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Physiochemical and bacteriological assessment of selected water sources in Calabar

R. U. B. EBANA, C. A. ETOK AND J. E. ALIKE

Department of Biological Sciences
University Of Calabar - Nigeria

ABSTRACT - Selected water sources were studied over a six-month period to ascertain their level of pollution. The water sources were Uwanse stream, Ikot Anwatim stream and Essien town tap water reservior. Results indicated that the physiochemical aparmeters BOD_5, Silica, and pH were positively correlated with indicator bacteria (Escherichia coli, Streptococcus faecalis and Klebsiella species with counts reaching a maximum of 520 faecal coliforms/100 mls in October in Uwanse stream. The importance of periodic monitoring of our water sources in order to prevent outbreaks of water borne diseases is discussed.

KEY WORDS: Physiochemical, Bacteriological assessment, water sources, periodic monitoring

INTRODUCTION

In the developing nations of the world, the water sources of a community are often polluted through rain run off and sewages, thereby increasing the load of chemical pollutants and dangerous micro-organisms in these waters, thus posing serious health hazards.

According to WHO 1971, potable waters are considered to be bacteria-free and recommended amount for physiochemical parameters have been set out. Ibok (1989) reported that a number of factors including light, nutrient concentration, pH, turbidity and temperature affect the bacterial population in aquatic environment. Adamson (1971) also pointed out that ecological factors in one way or other make the population of organism in the ecosystem to fluctuate. This study has investigated the physiochemical parameters of selected water sources and their effect on coliform count.

MATERIALS AND METHODS

Sources of Water/Sampling Sites

The sampling sites are illustrated in the Calabar Map (Appendix I)

Uwanse Stream:

This is located in a large swampy region south of the University of Calabar. It is formed by a tributary of the Kwa River. The stream is about 4 metres wide and 10 metres long with an average depth of 0.6 metres. It is shallow and slow flowing . The stream is used both for recreational and domestic purposes.

Ikot Anwatim Stream:

It is located at about 5km from Ikot Ansa Bus Stop along Murtala Mohammed Highway, south of Eastern match company in a large swampy region. The water emerge from a storm of bamboo trees on a highland. It is used by the villagers for domestic purposes. It is about 5 metres wide, 20 metres long with an average depth of 0.5 metres.

Essien Tap Water:

It is located west of Calabar Cement Company while the treatment plant and the reservoir are located in a valley down the naval base. The reservoir is an open stream covered by forest. It is exposed to dust from the cement factory. About half of the population of Calabar depend on this source for domestic use, (personal communication).

Collection of Samples:

The water samples were collected in sterile 250ml sampling bottles fitted with a screw cap and covered with aluminium foil. In the case of tap water, sodium thiosulphate was added to the bottle to neutralize residual chlorine. The tap was sterilized before any sample was taken. The temperature of the water was recorded. Samples were transported to the laboratory within 30 minutes and were analysed within 24 hours. Sampling was done once in a month.

Culture Procedure:

The membrane filtration media plates were prepared according to standard procedures recommended in Report No. 71 (1969). Total coliform cultures were incubated at 37° for 24 hours in an anhydride incubator. *E coil* culture were resuscitated at 30° for 4 hours followed by

incubation at 44°C for 20 hours. Cultures for faecal streptococci were resuscitated at 37°C for 4 hours then incubated at 44°C for additional 44 hours.

Counting Techniques:

At the end of incubation periods, all yellow colonies (lactose fermenters) on membrane lauryl sulphate broth incubated at 37° were counted as total coliforms while those incubated at 44°C were counted as presumptive *E. coli.* Colonies appearing red on membrane Enterococcus Agar were counted as faecal streptococci.

Confirmatory Test:

Yellow colonies from membrane lauryl sulphate broth incubated at 37°C were subcultured into tubes containing lactose peptone water fitted with Durham's tubes. These were incubated 37° for 48 hours to confirm acid and gas production. Colonies showing positive results were confirmed as coliforms. This test was repeated for presumptive *E. coli* colonies on lactose peptone water and tryptone water. Incubation was at 44°C for 24 hours. Colonies producing acid and gas as well as being indole positive at 44°C were confirmed as *E. coli.* The red colonies on Slanetz and Bartley medium were sub-cultured on MacConkey agar. Incubation was at 37°C for 48 hours. The red colonies (faecal stretptococci) were subcultured onto lactose fermentation tube and positive test was indicated by red colonies.

Differential Tests:

Indole test, methyl red, voges proskauer, and citrate utilization, oxidase, catalase and capsule tests were carried out as outlined by Chessbrough (1994).

Total Alkanlinity:

This was determined by indirect titration using a modified procedure of Wathenberg and Griepenberg (1952) cited by Grasshoff (1978). Biochemical Oxygen Demand (BOD_5) and Dissovled Oxygen (DO):The method of Grasshoff (1978) was used, BOD_5 was calculated as the difference in Dissolved Oxygen initially with final dissolved oxygen after 5 days. pH: pH was determined using a Lovibond pH meter.

Total Hardness:

Fifty millilitres of the sample was pipetted into a 250ml conical flask. 2ml of NH_4OH/NH_4Cl buffer was added using pipette filler and 0.1 to 0.2g Eriochrome Black T/Nacl indicator mixture was added using a clean spatula. The solution was titrated immediately with standard EDTA, stirring continuously until the last reddish tinge disappears. The end point is indicated by a clear blue colour. The burette reading was used to determine the hardness of the sample.

Determination of Colour:

This was carried out using the Lovibond tintometre in which colour is measured in Hazen units.

RESULTS

Table 1 shows the relationship between physiochemical parameters and coliforms over a six – month period (October 1996 to March 1997). It can be demonstrated that pH, BOD_5 and silica show relationship with indicator bacteria whereas ammonia showed no relationship whatsoever. Generally, there was a drop in the bacterial count which reached its lowest in February while in the month of March, there was a rise in the bacterial count with the exception of samples from Essien tap water.

The result of the correlation between physiochemical parameters and indicator bacteria as shown in Table 2 reveals that out of the four parameters tested, ammonia did not show correlation $p(> 0.1)$ to indicator bacteria in any of the samples. Similarly silica and BOD_5 in, Uwanse and pH in Ikot Anwatim did not correlate with faecal streptococci ($P > 0.1$). All other parameters showed correlation.

It is to be noted that the correlation for ammonia was negative indicating that ammonia decreases with increasing bacterial population. BOD_5 and silica are positive, though they showed no correlation indicating that with increasing population of bacteria, the parameters also increase but at a very minimal rate.

Figures 1,2,3 and 4 show the relationship between indicator bacterial content and physiochemical parameters (Ammonia, BOD_5, pH and Silica) respectively. Indicator bacterial count increased with increasing BOD, pH and silica in all three water sources whereas ammonia concentration was inversely proportional to the population of indicator bacteria in all the water sources examined.

The ratio of faecal coliform (*E. coli*) to faecal streptococci (Streptococcus faecalis) is presented on Table 3. It was observed that in

TABLE 1: RELATIONSHIP BETWEEN PHYSIOCHEMICAL PARAMETERS AND POPULATION OF INDICATOR BACTERIA OVER A SIX MONTH PERIOD.

Parameters	Oct.	Nov.	Dec.	Jan.	Feb.	March
			A			
PH	7.8	8.0	7.6	7.6	6.4	7.2
Silica	13.0	11.6	7.2	6.4	5.5	8.61
BOD_5	4.8	4.5	3.6	3.4	2.9	3.2
Ammonia	0.02	0.05	0.09	0.20	0.31	0.18
Total coliform	2800	2850	1910	1200	980	1240
Faecal coliform	520	380	360	260	180	220
Faecal streptococcus	340	220	80	60	40	50
			B			
PH	7.9	7.4	7.5	6.2	7.2	6.5
Silica	17.4	14.1	15.2	12.42	13.56	11.5
BOD_5	4.0	3.5	3.8	2.5	3.2	3.0
Ammonia	0.04	0.18	0.09	0.35	0.24	0.3
Total coliform	2300	1080	2010	880	1400	970
Faecal coliform	420	300	190	120	100	100
Faecal streptococcus	180	110	80	40	60	50
			C			
PH	7.8	7.6	7.3	6.8	6.6	7.2
Silica	13.8	12.5	10.2	8.60	5.2	7.4
BOD_5	4.9	4.5	3.8	3.2	3.0	3.7
Ammonia	0.04	0.07	0.08	0.95	0.21	0.06
Total coliform	3550	3000	2500	1800	900	2200
Faecal coliform	520	340	310	250	220	320
Faecal streptococcus	210	160	90	60	50	70

A, Ikot'Anwatim; B, Essien; C, Uwanse

Bacterial counts are expressed as per 100 ml

TABLE 2: CORRELATION BETWEEN PHYSIOCHEMICAL PARAMETERS AND INDICATOR BACTERIA CONTENT ANALYSED FROM THREE WATER SOURCES FROM OCTOBER TO MARCH

	A			B			C		
Parameters	**TC**	**FC**	**FS**	**TC**	**FC**	**FS**	**TC**	**FC**	**FS**
PH	••• 0.99	•• 0.89	•• 0.92	• 0.81	0.78	NS 0.65	• 0.85	• 0.83	• 0.83
Silica	••• 0.96	• 0.83	NS 0.46	•• 0.90	• 0.83	•• 0.93	•• 0.91	•• 0.88	•• 0.89
BOD_5	••• 0.96	•• 0.92	NS 0.48	••• 0.97	•• 0.94	••• 0.96	• 0.87	• 0.78	• 0.82
Ammonia	NS -0.88	NS -0.72	NS -0.63	NS -0.93	NS -0.92	NS -0.80	NS -0.92	NS -0.80	NS -0.84

A, Uwanse; B, Ikot Anwatim; C, Essien tap.

TC, Total coliform; FC, Faecal coliform; FS, Faecal streptococci;

+ + + Highly significant; + +, Very significant; + Significant; NS, Not significant

TABLE 3: FAECAL COLIFORM AND FAECAL STREPTOCOCCI RATIOS OF VARIOUS WATERS OVER A 6 - MONTH PERIOD.

	WATER SOURCES		
MONTH	**Uwanse stream**	**Ikot Anwatim stream**	**Essien Tap**
Oct.	**2.48**	**1.53**	**2.3**
Nov.	**2.83**	**1.73**	**2.7**
Dec.	**3.4**	**4.5**	**2.4**
Jan.	**4.17**	**4.3**	**3.0**
Feb.	**4.4**	**4.5**	**2.7**
March	**4.57**	**4.4**	**2.0**

TABLE 4: CHARACTERIZATION AND IDENTIFICATION OF ISOLATES ON MEMBRANE FILTRATION MEDIUM

S/N	Colonial morphology	Indole	Catalase	M. R.	V. P.	Oxidase	Capsule	Citrate	Motility	Eijkman	Lactose	Glucose	Sucrose	Gram Reaction	Organism
1.	Large, yellow	+	+	+	-		-	-	+	+	AG	AG	AG	-ve	*Escherichia coli* type I
2.	Large, yellow	+	+	+	-	-	-	-	+	+	AG	AG	AG	-ve	*E. coli* type III
3.	Small, yellow	-	+	+	-	-	-	-	+	-	AG	AG	AG	-ve	*E. coli* type II
4.	Large, yellow	-	+	-	+	-	-	+	+	+	AG	AG	AG	-ve	*Enterobacter aerogenes*
5.	Small, yellow	-	+	-	+	-	+	+	+	+	AG	AG	A	-ve	*Citrobacter freundii*
6.	Large, yellow	-	+	+	+	-	+	+	-	-	A	AG	AG	-ve	*Klebsiella cloacae*
7.	Small, mucoid	-	+	-	+	-	+	+	-	+	A	AG	AG	-ve	*Klebsiella aerogenes*
8.	Small, yellow	-	+	+	-	-	-	+	+	-	AG	AG	AG	-ve	Intermediate type I
9.	Large, yellow	+	+	+	-	-	-	+	+	-	AG	AG	AG	-ve	Intermediate tyoe II
10.	Maroon (Red) on Slanetz and Bartley medium	-	-	+	+	-	-	+	-	+	A	A	A	+ve	*Streptococcus faecalis*

\+ Positive; - Negative; A Acid only; AG acid and gas; -ve Gram negative: +ve Gram Positive.

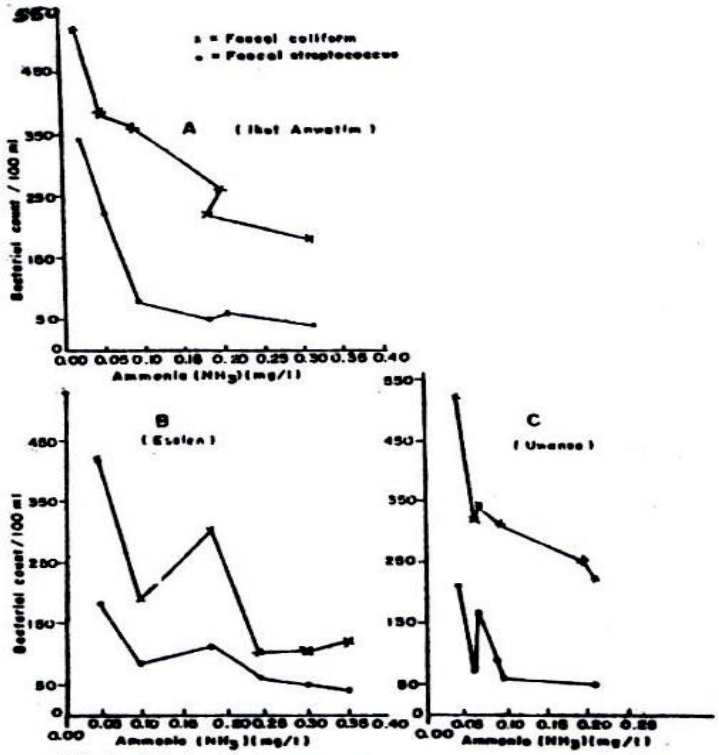

Fig. 1 : Relationship between Indicator Bacterial content and the concentration of Ammonia from Oct 1996 to March 1997.

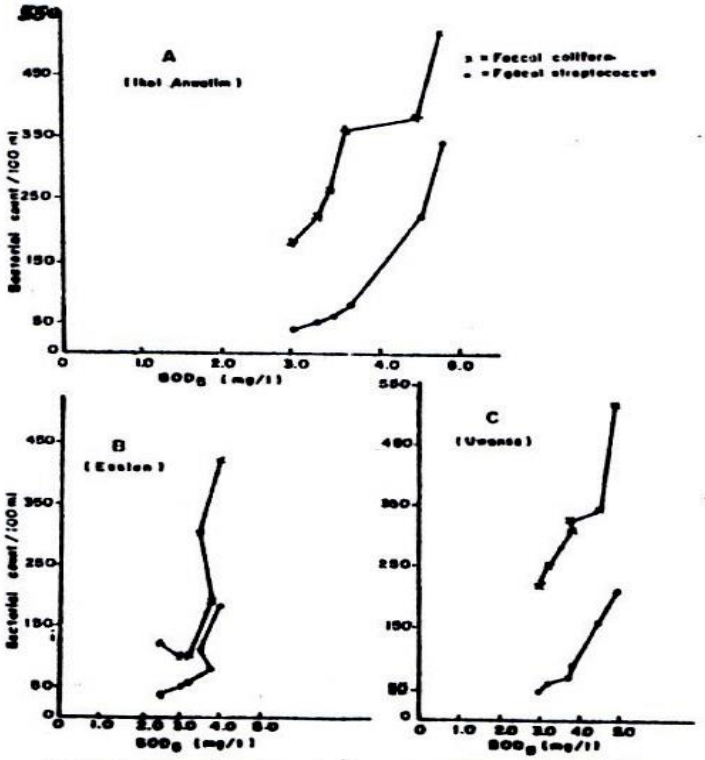

Fig.2 : Relationship between Indicator Bacterial content and BOD_5 of water sources from Oct. 1996 to March 1997.

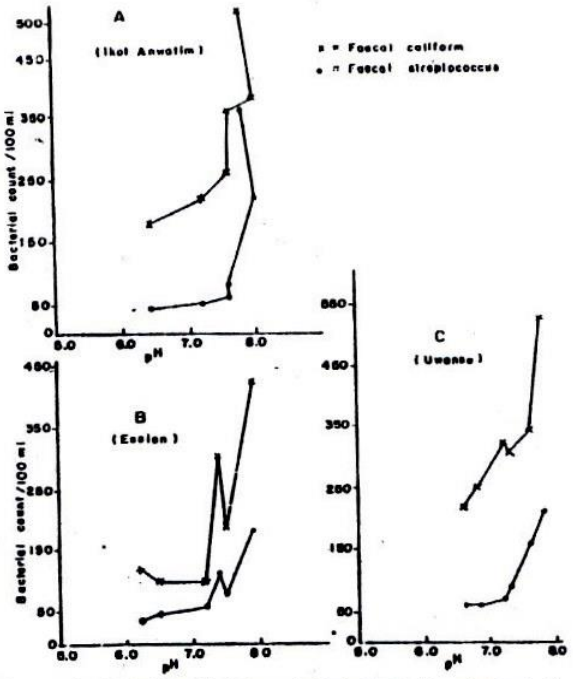

Fig. 3: Relationship between indicator Bacterial content and pH from Oct. 1996 to March 1997.

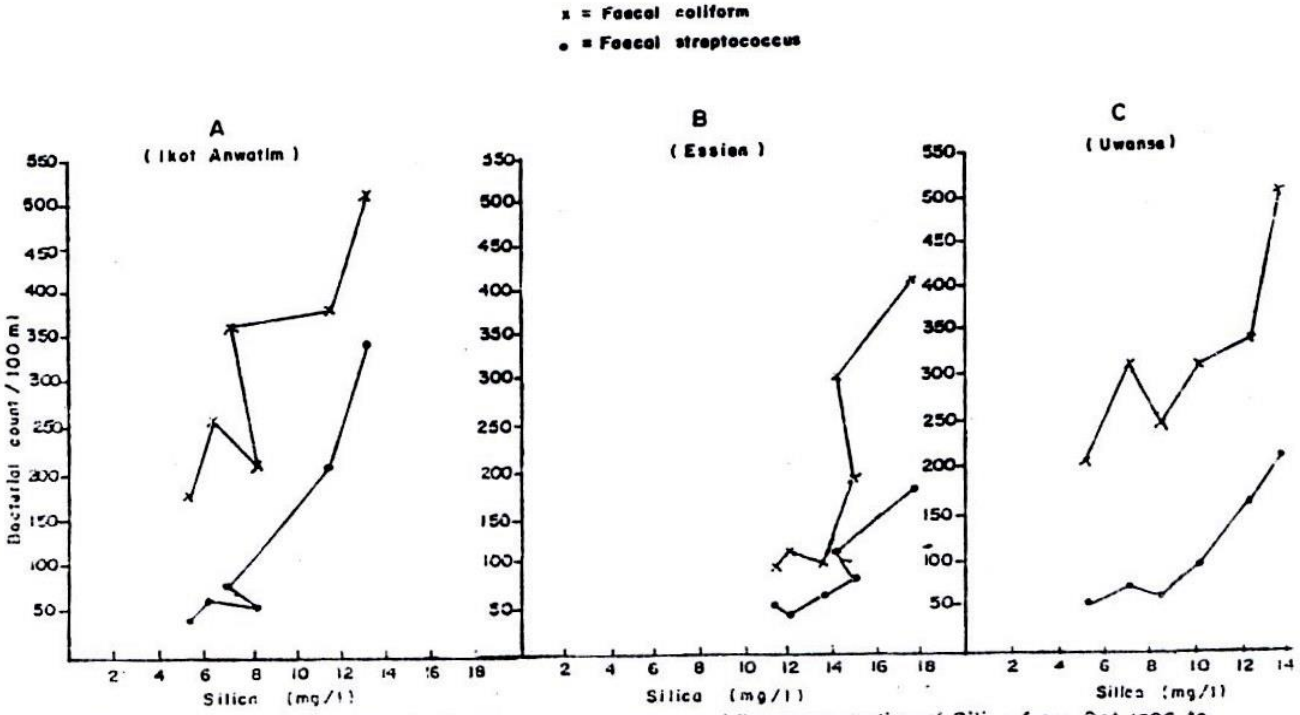

Fig. 4: Relationship between Indicator Bacterial content and the concentration of Silica from Oct. 1996 to March 1997.

Uwanse samples, the FC/FS ratio for the months of October, November and December were below 4, values above 4 occurred in Ikot Anwatim samples from January to March. The values for the months of October and November were 1.53 and 1.73 respectively, while from December to March, the values were above 4.

However in Essien samples the ratio were below 4 in all the months. The result of biochemical and physiological tests are shown in Table 4. Out of the 499 colonies tested, 161 were Eijkman negative. Eighty-two out of the Eijkman negative colonies belonged to the *E. coli* group. All the isolate were oxidase negative, and catalase positive. All except Klebsiella species and S. feacalis were motile. Klebsiella and Citrobacter were capsule positive. Furthermore, all isolates except S. faecalis were Gram negative.

DISCUSSION

. According to Kuznetsor (1970), a number of physiochemical parameters are responsible for the unstable population of indicator bacteria in aquatic environment. These factors include temperature, salinity, pH, dissolved oxygen, silica, nitrate etc. Results from this research have shown that indicator bacteria in water samples are closely correlated either positively or negatively with physiochemical parameters such as pH, BOD_5, silica and ammonia.

Silica serves as a nutrient for diatoms and plankton which in turn release dissolved organic carbon into the environment. The positive correlation between bacteria and silica might be used as an index of increased bacterial activity.

Ammonia is readily utilized by bacteria; the depletion of ammonia with increased bacterial count conforms with the report of Bell (1982). Results also confirms that an increase in the concentration of dissolved oxygen was inversely proportional to bacterial population and a corresponding decrease in BOD_5. (Babich 1980). This is because increased number facilitate the rate of degradation of organic matter thus depleting the dissolved oxygen content.

According to Geldreich (1970) a ratio of FC/FS greater than or equal to 4 indicate pollution by animal faecal matter. Thus the major source of contamination of Ikot Anwatim stream between October and November was by animal faecal matter (FC/FS ratios 1.53 and 1.73). From December to March, contamination was mainly by human faecal matter (FC/FS ratios 4.3 to 4.5). Contamination of Essien reservoir was mainly by animal faeces and this indicated either improper treatment of

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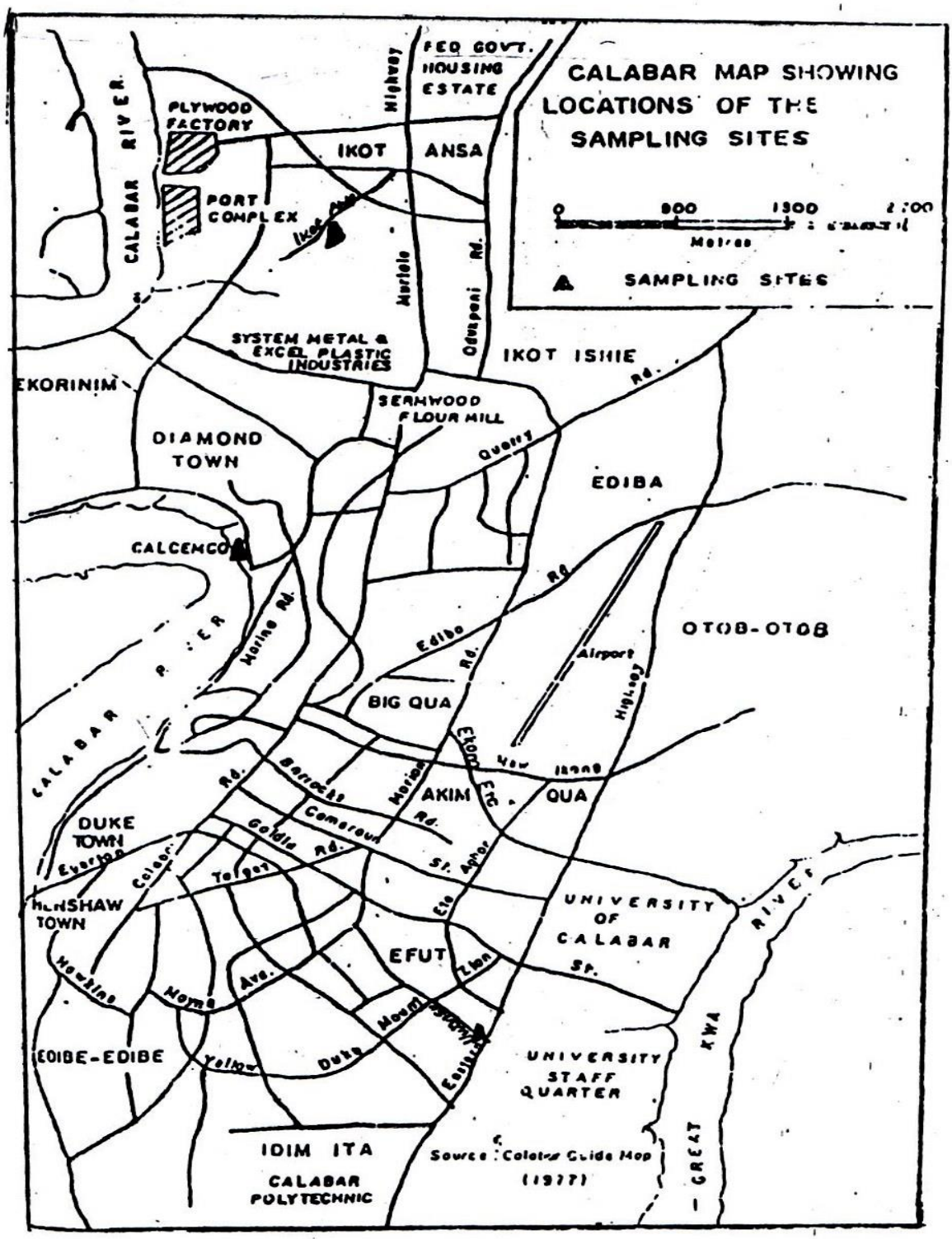

APPENDIX I: Map of Calabar showing the sampling sites