MICROBIAL AND PHYSIOCHEMICAL ANALYSES OF SEDIMENTS DEPTHS OF RIVER ALA, OGBESE AND OWENA IN ONDO STATE, NIGERIA

Olotu, T.M[1,2]. ; Adegunloye, D.V[2]; Ekundayo, F.O[2].; Ojo A. F[3].; Agunloye, O. G[2].; Ajibade, O. A[1] and Amao, F.A[4].

[1]Deparment of Microbiology, Faculty of Science, Adeleke University, P.M.B 250, Ede, Osun State, Nigeria.
[2]Department of Microbiology, School of Sciences, Federal University of Technology Akure, P.M.B 704, Akure, Ondo state, Nigeria.
[3]Department of Agriculture, Federal college of Agriculture, Mooro plantation, Ibadan.
[4]Deparment of Mathematics, Faculty of Science, Adeleke University, P.M.B 250, Ede, Osun State, Nigeria.

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Abstract – Urban Rivers represent a unique ecosystem in which pollution occurs regularly, leading to significantly altered of chemical and biological characteristics of the surface water and sediments. However, the impact of urbanization on the diversity and structure of the river microbial community physiochemical parameters has not been well documented particularly for Ogbese, Ala, and Owena. In this study microbial and physiochemical analyses of sediment depths of River Ogbese, Ala and Owena were analysed. Microbiological analyses of the sediments at various depths of the surface water, sediment surface, 50cm, 100cm and 150cm were analysed using standard microbiological procedures and so were the physiochemical parameters of the sediments were determined using standard methods. Generally, the microbial population of the aerobic bacteria decreases as the depth increases while the anaerobic bacteria increases as the depth increase. River Ala, Ogbese and Owena sediment surface had the highest aerobic bacterial population of 2.4x105cfu/ml, 2.3x105cfu/ml and 2.4x105cfu/ml respectively while Ogbese 100cm and 150cm depth has the lowest bacterial population of 0.4x104cfu/ml. In the anaerobic isolation Ogbese 150cm has the highest bacterial population of 0.5x 105cfu/ml while Owena 50cm and Owena 100cm has lowest anaerobic bacteria count of 0.2x104cfu/m. Ala control has the highest fungal population of 1.6x105sfu/ml while Ala 100cm has the lowest fungal population of 0.2x104sfu/ml. The microorganisms common to all the river sediments of the three rivers were Pseudomonas aeruginosa, Salmonella sp, Escherichia coli, Penicillium notatum, Aspergillus niger, and Rhizopus sp. The pH of the sediment at various depths ranged from 4.75 to 7.52. River Ogbses sediments had the highest organic matter content (5.5 %) while River Ala sediments had the lowest (1.5 %). River Ala had the highest ionization potential (53Mev), River Ala and Ogbese had the highest conductivity (172 µS, 119 µS, 209 µS; 344 µS, 192 µS, 79 µS) respectively and River Ala had the highest salinity (359 mg/kg) while River Owena had the lowest conductivity (12 µS) and salinity (2 mg/kg).

Keywords: Sediments depths, Microbial analyses and Physiochemical analysis

Background

An urban river is a unique ecosystem in where pollution occurs regularly which leads to significantly altered of chemical and biological characteristics of the surface water and sediments. However, the impact of urbanization on the diversity and structure of the river microbial community has not been well documented particularly for Ogbese, Ala and Owena. River Ala, Ogbese and Owena are located in the South Western, Nigeria, they majorly supply water to Ondo and Akure towns during the dry season. These rivers receive domestic wastes such as feces, laundry wastes and cassava peels. Run-offs from chemically sprayed agricultural farms enter the reservoir; human settlements along the Rivers have increased due to the growth of the city and population increase [1]. The bacterial diversity in sediments was significantly higher than their corresponding water samples. Additionally, archaeal communities showed obvious spatial variability in the surface water. Sediment comes from geologic, geomorphic, and organic factors. The nature of the sediment is dependent on location and geology of the location [2]. Sediments serve as major site for organic matter decomposition which is largely carried out by bacteria. However, river pollution by increasing untreated or partly treated wastewater is threatening human health and ecological systems worldwide [3]. Macronutrients such as nitrogen and phosphorous are continuously are been similarly between sediment and overlying water [4]. These sediment microorganisms play key roles in nutrient cycling, heavy metals immobilization

and degradation of organic compounds. In turn, their composition and activity are commonly sensitive to environmental pollutants. Nutrients and heavy metals contamination have also been shown to lead to changes in bacterial biomass, diversity and function. The physicochemical parameters of sediments such assalinity, dissolved oxygen, pH, and organic carbon influences the occurrence and abundance of species distributed in them [5]. Nitrogen settles to the sediment in organic forms, while phosphorus is either absorbed in clay minerals and settles with them on the bed sediments or reacts with Fe, Al, Mn and Ca and mineralizes [6].

Materials and Methods

The study areas are River Ala, Ogbese and Owena in Ondo State, located in the South Western geopolitical zone of Nigeria.

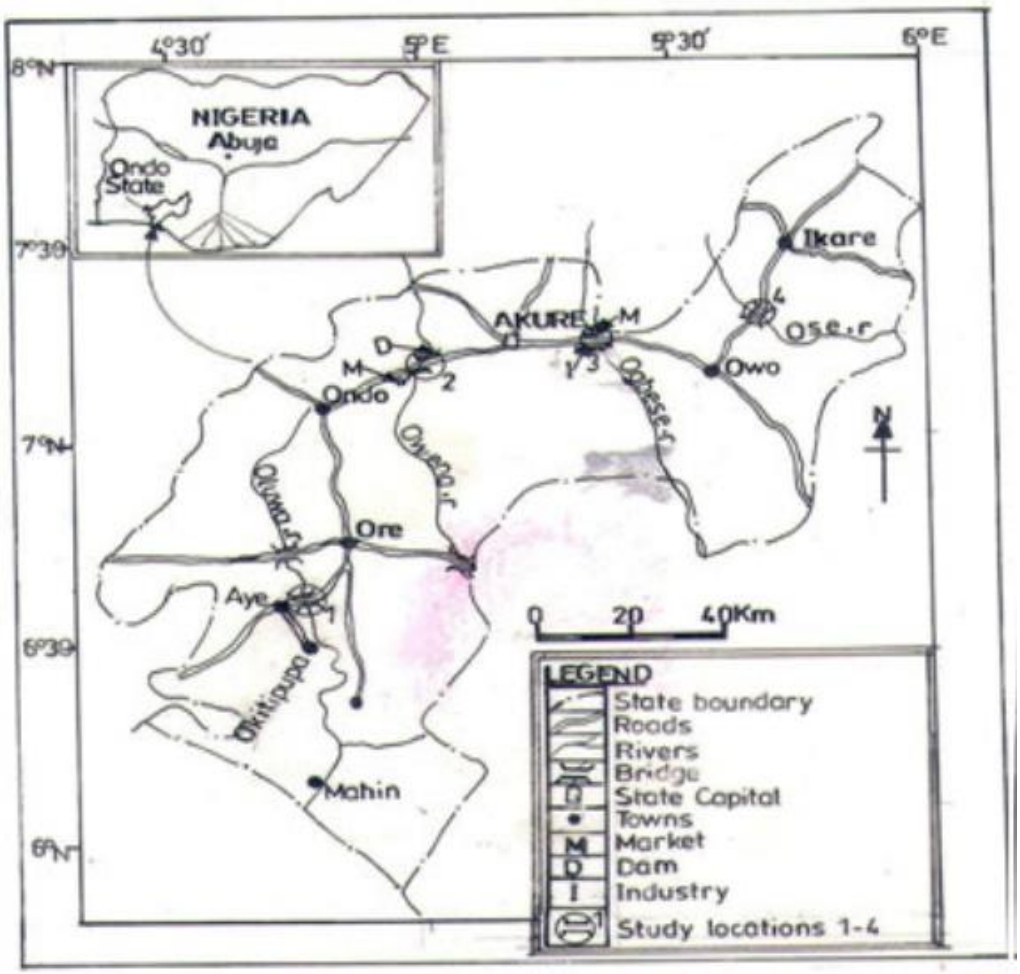

Figure 1: Map of Ondo State showing the River Ala, Owena and Ogbese [7].

Sample collection

The rich sediments samples were collected from River Owena, Ala and Ogbese in Akure, Ondo State. A soil auger was used to scoop the samples at various depths of 50m, 100m and 150m. The surface mud, the stream water as well as the soil at 10m away from the river was also collected. All the samples were used within 24 hours after collection.

Culture media preparation

The main types of media used were Nutrient agar (NA), MacConkey agar (MA), Eosin Methylene Blue agar (EMB), Potato Dextrose agar (PDA), *Salmonella-Shigella* agar (SSA), Manihot Salt agar (MSA). All media were sterilized in an autoclave at 121°C for 15 min. Inoculation, pour plating and sub culturing were all done near naked flame to

enhance aseptic condition. The media used for pour plating and subsequent culturing were Nutrient Agar (NA) and Potato Dextrose Agar (PDA), coliform counts were made on MacConkey Agar (MA) and Eosin Methylene Blue Agar (EMB) and were all sterilized at 121°C for 15min. Each medium was prepared according to the manufacturer's specification [8].

Microbial analysis

Serial dilution

The bacterial and fungal counts of the samples were determined; serial dilution of the sediment and water samples was made up to 10^{-5} dilutions. One gram of each of the sediment samples was added in 9ml of sterile distilled water which was shaken to make 10^{-1} and a sterile pipette was used to add 1ml of the 10^{-1} dilution into another test tube containing 9ml of sterile distilled water to make dilution up to 10^{-5}. 0.1 aliquot of the samples was used and are inoculated using pour plating method.

Physiochemical analysis

Determination of pH

Jenway's pH meter (that was standardized with buffer solution of 7) was used to check for the pH of the mud sediment samples, its meter was dipped into the samples and observed results were recorded [9].

Determination of moisture content

The moisture content of each of the samples was determined weekly by using oven-drying method. Clean and dry Petri-dishes were weighed by using meter balance and their respective weights were recorded (W1). 5 g of the sample was weighed into pre-weighed dry dishes (W2) spreading as much as possible. The dishes containing the sample were transferred into the oven maintained at 105°C and dried for three hours. After three hours they were transferred to the desiccator to cool and then weighed. This process was continued until a constant weight (W3) was taken to be the percentage moisture content [10]

$$\%\ \text{Moisture} = \frac{\text{loss in weight due to drying (W2} - \text{W3}}{\text{Weight of sample taken (W2} - \text{W1)}} \times \frac{100}{1}$$

Determination of organic carbon (O/C)

One gram of 2mm sieved soil was weight using Mettler Toledo PB602 weigh and the soil was poured into 250 mls conical flask. 10 mls of $K_2Cr_2O_7$ (Potassium dichromate), was added afterward 20 mls of H_2SO_4 (Sulphuric acid) was added and gently swirled immediately 100 mls of distilled water was added after it has cooled off for 30 mins. 3 drops ferroin indicator was added and titrated with 0.5 M of Iron II ammonium (ferrous sulphate). Colour change was noticed from green to dark green and then brownish red.

$$O/C = ((B - T) * M * 0.003 * 1.33)/wt * 100/1$$

Determination of organic matter sediment soil (O/M)

From walkley assumptions, soil organic matter contains 58% of carbon and the result can be expressed as % organic matter multiplied by 100/58 = 1.724. Organic Matter is thus calculated by $O.C * 1.724.$

Determination of salinity of the sediments samples

Soil salinity refers to the concentration of soluble inorganic salts in the soil. It is normally measured by extracting the soil sample with water (1:1 or 1:2.5 soil water ratio, w/v) or in an extract saturated paste. However, soil solution ratios of a 1:1 or wider are more convenient where the quantity of soil is limited. Such extracts are rapid, and salinity is measured by electrical conductivity (EC) using a conductivity bridge. The total salt content of a soil can be estimated from this measurement [11].

Determination of Conductivity of the sediments samples

This was determined with the aid of a digital Conductivity Mettler Toledo M400 measuring meter. The S'meter was put on to stabilize for about 15 minutes before meter electrode were put in the sample. The corresponding readings gave the conductivity in µS/cm [12].

Determination of Mineral content of each sediment sample

Half gram of the sample was pre-ash by putting the sample in a dish and heating gently on a bursen burner in a fume-cupboard until the cleared mass had to emit smoke. It was transferred to muffle furnace and ashed at 550°C. Heating was continued until the carbon got burnt off. The crucible plush ash was transferred to cool after which 0.1 Hmoz solution (10ml) was added to the content in the crucible to break up the ash, it was then filtered through acid washed Whatman Filter paper into 100ml volumetric flask containing diluted acid solution. Atomic Absorption Spectrophotometer was used for the analysis of the metals [12].

Result and Discussion

Population of microorganisms isolated from sediments of different depth of rivers

Figures 1- 3 show the aerobic bacteria, anaerobic bacteria population and fungi isolated from rivers sediment at various depths. Microorganisms were isolated from River Ala, Ogbese and Owena at various depth including control, surface water, mud surface, 50 cm, 100 cm and 150 cm depth. Generally, microbial population of the aerobic bacteria decreases as the depth increases while the anaerobic bacteria increases as the depth increases. Owena sediment surface and Ala sediment surface has the highest aerobic bacterial population of $2.4x10^5$cfu/ml while Ogbese 100 cm and 150 cm depth has the lowest bacterial population of $0.04x10^5$cfu/ml. In the anaerobic isolation Ogbese 150 cm has the highest bacterial population of $0.5x\ 10^5$cfu/ml while Owena 50 cm and Owena 100 cm has lowest anaerobic bacteria count of $0.2x10^4$cfu/m. Ala control has the highest fungal population of $1.6x10^5$sfu/ml while Ala 100 cm has the lowest fungal population of $0.2x10^4$sfu/ml. There was no growth observed for Ala control, Ala surface water, Ogbese control, Ogbese sediment surface, Owena control and Owena sediment surface and Owena surface water for anaerobic bacteria growth. Also there was no growth observed in Ala 150 cm, Ogbese 100 cm, Ogbese 150 cm, Owena 100 cm for fungi population.

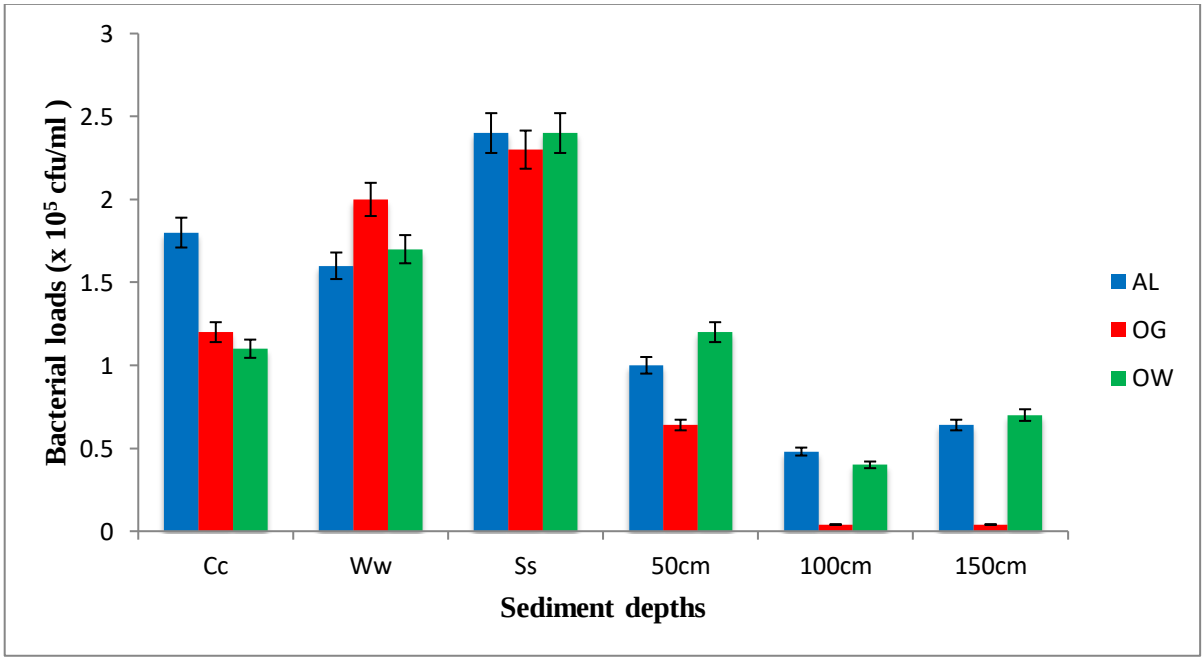

Figure 1: Aerobic bacterial population of sediment of Rivers Ala, Ogbese and Owena at different depths

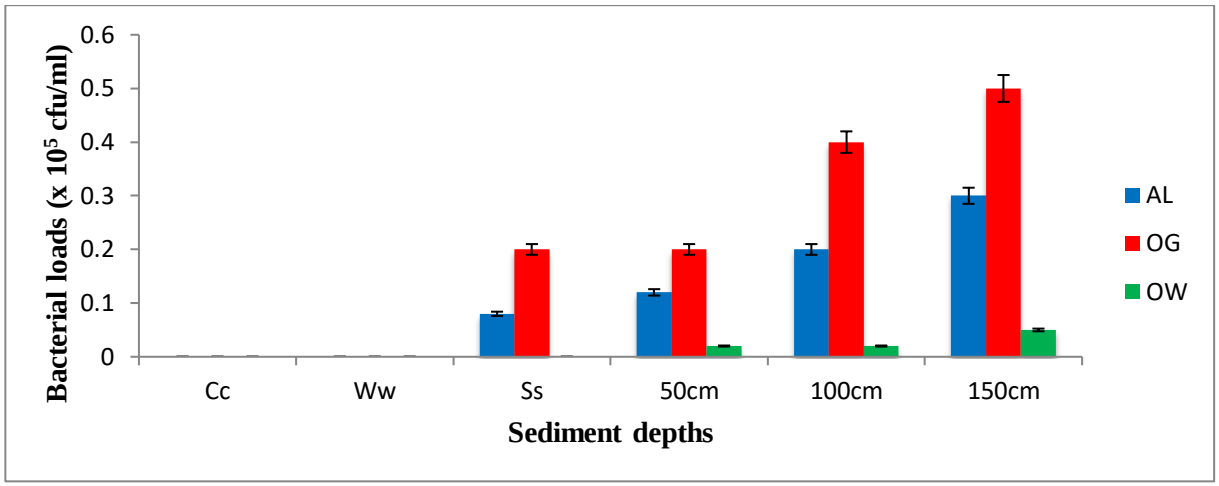

Figure 2: **Anaerobic population of sediment of Rivers Ala, Ogbese and Owena at different depths**

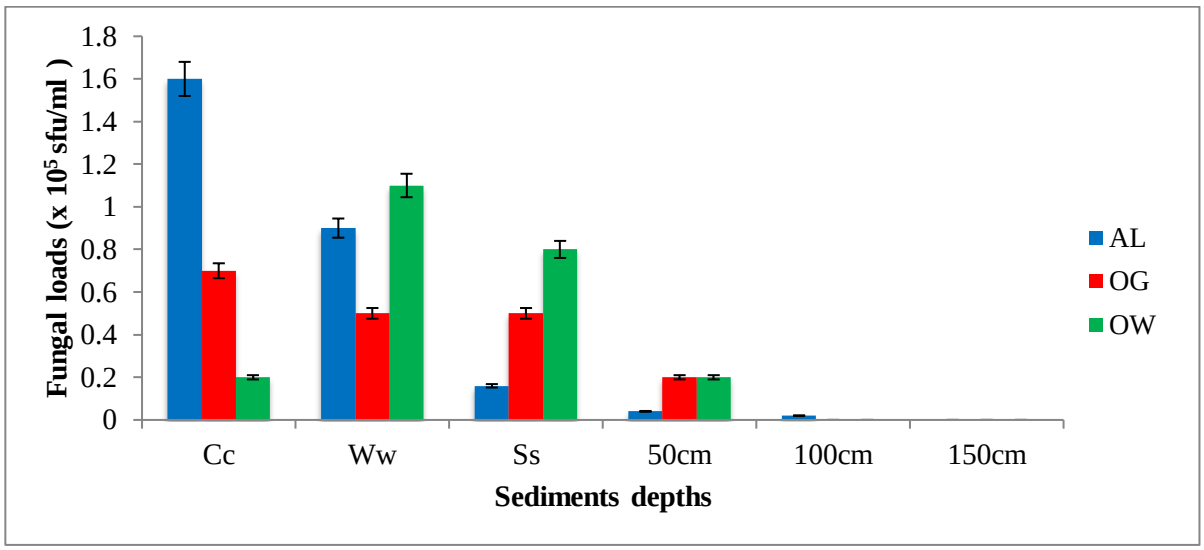

Figure 3: **Fungal population of sediment of Rivers Ala, Ogbese and Owena at different depths**

KEYS: AL- River Ala, OG- River Ogbese, OW-River Owena, Cc- Control, Ww-Surface water Ss- Surface mud, 50cm-Depth 50 cm, 100cm-Depth 100cm and 150cm- Depth 150 cm

Frequency of occurrence of Bacterial and fungal from the three rivers

Table 1 to 6 shows the percentage frequency of occurrence of the bacterial and fungal isolated from sediments of the three rivers, the microorganisms common to all the sediments were *Pseudomonas aeruginosa, Staphylococcus aureus*, *Escherichia coli, Penicillium aeruginosa*, *Aspergillus niger* and *Rhizopus nigricans*. In the bacteria population *Pseudomonas aeruginosa* had the highest percentage frequency of occurrence of 19% in River Ala sediment (Table 1), *Staphylococcus epidermidis* had the highest frequency of 20% for River Ogbese (Table 2) and *Staphylococcus aureus* shows the highest frequency of 20%. Among the fungal population, *Penicillium notatum* was the highest occurring organism with value of 50% for River Ala had (Table 4). River Ogbese shows *Rhizopus nigricans* as the highest occurring organism with the value of 27.3% (Table 5) and River Owena shows *Penicillium notatum* and *Aspergillus niger* as the highest occurring organism with frequency percentage of 25% (Table 6).

Table 1: Frequency of occurrence of bacteria from the sediment of Ala River

IS	ALC	ALW	ALS	AL50	AL100	AL150	TN	PI (%)
Escherichia coli	+	+	-	+	-	-	3	14.3
Staphylococcus aureus	+	+	-	-	+	-	3	14.3
Salmonella cholerasuis	+	+	+	-	-	-	3	14.3
Shigella flexneri	+	-	-	-	-	-	1	4.8
Pseudomonas aeruginosa	-	+	+	+	+	-	4	19
Enterobacter aerogenes	-	+	-	-	-	-	1	4.8
Enterococcus faecalis	-	+	-	-	-	-	1	4.8
Micrococcus luteus	-	-	+	-	-	-	1	4.8
Bacillus megaterium	-	-	-	+	-	-	1	4.8
Klebsiella pneumoniae	-	-	-	+	-	-	1	4.8
Erwinia carotovora	-	-	-	-	-	+	1	4.8
Clostridium tetani	-	-	-	-	-	+	1	4.8
							21	100

Table 2: Frequency of occurrence of bacteria from sediment of Ogbese River

IS	**OGC**	**OGW**	**OGS**	**OG50**	**OG100**	**OG150**	**TN**	**PI (%)**
Bacillus subtilis	+	-	-	+	-	-	2	8
Escherichia coli	+	-	-	+	-	-	2	8
Shigellaflexneri	+	+	-	-	-	-	2	8
Pseudomonas fluorescens	-	+	+	+	-	+	4	16
Salmonella cholerasuis	-	+	+	-	-	-	2	8
Proteus vulgaris	-	+	-	+	-	-	2	8
Streptococcus pyogenes	-	-	-	+	-	-	1	4
Micrococcus luteus	-	-	+	-	+	-	2	8
Staphylococcus epidermidis	-	+	+	+	+	+	5	20
Lactococcuslactis	-	-	+	-	-	-	1	4
Clostridium tetani	-	-	-	-	-	+	1	4
Klebsiellaflexneri	-	+	-	-	-	-	1	4
Escherichia coli							25	100

Table 3: Frequency of occurrence of bacteria from the sediment of Owena River

IS	**OWC**	**OWW**	**OWS**	**OW50**	**OW100**	**OW150**	**TN**	**PI (%)**
Bacillus thuringiensis	+	+	-	+	-	-	3	15
Pseudomonas aeruginosa	+	+	+	-	-	-	3	15
Escherichia coli	-	+	+	+	-	-	3	15
Staphylococcus aureus	-	+	+	-	+	+	4	20
Micrococcus luteus	-	+	+	-	-	+	3	15
Campylobacter jejuni	-	+	-	-	-	-	1	5
Corynebacterium pyogenes	-	+	-	-	-	-	1	5

Salmonella cholerasuis	-	-	-	-	+	-	1	5
Clostridium tetani	-	-	-	-	+	-	1	5
							20	100

Table 4: Frequency of occurrence of fungi from the sediment of Ala River

ALC	ALW	ALS	A50	A100	A150	TN	PI (%)	IS
+	-	+	+	-	-	3	50	*Penicillium notatum*
+	-	-	-	-	-	1	16.7	*Aspergillus niger*
-	-	+	-	-	-	1	16.7	*Trichoderma viride*
-	-	+	-	-	-	1	16.7	*Saccharomyces cerevisae*
						6	100	

Table 5: Frequency of occurrence of fungi from the sediment of Ogbese River

OGC	OGW	OGS	OG50	OG100	OG150	TN	PI (%)	IS
+	+	-	+	-	-	3	27.3	*Rhizopus nigricans*
+	-	-	+	-	-	2	18.2	*Aspergillus niger*
-	+	-	-	-	-	1	9.1	*Mucor mucedo*
-	+	+	-	-	-	2	18.2	*Fusarium Sporotrichoides*
-	-	+	-	-	-	1	9.1	*Cladosporium hebarum*
-	-	+	-	-	-	1	9.1	*Trichoderma viride*
-	+	-	-	-	-	1	9.1	*Penicillium notatum*
						11	100	

Table 6: Frequency of occurrence of fungi from the sediment of Owena River

OWC	OWW	OWS	OW50	OW100	OW150	TN	PI (%)	IS
+	+	-	-	-	-	2	25	*Penicillium notaum*
+	+	-	-	-	-	2	25	*Aspergillus niger*

+	-	-	-	-	-	1	12.5	*Fusarium sporotrichoides*
+	-	-	-	-	-	1	12.5	*Cladosporium hebarum*
-	-	+	-	-	-	1	12.5	*Mucor mucedo*
-	-	+	-	-	-	1	12.5	*Rhizopus nigricans*
						8	100	

Figure 4 shows the pH of the sediments of the three rivers at various depths. The pH all ranged from that ranged between 4.75 to 7.52 (slightly acidic to neutral pH). Figure 5 shows the Organic matter of the sediment at various depths, the organic matter contents were at close range and in minute quantity. River Ogbese sediments had the highest of 5.5%.

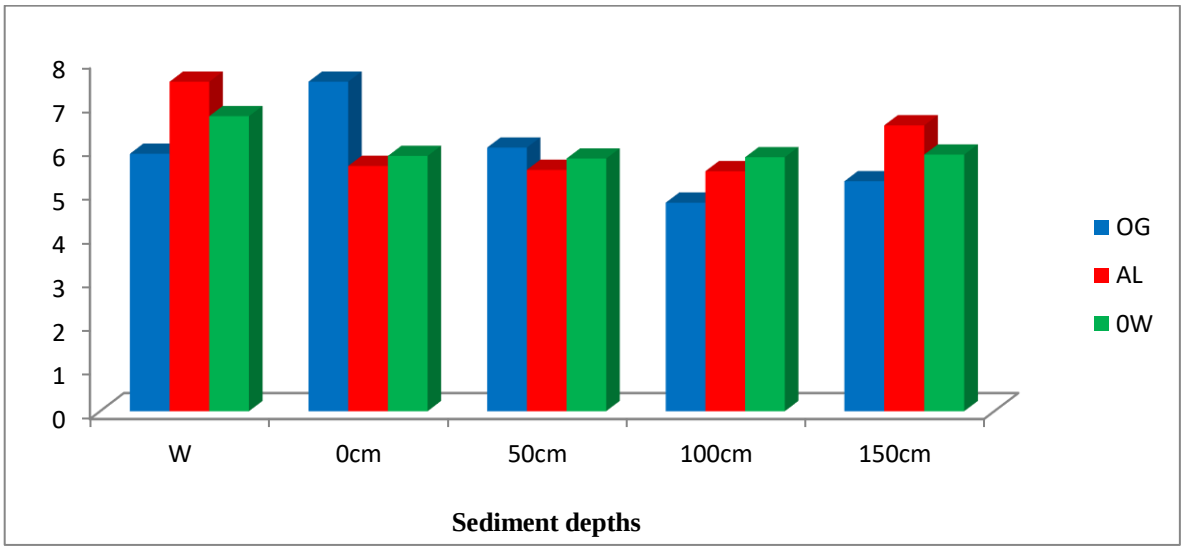

Figure 4: pH content of the sediment of the various Rivers

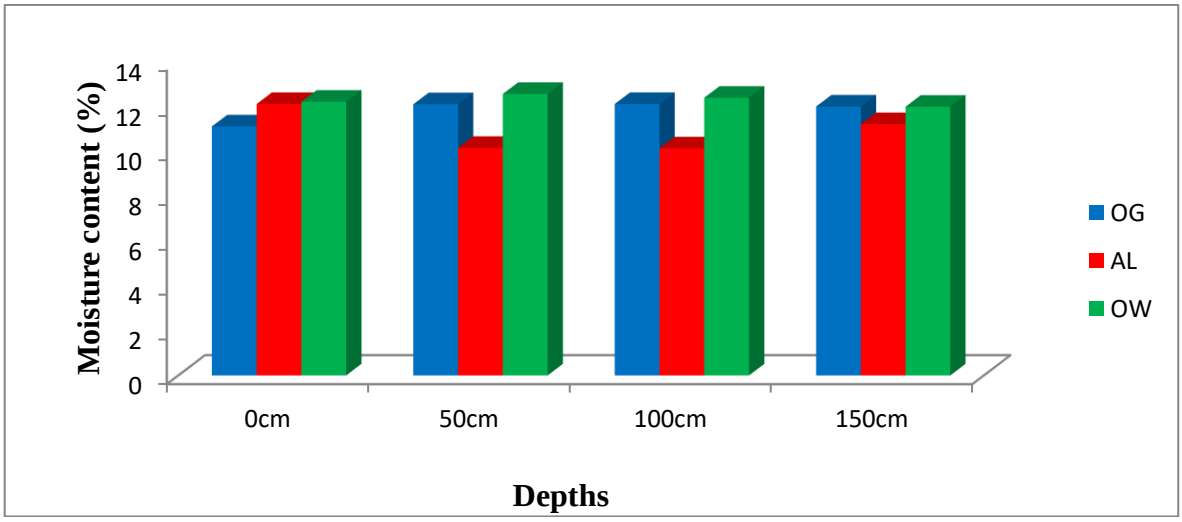

Figure: 5 Moisture content of River Ogbese, Ala and Owena sediment

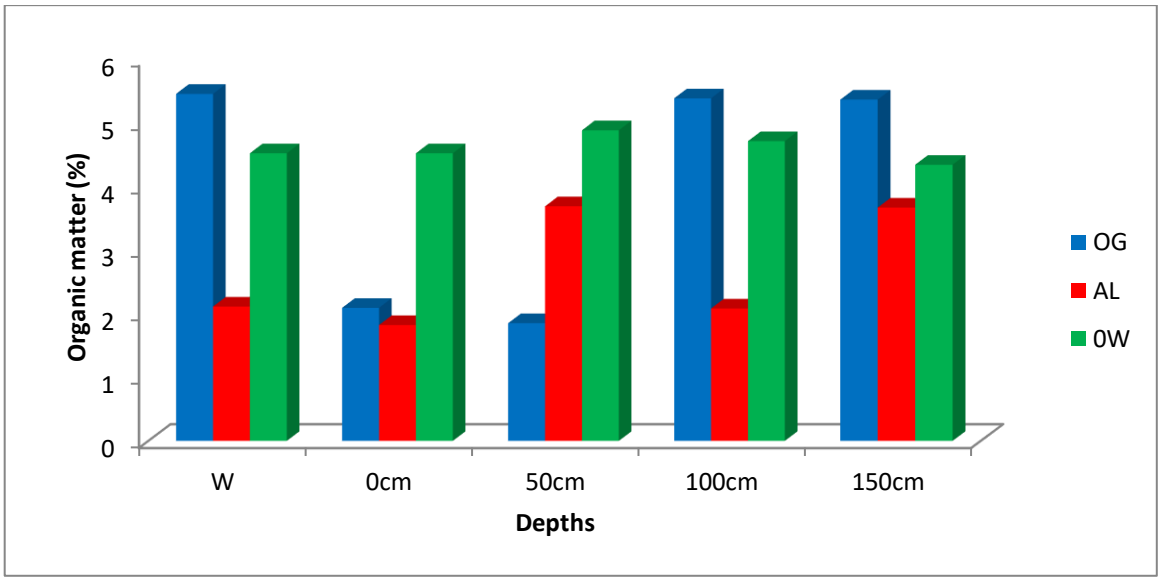

Figure 5: Organic matter content of the sediment of the various Rivers

Figure 6 indicates the Ionization potential of the sediment of River Ala, Ogbese and Owena, in the ionization potentials of the sediments, River Ala at sediment surface, River Ogbese at depth 50 cm and River Owena at its surface water had the highest ionization potential and River Ogbese had the lowest at its surface water. Figure 7 and 8 shows the conductivity and salinity content of River Ala, Ogbese and Owena respectively. The conductivity and salinity of the sediments decreases as the depth increases, River Ogbese and Ala has the highest conductivity at sediment surface to depth 100cm, River Ala had the highest salinity content at the surface water. Figure 9, 10 and 11 shows the Mineral composition of sediment of River Ala, Ogbese and Owena at different depths respectively. The minerals of the sediments at various depths were in minute quantity aside phosphorus content which was high.

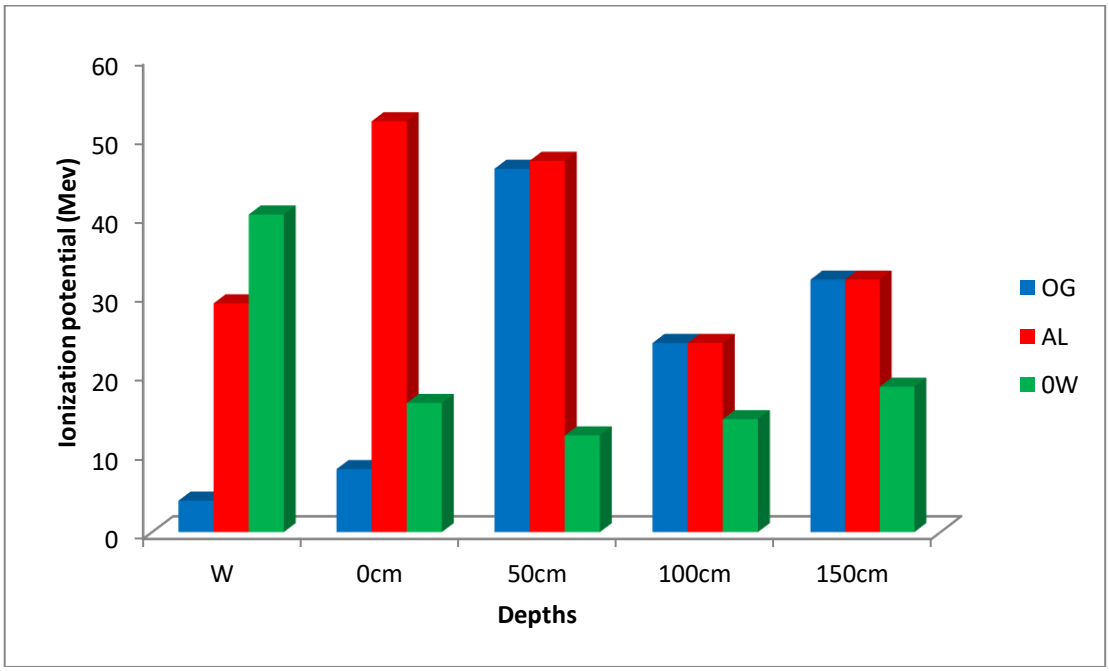

Figure 6: Ionization potential of the sediment of the various Rivers Conductivity of the sediment of River Ala, Ogbese and Owena

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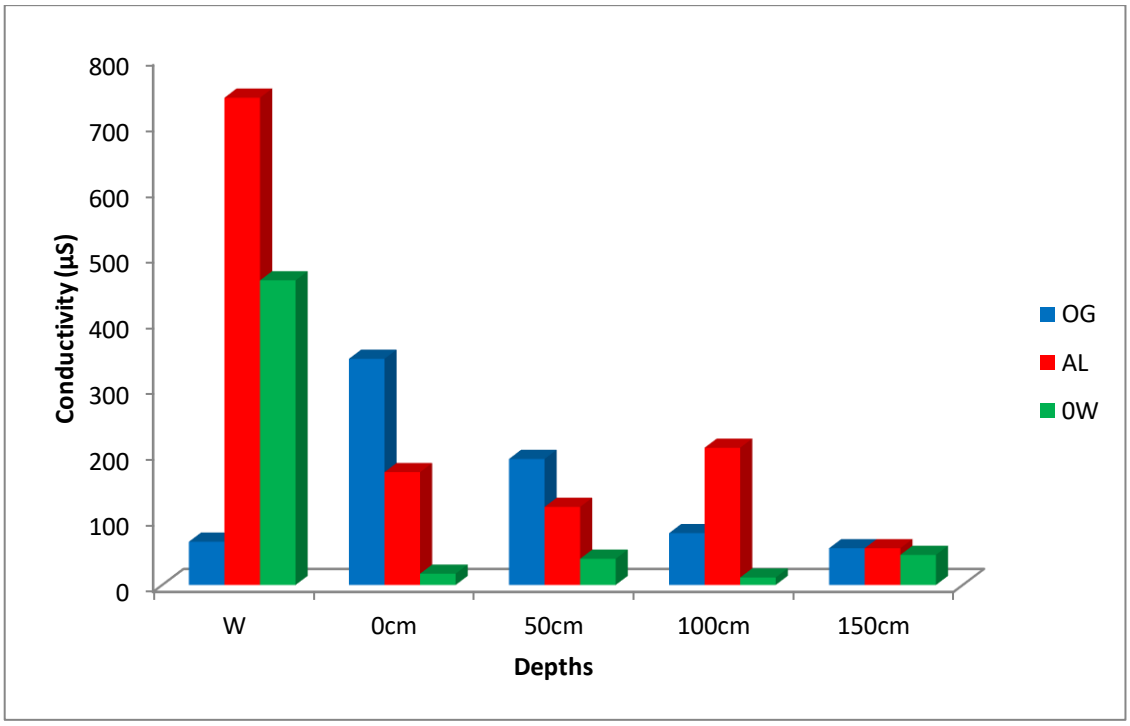

Figure 7: Conductivity of the sediment of the various Rivers

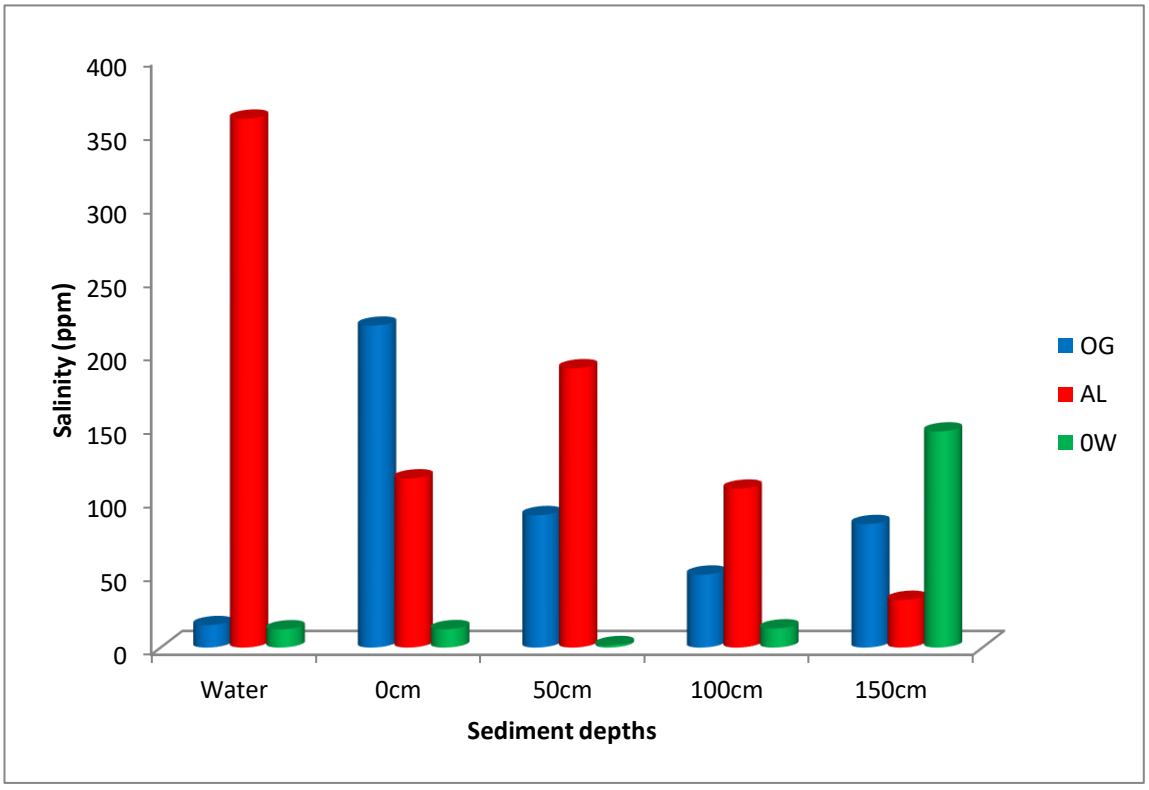

Figure 8: Salinity of the sediment of the various Rivers

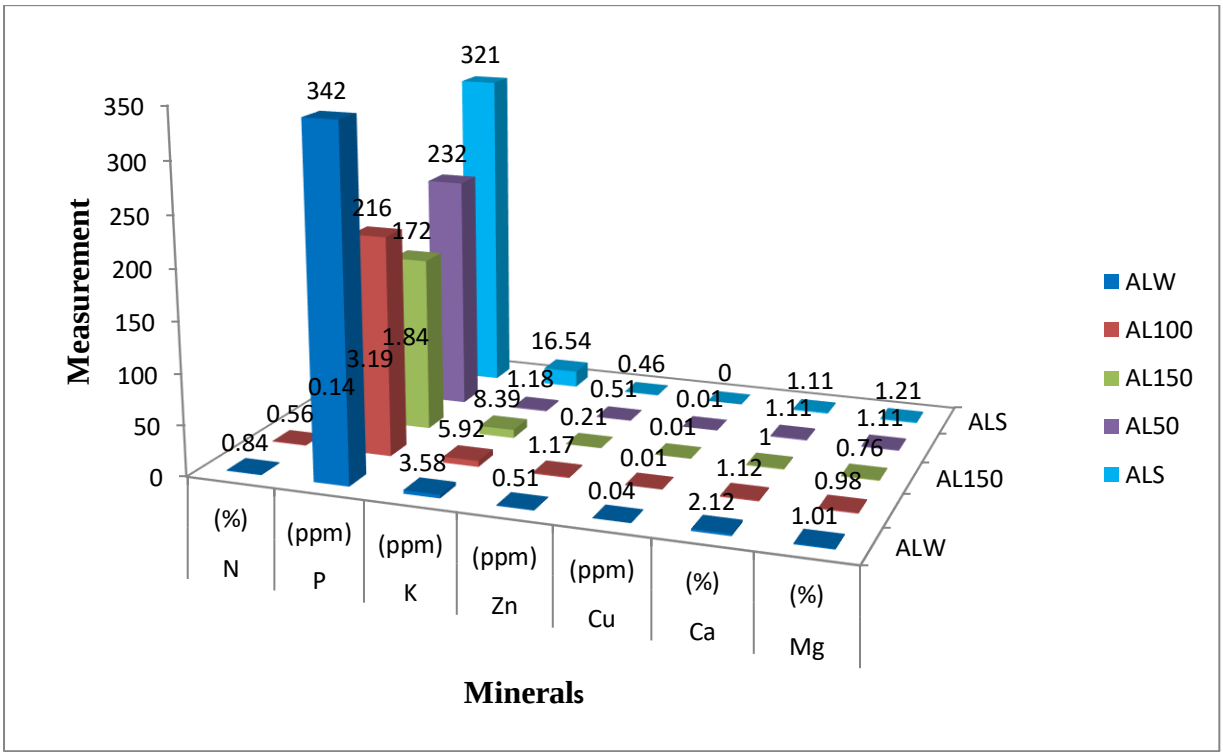

Figure 9: Mineral component of sediment of River Ala at different depths

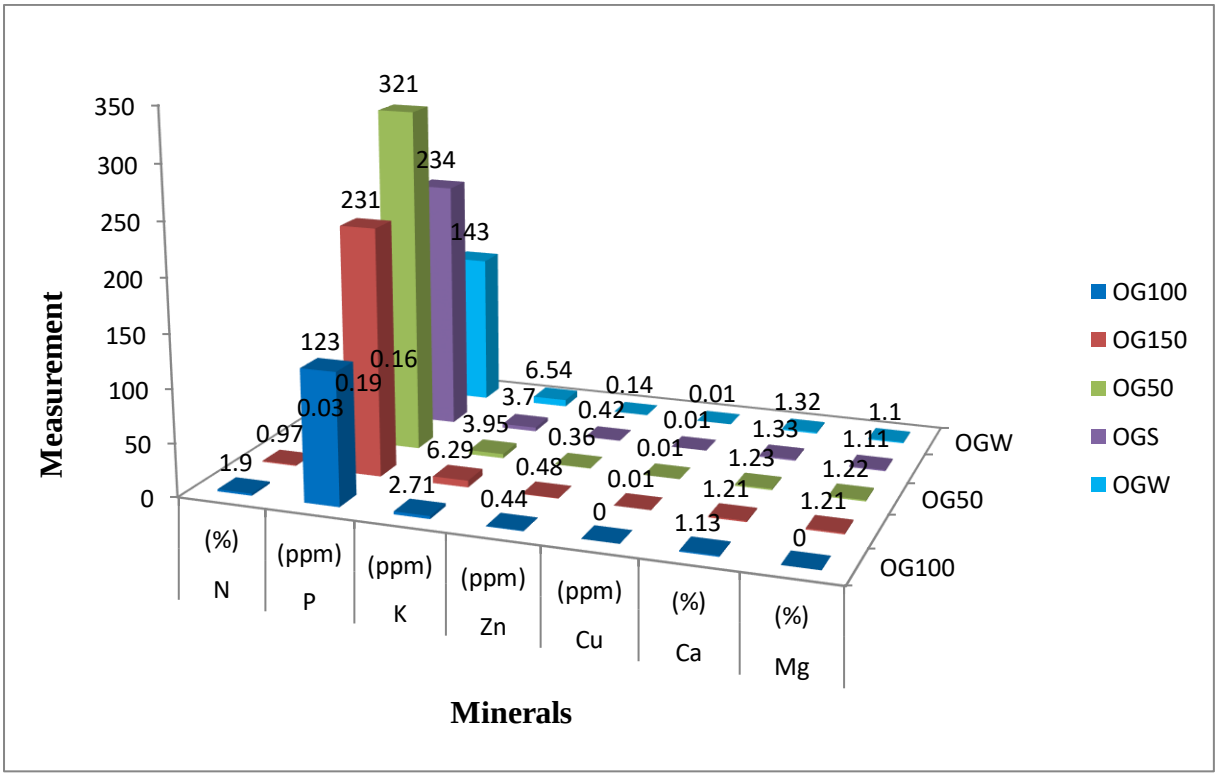

Figure 10: Mineral component of sediment of River Ogbese at different depths

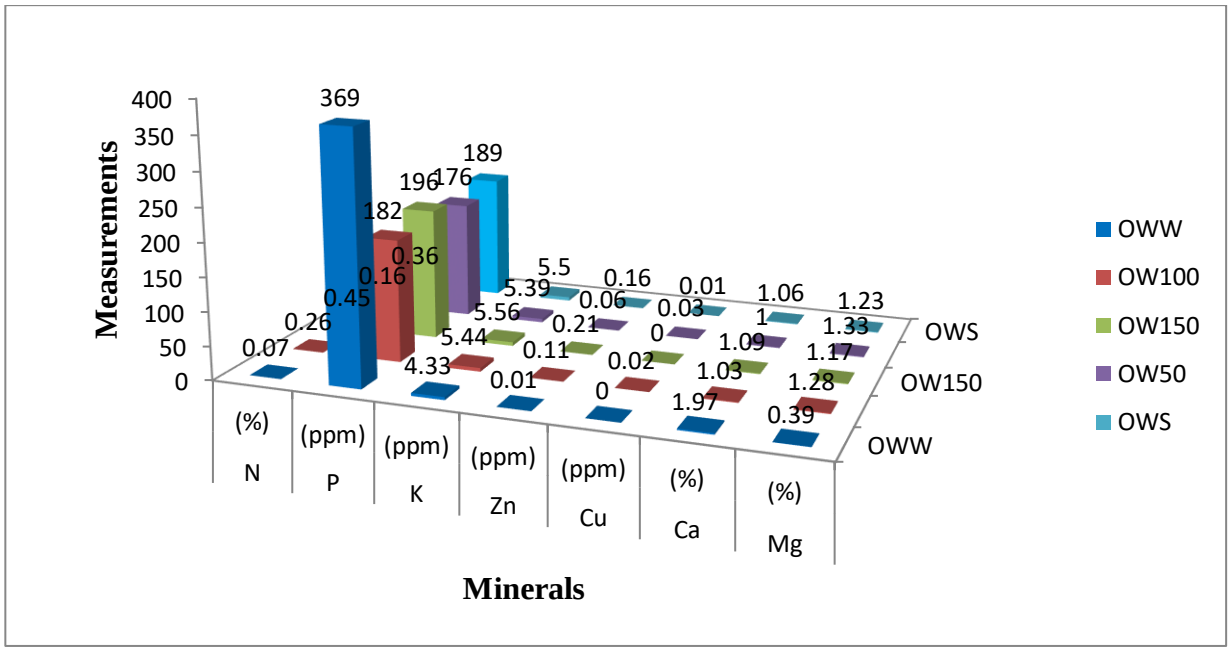

Figure 11: Mineral component of sediment of River Owena at different depths

Keys: ALW, OGW, OWW- Surface water, ALS, OGS, OWS-Sediment surface, AL50, OG50, OW50-50cm depth, AL100, OG100, OW100-100cm depth and AL150, OG150, OW150-100cm depth.

The decrease in the aerobic bacteria population down the depth might be due to oxygen retention that is lower at the lower depths which only permit the growth of only anaerobic organisms. Higher bacteria count observed at the sediment surface and in all depths of River Ogbese sediments showed, that these depths is richer in organic matter to support bacteria growth than other depths and rivers sediments. Fungal growth was not observed at the lower depths of the various river sediments, these findings agrees with findings of [14] who reported that aerobic microbial populations are restricted to zones where oxygen is available and that aerobic organisms become quiescent or die and new inhabitants, largely facultative and obligate anaerobic bacteria take over. He further explains that fungi are active in upland environment and are inhibited in the anaerobic environment which is primarily due to absence of oxygen and alteration in mud pH (acid to neutral) under anaerobic conditions [14] concluded that Bacterial abundance generally decreases with sediment depth independent of the method used, but the steepness of the decline differs which might have accounted for higher population of microorganisms at the upper layers of the sediments. Fungal isolated are lower in array and population to bacteria isolated and they are most found at the upper layer of the sediment which might be due to their aerobic and lower water activities [16]. Hydrogen ion concentration of the sediment of the three rivers at various depths was slightly acidic to neutral pH. pH that is highly acidic or alkaline would kill aquatic life. Aquatic organisms are sensitive to pH changes and biological treatment requires pH control [16]. River Ogbese recorded the highest organic matter content than other river sediments, as correlated with the findings of [17] who assess the organic matter and moisture content of river sediments. Generally, the ionization potential, conductivity and salinity of the sediments at various depths are within the same range which might be due to their organic matter content which is also in close range. River Ala and Owena surface water, River Ogbese sediment surface has the highest conductivity; River Ala sediment surface, River Ogbese at depth 50 cm and River Owena at its surface water has the highest ionization potential and River Owena at depth 150 cm has the highest salinity content. The more ions that are present, the higher the conductivity of water likewise, the fewer ions that are in the water, the less conductive it is and salinity is a strong contributor to conductivity [18]. Mineral composition of the sediment at various depths of the three rivers, generally across the depths of the rivers, River Ogbese has the highest mineral content it had the highest potassium, phosphorus, magnesium and zinc content). All the minerals across the three rivers at its various depths were in minute quantity aside phosphorus content which was very high in all the sediments. Phosphorus is the eleventh most abundant element in the earth's crust and is most associated with rivers that often expose to pollution.

CONCLUSION

River Ala, Ogbese and Owena sediments are associated with arrays of bacteria and fungi owing to its organic and mineral matter content and it's slightly acidic to neutral pH which makes it favourable for the sediments organisms to thrive well.

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