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Effects of global warming on microbiota of the great Kwa river in Cross River State, Nigeria

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Abstract: **The study was aimed at determining the effects of global warming on microbiota of the Great Kwa River in Cross River State. Water and sediment samples were collected from the river at different points and were subjected to standard microbiological and physicochemical protocols. The samples were analyzed for heterotrophic bacterial and fungal counts and physicochemical qualities of the river. The results obtained indicate that the water and sediment samples showed remarkable variations in physicochemical parameters during the wet and dry seasons. Nineteen microbial species with variable distribution and prevalence were isolated comprising of ten (10) bacterial and nine (9) fungal species. This study revealed valuable information about the periodic and seasonal changes in anthropogenic and environmental gradients that occur in the water samples of Great Kwa River.**

Keywords: **Global warming, great Kwa river, microbiota, greenhouse gases.**

1. INTRODUCTION

Global warming is a well – studied phenomenon referring to the current temperature of the earth's atmosphere and oceans and it is responsible for climate – driven habitats change that could influence survival and distribution. It is also referred to human – induced increase in global surface temperature (Roach, 2005).

Global warming can have many different causes, but it is most commonly associated with human interference, specifically the release of excessive amounts of greenhouse gases (EPA, 2010). The major cause of global warming which is the greenhousegases such as Carbon dioxide (CO_2), methane (CH_4), water vapor, Nitrous oxide (N_2O), Ozone (O_3) etc, act like a greenhouse around the earth surface. They trap the heat energy reflected by the earth's surface (Panikov, 2000; Bassey et al., 2016).

The Great Kwa River (also called Ibo River) flows through Cross River State, Nigeria, draining the east side of the city of Calabar. The river originates in the Oban Hills, in the to the Cross River National Park, and flows southwards tidal, with broad mud flats and drain the eastern coast of the city of Calabar.

The Great Kwa River covers a 17-hectare (42 acre) site between the University of Calabar and the Calabar River. The river ecology is under threat from human activity. Calabar municipality has no waste treatment facilities, and heavy rain wash human and industrial waste into the river.

Burning fossil fuels, cutting down forests and developing land for farm which release greenhouse gases into the atmosphere also causes global climate change. As suggested by some pioneering studies, global warming could have detrimental effects on the composition of micro-biota. Consequently global warming may pose a substantial challenge on many natural systems and in particular for micro-biota, living close to their upper critical thermal limits making themparticularly vulnerable to Global Warming (Jochum *et al.*, 2000; Thomas, 2010).

Rivers are very vulnerable to climate change. Some of the changes to the river are decrease in pH, an increase in sea surface temperature, rising sea levels, river acidification and oxygen depletion etc. The historical record clearly indicates that during previous periods of climate change, the distribution of rivers changed dramatically as the balance among

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precipitation, evapotranspiration and runoff shifted (Doney *et al.*, 2006). The main environmental concern about rising sea level is the effects on coastal wetland, if these wetlands are folded, the health of many sensitive ecosystems and unique species could be severely harmed or even destroyed (Wiltshire *et al.*,2008). Many marine species are threatened by the earth's changing climates. Global warming correlate to changes in body mass, growth rate, whereas the effect on immune function and micro-biota composition remained almost unexplored. Hence the need to study the effects this unprecedented warming and changes in the Great Kwa river on microbial species and activities.

2. MATERIALS AND METHODS

The study area and sampling site:

The study area is the Great Kwa River. The river is located in Cross River State in Nigeria. The river originates from the Oban Hills, in the Cross River National Park and flows southwards to the Cross River Estuary. It is located between latitude $4^0 46^0$N and longitude $8^0 23^0$E.

FIGURE 1: Map of Cross River Estuary showing the study area (Great Kwa River)

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Materials:

The materials used for this study includes; autoclave, Petri-dish, bunsen burner, pipettes, conical flask, weighing balance, cotton wool, masking tape, aluminum foil paper, incubator, beakers, distilled water, refrigerator, test tubes, syringes, inoculating loop, L-shape spreader, sterile bottles.

Culture media:

The culture media used for this study include; Nutrient agar, MacConkey agar and Sabouraud dextrose agar.

Reagents:

The reagents used include; hydrogen peroxide, crystal violet, gram iodine, 95% ethanol, Safranin, Kovacs indolereagents.

Methods:

Collection of water sample:

With the aid of sterile bottle, the water samples were collected from five different locationsof the Great Kwa River and then taken to the laboratory for analysis.

Preparation of diluents and media:

Appropriate quantity of the agar powder was weighed as specified by the manufacturer on the label using weighing balance and was pour into a conical flask.

Appropriate quantity of water was added into the conical flask containing the weighed agar powder and were stirred until the powder dissolve completely. The dissolved agar powder was sterilized using an autoclave for 15 minutes and was allowed to cool before dispensing into petri dishes.

Microbiological analysis of water samples:

Ten-fold serial dilution was prepared, 1ml of the water sample was dissolved into 9ml of the solvent and serial dilution was carried out. Using spread plat techniques, aliquots of 0.1ml of dilution 10^{-3} and 10^{-4} of the diluents were pipette into Petri dishes containing 20ml of molten nutrient, MacConkey agar (45^{o}C) and was mixed using the L-shape spreader.

Determination of bacterial load of the samples wasdone in triplicates. Plate were incubated at 37^{0}C for 24hours and was counted after incubation to obtain the total bacteria counts, which were calculated by multiplying the number of colony per plate by the dilution factors and recorded in colony forming unit (CFU) per ml.

Isolation, characterization and identification of microbes:

Pure culture of bacteria were obtained by aseptically streaking representative colonies of different morphology types which appeared on the culture plates onto freshly prepared nutrient agar plates which were incubated at 28^{0}C for 24 hours. Discrete bacterial colonies developed and were sub-cultured on nutrient agar slope, incubated at 28^{0}C for 24 hours. The nutrient agar slope served as pure culture stock for subsequent characterization tests. The pure cultures were identified based on their cultural, physiological and morphological characteristics.

3. RESULTS

Table 1 shows the frequency of occurrence of bacteria species isolated from water sample. Ten (10) species of bacteria identified from the sample included*Escherichia coli, Salmonella sp, Shigellasp, Staphyloccoccusaureus, Pseudomonas sp, Klebsiellasp, Bacillus sp, Enterobactersp, Proteus sp,* and *Micrococcusluteus.* The occurrence of bacterial species from different locations were from samples atriver bank, *Escherichia coli* had the highest frequency of (19.57%) followed by *Salmonella sp* (16.30%) while *Bacillussp* had the lowest frequency of (3.53%), 10 meters away from the river bank, *Escherichia coli* had the highest frequency of (21.88%) followed by *Staphylococcus aureus* (12.15%) while *Bacillus sp* had the lowest frequency of (2.78%). The water samples at 20 meters away from the river bank had *E. coli* with the highest frequency of (22.97%) followed by *Staphylococcus aureus* (12.61%) while *Bacillus sp* had the lowest frequency of (2.70%), from samples taken 30meters away from the river bank had *E. coli* with (25.42%) followed by *Staphylococcus aureus* (11.52%) while *Bacillus sp* had the lowest (2.42%) from samples taken from 40 meters away from the river bankhad *E. coli* with (27.10%) followed by *Salmonella sp* (12.15%) and *Staphylococcus aureus* (12.15%) while *Bacillus sp* had the lowest of (1.87%).

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TABLE 1: DISTRIBUTION AND FREQUENCY OF OCCURRENCE OF BACTERIAL SPECIES ISOLATED FROM WATER SAMPLES

Isolated bacterial species	Frequency of occurrence (%) of bacteria from different points				
	River bank	10m	20m	30m	40m
Escherichia coil	72 (19.57)	63 (21.88)	51 (22.97)	42(25.45)	29(27.10)
Salmonella sp	60 (16.30)	30 (10.42)	21 (9.46)	18(10.91)	13(12.15)
Shigellasp	33(8.97)	31 (10.76)	24 (10.81)	16(9.70)	11(10.28)
Staphyloccus aureus	44(11.96)	35(12.15)	28 (12.61)	19(11.52)	13(12.15)
Pseudomonas sp	35(9.51)	31 (10.76)	22 (9.91)	17(10.30)	9(8.41)
Klebsiellasp	28 (7.61)	23 (7.99)	18 (8.11)	12(7.27)	6(5.61)
Bacillus sp	13 (3.53)	8 (2.78)	6(2.70)	4(2.42)	2(1.87)
Enterobactersp	25 (6.79)	19 (6.60)	15(6.76)	11(6.67)	8(7.48)
Proteus sp	19 (5.16)	14 (4.86)	11(4.95)	9(5.45)	6(5.61)
Micrococcus luterus	39 (10.60)	34 (11.81)	26(11.71)	17(10.30)	10(9.35)
TOTAL	368	288	222	165	107

Table 2 shows the distribution and frequency of occurrence of fungal species isolated from water sample of the great kwa river. Nine (9) fungal species identified from the sample includes; *Aspergillus* sp, *Penicillium* sp, *Mucor* sp, *Fusarium* sp, *Rhizopus* sp, *Trichoderma sp*, *Helminthosporium sp*, *Neurosporas sp and Saccharomyces* sp. Their occurrence are as follows; from the river banks, 10meters 20meters, 30meters and 40meters away *Saccharomyces* sp had the highest frequency of 20.19%, 22.54%, 22.29%, 24.62% and 27.45% followed by *Rhizopus* sp with 16.83%, 19.08%, 19.11%, 20% and 20.59% while *Penicillum* sp were not isolated.

TABLE 2: DISTRIBUTION AND FREQUENCY OF OCCURRENCE OF FUNGI SPECIES IN GREAT KWA RIVER

Isolated fungal species	Frequency of occurrence (%) of fungal species from different points				
	River bank	10m	20m	30m	40m
Aspergillus	10 (4.81)	0 (0)	3 (1.91)	4(3.08)	6(5.88)
Penicillium	0 (0)	0 (0)	0 (0)	0(0)	0(0)
Mucor	22(10.58)	19 (10.98)	17 (10.83)	10(7.69)	6(5.88)
Fusarium	14(6.73)	11(6.36)	11 (7.01)	8(6.15)	5(4.90)
Rhizopus	35(16.83)	33 (19.08)	30 (9.11)	26(20)	21(20.59)
Trichoderma	29 (13.94)	21 (12.14)	19 (12.10)	11(8.46)	8(7.84)
Helminthosporium	31 (14.90)	30(17.34)	22(14.01)	21(16.15)	17(16.67)
Neurospora	25 (12.02)	120(11.56)	20(12.74)	18(13.85)	11(10.78)
Saccharomyces	42 (20.19)	39 (22.54)	35(22.29)	32(24.62)	28(27.45)
Total	208	173	157	130	102

Table 3 shows the effects of global warming on the physicochemical properties of the Great Kwa River. The result revealed that there was a significant ($p<0.05$) difference in pH levels between the wet and dry season with a mean percentage of (6.2±0.02%, 6.3±0.01%) in wet season and (5.2±0.03, 5.6±0.02) in dry season. Meaning that at increase acidity fungal species will thrive in the dry season than bacterial species. And also there are some significant difference ($p<0.05$) between mean percentage, mean level and mean concentration on the physicochemical properties of the Great Kwa River during both seasons.

Table 4 shows the effects of global warming on the distribution of microbial flora in the Great Kwa River. The result revealed that at sediment sample one(1) the mean density of total heterotrophic bacteria increase to ($3.53\pm 0{,}12x10^{7}$ $cfu/_{g}$) and that of total heterotrophic fungi is higher at sediment sample four (4) ($2.90\pm 0.6x10^{6}$ $cfu/_{g}$).

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TABLE 3: EFFECTS OF GLOBAL WARMING ON THE PHYSIOCHEMICAL PROPERTIES OF GREAT KWA RIVER

Parameters	Seasonal changes in Great Kwa River			
	Wet season		Dry season	
	April	May	November	December
pH	$6.2^{a}\pm0.02$	$6.3^{b}\pm0.01$	$5.2^{b}\pm0.03$	$5.6^{a}\pm0.02$
EC	$0.42^{a}\pm0.04$	$0.62^{a}\pm0.03$	$1.3^{c}\pm0.03$	$1.42^{a}\pm0.01$
Organic matter(%)	$5.3^{a}\pm0.21$	$3.3^{b}\pm0.09$	$3.9^{a}\pm0.04$	$3.5^{c}\pm0.03$
Total N(%)	$0.14^{a}\pm0.01$	$0.13^{a}\pm0.02$	$0.14^{a}\pm0.01$	$0.10^{a}\pm0.10$
Av. P (mg/kg)	$1.3^{a}\pm0.20$	$1.2^{c}\pm0.11$	$3.3^{a}\pm0.06$	$2.30^{a}\pm0.10$
Calcium (Ca)	$6.12^{b}\pm0.08$	$6.30^{c}\pm0.10$	$6.9^{b}\pm0.12$	$6.70^{a}\pm0.09$
Magnesium (Mg)	$2.3^{b}\pm0.03$	$2.45^{b}\pm0.06$	$2.6^{b}\pm0.04$	$2.83^{a}\pm0.05$
Potassium (K)	$0.23^{a}\pm0.03$	$0.91^{c}\pm0.06$	$0.46^{c}\pm0.02$	$0.55^{b}\pm0.04$
Sodium (Na)	$0.76^{a}\pm0.06$	$0.56^{a}\pm0.04$	$0.33^{a}\pm0.06$	$1.23^{a}\pm0.08$
EA ($cmolk^{-1}$)	$3.03^{a}\pm0.02$	$30.06^{a}\pm0.05$	$2.4^{a}\pm0.05$	$2.7^{a}\pm0.03$
ECEC (cmol/kg)	$12.37^{c}\pm0.02$	$13.31^{b}\pm0.34$	$12.52^{c}\pm0.08$	$14.23^{c}\pm0.03$
B.Saturation (%)	$75.51^{a}\pm0.02$	$76.15^{a}\pm0.42$	$80.17^{a}\pm0.30$	$81.07^{a}\pm0.55$
Sand	$40.6^{b}\pm0.02$	$40^{b}\pm0.18$	$65^{a}\pm0.62$	$64^{a}\pm0.32$
Silt	$37^{a}\pm0.39$	$35^{a}\pm0.08$	$27^{b}\pm0.13$	$23.4^{c}\pm0.23$
Clay	$22.4^{a}\pm0.08$	$24.3^{a}\pm0.23$	$8.3^{c}\pm0.03$	$16.5^{b}\pm0.07$

TABLE 4: EFFECTS OF GLOBAL WARMING ON MICROBIAL FLORA OF GREAT KWA RIVER SEDIMENTS

SOURCE OF SAMPLES AND SAMPLING LOCATION

	Ps	SS1	SS2	SS3	SS4
THB	$3.25^{b}\pm0.16x10^{6}$	$3.53^{a}\pm0.12x10^{7}$	$3.1^{a}\pm0.57x10^{6}$	$3.40^{a}\pm0.33x10^{7}$	$3.3^{a}\pm0.04x10^{7}$
THF	$2.34^{b}\pm1.52x10^{5}$	$2.53^{b}\pm0.53x10^{6}$	$2.70^{b}\pm0.24x10^{6}$	$2.30^{a}\pm0.61x10^{6}$	$2.90^{a}\pm0.06x10^{6}$

Means with same superscript along the horizontal arrays represent no significant difference (P>0.05)

Legend: THB= total heterotrophic bacteria, THF = total heterotrophic fungi, Ps= pristine sample, SSn – sediment samples.

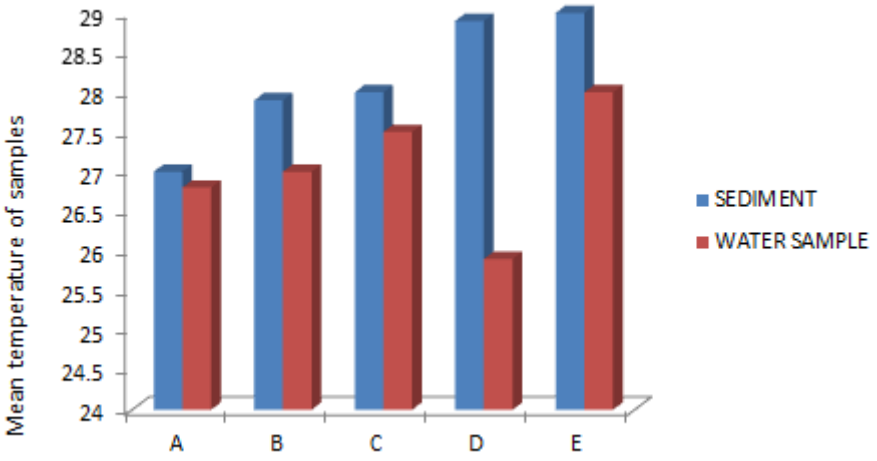

FIGURE 2: Fluctuation in temperature (^{0}C) levels in sediments and water samples of Great Kwa River

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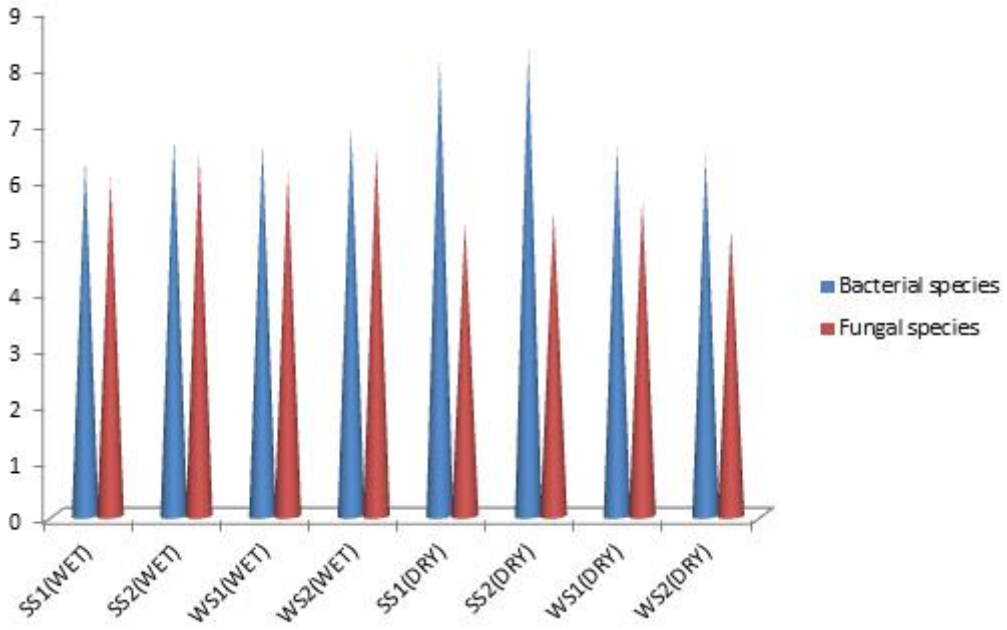

Legend: SS_n (wet/Dry)=sediment samples for wet season, WS_n (Wet/Dry)=water samples for dry season

FIGURE 3: Seasonal effects of Great Kwa River Acidification on Bacterial and Fungal species growth

4. DISCUSSION

In this research, the result showed that the season of study contributed greatly to the microbial proliferation. Significantly ($P<0.05$) higher counts were observed during the dry season Bassey *et al*., 2015, Bassey *et al*., 2016. This conformed to a similar study carried out by Unimke *et al.*, (2014).The increase in microbial density of heterotrophic microorganisms during the dry season can be linked with the slight increase in the temperature of the ecosystem during the research period. The slightly lower population of heterotrophic microorganisms during the wet season may be due to changes in biological oxygen (DO) level, temperature and salinity. Microbial loads were significantly high ($P<0.05$), but varied with sampling points. Similar patterns were observed in the study site and the pristine site. It was noted that inflow of the seawater provide high levels of nutrients in both the water column and sediments. The low microbial populations observed in Great Kwa River may be due to low levels of nutrients and productivity in the river environment.

In Great Kwa River (prisitine site), low densities of heterotrophic bacteria were observed. The results further showed that dry season had a significantly ($P<0.05$) higher counts of total heterotrophic bacteria (THB) than the wet season. This is in agreement with the report of Bassey *et al*., (2015).

Ten (10) bacterial species and nine (9) fungal species were isolated, characterized and identified. The bacterial species were *Escherichia coli, Samlonella sp, Shigella sp, Staphylococcus aureus, Pseudomonas sp, Klebsiella sp, Bacillus sp, Enterobacter sp, Proteus sp, Micrococcus luteus.*The fungal species isolated were *Aspergillus sp, Penicilium sp, Mucor sp, Fusariumsp, Rhizopus sp, Tr*ichoderma *sp, Helminthosporium sp, Neuropora sp,* and *Saccharomyces sp*. From the physicochemical properties, at pH level there is increase acidity during the dry season i.e (5.2± 0.03%, 5.6±0.026) than in wet season (6.2±0.02%, 6.3±0.0%). The observed pH levels are usual for unpolluted tropical rivers (Prati *et al*., 1974). The acidic nature of the pH values is attributable to geological and biochemical factors within the river catchment. The electrical conductivity during the dry season had a significantly ($p<0.05$) higher mean percentage (1.3±0.3%, 1.42±01%) than wet season (0.42±0.4%, 0.62±0.03%). The result of organic matter revealed that there was a significantly ($P<0.05$) higher mean percentage of (5.3±021%, 3.3±0.09%) during wet season than dry season 3.9±0.04%, 3.5±0.03%). The concentration of total nitrogen in the sample were quite low and similar, which revealed that there was no significant difference ($P>0.05$) in the mean level of total nitrogen in both season (0.14±0.01%, 0.13±0.02%) and (0.14±0.01%, 0.10±0.10%) during the wet and dry seasons respectively. The levels of available phosphorus were significantly ($P<0.05$) higher in dry season (3.3±0.06mg/kg, 2.3±0.10mg/kg) than wet season (1.3±0.20mg/kg,.2±0.11mg/kg). The concentration

of exchangeable bases (calcium, magnesium, potassium and Sodium) the mean concentration during the wet season were (6.12±0.08cmol/kg, 6.30±0.10cmol/kg) Ca, (2.3±0.03cmol/kg, 2.45±0.06cmol/kg) Mg, (0.23±0.03cmol/kg,0.19±0.06cmol/kg) K, and (0.76±0.06cmol/kg, 0.56±0.04cmol/kg) Na. during the dry season, the mean concentration were (6.9±0.12cmol/kg, 6.70±0.09cmol/kg) Ca,(2.6±0.04cmol/kg, 2.83±0.05cmol/kg) Mg, (0.46±0.02cmol/kg, 0.55±0.04cmol/kg) K, and (0.33±0.06cmolkg^{-1}, 1.23±0.08cmolkg^{-1}) Na.

The mean concentration of effective cation exchange capacity (ECEC) was (12.37±0.02cmol/kg, 13.31±0.34cmol/kg) during the wet season and (12.52±0.08cmol/kg, 14.23±0.03cmol/kg) during the dry season. From the result the mean percentage level of base saturation were (75.51±0.02%, 76.15±0.42%) during wet season and (80.17±0.30%, 81.07±0.55%) during dry season, which means that there is no significant difference ($p>0.05$) between both seasons. Particle size (sand, silt and clay) the mean values during wet season were (40.6±0.02%, 40±0.18%) sand, (37±0.39%, 35±0.08%) silt, (22.4±0.08%, 24.3±0.23%) clay and (65±0.62%, 64±0.32%) sand, (27±0.13%, 23.4±0.23%) silt, (8.3±0.03%, 16.5±0.07%) clay during dry season. However, the mean level observed during wet and dry season for sand was higher, and the textural class was sandy-loam.The observed seasonal variation is directly attributed to the climate of the study area which is usually characterized by a hot dry season and cold wet season (Akpan, 1999) From the result, the concentrations of exchangeable bases (Ca, Mg, K and Na) indicates their important as sources of micronutrient which ensure optimal primary and secondary productivity of the marine and brackish ecosystem. From the effects of global warming on microbial flora of the great kwa river, the result revealed that there is no significant different for the mean density of total heterotrophic bacteria and that of total heterotrophic fungi from the pristine sample, sediment sample 3 and 4 but there is significant difference in sediment sample 1 and 2.

From the frequency of occurrence of bacterial isolates from different locations *Escherichia coli* had the highest of frequencies of (19.57%) River bank, (21.88%) 10 meters, (22.97%)20meters, (25.45%)30meters and (27.10%)40meters and *Bacillus sp* had the lowest frequenciesof (3.53%) river bank, (2.78%) 10meters, (2.70%)20 meters, (2.42%)30meters, (1.87%)40meters. For the fungal species *Saccharomyces sp* had the highest frequencies of (20.19%) river banks, (22.54%) 10meters, (22.29%)20meters,(24.62%)30meters, and (27.45%)40meters but *Penicilliumspecies* were absent.

5. CONCLUSION

From the results obtained, it is pertinent to concluded that the higher frequencies of some microbial species is linked to the fact that Great Kwa River has some level of pollution due to anthropogenic origin. The low microbial population observed is due to low level of nutrient and productivity in the river environment leading to a structural shift in planktonic community resulting in the dominance of a few stress tolerant species. The lack of consistency in microbial community taxonomic response to warming current filed studies due to many confounding factors, not limited to edaphic and methodological variables. The consequences of human impacts on aquatic ecosystem activities have been far reaching, multiple stresses affect aquatic ecosystems globally thereby affecting physical, chemical andecological water status causing changes in species composition, ecosystem structure, function and biodiversity.

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