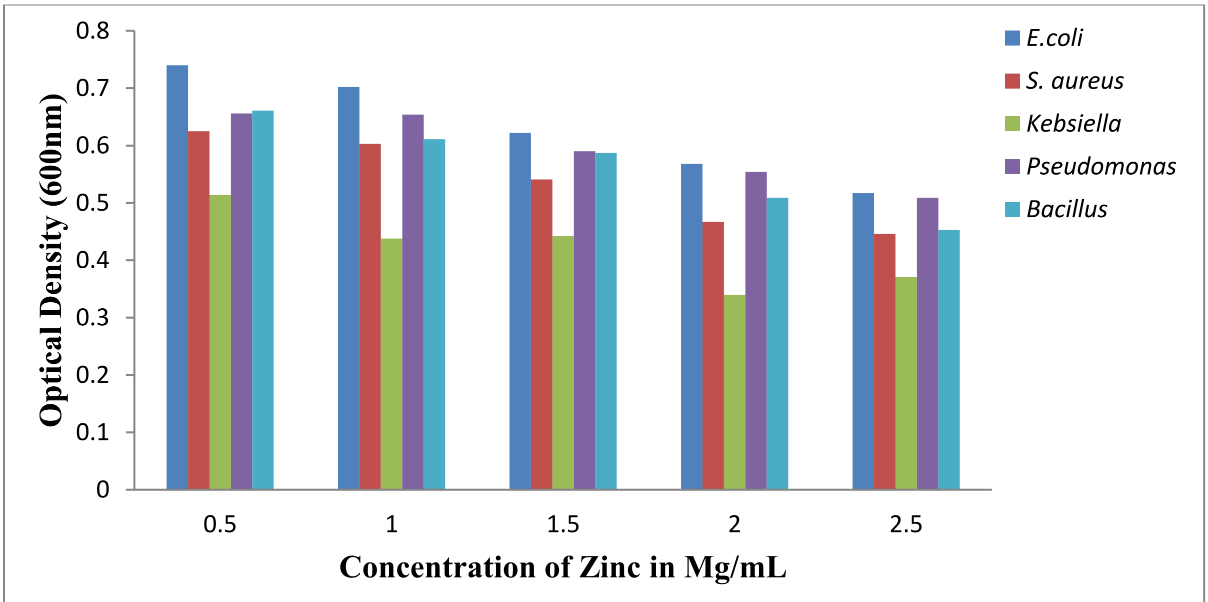

Fig 5: The bacterial isolates tolerance of the heavy metal.

The results of the physicochemical parameters of the wastewater samples investigated in the present study are presented in Table 1. The measured TDS value of 100 mg/L falls within the acceptable limit 500 Mg/L (WHO, 2014). The high value of total alkalinity, among other factors, implies that the water could corrode containers used for water storage (Adesina *et al.*, 2018). The observed pH value of 7.32 indicates that the water is within the neutral pH range. The value is above the findings of Abdullahi *et al.* (2020), who reported an acidic pH of 5.661 in abattoir wastewater. They observed that the pH is within the allowable limit set by the WHO. The reasons why the polluted effluent has a neutral pH could be attributed to several factors, such as effluent mixing with other water sources, buffering capacity, chemical reactions, biological treatment, and dilution volumes, among others. The alkalinity of the water sample was observed to be 245±7.214 mg/L, which exceeded the normal WHO standard. The high level of alkalinity makes the water unsuitable for irrigation, while its hardness is below the WHO standards. Water can have a high alkalinity level but a neutral pH due to the presence of alkaline substances, such as bicarbonates, carbonates, and hydroxides, which can neutralize the pH (Abdullahi *et al.*, 2020). The high level of CO_2 (80) in the wastewater sample could be a result of pollution by contaminated substances, which could also lead to the death of aquatic life, such as fish, through suffocation due to low oxygen in the water (Elemile *et al.*, 2019). The high level of CO_2 in wastewater exceeds the standards set by the WHO, which could be a result of pollution.

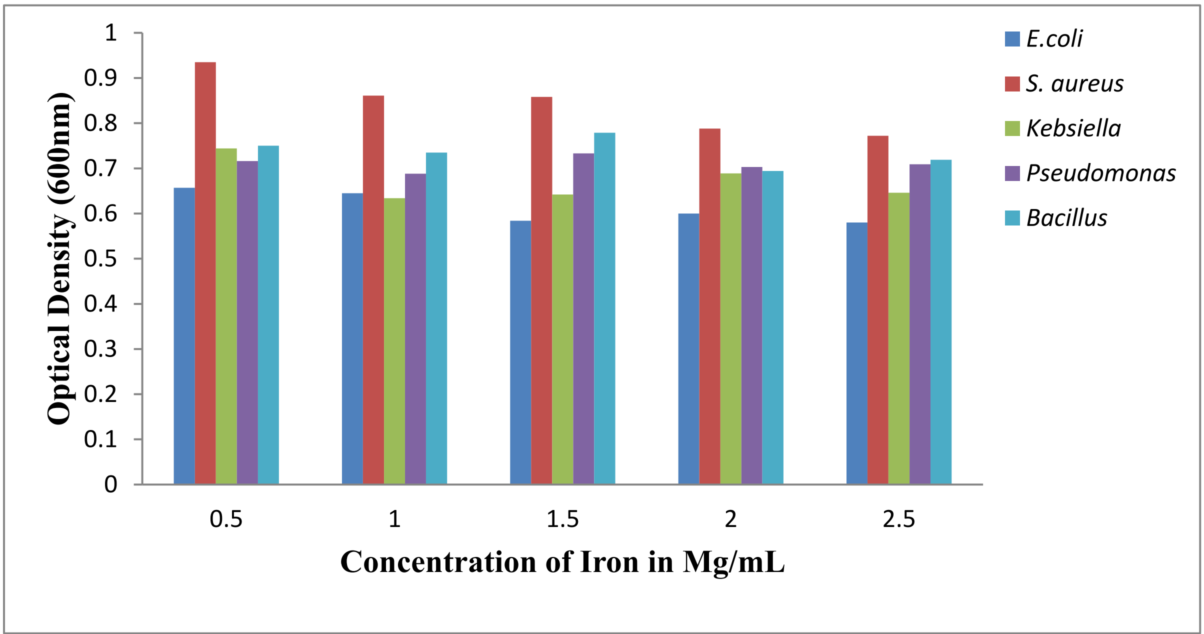

Figure 6: Effect of different iron concentration on the growth rate of bacteria isolates.

Heavy and trace metals are of importance in water, as living organisms require varying amounts of some of these metals as nutrients for proper growth and development, e.g., Cu, Fe, Mn, Ni, and Zn (Adeyebo *et al.*, 2019). On the other hand, metals such as Ag, Cd, Pb, and Hg have no known biological role and are therefore considered non-essential (Apkor and Muchie, 2011). Their presence in wastewater may be due to discharges from residential dwellings, groundwater infiltration, and industrial discharges. The concentration level of heavy metals in most Nigerian rivers is well above the acceptable and allowable limits for Pb, Cu, Zn, and Fe (Olayinka and Alo, 2014). The concentration of Iron stood at 0.32 mg/L. This value contradicts the reported concentrations of 0.54 mg/L from earlier findings (Adeyebo *et al.*, 2019). The differences could be due to the source of the sample analyzed, the extent to which the sample is polluted, among several other factors. This concentration exceeds the allowable limit set by the WHO (WHO, 2014). The observed level of zinc was 0.06 mg/L. The value falls within the allowable limit of 3.0 mg/L. The presence of zinc may be due to the infiltration of rainwater from nearby roofs. Excessively high levels may enhance susceptibility to carcinogenesis (WHO, 2017).

The level of Lead found in the wastewater sample was 0.95 mg/L, which exceeded the WHO limit. Lead has numerous negative effects on human health, including damage to the brain, kidney disease, and disorders of the circulatory and nervous systems (Gossuin and Vuong, 2018). The presence of Lead in wastewater may be a result of industrial processes such as mining, paint manufacturing, and battery electroplating (Gossuin and Vuong, 2018). The copper observed was in a small trace amount (0.79 mg/L), which was below the acceptable limit (2.00 mg/L). Copper can access wastewater through industrial activities (Jaishankar *et al.*, 2014). On the whole, the concentration of heavy metals present in the wastewater sample is in the following decreasing order: Pb>Fe> Zn>Cu. Five (5) different bacteria, namely: *E. coli*, *Klebsiella* species, *Pseudomonas* species *Staphylococcus aureus*, and *Bacillus* species were isolated from this study. The presence of these organisms indicates their ability to withstand environmental stress and pressure resulting from the low availability of nutrients. Moreover, their presence can be attributed to the fact that these organisms have the potential to utilize nutrients in the wastewater as a source of carbon for their proliferation and survival (Jaishankar *et al.*, 2014). This result is similar to the findings of Enimie *et al*. (2016), who also isolated *Staphylococcus aureus, E. coli, Pseudomonas* species, and *Klebsiella* species from petroleum refinery effluents. The percentage occurrence of the bacteria in this study revealed that *S. aureus* had the highest percentage occurrence (34.34%), while *Pseudomonas* species had the lowest (9.37%). The heavy metal tolerance of these bacterial isolates (*Escherichia coli, Klebsiella species, Pseudomonas* species, *Staphylococcus aureus*, and *Bacillus* species) from the wastewater sample conformed to the report by Kolawole and Obueh (2015), which isolated Staphylococcus species, E. coli, *Klebsiella, Salmonella*, and *Pseudomonas* species as metallotolerant bacteria. Similarly, Enimie *et al*. (2016) isolated *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Proteus vulgaris*, and *Klebsiella pneumonia* from petroleum refinery effluent. The bacterial isolates shows tolerance to four heavy metals (Cu, Pb, Fe, and Zn) as presented in Figures 3 to 6, respectively. The heavy metal tolerance of the bacterial isolates was assessed by their ability to grow in nutrient broth supplemented with various concentrations of heavy metals (0.5, 1.0, 1.5, 2.0, and 2.5 mg/mL). The growth of the five heavy metal-tolerant bacteria, as reflected in their optical density (600 nm) readings, was in the following order: Iron, Lead, Copper, and Zinc. The bacterial isolate showed that it exhibits high tolerance to Iron compared to other heavy metals. This is in agreement with the findings of Pandit (2013), who stated that Iron is an essential nutrient for living organisms due to its noticeable activity in electron transport reactions in biological systems. However, its insolubility and reactivity lead to problems of poor availability and toxicity, respectively. Wastewater containing a high concentration of Fe can increase soil acidity and diminish phosphorous in the soil (Abagale *et al*., 2013). When it comes to damaging effects on human health, Lead is a significant metal. It is a non-essential heavy metal. Lead causes oxidative stress and contributes to poisoning by disrupting the body's antioxidant balance. High-level accumulation of Pb in the body causes anaemia, colic, headaches, brain damage, and central nervous system disorders (Rehman *et al*., 2013). The ability of the isolates to grow in the presence of Lead, a non-essential heavy metal, could be due to the resistance mechanisms developed by the isolates. This finding is in agreement with Elizabeth *et al*. (2017), who reported the growth of *Escherichia coli* and *Staphylococcus aureus* at a concentration of 3.0 mg/mL. Among the bacterial isolates, Escherichia coli showed the greatest tolerance to the heavy metal zinc. These finding contradicted the report of Enimie *et al*. (2016), who reported the high maximum tolerance of *Pseudomonas aeruginosa* to zinc.

CONCLUSION

The total petroleum hydrocarbons in the effluents were high, with eicosane at 99% being the dominant hydrocarbon. Lead (Pb), CO_2, and alkalinity levels were key pollutants that exceeded the standards. Staphylococcus aureus was the predominant bacterium isolated from this study. Most metallotolerant isolates were *Pseudomonas* species for Cu and Bacillus species for Pb, respectively. Hence, these organisms were identified as good candidates for bioremediation and bioaugmentation of heavy metals in polluted industrial sites. This study deduced that wastewater from Panteka, Kaduna, is highly contaminated with the aforementioned compounds, and as such, immediate response strategies should be initiated, to safeguard environmental and public health.

CONFLICT OF INTEREST

The authors hereby declare that there is no conflict of interest.

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