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Research Article

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AN EVALUATION OF THE ABUNDANCE DYNAMICS OF SOME SPORULATING BACTERIA IN THE RAINWATER OF THE CITY OF YAOUNDE (CENTRAL AFRICA).

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Abstract

A study to evaluate the abundance dynamics of sporulating bacteria in rainwater was carried out in three sub-divisions of the city of Yaoundé during the period from February to August 2019. The bacteriological analysis were carried out after enrichment of the culture media by the technique of spreading on a Petri dish surface. The culture media used were the agars Luria-Bertani, Mossel and agar sulphitoreducer. The isolated bacteria were identified by standard methods for the assessment of water and wastewater.

The results of analysis of the abiotic variables showed air pollution. However, it has been noted that these waters are acid, in nature and are highly oxygenated. Some parameters such as temperature have been relatively stable, despite apparent spatial fluctuations. Bacteriological analysis revealed that these waters harbor a bacterial microflora consisting of bacteria of the genera Bacillus and Clostridium. Indeed, Bacillus cereus and Bacillus thuringiensis are the different Bacillus species identified with an average density of 8.29x10² CFU/100 mL for Bacillus thuringiensis and 8.13x10² CFU/100 mL for Bacillus cereus. In the Clostridium genus, only the species Clostridium perfringens has been identified with an average abundance of 2.93x10² CFU/100 mL. The monthly abundances of these bacteria undergo spatiotemporal fluctuations. Some physico-chemical parameters have influenced the distribution of these germs. Significant correlations between these germs have been obtained with temperature, electrical conductivity, turbidity and color. The degradation of the quality of these waters is favored by the atmospheric pollution due to the increase of the dust particles containing bacterial spores. These waters without any treatment, are not recommendable for the human consumption.

Keywords: rainwater, abiotic variables, bacterial spores, Yaounde.

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Introduction

Water is an essential element for all life on earth. It is a renewable resource, exhaustible and vulnerable to any contamination. It is essential for humans for their food needs, their agro-pastoral and industrial activities (Neveu et al., 2001). The water used for food is fresh water. They represent around 0.6% of the planet's water, or 8 million km³ distributed in rivers, lakes, reservoirs and groundwater (L'vovich, 1974; Vikram, 2005). In addition, the vast majority of this resource exists in the form of salt or brackish water, making the fraction of available fresh water limited. Today, a third of humanity lives in a situation known as "water stress", with less than 1700 cubic meters of fresh water available per capita and per year (WHO, 2004). Despite the importance of water, its quantity and quality are strongly influenced by the phenomenon of global warming on the one hand, and by pollution which can be physical, chemical or biological on the other (Vilaginès, 2003). The exponential population growth experienced by countries in general and in particular by emerging ones and their difficult economic conditions, lead to an anarchic urbanization that is difficult to control and a difficult supply of drinking water (Chippaux et al., 2002; Vermande and Emmanuel, 2002; August Boughrou, 2007). Faced with this situation, the populations have recourse to groundwater (well water, springs, boreholes) as a source of drinking water supply and to the rainwater which remains in all countries, a resource which cannot and should not be ignored or overlooked. The scarcity, the consequences of the lack of water make it necessary to avoid any waste and to move towards a balanced management (Nola et al., 1998; Perraud, 2005). Indeed, rainwater, often called meteoric, is an inexhaustible natural source

Material and methods

Description of the study area

and constitutes 93% of precipitation (Boudot, 2006). Their qualities depend on air contaminants and vary from place to place but also from season to season. This water for domestic use and human consumption harbors a varied microflora including among other faecal germs but also aerolized microorganisms which have not been killed by solar ultraviolet or dehydration. These aerolized microorganisms are spores that are able to germinate even after long periods of dormancy (Errington, 2003; Kaushik et al, 2014).

These spores effectively resist many environmental disturbances, but also most antimicrobial agents. They therefore represent a major concern due to their high resistance to physical and chemical water treatment processes. Several studies have already been conducted on food and environmental spores, it turns out that the bacterial spore is revealed as one of the most competitive ingredients to use as food supplements in adults who need it (Etoa, 2018). Despite these studies, research into sporulating bacteria from rainwater has often been little addressed. Little is known about the impact of some physicochemical parameters on the resistivity of rainwater spores. Likewise, the impact of industrialization on the transformation of bacteria into spores remains unclear.

The general objective of this study is to assess the abundance dynamics of sporulating bacteria in the rainwater of the city of Yaounde. Specifically, it involves isolating and identifying the spore forming bacteria present in rainwater; determine the physicochemical parameters that can influence the forms of resistance of these sporulating bacteria; to show the existing relationships between physicochemical parameters and sporulating bacteria.

The study was carried out in the city of Yaoundé. This city is generally limited between the parallels 3 ° and 5 ° North and by the meridians 11 ° and 13 ° East. It is located on the western edge of the

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plateau of South Cameroon, at an average altitude of 750 m and 250 km from the Atlantic coast (Ekodeck and Kamgang Kabeyene B., 2002). This city is subject to an equatorial climate with bimodal rainfall comprising 4 seasons which alternate throughout the year (Suchel, 1987): a long dry season from mid-November to mid-March, a short rainy season from mid- March to June, a short dry season from July to August and a long rainy season from September to mid-November. Yaoundé is found in the so-called Guinean-Congolese phytographic region (Villiers, 1995; Sabatier, 1997). The vegetation is varied and clearly influenced by human action. We find there the area of dense humid forest always green, Guinean-Congolese in the South and the area of dense semi-deciduous, Guinean-Congolese humid forest which covers the rest of the plateau. The relief of Yaounde is made up of several hills. The hydrographic network is made up of streams, rivers, streams, lakes and ponds. The soil of Yaounde, azonal, of ferralitic type, rests on a geological complex of metamorphic base of Precambrian age D (Kuété, 2000). This soil is rich in gravel and is acidic, the pH being between 4.9 and 5.8 (Bachelier, 1959; Yongueu-Fouateu, 1986; Foucault and Raoult, 1995). The particle size, porosity and permeability vary from 17 to 80%, from 5 to 71%, and from 50 to 300 cm.h-1 respectively (Bachelier, 1959; Humbel, 1996).

Collection of data

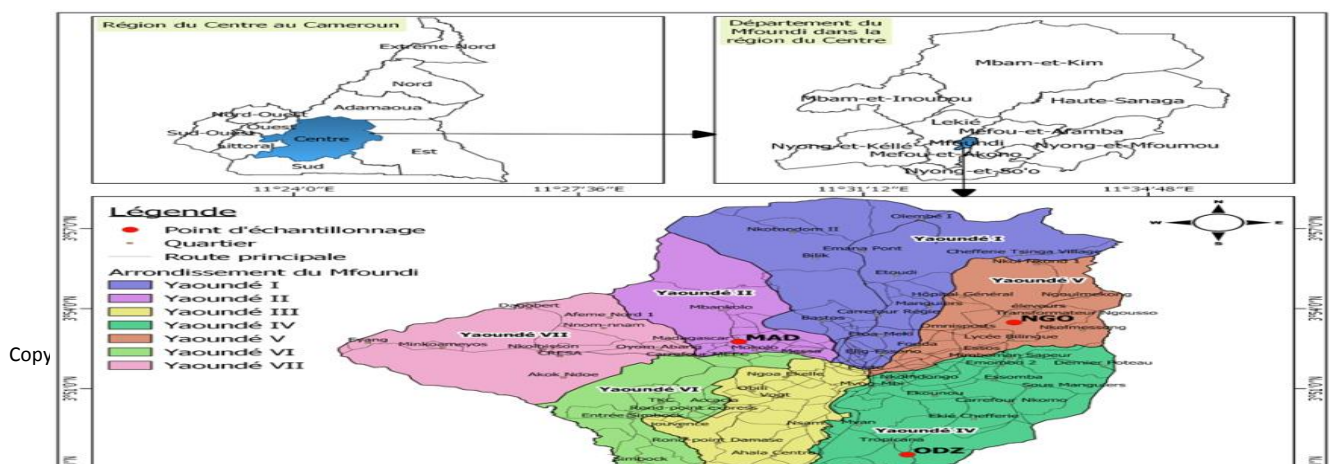
Samples were taken during rainy events. A rainwater sample was collected in a sterile 3L container. The water samples for physico-chemical

analyzes were collected in 1000 mL polyethylene bottles washed, rinsed and dried in advance in the laboratory. These bottles were first rinsed on the field with the water to be analyzed, then filled to the brim and capped in order to limit degassing (Rodier et al., 2009). The water samples intended for bacteriological analysis were collected in dry 500 mL glass vials, sterilized beforehand in an autoclave at 121 ° C for 15 min. These vials were 3/4 filled with water to allow homogenization before sowing (APHA, 1998 ; Rodier et al., 2009). All the samples were then transported to the laboratory in a refrigerated enclosure for analysis.

Choice and description of sampling points

Choice of sampling points

Study sites were randomly selected in the city of Yaoundé. This choice was motivated according to the preponderant activity carried out in the district, the **promiscuity** rate and the distance from the city center. The districts of the city of Yaoundé which were selected for this study are Ngousso, Madagascar and Odza. Ngousso is characterized by the presence of three reference hospitals (General, Obstetrics and Crasser), the presence of a large central transformer which supplies the city of Yaoundé. Madagascar is sandwiched between the Mokolo market, the Green City and the quarry district. A strong **promiscuity** is felt with the concomitant creation of drinking places, bakery and smoke bombs. The population is galloping. Odza is a peripheral zone of the city of Yaoundé, with a controlled development which sees the day. The main activity is family farming. Figure 1 shows the location of these points on the map of Yaounde.



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Figure 1: Positions of sampling stations in the Department of Mfoundi, city of Yaounde, Center Region (INC, 2019).

Geolocation of sampling points

Table I summarizes the geographic coordinates of the different districts. These geographic coordinates

| Neighborhoods | GPS coordinates | |
|---------------|-----------------|---------------|
| | Latitude | Longitude |
| Ngouso | 3°53'28,8" N | 11°33'6,3" E |
| Madagascar | 3°52'45,5" N | 11°29'37,6" E |
| Odza | 3°48'31,6" N | 11°31'45,3" E |

Analysis of physicochemical parameters in collected rainwater

The physicochemical parameters considered are those which the water takes in when it clears the atmosphere of aerosols. These parameters are likely to modify its quality and affect its chemical composition.

Physical variables

Temperature

The temperature was measured in situ, using a mercury column thermometer graduated to 1 / 10th of a degree Celsius. The thermometer was introduced vertically at 2/3 of the water column. After 1 to 2 minute (s) the figure corresponding to the rise in mercury has been noted and expressed in degrees Celsius (° C).

Suspended solids (SS)

The SS contents were determined directly using a HACH DR / 2000 spectrophotometer and the reading was carried out at the wavelength 810 nm. Values were given in mg / L.

Colour

were obtained in the field using a Garmin etrex 20x brand GPS and shows that overall, the area covered by the study is between 3 ° 48"31 " north latitude and 11 ° 33'7 " east longitude.

Table I: GPS (Global Positioning System) coordinates of the districts chosen in Yaoundé

The color was measured using a HACH DR / 2000 spectrophotometer. The reading was made at the wavelength 455 nm and the values obtained were expressed in Platinum Cobalt (Pt.Co).

Turbidity

The turbidity was measured in the laboratory using a spectrophotometer of HACH DR / 2010 brand at the wavelength $\lambda = 450$ nm and the results were expressed in Formazine Turbidity Unit (FTU).

Total Dissolved Solids (TDS)

The TDS water contents were measured in the laboratory using a HANNA brand HT 8733 portable TDS / Conductivity meter. The results were expressed in mg / L.

Chemical variables

pH

The pH was measured in the laboratory using a brand name portable multi-parameter. Indeed, an electrode of the device was introduced into the sample collected in the bottle at 1/3 for 2 to 3 minutes. After reading the values, the result was expressed in

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conventional unit (U.C).

Electrical conductivity

The electrical conductivity of the water was assessed in the laboratory using a HANNA brand HT 8733 portable TDS / Conductivity meter. Values were given in $\mu\text{S}/\text{cm}$.

Dissolved oxygen.

The dissolved O_2 content of the water was determined in the field using a HANNA brand oximeter, model HI 9146 and the result was expressed as a percentage of saturation.

Dissolved CO_2

The measurement of the dissolved CO_2 content was carried out in two stages:

- In the field, the CO_2 was fixed by introducing into a 200 ml volumetric flask, 20 ml of sodium hydroxide (NaOH) N / 20 plus 2 to 3 drops of phenolphthalein, this mixture was completed with the sample of raw water up to the gauge line. The mixture obtained with a pink color was stored in a 250 ml double-capped polyethylene bottle and then brought back to the laboratory.

- In the laboratory, 50 ml of this sample were titrated with hydrochloric acid (HCl) N / 10 until completely discolored. The CO_2 content of the water expressed in mg / l was then determined by the formula: $[\text{CO}_2] (\text{mg} / \text{L}) = (\text{descent of the control burette} - \text{descent of the sample burette}) \times 17.6$.

Nitrates

Nitrates were measured by colorimetry using a HACH brand DR / 2000 spectrophotometer, using Nitraver IV as a reagent. The nitrate content was read at a wavelength of 570 nm and the results were expressed in mg / L of NO_3^- .

Bacteriological analyzes

Isolation of spore forming bacteria

The isolation of the spore forming bacteria was carried out by the surface spreading technique on the agar enriched with Mossel, Luria-Bertani for the genus *Bacillus* and sufi-reducing agar for the genus *Clostridium*. Indeed, 100 μL of the sample was taken using a tensor pipette and spread on the surface of the culture medium poured into the 90 mm diameter petri dishes, until the drop of water (Marchal *et al.*, 1991). The Petri dishes were incubated for 24 h to 48 h at temperatures of 37 ° C and 44 ° C for the genera *Bacillus* and *Clostridium* respectively.

Enumeration of bacteria isolated

The quantitative analysis focused on the enumeration of isolated colonies and including the cultural characteristics of the suspect strains. This analysis was carried out using the direct agar counting method using a mechanical pointer (Holt *et al.*, 2000). The bacterial abundances were expressed in Colony-forming Units (CFU) / 100 ml of sample.

Macroscopic examination

The macroscopic examination consisted in describing the cultural characteristics of the bacterial colonies. These are the size, appearance, shape, color, contours and configuration of the colony surface (Diagnostic Pasteur, 1987).

Identification of enumerated germs

After isolating the germs, the identification was carried out after macroscopic examination of the colonies and by biochemical or enzymatic tests (classic gallery) which were carried out after a pure subculture of the strains on ordinary agar medium poured down in test tubes (Diagnostic Pasteur, 1987).

Biochemical or enzymatic tests

The biochemical identification of bacterial species is based on the revelation of certain particularly significant and stable aspects of the metabolism of the

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families, genera or species studied. It is a set of reactions whose results are equivalent to the phenotype and reveal the specific genetic configuration of the taxon under consideration. These tests can be grouped into three main sets: basic tests, orientation tests and differential tests. Thus, the search for enzymes such as catalase, glucase, lactase among others was carried out. In addition, the search for gas production, hydrogen sulfide, mannitol fermentation, mobility or transformation of citric acid was tested using the conventional gallery (Holt *et al.*, 2000; Marchal *et al.*, 2001).

Data analysis

Assessment of existing relationships between physicochemical parameters and densities spore forming bacteria

The abiotic and biological data recorded were analyzed using SPSS 20.0 software. To choose the statistical tests to be performed, the Kolmogorov Smirnov normality test K was used to verify the normality of the data collected during this study. This test revealed that the distributions of these data are not normal. To this end, non parametric tests were carried out.

Correlations between the recorded data

The Spearman rank correlation coefficient was determined from SPSS 20.0 software. This coefficient made it possible to establish the correlations between the physico-chemical variables, between the physico-chemical and biological variables and between the biological variables.

Data comparison

The Kruskal-Wallis H test compared the medians of different physico-chemical parameters and the abundances of sporulating bacteria over time and space to detect a possible difference. The Mann-Whitney U test compared the different parameters two by two to see exactly where there is a difference. These tests were performed using SPSS version 20.0 software

Results and discussion

Results

Evaluation of the physicochemical quality of the analyzed rainwater

Physical parameters

During the study, the water temperature varied from 21 to 25 ° C. The maximum value (25 ° C) was recorded in March in Madagascar and the minimum value (21 ° C) in May in ngouso and in July in Odza (Figure 2A). The SS variation profiles in the different districts reached 64 mg / L. The minimum SS value (07 mg / L) was observed in April and June in Madagascar and the maximum value (64 mg / L) was recorded in February in the same district (Figure 2B). The color variation profiles in the different districts varied between 6-220 Pt.Co. The minimum value (6 Pt.Co) was observed in June in Madagascar while the maximum value was recorded in February in the same district (Figure 2C). The profiles of variation of turbidity in the different districts varied between 10-136 FTU. The minimum value (10 FTU) was observed in Ngouso in June while the maximum value (136 FTU) was recorded in February in Madagascar (Figure 2E).

Chemical parameters

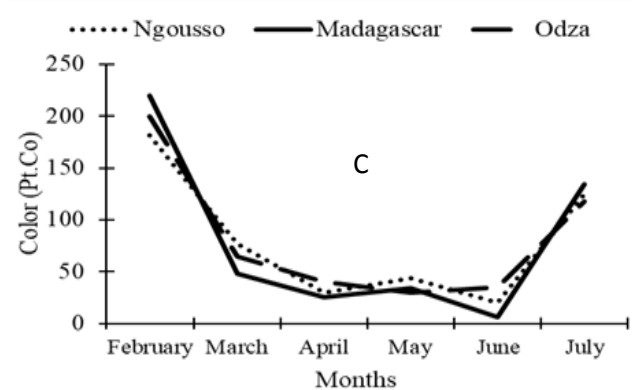
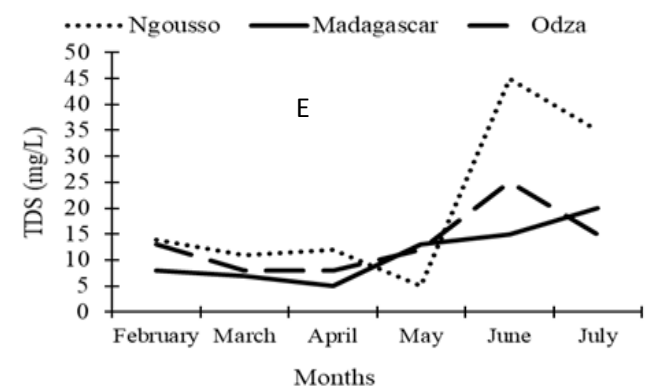
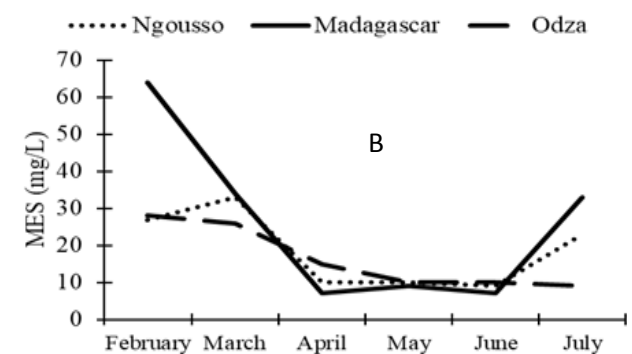
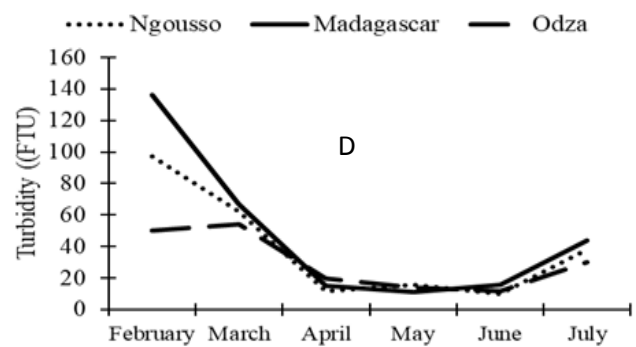
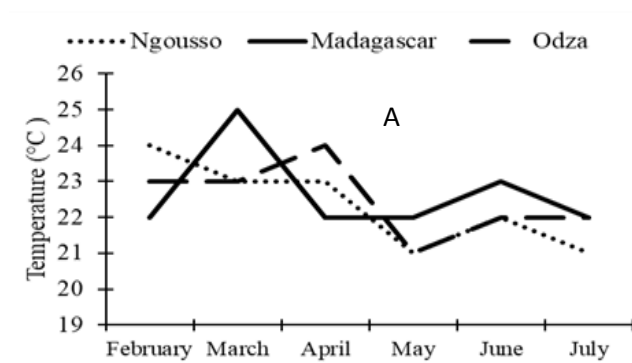
The profiles of variation of TDS in the different districts varied from 05-45 mg / L. The minimum value (05 mg / L) was observed in April in Madagascar and in May in Ngouso while the maximum value was recorded in June in Ngouso (Figure 2D). The pH values fluctuated between 5.04 and 7.70 CU throughout the study period. The maximum value (7.70 CU) was observed in July in Madagascar while the minimum value (5.04 CU) was recorded in May in Ngouso (Figure 3A). The CO₂ contents of the analyzed waters wavered between 5.28 and 17.6 mg / L. The maximum value (17.6 mg / L) was recorded in February, April and May in Ngouso and in June in Madagascar. The minimum value (5.28

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mg / L) was observed at all sampling points and almost every month (Figure 3B). The NO_3^- ion contents fluctuated between 0.01 and 7.2 mg / L. The minimum value (0.01 mg / L) was noted in June and July in Ngousso and Madagascar while the maximum value was observed in February in Madagascar (Figure 3C).

The dissolved O_2 contents varied between 57 and 82.5%. The maximum value (82.5%) was observed

in February in Ngousso and the minimum value (57%) was recorded in April in Madagascar (Figure 3D). The conductivity varies from 8.4-200 $\mu\text{S} / \text{cm}$. The minimum value (8.4 $\mu\text{S} / \text{cm}$) was recorded in March in Madagascar and the maximum value (200 $\mu\text{S} / \text{cm}$) in February in the same neighborhoods (Figure 3E).



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Figure 2: Spatio-temporal variations of the physical variables studied (A = Temperature; B = MES; C = Color; D = Turbidity; E = TDS).

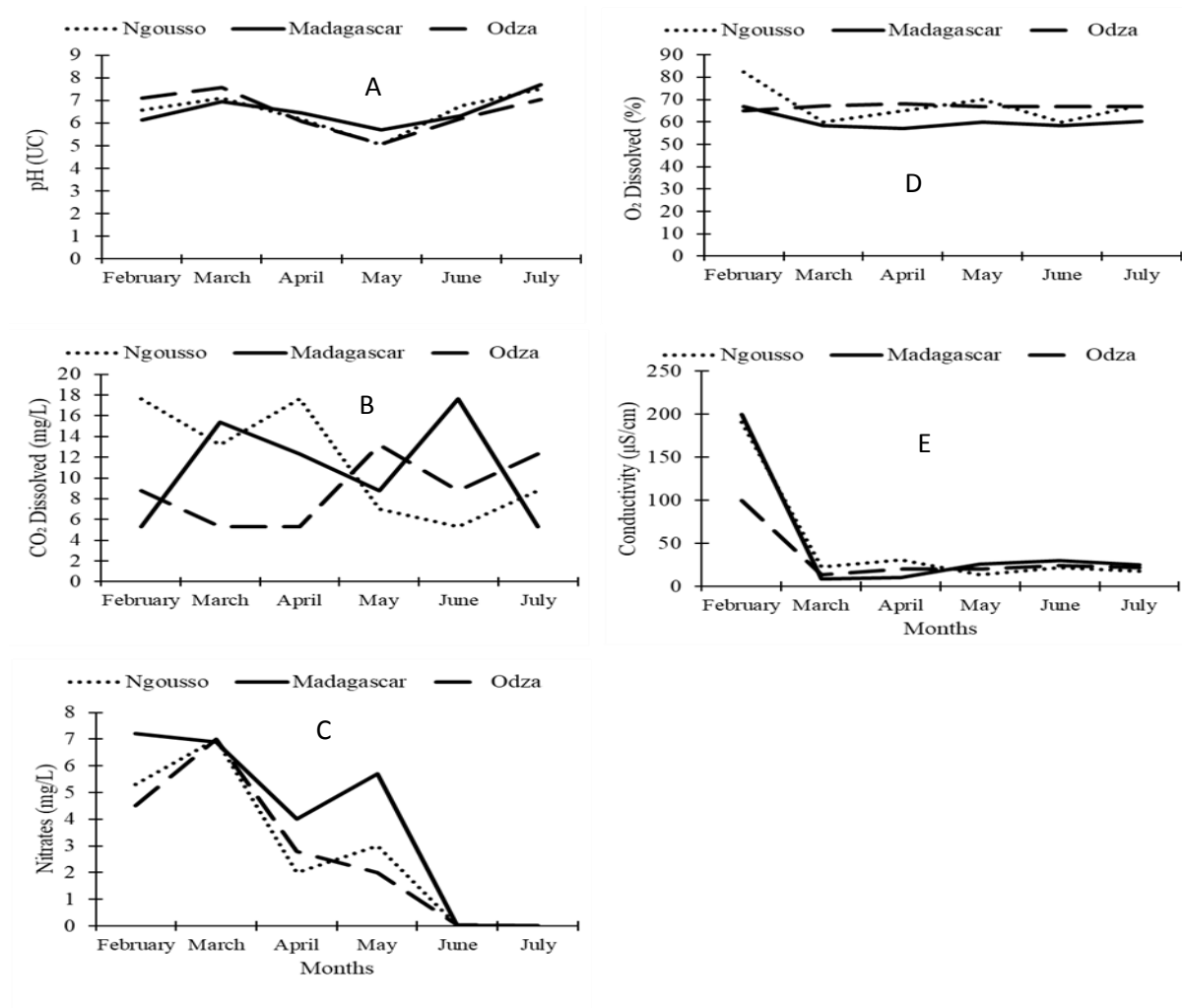


Figure 3: Spatio-temporal variations of the chemical variables studied (A = pH; B = Dissolved carbon dioxide; C = Nitrates; D = Dissolved oxygen; E = Conductivity).

Assessment of the abundance dynamics of sporulating bacteria from rainwater Isolation and characterization

Macroscopic examination of the bacterial colonies showed three types of colony: small white colonies (0.5-1mm in diameter) with irregular contours on Luria-Bertani agar; pink colonies of medium size

(0.5mm in diameter) with irregular contours, with a halo of whitish precipitate on Mossel agar; and black colonies of medium and small sizes (0.3-0.5mm in diameter) on agar for sulfite-reducers. Figure 4 shows the colonies of these bacteria on the culture media used.

Identification of isolated strains

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The biochemical tests carried out have made it possible to highlight a certain number of enzymatic reactions. It appears that the rainwater from the three districts of the city of Yaounde contains the sporulating bacteria made up of the species *Bacillus thuringiensis*,

Bacillus cereus and *Clostridium perfringens*. These three species have positive mobility and ferment glucose. *Bacillus cereus* is the only species to ferment lactose while *Clostridium perfringens* does not reduce citric acid (Table II).

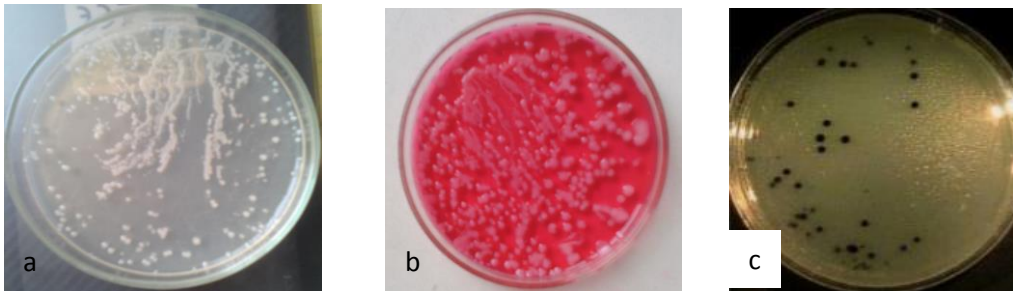


Figure 4: Photographs of colonies isolated on Luria-Bertani medium (a), Mossel agar (b) and sultoreductive agar (c).

Table II: Identification of isolated bacterial species

| Identification tests | Bacterial colonies | | |
|----------------------|-------------------------------|------------------------|--------------------------------|
| | A | B | C |
| Catalase | + | + | - |
| Mannitol | - | - | + |
| Mobility | + | + | + |
| Citrate | + | + | - |
| H ₂ S | - | - | - |
| AAF | + | + | - |
| Gaz | - | - | - |
| Lactose | - | + | - |
| Glucose | + | + | + |
| Suspended species | <i>Bacillus thuringiensis</i> | <i>Bacillus cereus</i> | <i>Clostridium perfringens</i> |

+: positive test -: negative test

Evaluation of the variation in the abundance of sporulating bacteria from rainwater

The abundances of bacterial cells varied according to the site of sampling and the month of sampling throughout the duration of the study. The cell density of *B. thuringiensis* reaches a maximum value of 24.70×10^2 CFU / 100 μ L in February in the Odza district and the minimum value (0.03×10^2 CFU / 100 μ L) was

recorded in the same district in July (Figure 5a). The cells of *B. cereus* reached 33.00×10^2 CFU / 100 μ L in February in the Madagascar district. The minimum value (0.05×10^2 CFU / 100 μ L) was recorded in June in the Odza district (Figure 5b). Cells of *C. perfringens* reached their maximum density (18.40×10^2 CFU / 100 μ L) in February in the Madagascar district and the minimum density (0.13×10^2 CFU / 100 μ L) was recorded in the same district in July (Figure 5c).

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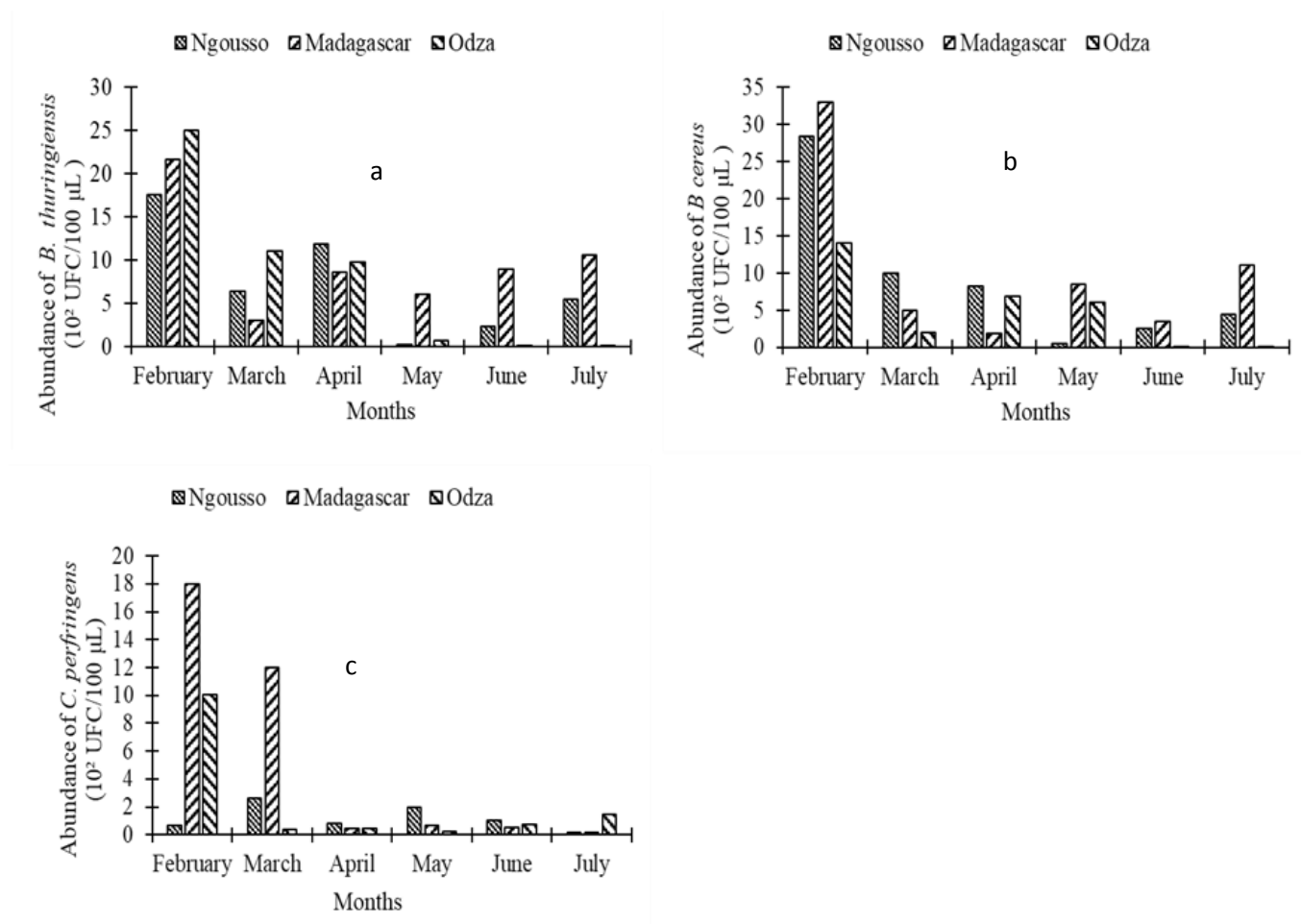


Figure 5: Spatio-temporal variations in the abundance of *B. thuringiensis* (a), *B. cereus* (b) and *C. perfringens* (c).

Data analysis

Correlations between the physicochemical and biological parameters analyzed

The degrees of binding were calculated between the bacteriological variables and the physico-chemical variables measured. It appears that in all of the areas studied, the increase in the turbidity of the medium leads to a significant increase ($P \leq 0.05$) in the density of *Bacillus thuringiensis* and *Bacillus cereus*. Likewise, the rise in temperature and electrical conductivity of rainwater leads to a significant increase ($P \leq 0.05$) in *Bacillus thuringiensis*. Very significant ($P < 0.01$) and positive correlations exist between the abundances of *Bacillus cereus* and parameters such as electrical conductivity and SS (Table III).

Table III: Correlations between the bacteria isolated and the physicochemical parameters measured

| Isolated germs | <i>Bacillus thuringiensis</i> | <i>Bacillus cereus</i> | <i>Clostridium perfringens</i> |
|----------------|-------------------------------|------------------------|--------------------------------|
|----------------|-------------------------------|------------------------|--------------------------------|

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| Physiological parameters | | | |
|-----------------------------------------------------|--------|---------|--------|
| Température (°C) | 0,510* | 0,337 | 0,270 |
| CO₂ dissolved (mg/l) | -0,042 | 0,045 | 0,110 |
| Nitrate (mg/l de NO₃⁻) | 0,383 | 0,388 | 0,463 |
| pH (UC) | 0,218 | 0,065 | -0,137 |
| Electrical conductivity (µs/cm) | 0,553* | 0,641** | 0,240 |
| TDS (mg/L) | -0,175 | -0,035 | -0,261 |
| Turbidity (FTU) | 0,477* | 0,483* | 0,280 |
| MES (mg/L) | 0,452 | 0,625** | 0,297 |
| Color (Pt.Co) | 0,399 | 0,515* | 0,253 |
| Dissolved oxygen (mg/L) | 0,062 | 0,036 | -0,143 |

Significant at the threshold $p \leq 0.05$ ** very significant at the threshold $p \leq 0.01$ DOF = 18

Correlations between the different bacteriological variables measured

The relationships between the different bacteriological variables were also assessed. In all the districts considered, very significant ($P \leq 0.01$) and positive correlations were recorded between the abundances of *Bacillus thuringiensis* and *Bacillus cereus* and would indicate the possibility of cohabitation between these two species (Table IV).

Table IV: Correlations between the different bacterial strains isolated

| | <i>Bacillus thuringiensis</i> | <i>Bacillus cereus</i> | <i>Clostridium perfringens</i> |
|--------------------------------|-------------------------------|------------------------|--------------------------------|
| <i>Bacillus thuringiensis</i> | 1,000 | 0,740** | 0,018 |
| <i>Bacillus cereus</i> | - | 1,000 | 0,152 |
| <i>Clostridium perfringens</i> | - | - | 1,000 |

** Very significant at the threshold $p \leq 0.01$ DOF = 5

Comparisons of means of physicochemical and biological variables as a function of sampling sites

The comparison between the means of the physicochemical and biological variables was carried out using the Kruskal-Wallis H test. It was noted that

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only dissolved O₂ presents a significant difference (p <0.05) between the districts considered.

Discussion

Physicochemical characteristics of the waters analyzed

Temperature values vary little from one neighborhood to another. They fluctuated between 21 and 25 ° C. This small thermal variation could be explained by the fact that the water temperature depends on the amount of sunshine and exchanges with the atmosphere. It would also be linked to that of ambient temperature. The pH of a water represents its acidity or alkalinity (Rodier *et al.*, 2009). An average pH value of 6.53 CU. was recorded. The water analyzed has an acidic pH, although a value of 7.59 CU was recorded in the Odza district in March. This acidity of the water is due to the fact that it is lowly mineralized. These results corroborate those of Mbega (2004) who points out that the pH between 6.0 and 8.5 CU is favorable for the expression of the biological potentials of many groups. This acidity of water could also be linked to the production of CO₂ in the atmosphere by cars and certain industries.

Even without pollution, because of its carbon dioxide content always present in the atmosphere, rainwater is naturally acidic. An average value of 64.83 mg / l of dissolved oxygen was also recorded during the study period. This average value recorded, reflects a fairly good oxygenation of the analyzed waters. The highest value (82.5 mg / l) recorded in the Ngousso district is due to the fact that during heavy rains, passive diffusion at the air-water interface promotes its reoxygenation (Ginet and Decou, 1977). The CO₂ content values range from 5.28 to 17.6 mg / L. According to (Rodier *et al.*, 2009), these levels are influenced by the production of CO₂ in the atmosphere by cars and certain industries.

Overall, the recorded electrical conductivity and TDS values are low. The average values recorded are 15.05 mg / L and 44.43 μS / cm respectively for the TDS and the electrical conductivity. It is the same for those of nitrates whose average value is 3.20 mg / L of NO₃⁻. These different values obtained indicate that these waters are soft, weakly mineralized and have a low organic matter load (Rodier *et al.*, 2009). Petrucci *et al.* (2017) point out to this effect that in industrialized and / or agricultural areas, rainwater is often polluted by various contaminants including nitrogen, nitrates, nitrites and various residues of human activity. However, the low values of nitrates observed in all the districts could be explained by the good oxygenation of the waters (Leynaud and Verrel, 1980). According to Rodier *et al.*, 2009, a concentration of 2 or 3 mg / L of NO₃⁻ can be considered normal in unpolluted natural waters.

Biological characteristics of the waters analyzed

Bacteriological analysis carried out on the water samples taken during the study period showed that these waters contain sporulating bacteria. During the study, 2 bacterial genera were identified. These are *Bacillus* and *Clostridium*. The densities of these two groups of bacteria have varied from point to point and over time. The average values of the abundances of *Bacillus thuringiensis* and *Bacillus cereus* recorded are 829 and 813 CFU / 100 ml respectively, while the average value of the abundances of *Clostridium perfringens* is 293 CFU / 100 ml. These values are all higher than the standards established by the WHO. In terms abundance, the genus *Bacillus* is the most represented with a proportion of 84.8% or 42.8% of *Bacillus thuringiensis* and 42% of *Bacillus cereus*, while the genus *Clostridium* (*Clostridium perfringens*) represents only 15.2%. The permanent presence of these pathogenic bacteria and their high abundance reflects the degree of pollution of these waters.

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A higher bacterial density in the water samples from the Madagascar district compared to those from the Odza and Ngousso districts was noted. This shows that the rainwater in the Madagascar district is more polluted than that in the other two districts. The presence of bacteria in rainwater is probably due to the fact that dust rises in the air when the wind blows and pollutes the atmosphere. According to Kaushik and Balasubramanian in 2012, rains from air masses from agricultural, urban, industrial contexts can also be significantly contaminated with bacteria, viruses and pathogenic spores. The predominance of bacteria of the genus *Bacillus* in this rainwater is due to the fact that, being very rich in oxygen, the medium is conducive to the growth of these bacterial spores to the detriment of the genus *Clostridium*. In this regard, (Avignonerossa *et al.*, 1992) stipulate that in the genus *Bacillus*, sporulation is closely linked to the oxygen supply.

Link between the parameters evaluated

The results of the correlations between the biological and physicochemical variables show that the physicochemical parameters analyzed, 4 variables (temperature, electrical conductivity, turbidity and color) significantly influenced the population and the distribution of bacteria throughout the study. The increase in water temperature significantly increases the abundance of bacteria of the genus *Bacillus* (*Bacillus thuringiensis*). Hong *et al.*, 2005 state for this purpose that only bacteria of the *Bacillus cereus* group are capable of growing in a temperature range of 4 ° C to 50 ° C. Turbidity and color are significantly positively correlated with *Bacillus thuringiensis* and *Bacillus cereus* species respectively. This difference would result in the fact that these bacteria react differently to organic matter and according to its composition. Indeed, organic matter influences the availability of nutrients by serving at the same time as

a source of energy and carbon for certain microorganisms (Nola *et al.*, 2004).

The rise in chemical compounds, in particular that of the electrical conductivity of water, leads to a significant increase in *Bacillus thuringiensis* and *Bacillus cereus* species. This would mean that the low abundances of *Bacillus cereus* would be due to the low values of the electrical conductivity. There is, however, a very significant correlation between suspended matter and spores of the genus *Bacillus cereus*. This would mean that bacteria react differently to chemical compounds. Indeed, the influence of chemical compounds on a bacterial species varies according to the ability of the bacterial species to degrade this chemical compound, either to neutralize its toxicity, or to make available the nutrients and the energy source, which are necessary for its biosyntheses.

Conclusion

Ultimately, it emerges from these works, the objective of which was to analyze the physicochemical parameters likely to influence the dynamics of abundance of spore forming bacteria contained in rainwater that the temperature of the analyzed rainwater varies according to the sites of Specimens. These waters are acidic, little mineralized and subject to average pollution, marking the average degradation of the physicochemical quality of these waters. Bacteriological analysis revealed the presence of sporulating bacteria in average proportions and not recommended for drinking water. Of the spore forming bacteria isolated, the genus *Bacillus* was the most represented (*Bacillus thuringiensis* and *Bacillus cereus*). The genus *Clostridium* (*Clostridium perfringens*) is less abundant in the rainwater analyzed.

The degree of connection between the variables considered varies from one physicochemical parameter to another and sometimes depending on the sampling site. Parameters such as temperature,

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electrical conductivity, turbidity, TSS and color influenced the spatial distribution of the bacteria. These bacteria are responsible in humans for gastroenteritis, watery diarrhea, abdominal pain and endophthalmitis.

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