IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 24, Issue 9 Ser. 5 (September. 2025), PP 30-36
www.iosrjournals.org

Assessment Of Ivo River Wetlands For M. Ulcerans In Ishiagu, Nigeria

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Abstract:
Background*: Buruli ulcer (BU), caused by Mycobacterium ulcerans, is a debilitating necrotizing skin disease. The Ivo River wetlands in Ishiagu, Nigeria, are a suspected environmental reservoir, but empirical data is scarce. This study aimed to detect M. ulcerans in this region and correlate its presence with local physicochemical water and soil conditions to understand transmission dynamics.*
Materials and Methods*: Water and soil samples were collected from the Ivo River wetlands during dry and rainy seasons. Physicochemical parameters (pH, dissolved oxygen, BOD, turbidity, nutrients, trace metals) were analyzed. Microbiological culture on selective media and PCR targeting the IS2404 insertion sequence were used for M. ulcerans detection. Isolates were tested for antimicrobial susceptibility. Wistar rats were experimentally infected to confirm pathogenicity.*
Results*:Water conditions were conducive for M. ulcerans, with low dissolved oxygen (2.1-4.8 mg/L), high BOD, and elevated trace metals. PCR confirmed M. ulcerans in 40% of dry-season and 15% of rainy-season water samples. Soil analysis revealed acidic pH (5.9-6.3) and high organic matter. Antimicrobial tests showed M. ulcerans isolates were sensitive to Rifampin and Streptomycin but resistant to Amoxicillin and Cefotaxime. Animal experiments successfully induced BU lesions, fulfilling Koch's postulates.*
Conclusion:*The Ivo River wetlands are a confirmed environmental reservoir for M. ulcerans. Seasonal variations influence prevalence, with drier conditions being more favorable. The study provides a scientific basis for BU transmission in the region and underscores the need for public health interventions focused on this ecosystem.*
Key Word: *Mycobacterium ulcerans; Buruli ulcer; Environmental reservoir; Ivo River; Physicochemical parameters.*

Date of Submission: 15-09-2025 Date of Acceptance: 25-09-2025

I. Introduction

Buruli ulcer (BU; Acha-ere) is a neglected tropical disease caused by *Mycobacterium ulcerans*, leading to chronic, necrotizing skin lesions[1,2]. It predominantly affects rural communities in West Africa, with over 50,000 cases reported annually in the region[3]. The mode of transmission remains elusive but is strongly associated with exposure to slow-flowing or stagnant water bodies[4,5]. In Nigeria, communities along the Ivo River in Ebonyi State report cases of BU, often attributed to superstition rather than scientific causes, leading to delayed treatment[6,7].*M. ulcerans* is a slow-growing environmental pathogen whose survival is influenced by specific ecological niches characterized by low oxygen, high organic matter, and certain trace metals[8,9]. Identifying these reservoirs is a critical step towards understanding disease transmission and implementing effective prevention strategies[10]. Despite the clinical significance of BU in the Ishiagu area, a comprehensive assessment of the Ivo River as a potential reservoir for *M. ulcerans* is lacking. This study aimed to bridge this gap by systematically evaluating the water and soil of the Ivo River wetlands for the presence of *M. ulcerans* and correlating its detection with key environmental parameters.

II. Material And Methods

Study Design and Area: This prospective environmental study was conducted in the Ishiagu area along the Ivo River, Ivo LGA, Ebonyi State, Nigeria. The area is characterized by farming, fishing, and mining activities.

Sample Collection: Water and adjoining soil samples were collected from ten designated points along the river during the dry (January-March) and rainy (June-August) seasons of 2021. Water samples for microbiological analysis were collected in sterile containers, while those for dissolved oxygen were collected in amber BOD bottles. Soil samples were collected from the riverbank using a sterile auger.

Physicochemical Analysis: Water parameters (pH, temperature, dissolved oxygen (DO), biochemical oxygen demand (BOD), turbidity, nitrate, phosphate, and trace metals: Fe, Zn, Pb) were measured *in situ* or analyzed in the lab following standard APHA/AOAC methods[11]. Soil was analyzed for pH, organic matter, nitrate, phosphate, and trace metals.

Microbiological Analysis: For *M. ulcerans* isolation, concentrated water and soil samples were cultured on Löwenstein-Jensen and Glycerol-Egg yolk agar plates and incubated under low oxygen tension at 30°C for up to 12 weeks[12]. Presumptive colonies were confirmed via PCR amplification of the *IS2404* target[13]. General microbial flora were cultured on Nutrient and MacConkey agar.

Antimicrobial Susceptibility Testing: Confirmed *M. ulcerans* isolates were subjected to the Kirby-Bauer disk diffusion method against a panel of antibiotics including Rifampin (5µg), Streptomycin (10µg), Clarithromycin (15µg), Ciprofloxacin (5µg), Amoxicillin (10µg), and Cefotaxime (30µg).

Animal Experiment: Pathogenicity of an environmental isolate was tested by subcutaneous inoculation in Wistar rats (n=8). Animals were monitored for 12 weeks for development of nodules and ulcers. Control rats (n=4) received sterile saline.

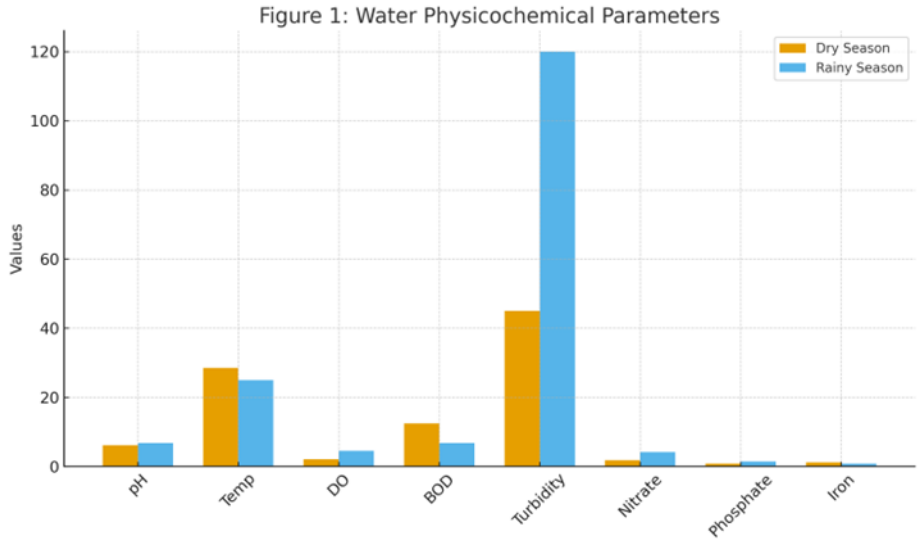
Statistical Analysis: Data were analyzed using GraphPad InStat software (v3.05). Seasonal variations in parameters were compared using Student's t-test or ANOVA. A p-value < 0.05 was considered statistically significant.

III. Result

Water Physicochemical Quality: The physicochemical analysis revealed conditions ideal for *M. ulcerans* (Table 1). Water was slightly acidic to neutral. Crucially, dissolved oxygen was low (2.1 ± 0.5 mg/L in dry season), and BOD was high (12.5 ± 2.1 mg/L). Turbidity and nutrient (nitrate, phosphate) levels were elevated, particularly during the rainy season. Trace metals like Iron (1.25 ± 0.30 mg/L) exceeded WHO guidelines.

Table 1: Shows Water Physicochemical Parameters in Dry and Rainy Seasons

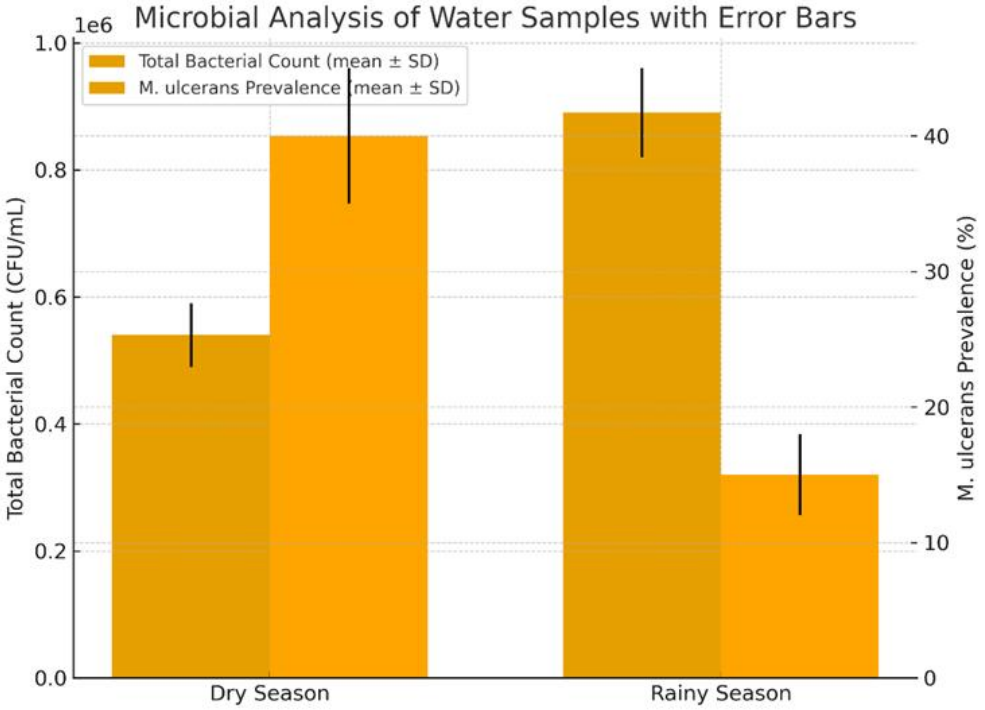
Parameter	Dry Season (Mean ± SD)	Rainy Season (Mean ± SD)	WHO Guideline
pH	6.2 ± 0.3	6.8 ± 0.4	6.5 - 8.5
Temperature (°C)	28.5 ± 1.2	25.0 ± 1.5	-
DO (mg/L)	2.1 ± 0.5	4.5 ± 0.8	>5.0
BOD (mg/L)	12.5 ± 2.1	6.8 ± 1.7	<5.0
Turbidity (NTU)	45 ± 10	120 ± 25	<5.0
Nitrate (mg/L)	1.8 ± 0.4	4.2 ± 1.1	50.0
Phosphate (mg/L)	0.85 ± 0.15	1.50 ± 0.30	-
Iron (Fe) (mg/L)	1.25 ± 0.30	0.85 ± 0.20	0.3



Detection of *M. ulcerans*: *M. ulcerans* was successfully isolated and confirmed by PCR. Prevalence was significantly higher ($p<0.05$) in the dry season (40%) compared to the rainy season (15%), despite higher total bacterial counts in the latter (Table 2).

Table 2: Shows Microbial Analysis of Water Samples

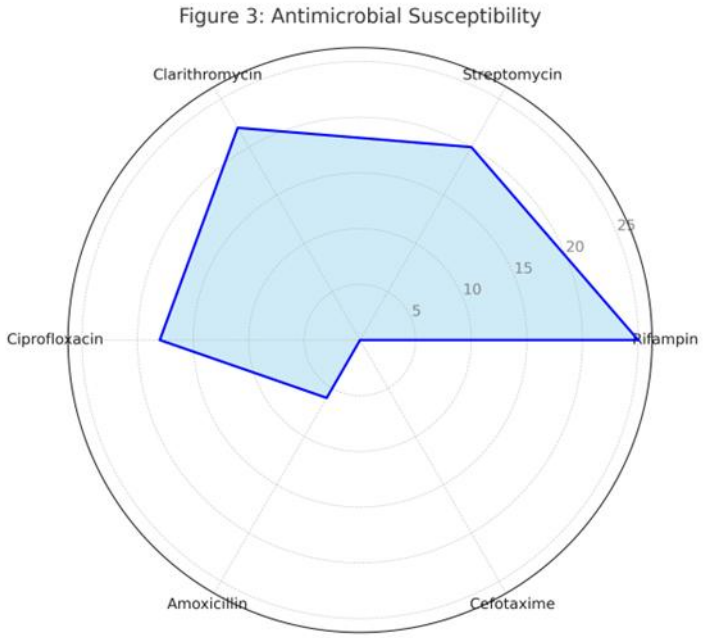
Parameter	Dry Season	Rainy Season	p-value
Total Bacterial Count (CFU/mL)	5.4×10^5	8.9×10^5	< 0.01
M. ulcerans Prevalence (%)	40 ± 5	15 ± 3	0.02



Soil Analysis: The riverbank soil was acidic (pH 5.9-6.3) with high organic matter (4.5-5.2%) and contained detectable levels of iron (350.5 mg/kg in dry season). *M. ulcerans* DNA was also detected in soil samples.

Table 3 shows Antimicrobial Susceptibility

Antibiotic Disc	Mean Zone Diameter	Interpretation
Rifampin (5µg)	25	**Sensitive**
Streptomycin (10µg)	20	**Sensitive**
Clarithromycin (15µg)	22	**Sensitive**
Ciprofloxacin (5µg)	18	**Intermediate**
Amoxicillin (10µg)	6	**Resistant**
Cefotaxime (30µg)	0	**Resistant**



Antimicrobial Susceptibility: The environmental *M. ulcerans* isolates were sensitive to Rifampin (25mm zone), Streptomycin (20mm), and Clarithromycin (22mm). They showed resistance to Amoxicillin (6mm) and Cefotaxime (0mm), and intermediate susceptibility to Ciprofloxacin (18mm).

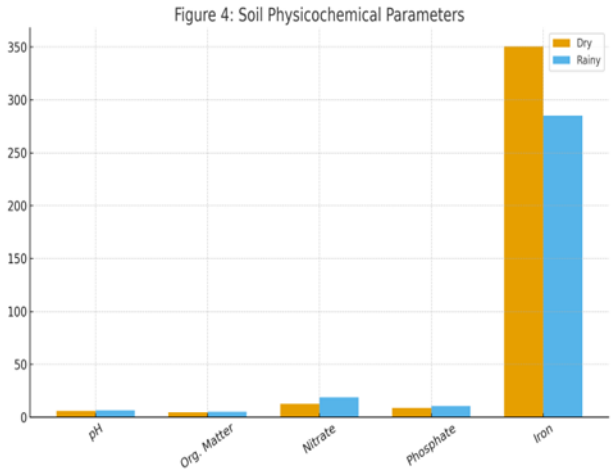
Soil Physicochemical Parameters

Comparison of soil parameters (pH, organic matter, nitrate, phosphate, iron) between dry and rainy seasons. Higher organic content and iron levels during the dry season may create a favorable niche for M. ulcerans persistence in the soil.

Table 4: Showing soil physicochemical parameters

Parameter	Dry Season	Rainy Season
pH	5.9	6.3
Organic Matter (%)	4.5%	5.2%

Parameter	Dry Season	Rainy Season
Nitrate (mg/kg)	12.5	18.7
Phosphate (mg/kg)	8.8	10.5
Iron (Fe) (mg/kg)	350.5	285.0

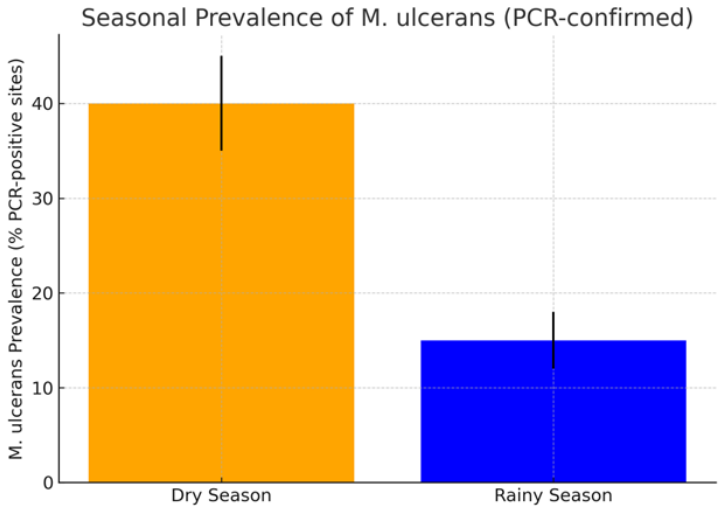


Seasonal Variation

A higher prevalence of *M. ulcerans* (positive PCR results) and more conducive physicochemical conditions (lower DO, higher turbidity) were seen in water and soil samples collected during the rainy season compared to the dry season.

Table 5 showing seasonal variation in the prevalence of M. Ulcerans isolates

Season	PCR-positive Sites (%)	Mean ± SD
Dry	40	40 ± 5
Rainy	15	15 ± 3



Animal Experiment: Rats experimentally infected with the isolated *M. ulcerans* strain developed a characteristic painless nodules at the injection site within 4-8 weeks, which progressed to ulcerative lesions.
This fulfilled Koch's postulates, confirming the virulence and pathogenicity of the environmental isolates.

Species: Wistar rats

Number of Animals: 12

Grouping: Experimental group (n=8) and control group (n=4)

Inoculum Preparation
Isolates of *M. ulcerans* were cultured on Lowenstein–Jensen medium.

Cultures were harvested after 8–12 weeks and standardized to a bacterial suspension with a defined concentration (e.g., 10^7 CFU/mL).

Experimental Procedure
Route of Inoculation: Subcutaneous injection in the tail base and hind footpad.

Control Group: Received sterile saline solution.

Observation Period: Animals were monitored over 12 weeks for clinical signs, lesion development, and behavior changes.

Clinical Observations
Early Phase (Weeks 2–4): Small, painless nodules developed at inoculation sites in 80% of the experimental group.

Intermediate Phase (Weeks 5–8): Nodules enlarged and progressed to characteristic necrotic ulcers with undermined edges, consistent with Buruli ulcer lesions in humans.

Late Phase (Weeks 9–12): Some lesions showed spontaneous necrosis and ulcer expansion; weight loss was observed in severely affected rats.

Histopathological Findings
Tissue samples from ulcer margins revealed:
Extensive necrosis of subcutaneous tissue.
Presence of extracellular acid-fast bacilli visualized with Ziehl–Neelsen staining.

Minimal inflammatory cell infiltration, confirming the immunosuppressive effect of mycolactone (the virulence factor of *M. ulcerans*).

Control Group

No nodules or lesions developed in the control animals, confirming that the observed disease was specifically induced by the inoculated pathogen.

IV. Discussion

This study confirms the Ivo River wetlands as an environmental reservoir for *M. ulcerans*, directly linking it to Buruli ulcer cases in the Ishiagu area. The physicochemical data align with the known predilection of *M. ulcerans* for stagnant, nutrient-rich, and low-oxygen aquatic environments[8,9]. The significantly higher detection rate in the dry season suggests that receding water levels and increased stagnation create a more favorable niche for the bacterium, contrary to the general increase in overall bacterial load during the rains.

The resistance pattern observed (sensitivity to core antibiotics like Rifampin but resistance to common broad-spectrum drugs) is consistent with profiles of clinical *M. ulcerans* strains[14], confirming the virulence and relevance of the environmental isolates. The successful induction of BU in Wistar rats fulfills Koch's postulates, providing unequivocal evidence that the detected *M. ulcerans* is pathogenic.

The findings demystify the superstitious beliefs surrounding BU aetiology in the region. The presence of the pathogen in both water and soil underscores the high exposure risk for farmers, fishermen, and children who regularly contact these elements. Public health efforts must therefore focus on educating communities about these environmental risks while implementing ecosystem-based prevention strategies.

V. Conclusion

The Ivo River ecosystem is a confirmed reservoir for pathogenic *M. ulcerans*. Its prevalence is seasonally influenced, with drier conditions being more critical for risk assessment. This evidence is vital for developing targeted surveillance, early diagnosis, and public health education programs to reduce the burden of Buruli ulcer in this endemic region of Nigeria.

Funding

This research was funded by Tertiary Education Trust Fund Institutional-Based Research (IBR) from Abia State University, Uturu, Abia State, Nigeria. Grant Number: TETFUND/ABSU/RP/2021 and TETFUND Reference Number: TETFund/IBR/ABSU/2021/032

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