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Microbial Assay of Otamiri River and Its Sediments in Parts of Owerri

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Abstract

The aim of the study was to analyse and identify microbial constituents in the water and sediment samples with comparison of the River to World Health Organisation (WHO, 2011) standard for drinking water and Federal Ministry of Environment (FME, 2006) and their public health implications with respect to water quality. The microbial assay of Otamiri River was investigated using Standard plate count. The result indicates that microbial constituents of Otamiri river obtained at five strategic gauge stations designated $SSWS_1$ (Egbu), $SSWS_2$ (Timber Market), $SSWS_3$ (FUTO), DOWNSTREAM (Mbirichi) and CONTROL POINT with mean Total coliform Count of 3.0×10^2, 3.0×10^3, 4.1×10^3 and 1.0×10^3 cfu/100ml with control point value of 0.5×10^3 respectively. The mean Total Bacteria Count was 3.0×10^4, 2.1×10^3, 1.1×10^3 and 0.8×10^3 cfu/100ml respectively with control point value of 0.2×10^3 while the mean values for Total *E. coli* Count were 1.1×10^2, 3.0×10^2, 4×10^3 and 2.0×10^3 cfu/100ml with control point value of 0.2×10^3. The biochemical identification of some organisms in water was *Escherichia coli*, *Vibro* spp., *Klebsiella* spp., and *Entrobacteria* spp. The result of stream sediment samples indicates that the mean Total Bacterial Count was 3.5×10^4, 5.0×10^4, 6.5×10^4 and 2.0×10^4 cfu/g respectively with 1.5×10^2 as control point value and that of Total Coliform Count was 6.5×10^3, 2.0×10^3, 2.5×10^3 and 0.8×10^3 cfu/g respectively with control point value of 0.5×10^2. While for the Total *E. coli* Count, the values were 2.5×10^3, 1.0×10^3, 2.5×10^3 and 0.5×10^5 cfu/g respective with control as 0.5×10^2. Biochemical identification of some organisms in sediments includes: *Escherichia coli*, *Vibro* spp., *Klebsiella* spp., *Entrobacteria* spp. and *Bacillus* spp. The mean total bacterial count, total coli form count and total *E. coli*, were not in conformity with both World Health

Organisation (WHO, 2011) Standard for drinking water and Federal Ministry of Environment (FME) 2006 standard for soil and thus constitute a threat to the River; these are attributed to waste dumps and anthropogenic activities around the five stations. The presence of bacteria in water can cause cholera, hepatitis, dysentery and typhoid. The microbial constituents can be reduced by chlorination.

Keywords

Coliforms, *E. coli*, Bacteria, Otamiri River, Sediments and Chlorination

1. Introduction

Water pollution is a major problem globally. It has been suggested that it is the leading worldwide cause of deaths and diseases and that it accounts for the deaths of more than 14,000 people daily (Mgbemena et al., 2012). Water pollution describes the presence of materials in water that interferes unreasonably with one or more beneficial uses of water (Akhionbare, 2009). Water pollution, especially surface water, can be caused by mining, recreational and agricultural activities. Agents of surface water pollution include bacteria, viruses and other substances present in such concentration or numbers to impair the quality of the water rendering it less suitable or unsuitable for its intended use and presenting a hazard to man or to his environment (Egereonu & Ibe, 1999; Egereonu et al., 2012).

The Otamiri River is a major fresh surface water resource of South-eastern Nigeria. The river takes its name from "Otamiri", a deity who owns all the water that is called by its name, and who is often the dominating god of Mban houses (Ihenyen & Aghimien, 2002; Fagorite et al., 2019). It is located on latitude 5˚23'N and 5˚30'N, and Longitude 6˚58'E and 7˚04'E (Figure 1). The river runs south from Egbu (its source) pass Owerri and through Nekede, Ihiagwa, Eziobodo, Obowuumuisu, Mgbirichi and Umuagwo (all in Imo state) to Ozuzu in Etche Local Government Area of Rivers state where it has a confluence with Oramirukwa River; both rivers flow from there into the Atlantic Ocean (Fagorite et al., 2019). According to Fagorite et al. (2019), Otamiri River is used for domestic, industrial and agricultural activities. The stream sediments on the river are used for various construction purposes. The quality of the river is exposed to threat from indiscriminate waste disposal, fishing, oil and natural gas activities to mention a few. Therefore, constant monitoring of the quality of surface water cannot be overstressed especially now that increase in population has resulted in generation of more waste thus exposing the water to more pollutants (Fagorite et al., 2019).

The aim of the study was to analyse and identify microbial constituents in the water and sediment samples with comparison of the River to World Health Organisation (WHO, 2011) standard for drinking water and Federal Ministry of
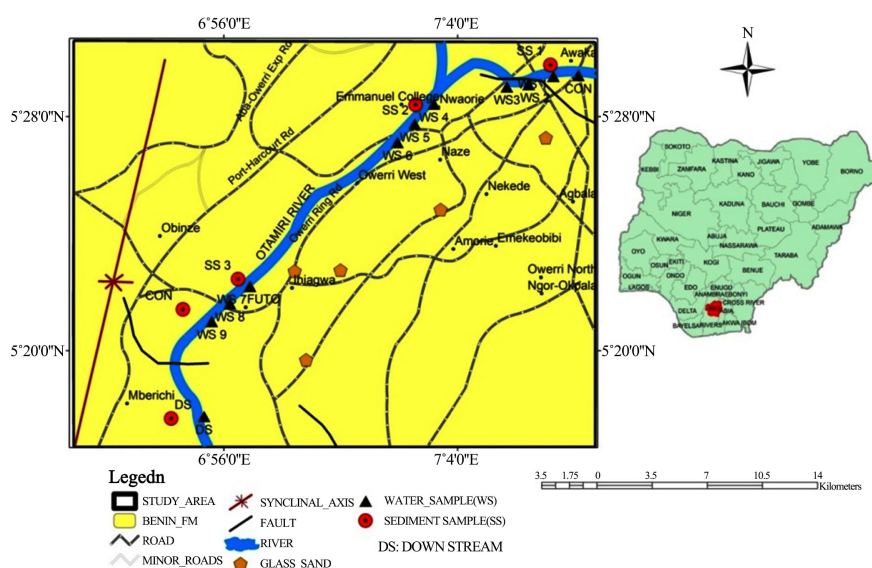
Figure 1. Geologic map of the study area.

Environment (FME, 2006) of the River and their public health implications. Recent studies on Otamiri River have been basically limited to just the water quality without relation with the sediment. Such study includes that of (Adieze et al., 2016), in which the effect of untreated sewage effluents on the water quality was investigated. Another study in India by Kora et al. (2017), on the water quality both physico-chemically and bacteriological parameters of Hussain Sagar lake. But the present study identified and analysed both water samples and sediment samples for microbial constituents in Otamiri River.

2. Geology and Hydrogeology of the Study Area

The geology of the area shows that it is underlain by the Benin Formation (Figure 1) which is an extensive stratigraphic unit in Southern Nigeria. The Benin Formation has variable thickness. It is underlain on land by the Oligocene—Miocene Ogwashi—Asaba Formation; on the subsurface by the petroleum bearing Agbada and Akata Formations (Ananaba et al., 1993). The Benin sands and sandstones are mainly deposits of continental upper deltaic plain environment. It is predominantly sandy with few shale intercalations becoming more abundant towards the base. The sands and sandstone are coarse grained pebbles, sub angular to well rounded and whitish or yellowish in colour probably because of limonite coating and bears lignite streaks and wood fragments (Ananaba et al., 1993). The sands may represent point bar deposits, channel fills or natural levees whereas the shales may be interpreted as back swamp deposits and oxbow fills. The thickness of the formation is variable and may be more than 1800 m.

3. Methodology

3.1. Sample Collection

Eleven water samples were obtained along the stretch of the Otamiri River at five

strategic stations designated SSWS$_1$ (Egbu), SSWS$_2$ (Timber Market), SSWS$_3$ (FUTO), DOWNSTREAM (Mbirichi) and CONTROL POINT using grab method Figure 2; the water samples were geo-referenced. Land use elements especially waste dump sites were visited and examined. Samples were taken at a distance of 300 meters and collected with the aid of sterilized 1.5 litres plastic containers. The samples were corked under water immediately after collection so as to avoid the oxidation of the constituents and later sent to the laboratory within 24 hours for analysis. Water samples collected from these sampling stations were analysed in accordance with the American Public Health Association (APHA) standard methods for examination of water and wastewater (APHA, 2012). The stream sediments were obtained at 5 strategic locations using manual dredging method Figure 2. The samples were collected into black polyethene bags and sent to the laboratory for analysis within 24 hours.

3.2. Laboratory Analysis

Serial dilution of the water samples were carried out using sterile distilled water as in (Chessbrough, 2002). The water and sediment samples were examined bacteriologically using culture techniques as in (Chessbrough, 2002). Each water sample was examined by culture technique using streak plate and spread plate technique Figure 3. A sterile wire loop was used to collect a loop full of each undiluted water sample and inoculated on the surface of nutrient agar, blood agar,

Figure 2. The grab method for water collection and collection of sediment through manual dredging.
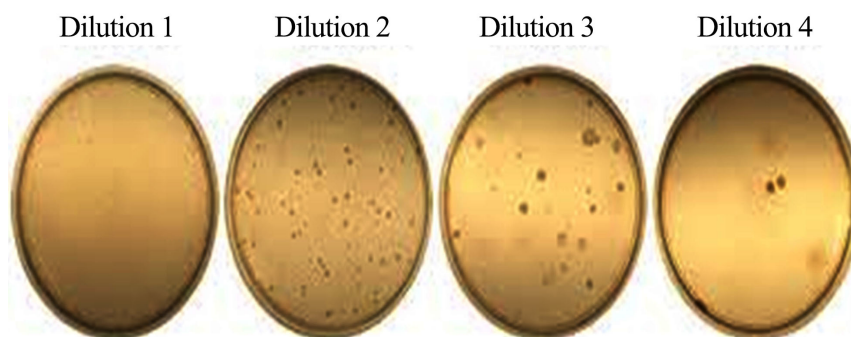

Figure 3. Standard plate method (Source: Wikipedia, 2019).

MacConkey agar and Eosine Methylene Blue agar (Mgbemena et al., 2012). The inoculated plates were subsequently sub-cultured on fresh nutrient agar, Mac-Conkey agar and blood agar plates to obtain pure cultures as well as study their morphological characteristics. They were incubated at 37˚C for 24 h. The pure culture isolates were sub-cultured in nutrient agar slant and incubated at 37˚C for 24 h and were then stored in the refrigerator until required for further use. All the different dilutions were properly labelled and used for total plate count. The bacteria isolates were subjected to various tests beginning from the study of their growth morphology on different agar media to different microbiological identification tests such as gram staining and motility tests and biochemical identification tests such as catalase, coagulase, oxidase, citrate utilization, urease, indole production, hydrogen sulphide production, nitrate and nitrite reduction, methyl red, Voges Proskeur and sugar fermentation tests (Table 5 and Table 6).

3.3. Parameters and Materials Used (Table 1 and Table 2)

Table 1. Materials ranging from the field tools, laboratory tools, reagents and software used.

Parameters	Materials
Total bacteria count, cfu/ml	Nutrient agar medium (Peptone 5 g/L, NaCl 5 g/L, beef extract1.5 g/L, yeast extract 1.5 g/L, agar 15 g/L, pH 7.4)
Total Coliform Count, cfu/ml and Total *E. coli* Count, cfu/ml	Selective medium HiCrome coliform agar (Peptone 3 g/L, NaCl 5 g/L, dipotassium hydrogen phosphate 3 g/L, potassium dihydrogen phosphate 1.7 g/L, sodium pyruvate 1 g/L, l-tryptophan 1 g/L, sodium lauryl sulphate 0.1 g/L, chromogenic mixture 0.2 g/L, agar 15 g/L, pH 6.8).

Table 2. Materials ranging from the field tools, laboratory tools, reagents and software used.

S/N	Field Tools	Laboratory Tools	Reagents	Softwares Used
1	Topographic map	pH meter	Sabourand Dextrose Agar	Microsoft word and excel
2	Global positioning system (E-Trex GPS)	Glass ware	Nutrient agar	
3	Sample bottles and sample bags	Hanna Hi 83200 photometer	Distilled Water	
4		Hox box oven	Mineral Salts Agar (medium)	
5		Burrette	Nitric acid	
6		Cornical flask	Perchloric acid	
7		Pipettes	Sulphuric acid	
8		Crucibles	Air oxidant gas	
9.		Incubator		
10.		Autoclave		
11.		Burnsen burner		

3.4. Method of Result Analysis

The average mean of three samples per station ($SSWS_1$-$SSWS_2$) was taken for three stations and one the reading for the remaining two stations (Control and Downstream). The results obtained were compared with World Health Organization (WHO, 2011) standard for drinking water while Federal Ministry of Environment (FME, 2006) was used for the sediment.

4. Results and Discussion

The mean Total Bacteria Counts of the Otamiri River at $SSWS_1$, $SSWS_2$, $SSWS_3$ and Downstream stations were 3.0×10^4, 2.1×10^3, 1.1×10^3 and 0.8×10^3 cfu/100 ml respectively with control point value of 0.2×10^3 cfu/100 ml. From the values indicated for Total Bacteria Count, it reveals that $SSWS_1$ (Egbu) was with the highest value. While the mean Total Coliform Count were 3.1×10^2, 3.0×10^3, 4.1×10^3 and 1.0×10^3 cfu/100ml respectively with control point value of 0.5×10^3 cfu/100ml. The value of $SSWS_3$ (FUTO) was the highest with respect to this parameter which is due to heavy activity within such as effluent disposal the school area (Adieze et al., 2016). The Total *E. coli*. Count were 1.1×10^2, 3.0×10^2, 4.0×10^3 and 2.0×10^3 cfu/100ml (**Table 4**) with control point value of 0.2×10^3 cfu/100ml (**Table 3** and **Figure 4**). $SSWS_3$ (FUTO) was with the highest value. Feresu & Vansickle (1990) recorded between 1.8×10^3 CFU/100ml and 3.3×10^4 CFU/100ml coliforms in Zambezi River receiving untreated sewage effluents. This agrees with the work of (Rheinheimer, 1991) which reported that the introduction of wastewater high in organic matter and essential nutrients bring about changes in the micro flora (Adieze et al., 2016). It was also observed that high counts of bacterial load reflected the level of water pollution as it gives indication of the amount of organic matter present (Anson & Ware, 1974; Adieze et al., 2016) (**Table 4**).
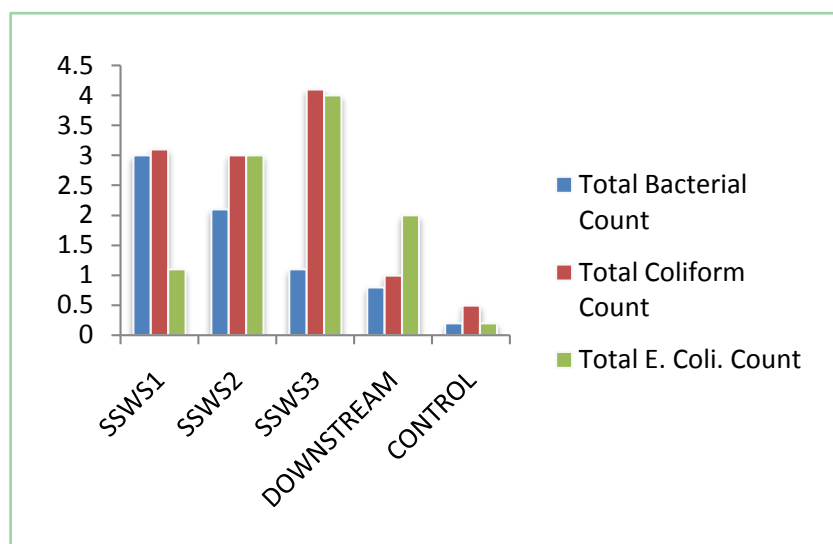

Figure 4. Microbial counts in water.

Table 3. Most probable organisms of water samples Otamiri River per stations.

Parameters	WHO 4th edition (2011) Guideline Value	SSWS$_1$ (Egbu)			Mean	SSWS$_2$ (Timber Market)			Mean	SSWS$_3$ (FUTO)			Mean	DOWNSTREAM (Mbirichi)	CONTROL POINT
Total bacteria count, cfu/ml	Absent	4.5×10^4	$3.5.0 \times 10^4$	4.0×10^4	3.0×10^4	2.0×10^3	3.0×10^3	1.3×10^3	2.1×10^3	1.0×10^3	1.0×10^3	1.3×10^3	1.1×10^3	0.8×10^3	0.2×10^3
Total Coliform Count, cfu/ml	Absent	2.3×10^2	3.0×10^2	5.0×10^2	3.1×10^2	2.0×10^3	3.0×10^3	4.0×10^3	3.0×10^3	3.3×10^3	3.0×10^3	$3. \times 10^3$	4.1×10^3	1.0×10^3	0.5×10^3
Total E. coli Count, cfu/ml	Absent	1.0×10^2	1.3×10^2	1.0×10^2	1.1×10^2	2.0×10^2	3.0×10^2	4.0×10^2	3.0×10^2	4.0×10^3	4.0×10^3	4.3×10^3	4×10^3	2.0×10^3	0.2×10^3

Table 4. Most probable organisms in sediment samples per stations from Otamiri River.

Parameters	FME Standard 2006	SSWS$_1$	SSWS$_2$	SSWS$_3$	DOWNSTREAM	CONTROL POINT
Total Bacteria Count, cfu/g	NS	3.5×10^4	5.0×10^4	6.5×10^4	2.0×10^4	1.5×10^2
Total Coliform Count, cfu/g	NS	6.4×10^3	2.0×10^3	2.5×10^3	0.8×10^3	0.5×10^2
Total E. coli Count, cfu/g	NS	2.0×10^3	1.0×10^3	1.1×10^3	0.5×10^3	0.5×10^2

Key: SSWS$_1$—Egbu, SSWS$_2$—Timber Market, SSWS$_3$—FUTO and DOWNSTREAM—Mbirichi WHO—World Health Organization (2011).

With respect to the sediments, the values for the Total Bacterial Count at SSWS$_1$, SSWS$_2$, SSWS$_3$ and Downstream station were 3.5×10^4, 5.0×10^4, 6.5×10^4 and 2.0×10^4 cfu/g respectively with control point value of 1.5×10^2 cfu/g. SSWS$_3$ (FUTO) was with the highest value again just as in the case of the water samples which is due to heavy activities within the school environment such as sewage disposal and defecation Figure 5. The Total Coliform Count was 6.5×10^3, 2.0×10^3, 2.5×10^3 and 0.8×10^3 cfu/g with a control point value of 0.5×10^2 cfu/g. SSWS$_1$ (Egbu) was with the highest value which is indicative of waste disposal within and around the station. For the Total E. Coli Count, the values were 2.5×10^3, 1.0×10^3, 2.5×10^3 and 0.5×10^3 cfu/g respectively with control point value of 0.5×10^2 cfu/g. In as much there is no standard assigned for the microbial constituents, the values all exceed the control point value due to the fact that rivers have a self-purifying capacity (Adieze et al., 2016). Therefore, the microbial constituents tend to reduce along the stretch of the river from the source to downstream Table 4 and Figure 6.

Although the WHO guideline indicates no standard, their presence in water indicates faecal contamination of the water. Escherichia coli, an indicator of these enterobacteria in water is harmless but the others have been implicated as the causative agent of one waterborne disease or the other (Duru et al., 2012). According to Ahiarakwem (2013), the indiscriminate disposal of diapers at dumpsites in addition to defecations within and around the watershed of the river is responsible for its poor microbial assay. The presence of bacteria in water can cause cholera, hepatitis, dysentery and typhoid (Ahiarakwem, 2013).

Table 5. Biochemical identification of some bacteria isolated from water samples from Otamiri River.

Stations	Most probable organism	Colony Morphology	Microscopic character	Gram Stain	Triple sugar ion agar (TSIA)						INDOLE	MOTILITY	OXIDASE	CITRATE	UREASE	CATALASE	V.P	METYL RED
					SLANT	BUTT	Glucose	Lactose	Gas	H₂S								
SSWS₁ₐ	Vibrio spp	Creamy colony on nutrient agar	Rod	−	B	A	−	+	+	+	+	+	+	+	−	+	+	−
SSWS₁ᵦ	Escherichia coli	Creamy colony on nutrient agar	Rod	−	A	A	−	−	+	+	+	+	−	−	−	+	−	+
SSWS₁C	Enterobacteria spp	Mucoidal Creamy colony on nutrient agar	Rod	−	A	A	+	+	+	+	−	−	−	+	−	+	+	−
SSWS₂ₐ	Klebsiella spp	Creamy colony on nutrient agar	Rod	−	A	A	−	−	+	+	−	−	−	+	−	+	−	+
SSWS₂ᵦ	Vibrio spp	Creamy colony on nutrient agar	Rod	−	B	A	−	+	−	−	+	+	−	+	−	+	+	−
SSWS₃C	Klebsiella spp	Creamy colony on nutrient agar	Rod	−	A	A	+	+	−	−	−	−	−	+	−	+	−	+
SSWS₃ₐ	Klebsiella spp	Creamy colony on nutrient agar	Rod	−	A	A	+	+	−	−	−	−	−	+	−	+	−	+
SSWS₃ᵦ	Escherichia coli	Creamy colony on nutrient agar	Rod	−	A	A	+	+	−	−	+	+	−	−	−	+	+	−
SSWS₃c	Vibrio spp	Creamy colony on nutrient agar	Rod	−	B	A	−	−	+	+	+	+	−	+	−	+	−	+
CONTROL POINT	Escherichia coli	Creamy colony on nutrient agar	Rod	−	A	A	−	−	−	−	−	−	−	−	−	+	−	+
DOWNSTREAM	Klebsiella spp	Creamy colony on nutrient agar	Rod	−	A	A	+	+	−	−	−	−	−	+	−	+	−	+

*SSWS1—Egbu, SSWS2—Timber Market, SSWS3—FUTO and DOWNSTREAM—Mbirichi WHO—World Health Organization (2011). Key: A = Acidic condition, B = Basic condition, + = positive, − = negative.

Table 6. Biochemical identification of some bacteria isolated from sediment samples from Otamiri River.

Location	Most probable organism	Colony Morphology	Microscopic character	Gram Stain	Triple sugar ion agar (TSIA)						INDOLE	MOTILITY	OXIDASE	CITRATE	UREASE	CATALASE	V.P	METYLRED
					SLANT	BUTT	Glucose	Lactose	Gas	H2S								
	Baccillus spp	Creamy colony on nutrient agar	Rod	+	B	A	+	−	−	−	−	+	−	+	−	+	−	+
SSWS1	Escherichia coli	Creamy colony on nutrient agar	Rod	−	A	A	+	+	−	−	+	+	−	−	−	+	−	+
	Enterobacteria spp	Mucoidal Creamy colony on nutrient agar	Rod	−	A	A	−	−	−	−	−	−	−	+	−	+	+	−
	Klebsiella spp	Creamy colony on nutrient agar	Rod	−	A	A	+	+	−	−	−	−	−	+	−	+	−	+
SSWS2	Vibrio spp	Creamy colony on nutrient agar	Rod	−	B	A	+	−	−	−	+	+	−	+	−	+	+	−
	Klebsiella spp	Creamy colony on nutrient agar	Rod	−	A	A	+	+	−	−	−	−	−	+	−	+	−	+
	Klebsiella spp	Creamy colony on nutrient agar	Rod	−	A	A	+	+	−	−	−	−	−	+	−	+	−	+
SSWS3	Escherichia coli	Creamy colony on nutrient agar	Rod	−	A	A	+	+	−	−	+	+	−	−	−	+	+	−
	Vibrio spp	Creamy colony on nutrient agar	Rod	−	B	A	+	−	−	−	+	+	−	+	−	+	−	+
C. Point	Escherichia coli	Creamy colony on nutrient agar	Rod	−	A	A	+	+	−	−	+	+	−	−	−	+	−	+
DOWNSTREAM	Vibrio spp	Creamy colony on nutrient agar	Rod	−	B	A	+	−	−	−	+	+	−	+	−	+	−	−

*SSWS1 —Egbu, SSWS2—Timber Market, SSWS3—FUTO and DOWNSTREAM—Mbirichi WHO—World Health Organization (2011), Key: A = Acidic condition, B = Basic condition, + = positive, − = negative.

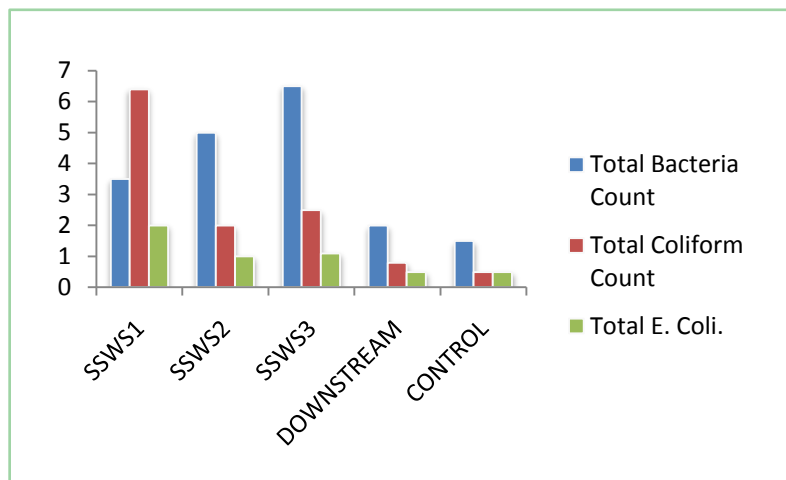**Figure 5.** Landfill along the Otamiri River channel.

Figure 6. Microbial counts in sediment.

5. Conclusion

The Otamiri River at the Five stations (**SSWS$_1$, SSWS$_2$, SSWS$_3$, Downstream** and **Control Point)** is polluted with microbial constituents (bacteria such as: *Escherichia coli*, *Vibro* spp., *Klebsiella* spp., and *Entrobacteria* spp.). For the sediment, it shows pollution with respect to microbial constituents (bacteria such as: *Escherichia coli*, *Vibro* spp*., Klebsiella* spp., *Entrobacteria* spp. and *Bacillus* spp.). These pollutants are attributed to the waste and effluents disposed from land of the five stations. This calls for proper waste management practices and pre-use treatment of the river water with chlorine as well as boiling before usage in order to reduce the microbial constituents of the water. Therefore, there is a need for constant monitoring of the microbial characteristics of the river. In addition, communities along the river bank need to ensure that untreated wastes are not disposed of improperly on land where it can be washed into the river. This work focused primarily on the sediments of the river. Therefore, subsequent research should be centered on the collection of samples per depth at other strategic locations of concern with respect to seasons. This is due to the over dredging of sands from the river which has resulted in erosion and sinkholes around the river.

Acknowledgements

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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