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The Physico-Chemical Factors of Water Influencing the Growth of Mycobacteria in Ivory Coast

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Abstract: *Environmental mycobacteria are responsible for skin infections in humans and animals. In Cote d'Ivoire, very little information is available concerning the presence of these environmental mycobacteria except for the one that causes Buruli ulcer (M. ulcerans). In Côte d'Ivoire almost all regions are affected. It is a real public health problem. The mode of transmission and environmental sources are not well known. The presence of these mycobacteria in the environment would be linked to several physical and chemical factors favoring their growth and proliferation. This would be a significant risk for people in permanent contact with the environment. This study was conducted in order to determine the environmental factors that may influence the presence of mycobacteria in Cote d'Ivoire. The results showed that the physical-chemical parameters influencing mycobacterial proliferation were: Conductivity ($p < 0.001$) ≤ 84, nitrite ($p = 0.001$) ≤ 98.5, free chlorine ($p < 0.001$) ≤ 13.1, and according to the type of sample ($p = 0.044$), the sediment was the most suitable.). The species M. peregrinum, M. chelonae, M. abscessus, M. mucogenicum, M. immunogenum, like M. smegmatis, like M. peregrinum and Mycobacterium sp. have been identified. At 50.76%, in hyper-endemic sites and 49.23% in hypo-endemic sites of Buruli Ulcer. This study discovered the presence of mycobacteria in all selected sites, hypo-endemic and hyper-endemic, corresponding to both environmental saprophytic species and potentially pathogenic species, as well as unidentified species*.

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I. Introduction

Environmental mycobacteria, also known as atypical mycobacteria or mycobacteria other than *M. tuberculosis* or NTM, are germs that cause lung, cutaneous or lymphatic infections [1]. They constitute a large taxonomic group distributed in various aquatic and terrestrial environments (**Winthrop et al., 2002, Sniezek et al., 2003, Marsollier et al., 2002 and 2004**). Most are saprophytes but some species are pathogens capable of infecting humans and animals. Compared to other bacterial species, environmental mycobacteria are exceptionally resistant to certain conventional disinfection products such as chlorine (**Carson et al., 1978, Kubalek and Komenda, 1995**).For that reason, they can escape water purification by the water distribution company and the pipeline network. Although water distribution companies are unfamiliar with these pathogens, many cases have shown that water plays a significant role in the transmission of NTM (**Wallace et al., 1998)**. They are capable of developing under extreme conditions such as acid ecosystems or alkaline waters (**Falkinham et al., 2004**). Their ubiquity is due to their ability to grow well on any ecological niches unoccupied by other microorganisms (**Falkinham et al., 2004**). The number of isolated environmental mycobacteria based on mycobacteria from clinical samples is increasing in the industrialized countries. The distribution of commonly isolated species is constantly changing in most of these countries and new species are emerging (**Martin-Casabona et al., 2004**). They would form part of the group of non-pigmentogenic atypical mycobacteria such as *M. abscessus, M chelonae, M fortuitum, M smegmatis* (**Brown and Wallace, 1992**). Buruli ulcer, caused by *M ulcerans*, is a disabling disease. In Côte d'Ivoire almost all regions are affected. It is a real public health problem. The mode of transmission and environmental sources are not well known. Atypical mycobacteria thus invade water that is generally polluted by various agents (chemicals, household waste) and the infections caused by them are increasing everywhere on the globe. In the environment, mycobacteria are found frequently in a very acidic environment (**Kirschner et al., 1992**). It is therefore extremely important to know the ecological conditions that could encourage the proliferation and persistence of NTM in aquatic environments (**Bland et al., 2005**). Therefore, in this study, some ecological factors that could influence the presence of mycobacteria in hypo-endemic and hyper-endemic sites in Côte d'Ivoire were studied. This

information would be necessary for the establishment and implementation of a preventive measures in case of possible outbreak of mycobacterial infections.

II. Materials And Methods

Equipment for measuring physical and chemical parameters of water

Various equipment has been used to measure the physical and chemical parameters of water and sediments. The pH values were measured using a Wagtech® portable pH meter. Measurement of conductivity, temperature and turbidity of water required the use of a Wagtech WTD® portable conductimeter and a Wagtech WTD® portable turbidimeter. Analysis of nutrient salts in water (nitrate, nitrite, phosphate, phosphorus, iron, etc.) was carried out using a photometer (7100 Wagtech WTD®). The analysis of nutrient salts in sediments (nitrate, nitrite, phosphate, phosphorus, iron, etc.) was carried out using a spectrophotometer (HACH LANGE DR 2800®), a GPS (Global Positioning System) (MLRSP 12X®) was used to obtain the geographic coordinates of the various sampling sites.

Biological Materials

The biological material consisted of water and sediment samples from the different environment studied.

III. Methodology

Sites and scope of the study.

The study was carried out in areas considered to be hyper-endemic to *Buruli ulcer* (Adiopodoumé, Tiassale, Adzopé) and hypo-endemic areas (Agboville, Bouaké, Aghien) according to the National Program for the Control of *Buruli ulcer* in Côte d`Ivoire. The sites were selected on the basis of the presence of at least one reported case of *Buruli ulcer* in the zone. They are: Adiopodoumé, Tiassale, Adzopé (hyperendemic zone) and Agboville, Bouaké, Aghien (hypoendemic zone) A monthly sampling was carried out from June 2014 to June 2015. A total of 22 collection stations was selected as follows: 11 from the Lagoon of Aghien, 3 from Adzopé water retention. 2 stations, each were selected from Sokrogbo sites, Bodo and Adiopodoumé respectively. 1station each were selected, at the entrance of Agboville and Loka in Bouake water retention.

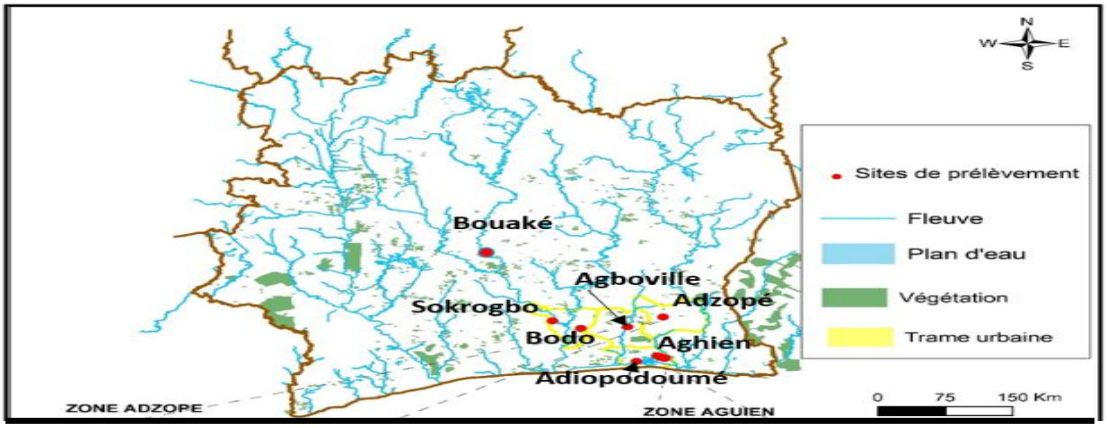

Figure 1: Sampling Sites

Protocol for sampling water and sediment samples

Special attention was given to the sampling equipment. Sterilized borosilicate glass bottles with Teflon stoppers were used. These vials were reused after adequate washing. For washing, detergents without phosphates or boron were used to avoid contaminating the samples. At the time of sampling collection, for the chemical analysis, the flasks were again rinsed 3 times with water to be analyzed and then filled to the top. The cap was inserted in such a way that there is no air bubble and not ejected during transportation in accordance with **NF EN ISO 1945 (2006).**

A 5 liter capacity bucket spouts allowed to draw water at the bank of water points and a hydrological bottle 1.5 L capacity for drawing water far away from the banks. For the samples of mycobacteria culture, a quantity of water was taken using the hydrological bottle. The sterilized Falcon 50 ml collecting tubes were used for the conditioning of the collected water samples. A cooler containing ice packs for the conservation of the samples to be conveyed to the laboratory. The sediment samples were collected using dump Eckmann at big water points and a sampler at the banks. Zip lock plastic bags were used for the treatment of the collected sediments. A mass of 500 g of sediment samples was collected about 5 cm deep (**Schiavone S and Coquery M., 2011**). All samples taken were marked with an indelible marker (sampling site, date, sample number,

sample type). For *in situ* measurements, 5 g of sediment was mixed in 5 mL of sterilized distilled water. Then, the supernatant was recovered in a glass tube and placed in a Wagtech instrument for reading the pH, temperature, conductivity and turbidity values. The samples were then kept refrigerated at 4°C during transportation, protected from light and taken to the laboratory within 24 hours of sampling in in accordance with **NF EN ISO 5667-15 (2009).** Transfer to the laboratory took place on the day of collection. A total of 473 samples was obtained, comprising of 251 water samples and 222 sediment samples distributed according to the sampling sites.

Physico-chemical analyzes

Measurements of temperature, conductivity, turbidity and pH values were carried out *in situ* between 9 and 10 am. The chemical analysis of the water and sediment samples were carried out on the same day or the following day, depending on the study site, taken to the Chemical and Environmental Microbiology department of the (Institut Pasteur-Côte d'Ivoire). The analysis was carried out using photometry (7100 Wagtech WTD®) with pellets adapted to each desired mineral element, namely nitrate, nitrite, phosphorus, phosphate, total iron, free chlorine, Calcium hardness, total chlorine. Analysis of mineral salts in sediments (nitrate, nitrite, phosphate, phosphorus, iron, free chlorine, calcium hardness, total chlorine) was carried out using a branded spectrophotometer (HACH LANGE DR 2800®) .

Culturing

In the laboratory, 500 g of each sediment sample and 100 mL of water were collected. In a Falcon tube of 50 mL capacity, 10 g of sediment was mixed with 40 mL of sterilized distilled water according to the method described by **Clovice et al. (2011)** slightly modified. After mixing the sediment and the sterilized distilled water, the supernatant was recovered in a new Falcon tube. The decontamination of the water samples and of the recovered supernatant was carried out with cetylpiridium chloride (CPC) (**Stinear et al., 2004**), followed by neutralization with the phosphate buffer. The different medium used for seeding are: Loweinstein Jensen (LJ), Mac Conkey without crystal violet, Ordinary agar and Middlebrook 7H10. The LJ and Middlebrook 7H10 medium samples were seeded in duplicate. A batch of each of these was packed in aluminum foil for the photo-induction test. Incubations were performed at 23^{o}C and 37^{o}C in ovens. A daily observation was made until obtaining a colony.

The phenotypic characteristics taken into account in the culture are those described by Runyon et al., namely the incubation temperature, the growth time (-8 days for the rapid-growing mycobacteria and + 8 days for the slow-growing mycobacteria) , The appearance of the colonies, detectable under the magnifying glass, either eugonic colonies (rough, progressive colonies up to 1 cm in diameter) of type r or s, or dysgonic colonies (smooth, colonies always small, (Sometimes colored in the agar) r or s type, photochromogens (colonies that produce pigment when exposed to the light), scotochromogens (colonies that produce pigment when exposed to the dark), achromogens (colonies Not pigmented), also according to pigmentation, in particular pale yellow, yellow, orange, pink or red or not (**David, 1986, Euzeby, 2010**).

To confirm the presence of a mycobacterium, the Ziehl neelsen(ZN) stain was systematically carried out on all colonies obtained. An optical microscope (Zeiss®) was used for the observation of resistant acido-alkalline bacilli after Ziehl-Neelsen staining (**Barksdale and Kim, 1977**).

After the microscopic observation of resistant acid-alkaline bacilli after Ziehl-Neelsen staining (**Barksdale and Kim, 1977**), the species classification was carried out according to the method described by **Runyon et al. (1959).** The biochemical identification of mycobacteria was performed according to the method described by **Nolte and Metchock (1995)**.

Biochemical identification of strains

All strains obtained were identified according to the identification characteristics of rapid- growing mycobacteria (Group IV). Several identification tests were performed, including growth on ordinary agar (soybean trypsin) at 37 ° C, 42 ° C, 45 ° C, 52 ° C, growth on Mac Conkey without Cristal violet agar, catalase For the detection of catalase activity at 22 and 68 ° C, the Urease test, growth in the presence of 5% sodium chloride (NaCl), ammonium ferric citrate (Tison), use of citrate Of sodium as the only source of carbon, as well as the use of mannitol as the only source of carbon.

Statistical analysis

To determine the link between physical-chemical parameters and the presence of mycobacteria in water bodies, we have used the "R" software to perform decision tree modeling. This decision tree allows us to know the favorable conditions for the presence of mycobacteria in the various sites studied. The analysis tool used for the various correlations is the Principal Component Analysis (PCA). The mean values of the various parameters studied were determined. The results are given in tables and graphs.

IV. Results And Discussion

A total of 473 samples was collected, comprising of 222 sediments and 251 waters. The number of water and sediment samples per sampling site is shown in Table 1.

Table 1: Number of samples per type and sampling site

	SAMPLING SITES							
	Endemic sites				non endemic sites			
	Adzopé	Adiopodoumé	Tiassalé		Agboville	Aghien	Bouake	TOTAL
			Sokrogbo	Bodo				
water	45	18	18	6	15	143	6	251
Sediments	16	18	18	6	15	143	6	222
TOTAL	61	36	36	12	30	286	12	473

Microbiological parameters

Based on morphological and biochemical characteristics, the isolated species were all rapid- growing mycobacteria. A total of 65 strains were isolated, that is 13.74% and 17 species identified from the isolated strains, about 26.15%. After performing various biochemical tests, 8 species of mycobacteria were identified and distributed according to the sampling sites.

Based on morphological and biochemical characteristics, the isolated species were all rapid- growing mycobacteria. The species *M. peregrinum, M. chelonae, M. abscessus, M. mucogenicum, M. immunogenum, like M. smegmatis, like M. peregrinum and Mycobacterium sp*. were identified in this study. However, with 50.76%, in hyper-endemic sites and 49.23% in hypo-endemic sites of *Buruli Ulcer*. The different species are distributed according to the sites in Table 2.

Table 2: Number of Mycobacteria Species Identified according to Site

Identified mycobacterial species	hyper -endemic sites				hypo –endemic sites			
	Adzopé(n = 61)	Adiopodoumé (n = 36)	Tiassalé		Bouake (n = 12)	Agboville (n = 30)	Aghien (n = 286)	Total
			Sokrogbo(n = 36)	Bodo(n = 12)				
M. peregrinum	4	1	1	0	0	1	2	9
Like m. smegmatis	1	0	0	1	0	1	0	3
Like m. peregrinum	1	0	0	0	0	1	2	4
m. immunogenicum	0	0	0	1	0	1	0	2
m.chelonae	1	0	0	0	0	0	1	2
m. mucogenicum	3	0	0	4	0	0	0	7
m. abscessus	1	0	0	0	0	0	0	1
Mycobacterium. sp	1	5	8	0	0	3	20	37
TOTAL	12	6	9	6	0	7	25	65

Physico-chemical parameters of water and sediments

Physical parameters

Temperature, Turbidity, Conductivity, pH

In water, on the average, the lowest temperature was 25.18 ° C (± 2.84) obtained at the site1 Sokrogbo and the highest 28.07 ° C (± 2.57) obtained at the site of 'Aghien. The lowest turbidity was found at the entrance of Agboville and was 18.01 NTU (± 7.12), but increased considerably to a value of 35.08 NTU (± 21.98) at Adiopodoumé . The lowest conductivity was 38.01 µS / cm (± 11.00) at Adzopé, and the highest 79.25 µS / cm (± 20.74) at Bouaké. The average pH value was between 6.86 (± 1.05) at Bouaké and 7.62 (± 0.92) at Aghien. As for the sediment, on the average, the lowest temperature was 23.59 ° C (± 2.09) at Adzopé and the highest 27.23 ° C (± 1.57) was obtained at the site of Aghien . The lowest turbidity value was 27.20 NTU (± 1.00) in Bouaké, but increased dramatically to a value of 52.28 NTU (± 27.92) at Aghien. The highest conductivity was 81.21 µS / cm (± 47.55) at Aghien, and the lowest 39.97 µS / cm (± 9.24) at Adzopé. The average pH values ranged from 6.81 (± 0.56) at Adzope to 7.58 (± 3.23) at Aghien.

Chemical parameters

Calcium / Calcium Hardness

On the average, Calcium was 7.67 mg / l (± 9.52) at the entrance of Agboville and the highest value (40.06 mg / l (± 34.41)) was obtained at the Site of Loka in Bouaké. In the sediments, the lowest mean value was 13.64 mg / l (± 29.48) at Aghien and the highest at Adiopodoumé was 27.16 mg / l (± 30.55). On average, the calcium hardness was highest at Adiopodoumé in water 52.24 mg / l (± 17.83) and in sediments 54.03 mg / l (± 12.79), against a lower value In Agboville 22.20 mg / l (± 22.72) and Bodo 28.74 mg / l (± 14.30) in water and sediments respectively.

Magnesium

Magnesium level in water samples was very high in Bodo with an average value of 29.73 mg / l (± 12.02) and very low in Adzope 2.02 mg / l (± 4.58). In the sediments, this value ranges between 8.43 mg / l (± 11.58) in Adzope and 33.88 mg / l (± 19.15) in Agboville.

Total iron

The mean total iron value in water samples was very low 1.8 mg / l (± 2.34) in Adzopé and very high in the site 2 of Sokrogbo, 258.76 mg / l (± 134.31) . In sediments, this value ranges from 15.79 mg / l (± 26.43) in Adzope and 223.71 mg / l (± 142.49) in Sokrogbo site 1.

Potassium

The average potassium value in the water was low at 4.70 mg / l (± 6.72) in Aghien and very high in Sokrogbo site 2,at 46,00 mg/l (± 17.10). In the sediments, the mean value was low at 25.43 mg / l (± 12.22) in Adzope and very high in Sokrogbo site 1 at 40,92 mg/l (± 15.99).

Sulfate

The average sulfate value was low at 2.57 mg / l (± 16.86) at Aghien and very high in Bodo 226.25 mg / l (± 105.62). It is still high in Bodo in sediments at 213.82 mg / l (± 92.36) compared with 29.09 mg / l (± 5.92) in Bouaké.

Phosphate

The average phosphate value was low at 0.14 mg / l (± 0.05) at Agboville and very high at Bouake 68.76 mg / l (± 56.17) in water in the sediments, Average in phosphate was low at Bouake 28.00 mg / l (± 5.76) and very high at Aghien 111.65 mg / l (± 54.08).

Manganese

In the water samples, the mean manganese value was low at 0.00 mg / l (± 0.00) in Agboville and very high at 49.02 mg / l (± 21.35) in Adiopodoumé. In the sediments, values ranged from 0.02 mg / l (± 0.09) in Adzope to 60.69 mg / l (± 160.11) in Agboville.

Total Chlorine

In water samples, the mean total chlorine value was low at 0.61 mg / l (± 1.20) in Agboville and very high at 74.00 mg / l (± 20.21) in Bodo. In the sediments, the values varied from 35.00 mg / l (± 11.17) in Agboville to 270.88 mg / l (± 117.39) in Aghien.

Nitrite / Nitrate

The mean nitrite value was low in both types of samples in Adzopé at 0.8 mg / l (± 2.18) for water and 10.99 mg / l (± 18.27) in the sediments, while the value was very high in water at 52.52 mg / l (± 19.90) in Bouaké and in the sediments at 90.90 mg / l (± 127.61) in Aghien.

The mean nitrate value in the water was low at 1.33 mg / l (± 2.31) in Aghien and very high at 37.60 mg / l (± 11.30) in site 1 of Sokrogbo , whereas in the Sediments, values ranged from 10.99 mg / l (± 18.27) in Adzopé and increased to 36.63 mg / l (± 11.98) in Sokrogbo site 1.

The mean values of physico-chemical parameters measured in water and sediments are shown in Table 3 and Table 4 (respectively).

Table 3: Mean values (standard deviations) of physico-chemical parameters of water (n = number of samples collected)

Parameters	hyper-endemic sites					hypo-endemic sites		
	Adzopé	Adiopodoumé	Tiassalé			Bouaké	Agboville	Aghien
	(n = 45)	(n = 18)	Sokrogbo 1 (n = 9)	Sokrogbo 2 (n = 9)	Bodo (n = 6)	(n = 6)	(n = 15)	(n = 143)
pH	7,08 ± 0,57	7,15±0,64	6,88±0,46	7,05±0,51	7,24±0,84	6,86±1,05	7,23± 0,72	7,62± 0,92
Temperature	26,60 ± 3,57	26,97±1,58	25,18±2,84	25,44±3,26	26,00± 1,26	25,83±0,75	25,39± 4,23	28,07± 2,57
Turbidity	19,36± 15,37	35,08±21,98	30,23±10,71	28,36±12,84	33,58±18,7	22,47±8,37	18,01± 7,12	32,27±31,53
Conductivity	38,01±11,00	60,61±18,35	44,63±11,20	53,98±16,30	54,04±16,33	79,25±20,74	42,61±9,91	71,42±21,30
Calcium	10,01±10,61	35,77±35,87	11,82±23,37	14,86±22,44	38,83±19,73	40,06±34,41	7,67±9,52	25,16±17,11
Magnesium	2,02± 4,58	23,08±8,85	19,62±12,84	15,00±13,57	29,73±12,02	21,78±4,21	13,99±17,90	18,04±25,85
Calcium hardness	22,61± 25,62	52,24±17,83	41,56±21,56	46,29±12,00	30,84±14,91	44,65±15,21	22,20±22,72	27,20±24,08
Total Iron	1,48± 2,34	197,77±111,89	149,50± 113,49	258,76±134,31	188,00±87,35	156,83±40,38	5,61±11,49	3,31±11,90
Total Chlorine	1,22±2,46	38,50±12,55	72,98±21,43	63,82±19,23	74,00±20,21	48,83±20,91	0,61±1,20	51,21±81,43
Nitrate	12,60± 14,36	26,11±5,67	37,60±11,30	29,00±10,74	30,33±12,40	28,83±8,21	12,69±15,99	1,33±2,31
Nitrite	0,8± 2,18	49,62±17,23	38,84±10,87	41,07±8,00	43,14±9,09	52,52±19,90	4,52± 5,35	3,51±26,60
Potassium	26,18±12,33	31,12±10,73	42,14±12,31	46,00±17,10	27,18±8,72	33,73±9,97	22,19±22,70	4,70±6,72
Sulfate	18,4± 24,71	33,28±6,97	89,88±117,65	108,36±127,54	226,25±105,62	37,00±6,66	33,80±41,01	2,57±16,86
Phosphate	20,32± 29,47	40,41±29,38	39,83±17,00	46,31±14,81	35,00±7,24	68,76±56,17	0,14±0,05	2,01±13,78
Manganese	0,05± 0,24	49,02±21,35	44,71±19,07	39,38±19,43	37,00±19,76	47,26±8,36	0,00±0,00	0,23±2,11

Table 4: Mean values (standard deviations) of the physico-chemical parameters of the sediments (n = number of samples collected)

Parameters	hyper-endemic site					hypo-endemic site		
	Adzopé	Adiopodoumé	Tiassalé			Bouaké	Agboville	Aghien
	(n = 16)	(n = 18)	Sokrogbo 1 (n = 9)	Sokrogbo 2 (n = 9)	Bodo (n = 6)	(n = 6)	(n = 15)	(n = 143)
pH	6,81±0,56	7,20±0,46	7,01±0,27	6,94± 0,29	7,10±0,73	7,08±0,79	7,31±0,85	7,58±3,23
Temperature	23,59± 2,09	26,72±2,02	27,04 ±2,32	26,30±1,68	25,50±2,59	26,83±1,17	26,93±2,11	27,23±1,57
Turbidity	30,36± 12,03	32,68±21,33	34,52±14,25	30,92±11,91	52,00±34,25	27,20±1,00	30,36±11,78	52,28±27,92
Conductivity	39,97± 9,24	57,26±21,47	60,21±13,11	47,40±14,88	60,17±16,51	81,00±16,92	41,51±11,57	81,21±47,55
Calcium	15,25±14,99	27,16±30,55	16,88 ±25,32	14,25±21,43	39,05±19,51	19,60±29,37	19,33±18,19	13,64±29,48
Magnesium	8,43± 11,58	20,31±2,74	18,33±12,22	21,86±14,79	35,07±12,04	22,70±3,76	33,88±19,15	19,38±23,49
Calcium hardness	41,56 ± 35,58	54,03±12,79	42,00±19,69	53,33±21,60	28,74±14,30	56,10±19,65	39,68±14,37	34,38±14,39
Total Iron	15,79±26,43	188,47± 81,27	223,71±142,49	133,59±109,60	187,00±87,09	189,53±77,13	40,39±53,08	68,37±32,97
Total Chlorine	46,95± 94,34	37,00 ±10,48	64,32±21,92	63,94±16,61	70,17±17,76	35,00±11,17	63,41±107,23	270,88±117,39
Nitrate	10,99±18,27	23,94 ±3,86	36,63±11,98	25,50±8,08	30,33±12,36	24,50±5,99	11,19±15,24	24,14±21,95
Nitrite	0,19±0,35	41,68±10,8	48,46±20,82	54,31±25,61	38,81±7,53	45,45±17,59	16,76±21,30	90,90±127,61
Potassium	25,43±12,22	31,74±10,31	40,92±15,99	37,82±9,06	27,25±8,68	29,67±10,73	37,13±11,27	26,18±26,80
Sulfate	33,61±66,60	36,42±6,53	113,76±125,63	99,07±109,74	213,82±92,36	29,09±5,92	152,99±211,01	148,67±85,46
Phosphate	40,13±52,72	51,22±44,80	44,60±16,45	40,87±13,11	36,50±3,73	28,00±5,76	52,39±51,65	111,65±54,08
Manganese	0,02±0,09	49,13±15,02	39,82±16,01	36,40±16,92	37,33±19,55	51,80±25,52	60,69±160,11	22,51±29,37

Correlation between physico-chemical parameters and the occurrence of environmental mycobacteria
The presence of mycobacteria is related to a Conductivity ($p < 0.001$) ≤ 84 and nitrite ($p = 0.001$) ≤ 98.5 and chlorine ($p < 0.001$) ≤ 13.1
Comparing the type of samples ($p = 0.044$), the sediment was most favorable for mycobacteria growth.

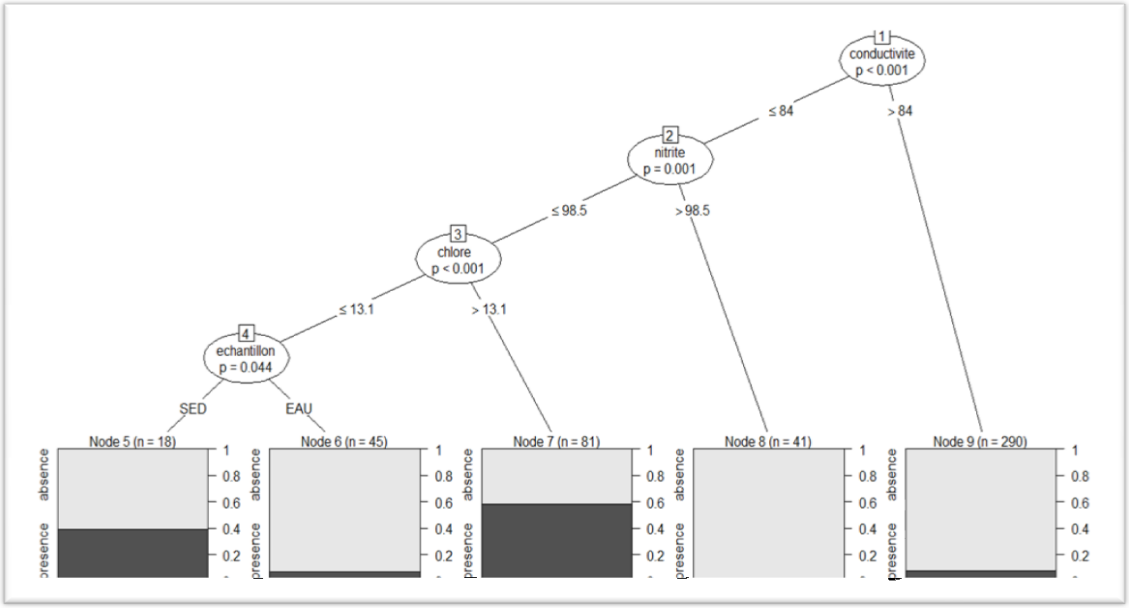

Figure 3: **Physico-chemical parameters influencing the presence and absence of mycobacteria**

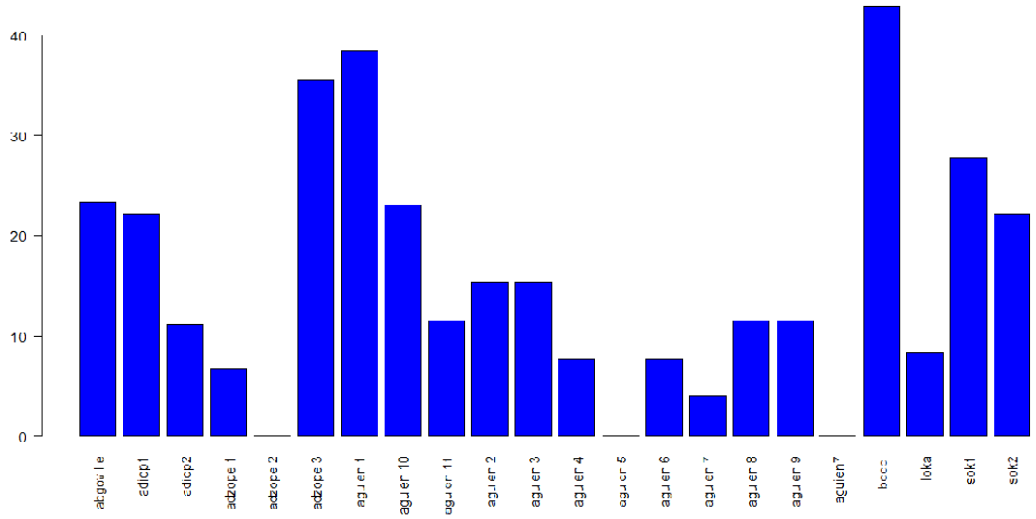

Figure 4: Proportion of samples containing mycobacteria according to site

Based on the proportions of samples containing mycobacteria per site, Bodo was the most favorable site for the proliferation of mycobacteria growth with a rate of 50% in mycobacteria obtained in the samples taken.

V. Discussion

In all a total of 473 water and sediment samples were analyzed. Sediments were the most contaminated by mycobacteria with a 60% presence, compared to 43.3% of water samples in hyper-endemic zones. In the hypo-endemic areas, water were the most contaminated with a rate of 53.57% against 43.24% in the sediments. Other studies like that of, **Mohammad et al. (2010)** found that the rate of mycobacterial isolates in water was lower than in the soils. This could be explained, due to the strong presence of parameters favorable to the growth of mycobacteria in the sediments (**Kirschner et al., 1992**). This difference could also be explained by the use of different decontaminants with variable effects on the growth of mycobacteria (**Stinear et al., 2007**). Thus, **Pickup et al. (2005**) noted that several NTM species in the Taff River in the United Kingdom could not

be recovered by culturing; some species of mycobacteria could be eliminated during the processing of the sample. According to the Runyon classification, all the mycobacteria isolated belonged to the group of rapidly growing mycobacteria (group IV). Several studies have also revealed the presence of rapid growing mycobacteria in soils (**Chilima et al., 2006**). The species *M. peregrinum, M. chelonae, M. abscessus, M. mucogenicum, M. immunogenum, like M. smegmatis, like M. peregrinum and Mycobacterium sp*. were identified in this study. However, with 50.76%, in hyper-endemic sites and 49.23% in hypo-endemic sites of *Buruli Ulcer*. This study discovered the presence of mycobacteria in all selected sites, hypo-endemic and hyper-endemic, corresponding to both environmental saprophytic species and potentially pathogenic species, as well as unidentified species. These facts can be explained by the environmental adaptability of these microorganisms to all environments where they can find nutrients. The effects of some physical-chemical parameters on these microorganisms have been studied in order to better understand the geographical distribution and the diversity of the atypical mycobacterial species in the water. The values of the different parameters vary considerably between the different sampling sites. Salts such as nitrate, chlorine, and sulfate have quite variable values. In this study the correlation between the occurrence of mycobacteria and the parameters studied is not really significant. Apart from a few rare cases such as conductivity ≤ 84, nitrite ≤ 98.5 and chlorine ≤ 13.1 added to the sediments that could be linked to the presence of mycobacteria. It was therefore in these conditions that the proportion of mycobacteria was high in the various sites. The variation in pH, as well as the turbidity, of the water samples in the sites was not correlated to the presence of mycobacteria. In this study, physico-chemical parameters influencing mycobacterial proliferation were : Conductivity ($p < 0.001$) ≤ 84 and nitrite ($p = 0.001$) ≤ 98.5 and free chlorine ($p < 0.001$) ≤ 13.1 and based on the type of sample ($p = 0.044$), the sediment was the most favorable to the growth of mycobacteria. These results are in contradiction to the results of **Iivanainen et al. (1993)**, they suggested that concentrations of iron, potassium, neutral pH, manganese, and chlorine influence the growth of mycobacteria in the rivers. Also, **Falkinham (2009**) suggested that NTM develop in swamps and low pH soils. Another study focusing on cattle breeding soils indicated that positive cultures of *M. gordonae, M. scrofulaceum, M. asiaticum and M. abscessus* species were significantly associated to low pH values, low calcium concentrations, and high concentrations of iron, zinc and manganese (**Norby et al., 2007**). Other factors not taken into account in this study may influence the presence of atypical mycobacteria in water, as shown by study of different streams in Finland (**Iivanainen et al., 1993**). Conditions and factors limiting the invasion of these microorganisms, constituting a serious risk to the environment, are not yet under control. Despite the existence of several studies on the microbial ecology of mycobacteria, discordant results are reported. Comparing the type of samples, the sediment was the most favorable medium for mycobacteria growth. Other studies, like that of **Mohammad et al. 2010** found that the rate of mycobacterial isolates in water was lower than in soils. This could be explained by the presence in the sediments of a strong presence of parameters favorable to the growth of mycobacteria (**Kirschner et al., 1992**).

VI. Conclusion

In this study, the analysis of the influence of environmental factors gave discordant results compared to those reported by some authors (**Iivanainen et al., 1993; Falkinham et al., 2001**). Some climatic factors such as precipitation may indirectly influence the occurrence of mycobacteria. Other environmental parameters such as climate, aquatic plants, humic and fulvic acid content of sediments should be taken into account in order to draw better conclusions. The prospective study on drinking water distribution system in relation to the occurrence of mycobacteria is strongly recommended. It would therefore be necessary to continue the sampling on a larger sample size with an emphasis on these parameters, also to test *in vitro* the effect of these parameters on mycobacteria. With a larger sample size, the study should allow us to take into account the evolution of potentially pathogenic species in the environments. These observations should be complemented by an epidemiological survey of mycobacterial diseases in order to assess the influence of environmental factors on mycobacteria.

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