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Impact assessment of proximity of local black soap industry on the bacteriological and physicochemical properties of Ebu stream in Ikere-Ekiti, Ekiti State, Nigeria

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ABSTRACT

Aims: The level of contamination of Afawo Stream located near Afawo Soap industry in Ikere-Ekiti were investigated by determining the total bacteria and coliform count with antibiotics susceptibility of the isolated bacteria and physico-chemical qualities of the water samples.
Methodology and results: The total bacteria and coliform count were determined using pour plate method, the antibiotic susceptibility were carried out using disc diffusion method, while physico-chemical and mineral studies were also carried out using standard methods. The mean total bacteria count of the water samples ranged 40.4×10^4 - 26.5×10^5 CFU/mL and 36.4×10^4 - 23.3×10^5 CFU/mL respectively. Percentage distribution of isolated bacteria include; *Escherichia coli* (17%), *Streptococcus* spp. (16%), *Bacillus* spp. (11%), *Nitrobacter* spp. (10%), *Aeromonas* spp. (9%), *Arthrobacter* spp. (9%), *Pseudomonas* spp. (5%), *Klebsiella* spp. (5%), *Enterococcus* spp. (5%), *Micrococcus* spp. (4%), *Staphylococcus* spp. (3%), *Vibro* spp. (3%), *Enterobacter* spp. (2%), *Salmonella* spp. (1%). Enteric microbes have high level of resistance to amoxicillin and augmentin, while nalixidic acid was most effective against the Gram negative isolates. Also the Gram positive isolates showed a high level of resistance to augmentin, cotrimoxazole and cloxacillin while streptomycin and gentamicin was most effective against the Gram positive isolates. Eighty four percent (84%) of the isolates exhibited multiple antibiotic resistance, some of which possess plasmids with very high molecular weight ranging between 10 and 21 kbp. The physico-chemical properties of the water samples revealed the presence of the some mineral element in the water samples; magnesium (15.60 mg/L), potassium (16.20 mg/L), calcium (8.75 mg/L), sodium (11.55 mg/L), zinc (0.34 mg/L), iron (0.76 mg/L), chloride (21.40 mg/L), sulphate (5.60 mg/L), nitrate (0.35 mg/L). Meanwhile, the mean values of soil minerals were; potassium (76.5 - 83.5) mg/kg, calcium (49.8 - 62.7) mg/kg, sodium (63.4 - 71.6) mg/kg, magnesium (65.8 - 72.4) mg/kg, phosphorus (266.5 - 275.3) mg/kg, zinc (8.28 - 12.22) mg/kg, copper (3.60 - 4.68) mg/kg.
Significance and impact of study: According to the international standard, the effect of effluent discharge from Afawo soap industry has made Ebu stream unfit for consumption and any domestic purposes unless being treated.

Keywords: Local black soap, enteric microbes, Ebu stream, effluent discharge

INTRODUCTION

Indiscriminate discharge/disposal of wastes generated from different anthropogenic activities has really been a challenge to developing countries till date (Omoleke, 2004), considering its direct effect on water bodies; rendering these natural resources unsuitable for both primary and/or secondary usage (Fakayode, 2005). More alarming is the contamination of these natural water bodies by industrial effluents, a scenario that seems like norm in many developing and densely populated countries like Nigeria (Ezeronye and Amogu, 1998); which has a lot to do with active industrialization, urbanization coupled with increased commercial activities in country Nigeria since 1960 (Ajayi and Osibanjo, 1981; Okoye *et al*., 2011). Meanwhile, the management of industrial effluents remained underdeveloped and very unsatisfactory leading to environmental pollution, depletion, global warning, deforestation and degradation of natural ecosystems (Okonkwo and Eboatu, 2006).

From times past, river system has been the major receptacle of treated, partially treated and/or untreated wastes, especially the effluents from industries that are near them, serving as factor of pollution to the water bodies (Sangodoyin, 1991). This menace could be as a result of primary or secondary organic pollution as in; the surplus of organic matter, which is the sum of un-decomposed organic material introduced into the water body resulting from an extremely increased bio-productivity within the polluted ecosystem itself (Nweke

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and Sander, 2009). Meanwhile, high level of pollutant in river systems causes an increase in biological oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS), total suspended solids (TSS), toxic metals such as cadmium, chromium, nickel, lead and fecal coliforms (Emongor *et al*., 2005) and hence upsetting the ecological balance.

The deterioration in the biological properties of water bodies subjected to pollution from runoff of waste dumps, mine tailings and industrial production sites, has thoroughly been revealed in several reviews (Barton, 1992; Moore *et al.*, 1998; Nduka *et al*., 2008; Okoye *et al*., 2011; Odeyemi *et al*., 2011). Those of industrial effluents attracting more attention, perhaps because they are rich in decomposable organic matters that enhance rapid growth of microorganisms and a consequent fall in the amount of dissolved oxygen in the water, causing the death of aquatic organisms (Cranford *et al*., 1998; Okoye *et al*., 2011). Perhaps, the resultant effect of these happenings on public health and the environment are usually of great magnitude (Osibanjo *et al*., 2011).

Industrial effluents from soap manufacturing industries are known to contain complex chemicals most of which are very toxic and harmful to the environment (EL-Gohary *et al*., 1987). Therefore the proximity of this type of industry to any natural water body could be very deleterious, a substantial reason for assessing the excesses in the physical, chemical and biological properties of Ebu stream located at about 50 m away from a local black soap industry; in relation to the possible pollution attributing to the effluent from the industry. Inference to the emphasis of Okoye *et al*. (2011), this work is also aimed at serving as relevant clue for the Federal Environmental Protection Agencies which shoulders the task of preparing the scientific base for the enactment of environmental protection laws in Nigeria, to aid her adequacy in discharging her expected functions

MATERIALS AND METHODS

Description of study site

Ebu stream (Figure 1) is located at about 50 m away from a local black soap industry (Figure 2) in Afawo community of Ikere-Ekiti, Ekiti State. The stream formerly serves as a source of water for the people in the community for consumption, domestic and recreational purposes. The soap industry is surrounded with a fence which demarcated it from the stream water. Soil samples were taken in between the stream water and the soap industry at an interval of 10 m; to make replicates.

Collection of water samples

Two hundred and fifty millilitre sterile sampling bottles (with cork) were used to collect water samples by placing it deep into the flowing water at about 120 mm below the water level, then corked tightly to avoid cross contamination from extraneous bacteria. Samples were transported in ice to the laboratory for microbiological analysis within 4 h of collection. Concentrated HNO_3 (5 mL/L) was added to another water samples for mineral analysis as chemical preservative.

Soil samples used in this work were collected using sterile polythene bags which were properly labeled and transferred to the laboratory and analyzed within 6 h of collection. The soil samples were placed in the refrigerator to avoid dryness.

Figure 1: Ebu stream, located 50 meters away from the black soap industry

Figure 2: Black soap industry located near Ebu stream at Afawo, Ikere-Ekiti

Determination of total bacterial count

Pour plate method was used in the enumeration of total bacterial and coliform count based on the serial dilution techniques (Olutiola *et al*., 1991). Ten-fold dilution was prepared, using 1 mL of each water samples following a vigorous shaking. Aliquots of 1 mL of dilution 10^{-4} and 10^{-5} of each sample were pipette into sterile labelled Petri-dish, which were then overlaid with about 20 mL of molten Nutrient and MacConkey agar (45 °C) respectively. Determination of bacterial load of the water samples were

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done in triplicates. Plates were allowed to set and incubated inverted at 37 °C for 24h. Pure cultures of isolates were kept on nutrient agar slants at 12 °C until used. The isolates were identified on the basis of cellular morphology following Gram stain, and results of biochemical testing, including catalase production, growth in 6.5% NaCl broth, haemolytic activity and motility (Devriese *et al*., 1992).

Antimicrobial susceptibility tests

The antibiotics susceptibility of the isolates was determined by the disk diffusion method on Mueller-Hilton agar according to Clinical and Laboratory Standard Institute (2005). The Gram negative bacterial isolates were tested against eight ABTEK antibiotic discs which comprised of Aug (30 µg), Tet (30 µg), Amx (25 µg), Cot (25 µg), Ofl (5 µg), Nal (30 µg), Nit (30 µg) and Gen (10 µg), While the Gram positive isolates were tested against; Cot (25 µg), Cxc (5 µg), Ery (5 µg), Gen (10 µg), Aug (30 µg), Str (10 µg), Tet (10 µg), Chl (10 µg). The inoculums was standardized by adjusting its density to equal the turbidity of a Barium sulphate ($BaSO_4$) (0.5 McFarland turbidity standard), and incubated at 35 °C for 18 h. The diameter of the zone of inhibition (including the diameter of the disk) was measured to the nearest whole millimeter and interpreted using CLSI guideline (CLSI, 2005).

Plasmid extraction and profiling

Plasmid profile analysis was done to determine the location of gene responsible for the resistance of some of the microbes towards some antibiotics. TENS (Tris 25 mM Ethyl-dimethyl tetra-amine; EDTA 10 mM, sodium hydroxide; NaOH 0.1 N and sodium dodecyl sulphate) protocol describe by Liu *et al*. (1995) was employed in plasmid extraction. Approximately 1.5 mL of overnight culture was spin for 1 min in a micro-centrifuge to pellet cells. Followed by gentle decant of the supernatant leaving 50 µL together with cell pellet and vortex mixed at high speed to re-suspend cells completely. A 300 µL of TENS was then added**.** An inverting tube was used to Mix for 3 times until the mixture becomes sticky. Approximately 150 µL of 3.0 M sodium acetate (pH 5.2) was then added to the preparation, followed by Vortex mixing. The preparation was spun for 5 min at 10,000 x g in micro-centrifuge to pellet cell debris and chromosomal DNA and then the supernatant was transferred into a fresh tube; and mixed well with 900 µL of ice-cold absolute ethanol. It was then spun again for 10 min to pellet plasmid DNA. (White pellet is observed) after which the supernatant was discarded; the pellet was rinsed twice with 1 mL of 70% ethanol and dry pellet. Pellet was re-suspending in 30 µL of buffer or distilled water for further use. The extracted plasmid DNA was electrophoresed on 0.8% agarose gel stained with ethidium bromide and visualized by UV-transillumination according to Robins-Browne *et al*. (2004). (TENS composition: Tris 25 mM, Ethyl-dimethyl tetra-amine; EDTA 10 mM, Sodium hydroxide; NaOH 0.1 N and Sodium dodecyl sulphate; SDS 0.5%).

Physicochemical analysis of water samples

The temperature of the water samples were taken at the sites of collection using a simple thermometer calibrated in °C as described by Edema *et al*. (2001) and Ademoroti (1996). Electrical conductivity was measured with a CDM 83 conductivity meter (Radio Meter A/S Copenhagen, Denmark). Turbidity and pH were determined at site using Water Proof Scan 3+ Double Junction and HI 98311-HI 98312 (Hanna) (Wagtech International, UK). The samples were stored under deep freezing conditions or temperature of –20 °C until it was analyzed. Other physicochemical characteristics determined were hardness determined by titrimetry; total dissolved solid and total suspended solid were determined by gravimetric method; acidity, alkalinity and sulphate were determined by titrimetry; both nitrate and phosphate were determined colorimetrically by Spectronic-20 (Gallenkamp, UK) as described by AOAC (2005). Metal analyses were carried out using flame atomic absorption spectrophotometer (GBC Avanta version 1.31). The instrument was set to zero by running the respective reagent blanks and the calibration curves were prepared separately for each metals by running different concentration of standard solution. Average values of three replicates were taken for each determination. Manganese was determined using atomic absorption spectrophotometer (Perkin-Elmer Model 403).

Digestion of soil sample and mineral analysis

Soil samples were digested by a method described by Kisku *et al*. (2000). One gram of soil sample was weighed into a dried 250 mL beaker and was digested with a mixture of (3:1 v/v) 40 mL of HNO_3 and HCl. Samples were digested on the hot plate until a clear solution was obtained, cooled and diluted with distilled water. It was then filtered through a Whatman No. 40 filter paper into a 100 mL volumetric flask. The residue was washed with warm distilled water and the solution made to mark with distilled water and transferred into 100 mL clean plastic container for the metal analysis using UNICAM 969 Atomic Absorption Spectrophotometer (AAS) as reported by Odeyemi *et al*. (2011).

RESULTS

The mean total bacterial counts and total coliform counts of the water samples at different times of collection ranged (40.4×10^4 - 26.5×10^5) CFU/mL and (36.4×10^4 - 23.3×10^5) CFU/mL respectively (Table 1). A total of one hundred (100) microbes were isolated from the water samples from Ebu stream in Afawo, Ikere-Ekiti. *Escherichia coli* showed the highest frequency of 17% while other bacteria followed in the following order; *Streptococcus* spp. with 16%, *Bacillus* spp. with 11%, *Nitrobacter* spp. with 10%, each of *Aeromonas* spp. and

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Arthrobacter spp. showed frequency of 9%, *Pseudomonas* spp., *Klebsiella* spp. and *Enterococcus* spp. had 5% each, *Micrococcus* spp. showed 4%, *Vibrio* spp. and *Staphylococcus* spp. had 3% each, *Enterobacter* spp. had 2% while *Salmonella* spp. showed 1% distribution (Figure 3).

Table 1: Microbial composition (CFU/mL) of water samples from Ebu stream in Afawo, Ikere-Ekiti.

	Total bacterial count		Total coliform count	
Water samples	10^4	10^5	10^4	10^5
A	53	48	51	41
B	18	12	17	14
C	52	28	35	25
D	16	10	32	12
E	37	14	24	13
F	46	11	63	13
G	48	39	38	30
H	45	30	36	26
I	42	31	35	29
J	49	38	33	37
Mean value	40.6	26.5	36.4	23.3

A, B, C, D, E, F, G, H, I, J- Period of collection at weekly interval.

The antibiotic resistance pattern (in percentages) revealed that the Gram positive isolates were more resistant to the antibiotics to which they were subjected, except for Nitrobacter spp. that were 100% susceptible to gentamycin and augmentin. Although gentamycin, streptomycin and tetracycline were observed to be less potent against the Gram positive bacteria; which had percentage resistance range of 18% to 67%. Apart from Salmonella spp. and Enterobacter spp. that were 100% resistance to augmentin, amoxicillin and cotrimaxole. The percentage resistance of other Gram negative bacterial isolates was variably below average as against antibiotics they were subjected to, signifying the relative potency of these antibiotics against isolated Gram negative bacteria (Tables 2 and 3).

A total of 84 isolates exhibited multiple antibiotic resistance, with all the *Bacillus* spp., *Enterobacter* spp., *Micrococcus* spp., *Staphylococcus* spp., *Salmonella* spp., *Arthrobacter* spp. and *Nitrobacter* spp. exhibiting multiple antibiotics resistance. The percentage incidence of multiple antibiotic resistance among other isolates include; *E. coli* (75%), *Aeromonas* spp. (78%), *Streptococcus* spp. (94%), *Enterococcus* spp. (80%), *Vibrio* spp. (67%), *Klebsiella* spp. (80%) (Table 4).

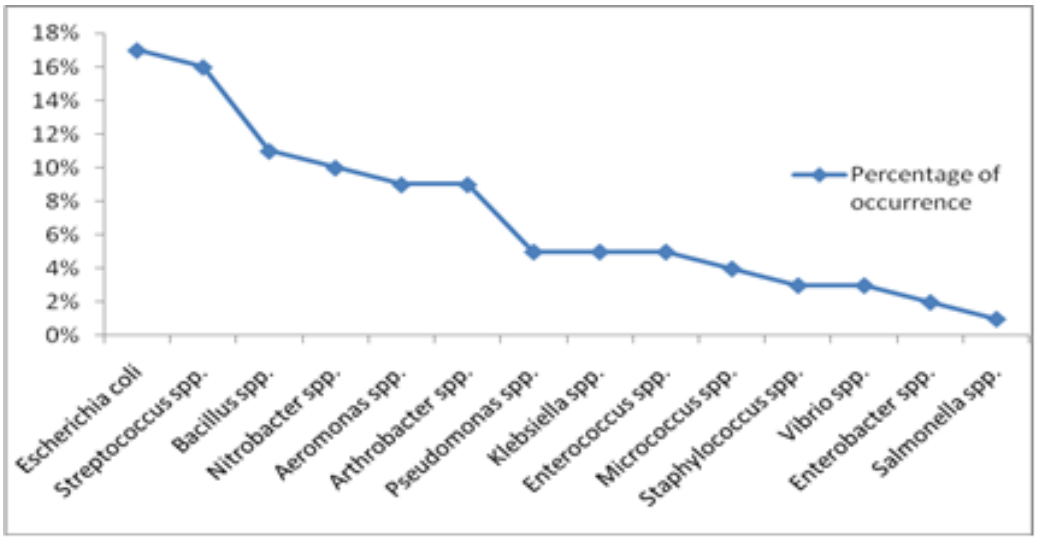

Figure 3: Percentage occurrence of bacterial isolates from Ebu stream in Afawo, Ikere-Ekiti.

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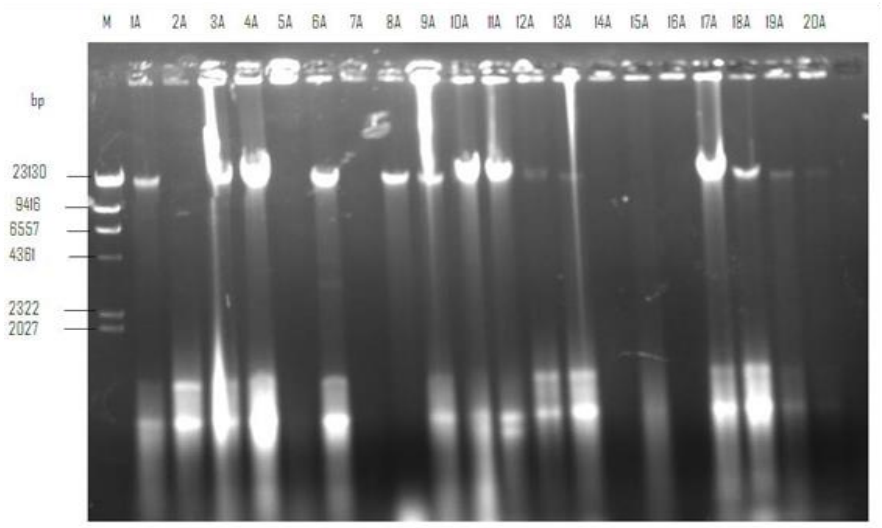

M, Hind III DNA Molecular Weight Marker

Figure 4: Plasmid profile of bacterial isolates from Ebu stream in Afawo, Ikere-Ekiti.

Twenty randomly selected MAR isolates: five of *Streptococcus* spp.; two each of *Enterococcus* spp., *Aeromonas* spp. and *Pseudomonas* spp.; and one each of *E. coli*, *Bacillus* sp., *Enterobacter* sp., *Arthrobacter* sp., *Micrococcus* sp., *Nitrobacter* sp*., Vibrio* sp., *Salmonella* sp., *Klebsiella* sp. were subjected to plasmid analysis, to detect the presence of extra-chromosomal DNA. There was absence of plasmid in four of this MAR isolates, while other sixteen isolates possessed one plasmid each. Molecular weight of the plasmids detected on all this MAR isolates ranged from 10 to 21 kbp (Table 5 and Figure 4).

The physicochemical analysis and metal concentration of the water sample from Ebu stream, Afawo in Ikere-Ekiti showed that the water was colourless and odourless, while the temperature of the water was averagely 25.4 °C and the water was minimally turbid at 0.75 NTU. The conductivity of the water was 11.4×10^2 µhoms/cm. The pH of the water was neutral (7.45), while concentrations of chloride was 21.40 mg/L, sulphate (5.60 mg/L), Nitrite (0.35 mg/L), the acidity of the calcium carbonate was 2.30 mg/L, the total dissolve solid was 21.56 mg/L, total solid (45.38 mg/L), total suspended solid (23.82 mg/L), total alkalinity was 28.50 mg/L, sodium (11.55 mg/L) potassium (16.20 mg/L), calcium (8.75 mg/L), magnesium (15.60 mg/L), zinc (0.34 mg/L) and iron (0.76 mg/L) (Table 6). The pH of soil samples (labelled A, B, C and D) were 7.62, 7.35, 6.97 and 7.4 respectively, thereby indicating that the soil pH were neutral. The organic carbon content and organic matter of the soil samples, in percentages ranged between (0.66-1.94) and (1.14-3.35) respectively. The percentages of the soil particles ranged as; clay (9-25) %, sand (80-84) % and silt (3-7) %. The analysis also revealed the presence of some metals at varying degree, these include; sodium ranged (63.4-71.6) mg/kg, calcium (49.8-62.7) mg/kg, magnesium (65.8-81.0) mg/kg, phosphorus (266.5-275.3) mg/kg, zinc (8.28-12.22) mg/kg, potassium (76.5-83.5) mg/kg, copper (3.60-4.68) mg/kg. The concentration of Exchangeable Hydrogen Ion was between 0.2 and 0.9, exchange aluminum Ion Concentration was between 0.5 and 0.8, while the Cation Exchange Capacity (CEC) of the soil samples ranged (2.53-4.13) mg/100g (Table 7).

DISCUSSION

As much as water is essential for life, it also mediates the survival of microorganisms and serves as home for pathogenic microorganisms responsible for endemic diseases that could befall the people relying on that source of water for daily activities. A good example is the scenario revealed from the study of excesses in the physicochemical and microbiological properties of Ebu stream, which is fifty meter (50 m) away from a black soap industry. The results however revealed that the stream is contaminated and threatened by environmental pollutants due to the activities of the black soap industry. Ebu stream happens to be one of the major sources of drinking water, domestic purposes and many people in Afawo and Oke'kere quarters depend on it for recreational purposes, because of inadequate supply of municipal tap water to Afawo community. The microbial composition of the water samples for the period of analysis was generally higher than expected to suit the aforementioned activities. The mean total bacterial counts (Table 1) were exceedingly higher than 1.0×10^2 CFU/mL stipulated as standard limit for bacterial count for drinking water (WHO, 2006).

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Table 2: Percentage resistance of Gram positive bacteria isolates from Ebu stream in Afawo, Ikere-Ekiti.

	Percentage resistance to different antibiotics (%)																							
	COT			CXC			ERY			GEN			AUG			STR			TET			CHL		
Isolates	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
Streptococcus spp.	88	-	13	94	-	6	75	-	25	31	-	69	87	13	-	25	6	69	37	50	13	62	13	25
Enterococcus spp.	60	20	20	80	-	20	60	20	20	40	-	60	80	-	20	-	60	40	-	40	60	60	20	20
Micrococcus spp.	100	-	-	100	-	-	100	-	-	50	-	50	100	-	-	50	-	50	50	50	-	100	-	-
Nitrobacter spp.	80	20	-	100	-	-	80	-	20	-	90	10	-	80	20	20	30	50	50	50	-	80	10	10
Staphylococcus spp.	100	-	-	100	-	-	67	33	-	-	67	33	67	-	33	67	33	-	33	67	-	67	-	33
Arthrobacter spp.	89	11	-	100	-	-	67	33	-	-	22	78	100	-	-	33	22	44	22	11	67	55	33	11
Bacillus spp.	100	-	-	100	-	-	64	-	36	18	27	55	73	27	-	36	36	27	55	45	-	45	18	36

Table 3: Percentage resistance of Gram negative bacteria isolates from Ebu stream in Afawo, Ikere-Ekiti.

	Percentage resistance to different antibiotics (%)																							
	NAL			OFL			AUG			TET			AMX			COT			NIT			GEN		
Isolates	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
Pseudomonas spp.	-	-	100	-	20	80	-	40	60	20	20	60	-	40	60	40	-	60	-	-	100	-	-	100
Klebsiella spp.	20	-	80	20	-	80	80	-	20	-	40	60	80	-	20	80	-	20	-	60	20	-	20	80
Vibrio spp.	-	-	100	-	-	100	33	67	-	67	-	33	67	-	33	33	33	33	33	33	33	-	33	67
Salmonella spp.	-	-	100	-	-	100	100	-	-	-	-	100	100	-	-	100	-	-	-	100	-	-	-	100
Enterobacter spp.	-	-	100	-	50	50	100	-	-	-	50	50	100	-	-	100	-	-	-	-	100	-	-	100
Aeromonas spp.	44	22	33	22	-	78	78	22	-	11	33	56	78	-	22	56	-	44	44	44	12	33	44	22
Escherichia coli	24	-	76	6	6	88	29	18	53	12	12	76	82	6	12	29	6	65	29	-	71	41	-	59

R, Resistance; S, Susceptibility; I, Intermediate; NAL, Nalixidic acid; OFL, Ofloxacin; AUG, Augmentin; TET, Tetracycline; AMX, Amoxicillin; COT, Cotrimaxole; NIT, Nitrofurantoin; GEN, Gentamycin; CXC, Cloxacillin; ERY, Erythromycin; STR, Streptomycin; CHL, Chloramphenicol.

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Table 4: Multiple antibiotics resistance (MAR) pattern of bacterial isolates from Ebu stream in Afawo, Ikere-Ekiti.

Isolates	Total number of isolates	Total number of MAR	Frequency of MAR isolate
Escherichia.coli	17	12	75%
Streptococcus spp.	16	15	94%
Bacillus spp.	11	11	100%
Nitrobacter spp.	10	10	100%
Arthrobacter spp.	9	9	100%
Aeromonas spp.	9	7	78%
Pseudomonas spp.	5	-	0%
Enterococcus spp.	5	4	80%
Klebsiella spp.	5	4	80%
Micrococcus spp.	4	4	100%
Staphylococcus spp.	3	3	100%
Vibrio spp.	3	2	67%
Enterobacter spp.	2	2	100%
Salmonella spp.	1	1	100%
Total	100	84	

Table 5: Plasmid profile of bacterial isolates with multiple antibiotic resistance.

Isolate	No of plasmid	Molecular weight of plasmid (kbp)
Streptococcus spp.	1	19
Streptococcus spp.	1	10
Enterococcus spp.	1	18
Escherichia coli	1	16
Pseudomonas spp.	-	-
Streptococcus spp.	1	18
Pseudomonas spp.	1	16
Aeromonas spp.	1	20
Micrococcus spp.	1	21
Vibrio spp.	1	17
Streptococcus spp.	1	18
Arthrobacter spp.	1	19
Nitrobacter spp.	1	20
Salmonella spp.	-	-
Enterobacter spp.	-	-
Streptococcus spp.	-	-
Aeromonas spp.	1	17
Bacillus spp.	1	20
Klebsiella spp.	1	20
Enterococcus spp.	1	20

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Table 6: Physicochemical and metal concentration result of water samples from Ebu stream Afawo, Ikere-Ekiti.

Parameters	Results	Standard for comparison	
		FEPA	WHO
Temperature (°C)	25.4	≤ 40	≤ 40
Odour	Odourless	Nil	Nil
Colour	Colourless	Nil	Nil
Turbidity (NTU)	0.75	-	5
Conductivity (µhoms/cm)	11.4×10^2	-	200
pH	7.45	6.0-6.9	6.5-8.5
Total dissolved solid (mg/L)	21.56	500	500
Total Solid (mg/L)	45.38	-	-
Total suspended solid (mg/L)	23.82	≤ 200	-
Total alkalinity (mg/L)	28.50	-	-
Acidity as $CaCO_3$ (mg/L)	2.30	-	-
Total hardness (mg/L)	23.20	500	125
Chloride (mg/L)	21.40	500	250
Sulphate (mg/L)	5.60	500	250
Phosphate (mg/L)	17.1	5.0	0.3-14
Nitrite (mg/L)	0.35	50	20
Sodium (Na) (mg/L)	11.55	-	-
Potassium (K) (mg/L)	16.20	-	-
Calcium (Ca) (mg/L)	8.75	200	75
Magnesium (Mg) (mg/L)	15.60	200	50
Zinc (Zn) (mg/L)	0.34	5.0	5.0
Iron (Fe) (mg/L)	0.76	20	0.3
Lead (Pb) (mg/L)	ND	< 1	0.01

Table 7: Mineral analysis of the soil samples from Ebu in Afawo, Ikere-Ekiti.

Parameters		Results			
		A	B	C	D
pH		7.62	7.35	6.97	7.41
Organic carbon (%)		1.24	0.66	0.98	1.94
Organic matter (%)		2.14	1.14	1.69	3.35
Particles size	(%) clay	9	25	15	15
	(%) sand	84	70	80	82
	(%) silt	7	5	5	3
Exchangeable H^+		0.90	0.50	0.20	0.50
Exchangeable Al^{3+}		0.50	0.70	0.80	0.60
CEC (mg/100g)		2.53	2.90	4.13	2.90
Potassium (mg/kg)		76.50	83.50	81.30	77.60
Sodium (mg/kg)		63.40	71.60	65.40	68.90
Calcium (mg/kg)		61.50	55.70	49.80	62.70
Magnesium (mg/kg)		72.40	65.80	69.50	81.00
Phosphorus (mg/kg)		266.5	275.3	268.5	270.6
Zinc (mg/kg)		11.54	12.22	10.31	8.28
Copper (mg/kg)		4.68	3.82	3.60	4.30

The high load of bacterial count could be attributed to availability of degradable nutrient materials and organic matters probably as a result of the industrial effluent. This tends to increase the demand for oxygen thereby resulting in the lowering of the dissolved oxygen. This is in agreement with the report of Obire *et al*. (2008) that relates the low dissolved oxygen concentration to increase in the bacterial population as a result of high flow of nutrient materials. The mean total coliform counts were also higher than zero coliform count per 100 mL of water sample, as stipulated by the Environmental Protection Agency as the Maximum Contamination Level (MCL) for coliform in water (EPA, 2003). According to Osuinde and Enezie (1999) and Odeyemi *et al*. (2012), the implication of the high load of coliform in this water could be directly linked to anthropogenical activities of either human or animal origin. Moreover, the condition surrounding the stream as shown in Figure 1 has a lot to do with the water likely to be polluted with its proximity to soap industry. The presence of bushes and shrubs makes it likely possible for animals to visit this source of water for drinking and consequently pass out faeces into the water bodies (Banwo, 2006).

The isolated organisms from Ebu stream entails fourteen genera, most of which have been frequently reported in several studies on surface water sources (Okonko *et al*., 2008; Odeyemi *et al*., 2011; 2012); and were indicated to as agent of alteration to the beneficial uses of surface water. Although, several researchers have drawn out minimal acceptable ranges of concentrations for different genera of bacteria from site to site and at different location and time. Pathogenic bacterial isolates like *Salmonella* sp., *Enterococcus* spp., *Escherichia coli* and *Vibrio* spp., occurring in this water source is of great significance to the public health because, their link to several gastrointestinal infections like dysentery, diarrhoea, typhoid fever and cholera, has long been documented (EPA, 2003). The presence of *Enterobacter* spp. and *Bacillus* spp. in the water could be linked to the bushes and soil surrounding the water body, corresponding to the report of Schlegel (2002); which related that *Enterobacter aerogenes* are non-faecal coliform that could be found in vegetables and soil. Moreover, many *Bacillus* spp. has long been documented to originate from soil and vegetation. Apparently, the exposure pathways through which contaminated water bodies can cause significant human health problems including swimming and recreational, drinking and consumption of seafood sourced from the water; makes it more alarming as it threatens their nearest community wellbeing. The occurrence and distribution of bacterial isolates from water samples used in this research work correspond to the results of other similar research (Pitt, 2007; Obire *et al*., 2008).

It becomes more deleterious to detect that the isolated bacteria from this water showed multiple antibiotic resistant (MAR). Among twenty antibiotic resistance bacteria tested, result showed that only four were plasmid- free while others carry a plasmid with exceedingly high molecular weight. This is in agreement with the finding of Odeyemi *et al*. (2013a) which reported the occurrence with high molecular weight of plasmids in the MAR bacteria isolated from a surface water "Arinta waterfall" in Ipole-Iloro Ekiti. Considering the wide range

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of hosts presented by microbial population in water environment, the potential health hazard is heightened knowing that the plasmid profile analysis indicated that the gene responsible for the resistance of some of the microbes to the antibiotics is located on the R-plasmid (Odeyemi *et al*., 2013b).

Although most of the physicochemical parameters recorded for the stream water could be said to be within the range of permissible limit documented by the Federal Protection Agency and World Health Organization for surface/natural water (FEPA, 1991; WHO, 2006). There are however some forms of correlation between the bacterial proliferation in Ebu stream and the physicochemical properties. The pH obtained for the stream water sample was within acceptable limit (WHO, 2006), which is very closer to the range (6.3-7.2) obtained in a similar studies on Eruvbi stream by Imoobe and Koye, (2011); and Odeyemi *et al*. (2012) also recorded 6.7 for Omisanjana stream. According to Ademoroti (1996), the circum-neutral values obtained for this water source could be attributed to CO_2/carbonate/bicarbonate equilibrium. The temperature of Ebu stream was though far below permissible limit, but the temperature must have been altered by factors traceable to the effluent discharge into the water body which is expected to be warm condensate in the water and increase in organic load leading to increase in absorption of heat (Ekhaise and Anyasi, 2005). More strong evidence for the aforementioned happening is the high value obtained for the turbidity of the water, hence the reason for slightly increased value for the suspended and total solids; since turbidity is a direct reflection of the concentration of suspended matters (Alabaster and Lloyd, 1980). Water temperature is influenced by substrate composition, turbidity, vegetation cover, run-off, inflows and heat exchange with the air (Umeham, 1989). The high electrical conductivity obtained for the water sample is directly a product of the concentration of ionic substances; magnesium, nitrate, chloride and calcium, which were all recorded with slightly high values though below the FEPA/WHO limit. This could be attributed to the industrial effluent discharged into the water system, in relation to a similar study by Obire *et al*. (2008). The biological significance of this is the disruption of the delicate ecological balance of the ecosystem, a reduction in population of organisms, and a subsequent loss of the already depleted biodiversity of the stream (Imoobe and Ohiozebau, 2009). A clear reflection of Afawo black soap industry as the major source of pollutant depriving Ebu stream its natural quality could be traced out from the level of alkalinity obtained for the water sampling. The alkalinity level could be attributed to the effluent containing concentrates of sodium-carbonate and potassium-hydroxide, both of which are major component used in the production of the soap (Schumann and Siekmann, 2005). Hence the reason for high value obtained for sodium and potassium in the water sample. Increased level of phosphate in the stream exceeding acceptable range limit of (0.3-14.0) mg/L, could be assumed to be as a result of vegetation surrounding the stream since phosphate according to Schmittner and Giresse (1999), is a major source of phosphorus for plant uptake. Apparently, the presence of nitrate, sulphate and phosphate will serve as nutrient for microorganisms to aid their proliferation, thereby altering the physicochemical properties of the water while microbial load increases (Okoye *et al*., 2011). This is in consonance with the report of Ovie (1997) that noted nitrate and phosphate as limiting nutrients in aquatic system and indices of eutrophication in rivers, lakes and reservoir.

The result of the mineral analysis obtained for the soil samples from Afawo black soap industry and Ebu stream, corroborate the presumed significant effect of the industrial discharge on the usage of the stream water. All parameter tested including ionic compounds and metals were more abundant in the soil, proving a relationship between their content in soil and in the stream water. High value obtained for phosphorus for example is as a result of vegetation surrounding the Ebu stream, in accordance to the report of Schmittner and Giresse (1999). According to the report of Geldreinch (1980), the possible means by which the composition of the soil between Afawo black soap industry and Ebu stream confer pollution on the water body could be primarily as a result of rainwater runoff/erosion and through foot transfer by the users of the stream water.

CONCLUSION

The study has indicated faults in the physical, chemical and microbiological properties of Ebu stream water, signifying the influence of the composition of the effluent discharge from Afawo black soap industry on the quality of the water and even the proximity; hence disclaiming its usability. The study thereby advocates the need for public enlightenment or awareness of the inherent dangers associated with the consumption and usage of this water in untreated form. Moreover, proper treatment of this factory effluent should be ensured before discharging into the water body. Dumping of waste products on the nearby soil should be disallowed and soil erosion should be controlled appropriately by proper channelization of wastewater and runoff from the industry. However, the result of multiple antibiotics resistance obtained in this study should be helpful for health care personnel in proper monitoring of rural waters and suggest possible solutions to problems that may arise from these resistance strains.

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