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Heavy metals tolerance in bacteria from industrial wastewater

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Abstract

The incidence of chemical stressors in industrial waste effluents has culminated in the re-engineering the genetic and metabolic characteristic of resident microbiota. Microbial adaptability enables them to tolerate these stressors however, propelling the phenomena of acquisition of heavy metal resistance which may also incite resistance to antibiotics. Waste water from industrial establishments may travel from site into surrounding communities via canals and waterways thus, disseminating these stressors as well as resistance in the environment. This study seeks to investigate the physicochemical and heavy metal composition of industrial effluent and its tolerance in resilient bacteria from the study area. Physiochemical analyses revealed pH level which ranged between (5.8-10.87), BOD (6.612-16.01 mg/l), TDS (937.226-2173.49 mg/l), Sulphates (658.72- 1342.28 mg/l), Nitrates (11.46-70.16 mg/l), Phosphate (3.03-8.43 mg/l) exceeded the NESRA limits; Cu (0.024-4.521 mg/l) Cd (0.002-6.41 mg/l), Pb (0.001-8.151mg/l), Zn (0.511-6.092 mg/l). All the isolates showed marked tolerance to Cu, Cr, Pb, Cd and Zn at concentrations between 200 and 500µg/ml, except *Alkanindiges* sp. *5-0-9* and *Bacillus altitudinis* which were not susceptible to all the heavy metals at all concentrations. This study revealed the incidence of heavy metal resistance among bacterial isolates from industrial wastewater, the incidence of which could give rise to co-occurrence with antibiotic resistance thus, aggravating a public health concern.

Keywords: Bacteria; Heavy metal; Tolerance; Resistance; Wastewater

1. Introduction

Industrial processes generate wastes with large quantities of chemicals which are discharged into a receptacle (soil or rivers), either treated or untreated. Liquid wastes are discharged in form of wastewater which requires some form of treatments to meet regulatory standards before discharge and prevent detrimental effect in the environment. Health and environmental concerns exist for wastewater because they tend to distort the chemical and biological balance of receiving milieu [1], which may have implications for aquatic lives and other organisms' dependent on them or make use of such water [2]. Untreated or partially treated wastes and effluents contain different quantities of heavy metals [2]. Heavy metals are natural constituents of the environment, as processes such as rock weathering and volcanic eruptions do release them into the environment, albeit at very trace amounts [1, 3]. They are pollutants of concern owing to their toxicity even at low concentrations, persistence and their bio-accumulative tendencies [3, 4]. Chemical toxicity is a major possible effect of wastewater in the environment, when the chemical constituent of the waste exceeds permissible limits or bio-accumulate in living cells over time. Industrialization and urbanization have caused increased contamination of the environment by heavy metals [3]. Oil and gas extraction and their refining process, metallurgical processes and agriculture are major sources of heavy metal pollution [3, 4]. Refinery effluents increase the heavy metal load of potable water which poses severe detrimental effect to communities residing near the refinery making water a very scarce commodity for their day-to-day activities [5]. Heavy metals are administered to animals to promote growth and resist disease [6], and because they cannot be fully absorbed, nor metabolized they are excreted into the

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environment [7]. Pesticides and inorganic fertilizers also contribute to the incidence of heavy metals from agricultural activities [8]. Aluminium smelting activities contaminate drinking water sources with heavy metals [9]. Exposure to heavy metals such as As, Cd, Co, Cr, Cu, Hg, Ni, Pb, Se and Zn can have effects on flora and fauna [10-13]. Heavy metals can inhibit metabolic functions of microorganisms and modulate their genetic composition [14].

Wastewater stimulates horizontal gene transfer amongst bacteria, resistant genes for antibiotics and heavy metals can be transferred in this medium [15]. Antibiotic resistance is worsened with heavy metal co-resistance which has been reported to trigger the expression of antibiotic resistance genes [16].

This study aimed to detect the presence of heavy metals from effluents from selected industries with in Trans-Amadi industrial layout, Port Harcourt, Rivers State, Nigeria and their resistance pattern among bacteria isolates.

2. Material and methods

2.1. Study Location

Wastewater samples were collected from 8 industrial establishments situated in Trans-Amadi industrial layout, an area which is a hub for medium to large scale industrial and servicing outfits located in Port Harcourt, Rivers State, Nigeria. The high concentration of industries in this area of the city was responsible for the selection of the area for sample collection. The effluent points sampled and the industrial activities taking place there are: FLS1 - aluminium manufacturing, LFS2 - cement manufacturing, TAS3 - main canal receiving effluents from Trans-Amadi Industrial Layout, HSS4 - heavy duty engine servicing, SMS5 - oil servicing industry, NRS6 - vegetable oil manufacturing, FAS7 - aluminium manufacturing, FIS8 - fish farming.

2.2. Collection of Samples

Wastewater samples were collected at eight (8) locations with sterile containers. Each sample bottle was rinsed with the appropriate sample before the final collection. The samples were transported to the laboratory for analyses within six (6) hours of collection.

2.3. Physicochemical Analysis of the Wastewater Samples

Physicochemical analysis was carried out using Eaton [17]. The physicochemical parameters monitored were pH, biological oxygen demand, chemical oxygen demand, total dissolved solid, nitrate, sulphate and phosphate.

2.4. pH

The pH of the wastewater samples was determined using the pH meter by immersing the electrode into in a beaker containing the test samples with stirring and the readings taken as displayed.

2.5. Biological Oxygen Demand

Using the dilution procedure for determination of BOD, which ensures equal conditions in the samples to be tested, with the seed (microorganism) diluted with de-ionized water, the dissolved oxygen was measured before sealing the sample which was kept at 20^0C in the dark for five days, afterward the dissolved oxygen was measured once more and the BOD determined as the difference between the two concentrations.

2.6. Chemical Oxygen Demand

To the wastewater sample (25ml) was added 1ml of 20% H_2SO_4, then 1 ml of $KMnO_4$ and left for 4 hours, after which 1 ml of 10% KI was as well as add starch to serve as indicator before titration with COD of 0.0125 Na.

2.7. Total Dissolved Solid

Wastewater sample was filtered via a 1.2 μm filter and the filtrate heated to dryness. The weight of the residue after drying in desiccators gives a measure of the TDS.

2.8. Nitrate

The Cadmium decrease test technique (ASTM D3867-09) was used to determine nitrate level in the waste water.

2.9. Sulphate

Turbidometric method (ASTM D516-07) was adopted to determine the level of sulphate in the wastewater using HACH spectrophotometer.

2.10. Phosphate

Phosphate level was ascertained as per ASTM D3456 utilizing HACH spectrophotometer

2.11. Determination of heavy Metals Concentration

The heavy metal composition of selected industrial wastewater was determined using the Atomic Absorption Spectrophotometer (AAS) (UNICAM 929, London) powered by SOLAAR software after digestion using the nitric acid method described by Hseu [18]. Ten millilitres (10 ml) of conc. HNO_3 were used to digest 1g of wastewater sample in a 250 ml digestion tube, which was heated for 45min at 90^oC, and then to 150^oC to boil for not less than 8 h till solution become clear. Five millilitres (5 ml) conc. HNO_3 was further added to the sample thrice and digested until the volume reached about 1 ml. The inner walls of the tube were sluiced with little distilled water and the tube swirled the whole duration of the digestion. Once cooled, 5 ml of 1% HNO_3 was added to the sample before filtration using filter paper and subsequently transferred into a 25 ml volumetric flask and topped with distilled water, before the concentrations of Cd, Cr, Cu, Pb and Zn were determined.

2.12. Determination of Minimum Inhibitory Concentration (MIC) of the Metals on Bacteria

To ascertain their tolerance to heavy metals, the bacterial isolates were subjected to varying concentrations of metals ($CuSO_4$, $CdCl_2$, $K_2Cr_2O_7$, $PbNO_3$ and $ZnSO_4$) on Mueller Hinton agar begining from $100\mu g/mL$ and increasing the concentration until no visible growth was noticed. The lowest concentration of each metal that prevented the growth of the bacteria was taken as the MIC.

Statistical analysis and graphs were created using GraphPad prism 9 software.

3. Results

3.1. Physicochemical Parameters of Wastewater

Results for the physicochemical parameters of the wastewater samples are presented in Figures 1-6. Figure 1 shows that the pH varied from 5.5-10.8. Figure 2 shows that the BOD varied from 10.6-17.1 mg/l. Figure 3 shows that the TDS values varied from 730-2173.9 mg/l. Figure 4 shows that the nitrate concentration varied from 11.4-95.5. Figure 5 shows that the sulphate concentration varied from 558.5-1332.8 mg/l. Figure 6 shows that the phosphate concentration varied from 2.7-8.4 mg/l.

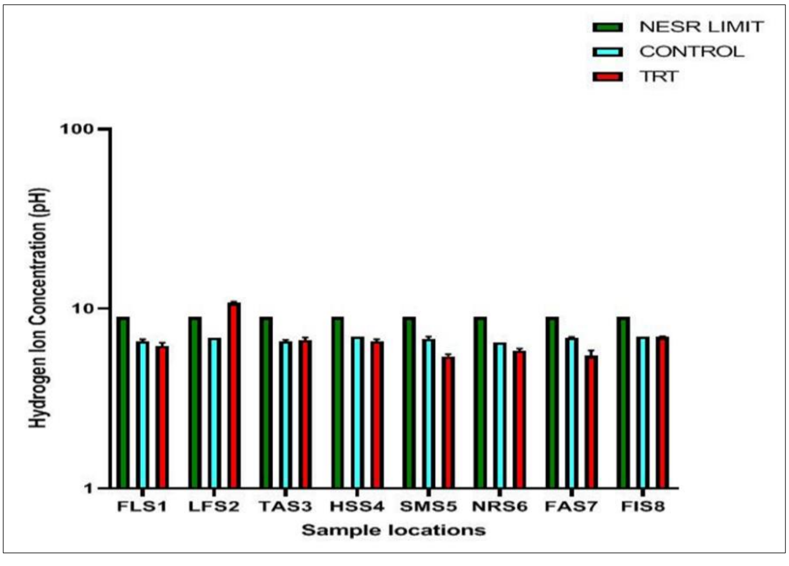

Figure 1 Hydrogen Ion Concentration (pH) Across the Study Locations

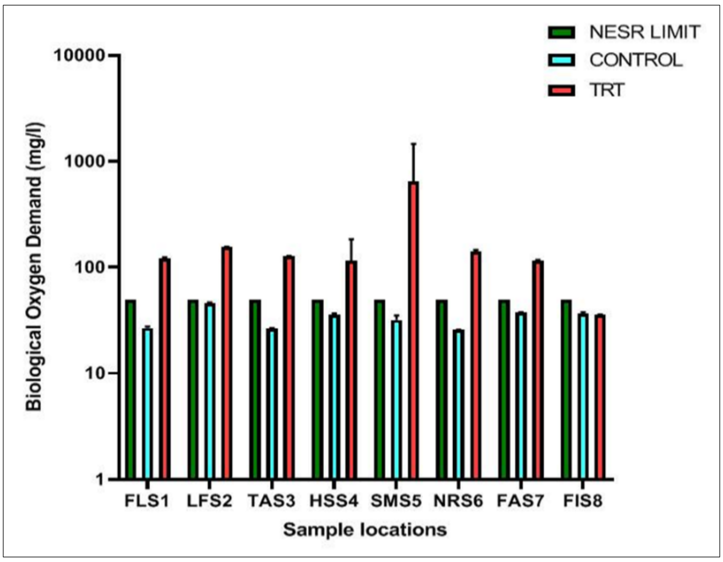

Figure 2 Biological Oxygen Demand across the Study Locations

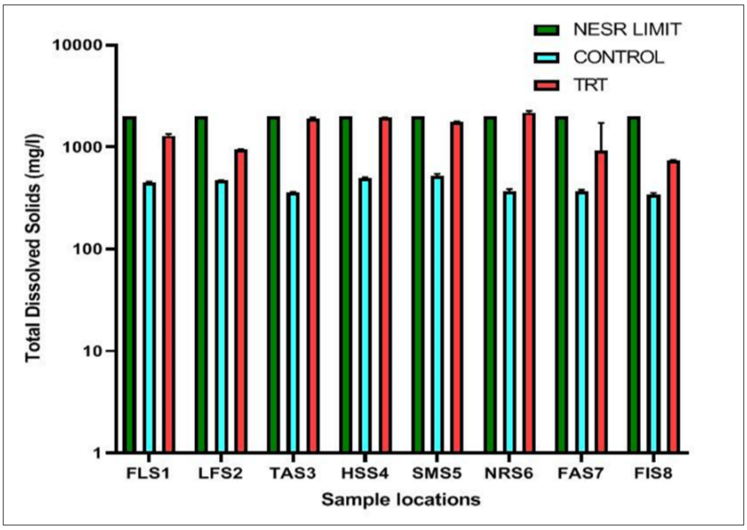

Figure 3 Total Dissolved Solids across the Study Locations

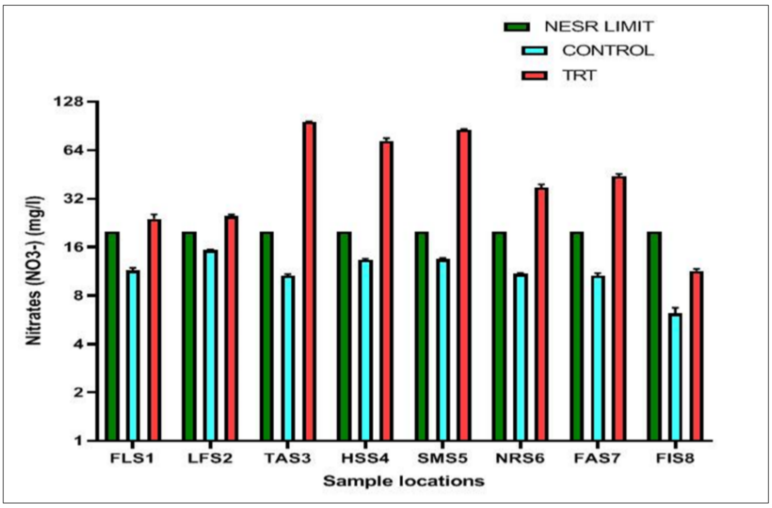

Figure 4 Nitrates (NO_3^-) Concentration across the Study Location

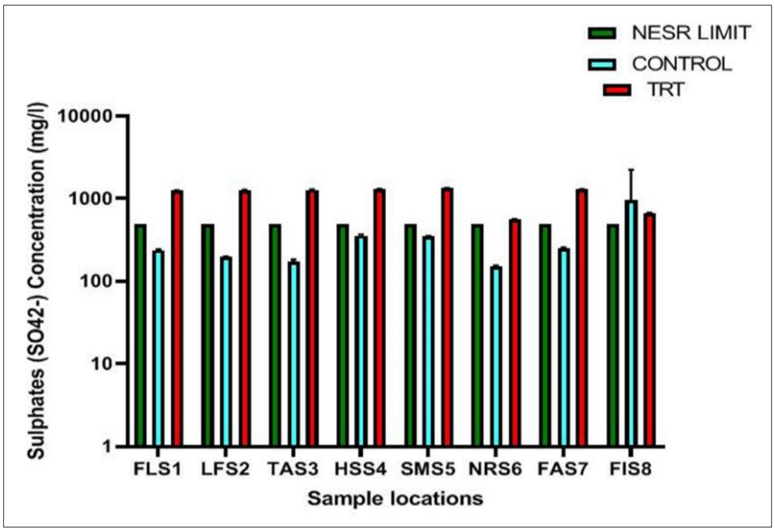

Figure 5 Sulphates (SO_4^{2-}) Concentration across the Study Locations

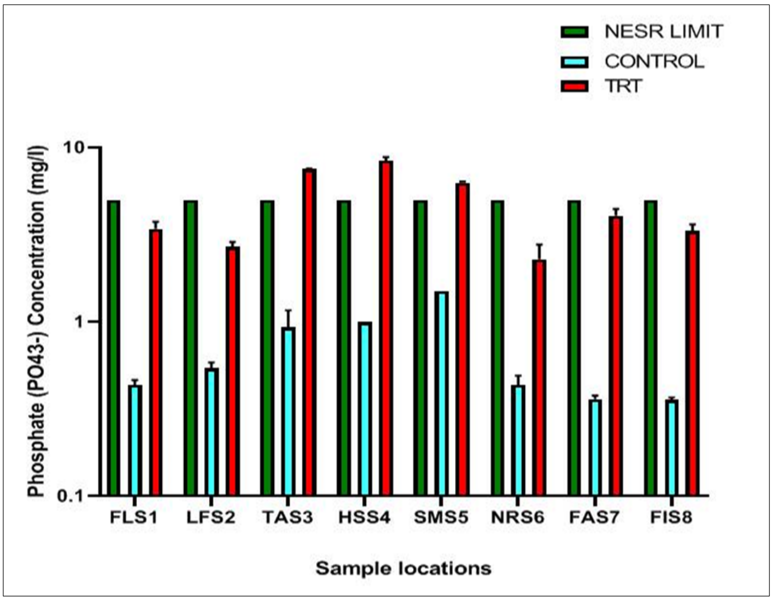

Figure 6 Phosphate (PO_4^{3-}) Concentration across the Study Locations

3.2. Heavy Metal Concentration in Wastewater from the Study Locations

Table 1 shows the heavy metal concentration in wastewater from the study locations. Cu concentration ranged from 0.024-4.521 mg/l; Cd concentration ranged from 0.002-6.41 mg/l. Cr concentration ranged from 0.005-6.203 mg/l; Pb concentration ranged from 0.001-8.151mg/l and Zn concentration ranged from 0.511-6.092 mg/l.

3.3. Minimum Inhibitory Concentration (MIC) of the Heavy Metals on the Bacterial Species

Table 2 shows the minimum inhibitory concentration (MIC) of the metals on the bacterial species. *Alkaligenes sp. 5-0-9* and *Bacillus altitudinis* were not susceptible to the metals. All the other isolates were susceptible to Cu, Cr, Pb, Cd and Zn at concentrations between 200 and 500µg/ml.

Table 1 Mean heavy metal concentration in wastewater from the study locations

Heavy metal	FLS1 (mg/l)	LFS2 (mg/l)	TAS3 (mg/l)	HSS4 (mg/l)	SMS5 (mg/l)	NRS6 (mg/l)	FLS7 (mg/l)	FLS8 (mg/l)	Nesra Limit (mg/l)
Cu	2.419 ± 0.02	3.183 ± 0.01	3.134± 0.02	4.521± 0.01	3.612 ± 0.01	0.024 ± 0.01	4.152± 0.01	0.0341± 0.01	0.5
Cd	0.512 ± 0.01	0.053 ± 0.03	0.562 ± 0.01	6.41 ± 0.002	2.001± 0.03	0.005± 0.001	0.162 ± 0.03	0.002 ± 0.00	0.1
Cr	6.203 ± 0.01	1.023 ± 0.005	1.083 ± 0.01	2.401± 0.001	4.55 ± 0.02	0.005 ± 0.001	4.023 ± 0.01	0.005 ± 0.001	0.1
Pb	4.231 ± 0.03	1.084 ± 0.02	6.093 ± 0.02	8.151 ± 0.02	6.023 ± 0.01	-	1.084± 0.02	0.001 ± 0.00	0.05
Zinc	6.092 ± 0.03	2.561 ± 0.003	3.013 ± 0.003	3.020 ± 0.00	4.301 ± 0.03	0.511 ± 0.03	5.541 ± 0.02	0.511 ± 0.03	2

Key: Cu – Copper; Cd – Cadmium; Cr – Chromium; Pb – Lead; Zn – Zinc; NESRA – Nigerian Environmental Regulation Agency limit for industrial discharge

Table 2 Minimum inhibitory concentration (MIC) of the metals on the bacterial species (μg/ml)

Bacteria	Cu	Cd	Cr	Pb	Zn
Baciilus licheniformis	500	200	300	300	500
Alkanindiges sp. 5-0-9	-	-	-	-	-
Bacillus thuringiensis	500	300	500	200	200
Bacillus altitudinis	-	-	-	-	-
Bacillus subtilis	500	500	200	400	300
Bacillus thuringiensis	500	300	500	300	300
Bacillus cereus	500	300	300	400	500
Bacillus thuringiensis	400	200	400	300	300

Key: Cu- Copper; Cd- Cadmium; Cr- Chromium; Pb- Lead; Zn- Zinc

4. Discussion

This study examined the physicochemical parameters of effluents from industries located at Trans-Amadi Industrial Layout, Port Harcourt, Rivers State, with the aim to ascertain the heavy metals resistance among the bacterial isolates. The pH of the wastewater samples varied significantly across locations. The values are either below or above the NESREA permissible limit of 6-9, except for samples FLS1, TAS3, SS4 and FIS8. The highest pH (10.8) was observed in LFS2. This could be attributed to the amount of basic compounds used by the companies in their operations or excess amount of basic compounds used in the treatment of the influents before discharge. High pH in water affects treatment in that it causes trace metals and insoluble solids to be immobilized [19, 20] and makes chlorination of water difficult [21]. Low pH on the other hand could cause corrosion of the piping network.

The BOD values for all the wastewater samples are higher than the NESREA limit of 10 mg/l. Sample FIS8 from a fish farm had the highest BOD (17.1 mg/l) followed TAS3 (16.1 mg/l) while sample FLS1 which is effluent sample from an aluminium manufacturing company had the least BOD (10.6 mg/l). The disparity in the BOD values could be as a result of the nature of waste generated from the companies, as fish farm waste is more likely to harbour more biodegradable waste and microorganisms involve in their oxidation than an aluminium processing plant. The BOD of the wastewater samples varied significantly across the eight locations. Otokunefor and Obiukwu [22] also reported a high BOD for treated refinery effluent discharged into the Bonny River. Similarly, Siyanbola *et al*. [23] reported a BOD range 340-560 mg/l in effluents collected from industries in Ikeja, Lagos State. The BOD values reported in this study are however higher than BOD (4.28 ± 1.08-6.11 ± 1.33 mg/l) reported by Edori and Nna [24] for wastewater samples discharged into the New Calabar River, in Port Harcourt, Rivers State, Nigeria.

The COD values for all the wastewater samples are higher than the NESRA limit of 50 mg/l except FIS8 with a value of 35.8 mg/l. Sample SMS5 from an oil servicing company had the highest COD (638.9 mg/l) which is several folds higher than in the other samples, an indication that it had more inorganic pollutants than all the other samples. There was no significant difference ($p < 0.05$) in COD across the locations but the value varied significantly between sample SMS5 and FIS8. Oil servicing companies generate wastes which contain heavy metals and other chemicals used in the oil and gas industry, which are known to increase the COD of water. Edori and Nna [24] reported COD values ranging from 13.54 ± 3.93-19.16 ± 2.10 mg/l for effluents discharged into the New Calabar River, in Port Harcourt, Rivers State. Otokunefor and Obiukwu [22] similarly reported a lower COD for treated refinery effluents discharged into the Bonny River estuary. Siyanbola *et al*. [23] reported a COD values ranging from 615-1254.50 mg/l, which is higher than reported in this study. The difference in values could suggest that probably the companies whose wastewaters were sampled do not adequately treat their wastewater before discharge into the river.

All the TDS values reported are within the NESRA limit of 2000 mg/l except NRS6 which was slightly higher at 2173.5 mg/l. The TDS values varied significantly across locations. The TDS values in this study are about ten folds higher than the TDS value of 209.23 mg/l reported by Otokunefor and Obiukwu [22] and about a hundred folds higher than 6.53 ± 0.56-8.89 ± 0.98 mg/l reported by Edori and Nna [24].

Wastewater sample TAS3 had the highest nitrate concentration (95.5) mg/l followed by SMS5 (85.7 mg/l) while FIS8 had the least (11.4 mg/l). All samples except FIS8 had values which are greater than the NESRA limit of 20 mg/l. The values varied significantly across locations. Fertilizers such as urea are used in the treatment of wastewater and could impact on the nitrate levels of the wastewater [25]. Ma *et al*. [26] posited that nitrate is an indirect source of food for fish. Meaning, fish can consume some of the nitrate in their aquatic environment, leaving only few in the wastewater from fish ponds. The relatively low nitrate concentration in FIS8 could also be attributed to utilization by microorganisms in the wastewater. The nitrate values recorded in this study are higher than the mean value (219.81 mg/l) reported by Edori and Nna [24] and the mean value (1.64 mg/l) reported by Otokunefor and Obiukwu [22].

Sulphate concentration in the wastewater samples which ranged from 558.5-1332.8 mg/l are all above the NESRA limit of 500 mg/l. Sample SMS5 had the highest value (1332.8 mg/l) while NRS6 had the least, and the difference between their values is statistically significant. Also, the sulphate concentrations differ significantly across locations. Akharame *et al*. [25] similarly reported high sulphate concentrations in effluents from industrial wastewater sampled in Benin City, Edo State. However, our finding is at variance with Otokune for and Obiukwu [22], Edori and Nna [24] and Siyanbola *et al*. [23] that reported low sulphate concentration of 39.08 mg/l, 0.46 mg/l and 24.18 respectively.

The sample HSS4 from a heavy-duty engine servicing company effluent discharge point had the highest phosphate concentration of 8.4 mg/l is above the NESREA limit of 5 mg/l while the least value of 2.7 mg/l was recorded in sample LFS2. The phosphate concentrations varied significantly in all eight the samples. Otokunefor and Obiukwu [22] reported phosphate level of 6.81 mg/l which is within the range reported in this study. Edori and Nna (2018) reported phosphate level of 0.46 mg/l which is below the range reported in this study. Akharame *et al*. [25] averred that phosphate-based cleaning agents for industrial sanitation impact on the phosphate level of wastewater from industries using them. High phosphate levels impinge on wastewater treatment [26].

Concentrations of the heavy metals in the samples varied significantly. FLS1, HSS4 and SMS5 had concentrations of Cu, Cd, Cr, Pd, Ni and Zn higher than the NESR limits. This shows that the wastewater is can endanger aquatic lives. LFS2, TAS3 and FL7 had concentrations of all the metals above the NESRA limits except for Cd. Only NRS6 and FLS8 had concentrations within the NESRA limits Siyanbola *et al*. [23] in their study of the characteristics of wastewater from industrial activities in Lagos, the centre of commerce in Nigeria, also showed Cu, Cd, Cr, Pd, and Zn concentrations did not meet compliances level. Akharame *et al.* [25] similarly reported the presence Cu, Cd, Cr, Pd, Ni and Zn in wastewater from industrial activities in Benin, but reported Zn as the only metal with concentration above the NESRA limit. This implies that the industries operating at the study location contribute more to heavy metal pollution than in the study by Akharame *et al.* [25]. However, it must be noted that the effluent produced is expected to vary by industry, which could explain the difference in the two studies.

The present study confirmed that heavy metals tolerance exhibited by the isolates. Bacterial strains occurring in the metal stressed environment may carry plasmid that encode for heavy metal resistance. Filali *et al*. [27] isolated bacterial species from wastewater of Casablanca, Morocco showing resistance to heavy metals, and the genes were plasmid borne. Rajbanshi [28] isolated 10 bacterial isolates from wastewater in Bagmati, Nepal, which include chromium resistant *Staphylococcus* sp., *Escherichia coli, Klebsiella* sp.; cadmium resistant *Acinetobacter* sp., *Flavobacterium* sp., *Citrobacter* sp.; nickel resistant *Staphylococcus* sp., *Bacillus* sp.; copper resistant *Pseudomonas* sp.; and cobalt resistant *Methylobacterium* sp., which all showed high resistance to the heavy metals with Minimum Inhibitor Concentration (MIC) ranging from 150 µg/ml to 500 µg/ml.

All the isolates except *Alkanindiges sp. 5-0-9* and *Bacillus altitudinis* were tolerant to heavy metals at concentrations between 200 and 500µg/ml. The isolates were more tolerant to Cu than the other metals since higher concentrations of its higher MIC range (400-500 µg /l). *Bacillus cereus* showed greater tolerance for all the heavy metals with MIC range of 300 – 500 µg/l. Kim *et al*. [29] showed in their studies that *Bacillus* sp. can tolerate concentrations of heavy metals including Cu, Cd, Cr, Pd, Ni and Zn as high as 100 mg/l. the ability of bacteria to tolerate high concentrations of heavy metals is considered beneficial in their application for bioremediation or biosorption of heavy metals from contaminated matrixes [30].

Antibiotic resistance is worsened with heavy metal co-resistance which has been reported to trigger the expression of antibiotic resistance genes [16]. Gao *et al*. [31] reported co-occurrence of erythromycin resistance with resistance to heavy metals such as Cd, Cr, Cu, Zn, Pd and Ni. Liu *et al*. [32] reported resistance to tetracycline (*tetA, tetC, tetM, tetO, tetS* and *tetW*) and heavy metals (Cu, Zn, and Cd) in study of wastewater obtained from the Yangtze River Delta region in China.

5. Conclusion

The physicochemical properties and heavy metals levels of wastewater in this study allowed for the induction of heavy metal resistance among bacterial species present in them. Due to the increasing reports of resistance to almost all known antibiotics, including those considered to be of last resort, serious attention should be paid to treatment of wastewater for the removal of heavy metals removal which triggers the expression of antibiotic resistance genes expression, which is a great public health concern.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflicts of interest.

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