

PHYSICO-CHEMICAL AND BACTERIOLOGICAL QUALITY OF DRINKING WATER SOURCES IN CALABAR MUNICIPALITY, NIGERIA

ABSTRACT

This study evaluated the Physico-Chemical and the Bacteriological quality of five different sources of drinking water in Calabar metropolis, Nigeria, to give a fair geographical representative of the town and to contribute to our understanding of the quality of drinking water in the metropolis. The Physico-Chemical characteristics such as pH, Temperature, Turbidity, Conductivity, Colour, Iron, Dissolved Oxygen, Calcium, Magnesium, Alkalinity, Total Hardness, Manganese, Sulphate, Chloride, Phosphate, Sodium, Zinc, Copper, Total Dissolved Solid, Nitrate, Nitrite, Ammonia, Ammonium and Potassium were determined following the procedures prescribed by American Public Health Association Standard Method. The Bacteriological Analysis was carried out using the standard microbiological standard for analysis of water for Total and faecal coliform count. The mean temperature of the evaluated waters ranged from 23.03-29.3⁰C, mean pH ranged from 4.37-6.76mg/l, while turbidity had a mean range of 0.16-4.13NTU. Conductivity ranged between 39.29-120.7µs/cm, dissolved Oxygen with 13.30-4.19mg/l, total Dissolved solids ranged from 72.4-23.5mg/l, while the mean for iron concentration ranged from 0.12-0.99mg/l. Similarly the mean for total hardness was 34.2-17.1mg/l and 7.93-6.71mg/l for total alkalinity. Others includes Manganese (0.88-0.02mg/l, magnesium 16.5-9.9mg/l, calcium 9.77-7.20mg/l, nitrate (14.6-3.66mg/l), nitrite (0.076-0.009mg/l), ammonia (0.89-0.25mg/l), ammonium (0.52-0.013mg/l), zinc (1.01-0.34mg/l), chloride (5.73-0.364mg/l), fluoride (0.76-0.277mg/l), copper (0.61-0.18)mg/l, sodium (2.73-0.180)mg/l, potassium (5.73-2.0)mg/l, sulphate (14.8-3.69)mg/l and phosphate with 4.8-3.69Tmg/l. The total coliform count for bottled water ranged between 2cfu/100ml-19cfu/100ml, the total coliform range for sachet water were 6cfu/100ml and 15cfu/100ml and no faecal coliform was detected. Public water had no growth at all, the stream and borehole bacteriological analysis ranged from 27x 10¹cfu/ml-55 x 10¹cfu/ml and 12cfu/100ml-33cfu/100ml for total coliform respectively and faecal coliform ranged from 15 x 10¹cfu/ml-52 x 10¹cfu/ml for stream and 9cfu/100ml-16 cfu/100ml for borehole. A total of seven (7) different bacteria species were isolated from the sampled drinking water sources. These included *Proteus spp*, *Streptococcus spp*, *Enterobacter spp*, *Pseudomonas spp*, *E.coli*, *Chromobacterspp*, *Salmonella spp* and *Enterococcus spp*. This study reveals a high level of poor quality sources of water in the metropolis and makes need for urgent health intervention.

Keywords: Physico-chemical, bacteriological, faecal coliform, hardness and total coliform

INTRODUCTION

Water is essential to sustain life; therefore a satisfactory (adequate, safe and accessible) supply of drinking water should be available to all. Every effort should be made to achieve a good quality of drinking water. Quality is of basic importance to human

44 physiology and man's continued existence depends very much on water availability;
45 (Lamikara, 1999, Okorafor *et al.*, 2012). Over the years human beings have in adequate
46 access to portable water and the used the ones that are contaminated with disease vectors,
47 pathogens or unacceptable level of toxins or suspended solid. Portable water is the water that
48 is free from disease producing microorganisms and chemicals substances that are dangerous
49 to health. (Lamikara, 1999). The provision of portable water to rural and urban population is
50 necessary to prevent health hazard. (Nikoladze *et al.*, 1989, Lee 1991; Okorafor *et al.*, 2012).

51 In Nigeria, majority of rural populace do not have access to portable water and
52 therefore, depends on well, streams and river water for domestic use (Shittu *et al.*, 2008), lack
53 of water has become a critical and urgent problem, and it is a matter of great concern to
54 families and communities that depends on non-public water supply system. (Okonko *et al.*,
55 2008, Adegoke *et al.*, 2012). Increase in the human population has enacted an enormous
56 pressure on the provision of safe drinking water in the developing countries, (Umeh *et al.*,
57 2005, Adegoke *et al.*, 2012). Drinking water is one of the oldest public health issues and is
58 associated with multitude of health related concerns. Access to safe drinking water is a
59 prerequisite to poverty reduction and prevention of the spread of water borne and sanitation
60 related diseases (Cosgrove *et al.*, 2000, Gomez *et al.*, 2002, Okorafor *et al.*, 2012, UNICEF
61 2005). Death due to water related diseases add up to more than three million people per year
62 (Opara, 2005, WHO 2003a). Infectious diarrhea alone claimed 1.7 million lives in 2002
63 (Opara, 2005, WHO, 2003b). The relation of disease to water is clearly established and the
64 mechanisms that link different diseases to water have been well described (Feachem, 1975,
65 Opara, 2005). It is well established that infectious diseases are transmitted primarily through
66 water supplies contaminated with human and animal excreta (i.e. faeces) (WHO, 1993). Out
67 breaks of water borne diseases continues to occur throughout the world but are especially
68 serious in the developing countries, disease contacted through drinking water kill about
69 5million children annually and make 1/6th of the world population sick (WHO, 2004, Shittu
70 *et al.*, 2008). The human pathogens that are present in drinking water includes; *Salmonella*
71 *species*, *Shigella species*, pathogenic *Escherichia coli*, *Vibro cholera*, *Yersinia enterocolitica*,
72 *Campylobacter species*, *Klebsiella* and various viruses such as *Hepatitis A*, *Hepatitis E*, Rota
73 virus and parasites such as *Entamoebahistolytica*, and *Giardia species* (Emde *et al.*, 1992 and
74 Joklik *et al.*, 1992). To curb this health problem of unsafe water, bottle water was introduced,
75 but only individuals who have good financial status can afford this product. Low income
76 earners are left with no option but to consume sachet water which is readily available and
77 affordable. (Adegoke *et al.*, 2012). The recognition that microbial infections can be

78 waterborne has led to the development of method of routine examination to ensure safety of
79 drinking water. It is impracticable to monitor drinking water for every possible microbial
80 pathogen. Therefore, normal intestinal organisms are used as indicator of faecal pollution.
81 (Lee, 1991, Catwright *et al.*, 1993). These include coliforms group of organisms as a whole.
82 Bacteriological quality of ground water, pipe borne water and other natural water supplies in
83 Nigeria, has been reported to be unsatisfactory, with coliforms counts far exceeding the level
84 recommendation by WHO (Dada *et al.*,1999a, 1999b, Edema *et al.*,2001). This happen
85 because of the highly toxic materials and domestic waste that are disposed by dumping them
86 into the earth-water, rivers and streams with total disregard for aquatic life and urban
87 dwellers: thus water becomes an important medium for transmission of enteric diseases.

88 In general, certain requirements must be met for water to be fit for human
89 consumption. These requirements are freedom from organisms and chemicals substances,
90 which might be injurious to health. Drinking water should be of such composition that
91 consumers do not question the safety of the water. This implies that turbidity, colour, taste
92 and odour should be low and micro-organism (e.g. worms, Asellus, aquatic and fly mymphs)
93 should not be present (Eja, 2002, Okorafor *et al.*, 2012). The world Health organization has
94 recommended continuous surveillance of water supplies, which should involve keeping a
95 careful watch at all safety and sustainability of water supplies. This is to be achieved through
96 sanitary inspection and water quality analysis while sanitary inspection identifies potential
97 risk factors of contamination and source of pollution. Water quality analysis confirms
98 whether the water supply is faecally contaminated (WHO, 2004, Cheesbrough 2000,
99 Okorafor *et al.*, 2012). The most preferable method used in analyzing faecal coliforms from
100 water is the membrane filtration technique. Water is the integral part of achieving all the UN
101 Millennium Development Goals. The Millennium Development Goals (MDG) target for
102 water is to halve by 2015 the proportion of people without sustainable access of safe drinking
103 water and basic sanitation. The WHO (2004) and Okorafor *et al.*,(2012) estimates that these
104 improvements were to be made in sub Saharan African alone, 434,000 child death due to
105 diarrhea would be averted annually. The inhabitants of Calabar municipality, Nigeria have
106 access to boreholes, streams, river and taps as the major source of water supply. The use the
107 water supplied from these sources for drinking and other domestic activities such as cooking,
108 washing bathing, poultry etc. among the inhabitant of Calabar municipality, Nigeria we also
109 see some other drinking water like bottle water and sachet water which are obtained from
110 boreholes and taps that are exposed to microbial contamination through rainfall runoffs, and
111 the fact that they are always or usually constructed very close to pit toilet or sewage tank.

112 Therefore the determination of the portability and sustainability of such supplies is of serious
113 concerns.

114 In some developing countries like Nigeria where dangerous and highly toxic industrial and
115 domestic wastes are disposed of by dumping them into the earth water, rivers and streams
116 with total disregard for aquatic life and urban dwellers, water becomes an important medium
117 for this transmission of enteric diseases in most communities. In Calabar municipality,
118 Nigeria most of the communities depends in the available stream, borehole during dry season.
119 Streams in Calabar, Nigeria may be polluted by chemical effluent from both industrial and
120 commercial establishments as well as organic and inorganic substances that Itah *et al.*, (1996)
121 observed. This may be responsible for intermittent outbreak of typhoid fever, paratyphoid
122 fever and cholera in recent years in Calabar. Apart from the toxic chemicals in domestic and
123 industrial waste discharged into these water bodies, the underground waters become
124 contaminated by pesticides, herbicides and fertilizer as they are applied by farmers and if this
125 water is been taken in by humans it will lead to serious health hazard. This study will
126 therefore evaluate the Physico- chemical and bacteriological quality of drinking water in
127 Calabar municipality, Nigeria and also seek to identify and characterize bacteria isolates
128 associated with drinking water in Calabar municipality, Nigeria.

129

130

131

MATERIALS AND METHODS

132 Study Area and Site

133 This study was carried out in Calabar municipality, Cross River State, Nigeria. Calabar
134 municipality lies between latitude $04^{\circ} 15'$ and $5^{\circ} N$ and longitude $8^{\circ} 25' E$. The municipality is
135 bounded in the north by Odukpani Local Government Area, in the northeast by great Qua
136 River (Akpabuyo Local Government Area). Its southern shores are bounded by the Calabar
137 River and Calabar South Local Government Area. It is politically divided into 10 wards. It
138 has a population of one hundred and seventy nine thousand three hundred and ninety two at
139 the 2006 census (NPC web) It has an area of 331.551 square kilometers. Calabar municipality
140 is a coastal town lying with the tropical region. The local government has two main seasons,
141 the rainy and dry seasons. Calabar municipality is made up of three tribes namely; Qua's,
142 Efut's and Efik's (Agbo & Mbotto, 2012).

143

144 The municipality has industries and establishments. E.g. Seaport, Free Trade Zones,
Airport, Export Processing Zone (EPZ), naval and Army Base, Tinapa, NNPC depot, Cement

149 **Sample collection**

150 Water samples for bacteriological and Physico-chemical analysis were collected
151 from five (5) different sources of drinking water in Calabar municipality, Nigeria and at
152 different location all in Calabar municipality. The sources of drinking water and their
153 designated sites: Stream, Boreholes, Pipe borne water, Sachet water and bottle water were
154 gotten from different water vendor outlet all in Calabar Municipality, Nigeria. The water
155 samples were collected in duplicate in two batches. Samples were analyzed in the laboratory
156 within two hours of collection.

157 **Borehole:** five different samples were collected at random at different streets in Calabar
158 municipality, Nigeria namely; EdimOtop Street, IkotEnebong Street, IkotOmin, Ekosin
159 Junction as shown in figure1. The samples were collected through convenience sampling into
160 a sterile bottle and put in an ice packed box, the water samples were analyzed for the listed
161 parameters, Temperature, conductivity, turbidity, colour, iron, dissolved oxygen, calcium,
162 magnesium, total hardness, alkalinity, manganese, sulphate, chloride, phosphate, sodium,
163 zinc, copper, total suspended solid, nitrate, nitrite, ammonia, potassium.

164 **Bottle water:** Five different brands of bottled water were collected in the study area (Calabar
165 municipality, Nigeria): **Ev, Ne, Qu, Cw,** and **Aq**. The following bottled water sample was
166 analyzed for the above parameters. Temperature, conductivity, turbidity, colour, iron,
167 dissolved oxygen, calcium, magnesium, total hardness, alkalinity, manganese, sulphate,
168 chloride, phosphate, sodium, zinc, copper, total suspended solid, nitrate, nitrite, ammonia,
169 potassium.

170 **Pipe borne water:** Five samples were collected through convenience sampling from five (5)
171 utility points around Calabar municipality and analyzed for all the above parameters.
172 Temperature, conductivity, turbidity, colour, iron, dissolved oxygen, calcium, magnesium,
173 total hardness, alkalinity, manganese, sulphate, chloride, phosphate, sodium, zinc, copper,
174 total suspended solid, nitrate, nitrite, ammonia, potassium.

175 **Sachet water:** Five different brands of sachet water; **UTWa, CW, ATW, ZTW** and **UTW**.
176 Were randomly selected, samples and analyzed for the above parameters. Temperature,
177 conductivity, turbidity, colour, iron, dissolved oxygen, calcium, magnesium, total hardness,
178 alkalinity, manganese, sulphate, chloride, phosphate, sodium, zinc, copper, total suspended
179 solid, nitrate, nitrite, ammonia, potassium.

180 **Stream:** Four different streams and one spring around Calabar municipality; Satellite town,
181 Parliamentary 1 and 2, NyakAsang stream and Unicem spring were collected using a sterile
182 bottle with about 20cm deep from the surface of the water. The stream were sampled and
183 analyzed for the above parameters. Temperature, conductivity, turbidity, colour, iron,
184 dissolved oxygen, calcium, magnesium, total hardness, alkalinity, manganese, sulphate,
185 chloride, phosphate, sodium, zinc, copper, total suspended solid, nitrate, nitrite, ammonia,
186 potassium.

187 **Physico-chemical analysis**

188 The conventional parameters used in assessing the quality and portability of water for
189 drinking are level of Suspended Solids, Total Dissolved Solid, Appearance, Hardness,
190 Conductivity, pH, Colour, Odour, etc. AWWA/APHA, 1998.

191

192 **Temperature:** The temperatures of the samples were taken, using a thermometer. The bulb
193 of the thermometer was dipped into the water sample in a beaker and allows standing for
194 some minute before the reading was taken.

195

196 **Conductivity:** Conductivity Meter (**Model: Hanna Instrument H18733**) was used. The
197 conductivity meter probe was rinsed with distilled water and inserted into the sample in a
198 beaker, conductivity reading was displayed.

199 **pH:** The pH was determined with a pH meter (**Model: Mettler Toledo Mp 220**). The pH
200 meter probe was inserted into the water sample in a beaker, the READ key was pressed and
201 the pH reading was taken.

202

203 **Turbidity:** A turbidity meter was used. (**Model: Hanna Instrument H193703**).

204 **Procedure:** The sample was placed in the turbidimeter bottle and the bottle wiped clean with
205 a cloth to erase any finger print that may affect the reading. The bottle was then placed on the
206 **turbidimeter** and the **read** key pressed, the turbidity reading was displayed.

207 **Colour:** The colour was determined using **Lovibond Comparator**. The test kit was
208 assembled and the water sample was poured into a tube and place in the right hand of the
209 comparator. The disc was place on the comparator and noted as the colour value.

210

211 **Iron** (Method: APHA, 2010.) using a Spectrophotometer

212 **Procedure:** 5ml of water sample was placed in a test tube and 0.3ml of iron reagent (Fe) was
213 added, shaken and allowed to stand for 3 minutes the iron concentration was determine at a
214 wave length of 420nm in the spectrophotometer.

215

216 **Dissolved Oxygen**

217 **Method** APHA, 2010. Using a Spectrophotometer

218 **Procedure:** A reaction cell was filled to overflow and 1 glass bead was added into it. Oxygen
219 reagent O2 – 1k was added (5 drops). Another 5 drops of oxygen reagent O2 – 2k were added
220 and mixed for 10 seconds Lastly 10 drops of oxygen reagent O2 – 3k was added mixed and
221 dissolved oxygen value read out in the spectrophotometer at a wave length of 498nm. The
222 summary of the result are presented in table 1 to 5 for the different water samples.

223

224 **Calcium:** Method: Spectrophotometry

225 **Procedure:** 0.1ml of the sample was placed in a test tube using pipette and 0.5ml of calcium
226 reagent Ca-1 was added and mixed. 0.4ml each of calcium reagent Ca-2 and Ca-3 were also
227 added to the test tube and mixed. The sample was allowed to stand for 8 minutes to elicit full
228 colour development and then filled into a reaction cell, placed in the spectrophotometer
229 where the calcium concentration was displayed.

230

231 **Magnesium:** Method: Spectrophotometry

232 **Procedure:** 1ml of the sample was placed in a reaction cell and mixed and 1ml of magnesium
233 reagent Mg-1k added to it. This was allowed to stand for 3 minutes and thereafter, 0.3ml of
234 magnesium reagent Mg-2k added, mixed and placed in the spectrophotometer. Magnesium
235 concentration was read at a wavelength of 568nm.

236 **Total Hardness:** Method: Spectrophotometry

237 **Procedure:** 1ml of the sample was placed in a reaction cell and 1ml of total hardness reagent
238 H-1k added with a pipette. Three minutes reaction time was allowed before total hardness
239 was determined in the spectrophotometer at a wavelength of 450nm.

240

241 **Alkalinity:** Method: Titrimetry

242 **Procedure:** The sample was placed up to the 5ml mark in the test tube and 1 drop of methyl
243 red indicator was added to it. The sample turns blue and a drop wise titration was carried out
244 using reagent TL AL7 until there was a colour change. The value in the syringe was taken as
245 the alkalinity value for the sample.

246

247

248 **Manganese:** Method: Spectrophotometry

249 **Procedure:** 5ml of the water sample was placed in a test tube and 4 drops of manganese
250 reagent Mn-1 was added and shaken. This was allowed to stand for 2 minutes. Thereafter,
251 0.2ml each of manganese reagents Mn-2 and Mn-3 were added, shaken and allowed to stand
252 for another 2 minutes before reading the manganese concentration from the
253 spectrophotometer at a wavelength of 520nm.

254

255 **Sulphate:** Method: Spectrophotometry

256 **Procedure:** 2.5ml of the water sample was placed in a test tube and 0.2ml of sulphate reagent
257 SO₄-1A added and mixed. 1 level spoonful of sulphate reagent SO₄-2A powder was added
258 and mixed. The solution was then tempered in a water bath at 40°C for 5 minutes. 2.5ml of
259 sulphate reagent SO₄-3A was added, mixed and the solution filtered using **Whatman No. 1**
260 filter paper. 0.4ml of sulphate reagent SO₄-4A was then added to the filtrate and mixed. The
261 solution was again tempered in a water bath for 7 minutes at 40°C. This was transferred into a
262 round cell and placed in the spectrophotometer to read off the concentration of sulphate in the
263 water sample. A wavelength of 520nm was used.

264

265 **Chloride:** Method: Spectrophotometry

266 **Procedure:** 5ml of the water sample was placed in a test tube and 2.5ml of chloride reagent
267 Cl-1 was added and mixed. Chloride reagent Cl-2 was also added, shaken and allowed to
268 stand for 1 minute before reading out the chloride concentration from the spectrophotometer
269 at a wavelength of 460nm.

270

271 **Phosphate:** Method: Spectrophotometry

272 **Procedure:** 5ml of the water sample was placed in a test tube and 0.5ml of the phosphate
273 reagent PO₄-1A added to it and mixed. This was followed by the addition of 1 level spoonful
274 of phosphate reagent PO₄-2A. 5 minutes reaction time was allowed before reading out the
275 phosphate concentration at a wavelength of 420nm.

276

277 **Sodium:** Method: Spectrophotometry

278 **Procedure:** 0.5ml of sodium reagent Na-1k was placed in a reaction cell and 0.5ml of the
279 water sample added to it and mixed. A reaction time of 1 minute was allowed before reading
280 the concentration of sodium from the spectrophotometer.

281

282 **Zinc:** Method: Spectrophotometry

283 **Procedure:** 10ml of the water sample was placed in a glass vessel and 1 micro-spoonful of
284 zinc reagent Zn-1k was added and shaken to dissolve (this is the pretreated sample). 0.5ml of
285 zinc reagent Zn-2k was placed in a reaction cell and 2.0ml of the pretreated water sample
286 added to it and mixed. 0.5ml of zinc reagent Zn-3k was also added into the reaction cell and
287 mixed. Zinc concentration was then determined in the spectrophotometer.

288

289 **Copper:** Method: Spectrophotometry

290 **Procedure:** 5ml of the water sample was placed in a reaction cell and 0.5ml of copper
291 reagent Cu-1k was added and mixed. 5 minutes reaction time was allowed before copper
292 concentration was determined.

293

294 **Total Dissolved Solids (TDS):** This was determined by multiplying through the conductivity
295 value. The conductivity of the sample was determined and the value multiplied by 0.6 to get
296 the TDS. $TDS = \text{Conductivity} \times 0.6$.

297

298 **Nitrate:** Method: Spectrophotometry

299 **Procedure:** 1 micro-spoonful of nitrate reagent NO₃-1A was placed in a dry test tube and
300 5ml of nitrate reagent NO₃-2A added into it and mixed to dissolve. 1.5ml of the sample was
301 added slowly and shaken. This was allowed to stand for 10minutes and nitrate concentration
302 was read out from the spectrophotometer at a wavelength of 520nm.

303

304 **Nitrite:** Method: Spectrophotometry

305 **Procedure:** 5ml of the water sample was placed in test tube and 1 micro-spoonful of nitrite
306 reagent NO₂-AN was added and shaken to dissolve. A time of 10 minutes was allowed before
307 reading out the nitrite concentration in the sample.

308

309 **Ammonia:** Method: Colorimetry

310 **Procedure:** 10ml of the water sample was placed in a calibrated plastic cup and 2 drops of
311 ammonia reagent 1 as well as 8 drops of ammonia reagent 2 (**Nessler Solution**) were each

312 added to the water sample and mixed. After 5 minutes, the solution was poured into the
313 colorimetry tube and the nearest colour match was used to determine ammonia concentration.

314

315

316 **Potassium:** Method: Spectrophotometry

317 **Procedure:** 2ml of the water sample was placed in a reaction cell and mixed. 0.6ml of
318 potassium reagent K-1k was added and mixed, 1 level micro-spoonful of potassium reagent
319 K-2k also added, mixed and allowed to stand for 5 minutes. The concentration of potassium
320 was read out from the spectrophotometer at a wavelength of 690nm.

321

322 **Bacteriological Analysis**

323 All the media used were prepared based on manufacturer's instruction and sterilized in the
324 autoclave at 121°C for 15mins. These were poured into sterile petri dishes (20ml each) and
325 allowed to cool before inoculation. The glass wares and the stainless steel filtration unit used
326 were also sterilized in the hot air oven at 150°C for 1hr.

327

328 **Inoculation Technique (REMOVE HIGHLIGHTED)**

329 The samples were shaken to mix and 100ml measured from it and filtered through membrane
330 filter (0.45µm pore size). This filter allows water particles to pass through but bacteria cells
331 are trapped.

332 After filtration, the membrane filter is carefully removed using a sterile forceps and placed on
333 the molten agar. Each sample had two (2) plates. These plates were incubated for 24 hours at
334 37°C. Emerging colonies after the period of incubation were enumerated using a colony
335 counter.

336 **Serial Dilution**

337 One milliliters of the water samples from (borehole and surface water) each were transferred
338 into nine (9ml) of sterile distilled water in a separate test tube. Logarithms dilution ranging
339 from 10^{-1} to 10^{-3} was made for each of the water samples. 1ml of the desired aliquot is
340 transferred into a sterile petri dishes and viable plate count was determined using pour plate
341 method. Faecal and total coliform counts were performed for each sample, and were
342 inoculated in the appropriate media (i.e. MF-C agar and MacConkey agar). The plates are
343 incubated at 37°C for 24 - 48 hours, and observed for growth, the colony counter is used in
344 counting the colonies, and those with 2- 22cfu/ml (colony forming unit) are counted.9

345 **REFERENCE?**

346

347 **Maintenance of Pure Culture**

348 The growth from the plates especially those from the MacConkey agar plates had mixed
349 colonies (culture) needed to be isolated in their pure form. The bacteria representatives (i.e.
350 from each colony) was picked and sub-cultured onto a fresh sterile nutrient agar medium.
351 Purity of isolates was enhanced and obtained through repeated streaking. **The pure culture
352 that was obtained now provides the pure culture of that isolates and were maintained on
353 nutrient agar slants as stock culture for characterization.**

354 **Characterization of Bacterial Isolates**

355 The bacteria isolates were characterized based on their cultural morphology and biochemical
356 test according to (Collins and Lyne, 1976, Cheesbrough, 2006). The identification was done
357 using the manual for identification of medical bacteria (Cowan and Steel, 1985). The
358 biochemical tests used for characterization and identification of bacteria includes; Grams
359 reaction, motility test, catalase test, coagulase test, oxidase test, methyl-red test,
360 Voges-Proskauer test and sugar fermentation test.

361

362 **Statistical Analysis**

363 Statistical analyses of the Physico-chemical result were carried out to deduce the range of
364 uncertainty to statistical test with the analysis of variance (ANOVA) to assess the drinking
365 water quality. The statistical analysis one way ANOVA was applied to estimate whether it is
366 statistically significant among the group in analysis and the significance reported at ($P < 0.05$).
367 The f test analysis was applied to find out the null hypothesis, the statistics were performed
368 within brands and between brands using SPSS ver. 20.

369

370

371

RESULTS AND DISCUSSIONS

372 **Mean of Physico-chemical Analysis of Sampled Bottled Water**

373 **In all, a total of five bottled water were sampled in duplicate. All the sampled water were
374 clear in appearance, the colour was less than 5.0, temperature ranges from 28.8°C to 27.1°C
375 with the highest from Ev water, Ne water had the highest pH of 6.89 and 6.68 from Aq water,
376 turbidity ranges from 0.69-0.111 NTU very low compared to the NIS and WHO standard.
377 Conductivity ranged between 235.6-12.8 $\mu\text{s}/\text{cm}$ while Dissolved Oxygen ranges from 7.25-
378 3.46 mg/l, total Dissolved Solids ranged between 141.4-7.7 mg/l, iron concentration ranges
379 from 0.24-0.09 mg/l, Total Hardness from 17.1 mg/l, 7.97-7.90 mg/l for Total Alkalinity, the**

380 range of Manganese, Magnesium, Calcium, Nitrate, Nitrite, Ammonia, Ammonium, Zinc,
 381 Chloride, Fluoride, Copper, Sodium, Potassium, Sulphate and Phosphate are as follows;
 382 0.045-0.01mg/l, 20.1-7.2mg/l, 13.7-5.0mg/l, 11.2-4.30mg/l, 0.014-0.004mg/l, 0.07-0.01mg/l,
 383 0.00mg/l, 0.65-0.18mg/l, 0.61-0.105mg/l, 0.60-0.045mg/l, 0.135-0.055mg/l, 0.30-0.08mg/l,
 384 3.12-2.05mg/l, 3.70-2.05mg/l and 6.53-3.30mg/l respectively. A summary of the result is
 385 shown in Table 1. (Reframe language scientifically)

386

387 Mean of Physico-Chemical Analysis of Sampled Sachets Water

388 All the sampled sachets water was clear in appearance. Their colour was less than 5.0
 389 compared to the standards, with temperature range of 27.3°C-26.3°C. The pH for CW had the
 390 highest pH (6.94), with turbidity ranging from 0.355-0.17NTU, and Conductivity ranged
 391 from 71.5-23.15µs/cm. The concentration of dissolved Oxygen ranged between 13.5-
 392 11.0mg/l with CW having the highest and ZTW with the least. The lowest concentration of
 393 total Dissolved Oxygen, was found in Aq (13.9mg/l) and the highest in ZTW (47.7mg/l). Iron
 394 concentration ranged from 0.25mg/l to 0.30mg/l and ZTW had the maximum value. Total
 395 Hardness concentration observed from sachets water ranged between 34.2-17.1mg/l. Total
 396 alkalinity varied from 7.98-6.59mg/l, Manganese concentration ranged from 0.055-0.03mg/l,
 397 Magnesium concentration varied from 26.3-9.0mg/l, Calcium concentration ranges from 9.4-
 398 5.05mg/l, Nitrate from 16.9-7.65mg/l, Nitrite, Ammonia, Ammonium, Zinc, chloride,
 399 Fluoride, copper, sodium, potassium, sulphate, and phosphate the values were from 0.0135-
 400 0.004mg/l, 0.60-0.45mg/l, 0.07-0.05mg/l, 0.44-0.65mg/l, 2.40-1.25mg/l, 0.70-0.45mg/l,
 401 0.40-0.20mg/l, 0.90-0.55mg/l, 2.1-4.15mg/l, 2.9-1.8mg/l and 6.1-3.75mg/l respectively. A
 402 Summary of this result is presented in Table 2.

403

404 Mean of Physico-Chemical Analysis of Sampled Public Water

405 All the sampled public water (tap) was clear in appearance. Their colour was less than 5.0
 406 compared to the standards, with temperature range of 26.0°C-24.75°C. Wb₄ (Marian road)
 407 had the highest pH (5.88) and the lowest pH (4.94) from Wb₁ (Edim Otop Street), turbidity
 408 ranged from 0.245-0.120NTU, Conductivity ranged from 51.1- 45.25µs/cm. The
 409 concentration of dissolved Oxygen ranged between 13.75-12.50mg/l. Total Dissolved
 410 Oxygen ranged from 30.5-27.1mg/l Iron concentration ranged from 0.145mg/l to 0.105mg/l,
 411 total hardness had from 34.2mg/l in all. Total alkalinity varied from 7.62-7.06mg/l,
 412 Manganese concentration ranged from 0.035-0.015mg/l, Magnesium concentration varied
 413 from 22.85-10.1mg/l, Calcium concentration ranges from 11.45-8.15mg/l, Nitrate from 4.55-

414 1.60mg/l, Nitrite, Ammonia, Ammonium, Zinc, chloride, Fluoride, copper, sodium,
 415 potassium, sulphate, and phosphate the values were from 0.045-0.0045mg/l, 0.81-0.61mg/l,
 416 0.35-0.25mg/l, 1.31-0.76mg/l, 6.81-5.11mg/l, 0.87-0.66mg/l, 0.81-0.31mg/l, 4.41-1.81mg/l,
 417 6.81-5.10mg/l, 25.2-18.1mg/l and 16.0-5.65mg/l respectively. A Summary of this result is
 418 presented in Table 3.

419

420 **Mean of Physico-Chemical Analysis of Stream Water Sampled**

421 All the sampled streams were clear in appearance. Their colour was less than 5.0 compared
 422 to the standards, with temperature range of 27.6-26.8⁰C. The pH for Nyakasang (Ny) water
 423 had the highest pH (6.11) and the lowest from Unicem spring (Us) with pH (4.37), with
 424 turbidity ranging from 5.72-3.39NTU, Conductivity ranged from 94.1-42.8 μ s/cm. The
 425 concentration of dissolved Oxygen ranged between 5.29-3.10mg/l. The lowest concentration
 426 of total dissolved oxygen was found in Nyakasang (Ny) (25.5mg/l) and the highest in
 427 Parliamentary stream (P₂) 56.6mg/l. Iron concentration ranged from 1.13mg/l to 0.76mg/l.
 428 Total Hardness concentration observed from sachets water ranged between 34.2-17.1mg/l.
 429 Total alkalinity varied from 7.79-7.07mg/l, Manganese concentration ranged from 1.21-
 430 0.55mg/l, Magnesium concentration varies from 10.7-9.25mg/l, Calcium concentration
 431 ranges from 8.15-6.35mg/l, Nitrate from 21.1-9.05mg/l, Nitrite, Ammonia, Ammonium,
 432 Zinc, chloride, Fluoride, copper, sodium, potassium, sulphate, and phosphate the values
 433 were from 0.014-0.0043mg/l, 1.05-0.71mg/l, 0.63-0.41mg/l, 1.21-0.81mg/l, 6.05-3.20mg/l,
 434 0.69-0.42mg/l, 0.81-0.31mg/l, 2.40-0.89mg/l, 4.50-2.10mg/l, 32.5-18.5mg/l and 19.4-
 435 11.2mg/l respectively. A Summary of this result is presented in Table 4.

436

437 **Mean of Physico-Chemical Analysis of Sampled Borehole Water**

438 Five samples were picked in duplicate; and the result is as follows; the appearance and colour
 439 were clear and less than 5.0 respectively, the range value for temperature, pH, turbidity and
 440 conductivity were as follows, 30.3⁰C-27.9⁰C, 4.63-4.14, 0.42-0.17NTU and 79.8-29.0 μ s/cm
 441 respectively. The value for Dissolved Oxygen and Total Dissolved Solid ranged from 14.1-
 442 11.2mg/l and 47.8-17.4mg/l, the value for total hardness is 17.1mg/l for all sampled water,
 443 total alkalinity ranged from 6.84-6.69mg/l, the value for manganese, magnesium and calcium
 444 ranges as follows, 0.04-0.02mg/l, 10.2-9.25mg/l and 8.15-6.55mg/l, the concentration of
 445 nitrate and nitrite ranged from, 6.35-4.30mg/l and 0.06-0.03mg/l respectively. The highest
 446 concentration in ammonia is found in BH₃ with 0.71mg/l and the least is from BH₄ with
 447 0.51mg/l, that of ammonium the highest and lowest was from BH_{3&5} and BH_{2&4} (0.05-

448 0.03mg/l). Zinc concentration ranged from 1.07-0.71mg/l, Chloride and Fluorides
449 concentration ranged from 5.80-2.05mg/l and 0.64-0.17mg/l. copper concentration ranged
450 from 0.42-0.11mg/l, sodium ranged from 1.15-0.78mg/l, the concentration of potassium
451 ranged from 2.75-1.35mg/l, sulphate 6.05-4.25mg/l while phosphate ranged between 4.75-
452 2.65mg/l. A summary of the result is presented in Table 5.

UNDER PEER REVIEW

Table 1: Summary of the Result of Physico-Chemical Analysis of Bottled Water Sold in Calabar Municipality

NIS	WHO	Aqu	Qu	Cw	Ev	Ne	Parameters/units
Clear	Clear	Clear	Clear	Clear	Clear	Clear	Appearance
15	20	<5.0	<5.0	<5.0	<5.0	<5.0	Colour (pt Co)
20-30	20-30	27.5	27.6	27.1	28.8	27.3	Temperature (°C)
6.5-8.5	6.5-8.5	6.72	6.68	6.81	6.72	6.89	pH
5.0	5.0	0.221	0.457	0.69	0.111	0.111	Turbidity (NTU)
500	500	12.8	235.6	107.9	83.6	163.5	Conductivity (µS/cm)
14	14	3.46	5.40	4.66	4.95	7.25	Dissolved Oxygen
1000	1000	64.8	141.4	7.7	50.2	98.1	Total dissolved solids
0.30	0.30	0.14	0.24	0.14	0.11	0.09	Iron (mg/l) Fe
150	500	17.1	17.1	17.1	17.1	17.1	Total hardness (mg/l)
-	400	7.92	7.90	7.94	7.92	7.97	Total alkalinity (mg/l)
0.20	0.05	0.045	0.04	0.03	0.02	0.01	Manganese (mg/l) Mn
100	20	7.7	20.1	7.20	11.1	11.85	Magnesium (mg/l) Mg
-	50	5.0	13.7	5.75	6.05	8.5	Calcium (mg/l) Ca
50	45	10.4	11.2	5.5	4.30	9.45	nitrate(mg/l) N
0.2	0.1	0.005	0.014	0.011	0.013	0.004	Nitrite (mg/l) N
1.0	1.0	0.025	0.01	0.03	0.07	0.035	Ammonia (mg/l) NH₃
0.50	0.50	0.00	0.00	0.00	0.00	0.00	Ammonium (mg/l)
5.0	3.0	0.45	0.65	0.21	0.215	0.18	Zinc (mg/l) Zn
250	250	0.40	0.30	0.40	0.61	0.105	Chloride (mg/l) Cl
1.50	1.50	0.60	0.50	0.17	0.045	0.07	Fluoride (mg/l) F
1.0	1.0	0.125	0.135	0.055	0.055	0.055	Copper (mg/l) Cu
200	200	0.155	0.255	0.30	0.12	0.08	Sodium (mg/l) Na
100	200	2.34	3.12	2.50	2.70	2.05	Potassium (mg/l) K
100	200	3.35	2.60	3.70	2.05	2.63	Sulphate (mg/l) SO₄
100	200	3.30	5.04	6.35	5.60	4.30	Phosphate (mg/l) PO₄

Table 2: Summary of the Result of Physico-Chemical Analysis of Sachet Water Sold in Calabar Municipality

NIS	WHO	ZTW	UTW _a	UTW	CW	ATW	Parameters/units
Clear	Clear	Clear	Clear	Clear	Clear	Clear	Appearance
15	20	<5.0	<5.0	<5.0	<5.0	<5.0	Colour (pt Co)
20-30	20-30	26.95	26.3	27.3	26.4	26.8	Temperature (°C)
6.5-8.5	6.5-8.5	4.82	6.94	5.09	4.2	5.99	pH
5.0	5.0	0.355	0.22	0.22	0.17	0.23	Turbidity (NTU)
500	500	27.12	65.9	54.4	71.5	23.15	Conductivity (µS/cm)
14	14	11	13	8.5	13.5	12	Dissolved Oxygen
1000	1000	47.7	32.7	39.51	16.3	13.9	Total dissolved solids
0.30	0.30	0.30	0.30	0.25	0.20	0.25	Iron (mg/l) Fe
150	500	17.1	34.2	34.2	17.1	17.1	Total hardness (mg/l)
-	400	6.59	7.98	7.25	6.67	7.66	Total alkalinity (mg/l)
0.20	0.05	0.055	0.055	0.04	0.03	0.03	Manganese (mg/l) Mn
100	20	9.0	26.3	24.8	12.05	10.4	Magnesium (mg/l) Mg
-	50	8.1	7.95	9.4	5.05	6.75	Calcium (mg/l) Ca
50	45	8.25	15.0	9.90	7.65	16.9	Nitrate (mg/l) N
0.2	0.1	0.005	0.0135	0.011	0.0125	0.004	Nitrite (mg/l) N
1.0	1.0	0.6	0.6	0.45	0.5	0.5	Ammonia (mg/l) NH ₃
0.50	0.50	0.06	0.055	0.06	0.05	0.07	Ammonium (mg/l) NH ₄
5.0	3.0	0.44	0.55	0.65	0.50	0.55	Zinc (mg/l) Zn
250	250	1.25	1.45	1.75	2.10	2.40	Chloride (mg/l) Cl
1.50	1.50	0.60	0.55	0.45	0.45	0.7	Fluoride (mg/l) F
1.0	1.0	0.35	0.30	0.40	0.20	0.30	Copper (mg/l) Cu
200	200	0.55	0.65	0.75	0.90	0.85	Sodium (mg/l) Na
100	200	2.1	3.1	2.65	3.25	4.15	Potassium (mg/l) K
100	200	2.4	1.8	2.2	2.3	2.9	Sulphate (mg/l) SO ₄
100	200	3.75	4.2	5.2	6.1	4.55	Phosphate (mg/l) PO ₄

456

Table 3: Summary of the Result of Physico-Chemical Analysis of Public Water Supply in Calabar Municipality

	NIS	WHO	Wb5	Wb4	Wb3	Wb2	Wb1	Parameters/units
	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Appearance
	15	20	<5.0	<5.0	<5.0	<5.0	<5.0	Colour (pt Co)
	20-30	20-30	24.75	25.90	24.85	26.10	25.05	Temperature (°C)
	6.5-	6.5-	5.75	5.88	4.94	5.47	5.47	pH
	5.0	5.0	0.236	0.245	0.123	0.120	0.120	Turbidity (NTU)
	500	500	45.25	45.75	50.4	51.1	51.1	Conductivity (µS/cm)
	14	14	12.65	13.50	13.75	12.50	12.50	Dissolved Oxygen
	1000	1000	27.18	27.41	30.35	27.50	30.3	Total dissolved solids
	0.30	0.30	0.125	0.145	0.105	0.120	0.125	Iron (mg/l) Fe
	150	500	34.2	34.2	34.2	34.2	34.2	Total hardness (mg/l)
	-	400	7.55	7.62	7.18	7.06	7.44	Total alkalinity (mg/l)
	0.20	0.05	0.015	0.025	0.030	0.025	0.035	Manganese (mg/l) Mn
	100	20	11.9	10.15	10.15	13.50	22.85	Magnesium (mg/l) Mg
	-	50	10.45	8.25	8.15	10.55	11.45	Calcium (mg/l) Ca
	50	45	3.85	4.25	4.05	4.55	1.60	Nitrate (mg/l) N
	0.2	0.1	0.045	0.012	0.0105	0.013	0.004	Nitrite (mg/l) N
	1.0	1.0	0.71	0.61	0.71	0.81	0.71	Ammonia (mg/l) NH₃
	0.50	0.50	0.31	0.35	0.31	0.35	0.25	Ammonium (mg/l) NH₄
	5.0	3.0	0.85	1.03	0.76	0.81	1.31	Zinc (mg/l) Zn
	250	250	5.11	5.31	6.81	5.11	6.31	Chloride (mg/l) Cl
	1.50	1.50	0.71	0.66	0.85	0.71	0.87	Fluoride (mg/l) F
	1.0	1.0	0.43	0.81	0.31	0.32	0.57	Copper (mg/l) Cu
	200	200	2.11	1.81	3.20	2.11	4.41	Sodium (mg/l) Na
	100	200	5.11	5.31	6.81	5.10	6.30	Potassium (mg/l) K
	100	200	11.35	8.70	10.6	18.1	25.2	Sulphate (mg/l) SO₄
	100	200	6.35	5.65	8.10	9.35	16.0	Phosphate (mg/l) PO₄

457

458

KEY: Wb₁=EdimOtop Street, Wb₂=Orok Street off Ediba, Wb₃= EdimOtop Close West, Wb₄= Marian Road Wb₅=IkotEfa Street.

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Table 4: Summary of the Result of Physico-Chemical Analysis of Stream Water in Calabar Municipality

	NIS	WHO	St	Us	Ny	P ₂	P ₁	Parameters/units
	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Appearance
	15	20	<5.0	<5.0	<5.0	<5.0	<5.0	Colour (pt Co)
	20-	20-30	26.8	27.1	27.2	27.6	27.5	Temperature (°C)
	6.5-	6.5-	4.81	4.37	6.11	5.28	5.15	pH
	5.0	5.0	3.39	5.49	5.72	3.67	2.41	Turbidity (NTU)
	500	500	69.4	91.6	42.8	93.4	94.1	Conductivity (µS/cm)
	14	14	3.25	3.10	5.05	4.39	5.29	Dissolved Oxygen (mg/l)
	1000	1000	45.8	53.6	25.5	56.6	55.4	Total dissolved solids (mg/l)
	0.30	0.30	0.76	1.21	0.81	1.13	1.06	Iron (mg/l) Fe
	150	500	17.1	17.1	17.1	17.1	17.1	Total hardness (mg/l) CaCO ₃
	-	400	7.07	7.79	7.73	7.33	7.77	Total alkalinity (mg/l)
	0.20	0.05	0.76	1.21	0.55	0.99	0.95	Manganese (mg/l) Mn
	100	20	10.3	9.65	9.85	9.20	10.7	Magnesium (mg/l) Mg
	-	50	6.85	7.35	7.30	8.15	6.35	Calcium (mg/l) Ca
	50	45	11.65	9.05	13.5	27.1	12.6	Nitrate (mg/l) N
	0.2	0.1	0.003	0.014	0.008	0.009	0.004	Nitrite (mg/l) N
	1.0	1.0	1.05	0.99	0.71	0.91	0.81	Ammonia (mg/l) NH ₃
	0.50	0.50	0.61	0.63	0.41	0.53	0.42	Ammonium (mg/l) NH ₄
	5.0	3.0	0.81	1.00	1.11	1.21	0.91	Zinc (mg/l) Zn
	250	250	3.85	5.75	3.20	5.55	6.05	Chloride (mg/l) Cl
	1.50	1.50	0.42	0.59	0.46	0.67	0.69	Fluoride (mg/l) F
	1.0	1.0	0.31	0.51	0.81	0.81	0.61	Copper (mg/l) Cu
	200	200	1.07	1.15	0.89	2.11	2.40	Sodium (mg/l) Na
	100	200	2.10	3.15	2.50	3.45	4.50	Potassium (mg/l) K
	100	200	20.8	18.4	22.1	32.5	28.1	Sulphate (mg/l) SO ₄
	100	200	13.5	15.3	19.4	14.6	11.4	Phosphate (mg/l) PO ₄

461

462

Key: P₁= Parliamentary Stream before the Bridge, P₂= Parliamentary after the SEMATEC, Ny=NyakAsang Stream, Us=Unicem Spring, St= Satellite Town Stream

463

Table 5: Summary of the Result of Physico-Chemical Analysis of Borehole Water in Calabar Municipality

NIS	WHO	BH ₅	BH ₄	BH ₃	BH ₂	BH ₁	Parameters/units
Clear	Clear	Clear	Clear	Clear	Clear	Clear	Appearance
15	20	<5.0	<5.0	<5.0	<5.0	<5.0	Colour (pt Co)
20-30	20-30	29.8	29.5	30.3	27.9	29.3	Temperature (°C)
6.5-	6.5-	4.63	4.30	4.44	4.13	4.37	pH
5.0	5.0	0.42	0.20	0.27	0.17	0.19	Turbidity (NTU)
500	500	29.1	36.2	29.0	79.8	22.4	Conductivity (µS/cm)
14	14	14.1	13.1	14.1	14.1	11.2	Dissolved Oxygen
1000	1000	17.4	21.7	17.4	47.8	13.4	Total dissolved solids (mg/l)
0.30	0.30	0.21	0.14	0.70	0.23	0.19	Iron (mg/l) Fe
150	500	17.1	17.1	17.1	17.1	17.1	Total hardness (mg/l)
-	400	6.69	6.82	6.84	6.74	6.89	Total alkalinity (mg/l)
0.20	0.05	0.03	0.02	0.03	0.04	0.03	Manganese (mg/l) Mn
100	20	10.4	10.6	10.3	9.80	9.25	Magnesium (mg/l) Mg
-	50	6.85	6.55	7.15	7.30	8.15	Calcium (mg/l) Ca
50	45	4.30	5.25	4.40	5.55	6.35	Nitrate (mg/l) N
0.2	0.1	0.04	0.05	0.03	0.03	0.06	Nitrite (mg/l) N
1.0	1.0	0.61	0.51	0.71	0.60	0.61	Ammonia (mg/l) NH ₃
0.50	0.50	0.05	0.03	0.05	0.03	0.04	Ammonium (mg/l) NH ₄
5.0	3.0	0.82	0.71	0.75	0.81	1.07	Zinc (mg/l) Zn
250	250	2.35	2.30	2.05	4.25	5.80	Chloride (mg/l) Cl
1.50	1.50	0.56	0.17	0.28	0.64	0.35	Fluoride (mg/l) F
1.0	1.0	0.12	0.13	0.11	0.13	0.42	Copper (mg/l) Cu
200	200	0.78	0.98	0.84	1.03	1.15	Sodium (mg/l) Na
100	200	1.35	1.80	2.70	1.45	2.75	Potassium (mg/l) K
100	200	5.25	4.85	6.05	4.25	5.65	Sulphate (mg/l) SO ₄
100	200	3.05	2.65	4.75	3.70	4.30	Phosphate (mg/l) PO ₄

464

465

Key: Bh₁=EdimOtop, Bh₂=IkotOmin, Bh₃=IkotEnebong, Bh₄=Ekosin Junction, Bh₅=NyakAsang

466 **Mean Bacteria Count for Sampled Bottled and Sachet Water**

467 The mean faecal count and total coliform bacteria count per 100ml of sampled water were
468 obtained from five different brands of bottle and sachets water collected in duplicate and the
469 mean result is presented in Table 6. The mean ranged for total coliform bacteria for sampled
470 bottled water ranged from 2 *cfu/100ml* to 19*cfu/100ml* and no coliform was detected. The total
471 coliform count for sachets water ranged from 6*cfu/100ml* to 15*cfu/100ml* and zero for faecal
472 coliform count. The zero faecal coliform shows that they have met the World Health
473 Organization standard for drinking water (\leq zero*cfu/100ml*). The summary of this result is
474 presented in Table 6.

475

476 **Mean Bacteria Count for Sampled Public Water**

477 Table 7 shows the bacterial count for sampled public water (tap), there was no growth on the
478 faecal and total coliform plates after 48 hours of incubation at appropriate temperature (35°C and
479 44±0.5°C) respectively. This result is satisfactory and it complies with the international standard
480 for drinking water set by the World Health Organization.

481

482 **Mean Bacteria Count for Sampled Stream Waters**

483 Table 8 shows the mean plate count for stream waters. The total and faecal coliform count per
484 millimeter (ml) of water obtained from serial dilution of sample to power 1 (10^{-1}). The samples
485 taken in duplicate from five different location in Calabar Municipality. The result ranged from
486 27×10^1 *cfu/ml* to 55×10^1 *cfu/ml* for total coliform and 15×10^1 *cfu/ml* to 52×10^1 *cfu/ml* for faecal
487 coliform (Satellite town) stream had the highest faecal coliform count, NyakAsang stream had
488 the least faecal coliform count. The highest total coliform count was from Unicem stream and the
489 least from NyakAsang stream.

490

491

492 **Mean Bacteria Count for Borehole Water**

493 Borehole water sampled from five different boreholes in different location in the study site. The
494 result ranged between 12 *cfu/100ml* to 33*cfu/100ml* for total coliform and 9*cfu/100ml* to
495 16*cfu/100ml* for faecal coliform. Ekosin junction (BH₄) had the highest value of total coliform
496 and IkotOmin (BH₂) with the least, while Nyakasang borehole (BH₅) and EdimOtop Street (BH₁)

497 had the lowest and the highest value respectively for faecal coliform. A summary of this is
498 presented in Table 9

499

500 **Characteristic and identification of bacterial isolates**

501 Based on the morphological and biochemical characteristics of bacteria isolated from the water
502 sources as presented in Table 10. The following bacteria were isolated, *Escherichia coli*,
503 *Enterobacter spp*, *Chromobacterspp*, *Proteus spp*, *Pseudomonas spp* and *Streptococcus spp*.

504

505 **Percentage Occurrence of Bacteria Isolate from Drinking Water**

506 The organism with the highest occurrence in the studied water sample *Pseudomonas spp*.23.5%,
507 followed by the *Proteus spp*.and *Escherichia coli* with 20.5%, *Streptococcus spp*.14.7%,
508 *Enterobacter spp*.8.8%, *Chromobacter spp*.2.9%, *Salmonella spp*2.9% and *Enterococcus*
509 *spp*.2.9%, stream water had the highest bacteria and bottled water with the least bacteria (2)
510 presented in Table 11.

511

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513

514

Table 6: Mean Bacterial Count for Bottled and Sachets Water Sample

Samples	Total Coliform Counts <i>cfu/100ml</i>	Feecal Coliform Counts <i>cfu/100ml</i>
Ev bottled water	4	-
Cw bottled water	3	-
Ne bottle water	2	-
Aq bottled water	8	-
Qu bottled water	19	-
Sachets		
UTWa	-	-
UTW	-	-
ATW	15	-
CW	10	-
ZTW	5	-

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Table 7: Mean Bacterial Count for Public (tap) Water Sample

Samples	Total Coliform Counts	Feecal Coliform Counts
	<i>cfu/100ml</i>	<i>cfu/100ml</i>
Wb1	–	–
Wb2	–	–
Wb3	–	–
Wb4	–	–
Wb5	–	–

520

521 **KEY:** Wb₁=EdimOtop Street, Wb₂=Orok Street off Ediba, Wb₃=EdimOtop Close West,522 Wb₄=Marian Road, Wb₅= IkotEfa Street, NG=No Growth

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Table 8: Mean Bacterial Count for Stream Water Sample

Samples	Total Coliform Counts $10^1 cfu/ml$	Feecal Coliform Counts $10^1 cfu/ml$
P ₁	49	25
P ₂	53	30
Ny	27	15
Us	55	28
St	43	52

533

534 **Key:** P₁=Parliamentary Stream before the Bridge, P₂= Parliamentary after the SEMATEC,

535 Ny=NyakAsang Steam, Us= Unicem Spring, St= Satellite Town Stream

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Table 9: Mean Bacterial Count for Borehole Water Sample

Samples	Total Coliform Counts <i>cfu/100ml</i>	Feecal Coliform Counts <i>cfu/100ml</i>
BH ₁	30	16
BH ₂	12	10
BH ₃	23	14
BH ₄	33	14
BH ₅	21	9

546

547 **Key:** Bh₁=EdimOtop, Bh₂=IkotOmin, Bh₃=IkotEnebong, Bh₄=Ekosin Junction, Bh₅=

548 NyakAsang

549

550 **Table 10: Summary of Morphological and Biochemical Characteristic of Bacteria Isolated from Drinking Water**

Cultural Characteristics	Gram's Reaction	Shape	Motility	Citrate	Indole	Oxidase	Methyl Red	VogesProskauer	Catalase	Lactose	Glucose	H ₂ S	Probable Organisms
Irregular, swarming and colourless	-	R	+	+	+	-	+	-	+	-	AG	-	<i>Proteus spp</i>
Creamy, circular, convex, smooth and moist	+	C	-	+	NR	NR	NR	NR	+	-	A	-	<i>Streptococcus spp</i>
Pale yellow, circular, convex and smooth	-	R	+	-	-	-	-	+	+	+	AG	+	<i>Enterobacter spp</i>
Translucent, moist and spreading	-	R	+	+	+	-	+	-	+	-	A	+	<i>Pseudomonas spp</i>
Pink, irregular, raised, moist, shiny	-	R	+	-	+	-	+	-	+	+	AG	-	<i>Escherichia coli</i>
Blue, round, circular, smooth	-	R	-	-	+	+	-	-	+	-	G	+	<i>Chromobacters pp</i>
Colourless and transparent	-	R	+	+	-	-	+	-	+	-	AG	+	<i>Salmonella spp</i>
Tiny pink and wrinkled	+	C	+	-	-	-	-	+	+	+	AG	-	<i>Enterococcus spp</i>

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552 **Key:** (+) positive, (-) negative, (NR) not relevant, (R) rod, (C) cocci, (A) acid fermentation, (G) gas production,

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554

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Table 11: Summary of Frequency of Occurrence of Bacteria Isolated From Drinking Water

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Probable Organism	Bottled Water	Borehole Water	Public Water	Sachets	Streams	% Occurrence
<i>Proteus spp.</i>	+	+	-	+	+	20.5
<i>Streptococcus spp.</i>	-	+	-	+	+	14.7
<i>Enterobacter spp.</i>	-	-	-	+	+	8.8
<i>Pseudomonas spp.</i>	+	+	-	+	+	23.5
<i>E. coli</i>	-	+	-	-	+	20.5
<i>Chromobacter spp.</i>	-	-	-	-	+	2.9
<i>Salmonella spp.</i>	-	-	-	-	+	2.9
<i>Enterococcus spp.</i>	-	+	-	-	-	2.9

557

558

Key:

-

(not

present),

+

(present)

559 Qualitative drinking water supply is often a major challenge to many developing countries
560 including Nigeria. In this study, the Physico-chemical and bacteriological quality of drinking
561 water sources in Calabar metropolis, Nigeria was investigated by collecting five different
562 sources, they include; Borehole, Streams, Public water (tap), bottled and Sachets waters.

563 The findings in the study of all the evaluated water sources have temperature within the
564 normal range that is in conformity with WHO (2011) standard and NIS. In a study reported by
565 Itah *et al.*, (2005), the temperature range of all the public water supplies investigated was within
566 the normal range.

567 Temperature is one of the most essential parameters in water. It has significant impact on
568 growth and acidity of ecological life and greatly affects the solubility of oxygen in water. When
569 the temperature is high the pH of the water will change thereby favouring the growth of some
570 organisms. The pH of the water sampled compared to the standards was with the specific range.
571 The turbidity of the water was within the standard except that of stream which was above the
572 standard. The ability of the sampled water to be able to conduct electricity has no health
573 implication on humans or organisms whether it is high or low like it is observed in this study.
574 Dissolved oxygen and Total dissolved solid of the five sampled water compared to the standard
575 were within the acceptable limit prescribed. Similarly the Iron concentrations of the studied
576 sample were within the normal limit and are in conformity with the findings of Okorafor *et al.*,
577 2012.

578 Total Hardness is caused by the presence of calcium and magnesium salt, all the water
579 samples have their values within the permissible limits. The quantitative capacity of an aqueous
580 media to react with H^+ ions (*i.e.* Total Alkalinity) for all sampled water were all within the limits.
581 The health implication for manganese if high causes neurological disorder and the study reveals
582 that they are all within the standard acceptable limit. Magnesium concentration of the water
583 sampled, as one of the causes of water hardness were within the Nigerian Standard and few
584 where within the World Health Organization Standard. Calcium is one of the compounds that
585 cause hardness of water if present. Their concentrations compared to the standards were all
586 within ranges for all the sampled water. The concentration of the different forms of nitrogen
587 (nitrate, nitrite, ammonia and ammonium) gives a useful indication of the level of micro-
588 nutrients in the water and hence the ability to support plant growths. The results obtained were
589 compared with the standard.

590 Nitrate concentration for all sampled water was within the acceptable limits that of Nitrite
591 also but, that of Unicem spring was slightly high above the limit, Thus high concentrations of
592 nitrite can cause cyanosis in infants less than 3months (NIS, 2007). Ammonia and Ammonium as
593 the derivatives of Nitrogen enters the water body through the organisms that survives or by
594 sewage effluent and runoff from land where manure has been applied or stored in the case of
595 surface water like stream that shows little slight vibration in the studies but still within the range
596 of acceptable limit. Zinc and copper are also a trace element that is needed in our system, but
597 excess amount of copper will cause gastrointestinal disorder (i.e. when it exceeds the limited
598 standards). Chloride are common constituents of all natural waters, higher value of it impacts a
599 salty taste to the water, making it unacceptable for human consumption.

600 The chloride value in this study is within the acceptable range. Fluoride is essential for
601 human beings as a trace element, higher concentration of this element causes toxic effect (Dental
602 Fluorosis), but a small amount of fluoride protects tooth decay and enhances bone development
603 (Kunduet *al.*, 2001). Sodium, Potassium and Sulphate were all within the acceptable range given
604 by the International and National standard. A high concentration of sulphate may induce diarrhea
605 and intestinal disorder.

606 Phosphate in water occurs in the form of orthophosphate, polyphosphate (Katariaet *al.*,
607 2011). This study reveals that all the sampled water was all within the acceptable limits that was
608 given by the standard and also fit for human consumption.

609 The result of the bacteriological analysis of the five sources of water sampled revealed
610 the unsanitary condition of most drinking water with some contaminated with coliforms and
611 pathogenic bacteria. This finding is similar to that reported by Adekunle *et al.*, (2004) in
612 Ibadan, Itahet *al.*, (2005), Ezeugwune *et al.*, (2009) in Nnewi, Oladipo *et al.*, (2009) in
613 Ogbomoso, Adegoke *et al.*, (2012). Similarly, Ademoroti (1996), which showed the presence of
614 some bacteria in sachet water, borehole water, stream water respectively while Zvidzai *et al.*,
615 (2001) in the study of microbial community analysis of drinking water sources from rural areas
616 of Zimbabwe detected faecal coliform and *Escherichia coli* which were attributed to poor
617 treatment handling of water. Some studies however have revealed that coliform bacteria are
618 widely found in nature and do not necessarily indicate faecal pollution (Binnie 2002, Griffith *et*
619 *al.*, 2003).

620 Adegoke *et al.*, (2012) observed that the presence of bacteria in some branded water like
621 bottle and sachet water could be as a result of poor environmental condition, poor treatment and
622 handling methods in processing industries. Others sources of possible contamination enumerated
623 includes; poor handling by distributors and seller, insufficient sterilization of the sachets and
624 bottles used in packaging the water, contamination with bacteria of the vending machine use in
625 packaging and the duration of the packaged (branded) water.

626 The study reveals that out of the five different brands of bottle water randomly selected
627 none had faecal coliform but all the five brands had total coliform, despite all having NAFDAC
628 approved numbers. NAFDAC standard states that total and faecal coliform levels must be
629 *zero cfu/100ml*. This means that the contaminants might not be of faecal origin but, it may be due
630 to some environmental factor (production, machine, staff or the container (Kolawole, 2009).

631 Adekunle, (2004) stated that the consumption of bottled water is increasing rapidly in
632 developing countries especially among the middle and high income earners as it is generally
633 perceived to be pure, clean and of good quality. This has led to the sales of different brands of
634 bottled water in the study area. Although disease outbreaks due to contaminated bottled water are
635 rare, bottled water has been found to cause traveler's diarrhea.

636 In this study all the samples investigated had zero faecal coliform count. Only 40%
637 (2/5) out of the 5 brands had zero total coliform count. As useful as sachets water is to the
638 society, the results of the analysis raised doubts as to its quality says Adekunle *et al.*, (2004).

639 The findings of no faecal and total coliforms in all the sampled public water is in line
640 with the international and Nigeria standard of drinking water (\leq *zero cfu.100ml*). Kolawole,
641 (2009) in the chemical and bacteriological quality of drinking water in Calabar municipality
642 reported no faecal and total coliform in public water.

643 The analyses of borehole water samples shows that none of the samples were portable for
644 drinking with the level of faecal and total coliforms which are observed from this study. This is
645 pathetic because borehole water is the most accessible source of drinking water in the study area.
646 The presence of faecal coliform suggests faecal contamination and the possible presence of
647 pathogenic bacteria like *Salmonella typhi* (Itah *et al.*, 2005). The result agrees with the earlier
648 reports by Itah *et al.*, (1996) in the study of bacteriological characteristics of rural water supply in
649 Calabar and that of Agbuet *et al.*; (1998) in Samaru, Zaria as well as Adesiyen in Katsina in terms
650 of high density coliforms obtained.

651 The faecal coliform and total coliform of streams water samples around the study area
652 was very high above the standard set for untreated water samples ($10cfu/100ml$). This is probably
653 due to contamination as this water flows from the source down to the fetching point and the
654 various activities (washing and bathing) being carried out by residents of the area. The analysis
655 also revealed the presence of *Proteus* species, *Streptococcus*spp, *Enterobacter* spp,
656 *Pseudomonas* spp, *Escherichia coli*, *Chromobacters*spp, *Salmonellas*spp and *Enterococcus* spp.
657 These organisms are of public health importance. Most organisms obtained from the drinking
658 water sources in Calabar have earlier been reported in drinking water sources in Calabar. Edema
659 *et al.*,(2001) reported that the presence of *Enterobacter* and *Proteus* species in water samples
660 suggest that these organisms could originate from burst pipes along distribution lines of drinking
661 water plant used in production of such water *Pseudomonas* species whose presence is of
662 significant value in determining the extent of water sample implies faecal pollution dating to
663 remote period (Itahet *et al.*, 2005).

664 The physical condition of an environment is one of the factors that can contribute to the
665 state of our drinking water supplies. In this environment of study (especially the stream
666 surrounding in satellite town) was dirty and bushy. Some people use it as dumping site which
667 may have contributed to the highest number of coliforms recorded in stream water sample. The
668 zero faecal coliform from public water sample and some brands of packaged water maybe due to
669 the routine treatment of their sources. This study does reveals that public water supply meets the
670 WHO standard for drinking water (\leq zero $cfu/100ml$).Schlegel, (2002) reported that *Enterobacter*
671 *spp* isolated from the water samples are example of non faecal coliforms and can be found in
672 vegetation and soil which serves as sources by which pathogens enter the water The British
673 Standard Institute specified that counts greater than 10^4 are considered unsatisfactory for
674 *Enterobacter* spp.

675 The presence of total coliforms and faecal coliform, *E.coli*, *Salmonellas*spp, *Shigellas*spp
676 and *Vibros*spp have been documented as criteria for evaluating drinking water and as a standard
677 for protecting the public by limiting the levels of contaminants in drinking water (EPA, 2002).

678 The result of statistical analysis of each analyzed parameter and the value of F test and
679 significance are presented in the tables above. The calculated F value were observed in the range
680 of 0.000-0.956, the F critical is 5.19 for stated level of confidence (typically 95%) which mean

681 that the difference being tested are statistically significant (*) and non- statistically significant
682 (**) at 95% confidence level.

683

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CONCLUSION

685 This study has revealed the unsanitary state of some drinking water consumed in this part
686 of the country under study. As most samples contain bacterial indicators of faecal pollution as
687 expected in streams. However the presences of bacteria in some brand of bottled, sachet and
688 borehole water sampled were not expected. This is pointer to the poor quality standard of some
689 waters that are being consumed by the inhabitant of Calabar Municipality. Tap water regulation
690 makes it mandatory that the public water supply is tested daily and that findings are freely
691 available for scrutiny. While most bottled and sachets water are safe, their bacteria contents
692 means that they are not as safe as tap water. The boreholes in the study area are not regulated or
693 monitored by any regulatory body, based on these findings. It has been known from this research
694 that the quality of drinking water sources in Calabar Municipality is poor and the majority of the
695 residents do not treat water. There should be awareness on effective household water treatment
696 on how to treat and maintain the microbial quality of water at the household level.

697

698

699 **RECOMMENDATIONS**

- 700 i. The Cross River State Water Board (CRSWB) Management should ensure periodic
701 check on their pipe line to avoid leakages and prevent future contamination of this
702 source of drinking water being the best in this study.
- 703 ii. They should also carryout proper enumeration, registration and regulation of boreholes
704 in the study area to enable periodic examination of this source of drinking water.
- 705 iii. CRSWB should make pipe borne water available and affordable for all residents of
706 Calabar Municipality, Nigeria.
- 707 iv. Due to poor quality of packaged drinking water (National Agency for Food Drugs
708 Administration and Control) NAFDAC should
- 709 a) Undergo periodic and regular visit to the packaged water factories for re-assessment of
710 their GMP not less than 4 times yearly.
- 711 b) Undergo periodic re-testing by randomly sampling and analyzing packaged water
712 being produced to ascertain if the quality still meet.
- 713 v. Water treatment by individual or household should be encouraged by government
714 through intensive campaign by workshops and seminar on the importance of water
715 treatment before use.

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REFERENCES

- 719
- 720 Adegoke, O. Adebayo, Bamigbowu, E. Olugbenga, Oni, E. Sunday, Ugbaja K. Nchedo. (2012).
721 *Microbiological Examination of Sachet water sold in Aba, Abia State, Nigeria. Global*
722 *Research Journal of Microbiology 2 (1): 062-066.*
723
- 724 Agbo, B. E. and Mbotto, C. I. (2012). Phytochemical and Antibacterial Evaluation of Selected
725 Locally Produced Herbal Medicines Sold in Calabar, Nigeria, *Archives of Applied Science*
726 *Research, 4 (5):1974-1990*
727
- 728 APHA- AWWA (1998) Standard Methods for the Examination of Water and Wastewater, *19th*
729 *Edition. American Publication Health Association, Washington,.pp.136.*
730
- 731 Catwright R.Y., Dadswell, I. V and Lewis M.J. (1993) .Laboratory investigation. The numbers
732 games. In Dawson A, west P (Eds). *Drinking water supplies. England, crown, pp22, 36.*
733
- 734 Cheesbrough, M. (2000).Bacteriological Testing of water in:*District laboratory practice in*
735 *tropical countries, Part 1,University Press, Cambridge, Pp 57-61.*
736
- 737 Cosgrove W. J. and Rysberman, F. R. (2000). World Water Vision: Making Water everybody's
738 Business. *Earth scan Publication Ltd; UK pp.108.*
739
- 740 Dada, O. O, Okufo, C.A and Obele, E (1990a). Faecal Pollution of Well Water in Zaria City,
741 Nigeria. *Savannah. 10; 1-5.*
742
- 743 Dada, O. O, Okufo, C. A and Yusuf, Z (1990b). The Relationship between Residual Chlorine
744 and Bacteriological Quality of Tap Water in the Water Distribution System of Zaria,
745 Nigeria Savannah. *10(2), 95-101.*
746
- 747 Edema, M. O, Omemu, A. M, and Fapetu, O. M (2001). Microbiological and Physico-Chemical
748 Analysis of Different Sources of Drinking Water. *Nigeria Journal of Microbiology. 15:57-*
749 *61.*
750
- 751 Emde, K.M.E., Mao, H. and Finch, G. R. (1992). Detection and occurrence of water borne
752 bacterial and viral pathogens. *Water environs. Res, 64:643*
753
- 754 Eja, M. E (2002). *Water Pollution and Sanitation for Developing Countries*, Seaprint Nigeria
755 Publishers, Calabar. Pp71.
756
- 757 Feachem R.(1975).An Improved Role for Faecal Coliform to Faecal Streptococci Ratios in the
758 Differentiation between Human and Non-Human Pollution Sources. *Water Res; 689-90.*
759
- 760 Itah, A. Y. Etukudo, S. M and Akpan, E. J. (1996). Bacteriological and Chemical Analysis of
761 Some Rural Water Supplies of Calabar. Nigeria. *West Africa Journal in Biological and*
762 *Applied Chemistry. 40:1-10.*
763

- 764 Lamikara, A. (1999). Essential microbiology for students and practitioners of pharmacy,
765 medicine and microbiology 2nd edition. *Amkra books*. 406p.
766
- 767 Lee, R. J. (1991). The Microbiology of Drinking Water. *Med. Lab. Set*, 48: 303-13.
768
- 769 Nikoladze, G.D.M and Akastal, S. (1989), Water Treatment for Public and Industrial Supply. *M I*
770 *R Publisher, Moscow*, pp 163.
771
- 772 Okorafor, K. A, Agbo, B. E, Johnson, A. M and Chiorlu, M (2012). Physico- Chemical and
773 Bacteriological Characteristics of Selected Streams and Boreholes in Akamkpa and
774 Calabar Municipality, Nigeria. *Achieves of Applied Science Research*, 4 (5):2115-2121.
775
- 776 Okonko, I. O., Ogunjobi, A. A, Adejoye, A. D, Ogunnusi, T. A and Olasogba, M.C. (2008).
777 Comparative Studies Used for Risk Assessment of Different Water Samples used for
778 Processing Frozen Sea Foods in Ijora-Olopa, Lagos State, Nigeria. *African Journal of*
779 *Biotechnology*, 7(16)2902 – 2907.
780
- 781 Shittu, O. B; Olaitan J. O and Amusa, T. S. (2008). Physico-Chemical and Bacteriological
782 Analysis of Water Uses for Drinking and Swimming Purposes in Abeokuta, Nigeria.
783 *African Journal of Biomedical Research*, 11: 258-290.
784
- 785 Umeh, C. N., Okorie O. I., and Emesiani, G. A (2005). Toward the provision of safe drinking
786 water. The Bacteriological Quality and Safe of Sachet Water in Akwa, Anambra State. In
787 the Book of Abstract of the 29th Annual Conference and General Meeting of Microbes. *As*
788 *Agents of Sustainable Development Organized by Nigeria Society for Microbiology*
789 *(NSM). University of Agriculture, Abeokuta*, pp22.
790
- 791 UNICEF (2005), *national rural water supply and sanitation investment programme. Final draft*,
792 *pp1*.
793
- 794 World Health Organization:(1993). *Guidelines for Drinking Water Quality. vol 1.*
795 *Recommendation. 2nd ed. Geneva. WHO*.
796
- 797 World Health Organization: (2003a). Water for Health Enshrined as a Human Right. *WHO:*
798 *Geneva [cited 2004 Nov 18]*.
799
- 800 World Health Organization: (2003b). The World Health Report 200,. Shaping the Future.
801 *Statistical Annex. Geneva*.
802
- 803 World Health Organization (2004). *Water Sanitation and Health Programme. Managing Water*
804 *in the Home: Accelerated Health Gains from Improved Water Sources. WHO.*
805 www.who.int.
806
807