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AQUATIC ENVIRONMENTAL ASSESSMENT IN RELATION TO BURULI ULCER EPIDEMIOLGY IN OGUN STATE, NIGERIA: THE PUBLIC HEALTH IMPLICATION

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ABSTRACT

This study attempted to access water quality status of aquatic habitats in BU identified communities in Ogun State and evaluated the heavy metal level in the rivers using fish as a bio-monitor. Water and fish samples were sourced from Eggua, Yewa and Whekan Topa rivers in Ogun State. Physico-chemical parameters and heavy metals were assessed using standardised methods for water and waste water analysis protocol. All the assessed physico-chemical parameters and heavy metals were within acceptable limits for fresh water bodies. However, WhekanTopa river showed highest level of total dissolved solids, conductivity and chemical oxygen demand which were significantly higher than those of Eggua and Yewa rivers. The limnology from this study revealed no obvious evidence of pollution of the study locations. This condition does not encourage growth sustenance of Mycobacterium ulcerans pathogen as it has been established that BU pathogen growth thrives in poor water quality environment. Therefore, it is of public health importance that the environments studied should be protected from environmental pollution to forestall possible M. ulcerans increased proliferation and concomitant outbreak of Buruli ulcer disease in the study areas.

Keywords: Water quality, Buruli ulcer, aquatic environment, public health, Ogun State.

INTRODUCTION

Water quality integrity of aquatic habitats is an indicator of the functionality of aquatic life (USEPA, 2016). Limnology is vital in the evaluation of optimum water quality through identification of specific factors affecting water quality for the purpose of proffering sustainable means of optimal utilization of water resources (Ovie *et al*., 2011). Aquatic life consists of myriads of biotic and abiotic components which contribute to the state of aquatic life (Ellis *et al*., 2011). The natural equilibrium of aquatic life is constantly disrupted by injurious activities originating from arrays of anthropogenic sources. Urbanization, agriculture and industrialization accompanied by flooding and landslides, all contribute to the distortion of natural interplays within aquatic life (Adeyemo, 2005; Otuh *et al*., 2011). These disturbances lead to increased favorable conditions for the proliferation of harmful biotic components such as pathogens of infectious diseases. Many of these diseases such as Buruli ulcer (BU) have been noted to be associated with aquatic life as some researchers hypothesized connection of the causative organism, *Mycobacterium ulcerans* with aquatic niches (Ross *et al*., 1997; Stinear *et al*.,

2000; Portaels *et al*., 2008). However, the exact mode of transmission of Buruli ulcer (BU) still remains evasive (Johnson *et al*., 2005; Merrit *et al*., 2010; Marion *et al*., 2015). Buruli ulcer is a disease with severe burden on children especially in Central and West Africa (Johnson *et al*., 2005; Aujoulat *et al*., 2003; Garchitorena *et al*., 2014). The devastating effects of this disease cause extensive abrasion and necrosis of skin and soft tissues with muscle atrophy leading to formation of enormous ulcers and bone disfiguration (Phanzu *et al*., 2006). Affected people suffer high disability burden and normal educational process for children hampered (Owusu and Adamba, 2012). Buruli ulcer causes untold suffering, hardship, economic loss with social discrimination hence requires more attention directed towards enlightenment and funding for research (Hagarty *et al*., 2015).Since the mode of transmission of Buruli ulcer (BU) is unknown, prevention and control strategies become difficult however, researches have postulated possible ways of *Mycobacterium ulcerans* infection from the environmental (ecology) and vector-borne sources (Garchitorena *et al*., 2015). Environmental sources show that *Mycobacterium ulcerans* pathogen can be detected from abiotic components; water; soil, sediments and biotic components; detritus, amoeba, frogs, snails, aquatic plants and fish (Marsorllier *et al*., 2002; Marsorllier *et al*., 2007; Eddyani *et al*., 2004; Willson *et al*., 2013, Dassi *et al*., 2015; Tian *et al*., 2016). These components have connections with water bodies. Vector-borne mechanism of transmission emanates from aquatic insects; *Naucoriscimicoides* and *Appasus species* (Carolan *et al*., 2014). Aquatic insects acquire *M. ulcerans* through the food chain from aquatic environments transmitting the pathogen to man and animal by bite on the intact skin (Carolan *et al*., 2014). Case control studies have shown that human interactions with aquatic sources; slow running waters, streams, ponds, and water logged farms through wading, bathing and agricultural activities are observable risk factors (Sopoh *et al*., 2011; Marion *et al*., 2014).

Water quality status is known to be closely related to BU transmission as poor water quality influences biological communities, leading to increased growth and proliferation of *M. ulcerans* in aquatic habitats (Hagarty *et al*., 2015). Assessment of some water bodies for oxygen, phosphorous, nitrogen and some heavy metals were known to have affected increased incidence of Buruli ulcer in some endemic communities (Duker *et al*., 2004; Garchitorena *et al*., 2015). Hagarty *et al*., 2015, explored the chemistry of natural waters in BU endemic and non-endemic regions of Ghana. Their finding revealed higher concentration of trace elements (arsenic, cadmium, copper, lead, selenium, and zinc) and low pH in Southern Ghana (BU endemic region) when compared with the Northern communities. In another study, while rainfall was key in the colonization of *M. ulcerans*, low dissolved oxygen and high temperature swamps favored the pathogen hence its maintenance in aquatic environment is seasonal, making the organism to thrive in some periods of the year displaying complex transmission interplay between biota and the ecology (Garchitorena *et al*., 2014). These findings imply that pollution of aquatic bodies in BU endemic areas would encourage growth and proliferations of *M. ulcerans* hence increase in BU incidence.

With the detection of *Mycobacterium ulcerans* DNA from several biotic and abiotic sources, an insight of linkage from environmental and human/animal possibility of transmission exists (Marsorllier *et al*., 2002; Marsorllier *et al*., 2007; Eddyani *et al*., 2004; Dassi *et al*., 2015; Tian *et al*., 2016). Therefore limnology and ecological studies are important channels to extensively explore in understanding the epidemiology of BU. Evidence of Buruli ulcer in Ogun State indicates closeness of the communities with BU endemic Benin republic (Otuh *et al*., 2018; Adeneye, 2015). These locations identified as BU hotspots are linked with network of

aquatic bodies constantly assessed by the rural people for source of water (Otuh *et al*., 2014). For this reason, this study was embarked upon to evaluate the physico-chemical factors of some aquatic bodies (rivers) within communities identified with cases of BU in Ogun State sharing proximity with BU endemic Benin Republic. The outcome will give an insight on whether the levels of physico-chemical parameters and heavy metals present in the water bodies studied, favor *M. ulcerans* growth and maintenance in the environments at risk.

MATERIALS AND METHODS

Study Area

The study area is located in Ogun State, southwestern Nigeria. Yewa north, Yewa south and Ipokia Local Government Areas (LGA) served as the study locations. Three rivers located within these LGAs, namely: Eggua, Yewa and WhekanTopa Rivers in the Eggua, Idogo/Ipaja/Oke-Odun and Whekan communities respectively were assessed.

Yewa river is a trans-boundary river between Republic of Benin and Nigeria connecting several communities in Yewa LGA while Eggua river is believed to have common source from Yewa river flowing into Whekan Topa river which empties into the Tongeji Island in the Republic of Benin. These rivers lie in the geographical grid reference of N 06.8333, E 002.9089 for Yewa river; N 06.4676, E 002.7518 for Whekan Topa river and N 07.0646, E 002.8969 for Eggua river. Inhabitants of these communities are mainly subsistent, and depend largely on the traversing river networks for both portable and domestic water supply. These rivers have high human activities providing means of livelihood to the artisanal fishermen and women engaging in varying degrees of commercial activities (preparation of local locust beans; iru).

Water and fish sample Collection/Preservation

Sampling was carried out by means of boat cruises/life raft (propel), and using a 12 Channel global positioning system navigator (GPS Magellan 315®) the flow chart of the Rivers were identified. Sample collection process spanned through October 2015 to March 2016 mainly during the dry season period of the year. Water samples were collected one meter below the surface water body in triplicates at three different points; upstream, midstream and downstream using one liter sized Lamotte water sampler [Model: JT-1 Dynamic Aqua-supply Ltd] from each of the rivers. Approximately 1.5L of water was collected into pre-cleaned plastic containers and airtight corked from all the points. From each river a total of 9 surface water samples were collected. They were subsequently kept cool on ice in a Coleman cooler at 4^0C and transported to the laboratory where analyses were conducted within 24hours.

The fish collected from the three rivers were all edible fish belonging to four different species and identified as *Chrysihthysauratus*, *Oreochronisniloticus*, *Sarotherodon (T.) galilaeus*, *Heterobranchusbidorsalis* and *Lamproluguscallipterus*. A total of 58 fish samples were collected during the period of study. They were filleted and only the musculature preserved in the freezer at − 4°C for subsequent laboratory analysis.

Chemical Analysis

Surface water quality parameters were determined according to standard procedures of the Society for Analytical Chemistry manual, 1973 and the APHA-AWWA-WPCF, 2010 manual as follows: pH was determined electronically using a pH meter, salinity and conductivity were determined electrometrically (JENWAY 3510 pH meter and PHILPS ECscan 40 conductivity tester), total hardness (EDTA titrimetric method); total dissolved solids (gravimetric method); dissolved oxygen (Winkler

titrimetric method); biochemical oxygen demand (dilution Winkler method); chemical oxygen demand (reflux oxidation titrimetric method); nitrate (phenoldisulphonic acid colorimetric method); phosphate (Ascorbic acid colorimetric method); sulphate (turbidimetric method); ammonia (Nessler's colorimetric method); carbondioxide and metal Pb, Cr, Cd, and Zn by atomic absorption spectrophotometric method (Buck Scientific Model 200A). Arsenic and mercury were determined in the water samples by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES-Perkin Elmer, Elan 4000). Commercial BDH stock standards were used for the instrument calibration.

The dissected fresh fish samples already gutted and filleted had their musculature weighed and oven dried at 105^0C to a constant weight. The weighed dried fish samples were milled with a centrifugal milling machine into which 2mm sieve had been incorporated. Two gram (2.00g) of the sample was weighed into a 60ml digestion tubes and digestion carried out using 10ml each of perchloric acid and nitric acid at the ratio of 1:1. The tubes were placed into the digestion blocks and slowly digested at 120^0C for 2 to 3 hours. The digest were washed into 100ml volumetric flask and made up with distilled water. The extract was analyzed for Pb, Cd, Cr and Zn using FAAS while As and Hg were analyzed for using ICP-OES as stated above. A blank sample was incorporated for every five fish samples processed.

Data Analysis

R© software statistical package for probability sampling was adopted in the analysis of data computed into means, with standard deviation. Analysis of variance (ANOVA) was used to compare group means while Tukey HSD postHoc test was used for multiple comparisons of means. Statistical significance was determined at $\alpha \leq 0.05$.

RESULT

Physical observations of the study locations

The three Rivers; Eggua, Whekan Topa and Yewa, were all situated in the interior rural settings. There were obvious extensive human activities (Figure 1). Such activities were for domestic (bathing, fetching water and washing cloth) and commercial purposes (fishing and preparation of locust beans by women).

Figure 1: Different human activities in the water bodies sampled

- **a.** Fishing activity in Yewa,
- **b.** Women washing locust beans while immersed in River Eggua,
- **c.** Eggua river showing a woman washing cloths

Physicochemical parameter levels of water samples

The mean plot of the physicochemical parameters of the three rivers (Whekan Topa, Odo Eggua and Odo Yewa) revealed pH (6.1-7), alkalinity (30-55mg$CaCO_3$), conductivity (90-853µhoms/cm), total hardness (26-125mg$CaCO_3$), total dissolved solids (44-425mg/L), DO (3.2-6.9mg/L), BOD (3.1-7.9mg/L), COD (66-143mg/L), sulphate (5.5-16.2mg/L), carbondioxide (2.1-3.3mg/L), salinity (0.04-0.4mg/L), ammonia-nitrogen (0.3-1.4mg/L), nitrate-nitrogen (1.3-1.4mg/L) and phosphate (0.2-1.1mg/L). Conductivity, total dissolved solids, total hardness, COD, and sulphates levels were highest in Whekan Topa River. However, pH and alkalinity were approximately the same level for the three rivers. There were significant differences in conductivity, total dissolved solids and COD levels of the three rivers at α (Pr ≥ F) 0.001, 0.008 and 0.001, respectively. However other detectable physico-chemical parameters were not significantly different (Figure 2).

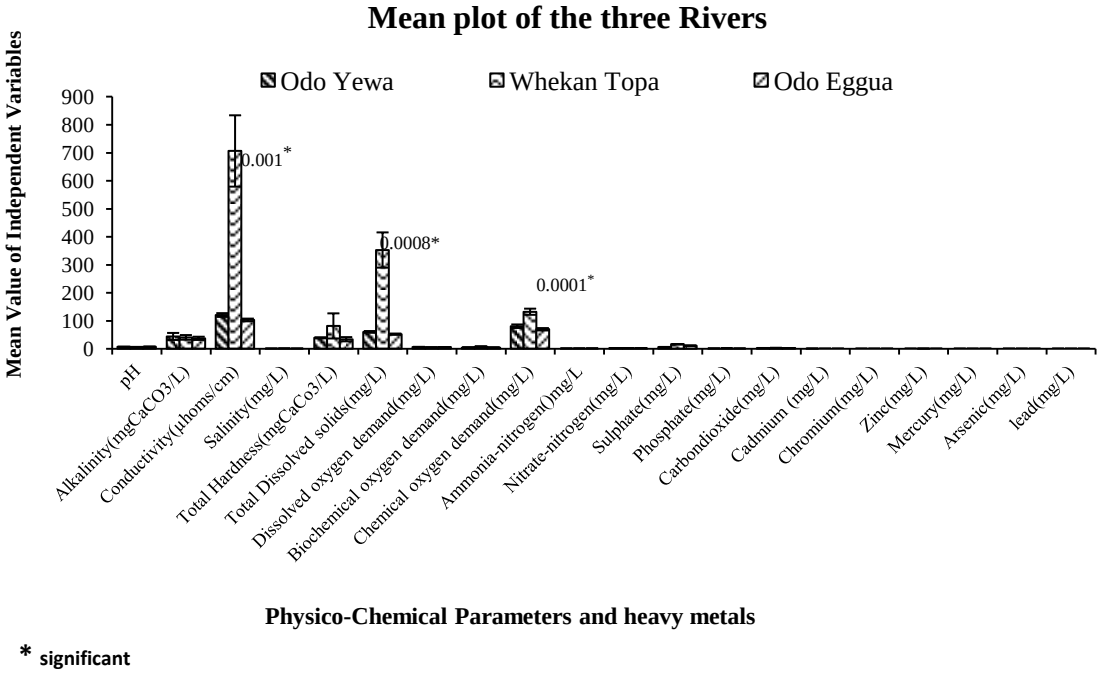

Figure 2: Mean plot of physicochemical parameters and heavy metals from the three rivers

The mean values of the physicochemical parameters sampled from different points (upstream, midstream and downstream) in each river confirmed that midstream values were higher in Whekan Topa and Eggua rivers when compared with upstream and downstream values. The contrast was the case with Yewa river which had a higher downstream value than the upstream and midstream (Table 1).

Table 1. Mean value of the physicochemical parameters of WhekanTopa, Eggua and Yewa rivers

Rivers	River points	Mean value	Standard Deviation
WhekanTopa	Upstream	62.96280	154.7333
	Midstream	81.07755	207.0424
	Downstream	58.77905	152.4280
Eggua	Upstream	15.987	27.56799
	Midstream	16.707	29.34713
	Downstream	16.274	28.79484
Yewa	Upstream	17.908	34.80643
	Midstream	18.146	32.30403
	Downstream	19.573	33.45411

In Whekan Topa, Odo Eggua and Odo Yewa no significant differences between the rivers were recorded respectively; α (Pr ≥ F) 0.911, 0.997 and 0.986. Multiple comparison, also affirmed this (Tables 2 and 3).

Table 2. Comparison of mean values of physicochemical parameters between and within the three rivers

Location(rivers)		Degree of freedom	Sum of square	Mean square	F-value	Pr≥ F
WhekanTopa	Rivers	2	5619	2810	0.094	0.911
	Residuals	57	1718021	30014		
Eggua	Rivers	2	5	2.6	0.003	0.997
	Residuals	57	46557	816.8		
Yewa	Rivers	2	32	16.2	0.014	0.986
	Residuals	57	64110	1124.7		

*ANOVA **Significant at* $\alpha_{\leq 0.05}$

Table 3. Multiple comparison of mean values of physicochemical parameters of the three rivers

Rivers	River points	Degree of freedom	95% family-wise Confidence interval		P-adj
			Upper region	Lower region	
WhekanTopa	Midstream/Downstream	22.30	154.13	-109.53	0.9129
	Upstream/Downstream	4.181	136.02	-127.65	0.9968
	Upstream/Midstream	-18.11	113.72	-149.95	0.9416
Eggua	Midstream/Downstream	0.433	22.18	-21.32	0.9986
	Upstream/Downstream	-0.287	21.46	-22.04	0.9994
	Upstream/Midstream	0.720	21.03	-22.47	0.9965
Yewa	Midstream/Downstream	-1.427	24.09	-27.18	0.9901
	Upstream/Downstream	-1.665	23.86	-27.19	0.9865
	Upstream/Midstream	-0.238	25.28	-25.28	0.9997

Tukeys Honest Significant Difference (HSD): Significant at α≤0.05

Heavy metal levels of water and fish samples

From the water samples, cadmium and zinc were detected at very low range; 0-0.01mg/l and 0.02-0.03mg/l respectively. Other heavy metals; chromium, arsenic, mercury and lead were not detected both from water and fish samples (Figure 2).

DISCUSSION

The findings from this study indicated that the three rivers within the BU identified communities had no obvious or deleterious environmental alteration of their natural state. There were no nearby sources of industrial waste pollution in those rural communities and serenity of rural settings was very apparent. Heavy metal levels in the rivers studied were not in the amounts that could be detected implying no or little anthropogenic influences. Above result is contrary to previous studies carried out in some fresh water bodies in the south western Nigeria where pollution was eminent, evidenced by elevated heavy metal levels (lead and mercury). These heavy metals with bioaccumulation potentials reported in both water and fish samples were present in levels deleterious to human population (Adeyemo, 2005; Otuh *et al*., 2011). Ducker *et al*., 2004, ascertained a connection between Arsenic enriched soil and water with the incidence of BU revealing obvious thriving of *M. ulcerans* pathogen; the etiology of BU. The report, however is different from this present study because the arsenic level in the three rivers studied were very low in such an extent that no trace was detected yet there were cases of BU in those study areas. On the other hand, the limnological results obtained from our study did not indicate favorable conditions for the maintenance of *M. ulcerans* pathogen since the levels of physicochemical parameters assessed were not indicative of pollution state which would have supported proliferation of the pathogen. In other words, pollution of the water bodies within these communities would support growth and eventual distribution of *M. ulcerans* organisms which will lead to outbreak of BU. It is also evident from this study that the water bodies still maintains relatively undisturbed ecological status attributable to low incidence of BU in those communities. Studies have shown that ecologically disturbed environments leads to increase in the incidence of BU (Johnson *et al*., 2005; Merritt *et al*., 2010; Garchitorena *et al*., 2015). Aquatic areas when disturbed by pollution gives rise to excess particulate matters reduced oxygen, subsequent proliferation of MU and increased incidence of BU.

The findings from this study showed that Whekan Topa River, Odo Eggua and Odo Yewa situated in the BU identified communities in Ipokia, Yewa North and Yewa South LGAs depicted unfavorable condition for proliferation of *M. ulcerans* pathogen as at the time of this research. All the assessed physicochemical parameters were within the levels of acceptable limits for natural fresh water habitats (USEPA, 2016). Water quality condition has in some ways been speculated to be closely connected with BU transmission; unlike other mycobacteria, *M. ulcerans* organisms thrive optimally in pH between 5.5 and 7.4 (Hagarty *et al*., 2015). Merritt *et al*., 2005, proposed that poor water quality enhances growth and proliferation of *M. ulcerans*. This assumption was supported by some studies presenting environments with low oxygen, high phosphorus and nitrogen concentrations as likely preferred for *M. ulcerans* growth, because emergence of other direct-transmission and vector-borne bacterial diseases are associated with environmental nutrient enrichment (Garchitorena *et al*., 2014; Johnson *et al*., 2010). The fact that BU had not become an epidemic situation in the study areas might not be unconnected to the fact that the environment has not been adversely distorted since this study has shown that levels of physicochemical parameters as well as the heavy metals were within recommended limits. Distortion of these identified BU endemic areas with pollution might potentiate the proliferation of *M. ulcerans* hence increasing incidence of Buruli ulcer cases in these localities.

CONCLUSION

Since the heavy metal levels were not in levels of threat in the assessed rivers, a pointer to non existence of pollution sources such as chemicals from heavy industrial activities within the communities, this portrays that the rural people are safe from harmful effects of some of these heavy metals. Water quality determines the biological, chemical and physical integrity of aquatic ecosystem hence its productivity reflected on the aquatic plant and animal biomass (Ovie *et al*., 2011). The three rivers studied in this research indicated apparently healthy status. Therefore supporting natural equilibrium of the aquatic life which if distorted may cause harmful changes and subsequent displacement of pathogens within the aquatic environments.

Recommendation

Communication and education of the inhabitants of these communities as well as the surrounding communities at risk is very important. They must be informed by the appropriate agencies of the need not to engage in activities that can cause upturn of the environment. The importance of public health activities involving environmental protection advocacy in the communities cannot be overemphasized. Adoption of the use of precautionary measures by the rural populace should be paramount during agricultural activities and its likes that enhance close contact of environmental and human interfaces.

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