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Physicochemical characterization, dynamics abundance bacteriological and aquatic fauna of groundwater in the City of Mbalmayo (Cameroon)

Tuekam Kayo Raoul Polycarpe*[1], **Nyamsi Tchatcho Nectaire Lié**[2],
Noah Ewoti Vivien Olive[3], **Chinche Sylvie Belengfe**[3], **Nziéleu Tchapgnouo Joseph Guy**[4],
Pountougnigni Oumarou Farikou[3], **Zébazé Togouet Serge Hubert**[3]

[1] *Department of Biological Science, Faculty of Science, University of Bamenda, Bambili, Cameroun*
[2] *Département de Gestion des Écosystèmes Aquatiques, Institut des Sciences Halieutiques de l'Université de Douala à Yabassi, Douala, Cameroun*
[3] *Département de Biologie et Physiologie Animales, Faculté des Sciences, Université de Yaoundé 1, Yaoundé, Cameroun*
[4] *École Nationale Supérieure Polytechnique, Département d'Hydraulique et Maîtrise des Eaux, Université de Maroua, Maroua, Cameroun*

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Abstract

In order to understand the potential impact of abiotic factors on the dynamics of bacterial abundance and diversity of the groundwater fauna in Cameroon, a study was carried out in 10 wells in the town of Mbalmayo. In each well, twelve samples were taken at monthly intervals. Analyses were carried out according to standard protocols. The results of the abiotic parameters show significant seasonal variations from one station to another. The principal component analysis (PCA) based on the mean values of the physicochemical and microbiological parameters groups the stations studied into 3 categories according to the chemical facies of the water. The first category includes wells with water of good physicochemical and microbiological quality, characterized in particular by low mineralization, low levels of nitrogen and phosphorus ions indicative of pollution, and concentrations of coliforms and streptococci below the WHO acceptable value for drinking water. From a faunistic point of view, the species richness is relatively high and is dominated by arthropods. It remains generally poorly correlated with water quality, but the species richness of the stygobite fauna, and even more so the abundance of stygobite species, decreases when water quality deteriorates. The Stygobite fauna, here the peracarid crustaceans, appear to be good indicators of groundwater quality.

***Corresponding Author:** Tuekam Kayo Raoul Polycarpe ✉ tuekamkayo@yahoo.fr

Introduction

One of the major concerns of ODD 6 (Sustainable Development Goals 6), is the supply of water, of which drinking water supply is still difficult for developing countries. On a global scale, the situation remains catastrophic for hundreds of millions of human beings as 1.3 to 1.5 billion people live without access to safe drinking water (WHO/Unicef, 2012). In Cameroon, this insufficiency forces a large part of the population to resort to groundwater without concern for its quality (Nola *et al.*, 1998; Zébazé Togouet, 2009; 2011). This water is an exhaustible resource that is vulnerable to pollution (Boutin, 1993). Moreover, the underground environment is one of the largest habitats on the planet, due to its extent and diversity, and offers considerable opportunities for animals to colonize it (Juberthie, 1995). This takes place where the physical and chemical conditions are acceptable for the development of organized life (Creuze Des Châteliers & Dole-Olivier, 1991).

Studies on underground aquatic environments carried out in several African and European countries provided data that can be used to assess water quality. They show that the underground aquatic fauna contains stygobite species which are generally much more sensitive than other aquatic species, especially epigobytic species, to global water pollution (Merzoug *et al.*, 2011, Di Lorenzo *et al.*, 2014, Marmonier *et al.*, 2018). Despite the work carried out in Cameroon on aquatic subterranean fauna (Nana Nkengmeni *et al.*, 2015; Chinche *et al.*, 2019), no studies associating physicochemical and microbiological quality with aquatic subterranean fauna have yet been carried out. The aim of this work is to contribute to the knowledge of the physicochemical, microbiological and faunal quality of groundwater in Cameroon and to assess the effect of abiotic factors on the organization of underground aquatic organisms.

Material and method

Presentation of the study area and choice of sampling sites

This study was carried out in the city of Mbalmayo, Nyong and So'o department, located in the Central region. It is located in the Nyong watershed, on a plateau with an average altitude of 750 m. It extends between 3°15'01" and 3°35'25" North latitude, 11°10'05" and 11°50'12" East longitude (INC, 1980). The soils are derived from a more or less micaceous quartz-feldspar material. There are three types of soils: ferralitic soils, hydromorphic soils, and poorly evolved soils (Sighomnu, 2004). Ferralitic soils have two variants: red ferralitic soils and yellow ferralitic soils. Red ferralitic soils, located on the tops of the interfluves, are very thick (over 15 m), clayey and acidic ($pH < 5.5$). Their main constituent minerals are: kaolinite, hematite, goethite, quartz and gibbsite (Yongué Fouateu, 1986). The town of Mbalmayo is subject to a particular equatorial climate known as the "Yaoundean climate" with four seasons of unequal distribution (Suchel, 1972). For this study, 10 wells were selected (referred to as PB1 to PB10) (Fig. 1.). The stations were chosen according to their accessibility, the interest shown by the population in these water points and the concern for a high geographical representativeness.

Sampling protocols

Twelve samples were monthly taken from June 2009 to May 2010 at each station. Sampling for physico-chemical analyses of the water was carried out using a 5 L bucket before wildlife harvesting and then transported in 1 L double-capped polyethylene bottles. Samples for microbiological analyses were stored in 250 mL sterile glass vials. Both types of samples were returned to the laboratory in a refrigerated insulated cabinet. The fauna was then harvested by making a minimum of 10 round trips in the water mass using a phreatobiological net, of the Cvetkov type modified by Mittelberg and Boutin (1984), and by installing a trap of the trap type, for a period of 10 to 18 h, in which a red meat bait was introduced, following the recommendations of Boutin and Boulanouar (1983). Samples containing the animal organisms were directly fixed on sitewith 96° alcohol before being brought back to the laboratory for extraction, enumeration and identification of the fauna with a stereomicroscope. The temperature, pH, electrical conductivity and dissolved oxygen content of the water were measured in the field using a mercury thermometer graduated to 1/10 of °C and portable electrical devices, Schöt-Gerate portable pH meter, HACH Conductivity meter and WTW Oxi 340 Oximeter (Anonyme, 1985) respectively. The Suspended Solids were measured in the laboratory by spectrophotometry using a HACH DR-EL 2010 spectrophotometer. Calcium hardness was measured in the laboratory by volumetry. The concentrations of ammonium, nitrate, nitrite, and orthophosphate ions were obtained by spectrophotometry with Nessler, Nitraver V, Nitriver III and Phosver III respectively. BOD was evaluated by respirometry in a suitable incubator at a temperature of 20°C (Rodier *et al.*, 2009). As for the microbiological analysis, the methods used were those recommended by AFNOR standard methods. In the laboratory, samples containing fauna were examined in successive fractions in Petri dishes to sort and extract animal organisms under a Wild M5 stereomicroscope equipped with an episcopic illumination. Taxonomic identifications were made using specific identification keys (Magniez, 1976, 1999; Tachet *et al.*, 2010; Zébaze Togouet *et al.*, 2013).

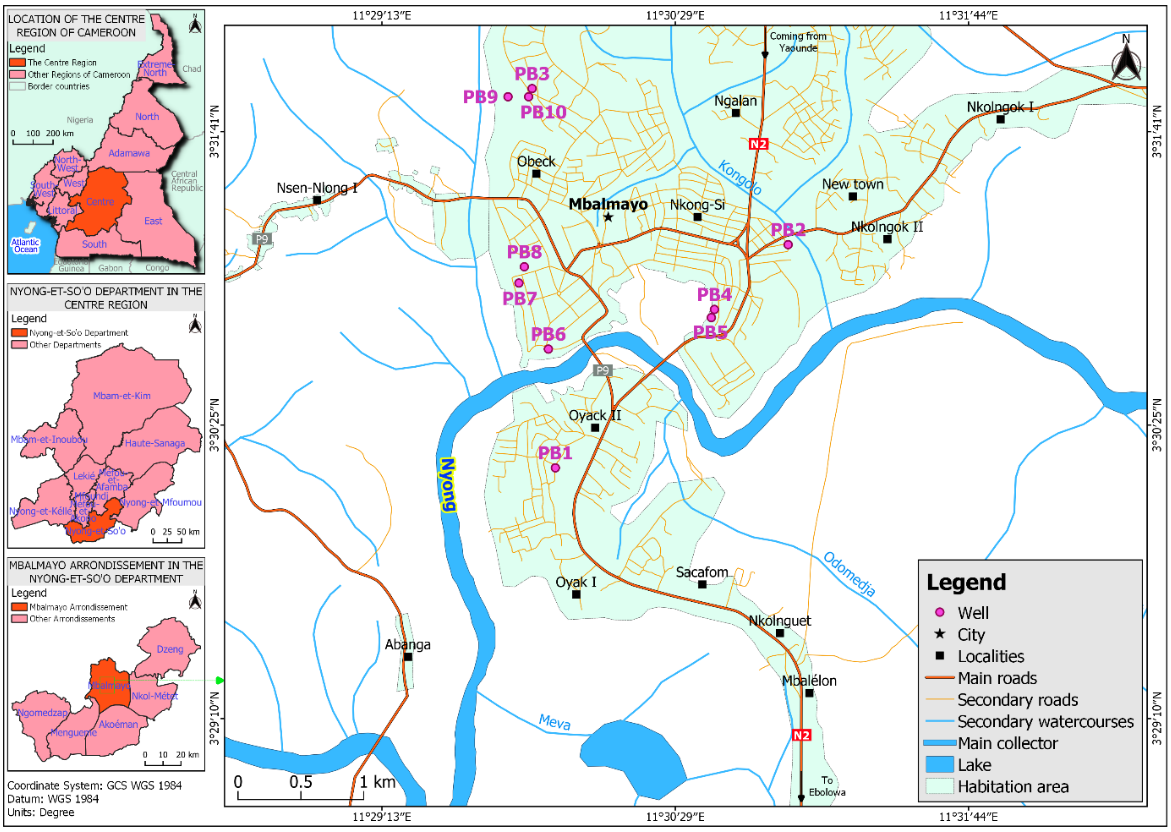

Fig. 1. Position of the wells studied in the different districts of the city of Mbalmayo (INC, 1980 modified).

Data processing

Spatial and temporal comparisons of the seasonal mean values of the different abiotic and microbiological variables obtained during the study were tested using one way ANOVA. The Principal Component Analysis (PCA) was carried out on the basis of all the annual mean values of the physico-chemical data in order to determine the classification of the sampled stations. The taxonomic richness which expresses the number of taxa present in a biotope was considered. The relative abundance (Nr) and frequency (F) measuring the regularity of each taxon was calculated at each station. This made it possible to obtain a hierarchical classification of certain harvested morphotypes. Data processing was carried out using SPSS 16.0 for Windows.

Results

Physico-chemical quality of the groundwater studied

Variations in the seasonal mean values of temperature and SS (Suspended Solids) were generally very small from well to well.

Seasonal temperature values ranged from 25 to 27.7°C (Fig. 2A.). Suspended Solids values ranged from 2mg/L to 94mg/L (Fig. 2B.). Significant differences were observed between the mean SS values during the short dry season (P = 0.003) and the long dry season (P = 0.001). Seasonal mean values for pH, alkalinity and calcium hardness remained relatively low in the groundwater sampled. The pH varied between 3.2 and 5.9 CU (Fig. 3A.). Seasonal mean alkalinity values fluctuated between 0.6 and 107.3mg/L (Fig. 3B.). Calcium hardness values ranged from 3 to 38.8mg/L (Fig. 3C.). Significant differences were obtained between the mean pH values during the short wet season, the short dry season and the long wet season at different stations (P = 0.037; P = 0.001).

As for alkalinity, it was significantly different between the different stations during the short dry season (P = 0.017), the long dry season and the long rainy season (P = 0.001). Calcium hardness was significantly different between stations during the short dry season (P = 0.003). Seasonal mean values of electrical conductivity were relatively highly variable among stations. They ranged from 39.9 to 705.5 µS/cm. Dissolved oxygen saturation percentages fluctuated between 34.1 and 91.2% (Fig. 4B.). There were significant differences between wells in mean electrical conductivity values during the short wet season, the long wet season and the long dry season (P = 0.001). Seasonal mean levels of the nitrogen forms were relatively variable from well to well. Seasonal mean values for nitrite ranged from 0.004 to 0.171mg/L NO_2^- (Fig. 5A.).

The highest value, 0.171mg/L NO_2^-, was recorded during the short dry season. Nitrates fluctuated between 0.09mg/L and 17.73mg/L of NO_3^- (Fig. 5B.). Seasonal mean values for ammonia nitrogen ranged from 0.07 to 24.88mg/L NH_4^+ (Fig. 5C.), with significant differences between the short wet season and the long dry season (P = 0.001). Seasonal mean values for Orthophosphates and BOD were relatively variable. The concentrations for orthophosphate ranged from 0.09 to 5.9mg/L PO_4^{3-} (Fig. 6A.). Those of BOD fluctuated between 38.7 and 342mg/L (Fig. 6B.) of oxygen with significant differences obtained during the short rainy season (P = 0.003).

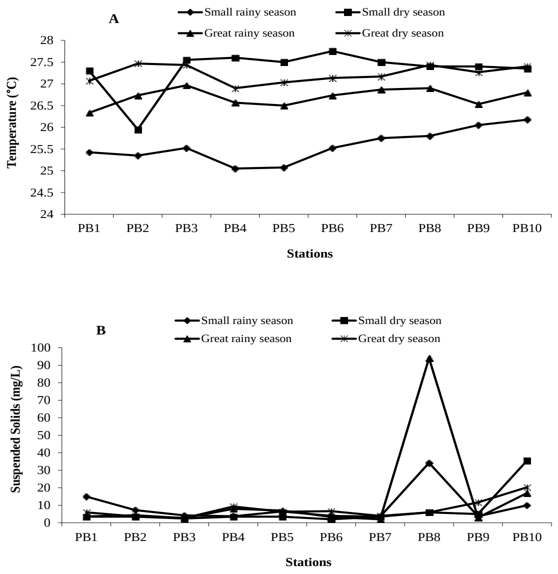

Fig. 2. Spatial variation of seasonal mean values of temperature (A) and Suspended Solids (B) in the groundwater studied in the city of Mbalmayo.

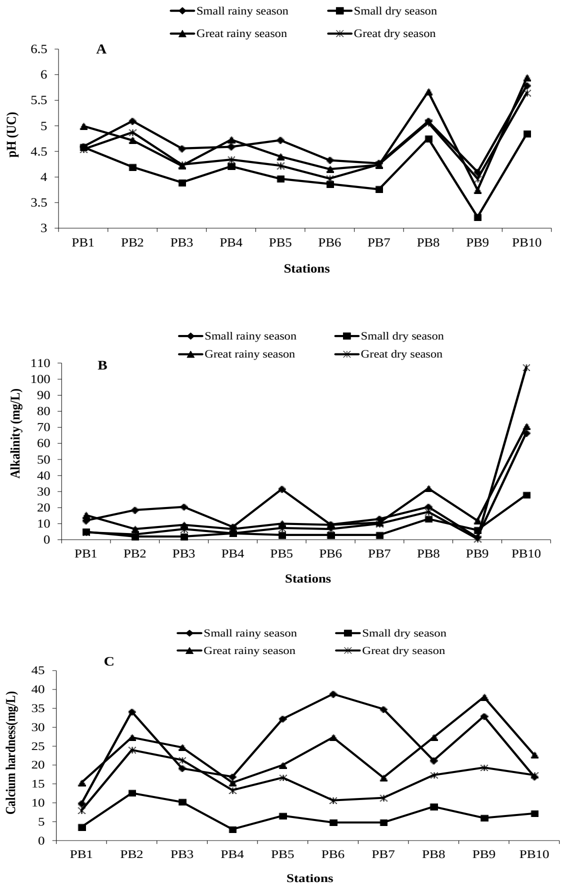

Fig. 3. Spatial variation of seasonal mean values of pH (A), Alkalinity (B) and Calcium hardness (C) in the groundwater prospected in the city of Mbalmayo during the study.

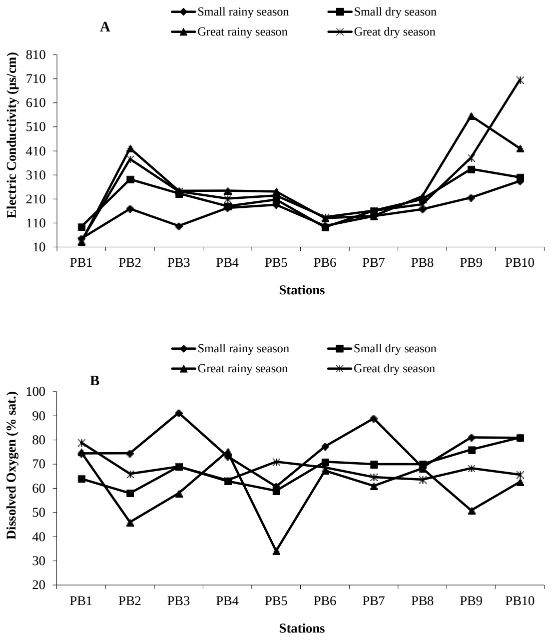

Fig. 4. Spatial variation of seasonal mean values of electrical conductivity (A) and dissolved oxygen (B) in the city of Mbalmayo during the study.

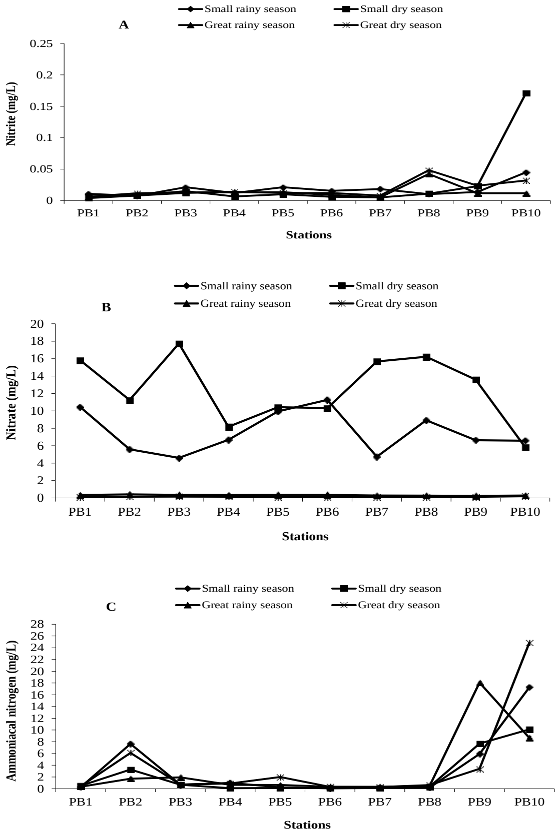

Fig. 5. Spatial variation of seasonal mean values of nitrite (A), nitrate (B) and ammoniacal nitrogen (C) in the groundwater prospected in the city of Mbalmayo during the study.

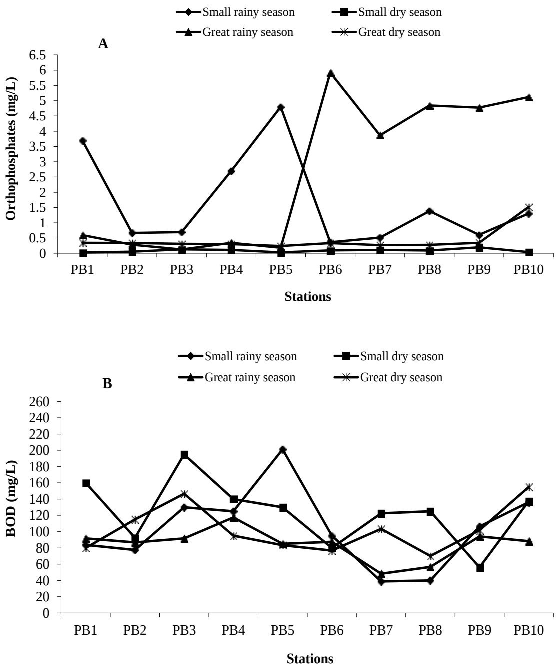

Fig. 6. Spatial variation in seasonal mean values of orthophosphates (A) and BOD (B) in the wells studied.

A principal component analysis was performed on the data matrix containing 10 variables and 12 elements. Only the two first principal components (PC1 and 2) that explain more than 65% of the total distribution were retained (Fig. 7A.). PC1, which explains 42.1% of the total variance, is strongly and positively correlated with pH, calcium hardness and negatively correlated with nitrate.

PC2 which expresses 22.9% correlates positively with calcium hardness and BOD, and negatively with temperature, alkalinity and dissolved oxygen. The projection of the stations on the factorial plan made on the basis of the physicochemical data made it possible to group the studied wells into 3 groups. Group I is composed of stations PB1, PB6 and PB7 with moderately oxygenated waters, characterized by low chemical and organic pollution. Group II is composed of the stations PB2, PB9 and PB10 with well-oxygenated, highly mineralized and alkaline waters. Group III, composed of the stations PB3, PB4, PB5 and PB8, therefore the waters are classified as poor quality, characterized by high concentrations of nitrate ion, ammonia nitrogen, and high SS values (Fig. 7B.).

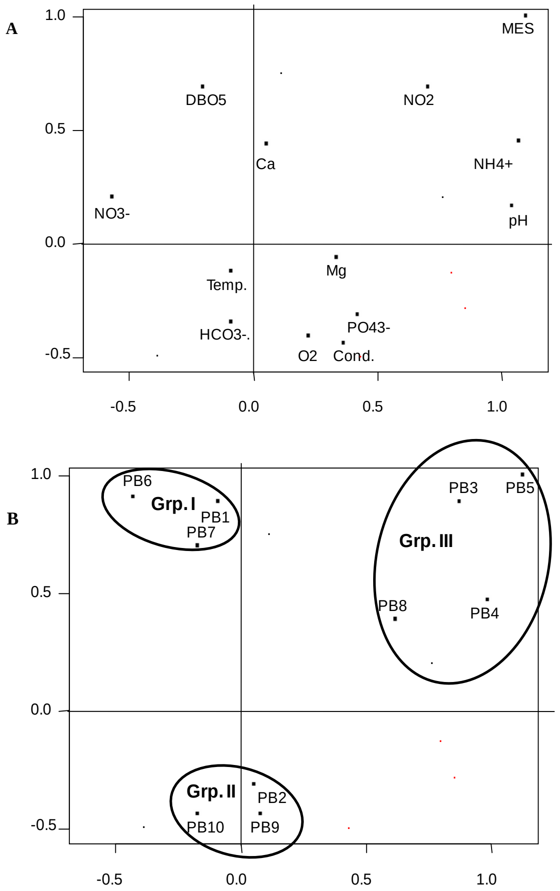

Fig. 7. Projection of the mean values of the physicochemical parameters of water (A) and stations (B) on the plane of the first two axes of the principal component analysis of the wells studied in the city of Mbalmayo (Grp. Group; Cond: Electric conductivity; O_2: Dissolved oxygen; NO_3^-: Nitrate; NO_2^-: Nitrite; NH_4^+: Ammoniacal nitrogen; PO_4^-: Orthophosphate; HCO_3: Alkalinity; Ca: Calcium hardness; MES: Suspended Solids; BOD5: Biochemical Oxygen Demand; pH: Hydrogen potential; Temp: Temperature).

Wells diversity

Bacterial Abundance

Overall, the seasonal mean concentrations of fecal coliforms and fecal streptococci were variable in the groundwater of Mbalmayo. Fecal coliform concentrations ranged from 12 to 148 CFU/100 mL (Fig. 8.A), and fecal streptococci concentrations ranged from 3 to 232CFU/100mL (Fig. 8.B). However, fecal coliform and fecal streptococcal concentrations remained below 20CFU/100mL at stations PB1, PB6 and PB7 during all seasons. Significant differences were obtained during the short and long dry season (P= 0.045; P= 0.031) and the short wet season (P= 0.021), respectively.

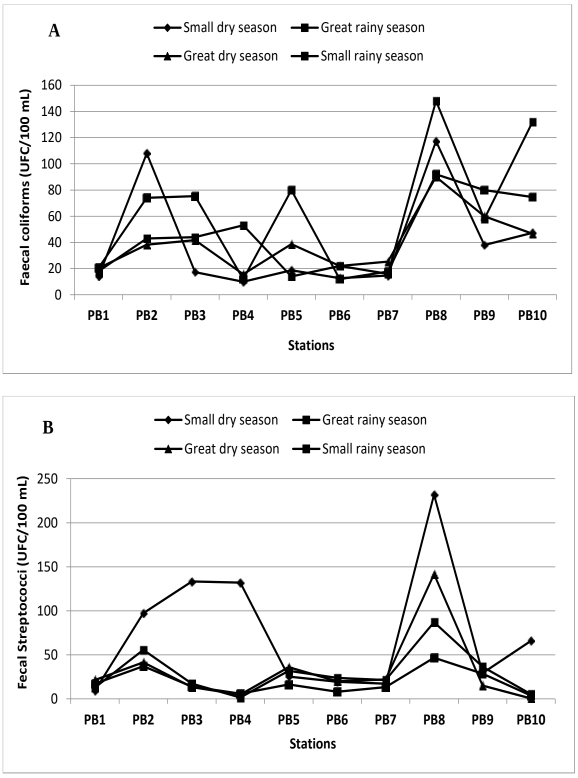

Fig. 8. Variation in mean seasonal abundance of fecal coliforms (A) and fecal streptococci (B) over the course of the study.

Faunal inventory

A total of 80 morphotypes belonging to 5 branches were harvested (Table 1). These were Arthropods (58.88%), Annelids (40.66%), Nemathelminths (0.08%), Plathelminths (0.05%) and Molluscs (0.03%). These taxa are divided into 6 classes, 16 orders, 46 families and more than 71 genera. The Hexapoda class predominates with 34.89% of the total abundance (Fig. 9.) with 9 orders, 24 families and 30 genus; it is followed by the Crustacea class (23.99%) with 4 orders, 9 families and 22 genus.

Of the 46 families collected, the Naididae class is the most represented with 7 genus: *Dero*, *Chaetogaster*, *Nais*, *Amplichaeta*, *Stylaria*, *Pristina* and *Aulophorus*. It is followed by the family Cyclopidae represented by 6 genus: *Tropocyclops*, *Ectocyclops*, *Mesocyclops*, *Afrocyclops*, *Allocyclops* and *Thermocyclops*.

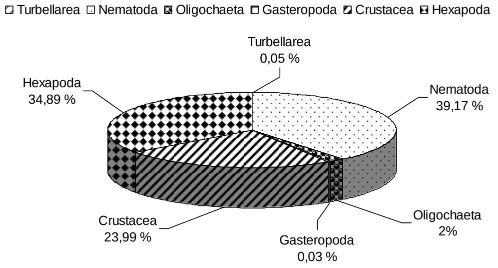

Fig. 9. Distribution of the relative abundances of the different classes of morphotypes collected in the groundwater of Mbalmayo.

Groundwater Richness

The taxonomic richness varied from 13 to 26 morphotypes. The poorest station was PB1 with 13 morphotypes, the richest was PB7 with 26 morphotypes harvested.

In the set of phreatobial species that constitute the real representatives of the water table, the number of morphotypes was two, present respectively in stations PB1, PB3, PB4, PB6 and PB7 (Table 1).

Table 1. Faunal list of taxa collected in the groundwater of the city of Mbalmayo.

			Stations									
Class	Family	Genus or Species	PB1	PB2	PB3	PB4	PB5	PB6	PB7	PB8	PB9	PB10
Turbellarea	Dugesiidae	*Dugesia* sp.		*	*							
	Dendrocoelidae	*Bdellocephala* sp.						*				
Nematoda	Merthidae	*Nematode* sp.										*
	Gordiacae	*Gordus* sp.		*								
Oligochaeta	Naididae	*Dero* sp.	*	*	*		*	*	*	*		*
		Chaetogaster sp.		*	*	*			*	*	*	
		Nais sp.						*	*	*		
		Amplichaeta sp.			*	*						
		Stylaria lacustris		*								
		Pristina pristinella						*				
		Aulophorus sp.							*			
		Potamothrix sp.		*						*		*
	Tubificidae	*Tubifex tubifex*		*					*			
		Aulodrilus sp.					*					
	Proppapidae	*Proppapus* sp.		*								
	Lumbriculidae	*Stylodrilus heringianus*	*									
		Trichodrilus sp.							*			
Gasteropoda	Lymnaeidae	*Galba truncatula*								*		*
	Physidae	*Physa* sp.										*
	Bithyniidae	*Bithynia* sp.		*								
		Melania tuberculata				*						
	Planorbidae	*Anisus* sp.					*					
		Segmentina sp.										*
		Ceratophallus natalensis										*
	Bulinudae	*Bulinus truncatus*					*					
Crustacea	Chydoridae	*Alonella* sp.		*								
		Kurzia latissima			*							
		Kurzia longirostris			*							
		Lidigia sp.										*
	Moinidae	Moinadaphnia				*						*
	Macrothricidae	*Ilyocryptus* sp.					*					
		Macrothrix triserilis					*					*
		Gurneyella monodi				*						
	Daphnidae	*Ceriodaphnia cornuta*									*	*
		Simocephalus latirostris									*	
	Candoninae	Candoninae sp.1										*
		Candoninae sp. 3		*								
	Harpacticoida	Harpacticoida				*		*				
	Cyclopoida	*Tropocyclops confinis*	*	*		*	*	*	*	*	*	
		Ectocycops hirtus			*			*		*		
		Mesocyclops sp.				*	*	*	*	*	*	
		Mesocyclops salinus					*	*	*			
		Mesocyclops inversus			*	*			*		*	
		Afrocyclops gibsoni					*			*	*	
		Allocyclops sp.	*					*	*			
		Thermocyclops sp.		*					*		*	*

			Stations									
Class	Family	Genus or Species	PB_1	PB_2	PB_3	PB_4	PB_5	PB_6	PB_7	PB_8	PB_9	PB_{10}
	Calanoïda	*Troglodiaptomus* sp.	*			*			*			
	Stenasellidae	*Metastenasellus camerounensis*			*			*				
		Metastenasellus n. sp.							*			
Hexapoda	Collembola	Anurida	*						*			
	Entomobryoïdae	Entomobryoïdae	*		*		*		*	*	*	
	Tomoceridae	*Tomocerus* sp.	*	*	*		*		*			
		Dicyrtomina				*						
		Pseudachorute							*		*	
	Ceratopogonidae	Ceratopogonidae	*	*		*	*	*	*	*	*	*
	Chironomidae (L + N)	*Crytochironomus deribea*	*	*	*	*	*	*	*	*	*	*
	Tanypodinae	Tanypodinae	*	*	*	*	*	*		*	*	*
	Culicidae (L + N)	Culicidae	*	*	*	*	*		*		*	
	Simulidae	Simulidae								*		
	Tipulidae	Tipulidae									*	
	Stratiomyidae	Stratiomyidae	*									
	Tabanidae	Tabanidae									*	
	Limmodiidae	Limmodiidae				*						
	Coleoptera sp	Coleoptera sp									*	
	Haliphidae	Haliphidae					*					
	Dytiscidae	*Aulonogyrus* sp.				*	*		*	*	*	*
		Eretes sp.					*			*		
		Gopelatus sp.				*					*	
	Blattoidae	Blattoidae			*				*	*	*	
	Elmidae	*Potamophilus* sp.								*		
	Noteridae	*Noterus* sp.							*			
	Ephémeroptère sp	*Ephémeroptère* sp.									*	
		Heptagenia sp.				*					*	
		Choropteopes sp.			*							
	Perlodidae	*Brachyptera* sp.		*								
	Hydrophilidae	*Hydrochara* sp.				*						*
	Heptageniidae	*Rhithrogena* sp.					*					
	Aeschnidae	Aeschnidae		*			*		*	*		*
	Cordulidae	*Epitheca bimaculata*							*			
	Total number of taxa = 80											
	Overall taxonomic richness		13	20	16	20	22	14	26	19	21	19
	Number of stygobite species		1	0	1	1	0	1	2	0	0	0

*present

Composition of fauna in groundwater

Relative abundance and frequency of taxa regularly present in the groundwater of the city of Mbalmayo

The relative abundances and relative frequencies of regularly harvested taxa are presented in Table 2. The genus *Metastenasellus* is represented by two species: *Metastenasellus camerounensis* and *Metastenasellus* n.sp. The first species *Metastenasellus camerounensis* was regularly present in our samples and the second, less abundant, species *Metastenasellus* n.sp. is new to science. In stations PB3, PB6 and PB7 the species *Metastenasellus camerounensis* was constant (Nr < 10% and F > 50%). The copepods *Tropocyclops confinis*, and *Mesocyclops* sp. were the most frequent in the stations. *Tropocyclops confinis* was a companion in stations PB4, PB8 (Nr < 10% and 20% < F < 50%), and constant in stations PB3 and PB6 (Nr < 10% and F > 50%). *Mesocyclops* sp. was abundant in stations PB6 and PB7 (Nr > 10% and F > 50%), constant in stations PB5 and PB9 (Nr < 10% and F > 50%), companion in stations PB4 and PB8 (Nr < 10% and 20% < F < 50%). Ostracods are represented by the family Candonidae so the species are stygophilic. This family is constant in station PB10 (Nr < 10% and F > 50%), and companion in station PB3 (Nr < 10% and 20% < F < 50%). The most frequent Oligochaetes belong to 3 genus: *Dero*, *Chaetogaster* and *Tubifex*. *Dero* sp. is absent in station PB1, PB4 and PB9 and abundant in stations PB5 and PB10 (Nr > 10% and F > 50%), then constant in stations PB2 and PB7 (Nr < 10% and F > 50%), and companion in station PB6 (Nr < 10% and 20% < F < 50%). *Chaetogaster* sp. is constant in stations PB7 and PB9 (Nr < 10% and F > 50%), companion in stations PB2 and PB4 (Nr < 10% and 20% < F < 50%) and accessory in stations PB3 and PB8 (Nr < 10% and 5% < F < 20%). *Tubifex tubifex* was constant in station PB2 (Nr < 10% and F > 50%) and companion in station PB10 (Nr < 10% and 20% < F < 50%).

Table 2. Relative abundance (Nr) and relative frequency (Fr) of taxa regularly harvested during the study.

Regular taxa		PB1	PB2	PB3	PB4	PB5	PB6	PB7	PB8	PB9	PB10
Dero sp.	Nr		3.9	0.6		65.4	0.4	3.1	2.4		58.3
	Fr		58.3	25		100	25	100	41.7		100
Chaetogaster sp.	Nr		1.1	0.6	0.8			1	0.5	2.9	
	Fr		41.7	8.3	41.7			58.3	8.3	66.7	
Tubificidae	Nr		4.5						0.5		0.5
	Fr		91.7						8.3		41.7
Tropocyclops conformis	Nr	1.3	0.1		1.4	0.1	3.1	0.6	2.0	0.2	
	Fr	8.3	8.3		41.7	8.3	66.7	16.7	25.0	8.3	
Mesocyclops sp.	Nr				12.5	6.7	42.1	84.0	2.9	10.2	
	Fr				41.7	91.7	91.7	100	41.7	91.7	
Mesocyclops salinus	Nr					0.2	10.9	5.4			
	Fr					33.3	58.3	100			
Candoninae	Nr			5.9							1.7
	Fr			41.7							58.3
Metastenasellus camerounensis	Nr			4.1			0.6	1.5			
	Fr			55.6			58.3	75			
Crytochironomus deribea	Nr	71.1	74.5	92.9	85.4	21.2	6.6	3.8	67.8	66.8	9.3
	Fr	75	50	75	100	100	66.7	66.7	100	91.7	100
Tanypus fuscus	Nr	5.3	4.3	10.7	3.1	1.8	0.3		5.9	1.3	0.3
	Fr	8.3	83.3	50	58.3	100	8.3		41.7	25	25

Discussion

The results of physico-chemical water analyses show that water quality varies from one station to another. The average temperature values ranged from 25 to 27.5°C overall. These values remain on the whole close to that of the ambient temperature and corroborate those of the works carried out in the ground water of Morocco by Idbennacer (1990), Boulal (1988, 2002). The groundwater studied remained acidic (pH < 6) during all seasons, which agrees with the results of some authors who had already found a pH between 4 and 6 in the groundwater of Yaoundé and showed that this acidity is related to the siliceous nature of the basement of the central region (Nola *et al.*, 1998; Zébazé Togouet *et al.* (2009, 2011). Overall, the groundwater of Mbalmayo is weakly mineralized with mean values of electrical conductivity below 500 µS/cm in all stations and all seasons, except for well PB10 where the mean values remained higher during the long dry season. These values are lower than those obtained by Zébazé Togouet *et al.* (2009, 2011) in the groundwater of the city of Yaoundé. This low mineralization could be linked to the acidity of the water likely to limit the presence of biodegradable organic matter and thus reduce the phenomenon of water mineralization. Oxygen saturation averaged between 34.1 and 91.2%. According to the grid of Nisbet & Verneaux (1970), the groundwater of Mbalmayo is located in class 3 corresponding to a doubtful situation. This oxygen undersaturation would probably result from the degradation of the organic matter present in these biotopes. Average calcium hardness levels remained below 30mg/L during the different seasons, except for wells PB2 and PB9 where they remained higher respectively during the short and long rainy seasons. These values remain on the whole low compared to those obtained in the groundwater of Morocco and Algeria. Indeed, this low calcium hardness content could testify to the absence of the limestone soils in the city of Mbalmayo that enrich the groundwater with carbonate ions by infiltration. Seasonal average concentrations of ammonium ions remained overall above 0.05mg/L in all stations. These values are above the WHO maximum allowable value for drinking water. They place the groundwater of the town of Mbalmayo in the "poor quality" category according to the grid of Guillemin & Roux (1992). According to Barres (1974) and Afonso (1992), this high ammonium ion concentration could be attributed to an organic load in the groundwater, the degradation of which generates nitrogen compounds, which enriches the water table with these elements. Similarly, the seasonal mean concentrations of nitrite were greater than 0.01mg/L.

According to Nisbet & Verneaux (1970) and Martin (1979), the presence of nitrites indicates a state of critical organic pollution. The presence of nitrites at our stations could be explained either by the oxidation of ammonium ions by nitrifying bacteria, the genus Nitrosomonas being the best known (Jayamohan *et al.*, 1988). Seasonal mean concentrations of nitrate ions ranged from 0.09mg/L to 17.73mg/L. However, they remain above 4mg/L at all stations during the short dry and short wet seasons, respectively. Overall, these values are low compared to those often observed in groundwater in Europe and the Maghreb. They are close to those obtained by Zébazé Togouet *et al.* (2011) in the groundwater of Yaoundé. The presence of nitrates in significant quantities in the groundwater of Mbalmayo could be linked to the use of fertilizers and fertilizers which are generally rich in nitrogen, which enrich the groundwater with nitrate ion, which is very mobile in the soil (Schepers *et al.*, 1991; Weir *et al.*, 1994). Mean concentrations of orthophosphates recorded were generally in the range of 0.09 to 5.9mg/L, and remained above 0.5mg/L at all stations during the short rainy season. According to Atteia, 2005, this high concentration of orthophosphate ion could be explained by the input of "black water" containing organic phosphates, fecal pollution and synthetic detergents since their concentration is higher than 0.1 or 0.2mg/L. The same is true for the 3 types of nitrogen ions considered above, which very rarely originate in the subsoil (Smith, 2016), although they can sometimes result from the more or less complete bacterial decomposition (depending on whether the medium is more or less oxidizing) of plant organic matter that may naturally reach well or spring water. At the study stations, BOD, which is an indicator of the organic load of water, does not differ significantly from the different nitrogen ions and phosphates. It is therefore very likely that these nitrogen ions, which are more abundant in the groundwater of Mbalmayo, come mainly from the natural decomposition of leaves or various plant debris brought by the wind to stations more or less protected from these external inputs. Seasonal mean concentrations of fecal coliforms, total coliforms and fecal streptococci are less than 20 CFU/100 mL at stations PB1, PB6 and PB7 during all seasons and greater than 100 CFU/100mL at other stations. These values for the three stations PB1, PB6 and PB7 are still within the range of those recommended for drinking water (WHO, 2014). The high values obtained in the other stations are not surprising since Nola *et al.*, 2005 recorded similar results in the groundwater of Yaoundé. The presence of these two bacteria in groundwater could be explained by human intervention in the deterioration of natural resources, generally through domestic urban discharges. On the other hand, groundwater contamination could be due to the infiltration of wastewater mainly favoured by the low thickness of the protective zone (soil and unsaturated zone) (Lyakhloufi *et al.*, 1999), which is generally less than 5m. However, given the adsorptive power of soil on bacteria, demonstrated by several authors (Nola *et al.*, 2012), the exceptional abundance of these germs in the waters of the wells studied suggests that much of the deterioration of the microbiological quality of water could also be due to local point source pollution on the surface, at or near the water point itself. The underground aquatic fauna of Mbalmayo is characterized by the dominance of epigean aquatic species, thus of external origin, compared to the small number of stygobite species, underground species whose complete life cycle is always and entirely carried out in the water table. This result is not surprising and confirms once again the statements of Vandel (1964) and Dalmas (1972, 1973) that wells constitute ecotones or cohabitate epigeous and endogenous species. This characteristic of the wells, first observed in Europe, has been verified everywhere in different regions of the world, particularly in Morocco during research carried out by Boulanouar (1986, 1995), Boulal (2002) and Aït Boughrous *et al.* (2007), in Algeria by Gagneur & Yadi (2000) and by Merzoug *et al.* (2010) and also in Cameroon during the first studies carried out in the Mfoundi Basin by Zébazé Togouet *et al.* (2009). The specific richness obtained ranging between 13 and 26 is much higher than that obtained by Zébazé *et al.* (2009) in Yaoundé groundwater, and that obtained (12 to 19 aquatic species) by Chinche *et al.* (2019) in the groundwater of Tiko in Cameroon. Overall, it can be noted that the most widespread organisms and especially the most abundant in number of individuals collected in the different stations (PB2, PB3, PB4, PB5, PB8 and PB9) are particularly the larvae of Diptera Chironomidae (*Crytochironomus deridae*). In wells PB1, PB6, PB7 and PB10 the most abundant organisms are copepods of the Cyclopoidae family represented by the species *Tropocyclops confinis*, *Mesocyclops* (*Mesocyclops* sp), Ostracods of the Cypridae family represented by the genus *Candonocypris* (*Candonocypris* sp) and Oligochaetes of the Naididae family represented by the genus *Nais*. Stygobite organisms representing aquatic subterranean fauna were present at three stations PB1, PB6 and PB7. They are represented by two species of Crustacea Isopods belonging to the family Stenasellidae and the genus *Metastenasellus*. One of the species *Metastenasellus camerounensis* is already known in the groundwater of Yaoundé (Zébazé Togouet *et al*., 2009, 2011, 2013), and the other *Metastenasellus* sp. is a species new to science. These two species have been harvested in PB1, PB6 and PB7, whose water quality appears to be good from a physicochemical and microbiological point of view. This demonstrative example suggests that there could be a positive correlation between the quality of the groundwater, the microbiological quality on the one hand, and the presence or abundance of these stygobite crustaceans on the other. It should be noted that Zébazé Togouet *et al.* (2009) having continuously prospected 14 wells all located in the Yaoundé agglomeration and Chinche *et al.* (2019) having prospected 10 wells in the city of Tiko found only one stygobite species, particularly in the wells containing the least polluted water.

The importance of stygobite species as indicators of well water quality had already been highlighted in Europe and the Maghreb by authors such as Camacho (1992), Juberthie and Decou (1994), Boutin (1993, 1994), Boutin & Coineau (2004), Montanari *et al.* (2021). We have no doubt that this relatively low number of stygobite organisms in the city of Mbalmayo compared to those demonstrated in Morocco by these authors is related to groundwater pollution due to the effect of anthropogenic pressure, which is at the origin of different forms of groundwater pollution. This result is in line with those of Boutin (1984) and Fakher *et al. (*1998), who had shown the sensitivity of stygobite crustaceans to water pollution and the idea had been put forward of the possibility of using these species as indicators (by their presence) of the relative quality of the water in a well or groundwater, or on the contrary (by their absence) of groundwater pollution (Boutin, 1984; Boutin & Dias, 1987; Montanari *et al.* (2021)).

Conclusion

This study allowed highlighting the physicochemical acidity of the groundwater of the city of Mbalmayo, characterized by high electrical conductivity values, high concentrations of ammonium and orthophosphate ions, and high contents of Suspended solids. The results of the faunistic study show a rich and diversified aquatic fauna of limnic origin, with stygobite species present in stations with low values of the physico-chemical parameters of the water and low concentrations of faecal coliforms and faecal streptococci. It therefore confirms the interest of research and study of the stygobionte fauna accessible at wells and springs, when one wishes to be able to quickly and simply formulate a global diagnosis of the quality of underground aquatic biotopes.

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