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Impact of heavy metal residues on the diversity of bacteria isolated from a few water points in areas of agricultural activity

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Abstract

A study aimed at evaluating the impact of heavy metal residues on the diversity of bacteria of the *Vibrio* and *Salmonella* genera in surface and groundwater in agricultural activity zones was conducted from January to July 2021. The microorganisms sought were the bacteria of the *Vibrio* and *Salmonella genera.* These bacteria were isolated from twelve (12) groundwater points and four (04) surface water points by the surface spreading technique and that of filter membranes on Thiosulfate Citrate Bile Sucrose (TCBS) and *Salmonella* - *Shigella* media. (SS) for *Vibrio* and *Salmonella* respectively. Some abiotic parameters like heavy metals (Lead, Mercury, etc.) were evaluated using the usual techniques.

The results show variations in the concentrations of certain heavy metals such as zinc and copper. The most identified heavy metals are zinc (59%) and copper (40%), which would be due to the use of agricultural inputs in this area. Bacteriological analyzes revealed that these waters have a high and varied bacterial load. Densities of pathogenic bacteria reached an average of 4.10^{2} CFU/100mL for *Vibrio* and $7.32.10^{3}$ CFU/100mL for *Salmonella.* These germs can be at the origin in this commune of the epidemics of cholera and typhoid. Highly significant correlations ($P<0.01$) between bacterial abundances and copper were observed. The degradation of the quality of these waters is caused by their proximity to sources of pollution. According to European Union standards, these waters are not recommended for human consumption without any prior treatment.

Keywords: Heavy metals; Pathogenic Bacteria; Groundwater; Abiotic Variables; Seasons.

1. Introduction

Today, aquatic environments are the receptacle for effluents and urban industrial waste that are often toxic to living organisms. Heavy metals are among the major pollutants of the environment both by their ubiquitous nature and by their toxicity and their potential bioaccumulation in several aquatic species, inducing devastating effects [1]. Because they are poorly metabolized, they can therefore be transferred into the food chain and accumulate in living matter [2] They are normal constituents of the environment in trace amounts but become toxic above a certain threshold [3,4]. Although industrial activity is less developed in most African countries, there is a gradual awareness of the need to preserve the environment by limiting the anarchic dumping of industrial waste. Specifically for Cameroon, its economic landscape is characterized by an industry in its infancy. But, as industrial and urban activities are to be expected to intensify, this issue now assumes even greater importance [5].

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In most cities, toxic waste is generally dumped in nature, without prior treatment, thus increasing the level of contamination of the environment. The consequences of the dispersion of pollutants in the environment have and continue to arouse great interest within the world scientific community because the protection of the environment requires knowledge of these pollutants as well as their fate in the environment. Pollution by heavy metals is currently a major problem due to the presence of these elements in the marine environment, especially in drinking water [1] Analyzes have revealed that unlike organic contaminants, heavy metals generated by anthropogenic activities, cannot be biologically degraded and persist indefinitely in the environment [4]. Similarly, polluted environments are generally subject to strong topographical, climatic and water constraints, strongly eroding the waste and inducing pollution for the surrounding waters and soils [5] In addition, heavy metals all have a toxic potential which depends mainly on their concentration in the medium considered and, on their bioavailability, that is to say on their fraction soluble and accessible by organisms [5]. The bioavailability of heavy metals defines their ability to be transferred from one compartment to another. The more the metal species is free and mobile, the more it is bioavailable and the more there is a risk of toxicity on living organisms. Indeed, heavy metals exist either in elemental form or in derived form. Being positively charged, they are then likely to interact with any negatively charged elements to form inorganic (salt, sulphide, oxide) or organic (with carbon bond) derivatives [6].

Other studies have focused on the impact of heavy metals present in water and in certain fishery products on human health and the evaluation of the heavy metal content of certain foods [7]. These studies, mostly conducted in large cities, took into account the effect of pollution by multiple heavy metals on bacterial diversity and community structure in agricultural soils [8]. Other work focuses on the impact of temperature, nutrients and heavy metals on bacterial diversity and the functioning of ecosystems studied by freshwater microcosms and high-throughput DNA sequencing [9] Despite these data, little information is available on the levels of heavy metals present in water intended for human consumption in rural agricultural areas. Moreover, little is known about the impact that these heavy metals would have on the variation in the diversity of bacteria present in drinking water. This work aims to determine the impact of heavy metal levels and bacterial diversity in some water points used for drinking water by the population of Mbam (Central Region of Cameroon).

2. Material and methods

2.1. Sample collection

The water samples intended for the bacteriological analyzes were collected in 500 ml glass bottles previously sterilized. For the analyzes of heavy metal content, the samples were taken using double-stoppered 500 ml polyethylene bottles, previously cleaned with 10% nitric acid, then rinsed with distilled water and dried. in the oven [10]

2.2. Choice and description of sampling points

Twelve (12) underground water points and four (04) surface water points were chosen in different districts of the towns of Ntui and Ombéssa, on the basis of criteria such as the accessibility of the points, the use of water point for drinking water by the populations, the presence of a possible source of pollution, the proximity of an agricultural plantation and with a view to covering the entire study area as well as possible. It was considered that a water point is all the more important when the volume of water drawn is high and/or when the water drawn is primarily intended for human consumption. Table 1 summarizes the codes of the groundwater points analyzed, their geographical coordinates and their average altitudes obtained in the field using a GPS map. The twelve (12) sampling points are coded from N1 to N12 for underground points and S1 to S4 for surface points (rivers). The sampling campaigns were carried out from January to July 2021, bringing together three climate seasons in these cities (long dry season (GSS): January to mid-March, short rainy season (PSP): mid-March to mid-June and small dry season (PSS): mid-June to July) following a monthly sampling step. Water was collected at each water point using different containers prepared in the laboratory for this purpose. Figure 1 shows the location of these points on the map of the sampling area.

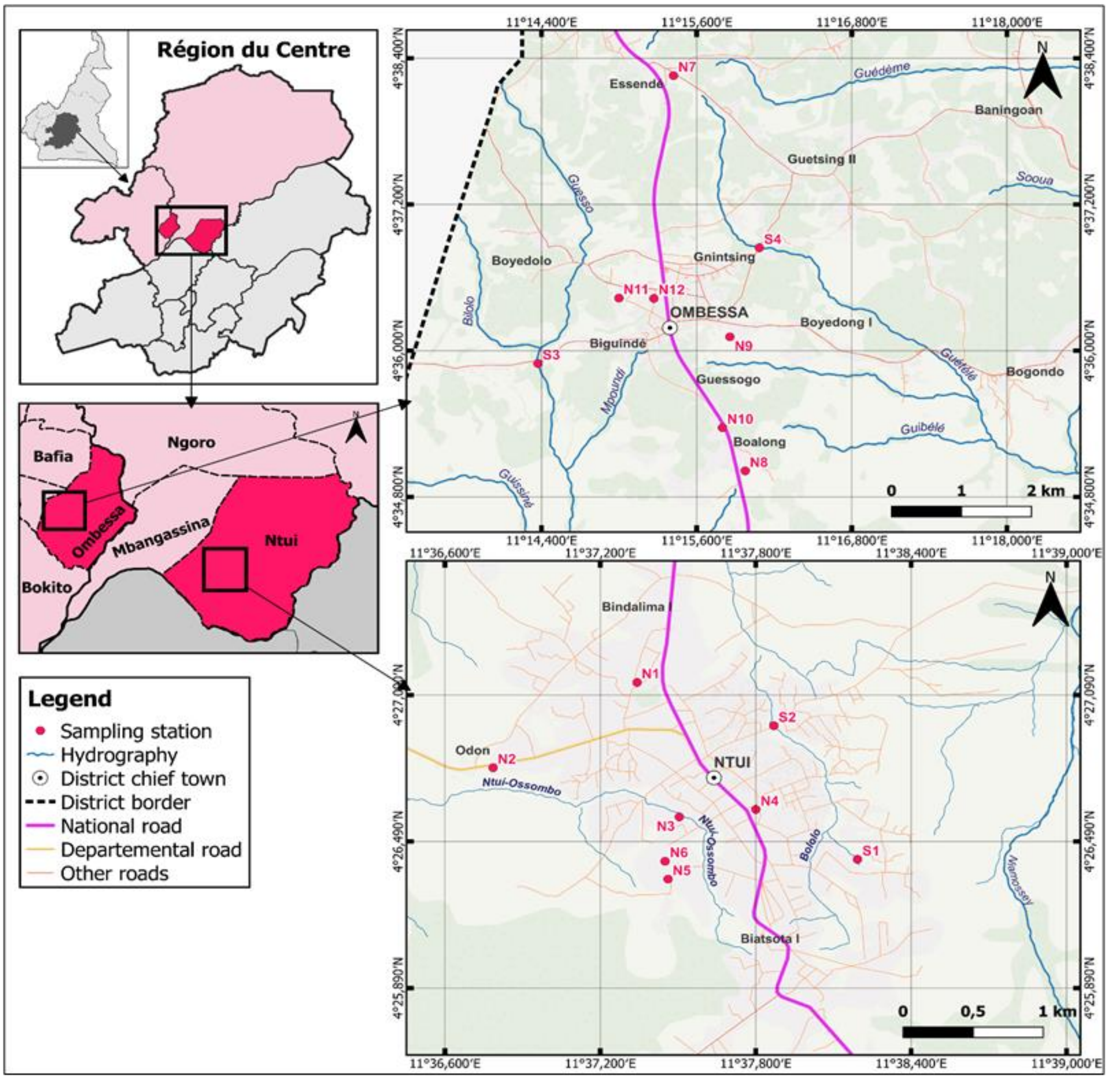

Figure 1 Locations of the different sampling points in the study area

Table 1 Geographical coordinates, average altitudes characteristic of sources of pollution of the water points studied

	Location: Websites	GPS coordinates		**Possible sources of pollution**
Resort codes		**Altitudes**	**Latitudes and Longitudes**	
N1	Mosque	532m	4 ⁰ 27' 8.4769''N 11 ⁰ 37'20.513''E	Agricultural activity 10m away, latrines nearby
N2	Odone district	493m	4 ⁰ 26' 47.529''N 11 ⁰ 36' 47.169''E	Agricultural activity, 15m
N3	Bami district	533m	4 ⁰ 26'35.4534''N 11 ⁰ 37' 30.30''E	Agricultural activity, 9m latrines nearby
N4	district hospital	528m	4 ⁰ 26'37.2818''N 11 ⁰ 37'48.016''E	Agricultural activity at 10 m
N5	Catholic Mission3(MC3)	523m	4 ⁰ 26'20.170''N 11 ⁰ 37'27.689''E	Agricultural activity at 8 m, latrines nearby

N6	Catholic Mission2 (MC2)	515m	4 ⁰ 26'24.5536''N 11 ⁰ 37'27.004''E	Agricultural activity at 13 m, latrines nearby
N7	Essende	486m	4°38'15.41512''N 11°15'25.239''E	Housing/ Agricultural activity at 14 m
N8	Boalong	443m	4°35'0.816''N 11°15'58.536''E	Marsh/field at 20 m
N9	Boyambois	437m	4°36'6.81146'' N 11°15'51.4483''E	Landfill/ Dwellings 15m
N10	Guessogo	460m	4°35'22.08'' N 11°15'47.982''E	Agricultural activity 10 m
N11	Sisters' house	466m	4°36'25.896'' N 11°14'59.904''E	Habitat/swamp/field 15 m
N12	Biaboo	467m	4°36'25.5"N, 11°15'16.1"E	Field/Habitat 8 m
S1	Bololo 1	545m	4°26'25.02''N 11°38'11.58''E	Proximity to fields
S2	Bololo 2	515m	4°26'57.84''N 11 °37'52.2''E	Proximity to fields
S3	Ofoue	443m	4°35'53.7"N, 11°14'21.3"E	Proximity to fields
S4	Bandama	453m	4°36'50.6"N, 11°16'5.1"E	Proximity to fields

2.3. Sample analysis

2.3.1. Choice of heavy metals and evaluation of the content in water (olive)

The heavy metals considered are Cu, Pb, Zn, Cd and Hg. These heavy metals were chosen because they can accumulate over time in organisms that ingest them, giving rise to a phenomenon of bioaccumulation [11, 12]. According to [13], bioaccumulation is the accumulation of pollutants in a living organism. The concentrations increase exponentially throughout the food chains, causing a phenomenon of biomagnification. In addition, high doses of these metals can cause irreversible damage to the kidneys and liver and consequently lead to death [14].

2.3.2. Assessment of the content in the waters

It is now recognized that monitoring certain contaminants in aquatic environments is a good way to assess environmental contamination [14]. These heavy metals (expressed in mg/L) were measured at the Soil, Plant, Water and Fertilizer Analysis Laboratory (LASPEE) of the Agricultural Research Institute for Development (IRAD) in Nkolbisson (Yaoundé). The method used was atomic absorption spectrometry, using a Perkin-Elmer type flame spectrometer [15].

2.4. Evaluation of the diversity of bacteria in the water sampled

2.4.1. Choice of germs

The germs sought were pathogenic bacteria of the genus *Vibrio* and *Salmonella.* These pathogenic bacteria were chosen because of their recurrent involvement in waterborne diseases and epidemics in emerging countries [15].

2.4.2. Isolation of the germs sought

The germs were isolated using the membrane filter technique 100 mL of water sample were taken and then filtered through a membrane filter Millipore cellulose ester membrane, Bedford, MA 01730 with a porosity of 0.45 µm [16], squared, sterile. Using fine tweezers previously passed through the flame of the bunsen burner, the membrane was then placed very gently in petri dishes containing TCBS and SS culture media for *Vibrio* and *Salmonella* respectively and incubated at 37°C for 24 h. [17], The manipulations were made in a diameter of 30 cm around the flame of the bunsen burner [16].

Determination of bacterial abundances

For each sampling campaign, the isolated bacteria were counted by direct counting using a colony counter pointer. This shows bacterial colonies of various shapes, colors, sizes and appearance. [18]. The concentrations were expressed in Colony Forming Units per 100mL (CFU/100mL).

Identification of germs

For the identification of these two genera, after gram staining, basic tests were carried out using the classic gallery. We can mention the search for oxidase, the search for the enzyme catalase, the fermentation of glucose, the production of gas, the affinity for oxygen (aerobic anaerobic facultative), the mobility, the fermentation of mannitol, the fermentation of lactose, the production of H_2S, the search for urease, the use of citrate among others [16,17]. The identifications were made on the *Salmonella genera Vibrio* in order to determine the species corresponding to the colonies thus isolated on petrie dishes and presenting satisfactory cultural characters [18], but also because of their recurrent involvement in waterborne diseases and epidemics [15].

2.5. Spearman rank correlation coefficient

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

2.5.1. Comparisons

The comparisons between the variables considered were carried out using the Kruskal -Wallis “H” comparison tests and the Mann-Whitney “U” tests using the PAST software.

2.5.2. PCA (Principal Component Analysis)

In this study, a PCA was carried out in order to characterize the sampling stations on the basis of the bacterial concentrations in relation to heavy metals. The objective of this descriptive analysis method is to present in the form of a graph, the maximum of the information contained in a large data table.

3. Results

3.1. Variation in the concentration of heavy metals in the water points studied

The variation in water content of metallic elements in the different sampling stations is shown in Figure 2. In general, the heavy metal content varies from one station to another and during the sampling period. The lowest values for all the heavy metals considered are obtained in groundwater compared to surface watercourses. Cadmium (Cd) concentrations vary between 0.001 mg/L and 0.014 mg/L, the minimum values are recorded at stations N7 and N9 during the PSP (short rainy season) and at station N12 during the PSS while the maximum value was recorded at station S4 during the PSS (small dry season) (Figure 2A). The minimum copper (Cu) content (0.5 mg/L) is recorded at station N4 during the PSP, while the maximum value (4.3 mg/L) is observed at station S4 during the same (season Figure 2B). Regarding Lead (Pb), the minimum value (0.003 mg/L) is recorded at station N6 during PSP while the maximum value (0.067 mg/L) is obtained at station S3 during the same period (Figure 2C). Zinc (Zn) concentrations vary between 1mg/L in stations N4, N5, N6 during the GSS, reaching its peak at 5 mg/L at station S2 during the PSS (Figure 2D). Regarding mercury (Hg), we note its rarity in some stations like N2 to N12 throughout the study period and during all seasons. The maximum value (0.0049mg/L) was recorded at station S3 during the PSS (Figure 2E).

In general, heavy metals are more present during the PSS which chronologically immediately follows the PSP (period of intense agricultural activity and use of inputs. Indeed, in the rainy season, agricultural activity forces the populations to use pesticides, the latter can thus make heavy metals available in the aquatic environment and even in the soil.Therefore, these metals can be either fixed in rocks and sediments, or mobile. first case, the quantities available are tiny and they have no meaning on the environment. But when conditions change in such a way that the metals become soluble again, the increased concentration then becomes a direct threat to the environment due to their increased availability to plants. Since few years, acid rain increases the mobility of metals in the soil and therefore causes an increase in their.

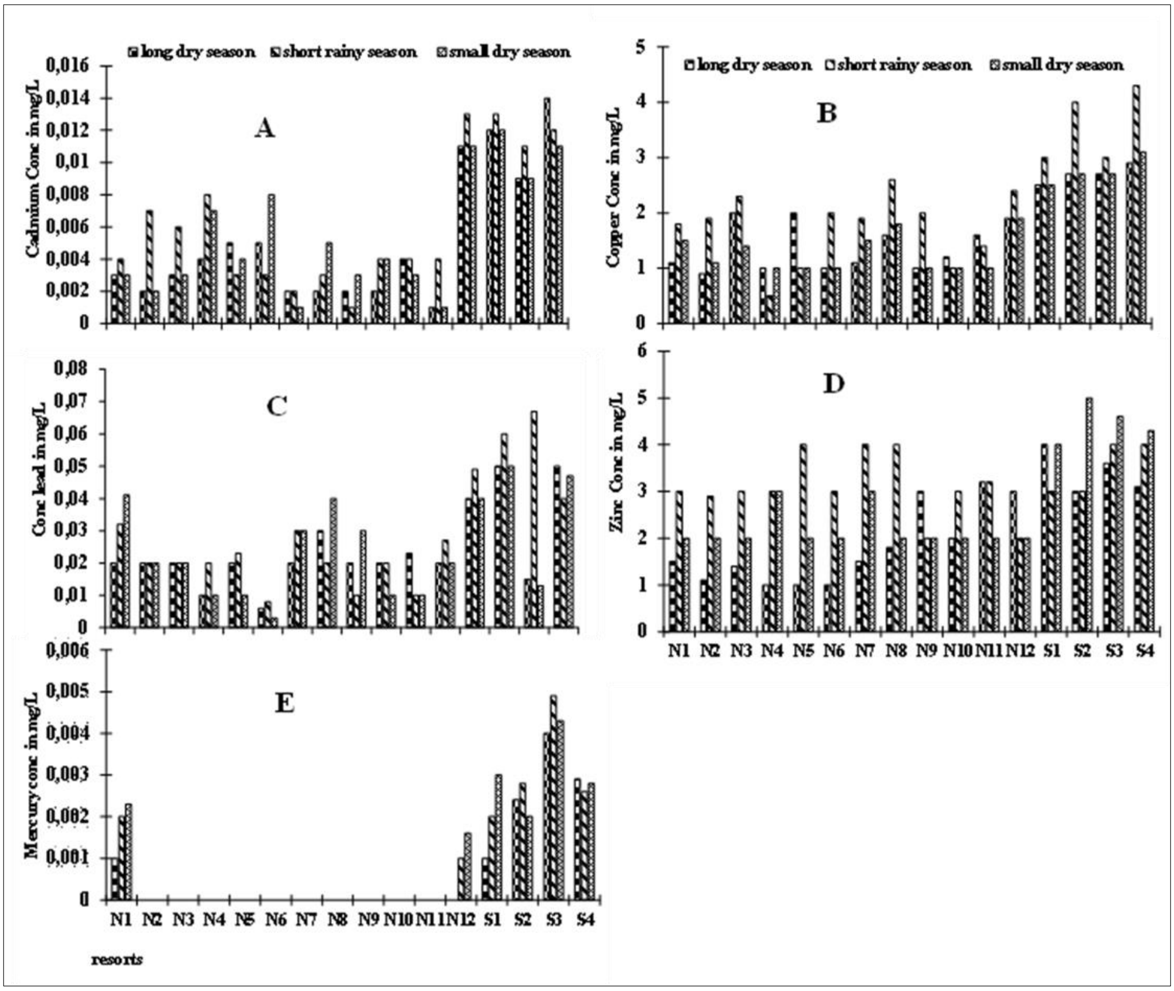

Figure 2 Variation in the levels of heavy metals in the waters sampled during the study period depending on the sampling seasons (A: Cadmium, B: Copper, C: Lead, D: Zinc, E: Mercury).

3.2. Distribution frequencies of heavy metals in waters during the sampling period

Throughout the sampling period, three heavy metals were frequently obtained at the sampling stations. It shows that the most represented is zinc (Zn) 59% followed by copper (Cu) 40%, and lead (Pb) (1%). Cadmium (Cd) and mercury (Hg) are classified as rare heavy metals in sampled waters (Figure 3).

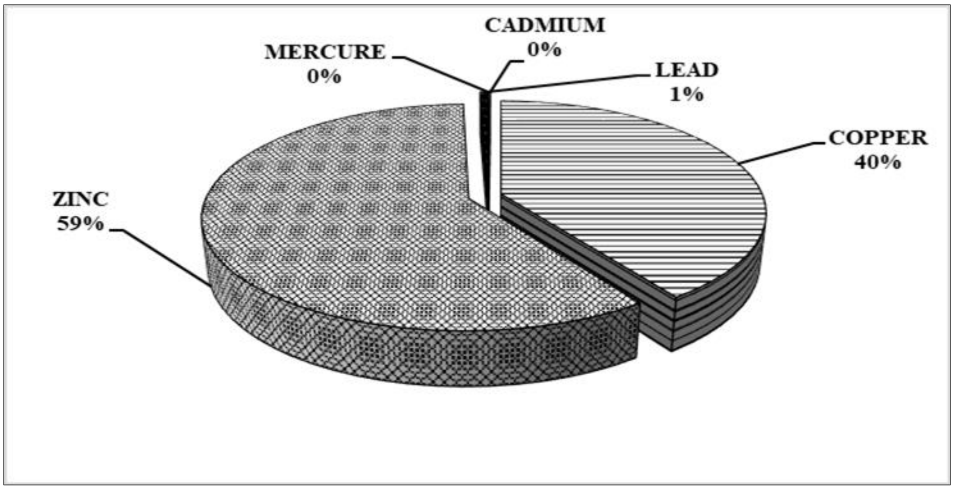

Figure 3 Quantitative distribution of heavy metals in the different stations during the study period

3.3. Evaluation of the seasonal variation of germs

3.3.1. Qualitative aspect

Macroscopic examination of bacterial colonies isolated on TCBS medium for bacteria of the genus *Vibrio* showed several aspects of the colonies, among which three presented the desired cultural characteristics: light yellow colonies with diameters varying from 2 to 3mm green colonies of medium size of diameters 2 to 3mm and the dark yellow colonies of large sizes of diameters varying from 3 to 5mm. The appearance of bacterial colonies isolated on SS medium for bacteria of the genus *Salmonella* and presenting satisfactory cultural characteristics after macroscopic examination are as follows: black colonies with diameters varying from 2 to 3mm, mauve colonies with diameters varying from 2 to 3mm and black colonies with diameters varying from 3 to 5mm.

3.3.2. Biochemical tests for the identification of microorganisms

The results of the enzymatic tests for identifying the isolated bacteria are presented in Table 2. *S. enterica* is the only species belonging to the genus *Salmonella* that has been identified. With regard to bacteria of the genus *Vibrio,* three species have been identified. These are *V. cholerae, V. parahaemolyticus* and *V. alginoliticus.* All the identified species do not produce H_2S, Urease and do not reduce lactose. *V. Cholerae* is indole positive catalase positive. The presence of catalase testifies that the isolated bacteria can live in the presence of oxygen dissolved in water.

Table 2 Identification tests carried out on the isolated bacterial strains and results

Tests carried out	**Bacterial strains**					
	A	B	C	D	E	F
Gram stain	-	-	-	-	-	-
Catalase	+	+	+	+	+	+
Oxidase	+	-	+	+	+	+
ONPG	-	-	+	+	+	-
Mannitol	+	+	+	+	+	+
Citrate	+	+	+	+	+	+
Mobility	+	+	+	+	+	+
Lactose	-	-	-	-	-	-
Glucose	+	+	+	+	+	+
H2S _ _	-	-	-	-	-	-
gas production	+	+	+	+	+	+
Urease	-	-	-	-	-	-
ADD	-	-	-	-	-	-
Indole	-	-	-	+	+	+
Species	*S. enterica*	*S. enterica*	*S. enterica*	*V. cholerae*	*V. parahaemolyticus*	*V.alginoliticus*

Legend : negative feedback ; (+): positive feedback ; test not performed : (/);(ONPG: orthonitrophenyl β -D - galacto - pyranoside ; ADH: arginine - dihydrolase ; LDC: lysine - de carboxylase; ODC: ornithine -decarboxylase ; H 2 S: hydrogen sulphide ; ADD: tryptophan deaminase (D: small rounded yellow colonies, bulging (2 to 3 mm); E: large rounded yellow colonies, bulging (3 to 5 mm); F: medium sized green colonies (2 to 3 mm);A: colonies of mauve appearance with black center of diameter varying from 2 to 3 mm, B: large and black colonies of diameter varying from 3 to 5mm, C: small colonies size, black in diameter varying from (2 to 3 mm)

3.4. Quantitative aspect

Overall, concentrations of bacteria of the genera *Vibrio* and *Salmonella* varied from station to station and within each season encompassing sampling months. In general, the concentrations of bacteria of the genus *Vibrio* varied between 4 and 3.9.10 2 CFU/100mL of water sample throughout the study period, the minimum concentration was obtained at station N12 during the PSP. While the maximum concentration was obtained at station N3 during the PSS (Figure 4A). With regard to bacteria of the genus *Salmonella,* the bacterial abundances varied from zero (0) thus representing the minimum value at station N1 during the PSS to reach a maximum concentration of 7.32.10 3 CFU/100mL at station N2 during the PSP (Figure 4B). Of the germs studied, bacteria of the genus *Salmonella* were the most abundant in terms of the number of colonies counted during the sampling period, thus encompassing the different seasons.

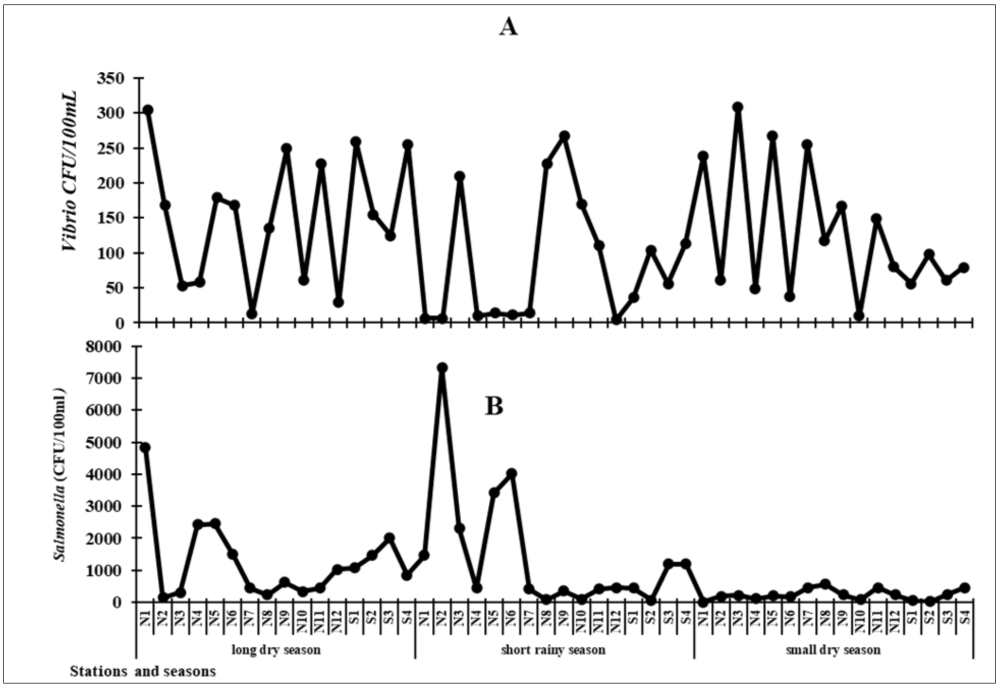

Figure 4 Spatio-temporal variation in the concentrations of *Vibrio* , *Salmonella* . A : *Vibrio* ; B : *Salmonella*.

The seasonal variations of bacteria of the *Vibrio* and *Salmonella genera* show that bacteria of the *Vibrio genera* are more present in water points during the short dry season, which would be due to the absence of leaching and runoff phenomena which would thus promote the concentration of these microorganisms in the given medium thus facilitating their proliferation. With regard to bacteria of the genus Salmonella, the highest abundances are observed during the short rainy season, which could be explained by the phenomena of leaching and runoff which will thus transport these microorganisms to the level of the points of 'water

3.5. *Vibrio* diversity identified during the study period

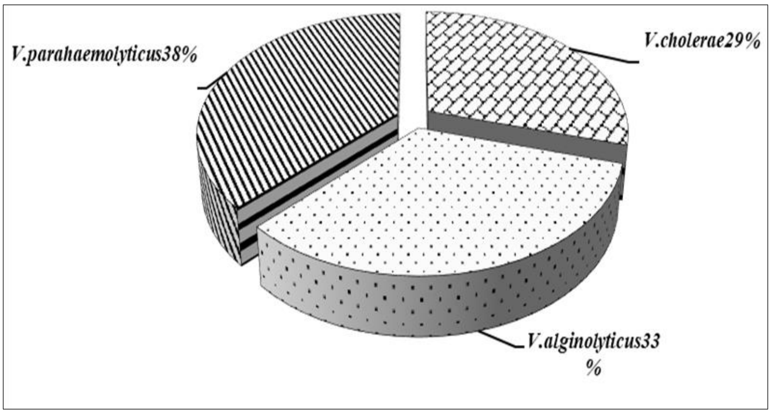

Figure 5 Diversity of identified *Vibrio* species

During the study period, 3 species of *Vibrio* were identified at all the sampling stations. Among the species identified, the most represented species was *V. parahaemolyticus* (38%) followed by *V. alginolyticus* (33%) and *V. cholerae* with a relative abundance of (29%), (Figure 5).

3.6. Seasonal variation in the concentrations of species belonging to the *Vibrio* and *Salmonella genera* thus identified during the study

Spearman's "r" correlation tests between heavy metals and the concentrations of isolated bacterial species show that there are very significant (P < 0.01) and positive correlations between the concentrations of the bacterial species V. cholerae *and* the heavy metals such as copper (r=0.369), Zinc (r=0.479) and Mercury (r=0.436). Between *V. parahaemolyticus* and heavy metals such as copper (r=0.449), lead (r=0.412) and zinc (r=0.392) (Table 3). A significant (P < 0.05) and positive correlation was observed between the concentrations of *V. parahaemolyticus* and Mercury (r=0.337) (Table 3).

Table 3 Correlations between bacteriological variables and heavy metals

	Cadmium	Copper	Zinc	Lead	Mercury
S. enterica	0.117	0.144	-0.064	-0.019	0.025
V. cholerae	0.235	**0.369** **	**0.479** **	0.099	**0.436** **
V. alginolyticus	0.096	0.227	0.045	0.231	0.190
V. parahaemolyticus	0.217	**0.449** **	**0.392** **	**0.412** **	**0.337** *

Legend: ***:** P < 0.05 ****:** P < 0.01 **P** = degree of significance **dof** = 48

3.7. Affinities between biotic and abiotic parameters

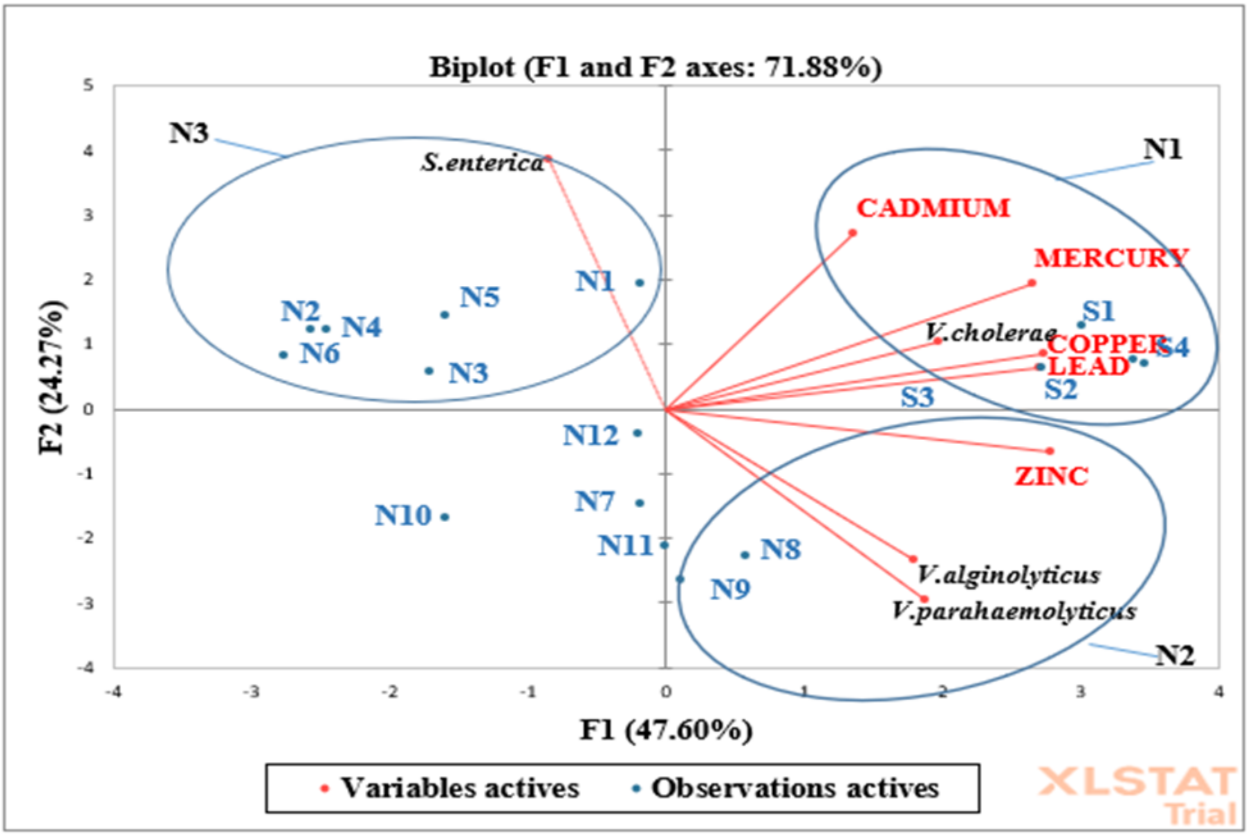

Figure 6 PCA values grouping the affinities between bacterial abundances, stations and heavy metals

Principal component analysis (PCA) applied to the various biological variables and heavy metals shows a grouping of the parameters into three nuclei (Figure 7). Nucleus 1 (N1) includes the stations, S1, S2, S3 and S4 in which *Vibrio cholerae* maintain strong affinities with heavy metals such as Cadmium, Mercury, Lead and Copper. In the nucleus (N2) containing the N9 and N8 stations, a strong affinity is observed between Zinc and bacterial species such as *V. alginolyticus* and *V. parahaemolyticus.* Regarding the nucleus (N3), it includes the stations N1, N2, N3, N4 and N6 and bacteria of the genus *Salmonella* there is however no affinity between heavy metals and the species belonging to this genus *Salmonella.*

3.8. Spatio-temporal comparisons between the concentrations of heavy metals and the diversities of bacterial species

Kruskal Wallis H comparison tests between the heavy metal contents according to the seasons and the sampling stations as well as the bacterial densities show that during the study period, among the heavy metals only the Zinc concentrations varied significantly. ($p<0.05$) between sampling seasons. These same results were also obtained for the abundances of the bacterial species *Salmonella enterica.*

In order to know precisely between which seasons these concentrations varied, the two-by-two Mann-Whitney comparison test was carried out. From the latter, it appears that these differences in zinc concentration are significant ($p < 0.05$) between GSS and PSS. The same is true for the abundances of *Salmonella bacterial species. enterica* between the short dry season and the short rainy season (Table 4).

Table 4 P values indicating the significance thresholds relating to the Mann-Whitney 2 to 2 comparison test between the bacterial abundances and the different sampling seasons**.**

Variations of the seasons	**Zinc**	***S. enterica***
Long dry season - Small dry season	0.025 *	0.043 *
Long dry season - Small rainy season	0.219	0.912
small rainy season - Long dry season	0.400	1.00

Legend: *****: significant at the threshold P < 0.05

3.9. Comparisons of variation in heavy metal levels and bacterial densities between sampling seasons

Kruskal Wallis H test, which compares the variations in heavy metal concentrations between the sampling stations during the study period, reveals significant differences ($p < 0.05$) in the concentrations of Cadmium and Mercury (Table 5). The same test applied to the abundances of diversity of bacterial species also shows significant differences ($p < 0.05$) in the abundances of *V. parahaemolyticus* between the sampling stations (Table 5)

Table 5 P values indicating the significance thresholds relating to the Mann-Whitney 2 to 2 comparison test between the bacterial abundances and the various sampling stations.

Stations	**Cadmium**	**Mercury**	***V. parahaemolyticus***
N1 - N6	0.458	0.982	0.049 *
N1 - N8	0.56	0.654	0.044 *
N2 - N4	0.459	0.876	0.048 *
N2-N12	0.857	0.654	0.027 *
N2 - N6	0.557	0.657	0.048 *
N3 - N8	0.455	0.765	0.016 *
N4 - S1	0.004 *	0.021 *	0.864
N4-N9	0.012 *	0.028 *	0.654
N5 - N10	0.857	0.457	0.674
N5 - N11	0.451	0.028 *	0.765

N5 - N12	0.569	0.009 *	0.643
N5 - S1	0.675	0.021 *	0.654
N6 - N12	0.014 *	0.345 *	0.432
N6 - S1	0.004 *	0.432	0.654
N6 - N10	0.006 *	0.632	0.762
N6 - N11	0.005 *	0.453	0.543

Legend: *: significant at the P < 0.05 threshold

4. Discussion

4.1. Abiotic parameters (heavy metals)

In general, heavy metals such as Mercury were rare in the waters of many stations (N2 to N12). This could be explained by the absence of industries in the areas from which these waters are abstracted. However, it is accepted that metallic pollution of surface waters is essentially of anthropogenic origin and that the flow of pollutant discharged per inhabitant and per day increases with urbanization, industrialization and population density [10]. Contrary results were obtained by [19] in the peri-urban watercourses of Douala (Cameroon coastal region). Indeed, this region constitutes the industrial zone of Cameroon. The presence of these industries in this region would thus promote the accumulation of these heavy metals in the waterways.

Heavy metal levels are generally higher in surface water compared to groundwater. This metal pollution would be due to the various and permanent contributions of wastewater from households, leaching by rainwater and runoff from garbage dumps, scrap metal dumps, hardware warehouses, automobile garages, and agricultural activities including the use of inputs among others. On this subject, [20,21] point out that household, municipal and industrial wastewater are the main sources of metal pollution in rivers. In addition, cadmium chlorides and sulphates, as well as zinc and copper chlorides, sulphates, sulphides and oxides are very soluble compounds widely used in industry (metallic, agriculture) and therefore liable to form found in aquatic environments [22, 23] In general, the levels of these heavy metals in the waters of the stations are higher than the standards established by the WHO, except for certain groundwater stations during certain seasons, this is the case for stations N7 and N7 for Cadmium , N4, N5, N6 for Zinc and N4 for copper.

4.2. Microbiological parameters

Bacteria of the *Salmonella genus* were present in all stations throughout the study period and dominate in terms of abundance compared to bacteria of the *Vibrio genus* thus identified. Indeed, the high abundance could be due to the fact that in the environment of these stations there are bacterial growth factors. Furthermore, the high bacterial load of these bacteria could also be due to contaminated runoff and seepage. In fact, according to [24], this factor favors the contamination of surface and underground waters, carrying bacteria along with them. However, this contamination depends on the pollution load of the contaminant and the permeability of the overlying soil. This result is similar to that obtained by [25], in groundwater and surface water in the city of Yaoundé.

Moreover, the high concentration and the permanent presence of *Salmonella* throughout the study and *Vibrio* particularly during the dry seasons, provides information on the degree of pollution of these waters and would confirm the idea of the permanent reappearance of epidemics and their monitoring [25],The finding that bacteria of the genus *Vibrio* were most often identified during dry seasons could be due to the fact that according to [10]; water in the dry season is rarely renewed, which weakens the rate of natural purification of watercourses. The content of these waters in bacteria of the *Vibrio* and *Salmonella* genera is higher than the maximum allowable concentrations for these microorganisms in drinking water. 0UFC/5L of water according to the [26], therefore they all require adequate treatment. before any use. The presence permanent of these bacteria pathogens as well as their high concentration reflect the degree of pollution of these waters. Results _ similar have summer obtained by [27] in Nkolafamba. These results have revealed the presence of several pathogens such as *Escherichia coli, Salmonella, Shigella* among others, witnesses of faecal contamination. Similar results were also obtained by [25], in Yaoundé and by [27] in Soa, who have analyzed drinking water and bacteria isolated witnesses of faecal contamination and opportunistic bacteria such as *E. coli, Vibrio, Klebsiella, Salmonella, Shigella* and *Pseudomonas.*

From the microbial flora studied, 1 species of the genus *Salmonella,* and 3 species of the genus *Vibrio* human pathogens were identified. These are *S. enterica* for the genus *Salmonella* and *V. cholerae, V. alginolyticus, V. parahaemolyticus* for the genus *Vibrio.* The results obtained are similar to those of [29], who worked in the surface waters of the city of Ntui and those of [30] in Douala, having analyzed drinking water, and isolated the pathogenic bacteria responsible for public health problems.

Their presence in groundwater would be due to the infiltration of contaminated water into the water table or linked to human activities by local populations. In addition, the infiltration rate depends on rainfall, grain size and porosity of the soil as well as the adsorption of bacteria in the solid matrix [31]. This could also be explained by the presence of landfills, latrines and fields located near the sampling stations [29]. The species of *V. alginolyticus* and *V. parahaemolyticus* are the least isolated from the genus *Vibrio* and present the lowest abundances in all the stations, which could be explained by the fact that these species, being halophiles, prefer marine environments. and coastal ones presenting as particularity their strong salinity to the detriment of fresh waters. This observation corroborates with that of [30], in the coastal zone in Cameroon where he concludes that *V. alginolyticus* does not develop in practically fresh waters but that it is brought by marine waters during the tides.

4.3. Affinity between variables during the study period

The results of the correlations between biological variables and heavy metals show that, of the heavy metals thus found in the waters analyzed, four metals (Copper, Zinc, Mercury and Lead) seem to significantly influence the population and distribution of bacteria throughout the process study. The increase in the levels of Copper, Zinc and Mercury in the water seems to significantly increase the density of the bacterial species *V. cholerae* and *V. parahaemolyticus* with regard to Lead the increase in its concentration seems to significantly increase the densities of the species bacteria *V. parahaemolyticus.* These results are contrary to those obtained by [32] who explain them by the fact that globally, heavy metals exert a selective pressure increasing the basic tolerance of certain microorganisms, but decrease their biodiversity [32]. However, the increase in bacterial diversities could be explained by the fact that these heavy metals combined with the organic matter present in these waters would constitute for these species a source of nutrients present in the water, thus promoting their proliferation in the environment. In addition, bacteria would develop resistance mechanisms to heavy metals to do so, they use, for example, active transport mechanisms to export toxic metals from their cytoplasm to the extracellular environment, thus promoting their growth in a given environment. [8].

As for the species of genus *Salmonella* heavy metals did not significantly influence their distribution during the study period. This would result in the fact that bacteria react differently to chemical compounds [8]. Indeed, the influence of chemical compounds on the microflora of the soil and subsoil and water 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 source of energy, which are necessary for its biosynthesis [33].

Regarding of the results of the correlations between the concentration of the different heavy metals thus identified in these station waters, they can be explained by the fact that the heavy metals would come from the dissolution of the rocks encountered by the water during its passage. These rocks would be made up of minerals and oxides containing Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+} and Pb^{2+} ions thus, the simultaneous dissolution of these rocks would lead to a simultaneous increase in the concentration of water in these oxides which would then be at the origin of the links thus observed between the different metals.

5. Conclusion

The objective of this work is to determine the levels of heavy metals and assess the impact of these metals on bacterial diversity in a few water points in an agricultural activity zone in Mbam (Central Cameroon). It emerges from this work that the analyzes of heavy metals have been made, which has shown that the groundwater and surface waters analyzed contain heavy metals such as Cadmium, Lead, Copper, Zinc and Mercury in high proportions. compared to WHO standards, thus reflecting average pollution. This result testifies to the considerable degradation of the physicochemical quality of these waters, thus presenting a considerable health risk, such as irreversible damage to the kidneys and liver that can affect the user populations. The levels of these heavy metals significantly influenced the distribution of bacteria. Bacteriological analyzes revealed the presence of pathogenic germs *S. enterica, V. cholerae, V. alginolyticus,* and *V. parahaemolyticus* at relatively high proportions and not recommended for drinking water before any prior treatment. These analyzes also revealed that the occurrence and abundance of these germs vary in time and space under the influence of heavy metal levels. According to WHO standards, the waters of certain points are not advisable for human consumption without any prior treatment.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no potential conflict of interest regarding the publication of this work. In addition, the ethical issues including plagiarism, informed consent, misconduct, data fabrication and, or falsification, double publication and, or submission, and redundancy have been completely witnessed by the authors.

Author contributions

Olive Vivien Noah Ewoti, Samuel Davy Baleng conceptualized, analyzed the data and prepared the manuscript. Serge Ronny Ott Song, Ulrich Kolkossok Badouana, Yves Yogne Poutoum, Pelagie Ladibé, Rodrigue Mboene Mboene, Stephane Arthur Noah and Morelle Raisa Djiala Tagne helped in collecting data, in analyzing and interpreting. The was supervised by Moïse Nola. All authors have read, agreed and approved the final manuscript.

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