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# Physicochemical, microbiological and heavy metal studies on water samples and bacteria obtained from Dandaru River in Ibadan, South-western Nigeria

**Adekanmbi, Abimbola Olumide and Falodun, Olutayo Israel***

Environmental Microbiology and Biotechnology Laboratory, Department of Microbiology, University of Ibadan, Oyo State, Nigeria.

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**The physicochemical, microbiological and heavy metal studies of water samples and bacteria obtained from the Dandaru River were investigated. Water samples were collected from six different sampling points along the course of the river and analysed using standard procedures. The physicochemical parameters showed that the water samples at point 5 had the highest value for all the parameters except Total Dissolved Solids. The heavy metal analysis revealed that the mean concentration of zinc, lead, chromium and iron were highest at point 5; 2.11, 0.02, 0.04 and 0.54 mg/l respectively, while that of copper and manganese concentration was highest at point 4 (0.59 and 0.41 mg/l respectively). The total heterotrophic bacteria count was between 3.6 - 64.0 $\times 10^1$ cfu/ml, with point 3 having the least value ($3.6\times 10^1$ cfu/ml); while the coliform count using Most Probable Number (MPN) tube method ranged from 350 - ≥1600 MPN index/100 ml. The bacteria obtained belonged to the genera *Bacillus, Enterobacter, Escherichia, Pseudomonas* and *Staphylococcus*. On exposure to increasing concentration of six heavy metals, 47.62% of the total bacteria obtained were observed to be growing at 500 µg/ml for all the six metals employed in this study, while the others showed varying degree of tolerance to the metals.**

**Key words:** Heavy metals, physicochemical, microbiological, river, bacteria.

## INTRODUCTION

Due to human and natural activities, contaminant enters the environment. These pollutants cause serious problems and risks to the environment and man himself (Shanbehzadeh et al., 2014; Espenoza-Quinones et al., 2005). Water resources are among the most critical resources and the importance of water resources, particularly surface water (rivers), in meeting the water need of humans, animals and industries underscores the need to protect them against contaminations (Shanbehzadeh et al., 2014). As municipal, industrial and agricultural wastes enter the water, biological and chemical contaminants including heavy metals also enter

*Corresponding author. E-mail: falod2013@gmail.com or oi.falodun@ui.edu.ng. Tel: +2348027342286.

**Plate 1a.** Sampling point 1.

**Plate 1b.** Sampling point 2.

**Plate 1c.** Sampling point 3.

water resources.

Rivers as water sources are very important in drinking water supply, recreational and sport activities such as water sports and fishing, and all these impact on human health (Shanbehzadeh et al., 2014). Humans may be contaminated by organic and inorganic pollutants associated with aquatic systems by consumption of contaminated fish and other aquatic foods from this environment (Mackay and Clarek, 1991) in addition to having a direct contact with the water body.

Heavy metals are intrinsic, natural constituents of our environment and are among the most common environmental pollutants and most harmful of the elemental pollutants and are of particular concern because of their toxicity to humans (Aderinola et al., 2009). Generally, they are present in small amounts in natural aquatic environments (Aderinola et al., 2009) and their occurrence in water biota indicates the presence of natural or anthropogenic sources. The main natural sources of metals in water are chemical weathering of minerals and soil leaching. The anthropogenic sources are associated mainly with industrial and domestic effluents, urban storm, water runoff, landfill, mining of coal and ore (El Bouraie et al., 2010). Furthermore, strong evidence linking the presence of heavy metals to the exacerbation of some microbial diseases in aquatic organisms is increasing (Boran and Altmok, 2010).

Dandaru River is located in Ibadan, and has a close proximity to a tertiary hospital. Activities carried out around the bank include car washing, laundry, bathing, swimming and fishing. These activities contribute significant amount of organic nutrients and other inputs to the river. Therefore the study aimed at investigating the physicochemical, microbiological and heavy metal constituent of water samples obtained from the river as well as determine the response of the isolated bacteria to increasing concentration of selected heavy metals.

## MATERIALS AND METHODS

### Study area

Dandaru River is located beside the popular Agodi zoological gardens in the ancient city of Ibadan, Nigeria. It receives input from the zoological gardens, a car wash, as well as from a University College Hospital (UCH). Six sampling points were chosen along the course of the Dandaru River. These points were strategically chosen because of the influx of wastewater and/or human activities/influence around the course of the river, which flows out to join the Ogunpa River. The description of the sampling points is shown in Plate 1a-f, while the GPS readings were also noted and recorded (Table 1).

### Water sampling

Water samples were collected from six points in pre-cleaned 1-L capacity plastic bottles and immediately delivered to the

**Plate 1d.** Sampling point 4.

**Plate 1e.** Sampling point 5.

**Plate 1f.** Sampling point 6.

Environmental Microbiology and Biotechnology Laboratory, Department of Microbiology, University of Ibadan, Nigeria in ice packs. The samples were processed immediately. Samples for the heavy metal analysis were acidified with $HNO_3$ to preserve the metal composition.

#### Physico-chemical analysis of the water samples

The water quality parameters which include colour, turbidity and conductivity were determined using the colour meter, turbidity meter, conductivity meter and the pH meter respectively. The classical titrimetric method was used for determination of acidity, alkalinity, total hardness, calcium hardness, magnesium hardness, chloride ions and sodium chloride levels of the water samples; while the total solids, total suspended solids and total dissolved solids were determined using the classical gravimetric methods. The spectrophotometric method was used in the determination of the phosphate and sulphate ions. The biochemical oxygen demand (BOD) and chemical oxygen demand (COD) were determined using the standard methods of APHA (1998).

#### Microbiological analysis

The water samples were analysed on different culture media using the standard pour plate technique. Nutrient agar was used for the heterotrophic plate count; Eosin Methylene Blue (EMB), for the isolation of *Escherichia coli*, Pseudomonas agar, for *Pseudomonas* species, mannitol salt agar (MSA), for the isolation of *Staphylococcus aureus*, MacConkey agar for *Enterobacter* and other coliforms; while the total coliforms was performed using the Most Probable Number (MPN) technique (APHA, 1998).

Colonies with distinct characteristics on each culture medium were identified on the basis of their morphological, sugar fermentation and biochemical properties using the scheme in the Bergey's manual of Determinative Bacteriology (Holt, 1994).

#### Exposure of the bacteria to 500 µg/ml concentration of heavy metals

The tolerance of each bacterial strain to metal ions from 0-500 µg/ml of the metal concentration was carried out on Nutrient Agar plates supplemented with varying concentration of the soluble metal salts ($K_2Cr_2O_7$, $Pb(CH_3COO)_2$, $CdCl_2$, $NiSO_4$ and $CuSO_4$, $ZnSO_4$) with 50 µg/ml as the starting concentration for each metal. The plates were incubated at 35±2°C and observed for growth. The culture growing on a previous concentration was transferred to the next higher concentration until the isolate failed to grow on the medium (Singh et al., 2010).

## RESULTS

### Heavy metal composition

The heavy metal composition of the water samples from the Dandaru River showed that the highest level of iron, lead, zinc and chromium was observed in the water sample from sampling point 5 with values of 0.54, 0.02, 2.11 and 0.04 mg/l, respectively. The lead level was 0.01 mg/l for all the sampling points except sampling point 5. The highest copper concentration was observed in the

**Table 1.** Location of the study sites with descriptions of the sampling points along the Dandaru River.

| Sampling point | Latitude | Longitude | Description |
|---|---|---|---|
| Point 1 | 7.44212N | 3.89766E | The entry of the Dandaru reservoir into the river course |
| Point 2 | 7.40421N | 3.89826E | The point at which fishing activities and other activities such as swimming is prevalent |
| Point 3 | 7.40405N | 3.89813E | The point of entry of wastewater from a college hospital into the river |
| Point 4 | 7.40372N | 3.89785E | The point at which human activities such as washing is undertaken |
| Point 5 | 7.39742N | 3.90583E | The point at which wastewater from a car-wash is discharged into the river |
| Point 6 | 7.40141N | 3.89649E | The point of connection of the river to the Dandaru community via a bridge. |

**Table 2a.** Metal composition of the water samples obtained from the six sampling points on Dandaru River (mg/l).

| Sampling points | Iron | Lead | Copper | Zinc |
|---|---|---|---|---|
| Point 1 | 0.44±0.01b | 0.01±0.00b | 0.45±0.02b | 1.16±0.02d |
| Point 2 | 0.43±0.00bc | 0.01±0.00b | 0.42±0.01b | 1.16±0.00d |
| Point 3 | 0.38±0.02d | 0.01±0.00b | 0.45±0.00b | 1.17±0.01d |
| Point 4 | 0.45±0.02b | 0.01±0.00b | 0.59±0.04a | 1.27±0.03c |
| Point 5 | 0.54±0.01a | 0.02±0.00a | 0.03±0.00d | 2.11±0.03a |
| Point 6 | 0.39±0.01cd | 0.01±0.00b | 0.32±0.00c | 1.37±0.01b |

Means with the same alphabet in the same column are not significantly different from each other.

**Table 2b.** Metal composition of the water samples obtained from the six sampling points on Dandaru River (mg/l).

| Sampling points | Chromium | Manganese | Nickel | Cadmium |
|---|---|---|---|---|
| Point 1 | 0.03±0.00b | 0.31±0.01c | <0.01±0.00a | <0.01±0.00a |
| Point 2 | 0.03±0.00b | 0.32±0.01c | <0.01±0.00a | <0.01±0.00a |
| Point 3 | 0.02±0.00c | 0.37±0.00b | <0.01±0.00a | <0.01±0.00a |
| Point 4 | 0.03±0.00b | 0.41±0.01a | <0.01±0.00a | <0.01±0.00a |
| Point 5 | 0.04±0.00a | 0.37±0.01b | <0.01±0.00a | <0.01±0.00a |
| Point 6 | 0.03±0.00b | 0.33±0.00c | <0.01±0.00a | <0.01±0.00a |

Means with the same alphabet in the same column are not significantly different from each other.

water sample from sampling point 4 (0.59 mg/l), while the nickel level was higher than 0.01 mg/l in the water sample collected from all the sampling points (Table 2).

## Physicochemical parameters

The physico-chemical parameters of the water samples are shown in Table 3a and Figure 1. The turbidity was highest at point 5 (37NTU[b]), than at all the other sampling points. Similar trend was observed for the colour, acidity, pH and alkalinity as the highest was observed at sampling point 5. However, the highest value for conductivity was at the sampling point 1 with a value of 348 µs/cm, whereas it was lowest at sampling point 5 with a value of 332 µs/cm.

Figure 1 shows that the total hardness, calcium hardness, magnesium hardness, total solids and total dissolved solids were highest at sampling point 5 with values of 145.45, 99.49, 42.97, 229.0 and 29.0 mg/l, respectively. However, the total dissolved solids was highest at both sampling points 1 and 2, with a value of 209 mg/l; while the lowest value was observed at sampling point 5 with a value of 200 mg/l.

The phosphate, nitrate, sulphate, chloride, sodium chloride, chemical oxygen demand (COD) and biochemical oxygen demand (BOD) were highest at sampling point 5 with values of 0.39, 0.16, 2.58, 52.04, 85.87, 63.49and 45.75 mg/l, respectively (Table 3b and Figure 1c).

## Total Heterotrophic Count (THC) and Coliform count

The THC is shown in Table 4. The highest THC value of 64.0×101 cfu/ml was observed at sampling point 5, while the lowest value of 3.6×101 cfu/ml was observed at

**Table 3a.** Physicochemical properties of the water samples collected from the six sampling points in Dandaru River.

| Sampling points | Turbidity (NTU[b]) | Conductivity (µS/cm) | Colour (TCU[a]) | pH | Acidity (mg/l) | Alkalinity (mg/l) |
|---|---|---|---|---|---|---|
| Point 1 | 28±0.00b | 348±0.00a | 16.43±0.00d | 7.05±0.00d | 25.66±0.04b | 172.79±0.02b |
| Point 2 | 28±0.00b | 347±0.00b | 16.55±0.00c | 7.04±0.00e | 25.84±0.22b | 152.19±1.01c |
| Point 3 | 26±0.00c | 344±0.00c | 16.00±0.00e | 7.05±0.00d | 26.37±0.75b | 153.19±2.01c |
| Point 4 | 23±0.00e | 342±0.00d | 16.60±0.00b | 7.06±0.00c | 25.66±0.04b | 130.32±0.74d |
| Point 5 | 37±0.00a | 332±0.00e | 24.00±0.00a | 7.11±0.00a | 52.14±0.91a | 183.82±0.25a |
| Point 6 | 24±0.00d | 344±0.00c | 16.60±0.00b | 7.07±0.00b | 26.34±0.72b | 152.63±1.45c |

Means with the same alphabet in the same column are not significantly different from each other. Each value is a mean of two replicates.

**Table 3b.** Physicochemical properties of the water samples collected from the six sampling points in Dandaru River.

| Sampling point | Phosphate (mg/l) | Nitrate (mg/l) | Sulphate (mg/l) | Chloride (mg/l) |
|---|---|---|---|---|
| Point 1 | 0.20±0.02d | 0.04±0.00d | 1.80±0.00c | 43.66±0.05c |
| Point 2 | 0.22±0.01bc | 0.05±0.00cd | 1.93±0.03b | 43.83±0.22c |
| Point 3 | 0.24±0.03bc | 0.06±0.01bc | 1.51±0.00d | 46.13±0.05b |
| Point 4 | 0.28±0.00b | 0.07±0.01b | 1.19±0.01e | 42.81±0.84c |
| Point 5 | 0.39±0.02a | 0.16±0.00a | 2.58±0.03a | 52.04±1.02a |
| Point 6 | 0.26±0.01bc | 0.06±0.00bc | 1.80±0.00c | 46.60±0.52b |

Means with the same alphabet in the same column are not significantly different from each other. Each value is a mean of two replicates.

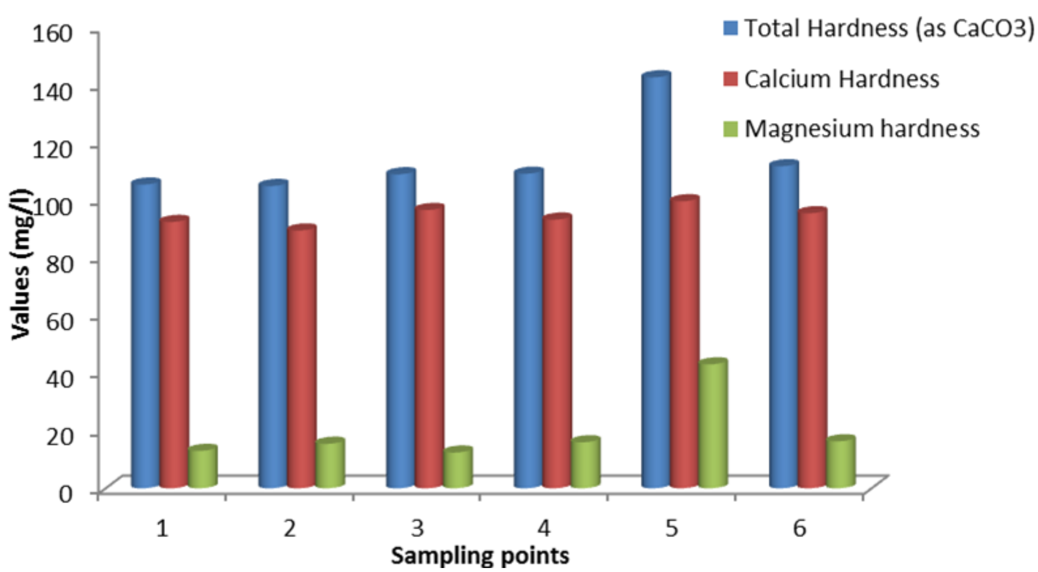


**Figure 1a.** Physicochemical properties of the water samples collected from the six sampling points in Dandaru River.

sampling point 3. The values for the remaining points were at point 1 (30.0×101 cfu/ml); point 2 (4.7×101 cfu/ml); point 4 (6.4×101 cfu/ml) and point 6 (15.2×101 cfu/ml). The coliform count which was done using the MPN technique showed that the lowest value was observed at sampling point 1 with a coliform count of 350/100 ml of

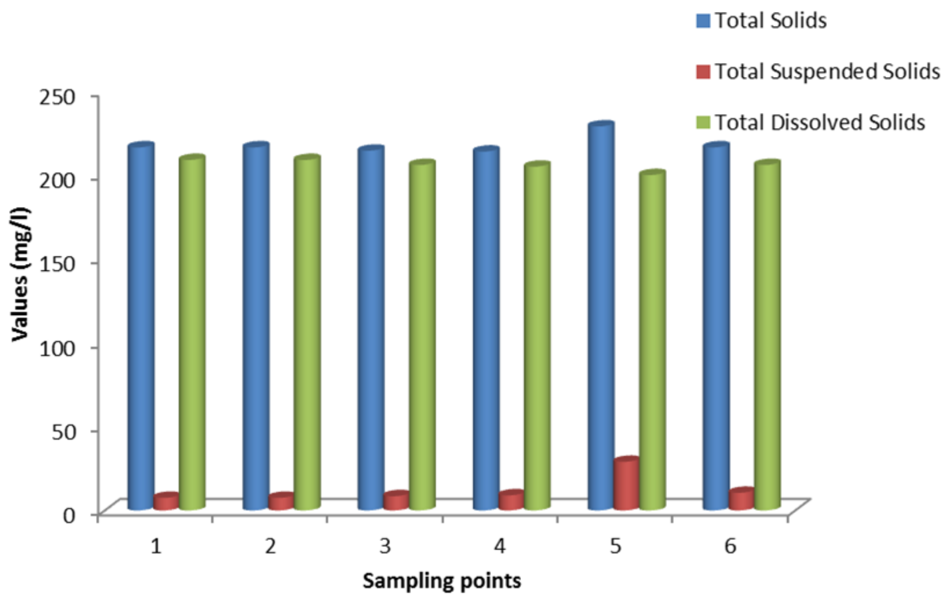


**Figure 1b.** Physicochemical properties of the water samples collected from the six sampling points on Dandaru River.

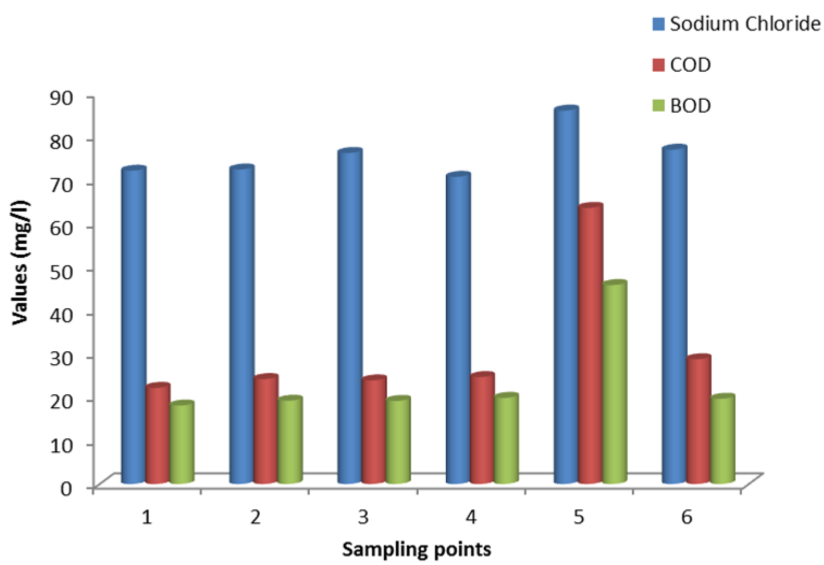


**Figure 1c.** Physicochemical properties of the water samples collected from the six sampling points on Dandaru River.

**Table 4.** Total heterotrophic and coliform count of the water samples.

| Sampling point | THC ($\times 10^1$ cfu/ml) | Coliform count (MPN/100 ml) |
|---|---|---|
| Point 1 | 30.0 | 350 |
| Point 2 | 4.7 | ≥1600 |
| Point 3 | 3.6 | 1600 |
| Point 4 | 6.4 | 1600 |
| Point 5 | 64.0 | ≥1600 |
| Point 6 | 15.2 | 1600 |

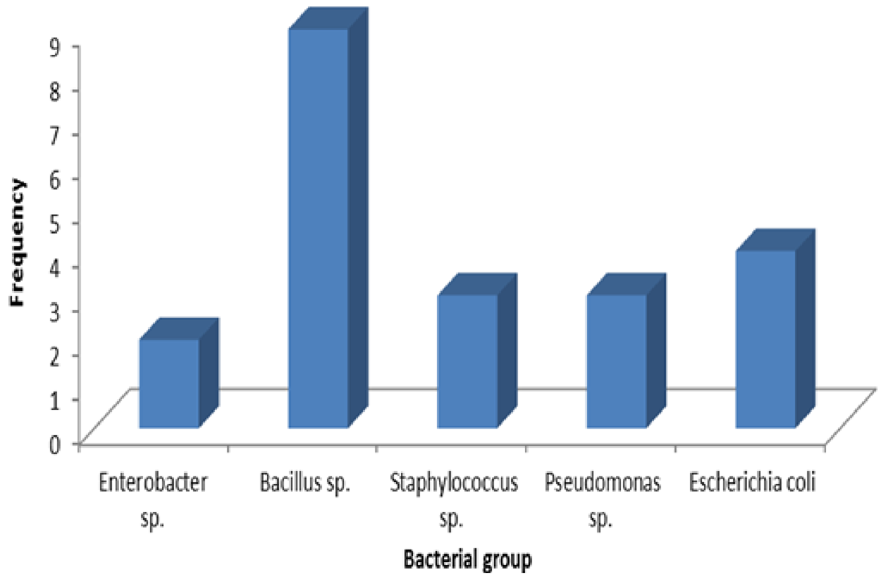


**Figure 2.** Frequency of distribution of the bacteria isolated from the water samples collected from the six sampling points in Dandaru River.

**Table 5.** Response of the bacterial isolates to increasing metal concentration (µg/ml).

| Bacterial isolates | Cr | Pb | Cd | Ni | Cu | Zn |
|---|---|---|---|---|---|---|
| *Staphylococcus* sp. (Dn001) | 500 | 500 | 500 | 500 | 500 | 500 |
| *Bacillus* sp. (Dn002) | 500 | 500 | 500 | 500 | 500 | 500 |
| *Bacillus* sp. (Dn003) | 500 | 500 | 500 | 500 | 500 | 500 |
| *Bacillus* sp. (Dn004) | 500 | 500 | 500 | 500 | 500 | 500 |
| *Bacillus* sp. (Dn005) | 500 | 500 | 500 | 500 | 500 | 500 |
| *Escherichia coli* (Dn006) | 500 | 500 | NG | 500 | NG | 500 |
| *Escherichia coli* (Dn007) | 500 | 500 | NG | 500 | 500 | 500 |
| *Enterobacter* sp. (Dn008) | 500 | 500 | 500 | 500 | 500 | 500 |
| *Bacillus* sp. (Dn009) | 500 | 500 | 500 | 500 | 500 | 500 |
| *Enterobacter* sp. (Dn010) | 500 | 500 | 500 | 500 | 500 | 500 |
| *Staphylococcus* sp. (Dn011) | 500 | 300 | 500 | 500 | 500 | 500 |
| *Bacillus* sp. (Dn012) | 500 | 500 | 500 | 500 | 500 | 500 |
| *Staphylococcus* sp. (Dn013) | 500 | 500 | NG | 500 | NG | NG |
| *Bacillus* sp. (Dn014) | 300 | 500 | 250 | 500 | 500 | 500 |
| *Pseudomonas* sp. (Dn015) | 250 | 500 | 500 | 200 | 500 | 500 |
| *Bacillus* sp. (Dn016) | 250 | 500 | 300 | 200 | 500 | NG |
| *Escherichia coli* (Dn017) | 300 | 500 | NG | 500 | 500 | NG |
| *Bacillus* sp. (Dn018) | 500 | 500 | 500 | 500 | 500 | 500 |
| *Pseudomonas* sp. (Dn019) | NG | 500 | 500 | 500 | 500 | 200 |
| *Escherichia coli* (Dn020) | NG | 500 | NG | NG | NG | NG |
| *Pseudomonas* sp. (Dn021) | 500 | 500 | NG | 200 | NG | 300 |

NG: No growth was observed at the 50 µg/ml initial concentration of the metal. 200: The bacterium was observed to grow till 200 µg/ml concentration of the metal. 250: The bacterium was observed to grow till 250 µg/ml concentration of the metal. 300: The bacterium was observed to grow till 300 µg/ml concentration of the metal. 500: The bacterium was observed to still be growing at 500 µg/ml concentration of the metal.

water. The value of 1600/100 ml of water sample was observed in the water samples from sampling points 3, 4 and 6; while the coliform count was greater than 1600 in water samples from sampling points 2 and 5 (Table 4).

### Frequency of occurrence of the bacterial isolates

A total of 21 morphologically distinct bacteria were obtained from the Dandaru River water samples. The bacterial isolates belongs to five genera; *Bacillus, Pseudomonas, Enterobacter, Escherichia* and *Staphylococcus*. The percentage frequency of occurrence is shown in Figure 2.

### Heavy metal exposure

The response of the bacteria obtained from the water samples is shown in Table 5. All the isolates with exception of *E. coli* Dn006, Dn007, Dn017 and Dn020; *Bacillus* Dn016; *Staphylococcus* Dn013 and *Pseudomonas* Dn021, were observed to be growing on all the metals at different concentration of the metals.

## DISCUSSION

The quality of Dandaru River was investigated and the results showed variation in the water quality. The results of the physicochemical parameters showed that turbidity, colour, acid, pH and alkalinity was highest at point 5 which receives wastewater from a car washing unit which must have influenced the higher level of these parameters as compared to other sampling points. However, the turbidity from other sampling points ranged between 24 and 28 NTU[b], which is far above the 5.0 NTU permissible limit of the Standard Organisation of Nigeria (SON). Except at sampling point 5 with a much higher value for colour (24 TCU[a]), the range for other sampling points (16.0-16.6) were only slightly higher than the 15 TCU permissible limit of SON. The pH range of all the sampling points was 7.04 – 7.11; and this is within the pH permissible limit of both the SON (6.5 -8.5) and (6.5 -9.5) of the World Health Organisation (WHO). This observation is also similar to the pH value range of 7.8-8.2 reported in Turkey (Atici et al., 2010) but lower compared to the range of 7.4 - 10.0 reported from another study in Turkey (Atici et al., 2008). The reason for the difference may be that the later study was from a dam reservoir. Moreover, the pH value obtained in this study is not in agreement with pH range of 6.5-9.4 that was reported from India (Begum et al., 2009). The reason for the variation may be attributed to the discharge of acidic water by agricultural and domestic activities into the river. Furthermore, total hardness, magnesium hardness, calcium hardness, total solids, and total dissolved solids fell below the permissible limit of SON and WHO. The range of the quantity of nitrate (0.04 - 0.16 mg/l), sulphate (1.18 - 2.55 mg/l) and chloride (46.08 – 51.02 mg/l) at different points of sample collection in the river was far lower as compared to the 50, 100 and 250 mg/l permissible limit of nitrate, sulphate and chloride, respectively, for SON and WHO. It was observed that almost all the physicochemical parameters considered were highest at sampling point 5 as compared to other sampling points; this may be as a result of the discharge of waste water from a car wash and the passage of human excreta at the point by workers at the car wash and other people in the neighbourhood. The range of the iron level of 0.36 – 0.53 in the river at different sampling points is above the permissible limit of 0.3 mg/l for both SON and WHO but lower as compared to the 1.14 mg/l reported in a study conducted in Lagos by Aderinola et al. (2009). The value was also lower as compared to the 0.06 mg/l reported in water and river bed sediment of Utra River in Poland (Wojtkowska, 2011). However, the result of the iron level in this study is similar to the ones reported in Egypt (El Bouraie et al., 2010; Saeed and Shaker, 2008) and black sea in Poland (Boran and Altmok, 2010).

Except at sampling point 5 with 0.2 mg/l, the lead content in the river was 0.1 mg/l and this is within the SON and WHO permissible limit. This observation was much lower as compared to the median level of 3.59 mg/l reported by Wostkowslan (2011). However, it was similar to the reports of studies carried out by Tole and Shitsama (2003), Shanbehzadeh et al. (2014) and El Bouraie (2010). Although, the range of the copper concentration of 0.03 – 0.55 mg/l in the river at different sampling points was lower as compared to the SON (1.0 mg/l) and WHO (2.0 mg/l) permissible limit, it was similar to the 0.26 – 0.62 mg/l reported from Tembi river in Iran by Shanbahzadeh et al. (2014); but slightly higher than the range (0.11 – 0.24 mg/l) reported from a study on surface river water and bed sediments at Nile Delta in Egypt (El Bouraie et al., 2010).

Furthermore, the level of nickel and cadmium of <0.01 observed in this study at all the sampling points was lower as compared to the maximum permissible limit of 0.02 and 0.03 mg/l of SON and WHO respectively for the metals. The result of the cadmium is similar to the result of Ogoyi et al. (2009), Saeed and Shaker (2011) and El Bouraie et al. (El 2010). It was lower as compared to those reported in Iran's Tembi River by Shanbehzadeh et al. (2014) and in Lagos lagoon surface water (Aderinola et al., 2009).

The level of zinc (1.14 – 2.08 mg/l) observed in this study was within the permissible limit of 3.0 mg/l for both SON and WHO. Likewise, the chromium level of 0.03 mg/l for all the sampling points, in addition to that of sampling point 5 (0.04 mg/l) were all within the permissible limit of 0.05 of both SON and WHO. This observation was lower as compared to the level of zinc and chromium of 0.33 and 0.06 respectively in another study in Lagos, Nigeria (Aderinola et al., 2009). The value was however higher than those reported in Egypt (El Bouraie et al., 2010; Saeed and Shaker, 2008) and lower than the 6.87 mg/l reported in Poland by Wastkowska (2011). The observation in this study that the highest value of heavy metals such as iron, lead, zinc and chromium at sampling point 5 is in agreement with the assertion that human activities has proved to elevate the levels of heavy metals in fresh water (Farkas et al., 2001)

The highest value of total heterotrophic count observed at sampling point 5 might have been influenced by the activities of the car wash, bathing, passage of human excreta and laundry at the point while the lowest count at sampling point 3 might be as a result of very little or no activity. More so, the high coliform count observed at points 2 and 5 may be as a result of the swimming, car wash activities and passage of human excreta at those points. The lowest count at the sampling point 1 may have been due to very little anthropogenic influence around the point.

The heavy metal exposure showed that most of the bacteria have the capacity to resist heavy metals at different concentrations. Of the metals tested, cadmium was the most toxic, followed by copper and zinc, chromium, nickel and lead in that order. All the bacteria grew in the presence of lead. The order of toxicity for the metals was Cd>Cu>Zn>Cr>Ni>Pb.

Except for iron and manganese, the concentration of all the metals in the water samples obtained from Dandaru River was within the permissible limits according to Standard Organisation of Nigeria and World Health Organisation. The higher values of most physicochemical and heavy metal content in the river at sampling point five may be due to the constant contamination from the car wash and other activities along the river. Generally, the physicochemical, heavy metal concentration, total heterotrophic and coliform count in the water could probably be as a result of the anthropogenic activities going on inside and around the Dandaru river. Though activities that give direct contact with human go on in the river, there is little to suggest that adequate wastewater treatment before discharge from adjoining sources is being carried out and this might be a potential source of transmission of infection and ingestion of metals which may pose a potential danger to public and human health.

## Conflict of Interests

The author(s) did not declare any conflict of interest.


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