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Abundance of Faecal Coliforms and Pathogenic *E. coli* Strains in Groundwater in the Coastal Zone of Cameroon (Central Africa), and Relationships with Some Abiotic Parameters

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Abstract: This study aimed to assess the impact of many pollution sources on the microbiological water quality of 20 wells in the coastal zone of Cameroon (Central Africa) and the relationships with some physico-chemical parameters that can influence the bacterial growth. The average values of faecal coliforms obtained during this study reached 19×10^3 CFU/L. The abundance of pathogenic strains of Enteropathogenic *E. coli* (EPEC) and Enterotoxigenic *E. coli* (ETEC) sometimes reached 3 CFU/L of water. The water temperature ranged from 21.2 to 29ºC, the pH from 3.55 to 12, and the electrical conductivity from 11.80 to 2189 µS/cm. The concentration of the dissolved oxygen ranged from 0.5 to 7 mg/L while that of CO_2 ranged from 0.6 to 188.3 mg/L. The concentrations of ions ranged from 0.01 to 28.64 mg/L for orthophosphate, from 0 to 23.10 mg/L for nitrogen ammonia, from 0.01 to 3.20 mg/L for nitrates, from 1 to 225 Pt. Co for the color, and from 1 to 39 FTU for the turbidity. The relationships between the abundance of each bacterial group identified and each physico-chemical parameter considered varied from with respect to the sampling site and are non significant in most cases ($p>0.05$). This suggests the involvement of a large numbers of environmental factors in the distribution of species and strains of bacteria isolated from this groundwater points. The consumption of groundwater from this region is a health risk in the short term.

Key words: Enteropathogenic *E. coli*, enterotoxigenic *E. coli*, physico-chemical parameters, well water

INTRODUCTION

In many regions of the world and especially in developing countries, waste waters containing biodegradable organic compound are often discharged to the environment without any pre-treatment, and therefore, can potentially pollute soil and groundwater. Numerous infection cases due to groundwater contamination by microorganisms are often reported around the world. In those regions, groundwater is one of the main sources of water supply. Contamination of this resource constitutes a real threat to public health. Poor chemical quality of water supply is responsible of health risk in the long term for consumers while, poor microbiological quality is considered as being responsible for health risks in the short term. Poor quality of water is responsible for several million cases of diarrhea each year worldwide (Rijal and Fujiokal, 2001).

In Douala, the economic capital of Cameroon, public drinking water supply is insufficient for the populations who resort to groundwater without worries about its physico-chemical and microbiological qualities (Cronin *et al*., 2006; Ndjama *et al*., 2008; Takem *et al*., 2010). This groundwater's are opened to public in most cases through wells and used for many purposes. The microorganisms found in groundwater can be commensal, opportunistic pathogens or pathogens strict (Banton and Bangoy, 1997; Nola *et al*., 2001, 2002). Moreover, in the Douala region, the sanitation is practically nonexistent. Wastewaters are discharged in the environment without any treatment, polluting the soil and subsoil, and therefore groundwater. However, the pollution of groundwater depends on several properties of seepage (Nola *et al*., 2006a), the physico-chemical and mineralogical characteristics of the surrounding soil (Nola *et al*., 2006b, 2011). Several cases of infections due to consumption of contaminated water by pathogenic bacteria have been reported in many parts of the world, sometimes causing epidemics followed by loss of human life (Angulo *et al*., 1997).

Some studies conducted on groundwater in the Douala region showed that these waters harbored faecal coliforms and faecal streptococci and their abundances undergoes spatio-temporal variations (Djuikom *et al*.,

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2009, 2011). Most bacteria from these two groups usually known as commensal belong to *Escherichia* and *Streptococus* genus; their presence simply indicates faecal contamination of water. The species mostly used as indicators of the faecal contamination of water are *E. coli* and *S. feacalis* (WHO, 2004). Some strains of *Escherichia* are often pathogen (Holt *et al*., 2000). Little or no studies have been focused on the characterization of pathogenic strains of *Escherichia coli* contained in groundwater for household purposes. Physico-chemical factors have sometimes been indicated as significantly impacts the distribution of some bacteria species in the ground and surface water. Few data are available on the potential influence of these parameters on the distribution of pathogenic strains of *E. coli* in groundwater. The main purpose of this study aims to assess the relative abundance of the pathogenic strains of *E. coli* in groundwater of the Douala region and to evaluate the influence water physico-chemical parameters on their distribution.

MATERIALS AND METHODS

Description of the study site: The Douala region is located between 4º04′ N latitude and 9º45′ E longitude, 60 m above mean sea level, at about 30 km from the Gulf of Guinea. It is divided into five districts and is drained by several streams (Fig. 1). The region has a humid equatorial climate which is characterized by a long rainy season from April to October and a short dry season from November to March (Suchel, 1988). The peak rainfall occurs from July to September, with annual averages of 4000 mm rainfalls. The average annual temperature is between 23º and 33ºC, January and February being the hottest months of the year. The soil type varies from brown to black shallow ferralitic to deep sandy clayey (Asaah *et al*., 2006). The groundwater table is generally located less than 10 m below the surface (Mafany *et al*., 2006), the aquifer being continuously recharged by rainfalls. Wastewater from drainage channels also infiltrates into this aquifer. Several streams run along the area and may also recharge the aquifer depending on the season and water levels. The fluctuations of the average groundwater levels ranged from 0.3 to 2.1 m between the dry and wet seasons.

Sampling and water analysis: Twenty wells were chosen and numbered W_1, W_2,…, W_{20} (Fig. 1). Each well was sampled from January 2009 to January 2010 once a month. Samples were manually collected at 50 cm below the surface in 500 mL sterile glass bottles and in polyethylene clean bottles of 1 L. The Samples in the glass bottles were used for bacteriological analyses while those in polyethylene bottles were used for physico-chemical analyses. The samples were then transported to the laboratory and kept in dark refrigerated conditions for laboratory analyses. The time lapse between the sample collection and laboratory analyses was in all cases lower than 2 h.

The Physico-chemical parameters considered were the temperature, pH, dissolved oxygen, nitrates, electrical conductivity, orthophosphates, color, turbidity, nitrogen ammonia and dissolved CO_2. The analyses were carried out using standard methods (Rodier, 1996; APHA, 1998).

The bacterial analysis considered the faecal coliforms after appropriate dilutions using NaCl solution (0.85 g/L). Each analysis was performed in triplicate. The membrane filtration technique was used for bacterial counts (Ford, 1994). The filter membranes (Millipore Corporation, Bedford, MA 01730 MQ) of porosity 0.45 μm and 47 mm diameter were used (Apha, 1998). The Endo agar culture medium was used (Marchal *et al*., 1991). After 24 h of incubation at 44ºC, red and metallic green sheen Colony Forming Units (CFU) were considered as faecal coliforms and then counted. The CFU from faecal coliforms on this culture medium are red in color due to lactose degradation (Marchal *et al*., 1991; Le Minor and Richard, 1993; Holt *et al*., 2000). Results were expressed as number of CFU/L of water. Each metallic green sheen CFU was subsequently identified after a sub-culture on a standard agar medium, according to Holt *et al*. (2000).

Identification and characterization of pathogenic *E. coli* strains: Only metallic green sheen CFUs were identified according to Holt *et al*. (2000). Characterization tests were done in two steps. The first step was based on the property that pathogenic strains of the *E. coli* species possess adhesion factors that have an important affinity for the cellular receptors with their carbohydrate residues present on the α-D-mannose. Washed red cells were used for reagent preparation. Three volumes of physiological saline solution were used in the presence of a volume of human blood group A Rhesus positive freshly collected in a test tube. The mixture was centrifuged three times at 3000 revolutions/min for 5 min, throwing each time the supernatant and adding a physiological saline solution. The pellet was recovered at the end. To the suspension necessary for haemagglutination reactions, is brought into it phosphate buffer (pH 7.4), washed red blood cells, and D-mannose in order to obtain a final concentration of 2.5% D-mannose (trace of D-mannose may be sufficient to this).

As for the test, a drop of red blood cell was deposited on a clean glass slide next to one or three colonies of bacterial culture taken from Mueller Hinton agar after 24 hours of incubation at 37ºC and emulsification. This mixture on the slide was rotated manually for 1-2 min and observed for haemagglutination macroscopically. When the suspension remained consistent after 2 min, the test

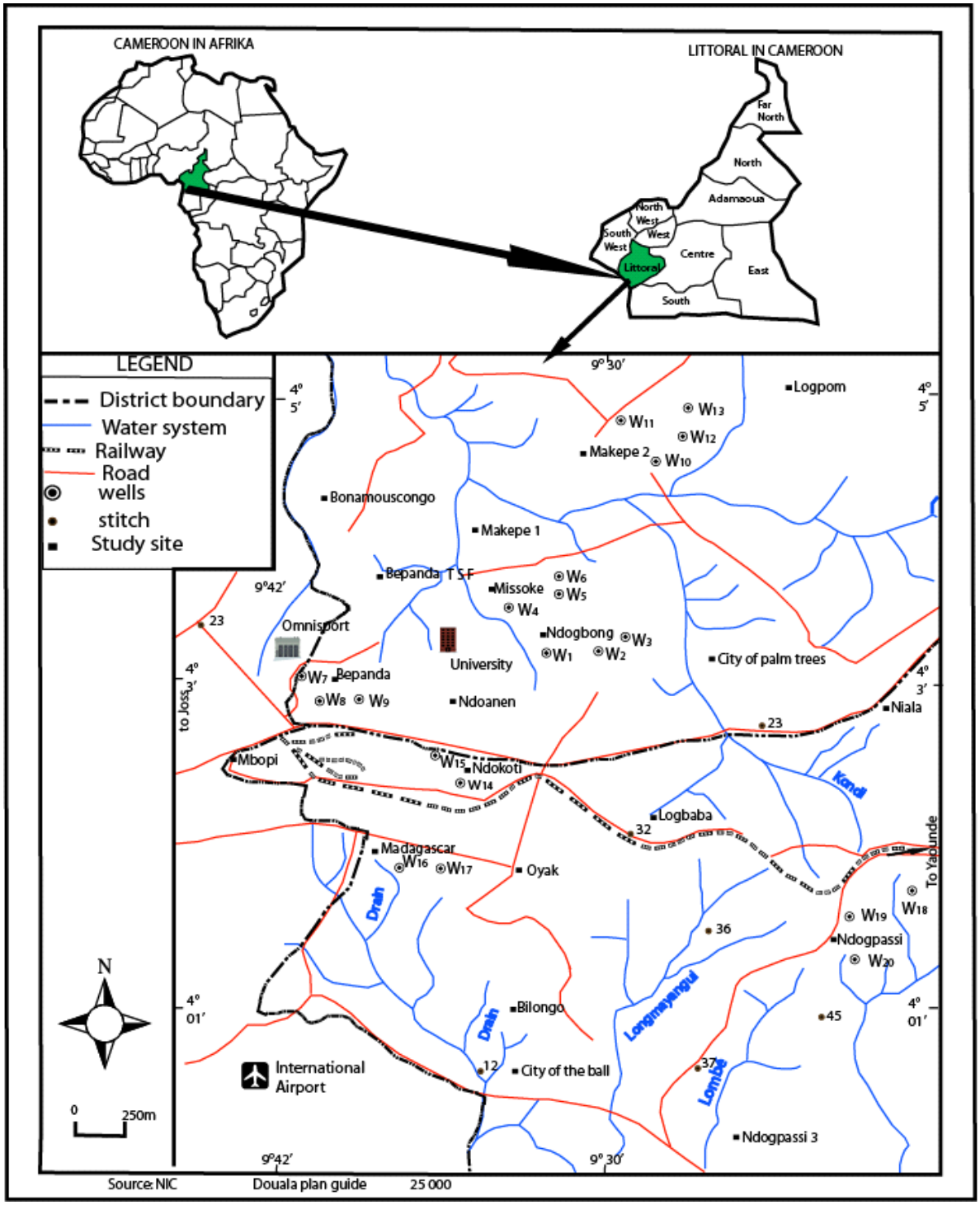

Fig. 1: Location of sampling points

was negative and is considered as Mannose Sensitive Haemagglutination (MSHA). The test was positive when agglutination occurred in two minutes; it was therefore considered as mannose resistant haemagglutination (MRHA). This test is a phenotypic marker complementary to the selection of potentially pathogenic strains (Bouhaddioui *et al*., 1998; Okeke *et al*., 2000a; Yasmeen *et al*., 2009).

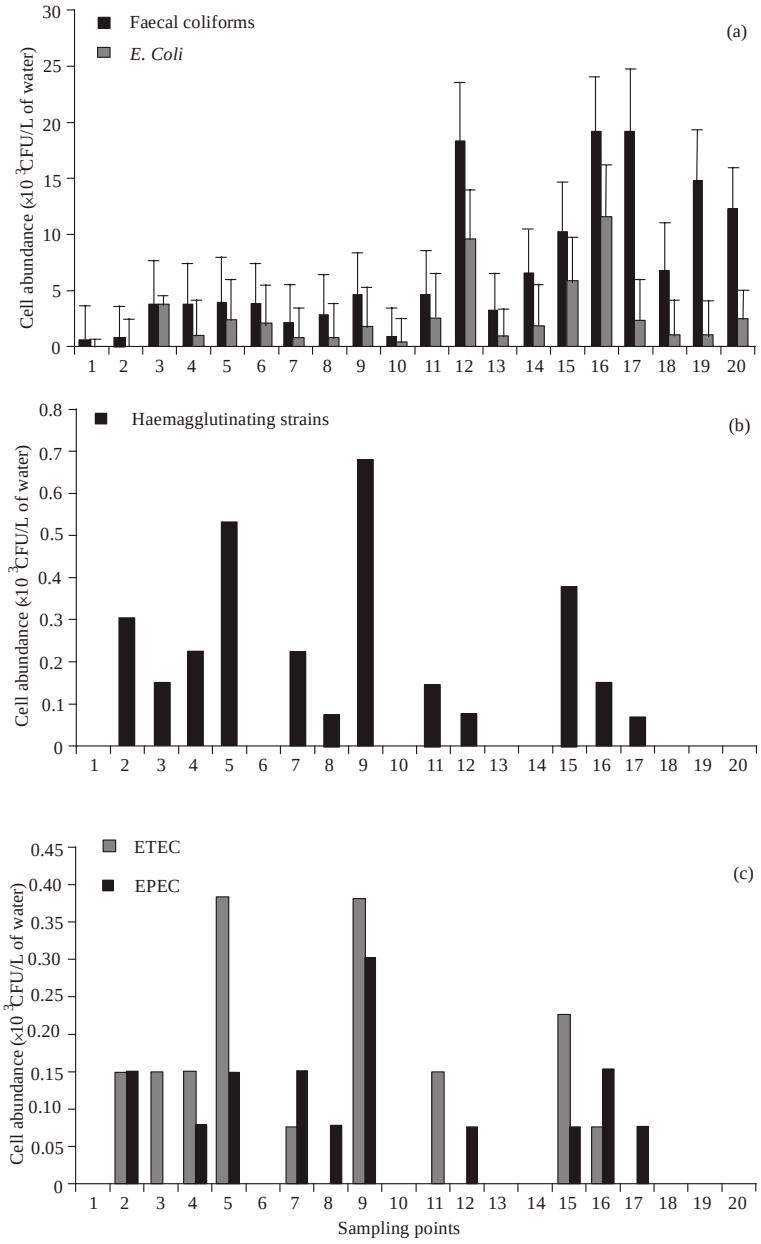

Fig. 2: Variation with respect to the sampling site of the abundance of faecal coliforms and *E. coli* (a), Haemagglutinating strains of *E. coli* (b), EPEC and ETEC (c)

Secondly, the antisera determining the Enteropathogenic *E. coli* (EPEC) group (Bio-Rad) was used to determine the serotype of different pathogenic strains obtained after the haemagglutination tests. Trivalent sera I, II, III, IV and a mixture of Nonavalent and Trivalent IV serums were used. Identification of Enterotoxigenic strains of *E.coli* (ETEC) was made by differentiation of stem Mannose-sensitive haemagglutinins, but negative in contact with the serum (Bouhaddioui *et al.*, 1998).

Data analysis: In total, 260 samples were analyzed for biotic and abiotic factors. The variations of values of abiotic parameters and the abundances of identified microorganism were illustrated respectively by curves and histograms. The isolation frequency of the identified pathogenic strain with respect to each serum was assessed. The relationships between the abundance of isolated microorganisms and abiotic parameters were also assessed using Spearman correlation test "r". The

comparison among the abundances of microorganisms isolated was performed using the Kruskal Wallis test.

RESULTS

The annual average abundance of faecal coliforms reached 19×10^3 CFU/L and those of *E. coli* 12×10^3 CFU/L (Fig. 2a). The highest abundance of faecal coliforms was recorded in wells W_{16} and W_{17} (Fig. 2a) located geographically very close each other (Fig. 1). For *E. coli,* its highest abundance was recorded in well W_{16}. These microorganisms were sometimes scarcely isolated in some water sources (Fig. 2a).

The abundance of the Enteropathogenic strain of *E. coli* sometimes reached 3 CFU/L of water. The haemagglutination test showed 40 potentially pathogenic strains of *E. coli* (strains agglutinating red blood cells of human blood). These strains were isolated from 12 water points (W_2, W_3, W_4, W_5, W_7, W_8, W_9, W_{11}, W_{12}, W_{15}, W_{16} and W_{17}). For all the sampling sites, the highest frequency isolation of the potentially pathogenic strains was 4, recorded in well W_9. In this site, this strain was isolated in February, April, July and December. During these months, a total of 9 potentially pathogenic strains were isolated. In well W_5, 4 potentially pathogenic strains were isolated in August and 3 in September, while in well W_2, 2 strains were isolated in February and 2 in April. In well W_{15}, 2 strains were isolated in January and 3 in November. In well W_4, 1 strain was isolated in April and 2 in January of the following year. In W_7, 1 strain was isolated in August and 2 in September while in W_3, 2 strains were isolated in April. In W_{11}, 1 strain was isolated in March and one in July. In W_{16}, 1 strain was isolated in June and another one in July. In W_8, W_{12} and W_{17}, the frequency of isolation of haemagglutinating strain of *E. coli* was 1. The number of haemagglutinating strains isolated in each water point is shown in Fig. 2b.

Serotyping tests were performed using trivalent I (O111, O55, 026), II (O86, O119, O127), III (O125, O126, O128), IV (O114, O124, O142) sera and a mixture of Nonavalent and Trivalent IV serum. It was noted that 19 of the 40 pathogenic strains were identified as Enteropathogenic and the 21 others identified as Enterotoxigenic. Their distributions are shown in Fig. 2c. The highest abundance of Enteropathogenic strains was recorded in well W_9, while in wells W_5 and W_9, the number of Enterotoxigenic strain of *E. coli* was 5 (Fig. 2c).

The Enteropathogenic strains of *E. coli* as a whole showed various sensitivities towards the sera used. A large proportion of identified strains (36%) was sensitive to Trivalent I serum and a very small proportion (11%) sensitive to Trivalent II serum (Fig. 3). The Trivalent I serum was sensitive to Enteropathogenic strains of group O111 B4, B5 O55, O26 B6. The Trivalent III, IV sera and the mixture of Nonavalent and Trivalent IV serum respectively enabled the identification of 16%, 21 and 16% of the Enteropathogenic strains of *E. coli* (Fig. 3).

The annual mean of each biological parameter was computed in each sampling site. The pathogenic faecal population and the abundances of *E. coli* varied significantly amongst sampling sites ($p<0.01$) (Table 1). Moreover, the distribution of Pathogenic strains of *E. coli* globally did not varied significantly with sampling site (Table 1). Concerning the physico-chemical parameters, although no significant difference was observed among sites for the dissolved CO_2, the mean values of temperature, phosphorus, turbidity, NH_4+, electrical conductivity and color showed significant variation amongst wells sampled ($p<0.01$) (Table 1).

It is noted that the mean annual value of water temperature ranged from 21.2º to 29º C, pH from 3.55 to 12, electrical conductivity from 11.80 to 2189 µS/cm, and dissolved oxygen from 0.5 to 7 mg/L. The concentrations of dissolved CO_2 fluctuated from 0.6 to 188.3 mg/L. While the concentrations of phosphates, nitrogen ammonia and nitrates ranged respectively from 0.01 to 28.64, 0 to 23.10 and 0.01 to 3.20 7 mg/L. The color of water ranged from 1 to 225 Pt. Co and the turbidity from 1 to 39 FTU. Figure 4 presents the spatial variations of the mean annual values of each parameter recorded in all sampling sites.

The Spearman correlation test showed a heterogeneous degree of relationship between the abundance dynamic of faecal coliforms and the concentration of physico-chemical parameters (Table 2). The rise in pH led to a decrease of abundance of faecal coliforms and *E. coli* respectively in wells W_2 and W_{11} ($p<0.05$). In the well W_{20}, the rise in pH seemed rather to promote the development of faecal coliforms (Table 2). The increase in the concentration of nitrate ions seemed to favor ($p<0.01$) the increase of *E. coli* abundance in wells W_{17}. In wells W_3 and W_{20}, the increase in electrical conductivity was concomitant to an increase in the abundance of faecal coliform ($p<0.05$), but the increase of this factor led to a decrease of chemical abundance of *E. coli* in wells W_{12} (Table 2). In the well W_2, the increase

Table 1: Comparison of mean abundance of physico-chemical parameters and faecal coliforms in the different stations

	Considered parameters										
	T°	PO_4^{3-}	Turb	NH_4^+	CO_2	NO_3^-	E. C	Color	Faecal colif	*E. coli* strains	Pathogenic *E. coli* strains
p-values	0.000**	0.000**	0.000**	0.000**	0.995	0.000**	0.000**	0.000**	0.000**	0.000**	0.198*

**: $p<0.01$; *: $p<0.05$; ddl: 19; T°: Temperature; Turb: Turbidity; E.C: Electrical conductivity; Faecal colif: Faecal coliforms

Table 2: Correlation coefficients between the monthly averages of bacteria abundances and those of physico chemical parameter

Sampling sites	Considered parameters: PH			Color			Nitrates			Electrical conductivity			Dissolved oxygen		
	F. colif	*E. coli* strains	Path *E. coli*	*F. colif.*	*E. coli* strains	Path *E. coli*	*F. colif.*	*E. coli* strains	Path *E. coli*	*F. colif.*	*E. coli* strains	Path *E. coli*	*F. colif.*	*E. coli* strains	Path *E. coli*
W_1	- 0.483	- 0.463	NE	0.472	- 0.284	NE	0.095	0.097	NE	0.910	0.192	NE	- 0.166	0.311	NE
W_2	- 0.591	*-0.459	- 0.114	0.269	- 0.405	0.139	0.239	- 0.025	0.289	0.518	0.340	0.228	- 0.265	- 0.055	- 0.343
W_3	- 0.200	0.010	- 0.387	0.215	0.244	0.313	0.069	- 0.175	0.040	0.640*	0.387	- 0.116	- 0.488	- 0.306	0.387
W_4	0.216	- 0.039	0.061	0.132	- 0.449	- 0.104	- 0.544	- 0.279	0.148	0.089	0.350	- 0.420	- 0.249	- 0.344	0.092
W_5	- 0.104	- 0.068	- 0.070	- 0.189	0.194	0.403	0.231	0.095	0.080	0.275	0.331	- 0.074	- 0.079	- 0.142	0.262
W_6	- 0.435	- 0.262	NE	- 0.532	- 0.604*	NE	- 0.046	- 0.056	NE	0.177	0.203	NE	0.057	0.051	NE
W_7	- 0.092	- 0.074	- 0.472	- 0.086	- 0.597*	- 0.468	- 0.244	- 0.022	- 0.058	- 0.362	0.072	- 0.206	0.063	0.227	0.162
W_8	0.552	0.481	-.0386	0.370	0.013	- 0.188	0.051	0.096	0.192	0.008	0.088	0.000	0.072	0.185	0.465
W_9	- 0.223	0.036	0.190	- 0.329	- 0.403	- 0.111	- 0.288	- 0.172	0.251	- 0.346	- 0.231	0.495	- 0.012	0.141	0.041
W_{10}	0.047	0.118	NE	- 0.295	0.089	NE	0.193	- 0.141	NE	- 0.104	0.161	NE	0.341	0.080	NE
W_{11}	- 0.394	- 0.568*	- 0.143	- 0.072	- 0.325	- 0.373	0.061	0.128	0.460	0.189	- 0.168	- 0.462	0.111	- 0.128	- 0.516
W_{12}	0.327	- 0.123	0.039	- 0.208	- 0.056	- 0.157	0.327	- 0.092	0.196	- 0.218	- 0.593*	- 0.426	- 0.058	- 0.468	- 0.233
W_{13}	- 0.055	- 0.066	NE	- 0.315	0.144	NE	0.106	- 0.210	NE	- 0.050	- 0.145	NE	- 0.069	- 0.245	NE
W_{14}	- 0.332	0.330	NE	NE	NE	NE	- 0.071	- 0.114	NE	0.094	0.463	NE	0.259	0.056	NE
W_{15}	0.354	0.160	0.307	0.187	0.248	0.035	0.447	0.967	0.561	0.130	0.473	0.328	- 0.319	0.010	0.119
W_{16}	0.160	- 0.110	- 0.342	- 0.243	- 0.238	0.097	- 0.135	- 0.302	- 0.144	- 0.247	- 0.099	- 0.200	- 0.094	- 0.085	- 0.411
W_{17}	- 0.124	0.146	0.232	0.011	- 0.060	0.478	0.199	0.755**	- 0.156	0.435	0.524	0.232	0.444	0.092	0.468
W_{18}	0.199	0.091	NE	- 0.188	- 0.281	NE	0.515	0.298	NE	0.096	0.073	NE	- 0.387	- 0.212	NE
W_{19}	- 0.276	- 0.100	NE	0.190	0.390	NE	- 0.083	- 0.089	NE	0.795	0.594	NE	- 0.426	- 0.300	NE
W_{20}	0.567*	0.430	NE	0.393	0.240	NE	0.348	0.235	NE	0.625*	0.413	NE	- 0.227	- 0.183	NE

*: p<0.05; **: p<0.01; n: 13; NE: Non evaluated; F. colif. : Faecal coliforms; Path. *E. coli*: Pathogenic *E.coli*

Table 2: (Continuse)

Sampling sites	Considered parameters: T°			PO_4^{3-}			Turbidity			NH_4^+			Dissolved CO_2		
	E. colif	*E. coli* strains	Path *E. coli*	*E. coli colif.*	Path strains	*F. E. coli*	*E. coli colif.*	Path strains	*F. E. coli*	*E. coli colif.*	Path strains	*F. E. coli*	*E. coli colif.*	Path strains	*E. coli*
W_1	0.326	0.390	NE	- 0.348	0.464	NE	0.175	- 0.220	NE	0.961	0.240	NE	- 0.478	0.077	NE
W_2	0.017	0.017	0.602*	- 0.158	0.108	- 0.115	0.415	- 0.333	- 0.231	0.099	- 0.103	0.000	0.152	0.379	0.057
W_3	0.132	0.274	0.390	- 0.599*	- 0.440	- 0.155	0.235	0.136	- 0.157	- 0.636*	- 0.301	- 0.311	- 0.329	- 0.286	0.389
W_4	0.045	- 0.111	0.531	0.124	- 0.200	0.079	0.035	- 0.436	- 0.098	- 0.014	0.048	- 0.022	0.044	0.192	0.561
W_5	0.237	- 0.008	- 0.176	- 0.280	- 0.225	0.206	- 0.039	0.311	- 0.123	0.312	0.257	- 0.238	- 0.130	- 0.260	0.306
W_6	0.348	0.297	NE	- 0.196	- 0.219	NE	- 0.417	- 0.615*	NE	0.605*	0.669*	NE	- 0.030	0.113	NE
W_7	-0.150	- 0.452	- 0.496	- 0.089	0.410	0.322	0.146	- 0.234	- 0.210	- 0.412	- 0.396	- 0.472	0.226	0.146	0.416
W_8	0.334	0.127	0.388	0.019	0.206	- 0.465	0.181	0.174	- 0.220	- 0.315	- 0.202	- 0.318	- 0.163	0.114	- 0.155
W_9	-0.032	0.086	0.186	- 0.329	- 0.077	- 0.040	0.080	0.107	0.236	0.325	- 0.052	- 0.366	- 0.229	0.011	0.024
W_{10}	-0.184	- 0.010	NE	0.224	- 0.010	NE	- 0.386	0.239	NE	0.030	- 0.189	NE	0.194	0.085	NE
W_{11}	0.354	0.276	0.115	- 0.634*	- 0.721**	-0.431	0.862	- 0.256	- 0.326	0.072	0.507	- 0.029	- 0.627*	- 0.485	- 0.173
W_{12}	0.244	0.461	0.085	- 0.025	- 0.008	0.350	0.004	0.190	0.575*	- 0.187	- 0.486	- 0.118	- 0.133	-0.431	0.039
W_{13}	0.354	0.641*	NE	0.119	0.075	NE	- 0.402	- 0.397	NE	- 0.053	0.006	NE	- 0.274	- 0.090	NE
W_{14}	0.078	- 0.515	NE	0.169	- 0.071	NE	0.171	0.029	NE	- 0.290	- 0.244	NE	- 0.583*	0.011	NE
W_{15}	0.383	0.283	- 0.078	- 0.515	- 0.010	-0.215	0.194	0.300	0.423	- 0.091	- 088	- 0.382	- 0.074	- 0.021	0.131
W_{16}	0.237	0.157	- 0.295	- 0.213	- 0.372	-0.629*	- 0.335	- 0.325	0.148	- 0.355	- 0.286	- 0.176	0.045	- 0.366	0.058
W_{17}	-0.483	- 0.267	0.196	0.389	0.134	0.465	0.126	0.075	0.237	0.128	0.008	0.330	0.149	0.084	0.116
W_{18}	0.188	0.203	NE	- 0.719**	- 0.628	*NE	- 0.142	- 0.377	NE	0.179	0.359	NE	- 0.241	- 0.331	NE
W_{19}	0.303	- 0.008	NE	- 0.287	- 0.244	NE	0.043	0.276	NE	0.047	0.118	NE	- 0.403	- 0.211	NE
W_{20}	0.024	- 0.053	NE	0.448	0.349	NE	0.179	0.201	NE	0.497	0.218	NE	0.167	- 0.226	NE

* P < 0.05; ** P < 0.01; n= 13; NE: Non Evaluated; F. colif. : Faecal coliforms. Path. *E. coli*: Pathogenic *E.coli*

of temperature favored (p<0.05) the increase in abundance of pathogenic strains of *E. coli*. The increase of phosphates concentration seemed to favor the reduction of bacterial abundance (p<0.05) in wells W_3, W_{11}, W_{16} and W_{18} (Table 2). The increase of turbidity in well W_6 reduced the abundance of *E. coli* (p<0.05). However, the decrease in value of this parameter is concomitant to an increase in the abundance of pathogenic strains of *E. coli* in wells W_{12}. The nitrogen ammonia ion appeared to have an influence on the abundance of coliform bacteria in wells W_3 and W_6, and that of *E. coli* in well W_6 (Table 2). The increase in dissolved CO_2 promoted the reduction of coliforms bacteria in wells W_{11} and W_{14}, but was nevertheless concomitant to the increase in the abundance of pathogenic *E. coli* in well W_4 (p<0.05).

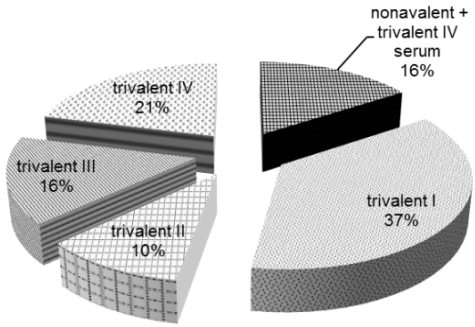

Fig. 3: Percentage of Enteropathogenic *E. coli* (EPEC) strains obtained according to the different sera used

DISCUSSION

Wells in Douala region harbors faecal coliforms in relatively high abundance, consisting among others of *E. coli* (Fig. 2). This species is very abundant in animal and human fecal flora. Although it has the disadvantage of being generally less persistent in the environment, it is considered the best indicator of fecal contamination which could be due to the proximity of contamination sources and the shallow groundwater. Boutin (1987) indicated that the underground water generally appears as vulnerable as the top of the water table is near the soil surface, the soils layers topping the water table permeable, and the superficial sources of pollution numerous, important and close to the study site. The number of this bioindicator obtained in different samples of the analyzed water was above the norm, especially in water points W_{12}, W_{16} and W_{17} (Fig. 2). The presence of this bacterial species reflects the high probability of pathogenic bacteria presence. The high faecal coliforms recorded are similar to those recorded by Nola *et al.* (2002) in the groundwater of Yaoundé.

The strains of pathogenic *E. coli* belonging to the EPEC and ETEC groups were isolated (Fig. 2c). From the 40 pathogenic strains isolated 19 (47.5%) were positive to different EPEC antisera (Bio-Rad). Most of positive strains with EPEC antisera were sensitive to Trivalent I serum (Fig. 3). Fotsing-Kwetche (2008) also observed in the Yaoundé region some serogroups belonging to EPEC; the most encountered were classified in decreasing order O119B14, O111B4, O126B16, O127B8, O55B5 and O26B6. In this study most of EPEC strains identified which were sensitive to Trivalent I serum were the O111 B4, B5 O55 and O26B6 groups.

A temporal variation of the abundances of EPEC and ETEC was noted in this study. In the Yaoundé region, a seasonal variation in the abundances of EPEC strain sensitive to Trivalent I serum has also been noted (Fotsing-Kwetche, 2008). The temporal variation of the cells abundances noted could be related to differences in pollutant loads contained in the runoff and wastewater seeping into the ground. This could also be attributed to the variability of the microbial retention potential on soil particles during infiltration (Kravitz *et al*., 1999; Nola *et al*., 2006a). This bacterial retention process on solids particles is sometimes selective (Nola *et al*., 2005, 2006a). It has been indicated that the inactivation rate of pathogenic strains of *E. coli* in sand-free groundwater was approximately 0.03 log unit per day, and 0.04 and 0.2 log unit per day for *Salmonella typhimurium* and *Staphylococcus aureus* respectively (John and Rose, (2005). In addition, it has been indicated that *E. coli* populations themselves may be heterogeneous in terms of their attachment characteristics; moreover in each *E. coli* plume travelling through an aquifer, few strains would survive for a relatively long time and would not be attached, giving rise to relatively low sticking efficiencies (Hijnen *et al*., 2004).

Numerous factors have been identified to influence the flow of bacteria and viruses in groundwater. Among them are the size and the isoelectric point of virus particles, organic content of groundwater, saturated versus unsaturated groundwater flow, pH of groundwater, aquifer substrate grain size and some other size-dependent exclusion factors such as filtration and cell size, and hydrological factors such as flow velocity and the heterogeneity of the aquifer substrate (John and Rose, 2005). In addition, Pang *et al.* (2004), Roslev *et al.* (2004) and Stevik *et al.* (2004) reported that bacterial inactivation in groundwater, in absorbed or liquid phases, can be caused by native groundwater organisms.

Regarding *E. coli* transport in sand column with constant and changing water contents, Powelson and Mills (2001) stated that unsaturated flow regimes resulted in a significantly lower maximum outflow relative concentration in comparison to saturated flow regimes, although unsaturated filtration coefficient was not significantly different from that of saturated flow regimes. McClaine and Ford (2002) indicated that the reversal of flagellar rotation of *E. coli* is important in its initial attachment in dynamic system with high and low ionic strength environment.

The adverse effects of wastewater on groundwater quality and human health have been demonstrated by some studies (Kholtei *et al*., 2003; Montiel, 2004; El Kettani and Azzouzi, 2006). The urban wastewaters often are of high in nutrients concentrations (macronutrients N, P, K, Ca, Mg and micronutrients Fe, Zn, Cu, Mn) and other chemicals which can stress the bacterial populations (Thomas, 1995).

The wells analyzed are of pH ranging from acidic to alkaline. Their degree of mineralization varied from weak to strong and contained large concentrations of dissolved CO_2 (Fig. 4). Korkka-Niemi and Laikari (1994) indicated that chemical composition of groundwater is relatively close to that of the soil topping. The water acidity observed in this study is related to the soil pH of the region concerned, which is acidic (Takem *et al*., 2010). According to Chapman and Kimstach, (1996), the acidic nature of groundwater is due to the presence of organic acids in the soil as well as those of atmospheric origin infiltrated to the water. The spatial fluctuations of the electrical conductivity values (11.80-2189 μS/cm) would be according to Niquette *et al*. (2001), related to the spatial variations in the solubility of mineral soil and the importance of inputs from the soil surface resulting from human activity.

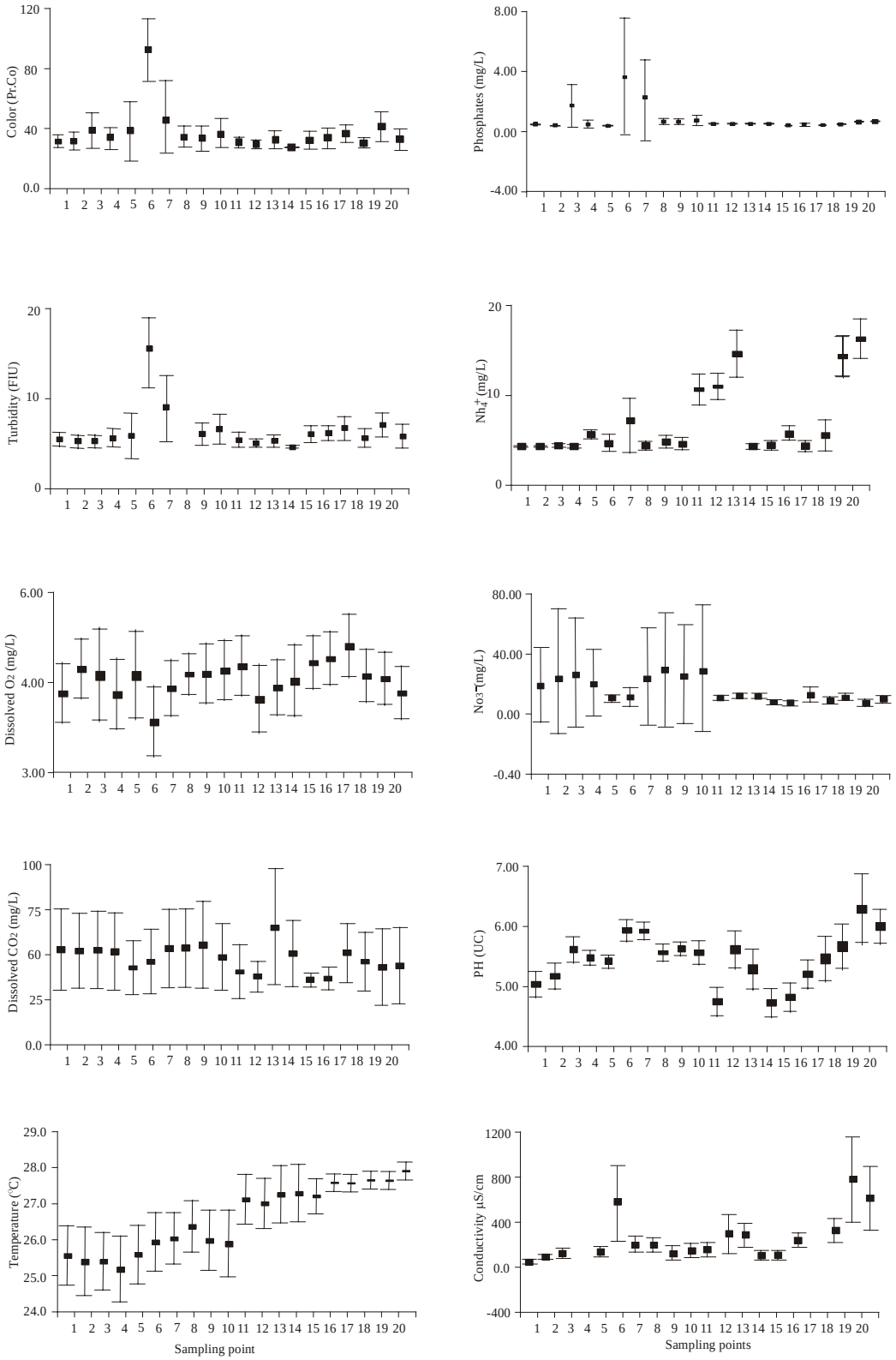

Fig. 4: Variation with respect to the sampling site of the annual mean (and standard error) of the temperature, pH, electrical conductivity, color, turbidity, and concentration of Nitrates, Ammonium, Phosphates, Dissolved Oxygen and CO_2

The concentrations 0-23.10 mg/L of nitrogen ammonia and nitrates are relatively low in most water sampling points analyzed. In general, ground waters are naturally free of nitrogen compounds. Those may derive from the decomposition of living matter by soil microorganisms that can be mineralized to molecular nitrogen or stay in very small quantities in the soil (Beauchamp, 2003). This leads to artificial increase of the combined nitrogen in the soil, creating a balance between supply and consumption producing an excess of nitrogen that is ultimately transferred to the ground.

The solubility of CO_2 and oxygen in well's water depends on the partial pressure of gases in the atmosphere, water temperature (solubility decreasing as temperature rises) and the content of electrolytes, that reduce the solubility of gases (Rodier, 1996). It is reported that low levels of dissolved O_2 enhance the inhibitory effect of CO_2 in the environment (Dixon *et al*., 1987). High concentrations can sometimes in turn hinder the development of *E. coli* in aquatic surface (Kaper *et al*., 1981). High concentration of dissolved CO_2 recorded would result from metabolic processes, mainly respiration of the water microbial flora.

WHO (2000) sets the values of turbidity to 5 NTU (FTU) for human water consumption. The turbidity values obtained in this study fully meet these standards, except for sampling points W_6 and W_7 where the average values fluctuated between 17 and 7.54 (FTU). According to GSE (2003), this is related to the presence of various organic particles, clay or colloids. The presence of these particles may have important effects on microbial quality of drinking water. Several studies showed a relationship between turbidity and the presence of microorganisms (viruses, bacteria and protozoa) in drinking water. Water with low turbidity is generally being of low microbial abundance (GSE, 2003). In our study, it was noted that the increase of turbidity in well W_6 reduced the abundance ($p<0.05$) of *E. coli* while low turbidity values seemed to favor the abundance of pathogenic strains in point W_{12}.

The water color often refers to the appearance of water that is free of suspended matter. It almost results entirely from the extraction of colored matters and decaying organic materials, as well as the concentration of dissolved chemicals such as Fe, Mn (Olanezuk-Neyman and Bray, 2000). Some of the dissolved ions can promote the bacterial growth whereas other increased the speed of cell inhibition (Pelmont, 1993).

Correlations between physico-chemical parameters and the isolation frequency of the pathogenic strains identified are not significant in most cases. The nature of the impact of some physico-chemical factors such as the turbidity varied with respect to the sampling points. According to Jamieson *et al*. (2005), this may be caused by « confusion factors » that mask the influence of parameters considered in this study and which influence the bacterial survival and its growth.

CONCLUSION

Groundwater in the region concerned harbored faecal coliforms. The presence of EPEC and ETEC is a major sanitary risk for the water users, as these bacteria are known to be responsible for many cases of diarrhea, especially in children. The persistence of these microorganisms can be attributed to human actions that occur when fetching. Public health authorities should promote public awareness for the potential danger of public water supply, while encouraging in-house treatment of water boiling and chlorination before consumption. Physico-chemical characteristics of this resource meted the international standards, and therefore could eventually become a threat for human health.

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