Journal of Bioresource Management

Volume 9 | Issue 1 Article 10

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Recommended Citation

Okafor-Elenwo, E. J., Imade, O. S., & Izevbuwa, O. E. (2022). Microbiological Contamination Associated with the Proximity of A Refuse Dumpsite to a River Situated in Okada, Edo State, Nigeria, *Journal of Bioresource Management, 9* (1).
ISSN: 2309-3854 online
(Received: Aug 30, 2021; Accepted: Nov 8, 2021; Published: Mar 24, 2022)

Microbiological Contamination Associated with the Proximity of A Refuse Dumpsite to a River Situated in Okada, Edo State, Nigeria

MICROBIOLOGICAL CONTAMINATION ASSOCIATED WITH THE PROXIMITY OF A REFUSE DUMPSITE TO A RIVER SITUATED IN OKADA, EDO STATE, NIGERIA

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ABSTRACT

This study was performed to evaluate the potential contamination of the Okponha river situated near a dumpsite in Okada, Edo State, Nigeria. Water samples were collected and analyzed for bacteriological and parasitological quality using standard procedures. Isolation and enumeration of bacterial colonies were performed by pour plate technique and the isolated bacteria were identified by standard phenotypic tests. Helminths and protozoa were screened by the direct smear technique. The values of HPC (3.79 ± 0.12 $\log_{10}$ CFU/ml) and TCC (2.20 ± 0.14 $\log_{10}$ CFU/ml) obtained from the river water samples exceeded WHO and NAFDAC recommended limits (≤ 2 $\log_{10}$ CFU/ml and ≤ 1 $\log_{10}$ CFU/ml for HPC and TCC respectively). *Bacillus* spp., *Enterobacter* spp., *Staphylococcus aureus* and *Chromatium* spp. were the bacteria that were found in the river water samples. Except for the *Chromatium* spp., the same bacteria present in the river water were also found in the dumpsite soil, thus indicating a potential runoff from the dumpsite. *Ascaris lumbricoides* and *Trichuris trichiura* were the main helminth species that were seen in the river water and dumpsite samples, while the main protists that were identified included *Entamoeba coli* and *Giardia lamblia*. The high bacterial load seen in the river water is a source of concern because the water is used for a wide range of domestic purposes by inhabitants. Therefore, health authorities should make the public aware of the potential danger in using untreated water as a source of drinking water and also encourage in-house treatment of the raw water.

Keywords: Dumpsites, river, microorganisms, proximity, solid waste.

INTRODUCTION

A refuse dump is a place or an area where refuse and other solid waste is put (Banga, 2011). Solid waste is generally referred to as any garbage or refuse, sludge from a wastewater treatment plant, water supply treatment plant, or an air pollution control facility and other discarded material, including solid, liquid, semi-solid, or contained gaseous material resulting from industrial, commercial, mining, agricultural operations and community activities (US EPA, 2012). In the words of Misra and Panday (2005), "*a material becomes waste when it is discarded without expecting to be compensated for its inherent value"*.

Refuse or solid waste is composed of combustibles and non-combustible materials. The combustible materials include paper, plastics, yard debris, food waste, wood, textiles, disposable diapers and other organics. Non-combustibles also include glass, metal, bones, leather and aluminium (Srivastava et al., 2014).

Refuse contains waste products from all aspects of human activity and as such is an extremely complex and heterogeneous material. Increasingly, it has been shown that a few chemical compounds within municipal solid waste contribute significantly to environmental and health impacts. The characteristics of refuse vary from place to place. Factors that influence the composition is the

average income level, the sources, the population, social behaviour, climate, industrial production and the market for waste materials (Malav et al., 2020). Several microbial pathogens such as *Vibrio cholerae*, *Salmonella* spp., *Shigella* spp. *Legionella* spp., *Aeromonas* spp., *Pseudomonasaeruginosa* and *Mycobacterium avium* and *Cryptosporidium spp.* have natural reservoirs in the aquatic environment and soil. These organisms are introduced from the surface water into the drinking water system usually in low numbers and may survive and grow within the distribution system biofilm (Wilson et al., 1983). The present study was performed to evaluate the potential contamination of Okponha river located in Okada, Ovia North East Local Government, Edo State, Nigeria by the refuse dumpsite situated about 50 meters away from this river.

MATERIALS AND METHODS

Study Area

The study area was located in Okada, Ovia North East Local Government, Edo State, Nigeria at a Longitude of 6.6342^{o}N and Latitude 5.9304^{o}E with an altitude of 300 meters above sea level. It has a tropical climate with a well-defined rainy season which occurs from April to October and the dry season from November to March. The residents of this area depend on water from the river as a major domestic water source due to the low affordability of potable water in this area as a result of poor socioeconomic status.

Sample Collection

Okponha river in Okada, Ovia North-East local government area, Edo State, was visited in the months of April to June 2021 for sample collection at upstream and downstream points along the river. At each visitation, five water samples were collected consisting of three water samples from the downstream and two water samples from the upstream. The water samples were collected with sterile 25 ml bottles, stored in a cooler containing an icebox to maintain a stable temperature of 4^{o}C and immediately transported to the laboratory for bacteriological and parasitological analysis that was performed within six hours of sample collection. Soil samples from Okponha dumpsite about 50 meters away from the river were aseptically collected using a soil sampler to a depth of 20 cm, stored in sterile aluminium foils and transported to the laboratory within 6 hours of collection for bacteriological and parasitological analysis.

Bacteriological Analysis of River and Dumpsite Samples

Bacterial isolation and enumeration of the river water samples and soil samples collected from the dumpsites were performed with the pour plate technique (Public Health England, 2014). Serial dilution of river water and soil samples were made up to 10^{-7}, with the first dilution of the river water samples made by mixing 25 ml of river water with 225 ml of sterile 1.5% peptone water in a sterile tube. For the soil samples, the first dilution was made by mixing 25 grams of soil with 225 ml of sterile 1.5% peptone water in a sterile tube. One millilitre of each the serially-diluted river water and soil samples was separately poured into two different duplicates sterile Petri dishes and then respectively mixed with 15 ml of sterile tryptic soy agar (TSA) and MacConkey agar (MA) media (Himedia Laboratories, India). The TSA and MA Petri dishes were subsequently incubated at room temperature for 48 hours. After incubation, bacterial colonies were counted with the colony counter and counts on TSA and MA Petri plates were respectively reported as total heterotrophic plate count (HPC) and total coliform count (TCC). The counts were expressed as

colony-forming units per millilitre (CFU/ml) of the river water sample and colony-forming units per gram (CFU/g) of the soil sample.

Genus-level Identification of Bacterial Colonies

The phenotypic techniques employed for the genus-level identification of bacterial isolates obtained from the river water samples were performed with standard methods (Krieg and Holt, 1984). The colonial morphology, biochemical testing as well as the fermentation of various sugars were performed to identify each unique colony from the prepared purity plate.

Parasitological Examination

The water samples were checked for parasitic infections using the direct smear technique (Desowitz, 1980) to screen for helminths and protozoa. Lugol's iodine was added in simple smears, which helps to stain and produce better visualization for protists identification.

Statistical Analysis

The NCSS ver. 12 data analysis software was used to carry out descriptive statistics of the datasets obtained from the bacteriological analysis. Shapiro-Wilk normality test and Fisher parametric one-way ANOVA test were also performed with NCSS ver. 12. The test of the hypothesis was considered statistically significant if the achieved level of significance (p) was less than 0.05.

RESULTS

Bacterial Load in the River

Tables 1 and 2 represent the HPC and TCC in the river water samples. Mean HPC ranged between 3.69 ± 0.14 $\log_{10}$CFU/ml to 3.86 ± 0.62 $\log_{10}$ CFU/ml across all visitations, while mean TCC ranged from 2.04 ± 0.23 $\log_{10}$CFU/ml to 2.29 ± 0.56$\log_{10}$ CFU/ml across all visitations. Overall mean HPC and TCC from all the 15 river water samples were estimated at 3.79 ± 0.12 $\log_{10}$ CFU/ml and 2.20 ± 0.14 $\log_{10}$ CFU/ml respectively. Shapiro Wilk test showed that the HPC and TCC datasets of the river water samples across all visitations was normally distributed ($p = 0.97$; $\alpha = 0.05$) for HPC and not normally distributed ($p = 0.002$; $\alpha = 0.05$) for TCC. Based on the outcome of the normality tests, the Fisher test, the preferred ANOVA technique, indicated no statistically significant difference ($p = 0.84$; $\alpha = 0.05$) in the HPC; while the Kruskal-Wallis test, using Chi-Square distribution, indicated no statistically significant difference ($p = 0.38$; $\alpha = 0.05$) in the TCC of the river water samples across all visitations.

Bacterial Load in the Dumpsite

Tables 3 and 4 respectively represent the HPC and TCC in the dumpsite soil samples. The distance of the dumpsite from the river was estimated at 50 meters. Mean HPC ranged from 7.93 ± 0.35 CFU/g to 8.02 ± 0.40 CFU/g across all visitations, while mean TCC was between 3.78 ± 0.61 CFU/g and 3.95 ± 0.49 CFU/gacross all visitations. The HPC and TCC datasets were normally distributed ($p = 0.07$; $\alpha = 0.05$ for HPC and $p = 0.17$; $\alpha = 0.05$ for TCC). Based on the outcome of the normality tests, Fisher test indicated no statistically significant difference in both the HPC and TCC ($p = 0.98$; $\alpha = 0.05$ for HPC and $p = 0.97$; $\alpha = 0.05$ for TCC) across all visitations.

Identified Bacteria in the River and Dumpsite

Table 5 presents the main bacterial isolates that were found in the river and dumpsite samples.

Table 1: Total heterotrophic count obtained from the river water.

River water samples	Visitations					
	1st visitation		2nd visitation		3rd visitation	
	HPC Log_{10} CFU/ml	Mean HPC N = 5 Log_{10} CFU/ml	HPC Log_{10} CFU/ml	Mean HPC N = 5 Log_{10} CFU/ml	HPCC Log_{10} CFU/ml	Mean HPC N = 5 Log_{10} CFU/ml
		3.81 ± 0.21		3.69 ± 0.14		3.86 ± 0.62
1	3.54		3.26		3.84	
2	3.30		3.50		2.95	
3	4.03		3.75		3.78	
4	4.48		3.98		4.66	
5	3.70		3.95		4.07	

HPC: total heterotrophic count; Mean values are reported as mean ± standard error of mean.

Table 2: Total Coliform count obtained from the river water.

River water samples	Visitations					
	1st visitation		2nd visitation		3rd visitation	
	TCC Log_{10} CFU/ml	Mean TCC N = 5 Log_{10} CFU/ml	TCC Log_{10} CFU/ml	Mean TCC N = 5 Log_{10} CFU/ml	TCC Log_{10} CFU/ml	Mean TCC N = 5 Log_{10} CFU/ml
		2.04 ± 0.23		2.29 ± 0.56		2.17 ± 0.25
1	1.45		1.59		1.60	
2	2.50		2.73		2.66	
3	2.54		2.64		2.57	
4	1.59		1.78		1.51	
5	2.55		2.72		2.50	

TCC: total Coliform count; Mean values are reported as mean ± standard error of mean.

Table 3: Total heterotrophic count obtained from the dumpsite soil.

Soil samples	Visitations					
	1st visitation		2nd visitation		3rd visitation	
	HPC Log_{10} CFU/g	Mean HPC N = 5 Log_{10} CFU/g	HPC Log_{10} CFU/g	Mean HPC N = 5 Log_{10} CFU/g	HPC Log_{10} CFU/g	Mean HPC N = 5 Log_{10} CFU/g
		7.93 ± 0.39		7.93 ± 0.35		8.02 ± 0.40
1	8.81		8.67		8.92	
2	6.69		6.88		6.84	
3	7.81		7.60		7.88	
4	7.68		7.75		7.57	
5	8.67		8.73		8.88	

HPC: total heterotrophic count; Mean values are reported as mean ± standard error of mean.

Table 4: Total Coliform count obtained from the dumpsite soil.

Soil samples	Visitations					
	1st visitation		2nd visitation		3rd visitation	
	TCC Log_{10} CFU/g	Mean TCC N = 5 Log_{10} CFU/g	TCC Log_{10} CFU/g	Mean TCC N = 5 Log_{10} CFU/g	TCC Log_{10} CFU/g	Mean TCC N = 5 Log_{10} CFU/g
		3.78 ± 0.61		3.29 ± 0.48		3.95 ± 0.49
1	5.51		5.59		5.48	
2	3.04		3.04		3.03	
3	3.67		3.60		3.67	
4	4.66		4.37		4.63	
5	2.04		3.02		2.95	

TCC: total Coliform count; Mean values are reported as mean ± standard error of mean.

Table 5: Characterization of bacterial colonies obtained from the river water and dumpsite soil.

Sample source	Rep. isolates	Morphological examinations			Biochemical examinations									Suspected organisms	Frequency of occurrence	
		Colony characteristics on TSA plates	Gram staining	Haemolysis test	CO	CA	OX	MR	VP	IN	CI	LA	MA		F	P (%)
River water	1	Purple colony	Negative rods	γ-haemolytic	NP	+	-	-	+	-	+	-	-	*Chromatium* sp.	16/90	17.78
	2	Mucoid colony	Positive cocci	β-haemolytic	+	+	-	-	+	-	+	-	+	*Staphylococcus aureus*	17/90	18.89
	3	Mucoid colony	Negative rods	γ-haemolytic	NP	-	-	-	+	-	+	+	-	*Enterobacter* sp.	25/90	27.78
	4	Dry colony	Positive rods	V	NP	+	+	-	+	-	+	-	-	*Bacillus* sp.	32/90	35.56
Dumpsite soil	1	Dry colony	Positive rods	V	NP	+	+	-	+	-	+	-	-	*Bacillus* sp.	62/90	68.89
	2	Mucoid colony	Negative rods	γ-haemolytic	NP	-	-	-	+	-	+	+	-	*Enterobacter* sp.	23/90	25.56
	3	Mucoid colony	Positive cocci	β-haemolytic	+	+	-	-	+	-	+	-	+	*Staphylococcus aureus*	5/90	5.56

TSA: tryptic soy agar. HM: haemolysis test. CO: coagulase test. CA: catalase test. OX: Oxidase test. CI: Citrate test. IN: Indole test. MR: Methyl red test. VP: Voges-Proskauer test. LA: Lactose fermentation test. MA: Mannitol fermentation test. V: Variable result. +: Positive results. -: Negative results. β: Complete zone of haemolysis. γ: No zone of haemolysis. NP: Not performed. F: Fractional prevalence. P: Percentage prevalence.

Bacillus spp., *Enterobacter* spp., *Staphylococcus aureus* and *Chromatium* spp. were the bacteria that were found in the river water. *Bacillus* spp. were the most abundant bacteria in the river, while *S. aureus* least frequently occurred. The increasing order of occurrence of bacteria in the river was as follows: *Bacillus* spp. >*Enterobacter* spp. >*S. aureus*>*Chromatium* spp. In the dumpsite soil samples, *Bacillus* spp., *Enterobacter* spp. and *Staphylococcus aureus* were the main isolates that were found. *Bacillus* spp. were also the most abundant bacteria in the dumpsite soil, while *S. aureus* least frequently occurred. The increasing order of occurrence of bacteria in the dumpsite soil was as follows: *Bacillus* spp. >*Enterobacter* spp. >*S. aureus*.

Identified Parasites

The main helminth species that were identified in the river water and dumpsite soil samples were *Ascaris lumbricoides, Trichuris trichiura*, while the main protists that were identified included *Entamoeba coli* and *Giardia lamblia*.

DISCUSSION

Contamination of river water is largely caused by improper waste disposal with high turbidity of the water attributed to leachate run-off from dumpsites. Urban wastes exert an impact on the ecosystem because it constitutes a large source of pollution. The mean HPC and TCC counts (Tables 1 and 2) reported in the river water samples were above the World Health Organization (WHO) and National Agency for Food and Drug and Control (NAFDAC) limits stipulated for drinking water. This study agreed with the report of Doughari et al. (2007) and Adesakin et al. (2020) that a high total bacterial load in water is suggestive of probable contamination by the potentially dangerous microorganisms, thus, rendering the water unfit for human consumption. Dumpsite soil samples also had a high load of bacteria (Tables 3 and 4). The high bacterial load seen in the river water examined in this study may be attributed to runoff from faecal and decaying materials from the dumpsite. The bacterial species identified from the river water may also be due to farming activities occurring near the surface water by inhabitants of the community living around this water body. Other sources may include human activities like bathing, washing and other recreational activities (Anyanwu and Okoli, 2012; Schweitzer and Noblet, 2018). *Bacillus* spp., *Staphylococcus aureus*, *Enterobacter* and *Chromatium* spp. were the main bacteria that were isolated from the river water (Table 5). Except for the *Chromatium* spp., the same bacteria present in the river water were also found in the dumpsite soil. The presence of *Enterobacter* species in the stream is suggestive of the presence of faecal contamination and potential health risks due to a probable pathogen presence in the stream (Tamungang et al., 2016). The coliforms are the primary bacterial indicator for faecal pollution in water and they are the most abundant bacteria in water responsible for waterborne diseases such as typhoid, dysentery, diarrhoea and have also been implicated in mortality across the world (WHO, 2011). The high abundance of coliforms recorded in the river water could be related to one or to a combination of sewage effluents, such as agricultural run-off and direct faecal contamination from natural fauna. The presence of *S. aureus* in the stream is of significant health concern because this bacterium produces enterotoxins that can pose a potential adverse health effect to humans (Dong et al., 2019). The major diseases that could arise from bacteriological contamination of the river water include waterborne diseases such as typhoid, diarrhoea and cholera, as well as meningitis, pneumonia and urinary tract infections in humans (Bhasin et al., 2020).

Ascaris lumbricoides and *Trichuris trichiura* were the main helminth species that were seen in the river water and dumpsite samples examined in the present study, while the main protists that were identified included *Entamoeba coli* and *Giardia lamblia*. The protists identified in this study have been implicated as agents of diarrheal diseases that have routinely been associated with the consumption of contaminated water which contained traces of faeces.

CONCLUSION

The findings of this study showed that HPC and TCC of the river water exceeded the limits set by WHO and NAFDAC for drinking water. Dumpsite soil samples also had a high load of bacteria. The high bacterial load seen in the river water examined in this study may be attributed to runoff from faecal and decaying materials in the dumpsite. The bacteria that were isolated from the river water were similar to those found in the dumpsite soil, except for the presence of *Chromatium* spp. in the river water.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHOR'S CONTRIBUTIONS

EJO conceived the study and participated in its design and coordination. OEI participated in the laboratory investigations and helped to draft the manuscript. OSI participated in the laboratory investigations, performed the statistical analysis and helped to draft the manuscript. All authors read and approved the final manuscript.

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