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Abundance Dynamics of 2 *Bacillus* Species in Rain and Underground Water in an Urbanized Area in Cameroon (Central Africa) and Impact of Some Abiotic Parameters

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Abstract: The present study aimed to assess the abundance dynamics 2 spore forming bacteria of sanitary importance *Bacillus cereus* and *B. thuringiensis,* in the rain and groundwater in urbanized area in Cameroon (Central Africa) and potential impact of some abiotic parameters. The bacteriological analyzes were made by cultures on agar media and the chemical analyzes by spectrophotometry. It appears that heterotrophic aerobic mesophilic bacterial abundances ranged from $1x10^6$ to $1x10^8$ CFU/100µL in wells and from $9x10^6$ to $196x10^6$ CFU/100µL in rainwater. The abundances of *B. thuringiensis* reached 320 CFU/100µL in wells, and 730 CFU/100µL in rainwater. That of *B. cereus* reached 340 CFU/100µL in wells, and $12x10^2$ CFU/100µL in rainwater. The pH of wells fluctuated between 5.05 and 7.33 whereas that of rainwater varied from 6.12 to 6.88. Electrical conductivity values ranged from 111 to 885 µS/cm in wells, and varied from 3 to 92 µS/cm in rainwater. Both media contains nitrate, nitrogen ammonia, phosphate, dissolved CO_2 and O_2 and their concentration undergoes spatio-temporal variations. Correlations coefficients between meteorological/chemical parameters and the bacterial abundance dynamics undergoes spatial variation on one hand, and varied according to a given abiotic parameter and the bacterial species considered on the other hand. The relationships between the properties of the previous month's rainwater on the abundance dynamics of the microflora in sampled wells during the current month, referred to as a delayed impact, showed a various degrees of influence, suggesting that the properties of the sampled groundwater would mainly result from the interactions of the confounding factors, and not only due to the rainfall or rainwater properties.

Keywords: Rainwater, Wells, *Bacillus cereus*, *B. thuringiensis*, Abundance Dynamics, Abiotic Factors

1. Introduction

The exponential population growth in several countries in the world coupled with their sometimes difficult economic conditions is causing difficulties in the supply of drinking water. Rainwater, often referred to as meteoric water, is thus increasingly used. As a means of water conservation, rainwater is collected and stored rainwater serves as a potential source of water for various beneficial purposes in many countries [1, 2]. The atmosphere is an important route for transporting and disseminating microorganisms over short and long distances, and they have impact in public health, meteorology and atmospheric chemistry [3]. Various particles suspended in the air and constituting aerosols can be contained in this water. The particle diameters in the air often ranged from 100 µm for the largest to less than 100 nm for the smallest [4].

Investigation on biological particles into atmospheric aerosols has been known as aerobiology, and the term “primary biological aerosol particles” has been defined to describe solid airborne particles derived from biological organisms, including microorganisms and fragments of biological materials [5]. Airborne biological particles as a whole are also denoted as bio-aerosols. They are a complex mixture consisting of different components, from simple organic molecules with dimensions in the nanometer range, through to viruses, bacteria, bacteria spores, fungal spores, mould spores and hyphae [6, 7]. According to Bowers *et al.* [8], bacteria and fungi are ubiquitous throughout the Earth’s lower atmosphere where they often represent an important component of atmospheric aerosols with the potential to impact human health and atmospheric dynamics.

The role of *Bacillus* spores in the environment is to preserve and to propagate the genetic information contained within the bacterium. It is known that spore formation evolved as a mechanism for both spatial and temporal escape from local conditions unfavorable to rapid growth [9, 10]. They can be found in the air, in water or in the soil [11], and are known, among other things, to be the cause of many infections in humans. Besides food poisoning, these bacteria induces local and systemic infections, and the main described conditions are septicemia, endophthalmitis, pneumonia, endocarditis, meningititis and encephalitis, especially in immunosuppressed individuals such as neonates, resulting in the patient death in about 10% of cases [12-14].

It has been indicated that most viable airborne particles are spores which are to some extent suited for survival in such an environment for a limited period [3]. The study of the influence of abiotic factors on the distribution of airborne microbes often gives contradictory results. The degree of impact depends on the parameter concerned and on all of the other abiotic parameters (rainfall, temperature, sunshine, UV index, relative humidity, carbon dioxide concentration, dust levels, and airborne bacterial counts, surrounding landscapes, wind conditions, among others) which prevail in a given geographical space [3, 15].

Several studies have already been carried out on non-sporulating bacteria in groundwater in the equatorial region of Cameroon (Central Africa). It appears that these waters harbor a varied bacterial microflora consisting among others of Vibrionaceae, Enterobactriaceae, Pseudomonadaceae, Aeromonadaceae with some strains being pathogen [16-18]. The abundance of these germs is influenced in part by meteorological factors, and in part by factors related to bacterial species [19-21]. Their sensitivity to antibiotics varies depending on the species or strain concerned [22, 23]. The recharge of this groundwater occurs with a delay of one month in relation to rainfall, time needed to saturate the soil layers upper groundwater table [16]. This period can sometimes be shortened in the case of shallow groundwater [24].

The transfer of bacteria-contaminants from the soil surface to the water table is influenced by the nature of the soil layers crossed [25-27]. The intrinsic properties of bacteria are also involved, and microbiological and chemical properties of this groundwater undergoes temporal variation when stored in households conditions [28]. If some data are thus available on the diversity and the abundance dynamics of non-sporulating bacteria and the mechanisms of their transfer into groundwater in the equatorial region of Cameroon, little information is however available concerning spores or sporulating bacteria, both in these underground aquatic biotopes and in the atmosphere.

The spore longevity and survival in the environment are due to many factors [29]. It is resistant to heat, (wet-heat resistance and dry-heat resistance), to desiccation, chemicals, UV radiation, ɣ-Radiation and to ultrahigh hydrostatic pressure. Its DNA repair protective mechanisms aims to prevent or dramatically slow the rate of formation of certain types of DNA damage or alter the type of damage formed in spore DNA. Its general DNA repair systems included the nucleotide excision and the recombination-mediated mechanisms [29].

The spores contained in the atmosphere are liable to be carried to the soil surface by rainfall. Few studies so far have been carried out on the diversity and abundance of sporulating bacteria in groundwater as well as in the rainwater of this geographic area. Little information is also available on the meteorological factors that can control their distribution and specific diversity. The present study aimed to assess the abundance dynamics of 2 spores forming bacteria *Bacillus cereus* and *B. thuringiensisi* in the rainwater and groundwater in Yaounde and the impact of some abiotic parameters on this spore-forming bacteria abundance dynamics.

2. Material and Methods

2.1. Study Area and Sampling Sites

Yaounde is the capital of Cameroon. It is located 300 km from the Atlantic coast, between 3°5' North latitude and 11°31' East longitude. The climate is equatorial, characterized by the alternation of two dry seasons and two rainy seasons: a long dry season from December 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 November. The annual average temperature is 23.5°C, varying between 16 and 31°C depending on the season, and 1650 mm of rainfalls per year [30]. The city's hydrographic network is very dense and essentially composed of the Mfoundi river and its tributaries.

Six wells points encoded W1-D5, W2-D5, W3-D3, W4-D3, W5-D6 and W6-D6 were chosen in 3 different administrative districts of Yaounde. Wells W1-D5 and W2-D5 were choose in N°5 district area, wells W3-D3 and W4-D3 in N°3 district area, and W5-D6 and W6-D6 in N°6 district area. At the same time, one station was installed in each of the 3 districts to collect rainwater. It was named R1-D5 in N°5 district area, R2-D3 in N°3 district area and R3-D6 in N°6 district area. The location of each sampling wells and each station for rainwater collection is indicated in Figure 1. The geographic coordinates of each sampling site are indicated in Table 1.

The meteorological data during the sampling period have been downloaded from the NASA web site (https://power.larc.nasa.gov/data.access.viewer/). During this sampling period, the rainfall varied from 2.2 to 11.8 mm/day. The air temperature and the air relative humidity varied from 20.02 to 24.28°C and from 82.97 to 91.25% respectively. The insolation varied from 2.59 to 5.54 Kw-h/m^2/day. The temporal variation of these data is presented in Figure 2.

Table 1. *Geographic coordinates of sampling sites.*

Location of the sampling site and nature of the water sampled		Geographic coordinates of the sampling site and total depth of well			
Location of the sampling site	**Nature of the water sampled**	**Latitude**	**Longitude**	**Altitude (m)**	**Total well depth (m)**
W1-SD5	Wells	03°54'19.9"N	11°33'12.9"E	692	9.5
W2-SD5	Wells	03°54'18.0"N	11°33'11.3"E	707	17.3
W3-SD3	Wells	03°51'14.4"N	11°29'43.9"E	728	11.5
W4-SD3	Wells	03°51'04.2"N	11°29'48.5"E	725	5
W5-SD6	Wells	03°50'31.0"N	11°29'07.1"E	711	20
W6-SD6	Wells	03°50'31.5"N	11°29'10.2"E	720	17
R1-SD5	Rainwater	03°50'31.5"N	11°33'06.3"E	729	/
R2-SD3	Rainwater	03°51'04.2"N	11°29'48.5"E	725	/
R3-SD6	Rainwater	03°50'31.0"N	11°29'07.1"E	711	/

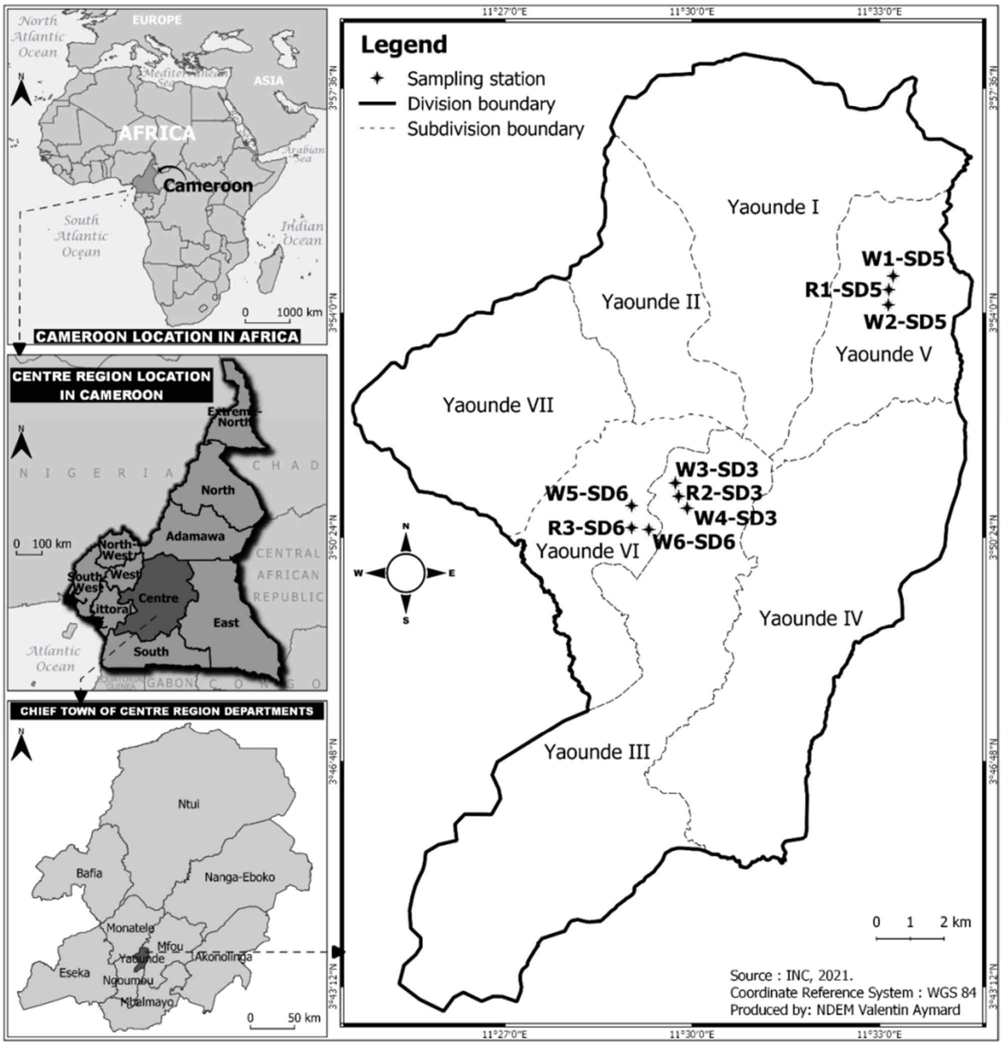

Figure 1. *Sampling sites of the water samplings (National Institute of Cartography, Cameroon, 2020, modified).*

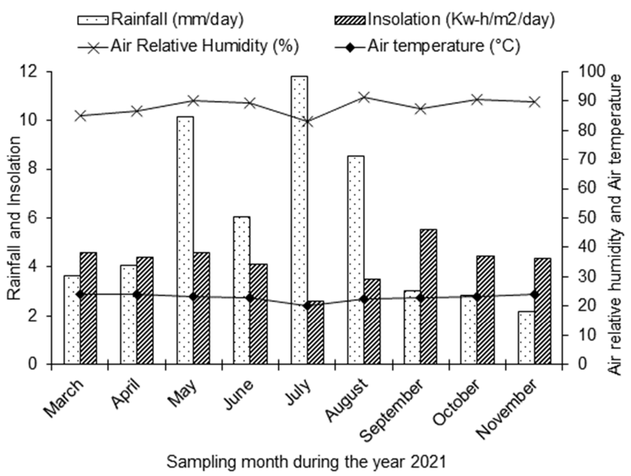

Figure 2. *Temporal variation of rainfall, insolation, air relative humidity and air temperature during the sampling period.*

2.2. Water Sampling

Rainwater and wells were analyzed once a month, from March to November 2021. Sample was collected once a month. During the short rainy season, it rains at least once a week, while during the short dry season it rains it rains 1 to 2 every 15 days. During the long rainy season, it rains 3 to 5 times a week.

Each rainwater sample was collected using an autoclaved glass container and placed in the yard of a specific dwelling. Said container was opened just when the rain started to fall. The collected water was poured into 2 vials: a sterile 500 mL glass vial and a clean 1 liter polyethylene vial. After the rain, they were then immediately transported in a refrigerated enclosure for the laboratory.

For each well chosen, the water sample was taken sterile using a sterile bucket attached to a rope. The collected water was poured into 2 vials: a sterile 500 mL glass vial and a clean 1L polyethylene vial. After taking this sample, the hydrological parameters of the well were measured using a graduated metal rope, also sterilized in an autoclave and connected to ballast at its end.

For the both rainwater and wells sample, the water temperature of each sample was measured *in situ*.

2.3. Laboratory Analysis

The physico-chemical parameters considered were the water temperature, the pH, electrical conductivity, water color, and concentration of dissolved CO_2, O_2, TDS (Total Dissolved Solids), nitrates, nitrogen ammonia and phosphate.

Bacteriological analyzes consisted of the isolation and counting of heterotrophic aerobic mesophilic bacteria (HAMB), *Bacillus cereus* and *Bacillus thuringiensis.* For the enumeration of HAMB, 100 µL of a diluted water sample was spread on standard agar culture medium contained in the Petri dishes. Each analysis was done in triplicate. The dishes were then incubated at laboratory temperature (24 ± 2°C) for 5 days.

Bacillus cereus was isolated on Mossel agar medium (Humeau laboratories) containing Polymyxin and the egg yolk added after autoclaving. *Bacillus thuringiensis* was isolated on Luria Bertani agar culture medium (Sigma-Aldrich) [31]. For that, 100 µl or a diluted water sample was analyzed, by the surface plating method. Petri dishes were then incubated at 36 ± 1°C for 24 hours. The morphological identification of the colonies considered the size and the outlines the color. *B. cereus* on Mossel agar medium formes colonies of around 5mm diameter, pink colour (mannitol negative) and typically sorrounded by an opaque halo due to egg precipitation (lecethinase positive) [32, 33]. On Luria Bertali agar, the colonies of *B. thuringiensis* are white, with a diameter varying from 0.5 to 1 mm [32]. Biochemical identification was further performed using API 20^E system [32-34]. Each analysis was done in triplicate. For all bacteriological analyzes, the results were expressed in Colony Forming Units (CFU) per 100µL.

2.4. Data Analysis

The abundances of isolated bacteria were expressed in CFU/100µL of water sample. The relationships between the considered parameters were assessed using Spearman's correlation test. A comparison of cell abundances between different stations was made using the Kruskal Wallis test.

3. Results

3.1. Physico-chemical Parameters

The values of the physico-chemical parameters are presented in Figure 3. This shows a spatio-temporal fluctuation of the values of each parameter, whether in wells or in rainwater.

3.1.1. Physico-chemical Parameters in Wells

The pH fluctuated between 5.05 and 7.33. The lowest value was observed in wells W1-SD5 during July and the highest in wells W5-SD6 during August (Figure 3). The electrical conductivity values ranged from 111 to 885 µS/cm. The smallest value was recorded in the wells W2-SD5 during August and the highest in the wells W4-SD3 during September (Figure 3). Water temperature ranged from 24.1 to 26.9°C, with the highest value recorded in wells W5-SD6 during June and W4-SD3 during July (Figure 3).

Dissolved CO_2 values fluctuated between 3.52 and 31.68 mg/L. The highest value was recorded in wells W4-SD3 during May and the lowest in wells W2-SD5 during April and W5-SD6 during June (Figure 3). Dissolved oxygen concentrations fluctuated between 1.11 (registered in wells W4-SD3 during November) to 7 mg/L (registered in wells W5-SD6 during July) (Figure 3). TDS concentrations fluctuated between 52mg/L (in wells W1-SD5 during March) and 444 mg/L (in wells W4-SD3 during November) (Figure 3).

The water color sometimes reached 200 Pt-Co. This highest value was recorded in the wells W2-SD5 during November.

However, water of several wells was sometimes very clear for several months (Figure 3). The nitrate contents varied from 0.08 to 43.1 mg/L. The lowest value was recorded in wells W2-SD5 during September and the highest in wells W6-SD6 during April (Figure 3). The phosphate and nitrogen ammonia contents varied respectively from 0.09 to 7.32 mg/L and from 0 to 6.04 mg/L. For phosphates, the highest value was recorded in wells W2-SD5 during October and the lowest in wells W3-SD3 during March. For nitrogen ammonia, the lowest value was registered in wells W2-SD5 during June and the highest in wells W4-SD3 during May (Figure 3).

3.1.2. Physico-chemical Parameters of Rainwaters

The pH fluctuates from 6.12 to 6.88. The lowest value was observed at station R1-SD5 and the highest at station R2-SD3. All these 2 values were recorded during September (Figure 3). Electrical conductivity values varied from 3 µS/cm (recorded at station R1-SD5 during May) to 92 µS/cm (at R3-SD6 station during September) (Figure 3). The water temperature fluctuated between 22.1 and 29.7°C, with the lowest value recorded at station R2-SD3 during March and the highest value at station R3-SD6 during April (Figure 3).

Dissolved CO_2 was rare during April at R2-SD3 station. Its highest concentration was 15.84 mg/L registered at station R3-SD6 during July (Figure 3). Dissolved oxygen concentrations fluctuated from 1.5 to 7.18 mg/L. The lowest value was noted at stations R1-SD5 during March and R2-SD3 during May; the highest value was recorded at station R3-SD6 during September (Figure 3). TDS concentrations varied from 3 mg/L (station R1-SD5 during May) to 46 mg/L (station R3-SD6 during April) (Figure 3).

The color of the water sometimes reached 83 Pt-Co. This value was recorded at station R3-SD6 during May. Water at station R1-SD5 was sometimes very clear (Figure 3). Nitrate concentrations varied from 0.1 mg/L (station R1-SD5 during September) to 15.6 mg/L (station R2-SD3 during June) (Figure 3). The phosphate and nitrogen ammonia contents varied respectively from 0.07 to 4.66 mg/L and from 0 to 2.01 mg/L. The highest value for phosphates was recorded at station R2-SD3 during April and the highest for nitrogen ammonia at station R3-SD6 during April (Figure 3).

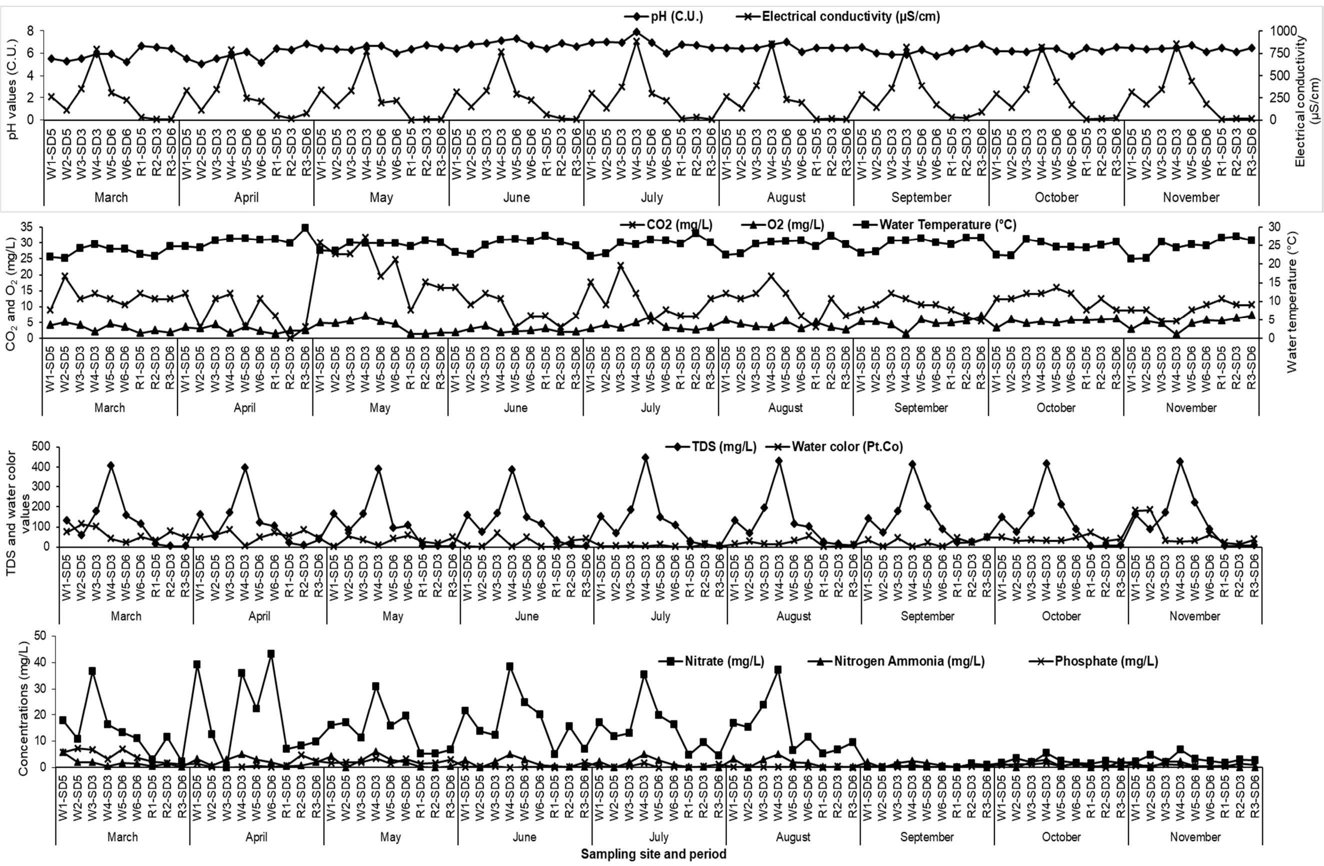

Figure 3. *Variation of the abiotic parameters values in each wells and each rainwater sampled with respect to the sampling month.*

3.2. Bacteriological Parameters

The variation in the abundance of heterotrophic aerobic mesophilic bacteria (HAMB) and *Bacillus* recorded in rainwater and wells is shown in Figure 4.

3.2.1. Bacteriological Parameters in Wells

In groundwater, HAMB abundances ranged from $1x10^6$ to $1x10^8$ CFU/100µL. The highest value was recorded in wells W2-SD5 during June (Figure 4), and the lowest in wells W4-SD3 during March (Figure 4). The abundances of *Bacillus thuringiensis* varied from 1 to 320 CFU/100µL. The highest value was recorded in wells W3-SD3 during November (Figure 4) and the lowest in wells W2-SD5, W3-SD3, W4-SD3 and W5-SD6 (Figure 4). The abundances of *Bacillus cereus* reached 340 CFU/100µL. This was recorded in wells W4-SD3 during September. This bacterium was rare in wells W4-SD3 during October (Figure 4) and W5-SD6 during April (Figure 4).

3.2.2. Bacteriological Parameters in Rainwater

In rainwater, the abundances of HAMB varied from $9x10^6$ to $196x10^6$ CFU/100µL. The highest value was recorded at station R3-SD6 (Figure 4), and the lowest at station R1-SD5 (Figure 4). Both values were recorded during May (Figure 4). Abundance of *Bacillus thuringiensis* varied from 30 to 730 CFU/100µL, and that of *B. cereus* varied from 10 to $12x10^2$ CFU/100µL (Figure 4). For *B. thuringiensis* the highest abundance was recorded at station R1-SD5 during March (Figure 4) and the lowest at station R2-SD3 during June (Figure 4). For *B. cereus* the lowest abundance was observed at station R3-SD6 during March (Figure 4) and the highest at station R1-SD5 during April (Figure 4).

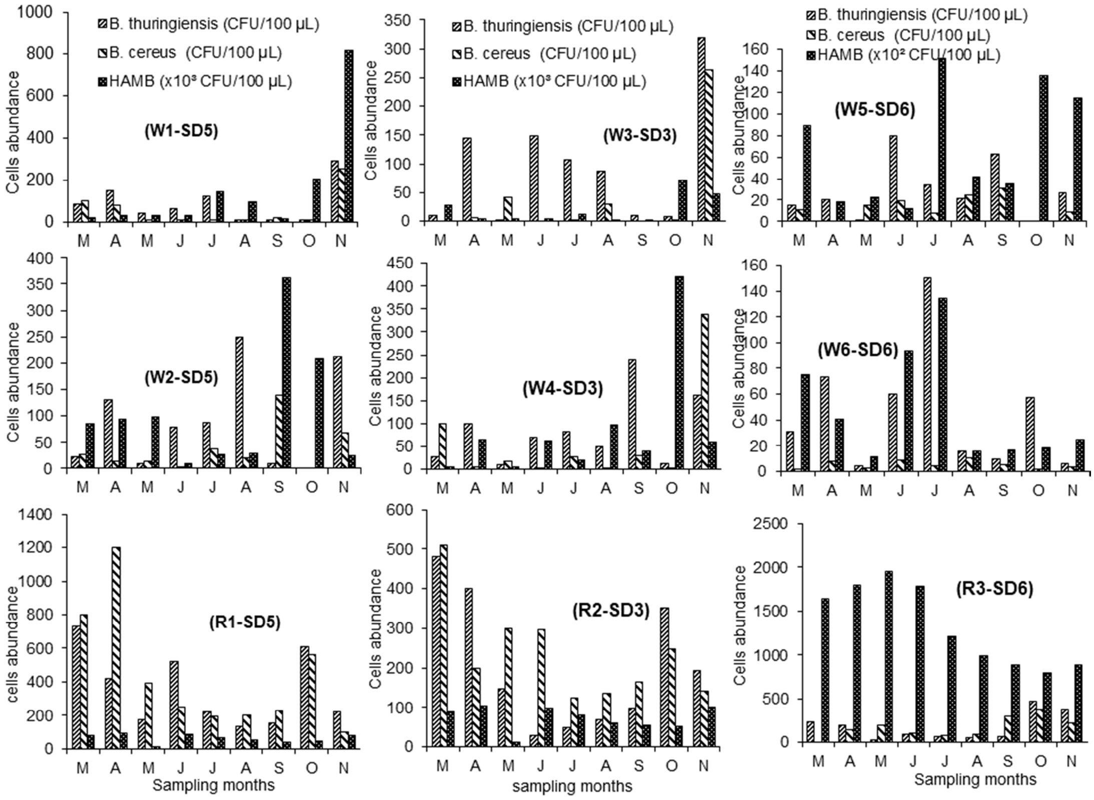

Figure 4. Variation of the bacterial abundance in each wells and each rainwater sampled with respect to the sampling month.

3.3. Correlations Amongst the Considered Parameters

The Spearman correlation coefficients have been calculated between the bacterial abundances and the abiotic parameters considered. The relationships between the parameters have then been classified into 4 categories: no significant correlation (P>0.1), significant correlations (P≤0.1), high significant correlations (P≤0.05) and higher significant correlations (P≤0.01). This has been done with wells as wells as with rainwater collected data.

3.3.1. Correlation Coefficients Between Bacterial Abundances in Wells and Wells Physicochemical/Meteorological Parameters

The 2 well water points of each subdivision were assumed to belong to the same underground watershed. Sampling was then assumed to be carried out twice a month, for the entire duration of the study. The number of samples was thus 18. This aimed to assess the impact of some meteorological and abiotic parameters of wells on the abundance dynamics of the bacteria contained in these wells. The Spearman correlation coefficients were then calculated are shown in Table 2.

It can be noted that in SD3, the content in dissolved CO_2, dissolved O_2 and phosphates as well as the insolation negatively impact the abundance of *B. thuringiensis* (P≤0.1). The abundance of this bacteria species is also negatively impacted in SD5 by the content in dissolved O_2 (P≤0.05), and in SD6 by dissolved CO_2, phosphates and the air relative humidity (P≤0.05) (Table 2). The water temperature is positively correlated with this cells abundance in SD6. The *B. cereus* abundance is negatively impacted by the content in dissolved CO_2 in SD5 (P≤0.1) and phosphates in SD6 (P≤0.1), and it is positively correlated with water pH in SD6 (P≤0.05). The HAMB's abundance is significantly impacted by the water electrical conductivity, and the content in TDS and nitrogen ammonia (P≤0.05) in SD3 and SD5, although the positive or negative role of the abiotic parameter on the bacterial abundance varies with respect to the sampling site (Table 2).

Table 2. Spearman correlation coefficients between bacterial abundances in wells and wells physicochemical/meteorological parameters in each considers subdivision.

Abiotic parameters considered	Sub-divisions (SD) and bacteria considered								
	SD3			SD5			SD6		
	B. thuringiensis	*B. cereus*	HAMB	*B. thuringiensis*	*B. cereus*	HAMB	*B. thuringiensis*	*B. cereus*	HAMB
Water T°	-0.030	-0.200	0.045	-0.057	-0.054	-0.088	0.469**	0.312	-0.061
Water pH	0.141	-0.004	0.278	-0.011	-0.272	-0.248	0.044	0.538**	0.224
Dissolved CO_2	-0.533**	-0.265	-0.109	-0.039	-0.442*	-0.018	-0.491**	-0.074	-0.249
Dissolved O_2	-0.407*	0.009	0.246	-0.488**	-0.059	-0.092	-0.312	0.057	0.006
Nitrates	-0.237	-0.205	0.060	0.234	-0.170	-0.224	0.345	0.020	0.014
Phosphates	-0.409*	0.248	0.213	-0.146	0.159	0.021	-0.512**	-0.444*	-0.286
TDS	0.176	0.274	0.456**	0.004	-0.054	-0.498**	0.125	0.250	0.372
Nitrogen ammonia	-0.015	0.130	0.456**	-0.059	0.037	-0.603***	0.170	0.264	-0.313
Water Color	-0.319	-0.262	-0.285	0.273	0.403*	0.171	-0.202	-0.271	-0.331
Elec. conductivity	0.157	0.280	0.448*	-0.017	-0.086	-0.487**	0.138	0.274	0.410*
Rainfall	-0.164	-0.092	-0.305	0.006	-0.368	-0.127	0.166	0.239	0.137
Air relative humidity	-0.207	0.083	0.380	-0.197	-0.356	0.195	-0.481**	0.121	-0.153
Air T°	-0.083	0.208	0.135	0.305	0.401*	0.222	-0.245	-0.351	-0.278
Insolation	-0.405*	-0.025	-0.214	-0.382	0.306	-0.004	-0.344	-0.125	-0.485**

ddl=17; ***: P ≤ 0.01; **: P ≤ 0.05; *: P ≤ 0.1; Water T°= water temperature; Air T°= air temperature; Elec. conductivity= electrical conductivity.

3.3.2. Correlation Coefficients Between Bacterial Abundances in Rainwater and Rainwater Physicochemical/Meteorological Parameters

The Spearman correlation coefficients have then calculated between the bacterial abundances registered during the 9 months in rainwater and the abiotic parameters considered. This aimed to evaluate the impact of some meteorological and abiotic parameters of rainwater on the abundance dynamics of the bacteria contained in this rainwater. The Spearman correlation coefficients calculated are shown in Table 3.

It appears that the water pH and the dissolved CO_2 are positively correlated (P≤0.1) with the dynamics abundance of *B. thuringiensis* at the station R1-SD5. At the station R2-SD3, the abundance variations of this bacteria species are negatively correlated (P≤0.1) with the water temperature, pH and electrical conductivity. However, phosphates, air temperature and insolation are positively correlated with this bacterial abundance dynamics (P≤0.1). At station R3-SD6, the profile of the abundance of this bacterium is strongly and negatively correlated (P≤0.01) with rainfall (Table 3).

Table 3. Spearman correlation coefficients between bacterial abundances in rainwater and rainwaters physicochemical/meteorological parameters at each collection's station in each considers subdivision.

Abiotic parameters considered	Rainwater collection's station and bacteria considered								
	R2-SD3			R1-SD5			R3-SD6		
	B. thuringiensis	*B. cereus*	HAMB	*B. thuringiensis*	*B. cereus*	HAMB	*B. thuringiensis*	*B. cereus*	HAMB
Water T°	- 0.683**	-0.850***	-0.017	-0.268	-0.367	0.450	0.172	0.686**	0.159
Water pH	-0.577*	0.259	-0.075	0.601*	-0.151	0.251	0.038	0.577*	0.460
Dissolved CO_2	0.238	0.298	-0.064	0.563*	0.138	-0.138	-0.114	-0.269	-0.092
Dissolved O_2	0.117	-0.600*	-0.083	0.232	-0.521	-0.261	0.336	0.577*	-0.385
Nitrates	-0.183	0.233	0.533	-0.504	0.276	0.343	-0.368	-0.417	0.717**
Phosphates	0.617*	0.283	0.450	0.385	0.700**	0.067	0.084	0.201	0.485
TDS	-0.471	-0.723**	-0.084	-0.160	-0.142	0.268	0.256	0.498	-0.236
Nitrogen ammonia	0.450	0.250	-0.083	-0.151	0.050	0.695**	-0.131	-0.287	0.523
Water Color	0.519	0.686**	0.310	0.017	0.678**	-0.119	-0.101	0.326	0.059
Elec. conductivity	-0.571*	-0.790***	0.084	-0.025	0.351	0.628*	0.324	0.561*	-0.310
Rainfall	-0.533	-0.133	-0.100	-0.268	-0.067	-0.083	-0.787***	-0.600*	0.683**
Air rel. humidity	-0.100	0.000	-0.467	-0.142	-0.133	-0.467	-0.092	0.483	0
Air T°	0.850***	0.667**	0.400	0.218	0.617*	0.400	0.519	0.033	-0.183
Insolation	0.583*	0.633**	-0.317	0.126	0.517	-0.033	0.134	0.400	-0.283

ddl=8; ***: P ≤ 0.01; **: P ≤ 0.05; *: P ≤ 0.1; Water T°= water temperature; Air T°= air temperature; Elec. conductivity= electrical conductivity; Air rel. Humidity= Air relative humidity.

For the bacterium *B. cereus*, its abundance dynamics is negatively correlated (P≤0.1) with dissolved O_2, TDS, water electrical conductivity and the air temperature, at the station R2-SD3, and with rainfall at the station R3-SD6 (P≤0.1). This bacterial abundance's variation is strongly and positively correlated (P≤0.01) with the water color, the air temperature and the insolation at R2-SD3, with the phosphates concentration, water color and air insolation (P≤0.1) at R1-SD5, and with water temperature, pH, dissolved O_2 and electrical conductivity (P≤0.1) at R3-SD6 (Table 3).

The abundance dynamics of the HAMB is positively impacted (P≤0.1) by the color and electrical conductivity of the water at station R1-SD5, and nitrate concentration and rainfall (P≤0.1) in station R3-SD6 (Table 3).

3.3.3. Delay Correlations Between Groundwater and Rainwater Data

The water from the 2 wells in each borough was assumed to belong to the same underground watershed. In this way, the average values of each parameter analyzed were calculated for each underground watershed. It was indicated that a period of one month is necessary in the study area, to observe the impact of rainwater on the piezometric height of a water table [16, 24]. Correlation coefficients were thus calculated between the current month's data collected in groundwater and the previous month's data collected in rainwater. These correlations have thus been qualified as correlations with delay. The number of samples considered thus was 8. This aimed to assess the impact of some meteorological parameters and as well as some biotic and abiotic properties of rainwater on the dynamics of abundance of the bacteria considered in well water. The results are presented in Table 4.

It appears that the pH of rainwater positively impacts the abundances of *B. thuringiensis* and *B. cereus* in the waters of the W-SD5 underground watershed (P≤0.05) (Table 4); similarly, the abundances of *B. thuringiensis* in rainwater positively impact that of *B. thuringiensis* in the waters of the underground watershed in the same district (P≤0.01). It is also noted that dissolved CO_2, phosphates, air temperature and the abundances of *B. cereus* in rainwater are positively and significantly correlated with the abundances of HAMB in the waters of the W-SD5 underground watershed (P≤0.1) (Table 4). The abundances of HAMB in the waters of the underground watershed W-SD3 are significantly and negatively impacted by the rainfall of the previous month (P≤0.05) (Table 4). In the waters of the W-SD6 underground watershed, the dissolved oxygen and electrical conductivity of rainwater are negatively and significantly related to the abundances of *B. thuringiensis* (P≤0.1). On the other hand, the nitrate contents of rainwater and rainfall are positively and significantly linked to the abundances of *B. cereus* in the waters of this W-SD6 underground watershed (P≤0.1). Insolation is negatively related to the abundances of this bacterium in this underground watershed (P≤0.05) (Table 4).

Table 4. *Correlation coefficients between the current month's data collected in underground water (W) and the previous month's data collected in rainwater, in each district.*

Rainwater parameters	Underground water in each sub-division and bacteria considered								
	W-SD3			W-SD5			W-SD6		
	B. thuringiensis	***B. cereus***	**HAMB**	***B. thuringiensis***	***B. cereus***	**HAMB**	***B. thuringiensis***	***B. cereus***	**HAMB**
pH (UC)	-0.084	-0.470	-0.299	0.690**	0.690**	-0.119	-0.357	-0.323	0.119
Dissolved CO_2	0.540	-0.420	-0.393	0.476	-0.125	0.601*	0.410	0.224	0.193
Dissolved O_2	0.143	0.479	0.500	0.000	0.054	-0.181	-0.611*	-0.120	0.275
Nitrates	0.119	0.120	-0.238	-0.252	-0.084	-0.084	0.048	0.647*	-0.452
Phosphates	-0.476	-0.383	-0.548	0.524	0.238	0.619*	-0.084	0.066	0.000
Nitrogen ammonia	0.095	0.084	0.310	-0.335	-0.108	-0.252	0.157	0.133	-0.506
Elec. conductivity	-0.563	0.247	0.228	-0.335	-0.228	-0.455	-0.778**	-0.265	-0.383
B. thuringiensis	0.095	0.048	-0.119	0.810***	0.476	0.119	-0.395	-0.542	-0.036
B. cereus	-0.286	-0.359	-0.095	0.310	0.071	0.643*	-0.286	-0.287	0.095
HAMB	-0.214	0.347	-0.048	0.024	0.214	-0.357	0.381	0.228	-0.214
Rainfall	-0.167	-0.204	-0.595*	-0.095	-0.214	-0.405	0.310	0.790**	0.095
Air rel. Humidity	0.571	-0.012	-0.119	-0.167	0.381	0.095	0.190	0.240	-0.167
Air T°	0.095	-0.096	-0.143	0.033	0.000	0.595*	0.048	-0.419	-0.500
Insolation	-0.190	-0.539	0.190	-0.024	-0.357	0.500	0.119	-0.790**	-0.119

ddl=7; ***: P ≤ 0.01; **: P ≤ 0.05; *: P ≤ 0.1; Air T°= air temperature; Elec. conductivity= electrical conductivity; Air rel. Humidity= Air relative humidity.

3.3.4. Spearman Correlation Coefficients Between the Bacterial Abundances and the Abiotic Parameters at the Whole Rainwater Collection's Stations

The 3 rainwater collection's stations were assumed to belong to the same geographical area. Sampling was then assumed to be carried out 3 times a month, for the entire duration of the study. The number of samples was thus 27. The Spearman correlation coefficients were then calculated and are shown in Table 5. For the whole rainwater collection's stations, the abundance dynamics of *B. thuringiensis* is negatively impacted (P≤0.1) by water temperature, nitrates and rainfall, and a positive impact (P≤0.01) by air temperature. The variations of the abundance of *B. cereus* are positively impacted (P≤0.1) by water color, air temperature and insolation. For HAMB, nitrates and phosphates have a positive impact (P≤0.05) on their abundance, while the air relative humidity has a negative impact (P≤0.1) (Table 5).

Table 5. *Spearman correlation coefficients between the bacterial abundances and the abiotic parameters considered registered at the whole rainwater collection's stations.*

Abiotic parameters considered	Bacteria considered		
	Bacillus thuringiensis	***B. cereus***	**HAMB**
Water T°	-0.339*	-0.265	0.119
Water pH	-0.162	0.103	0.172
Dissolved CO_2	0.197	0.016	-0.126
Dissolved O_2	0.219	-0.119	-0.210
Nitrates	-0.355*	-0.080	0.412**
Phosphates	0.221	0.147	0.454**
TDS	-0.070	0.068	-0.067
Nitrogen ammonia	0.112	-0.018	0.197
Water Color	0.177	0.393**	0.316
Elec. conductivity	-0.029	0.219	0.033
Rainfall	-0.497***	-0.218	0.051
Air relative humidity	-0.061	0.104	-0.333*
Air T°	0.481**	0.336*	0.289
Insolation	0.268	0.466*	-0.157

ddl=26; ***: $P \leq 0.01$; **: $P \leq 0.05$; *: $P \leq 0.1$; Water T°= water temperature; Air T°= air temperature; Elec. conductivity= electrical conductivity.

4. Discussion

It is noted that rain and well water sampled harbors HAMB and specially *B. thuringiensis* and *B. cereus* which are spores forming bacteria. Microorganisms found in rainwater should be contained in aerosols. According to Tignat-Perrier *et al*. [3], airborne microbial communities appear to be the result of large inputs from nearby sources with possible low and diluted inputs from distant sources. Most airborne micro-organisms would be carried with particle size fractions higher than 10 µm aerodynamic diameters. Temporal stability in the composition of airborne microbial communities is mainly explained by the diversity and evenness of the surrounding landscapes and the wind direction variability over time [3]. Cheol Cho and Jang [2], analysed ATP content in bacterial fraction of the rainwater and the result suggested that the rainwater bacteria were metabolically active. Rainwater bacteria isolated showed potentials of fast growth.

In this study, it has been noted that bacterial abundance undergoes spatio-temporal variations in both media. Airborne bacterial communities seem to be in permanent change and this change is according to location and land-use around sampling point [35], and a complex set of environmental factors, including changes in atmospheric conditions and shifts in the relative importance of available microbial sources, may act to control its composition [8]. Temporal shifts in microbial community composition could sometimes be related to the different origin of air masses [36-38], but this seems possible only when air masses at the same location originated from clearly different environments. The spatio-temporal variation of the bacterial composition of rainwater samples has also been noted by Cheol Cho and Jang [2] and they suggested that it could be attributed to a local phenomenon and the spatial variability of aerosolized gamma-bacteria captured by rain which is seasonally dependent.

Variation of the rainwater bacterial abundance noted in this study could be link to diel cycle environmental factors. Gusareva *et al.* [39] noted in fact that the airborne microbial organisms followed a clear diel cycle, possibly driven by environmental factors. Interday taxonomic diversity exceeded day-to-day and month-to-month variation. Some specific fungal and bacterial species can be strongly correlated with temperature, humidity, and CO_2 concentration, making them suitable biomarkers for studying the bioaerosol dynamics of the atmosphere [39].

It has been noted in this study that pH of wells fluctuated between 5.05 and 7.33 whereas that of rainwater varied from 6.12 to 6.88. Electrical conductivity values ranged from 111 to 885 µS/cm in wells, and varied from 3 to 92 µS/cm in rainwater. Both media contains nitrate, nitrogen ammonia, phosphate, dissolved CO_2 and O_2 and their concentration undergoes spatio-temporal variations. Other authors indicated that the aerosol contains H^+, Li^+, Na^+, K^+, NH_4^+, Mg^{2+}, Ca^{2+}, Cl^-, Br^-, NO_3^-, HSO_4^{2-}, $SO4^2$, CO_3^{2-}, $CH_3SO_3^-$ [40-42]. The gas-phase species that can partition to the particle phase include H_2SO_4, CH_3SO_3H (methanesulfonic acid), HNO_3, HCl, NH_3, and any number of secondary organic, and their concentrations undergoes spatio-temporal variation [40-42].

The hydrogen ion activity in aqueous aerosols affects the partitioning of total nitrate and total ammonium between the gas and aerosol phases [42]. In the atmosphere, the acidity of condensed phases (aerosol particles, cloud water, and fog droplets) governs the phase partitioning of semivolatile gases such as HNO_3, NH_3, HCl, and organic acids and bases as well as chemical reaction rates [42]. It has implications for the atmospheric lifetime of pollutants and deposition. Changes in acidity also affect the number of chromophores contained within aerosol and their efficiency in absorbing sunlight in the near-UV range, and acidity-induced changes in aerosol contribute to the formation of droplets in warm and mixed-phase clouds [42-44].

Dust supplies the majority of the total phosphorus of aerosols and air particulate contain in phosphorus increase exponentially with daily temperature [45, 46]. The temperature and precipitation pattern dependence indicate a possible climate change influence on the aerosol phosphorus [46].

It has been noted in this study that some meteorological such as insolation, air temperature and rainfall, and some chemical parameters such as water temperature, water pH, dissolved CO_2, dissolved O_2, TDS, nitrogen ammonia, nitrate, phosphates, and electrical conductivity significantly impact bacterial abundance in rainwater although this varies with respect to the bacteria. It is indicated that air temperature and relative humidity are most often the factors that significantly shaped the microbial communities [47, 48]. The effects of relative humidity are more complex, and varies with environmental conditions. At higher temperatures, air can hold more water vapour, and the relationship is roughly exponential-air at high temperatures can hold much more water vapour than air at lower temperatures [49, 50]. When the organisms are dry-disseminated they tended to absorb water from the environment (i.e. they partially rehydrated), and when wet-disseminated, the opposite occurred, i.e. they desiccated [50]. Air temperature and relative humidity also affects virus survival, as they can affect the state of viral proteins (including enzymes) and the virus genome (RNA or DNA) [50].

Working on spores of *Bacillus subtilis* exposed to a series of stratosphere simulations, Smith *et al*. [51] noted that the stratosphere can be a critical barrier to long-distance microbial dispersal and cell survival in the upper atmosphere may be constrained by UV irradiation.

About the groundwater bacterial abundance, some authors indicated that microorganisms retrieved at ground level are not significantly different from those found at relatively higher elevation (238 m), thus suggesting the occurrence of a wide atmospheric mixing [52]. Brillard *et al.* [11] investigated the rain and groundwater closely linked to the *B. cereus***.** They noted the presence of this bacteria species primarily as spores, in all of the tested compartments. During rain events, leachates collected after transfer through the soil eventually reached the groundwater and were loaded with *B. cereus*. In groundwater samples, newly introduced spores of a *B. cereus* strain were able to germinate, and vegetative cells arising from this event were detected for up to 50 days [11].

It has been stated that the impact of rainwater properties on those of groundwater is significant after a period of a few weeks to a month, although depending on the depth of the water table [16, 24]. This is sometimes referred to as a delayed impact. But in the present study, the significant impact of rainwater microbiological and chemical parameters on the biotic properties of groundwater varies from one parameter to another. The impact of several rainwater parameters on the dynamics of groundwater microflora would be drowned in what Elias *et al*. [53] qualified as confounding factors. This would include cells anatomy physiology, soil structure and chemical properties.

5. Conclusion

The abundances of *B. cereus* and *B. thuregiensis* in analysed water samples undergo spatio-temporal variations. In rain and well water, the degrees of correlation between meteorological or chemical factors and the dynamics of bacterial abundance varied according to the abiotic parameter and the bacterial species considered. The relationships between the meteorological data and the properties of the previous month's rainwater on the abundance dynamics of the microflora in the well waters of the current month showed a diversity of degrees of impact. This would mean that even if the rainwater feeds the groundwater, the general properties of this groundwater would result from the interactions of the confounding factors. Due to the presence of these 2 bacteria species, the use of these waters presents short-term health risks for local populations.

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