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BIOCHEMICAL AND PHYSICO-CHEMICAL CHARACTERIZATION OF MAJIDUN RIVER WATER, SOUTH-WEST, NIGERIA

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ABSTRACTS: *Biochemical and physico-chemical characteristics of Majidun River were studied to investigate its microbial composition in relation to bacteria load and physico-chemical parameters. Water samples were weekly collected (October, 2013 – January, 2014) for analysis on total bacterial count, biochemical test and physico-chemical parameters following standard procedures. Data obtained were subjected to analysis of variance to find out the significant difference of the total microbial load and chemical contaminants in the water body. Total colony forming unit (Cfu) of bacterial in the river was highest in descending order CA > MCA > PCA > MSA and ascending order as MCA < MSA < CA < PCA at the first and second serial dilution respectively. Biochemical test showed the presence of Staphylococcus species, Escherichia coli, Pseudomonas aeruginosa, Proteus species, Salmonella species and Baccillus species in the river. Physico-chemical parameters mean concentrations was recorded for pH (6.200 ± 0.12 - 6.948 ± 0.08), total alkalinity (9.300 ± 0.03 - 9.352 ± 0.04), TDS (2647.000 ± 12.81 - 2666.000 ± 4.30), salinity (2073.000 ± 22.23 - 2117.000 ± 27.09), conductivity (3.712 ± 0.03 - 3.734 ± 0.03), COD (98.774 ± 0.03 - 98.816 ± 0.03), BOD (25.616 ± 0.04 - 25.634 ± 0.02), DO (7.056 ± 0.01 - 7.098 ± 0.01), chloride (4250.000 ± 1.58 - 4252.000 ± 4.04), total hardness (344.000 ± 1.05 - 347.000 ± 3.58), phosphate (11.902 ± 0.03 - 11.942 ± 0.02), sulphate (6.204 ± 0.01 - 6.248 ± 0.02) and nitrate (8.614 ± 0.01 - 8.656 ± 0.03). There were great variabilities in physico-chemical parameters and microbial load in Majidun River as a result of different human activities around the river water which might makes it to be unsafe for human consumption if not adequately purified.*

KEYWORDS: Microbial Load, Aquatic Characterization, Coliform, Agar, Salinity

INTRODUCTION

In the last decades, aquatic contamination has become a global problem which enters aquatic body from different natural and anthropogenic sources which can either be chemical or microbiological. Polluted surface waters can contain large variety of pathogenic microorganisms including bacteria, viruses and protozoa. The main origin of these pathogenic microorganisms is the faeces of human and animals which are brought to the aquatic environments through the release of wastewater effluents, surface runoff and soil leaching.

Human sanitary risk linked to the presence of these pathogens depends on the use of the water (drinking, bathing, irrigation, fishing, transportation, recreational activities) and on the pathogen concentration in water. In aquatic systems, enumeration of pathogenic microorganisms is potentially impossible due to the large diversity of the pathogens, the low abundance of each species and the absence of standardized low-cost methods for the detection of each of them. Thus, faecal indicator bacteria (FIB) are usually enumerated to evaluate the level of microbial water contamination.

The abundance of this FIB is supposed to be correlated with the density of pathogenic microorganisms from faecal origin and is thus an indication of the sanitary risk associated with the various water utilizations. For more than a century, total coliforms and faecal coliforms were main organisms used as bacterial indicators. Nowadays, *Escherichia coli* and intestinal entero- cocci (IE) are the most frequently used indicators of faecal pollution as it was demonstrated by epidemiological studies that they were better indicators of the human risk associated with waters than coliforms (Fewtrell and Bartram, 2001).

Many of these micro-organisms give rise to ugly situations that can affect taste, odour and drinking quality. This makes the drinking water treatment necessary to public water supplies according to the requirements of the supply, nature and vulnerability of the sources which broadly comprise coagulation, flocculation, filtration and oxidation system. Residual measurements that can act as a bio-marker to show disinfection and preservation in water distribution using the most common oxidative disinfectant (chlorine) to provide effective and robust barrier to pathogens (Farombi *et al.,* 2007).

Ground and surface water such as rivers and reservoirs contain natural contaminants, particularly inorganic contaminants that arise from the geological strata through which the water flows and to a varying extent, anthropogenic pollution by both microorganisms and chemicals (Storelli *et al.,* 2005). The run-off or leaching of nutrients into flowing or still surface waters can result in excessive growth of cyanobacteria or blue-green algae which makes surface water to be more vulnerable to pollution than groundwater (Gupta *et al*., 2009).

Since Majidun river is useful to the community and its environments ranging from swimming, transportation, fishing, domestic use, recreational, sand digging and many more. This study was aimed to assess the microbial constituents, water characteristics using physico-chemical parameter and identify the bacterial compositions of Majidun River using biochemical tests in addition to agar isolation. This will also enlighten the inhabitant and general public on the toxic level of the river on human consumption or general usage.

MATERIALS AND METHODS

Study Area

This study was carried-out on Majidun river water body which was located in Ikorodu, a city and Local Government Areas in Lagos State along Lagos Lagoon that share boundary with Ogun State (Figure 1). The river was located on latitude 6°36′N 3°30′E and longitude 6.600°N 3.500°E is a major resource of the local government with wide front at Lagos Lagoon, numerous streams and distributaries (Figure 1). It is also known as Majidun Ilaje creek because the major inhabitants are mainly from Ilaje, Ondo State (Ayejuyo *et al.,* 2003). This definitely pose a lot of potentials for development with vast area of uncultivated land while the industrial situation is yet to be fully exploited and if all the resources were properly use, it will grow not only in size but also in importance (Balogun, 1991).

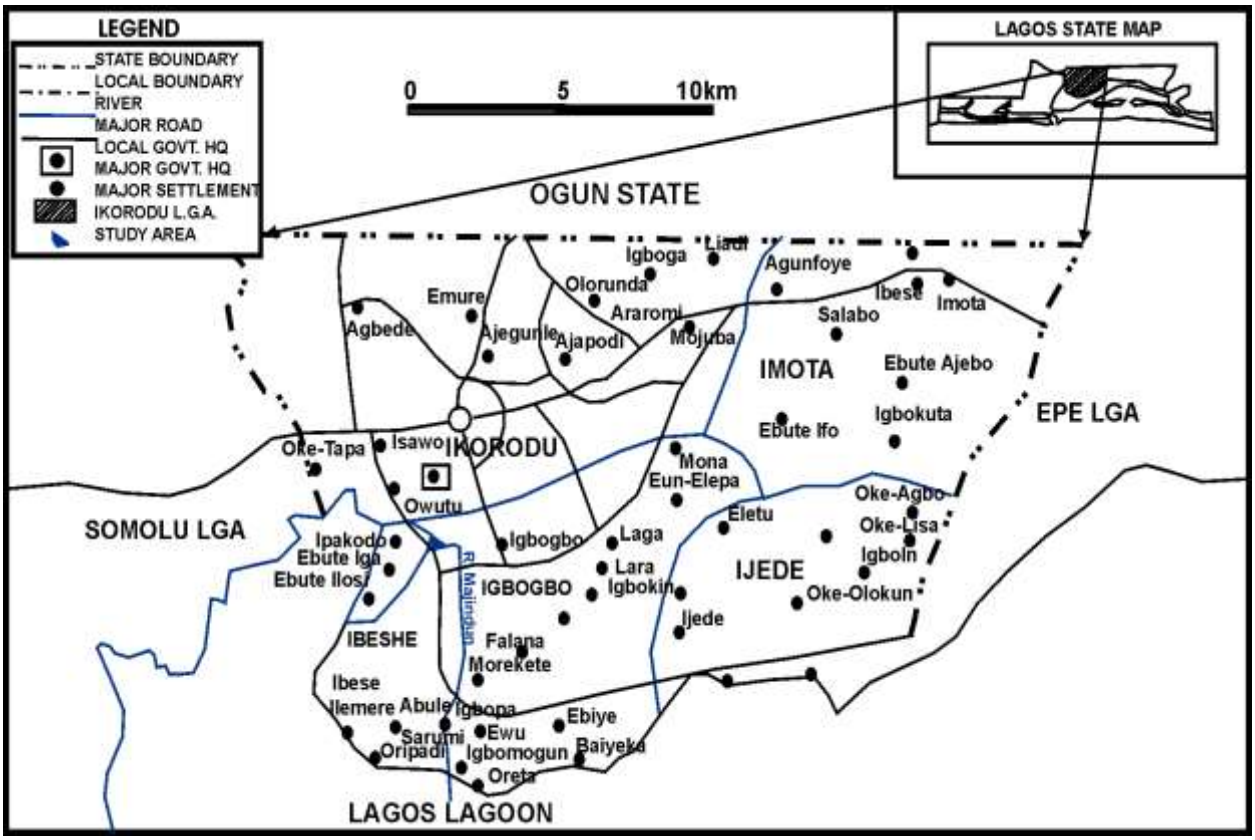

Figure 1: Political Map of Ikorodu, Lagos State Showing the Study Area

Biochemical Analysis

Five agar media {Mannitol Salt Agar (MSA), Centrimide Agar (CA), Mac-Conkey Agar (MCA), Eosin Methylene Blue Agar (EMB) and Plate Count Agar (PCA)} were used for microorganism isolation and prepared according to the manufacturer's instructions and standard laboratory procedure. 50g powder of anhydrous Agar was dissolved in 1 litre of distilled water, then sterilized in an autoclave at 121^0C for 15 minutes, allowed to cool to about 45^0C, EMB and PCA were left to cool to 45˚C by using water bath before pouring into Petri dishes.

100µl of the water sample was introduced into each of the 10 sterile eppendorf tube arranged on tube rack containing 900µl of distilled water and mixed properly using the vortex mixer. Then, 100µl of the diluted sample from each tube was transferred into another tube to repeat the above procedure until tenfold serial dilution was completed. 100µl of diluted sample of dilutions 10^{-1} ml and 10^{-2} ml was dispensed into sterile Petri dishes, cooled to 45^0C and the media were dispensed into each of the inoculated plate. The plates were swirled clockwise and anticlockwise for homogenization. The plates were incubated at 37^0C for 24hrs in an incubator and the colony forming unit in each plate was counted, recorded and calculated (Heath, 1991)

$$Cfu/ml = \frac{Number\ of\ colony \times dilution}{Volume\ inoculated}$$

Key:

Cfu = Colony Forming Unit
ml = Milliliters

All glass wares (petri dishes, test tubes, universal bottles, beakers, conical flasks, MacCartney bottles, media bottles) were sterilized in an hot air oven at 160^0C for 1 hour before use. The media (Mac-Conkey agar (Oxoid®), Plate Count Agar (Oxoid®), Mannitol Salt Agar, Centrimide Agar, Eosin Methylene Blue Agar, Simmon's citrate medium, Sulphide Indole Molitity Medium (SIM) and test reagent, Urea agar (Christensen's medium) and sensitivity testing agar (Oxoid®) and others) were sterilized by moist heat under pressure in an autoclave at 121^0C for 15minutes.

Each growth on this agar was later subjected to biochemical test (Table 2) such as Glucose, Lactose, Sucrose, Citrase, Gelatinase, Indole, Methyl Red, Urease, Catalase, Oxidase, Motility test and Gram Staining Reaction to identify likely microbes therein as described by Antharam *et al.* (2013).

Physico-chemical Analysis

Water samples were collected from the study area (Majidun River, Ikorodu, Lagos) on weekly basis for a period of fifteen (15) weeks between October 2013 and January 2014. The water samples were collected in a covered water tight lid plastic keg which had been detergent washed, rinsed with dilute HNO_3, double de-ionized distilled water and the sample water with caps three times prior to collection. The container was immersed into the river to about 15 cm below its surface as care was taken as the container was appropriately labeled.

Method employed by Gregg, 1989 as reported by USEPA, (2007) was adopted for the collection of all the water samples. Standard procedure for each physico-chemical procedure as described by APHA, (1998) was followed and appropriate instruments were used as for pH: pH meter (LaMotte tracer), total alkalinity: titrimetric method, electrical conductivity: LaMotte trace pocket ester meter, chemical oxygen demand (COD): titration method, biochemical oxygen demand (BOD): titration method, dissolved oxygen (DO): Winkler's method, chloride: titration method, total hardness: titration method, phosphate: Phosphate Spectronic (20D), sulphate: turbidimetric method and nitrate: calorimetric method.

Statistical Analysis

The data obtained were subjected to statistical analysis using Statistical Package for Social Sciences (SPSS) version 16.0. This include descriptive statistics and one-way analysis of variance (ANOVA) to find out the significant difference in the concentrations of physico-chemical contaminants and the metals in the water body as described by Ayejuyo *et al*. (2003). Variations were determined using DMRT of variance due to sampling errors and mean values were also separated which were accepted being significantly different if $p < 0.05$.

RESULTS AND DISCUSSION

Biochemical Characterization

The total bacterial count in Majidun River at the first serial dilution (Table 1) makes the highest number of total colony forming unit (Cfu) count except in PCA which is general microbial detection agar (Fig. 2). Total colony forming unit (Cfu) of bacterial in the river was highest in descending order as CA > MCA > PCA > MSA for the first serial dilution which is

the highest dilution (10^{-1}) and the second serial dilution (Table) also present a maximum bacterial growth in ascending order as MCA < MSA < CA < PCA (Fig. 2).

The biochemical tests on sub-culture of MSA off-white, MSA H_2O 2, MSA H_2O 3 and those that showed positive to catalase test (Table 2) indicate the presence of *Staphylococcus* species as set-up by Barrow and Feltman, (1993) and described by Antharam *et al.* (2013). *Staphylococcus* species are small component of soil microbial flora (Madigan and Martinko, 2005) that are round (cocci) in shape, grape-like clusters, gram-positive bacteria and produce catalase frequently (Ryan and Ray, 2004). *Staphylococcus* can they can cause a wide variety of diseases in humans and animals through either toxin production or penetration as they colonize the skin and upper respiratory tracts of mammals and birds (Chan *et. al*., 2011).

Escherichia coli is a gram-negative, facultative anaerobic, rod-shaped bacterium of the genus Escherichia that is commonly found in water and lower intestine of warm-blooded organisms (Singleton, 1999). Antharam *et al.* (2013) indicated positive indole test on EMB greenish and EMB greenish Metallic River water sub-cultured (Table 2) as the presence of *Escherichia coli*. Though, most E. coli strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination (Vogt and Dippold, 2005).

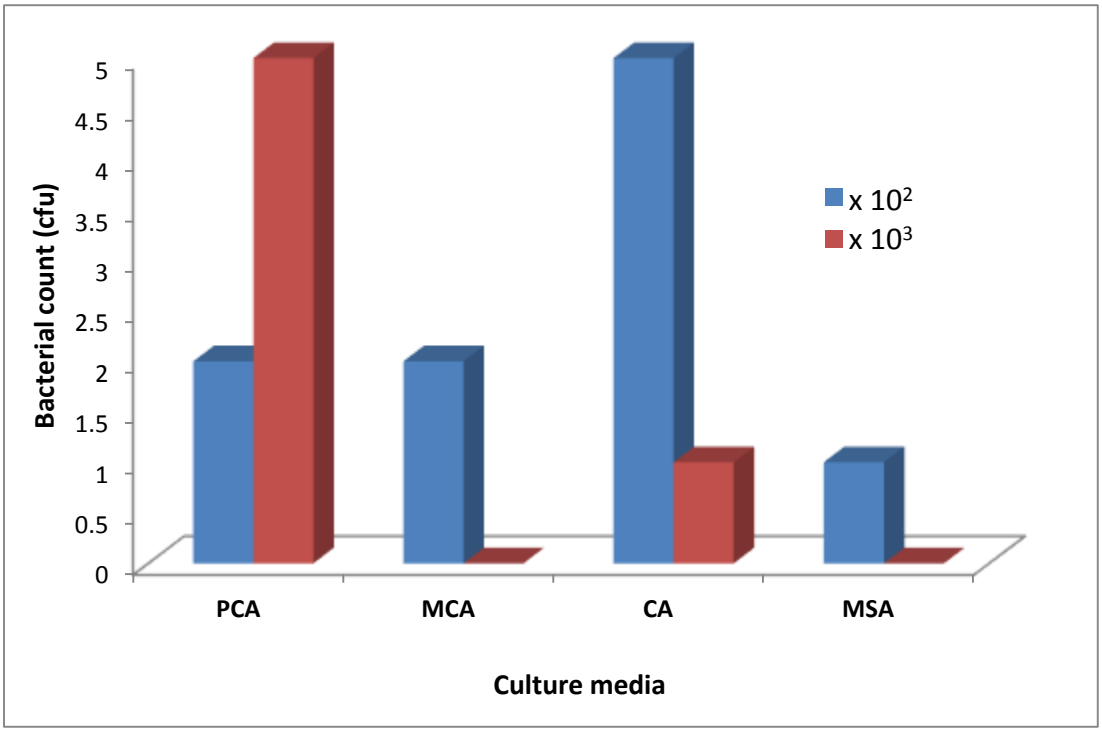

Figure 2: Total Bacterial Count in Majidun River Water.

Key; PCA - Plate Count Agar; MCA - Mac-Conkey Agar; CA - Centrimide Agar; MSA – Mannitol Salt Agar

Table 1: Serial Dilutions of the Agar Used and the Total Coliform Counts

Sample	Media used	10^{-1}	10^{-2}	CFU/ml10^{2}	CFU/ml10^{2}
WATER	Plate Count Agar	20	5	2.0×10^{3}	5.0×10^{3}
	Mac-Conkey Agar	2	NG	2.0×10^{2}	NG
	Centrimide Agar	5	1	5.0×10^{2}	1.0×10^{3}
	Eosin Methylene Blue Agar	5	NG	5.0×10^{2}	NG
	Mannittol Salt Agar	1	NG	1.0×10^{2}	NG

Key; NG – No Growth, CFU – Colony Forming Unit

Table 2: Effects of Biochemical Tests Carried on Majidun River Water

Sample ID	Catalase	Oxidase	M – red	Urease	Motil	H_2S	Indole	Citra	Sucro	Gluco	Lacto	Gelatinase	G- rxtn	Shape
EMB H_2O	**+ve**	**-ve**	**+ve**	**-ve**	**+ve**	**+ve**	**-ve**	**+ve**	**+ve**	**+ve**	**-ve**	**+ve**	**-ve**	**Cocci**
EMB Pink Centre	**+ ve**	**+ ve**	**+ve**	**-ve**	**+ve**	**+ve**	**-ve**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**	**Cocci**
EMB Grenish Metallic	**+ ve**	**- ve**	**+ve**	**-ve**	**+ve**	**+ve**	**+ve**	**-ve**	**+ve**	**+ve**	**+ve**	**-ve**	**-ve**	**Cocci**
EMB Grenish	**+ ve**	**+ ve**	**+ve**	**-ve**	**+ve**	**+ve**	**+ve**	**-ve**	**+ve**	**+ve**	**+ve**	**-ve**	**-ve**	**Cocci**
MAC H_2O	**+ ve**	**+ ve**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**+ve**	**+ve**	**-ve**	**-ve**	**-ve**	**+ve**	**Rod**
CA H_2O	**+ ve**	**+ ve**	**+ve**	**-ve**	**+ve**	**+ve**	**-ve**	**-ve**	**+ve**	**+ve**	**-ve**	**-ve**	**-ve**	**Cocci**
MSA Off-White	**+ ve**	**- ve**	**+ve**	**-ve**	**+ve**	**+ve**	**-ve**	**-ve**	**-ve**	**+ve**	**-ve**	**-ve**	**-ve**	**Cocci**
MSA Yellow	**+ ve**	**- ve**	**-ve**	**-ve**	**+ve**	**+ve**	**-ve**	**+ve**	**+ve**	**-ve**	**-ve**	**-ve**	**+ve**	**Rod**
MSA H_2O (2)	**+ ve**	**+ ve**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**	**-ve**	**-ve**	**-ve**	**Cocci**
MSA H_2O (3)	**+ ve**	**- ve**	**+ve**	**+ve**	**+ve**	**+ve**	**-ve**	**+ve**	**+ve**	**+ve**	**-ve**	**-ve**	**-ve**	**Cocci**

Key:- M-red: Methyl Red, **EMB:** Eosin Methylene Blue Agar, **Motil:** Motility, **MCA:** Mac-Conkey Agar, **H_2S:** Hydrogen sulphide production, **CA:** Centrimide Agar, **Citra:** Citrase, **MSA:** Mannittol Salt Agar, **Sucro:** Sucrose, **H_2O:** Water, **Gluco:** Glucose, **Lacto:** Lactose, **G-rxtn:** Gram staining reaction**, +ve:** Positive, **-ve:** Negative

Pseudomonas aeruginosa is a common gram-negative, aerobic bacterium with unipolar motility that can cause disease in animals including human. It is citrate, catalase and oxidase positive that are found in soil, water, skin flora and most man-made environments throughout the world (Ryan and Ray, 2004). EMB pink center, MAC H_2O and MSA H_2O 2 river water sub-culture show positive to oxidase, citrase and catalase test (Table 2) which indicated the presence of *Pseudomonas aeruginosa* as set-up by Barrow and Feltman, (1993) and described by Antharam *et al.* (2013). *P. aeruginosa* is an opportunistic human and plant pathogen which can be isolated as clear colonies on Mac-Conkey agar which will test positive for oxidase and confirmatory tests include production of the blue-green pigment on centrimide agar (Mathee *et. al.*, 2008).

Proteus is a gram-negative Proteo-bacteria which are widely distributed in nature as saprophytes and found in decomposing animal matter, sewage, soil, water, human and animal faeces (Matsuyama *et. al*., 2000). MSA H_2O and MSA H_2O 3 were found positive to urease test (Table 2) which showed the existence of *Proteus* species in the river as they are oxidase-negative, catalase and nitrate positive but specific tests include positive urease (Rauprich *et. al.*, 1996) which is the fundamental test to differentiate *Proteus* spp from *Salmonella* spp. *Proteus* are opportunistic pathogens which responsible for many human urinary tract and wound infections (Guentzel, 1996).

Salmonella species is a rod-shaped, gram-negative bacteria which are found in animals, water and environment that can cause illnesses such as typhoid fever, paratyphoid fever and food poisoning (Ryan and Ray, 2004). Most subspecies of *Salmonella* produce hydrogen sulphide (Fabrega and Vila, 2013), which can be readily detected on host-associated lifestyles and are frequently isolated from water sources which act as bacterial reservoirs to facilitate transmission between hosts (Winfield and Eduardo, 2003). Positive hydrogen sulphide (H_2S) production in all the sub-cultured river water except MAC H_2O (Table 2) established the occurrence of *Salmonella* species (Antharam *et al.*, 2013).

All biochemical tests on MSA yellow (Table 2) affirmed the presence of *Baccillus* species in Majidun River as proved by Antharam *et al.* (2013). *Baccillus* are ubiquitous in nature which include both free-living (non-parasitic) and parasitic pathogenic species that test positive for catalase when there has been oxygen used or present (Turnbull, 1996). They are considered to be significant as they cause anthrax, food poisoning, insect pathogen and sometimes to control insect pests (Ryan and Ray, 2004). Some environmental and commercial strains play a role in food spoilage of highly acidic, tomato based products (Madigan and Martinko, 2005).

Physico-chemical Characterization

Weekly mean concentrations of physico-chemical parameters of Majidun River were presented in Table 3. All the mean concentrations contained were not significantly different to each other ($p > 0.05$) except in pH, DO and Pb (Table 3).

Mean concentration of total alkalinity, electrical conductivity, COD, BOD, DO, phosphate, sulphate and nitrate in Majidun River were significantly different ($p < 0.05$) to each other (Table 3). This showed that Majidun river retain its physico-chemical properties except pH, TDS, Salinity, THD and Chloride which may be as a result of rainy and dry seasonal period. The little fluctuation in the physico-chemical characteristics of the water will produce mild effects on fish mortality (Fafioye, 2002) and these parameters serve as variables since fluctuation of one affect the values of others (Fafioye *et al*., 2005).

Table 3: Mean concentrations of physico-chemical parameters of Majidun River Water

Parameters	Weeks 1 – 5	Weeks 6 – 10	Weeks 11 – 15
pH	6.200±0.12[b]	6.948±0.08[a]	6.880±0.21[a]
Total Alkalinity (mg/l)	9.300±0.03[a]	9.314±0.0[a]	9.352±0.04[a]
Total Dissolved Solids (ppm)	2647.000±12.81[a]	2660.000±6.32[a]	2666.000±4.30[a]
Salinity (ppm)	2108.000±30.89[a]	2073.000±22.23[a]	2117.000±27.09[a]
Conductivity (ms/cm)	3.712±0.03[a]	3.724±0.01[a]	3.734±0.03[a]
Chemical Oxygen Demand (mg/l)	98.774±0.03[a]	98.810±0.01[a]	98.816±0.03[a]
Biochemical Oxygen Demand (mg/l)	25.616±0.04[a]	25.622±0.01[a]	25.634±0.02[a]
Dissolved Oxygen (mg/l)	7.068±0.01[ab]	7.056±0.01[b]	7.098±0.01[a]
Chloride (mg/l)	4250.400±4.25[a]	4250.000±1.58[a]	4252.000±4.04[a]
Total Hardness ($mCaCO_3$/l)	347.000±3.58[a]	344.000±1.05[a]	350.600±3.34[a]
Phosphate (mg/l)	11.926±0.03[a]	11.942±0.02[a]	11.902±0.03[a]
Sulphate (mg/l)	6.204±0.01[a]	6.206±0.01[a]	6.248±0.02[a]
Nitrate (mg/l)	8.614±0.01[a]	8.620±0.01[a]	8.656±0.03[a]

Mean values (±Standard Error) in the same row with the same superscripts are not significantly different ($p > 0.05$)

When salinity increases, it increases the chloride content and the total hardness, meanwhile, if TDS increases, the electrical conductivity of the water also increases and vice versa. When the sulphate and nitrates increases, the total alkalinity of the water increases, while DO content increases, BOD and COD increase and vice versa. There was significant difference in DO content, while pH was not (Fafioye *et al.*, 2008).

There were great variabilities in the concentrations of physico-chemical parameters and microbial load in the river as a result of different human activities around the river water which might makes it to be unsafe for human consumption. Consumption of Majidun river water without adequate purification could exert toxic effects on life and hazardous to their health. This shown that Majidun River has been greatly polluted by anthropological activities such as discharge of domestics wastes, organic wastes (faecal matters), commercial water

transportation by boat, construction, heavy motor traffic and uncontrolled fishing methods among others.

Periodic monitoring of the river should be strengthened to avoid diseases outbreak in view of the nutritional and socio-economic importance of the river. Also, Wide education and strict enforcement of rules and regulations in relation to international guidelines for the Majidun community inhabitants and the general public on proper discharge of wastes and the general usage of water ways.

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