Research Article

PHYSICAL, CHEMICAL AND MICROBIOLOGICAL ANALYSIS OF IKOGOSI WARM SPRING IN NIGERIA

Adeyeye Emmanuel I*, Fagbohun Emmanuel D[+]
*Department of Chemistry and [+]Department of Microbiology, University of Ado Ekiti, P.M.B. 5363, Ado Ekiti, Nigeria.
***Corresponding author's E-mail:** eiadeyeye@yahoo.com

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ABSTRACT

Water and soil samples were taken for analyses from Ikogosi Warm Spring and for purposes of comparison analyses were made of the adjoining cold stream, mixture of warm and cold water and their intersect. All water samples were tasteless and odourless; all cold and warm had clear appearance; intersect was turbid as well as one of mixture samples; conductivity (µmS/cm) was low (0.5 x 100, cold; 1.5 x 100, warm), temperature was close (25-26, cold to 34-35^{O} C, warm). Alkalinity, hardness, total solid, PO_4^{2-} and SO_4^{2-} were all far below the WHO guideline values. NO_3^- range was 24-76 ppm, very much above the limit of 10 mg/litre of the WHO. Average ranges of the minerals in water were (ppm): Fe 3. 1-6.1, Zn 2.0-2.7, Mn 9.1-11.9, Cu (ND), Mg 7.9-21.0, Na 4.6-6.2, Ca 9.4-13.1, Pb (ND), K 3.0-9.2, Cr (ND), Cl 30.8-56.9, Ni (ND). Fe and Mn might affect the aesthetic value of the water. In soils, mineral ranges were (ppm): Fe 0.3-0.5, Zn 0.27-0.37, Mn 0.05-0.3, Cu 0.04-0.06, Mg 1.11-1.4, Na 1.19-1.4, Ca 0.87-1.3, Pb 0.22-0.29, K 1.13-1.5, Cr 0.03-0.08, Cl 0.007-0.02, Ni 0.03-0.044; the soils would serve as reservoirs for Pb, Cr and Ni. MPN in 100 ml of water ranged as follows: 4-250 (cold), 1-40 (warm), 1.0 (intersect), 12-1600 (mixture). Bacteria majorly found in water samples were *Escherichia coli* and *Aerobacter aerogenes* while the fungi in the soils were mostly *Aspergillus niger*. Water would be unsafe microbiologically for human consumption.

Keywords: Ikogosi Warm Spring, adjoining cold stream, Physico-chemical and microbiological analysis.

INTRODUCTION

The small town of Ikogosi-Ekiti in Ekiti State in Western Nigeria is situated between lofty, steep-sided and heavily-wooded, north-south trending hills about 27.4 km east of Ilesha (Osun State) , and about 10.5 km southeast of Effon Alaye (also in Ekiti State). It is located just north of the 7^{o} 35'N latitude and slightly west of the 5^{o} 00' E longitude. The elevation of the general area is between 457.0-487.5 m[1].

The Ikogosi Warm Spring is located about 1.61 km west of Ikogosi town. A Rest Camp has been built near the spring by the Baptist Mission. The mission has developed the spring for domestic use and constructed a swimming pool for recreational purposes[1]. At Ikogosi Warm Spring, warm and cold springs ooze out of hills from different sources, flow side by side and meet-the first of such occurrence in the whole world. The natural quiet environment is left untouched for eco-tourism appeal[2].

Natural water supply is very plenty in Ekiti State, which has several water schemes [Ero dam, Little Osse (Egbe) dam, Itapaji dam, Ado waterworks, Ikere artesian well and mini water schemes at Efon, Okemesi, Igbara-Odo and Ido-Ajinare] but none is working effectively owing to lack of electricity, obsolete equipment or inadequate water pipelines. Therefore, access to potable water is poor and this is the major cause of water-borne disease in Ekiti State of Nigeria. Access to potable water will improve health thereby reducing child and adult mortality and healthcare cost [3].

The 1991 National Census puts the population of Ekiti State at 1,647,822 [2]. At present, potable water production per annum is 20,000 M3 [3].

Rogers *et al.* [1] have described the geological setting of the spring and its physical and chemical properties discussed in relation to that of the surrounding rocks. A provisional check list of organisms inhabiting the spring is also included. In this present study, samples of soil sediments and water were taken for comparative analyses at three different spots for the cold stream, four different spots for the warm spring, one spot for the intersect of warm and cold water; and three spots for the mixture. Parameters evaluated for were physico-chemical characteristics of the water samples, mineral levels of water and soil sediments, estimated number of bacteria of the coilform group present in 100 ml of water sample, and isolated micro-organisms (bacteria and fungi) from water and related cold and warm soils. The spring was selected for study because of the interest which geochemists, food chemists, ecologists, and the public show towards such environments. Further, a great number of people within and outside Ikogosi Ekiti still consume the spring water untreated because it is believed that the water has a lot of therapeutic properties such as ability to cure hypertension, guinea worm, hook worm, kidney stone, rheumatism, body rashes and pimples by either drinking it or bathing with it. The warm water is also used for other domestic chores.

MATERIALS AND METHODS

Sampling and Sample Treatments:

Eleven different samples of water and underlying soil sediments (5.0 cm below the surface) were collected along the sampling sites (at intervals) which lied along the course of stream of both warm and cold water. Details of interval of sample collections are shown in Table 1. Figure 1 also shows the relative positioning of warm, cold and mixed water.

Table 1: Sampling information details

Code	Sample	Distance from F_1 (metres)*
A_1	Cold stream water	7.6
A_{11}	Cold stream soil sediment	7.6
A_2	Cold stream water	18.3
A_{22}	Cold stream soil sediment	18.3
B_1	Cold stream water	28.9
B_{11}	Cold stream soil sediment	28.9
B_2	Warm spring water	18.3
B_{22}	Warm spring bed soil	18.3
C_1	Warm spring water	32.0
C_{11}	Warm spring bed soil	32.0
C_2	Warm spring water	50.3
C_{22}	Warm spring bed soil	50.3
D_1	Warm spring water	108.4
D_{11}	Warm spring bed soil	108.4
F_1	Mixed region (intersect) water	0
F_{11}	Mixed region soil sediment	0
D_2	Mixed water	18.3
D_{22}	Mixed water soil sediment	18.3
E_1	Mixed water	30.5
E_{11}	Mixed water soil sediment	30.5
E_2	Mixed water	54.8
E_{22}	Mixed water soil sediment	54.8

*See Fig.1.

Water samples were collected with clean, sterile one litre wide mouthed plastic bottles previously leached with 1:1 HCl. The bottles were fitted with screw caps and the stopper and necks of the bottles were protected with aluminium foil. Bottles were rinsed with appropriate samples before being filled. The soil samples were collected into acid leached polyethylene bags. Appropriate labels are as shown in Table 1. The work was carried out under aseptic techniques.

The water sample temperature was taken immediately at the site of collection using a simple thermometer calibrated in ^{o}C; electrical conductivity was measured with a CDM 83 conductivity meter (Radio meter A/S Copenhagen, Denmark). The water samples were then stored in the deep freezer until analysis was carried out.

Once in the laboratory, the soil sediments were air dried. After drying the soil particles were ground into fine particles using the laboratory mortar and pestle. The fine soil particles were sieved using a 200 mm-mesh sieve. The finer soil particles were packed in sample bottles and labeled.

A portion, 0.5 g of each soil sediment was weighed using metler balance AE 160 weighing machine in 50 ml beakers. Concentrated HCl, HNO_3, $HClO_4$ and HF were added in that order to each of the samples in 5 ml applications. The beakers containing each of the samples were placed on the heater for about 3 h [4]. For a portion of water sample, 5 ml of concentrated hydrochloric acid was added to 250 ml of water and evaporated to 100 ml. The concentrate was transferred into labeled bottle containers after cooling [5].

Preparation of Culture Media:

Nutrient agar was used for the isolation of bacteria. This was prepared and autoclaved at 121^{0}C for 15 min. The plates were inverted after cooling to prevent steam formation [6]. Potato dextrose agar (DPA) (Oxiod) was used in isolation of fungi.

Single strength MacConkey broth was prepared and about 5 ml was poured into each of the five test tubes used. An inverted Durham tube was carefully dropped inside each of the five test tubes and sterilized by autoclaving at 121^{o}C for 15 min and cooled. Water samples to be analysed were then inoculated into the prepared medium under aseptic condition [6].

Double strength Macconkey broth was prepared according to the procedure used for single strength MacConkey broth but using 70.0 g powder in 1000 ml sterilized distilled water instead of 35.0 g powder in 1000 ml distilled water.

Eosin methylene blue (EMB) agar was prepared by weighing about 34.5 g of the agar and dissolved in 1000 ml sterilized water, boiled for complete dissolution and sterilized at 121^{o}C for 15 min and allowed to cool before pouring.

Analyses of Samples:

The physical parameters determined/observed were the temperature, appearance, odour, taste and conductivity. Other physico-chemical characteristics determined were: pH by the use of pH meter; hardness was determined by titrimetry[7]; total solid, total dissolved solid and total suspended solid were determined by gravimetric method[7]; alkalinity and sulphate were determined by titrimetry[7]; both nitrate and phosphate were determined colorimetrically by Spectronic 20 (Gallenkamp, UK)[7]. Atomic absorption spectrophotometer (Perkin-Elmer Model 403, Norwalk, CT, USA) was used to determine the levels of Fe, Zn, Mn, Cu, Mg, Ca, Pb, Cr, and Ni while Na and K were determined using a flame photometer (Corning, UK, Model 405) using NaCl and KCl to prepare standards[8]. Chloride was determined by Mohr's method[9].

Determination of Total Bacteria:

This test was used to estimate the bacterial population present in the water samples. Serial dilution of water sample to 10^{-2} was made for each sample. The samples were inoculated on nutrient agar using pour plate method[6]. [Viable counts are usually subject to large error,

to more clearly state the result, viable counts were expressed as the number of *colony-forming units* rather than as the number of *viable cells* since a colony-forming unit may contain one or more cells.]

Determination of Coliform Bacteria:

This is a standard method of water analysis for the detection of presence of coliform bacteria [10]. It is a 3-part procedure namely: presumptive test, confirmed test and the completed test.

In presumptive test, each of the five tubes containing 5 ml of single strength MacConkey broth (with added bromocresol purple indicator) and another five set was inoculated with 0.1 ml, 1.0 ml and 10 ml of the water, and incubated at 37 ^{o} C for 24 h. At 24 h, the tubes were examined for acid and gas production. Acid production changed the MacConkey purple colour to yellow and gas production was seen inside the inverted Durham tubes. The number of positive tubes was noted. These processes were repeated with sterile water to serve as control. The number of positive tubes were counted, recorded and compared to the McCrady probability table to determine the most probable number (MPN) of organism(s) present [11]. This number, based on certain probability formulae, was an estimate of the mean density of coliform in the water sample.

The confirmatory test was used to determine the type of organism present after the presumptive test. Any positive tube from the presumptive test was inoculated into a medium of Brilliant Green Lactose-Bile Broth (BGLB) and incubated for 24 h. This medium inhibits growth of lactose fermenters other than coliforms; thus, gas formation in the BGLB medium constituted a confirmed test of coliform presence.

In the completed test about 1 ml of the broth culture from positive tube (presumptive) was first streaked onto EMB agar to obtain colonies. This was incubated at 37^{o}C for 24 h. Fermentation of lactose broth and demonstration of gram-negative, non- sporulating bacilli constituted a positive complete test demonstrating that some members of the coliform group were in the volume of samples examined. Coli-aerogenes organisms produced characteristic colonies.

Escherichia: small colonies, dark, almost black centers, with greenish metallic sheen; *Enterobacter:* large, pinkish mucoid colonies, dark centers, rarely show metallic sheen.

Isolation and Identification of Soil Organisms:

One gram of each soil sample was suspended in test tube with cooled sterile distilled water, shaken properly and serial dilutions made up to 1:10^{7}. Three different methods were used for isolation of organisms: direct plating, spread plate and pour plate methods [12].

For detailed identification of bacteria isolates, the shape, elevation and colour, were observed and recorded. The isolates were determined by use of gram staining, spore staining, motility test and biochemical tests (catalase, oxidase, sugar fermentation and culture).

For fungal isolates, the properties used for identification were cultural characterization in which the colonies on media were observed and recorded. Identification procedures included the microscopic examination of isolates for the presence of different hyphae types, shape and type of spores produced.

Fungal staining was also carried out using lactophenol blue stain, observed under the microscope and observations recorded.

Statistical Analysis:

Grand means, standard deviations and coefficients of variation precent were calculated as appropriate [13].

RESULTS AND DISCUSSION

Table 1 shows the distances of sample collections for the cold, warm and mix water and their corresponding soil sediments. The distance of cold water collection ranged from 7.6-18.3 meters; distance of warm water collection ranged from 32.0-108.4 metres; the point of intersect of warm and cold water was labeled O metre and all measurements were from this point; mixed water distance collections were from 18.3-54.8 meters. Site of collection was determined by the ease of collection. The exact sites of collection along the spring flow are shown in Figure 1 with the appropriate sites shown by A, B, C, F and E with appropriate subscripts. Total numbers of samples for water and soil sediments were eleven each, making a total of twenty-two samples. Of these, cold water/soil sediments = 3/3 sites or 13.6/13. 6 %; warm water/soil sediments = 4/4 sites or 18.2/18.2 %; mixture of water/soil sediments = 3/3 or 13.6/13.6 %; intersect of water/soil sediments = 1/1 or 4.5/4.5 %. The cold/warm water and cold/warm soil sediments as well as the mixtures were analysed on comparative basis. While the samples of warm water/warm soil were taken from sites including about the source (108.4 metres from intersect, O metre, see Fig. 1), the easiest site of collection for cold water/soil was 60.93 metres from source or 28.9 metres from intersect (O metre).

Table 2 depicts the aesthetic qualities of water samples. They were all odourless, tasteless and of clear appearance. The conductivities were all generally low with cold water (A_1-B_1) ranging from 0.5-0.6 x 100 µScm^{-1}, warm water (B_2-D_1) ranging from 1.3-1.45 x 100 µScm^{-1}, intersect (F_1) was 1.0 x 100 µScm^{-1} and mixture (D_2 -E_2) ranged from 1.0-1.0 x 100 µScm^{-1} showing that the warm had the highest conductivities. This would be expected since conductivity depicts the levels of mineral suspension in the water and warmer temperature water would be able to dissolve more inorganic matter than the cold water. Also the influence of the warm water was manifested in the mix water whose conductivity increased by about 43.0 %. Table 2 shows that the temperature followed similar trends with cold water

range of 25-26 ^{o}C, warm range of 34-35^{o}C, intersect of 32^{o}C and mix of 31-31.5^{o}C. Again the mixed region showed intermediate values. Our results were very close to those of Rogers *et al*. [1] whose values were 35.5 ^{o}C (warm), 26.0^{o}C (cold) and 32.5^{o}C (mixed region).

Table 2: Physical properties of the water samples

Sample	Appearance	Odour	Taste	Conductivity X100 (µS/cm)	Temp. ^{o}C
A_1	Clear liquid	Odourless	Tasteless	0.6	26
A_2	Clear liquid	Odourless	Tasteless	0.6	25.5
B_1	Clear liquid	Odourless	Tasteless	0.5	25
B_2	Clear liquid	Odourless	Tasteless	1.3	34
C_1	Clear liquid	Odourless	Tasteless	1.3	34
C_2	Clear liquid	Odourless	Tasteless	1.45	34
D_1	Clear liquid	Odourless	Tasteless	1.3	35
F_1	Slightly turbid	Odourless	Tasteless	1.0	32
D_2	Clear liquid	Odourless	Tasteless	1.0	31.5
E_1	Slightly turbid	Odourless	Tasteless	1.0	31
E_2	Clear liquid	Odourless	Tasteless	1.0	31

Table 3: Some physico-chemical characteristics of the different water samples (ppm)

Sample	pH[a]	Alkalinity	Hardness	TS[b]	TSS[c]	TDS[d]	PO_4^{2-}	NO_3^-	SO_4^{2-}
A_1	8.1	142.3	20	0.03	0.02	0.01	ND[e]	50	14
A_2	8.0	101.7	24	0.04	0.02	0.02	ND	24	12
B_1	7.9	122	23	0.28	0.10	0.18	ND	44	14
$\bar{x}$[f]	8.0	122	22.3	0.12	0.05	0.07	-[i]	39.3	13.3
SD[g]	0.1	20.3	2.1	0.14	0.05	0.10	-	13.6	1.2
CV %[h]	1.3	16.6	9.3	118	92.4	136	-	34.6	8.7
B_2	8.2	122	52	0.07	0.04	0.03	ND	28	12
C_1	8.2	122	50	0.10	0.06	0.04	ND	76	12
C_2	8.3	183	52	0.02	0.07	0.01	ND	25	10
D_1	8.1	142.3	46	0.04	0.03	0.01	ND	60	8
$\bar{x}$	8.2	142.3	50	0.06	0.05	0.02	-	47.3	10.5
SD	0.08	28.8	2.8	0.04	0.02	0.02	-	24.9	1.9
CV %	1.0	20.2	5.7	58.3	36.5	75	-	52.6	18.2
F_1	7.9	101.7	42	0.09	0.06	0.03	ND	28	8
D_2	8.1	183	44	0.12	0.12	0.02	ND	64	12
E_1	8.1	101.7	42	0.20	0.10	0.10	ND	26	16
E_2	8.2	162.7	42	0.09	0.06	0.03	ND	29	12
$\bar{x}$	8.13	149.1	42.7	0.14	0.09	0.05	-	39.7	13.3
SD	0.06	42.3	1.2	0.06	0.03	0.04	-	21.1	2.3
CV %	0.71	28.4	2.7	40.6	33.9	87.2	-	53.2	17.4
$G\bar{x}$[j]	8.1	134.9	39.7	0.10	0.06	0.04	-	41.3	11.8
GSD[k]	0.13	30.5	11.8	0.08	0.03	0.05	-	18.6	2.4
GCV%[L]	1.6	22.6	29.8	79	56.2	129.6	-	45.0	20.3
WHO	6.5-8.5	200	500	1000	1000	1000	0.4	10	200

[a]pH has no unit; [b]Total solid; [c]Total soluble solid; [d]Total dissolved solid; [e]Not detected; [f]Mean; [g]Standard deviation; [h]Coefficient of variation percent; [i]Not applicable; [j]Grand mean; [k]Grand standard deviation; [l]Grand coefficient of variation percent.

Table 4: Mineral levels in water and underlying soil (ppm)[a]

Mineral	Sample																				
	A_1	A_{11}	A_2	A_{22}	B_1	B_{11}	B_2	B_{22}	C_1	C_{11}	C_2	C_{22}	D_1	D_{11}	F_1	F_{11}	D_2	D_{22}	E_1	E_{11}	E_{22}	E_{22}
Fe	2.5	0.4	2.7	0.4	4.4	0.61	1.2	0.56	2.3	0.36	4.2	0.36	4.7	0.7	6.1	0.3	3.6	0.5	4.4	0.4	4.8	0.44
Zn	2.2	0.31	1.8	0.4	2.4	0.4	2.1	0.33	4.5	0.29	2.6	0.37	1.1	0.1	2.1	0.33	1.6	0.3	2.4	0.44	4.1	0.15
Mn	9.3	0.02	10.3	0.03	11.3	0.05	8.3	0.03	5.6	0.08	12.2	0.01	12.5	0.06	9.1	0.05	9.2	0.02	19.2	0.04	7.3	0.04
Cu	ND	0.01	ND	0.03	ND	0.07	ND	0.05	ND	0.02	ND	0.05	ND	0.08	ND	0.04	ND	0.05	ND	0.06	ND	0.07
Mg	8.9	1.44	7.8	1.28	6.9	1.44	22	0.93	23.5	1.13	18.3	1.01	20	1.42	11.2	1.27	17	1.02	15.5	1.22	14.1	1.1
Na	6.6	0.98	4.3	1.21	5.6	1.3	4.2	1.45	12.9	1.33	4.0	1.19	3.5	1.44	5.1	1.28	4.3	1.21	4.6	1.28	4.9	1.08
Ca	9.5	1.12	13.2	1.28	13	0.96	10.1	1.4	8.1	0.78	12.2	1.22	7.3	0.94	10.2	1.3	11.2	0.81	19.2	0.8	8.8	1.01
Pb	ND	0.19	ND	0.35	ND	0.33	ND	0.25	ND	0.02	ND	0.25	ND	0.35	ND	0.25	ND	0.24	ND	0.24	ND	0.23
K	2.4	1.29	2.2	1.51	4.4	1.62	3.8	1.18	4.7	1.0	7.6	1.32	8.1	1.01	5.8	1.36	7.7	0.89	7.0	1.26	13	1.26
Cr	ND	0.01	ND	0.06	ND	0.01	ND	0.05	ND	0.05	ND	0.01	ND	0.004	ND	0.03	ND	0.004	ND	0.02	ND	0.23
Cl	48.7	0.01	49.6	0.008	72.3	0.004	35.5	0.01	42.5	0.01	28.4	0.01	49.6	0.004	49.6	0.02	21.3	0.02	35.5	0.004	35.5	0.01
Ni	ND	0.005	ND	0.04	ND	0.03	ND	0.03	ND	0.03	ND	0.005	ND	0.05	ND	0.044	ND	0.05	ND	0.005	ND	0.05

[a]Soil samples were analysed on dry weight basis.

Table 5: Statistical variation between the water mineral levels and between the soil sediment mineral levels

Sample	Mineral											
	Fe	Zn	Mn	Cu	Mg	Na	Ca	Pb	K	Cr	Cl	Ni
A_1-B_1												
$\bar{X}$	3.2	2.1	10.3	-a	7.9	5.5	11.9	-	3.0	-	56.9	-
SD	1.0	0.3	1.0	-	1.0	1.2	2.1	-	1.2	-	13.4	-
CV %	32.6	14.5	9.7	-	12.7	21.0	17.5	-	40.6	-	23.5	-
A_{11}-B_{11}												
X	0.47	0.37	0.03	0.04	1.4	1.2	1.12	0.29	1.5	0.03	0.007	0.03
SD	0.12	0.05	0.02	0.03	0.09	0.17	0.16	0.09	0.17	0.03	0.003	0.02
CV %	25.8	14.0	50.9	76.4	6.6	13.8	14.3	30.1	11.2	100	43.6	60.1
B_2-D_1												
$\bar{X}$	3.1	2.0	9.7	-	21.0	6.2	9.4	-	6.05	-	39.0	-
SD	1.6	0.7	3.3	-	2.3	4.5	2.2	-	2.12	-	9.1	-
CV %	52.7	32.5	34.1	-	10.8	72.7	23.3	-	35.1	-	23.3	-
B_{22}-D_{11}												
$\bar{X}$	0.5	0.27	0.05	0.05	1.12	1.4	1.09	0.22	1.13	0.03	0.009	0.03
SD	0.17	0.12	0.05	0.02	0.21	0.12	0.28	0.14	0.15	0.02	0.003	0.02
CV %	0.33	44.3	69.1	49.0	19.2	8.7	25.5	63.6	13.5	83.2	33.3	92.1
F_1												
X	-	-	-	-	-	-	-	-	-	-	-	-
SD	-	-	-	-	-	-	-	-	-	-	-	-
CV %	-	-	-	-	-	-	-	-	-	-	-	-
F_{11}												
X	-	-	-	-	-	-	-	-	-	-	-	-
SD	-	-	-	-	-	-	-	-	-	-	-	-
CV %	-	-	-	-	-	-	-	-	-	-	-	-
D_2-E_2												
$\bar{X}$	4.3	2.7	11.9	-	15.5	4.6	13.1	-	9.2	-	30.8	-
SD	0.61	1.3	6.4	-	1.5	0.3	5.4	-	3.3	-	8.2	-
CV %	14.2	47.3	53.7	-	9.4	6.5	41.6	-	35.5	-	26.6	-
D_{22}-E_{22}												
$\bar{X}$	0.45	0.30	0.03	0.06	1.11	1.19	0.87	0.24	1.14	0.08	0.01	0.035
SD	0.05	0.15	0.01	0.01	0.10	0.10	0.12	0.006	0.21	0.13	0.008	0.03
CV %	11.2	48.3	38.5	16.7	9.1	8.5	13.6	2.4	18.7	157.6	80.8	74.2
WHO (ppm)	0.3**	5.0**	0.1**	1.0**	-	200**	-	0.05*	-	0.05*	250**	-

[a]Not calculated; *Health-related guideline value; **Characteristic that may affect the aesthetic quality of drinking-water.

Table 6: Estimated number of bacteria of the coliform group present in 100 ml of water sample

Sample	Numbers of tubes giving positive reaction (acid and gas)				MPN[a] per 100 ml
	Quantity of water	10 ml	1 ml	0.1 ml	
		No. of samples of each quantity tested			
		5	5	5	
A_1		5	4	0	130
A_2		5	5	0	250
B_1		1	1	0	4
B		2	0	0	5
C_1		3	2	1	17
C_2		2	2	0	9
D_1		4	3	2	40
F_1		1	0	0	1
D_2		2	2	1	12
E_1		5	5	4	1600
E_2		5	5	4	1600

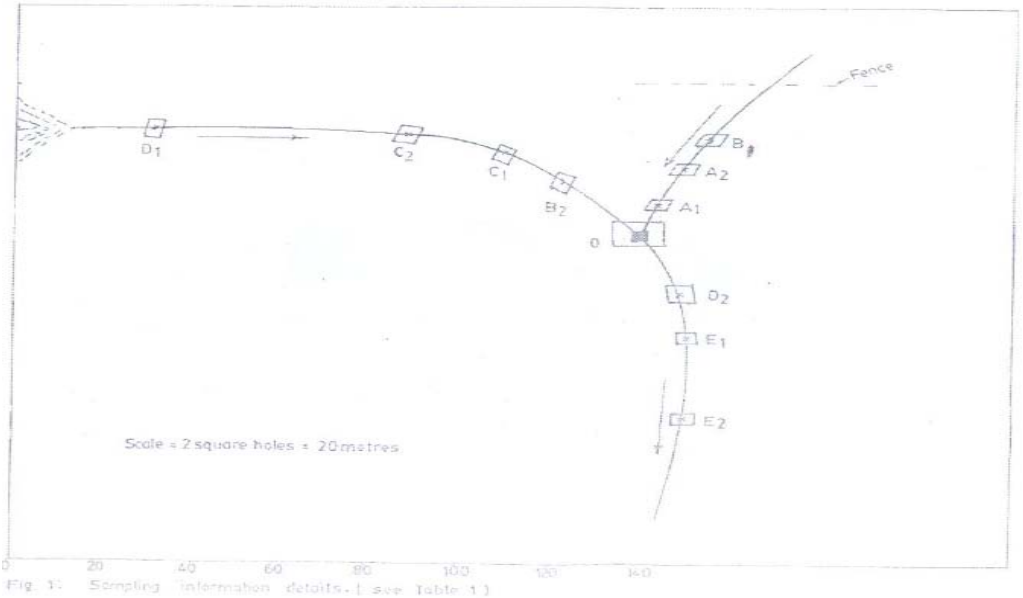
[a]Most probable number of coilform organisms in 100 ml of the original water.

Table 7: Isolated micro-organisms from water and related cold warm soils

Sample	Isolated Bacteria[a] (in water)	Bacteria[a] (in soil)	Isolated fungi (in soil)
A_1, A_{11}	*E. coil and A. aerogenes*	*Corynebacterium* sp	*Trichoderma* sp
A_2, A_{22}	*A. aerogenes*	*Proteus* sp	*Rhizopus* sp
B_1, B_{11}	*E. coli and A. aerogenes*	*Micrococcus*	*Aspergillus niger*
C_1, C_{11}	*E. coli and A.aerogenes*	*A. aerogenes*	*Aspergillus niger*
C_2, C_{22}	*E. coli and A.aerogenes*	*A.aerogenes*	*Aspergillus niger*
D_1, D_{11}	*E. coli and A.aerogenes*	*E. coli*	*Aspergillus niger*
D_2, D_{22}	*E. coli and A.aerogenes*	*A.aerogenes*	*Aspergillus niger*
E_1, E_{11}	*E. coli and A.aerogenes*	*A.aerogenes*	*Fusarium* sp
E_2, E_{22}	*E. coli and A.aerogenes*	*A.aerogenes*	*Rhizopus* sp
F_1, F_{11}	*A.aerogenes*	*Pseudomonas* sp	*Aspergillus niger*

[a]*E. coli* = *Escherichia coli*; *A.aerogenes* = *Aerobacter aerogenes*.

Figure 1: Sampling information details



The pH, alkalinity, hardness, total solid, total suspended solid, total dissolved solid, phosphate, nitrate and sulphate values of the water samples are depicted in Table 3. The average pH for cold water was 8.0 ± 0.1 with a coefficient of variation percent (CV %) of 1.3; in warm water it was 8.2 ± 0.08 with CV % 1.0; intersect was 7.9 and mixed region 8.13 ± 0.06 with CV % 0.71. In Rogers *et al*. [1] pH was 7.45 (warm), 7.50 (cold) and 7.65 (mixed); our own results were slightly higher and of different trend. The alkalinity values (ppm) on the average were: cold water 122 ± 20.3 with CV % 16.6; warm 142.3 ± 28.8 with CV % 20.2; intersect 101.7 and mixed 149.1 ± 42.3 with CV % 28.4. The alkalinity for the mixed region was the most varied and the alkalinity was the highest; this could have been contributed by the roots of decaying plants in the course of the mixed region. Also the intersect bore no relationship with both the warm and the cold water. The hardness average values (ppm) were: 22.3 ± 2.1 with CV % 9.3 (cold); 50 ± 2.8 with CV % 5.7 (warm); 42 (intersect); 42.7 ± 1.2 with CV % 2.7 (mixed). Hardness was highest in the warm, lowest in the cold and virtually similar in intersect (42 ppm) and mixed (42.7 ppm). Our values for hardness were much higher than the value of 7.5 ppm and 11.5 ppm as reported by du Preez and Barber [14] for Wikki Spring water. The total solids (TS), total suspended solids (TSS) and total dissolved solids (TDS) were all very low (Table 3) and they were all widely varied with low (33.9) to high (136) CV %. The TDS and TS in our results were much lower than the literature results [1, 14]. Phosphate was not detected in any of our water samples but Roggers *et al*. [1] recorded low level of 0.11-0.13 ppm. The average nitrate levels (ppm) were: cold water 39.3 ± 13.6 with CV % 34.6; warm 47.3 ± 24.9 with CV % 52.6; intersect was 28; mixed 39.7 ± 21.1 with CV % 17.4. Nitrate levels here could be traced to percolating nitrate from sources such as decaying plant and animal material and agricultural fertilizer. The grand coefficients of variation percent were determined for the parameters in Table 3. It showed that the pH was the least varied with CV % of 1.6. In descending order of variation, the followings were obtained: TDS>TS>TSS>NO_3^-> hardness>alkalinity>sulphate>pH. The World Health Organization (WHO) [15] guideline values were higher than our results in pH, alkalinity, and total hardness, TS, TSS, TDS, PO_4^{2-} and SO_4^{2-}. However the NO_3^- level of 10 ppm was much lower than our values. Nitrate is toxic when present in excessive amounts in drinking water, and in some cases causes methamoglobinaemia in bottle-fed infants; for older age groups, this problem does not arise, but there is a possibility that certain forms of cancer might be associated with very high nitrate concentrations [15].

Table 4 contains the pair wise analytical results of the minerals for both water and soil sediment collected about the same spots along the course of the streams. While the subscript of the lower Arabic figure represents the minerals in water, the higher figure represents the mineral in soil of the same alphabet. Highest level of Fe was observed in the intersect with a value of 6.1 ppm and generally higher in the mixed region than both the cold and warm water zones. Zn was generally lower than Fe but the highest level of 4.5 ppm was observed in C1 in the warm water region. Mn was the most concentrated metal mineral among the trace metals with values as high as 5.6-19.2 ppm in the water samples. However Mn levels in the soil sediments were lower than in the soil sediments of Fe and Zn. The following minerals were not detected in the water samples: Cu, Pb, Cr and Ni; on the other hand, all these trace metals (Cu, Pb, Cr, Ni) were found at various levels in the soil sediments. Also, none of the metal levels was up to 1.0 ppm in the soil sediments but the results showed that the soil sediments could serve as reservoir for those minerals. Roggers *et al*. [1] had adduced the probable reasons for the low soil sediment contents of Cr, Mn and Cu as being due to scarcity of magnetite, and calcium- and iron-bearing silicates in the rock samples. The values of Fe being less than those of Mn might be explained thus: Fe^{3+} will be carried by acidic solutions but a slight increase in hydroxyl ion concentration would result in its precipitation as the hydroxide thereby making it unavailable [16]; if a solution contained both Fe^{2+} and Mn^{3+} then the following reaction is possible:

$$Fe^{2+}(aq) + Mn^{3+}(aq) \rightleftharpoons Fe^{3+}(aq) + Mn^{2+}(aq)$$

and the values for Fe suggest that it would indeed proceed to the right; thus further reducing the level of Fe in water. Among the major metals Mg was the most concentrated across board followed by Ca, Na and finally K. The soil sediments major metal minerals were better concentrated than in the trace metals. The chloride concentration ranged as follows: 28. 4-49.6 ppm (warm), 49.6 ppm (intersect), 21.3-35.5 ppm (mixed); these values were greater than those of Roggers *et al*. [1] with values of 3.72-3.26 ppm but within the range of 24.0-55.5 ppm from ground water results [17].

Table 5 shows the statistical variations between the water mineral levels. The average Fe distribution was D_2-E_2 (mixed, 4.3 ppm) > A_1 –B_1 (cold, 3.2 ppm) > B_2 –D_1 (warm 3.1 ppm). This trend was followed in Zn and Mn. For Mg, B_2 - D_1 (warm water, 21.0 ppm) > D_2 – E_2 (mixed, 15.5 ppm) > A_1 – B_1 (5.5 ppm); Ca, A_1 – B_1 (11.9 ppm) > B_2 – D_1 (9.4 ppm) < D_2 – E_2 (13.1 ppm); K, D_2 –E_2 (9.2 ppm) > B_2 – D_1 (6.05 ppm) > A_1 – B_1 (3.0 ppm) and Cl, A_1 –B_1 (56.9 ppm) > B_2 –D_1 (39.0 ppm) > D_2 –E_2 (30.8 ppm). A_1 –B_1 (cold) was best concentrated in Cl, second best in Fe, Zn, Mn, Na and Ca and third best in Mg and K; B_2-D_1 (warm) was best concentrated in Na and Mg, second best in Cl and K and third best in Fe, Zn, Mn, Ca and K, second best in Mg but third best in Na and Cl. The mixed region actually balanced the warm and cold region characteristics in the mineral composition. The soil sediments on the other hand did not follow similar trends as we have for the minerals already discussed for the water samples, Pb, Cr, Cu and Ni have detectable levels in the soil sediments, hence were involved in these various calculations. Pb, Cr, Cu, Cl, Zn, Ni and Na were all below

the guideline values set by the WHO [15] either health-related or on aesthetic quality so the water samples were save on those parameters. On the other hand, Fe and Mn were all outside the aesthetic quality of 0.3 ppm and 0.1 ppm respectively. Although Fe is an essential element in human nutrition, drinking water is not considered to be an important source. At levels of about 0.3 ppm, Fe stains laundry and plumbing fixtures and causes an undesirable taste in beverages. The precipitation of excess Fe gives an objectionable reddish-brown colour to the water [15]. At levels exceeding 0.15 ppm, Mn in water supplies stains plumbing fixture and laundry. At higher concentrations, it causes an undesirable taste in beverages.

The total bacteria count after 24 h incubation on nutrient agar at 10^{-1} and 10^{-2} dilutions showed that all the cold water samples had 300 + and for mixed, D_2-E_2 had 300 + for undiluted and 10^{-1} but 215-285 for 10^{-2}; in the intersect, undiluted (300+), 10^{-1} (297) and 10^{-2} (253). The colony forming unit per ml (cfu/ml) ranged between 3.0x 10^3-2.97 x10^3 at 10^{-1} for all the samples and 3.0 x 10^4 -2.2 x 10^4 at 10^{-2} dilution. All these were not compatible with the guideline value of 3 cfu/100 ml [15]. Colony counts provide an estimate of general bacteria purity, which is of particular value when water is used industrially for the preparation of food and drink. They may also give forewarning of pollution[11]. Colony counts are not essential for assessing the safety of domestic water supplies, they are however useful for indicating the efficiency of certain processes in water treatment, for example coagulation, filtration, and chlorination, and the cleanliness of the distribution system[11].

Table 6 shows the most probable number (MPN) of coliform organisms in 100 ml original water samples. The McCrady Table expressing the MPN in 100 ml of water which correspond with the various combinations of positive and negative tubes in the series used were indicated. The MPN represent only an estimate of the number of bacteria present in any sample. The McCrady Table showed that MPN in cold water was 4-250; warm water was 5-40; intersect was 1 while mixed region water was 12-1600. The fact that we had values for MPN in any of the multiple tube method meant that the water should not be useful for human consumption.

Table 7 depicts the bacteria isolated from the water and soil samples as well as the isolated fungi from the soil sediments. The two major bacteria detected in the water samples were *Eschericha coli* and *Aerobacter aerogenes.* In the cold water, *E. coli* was found in two samples (A_1, B_1) and *A. aerogenes* in three samples (A_1, A_2 B_1). In the warm water, all the samples (B_2, C_1, C_2, D_1) contained *E. coli* while C_1, C_2 and D_1 contained *A. aerogenes* in addition. For the mixed water (D_2, E_1, E_2) all contained both *E. coli* and *A. aerogenes*. The intersect (F_1) contained only *A. aerogenes*. The presence of *E. coli* in a water sample indicates excretal pollution of either human or animal origin. Other sources of *E. coli* could be soil, and vegetation[11, 15]. High counts indicate heavy and recent pollution, whereas low counts slight or relatively remote pollution. Since there is no satisfactory method for determining whether *E. coli* is of human or animal origin, its presence should always be regarded as indicating potentially dangerous pollution. Coliform organisms other than *E. coli* can also occur in water sources as a result of contamination by soil washings or from growth on decaying vegetation especially in warm weather [11]; this might be the reason for the results obtained in the present report.

The bacteria found in the soil sediments were of varied forms; *Corynebacterium* sp in A_{11}; *Proteus* sp in A_{22}; *Micrococcus* sp in B_{11}; *A. aerogenes* in C_{11},C_{22}, D_{22}, E_{11} and E_{22} whereas *E.coli* was in D_{11} and *Pseudomonas* sp in F_{11}. The soil fungi were also varied: *Trichoderma* sp (A_{11}), *Rhizopus* sp (A_{22}, E_{22}), *Aspergillus niger* (B_{11}, B_{22}, C_{11}, C_{22}, D_{11} and E_{11}) while *Fusarium* sp was in E_{11}. While *A. aerogenes* was the most prominent bacterium in the soil sediments, it was the *Aspergillus niger* fungus in the soil sediments. The free-living organisms that may occur in water supplies include fungi, algae, etc. These organisms may be of public health significance as carriers of disease-causing organisms or because they produce toxins [15]. It is desirable that these free-living organisms should be absent from drinking-water. Guideline values are not available yet for free-living organisms in water [15]. Knowledge of the identity and abundance of organisms in raw water supplies is valuable in water resource management.

REFERENCES

1. Rogger AS, Imevbore AMA, Adegoke OS. Physical and Chemical Properties of the Ikogosi Warm Spring, Western Nigeria. J Min Geol 1969; 4: 69-81.
2. Ekiti State of Nigeria, Tourism Attractions. Ekiti State Tourism Board, Ado-Ekiti, 2004; pp 2-3.
3. Ekiti State of Nigeria, State Economic Empowerment and Development Strategy [SEEDS]. Ekiti State Government of Nigeria, Ado-Ekiti, 2004; pp 42 - 53.
4. Udo EJ, Ogunwale AJ. Laboratory Manual For The Analysis of Soil, Plant and Water Samples Department of Agronomy, University of Ibadan, Ibadan, 1979; pp 70-76.
5. Parker RC. Water Analysis by Atomic Absorption Spectroscopy. Varian Techtron Geneva, 1972; pp 15-30.
6. Olutiola PO, Famurewa O, Scontag HG. An Introduction to General Microbiology – A Practical Approach. Bolabay Publications Lagos, 2000; pp 90-111.
7. Vogel AI. A Textbook of Quantitative Inorganic Analysis Including Elementary Instrumental Analysis, 4th edn. Longman Inc., New York, 1978; pp 223-511.
8. Association of Official Analytical Chemists (AOAC), Official Methods of Analysis. AOAC, Washington, DC, 1990; Section 968-08.

9. American Public Health Association (APHA), Standard Methods For the Examination of Water and Wastewater, 15th edn. APHA, Washington, DC, 1980; 1134 pp.

10. Pelzer MJ, Chan E, Krieg NR. General Microbiology, 5th edn. McGrawHill Books Company, New York, 1999; pp 593-617.

11. Department of Health and Social Security, The Bacteriological Examination of Water Supplies. Her Majesty's Stationary Office, Reports on Public Health and Medical Subjects No. 71, London, 1969; pp 1-52.

12. Fawole MO, Oso BA. Laboratory Manual of Microbiology. Spectrum Books Ltd., Ibadan, 1995; pp 34-56; 71-81.

13. Steel R G D, Torrie JH. Principle of Procedures of Statistics. McGraw-Hill, London, 1980; pp 7-30.

14. du Preez JW, Barber W. The distribution and chemical quality of groundwater in Northern Nigeria. Geol Surv Nigeria Bull 1965; 36: 1-93.

15. World Health Organization (WHO), Guideline Foe Drinking-Water Quality, Volume 1, Recommendations. WHO, Geneva, 1984; pp 17-101.

16. Adeyeye EI. Water quality criteria and the relationship between the distribution and concentration of some mineral elements in soil sediments, ambient water and the body parts of *Clarias gariepinus* fish in a freshwater pond. *Ghana Journal of Chemistry* 1997; 3 (2): 42-50.

17. Adeyeye EI, Abulude FO. Analytical assessments of some surface and ground water resources in Ile-Ife, Nigeria. *J Chem Soc Nigeria* 2004; 29(2): 98-103.

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