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Survival and Speed of *Escherichia coli* Infiltration in a Hydromorphic Soil in Wet Tropical Zone

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Authors' contributions

This work was carried out in collaboration between all authors. Author NTH designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author TE performed the statistical analysis. Authors TE and NT managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: Untreated wastewater is usually used for crop irrigation in developing countries; however it contains a lot of pathogenic bacteria. This study was carried out to determine the fate of *E. coli* contained in wastewater in a hydromorphic soil.
Study Design: Environmental microbiology
Place and Duration of the Study: This study was carried out in the experimental field of the Dschang University, during the dry season (November 2011- Mars 2012) and the rainy season (June 2012-September 2012).
Methodology: Six plots of 4 m^2 each were tilled in 400 m^2 surface area in the dry and in the rainy seasons. Wastewater was collected from the experimental wastewater treatment station in the University of Dschang; it was applied on three plots, and three other plots were used as controls. Once every week, soil samples were taken on the surface (0 - 10 cm), in the medium (20 - 30 cm) and at the water table level (40 - 50 cm). Levels of *E. coli* in soil samples were determined on "Lactose Tergitol® 7 Agar with TTC" medium, and

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biochemical confirmation tests were carried out (tests of indol, Simmons citrate, gas production, mobility, fermentation of mannitol, glucose and lactose).
Results: In the rainy season, *E. coli* was detected on the soil surface until the 112th day, while in the dry season detection did not exceed the 63rd day. *E. coli* was detected in the deeper layers of the soil (20 - 30 and 40 - 50 cm) from the 14th and the 70th day respectively. This helps to estimate the speed of vertical migration to be between 5 and 18 mm per day.
Conclusion: *E. coli* bacteria contained in urban wastewater survive for a long-time in hydromorphic soils and reach significant depths, and can consequently pose serious problems of public health.

Keywords: E. coli; hydromorphic soil; wastewater; wet tropical zone.

1. INTRODUCTION

Most African cities suffer from rural exodus, and the urban population increase rate ranges between 4 and 6% per year since 1960 [1 - 3]. The direct consequence is food insecurity and unemployment in the city. Urban agriculture partly solves this problem of food insecurity, and the immigrant’s unemployment in the city. This activity is mostly developed in swamp zones and the plots receive polluted urban water. The latter indeed constitutes one of the principal water resources used in agriculture in the developing countries [4 - 6]. Food crisis, limited access to good water, infertility of soil, and high cost of fertilizers are the main reasons which explain the use of wastewater in urban agriculture. The fertilizing capacity of this water is however a reason frequently arising from the interviews carried out from the urban market-gardeners [7, 8]. Indeed, this water contains fertilizing elements; their organic matter content contributes to the soil humus enrichment, and this water is always available [4,9]. However, these polluted urban waters are very rarely treated before use; they contain significant quantities of pathogenic microorganisms [10, 11]. Their use in agriculture causes a risk on human and environmental health, due to the capacity of these microorganisms to survive on the crops, the soil, and to migrate towards the underground water table [12]. Studies in this domain remain limited in Africa; the time of survival and vertical migration of polluting microorganisms in swamp soils in the wet tropical zone is almost unknown. Consequently, the objective of this study was the evaluation *in situ* of the duration of survival and the speed of infiltration of *E. coli* in a hydromorphic soil. More specifically, this study would like to: 1) determine the levels of *E. coli* in soil and wastewater used for irrigation, 2) follow the changes in levels over time (from wastewater inoculation period up to 4 months afterwards) and in space (to various soil depths), 3) estimate the speed of infiltration of *E. coli* in a hydromorphic soil.

2. MATERIALS AND METHODS

2.1 Presentation of the Study Zone

Geographically, Dschang is located at the 15thmeridian East, between latitudes 5º10' and 5°38' North, and between longitudes 9º50' and 10º20' East; it is found at a mean altitude of 1400 m above sea level. This town is located on the South–Western slope of the Bambouto Mountain, and dominated by low plateaus highly dissected by small valleys which are sometimes marshy. The climate is characterized by one dry season that goes from mid-

November to mid-March and a rainy season going from mid-March to mid-November. Fig. 1 shows the localisation of the Dschang municipality.

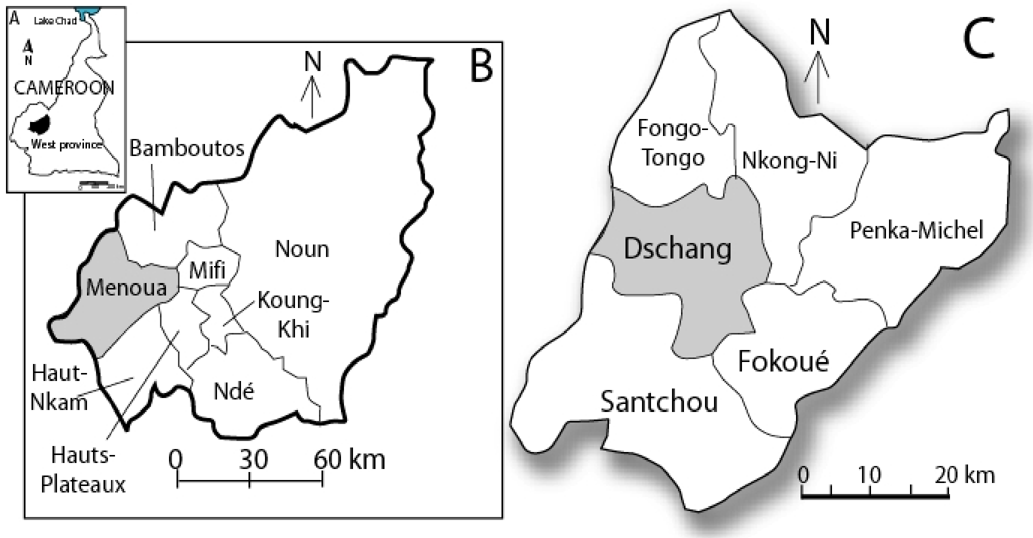

Fig. 1. Localisation of the Dschang council in the Menoua division (C), West region (B), in Cameroon (A)

2.2 Characterization of the Wastewater and Soil Used

The experimental soil, situated in a wetland in the University of Dschang Campus, was characterised. These soils remained fallow for five years. After clearing the grass, composite soil samples were collected for physico-chemical analyses. For bacteriological characterisation of soil, four surfaces (1 m^2 each) were selected randomly in the experimental surface (400 m^2), and soil samples were collected from these surfaces for analysis every week for one month (four different points on each surface). For the initial characterisation of wastewater used, a physico-chemical and a bacteriological characterisation of wastewater from the experimental treatment station in the University of Dschang, were made once every week for one month in the dry and in rainy season.

The pH of the water was measured with handhold Cyberscam PD 300 of WTW. Five days biochemical oxygen demand (BOD_5) was measured using the BOD Track^{TM} of Hach Company. Chemical oxygen demand (COD) was read using the DR2500 spectrophotometer after the dichromate digestion of the samples on the Hach DRB200™ reactor. PO_4^{2-}, $NO_3^{+}$and K^{+} were measured using RQflex plus 10 Reflectometer of Merk. *E. coli* were counted with the membrane filtration technique.

E. coli in the soil was enumerated using the method used by Malkawi & Mohammad [13] modified: 20 mg of soil was added to 180 ml of distilled water, then agitated during 2 minutes. 0.1 ml of the suspension was inoculated on the "Lactose Tergitol® 7 Agar with TTC" medium and incubated at 44°C during 24 hours. After incubation, five characteristic colonies, ie yellow colonies surrounded by a yellow halo, were recultivated on the non selective standard medium (Plate Count Agar^{TM} of Merck), in inclined test tubes, for the biochemical identification. Mannitol-Mobility medium of Bio-Rad was used to study the fermentation of mannitol by *E. coli* and its mobility; peptoned water was used to seek the production of indol; Kligler-Hajna™ medium was used to evaluate the fermentation of glucose, lactose and gas production by *E. coli*; Simmons™ medium was used to evaluate the effect "Citrate negative" by *E. coli.* Soil physicochemical characteristics were analysed on fine soil (less than 2 mm air-drying soil) according to the methods descibe by Pansu and Gautheyrou [14] (particles size by Robbinson technique, CEC by ammonium citrate extraction at soil pH, total organic carbon by iron sulfate extraction, total nitrogen by H_2SO_4 extraction, pH and electrical conductivity by 1: 2.5 (soil / solution) proportion. For residual soil moisture, 20 g of air-dried soil were weighted, dried at 105°C for 24 hours in side an oven, and weighed again, and the result is expressed as wet weight.

2.3 Experimental Steps

Six plots of 2×2 m each, randomly laid were thereafter tilled on this enclosed pilot plot of 400 m^2, in the dry season (November to March) and in the rainy season (June to September). Wastewater was applied only once to three of the plots, at the rate of 40 litres per plots. These plots subsequently received only rainwater in rainy season and drinking water in dry season (twice weekly). The three control plots also received only drinking and rain waters in the dry and rainy seasons respectively. Three soil samples from the surface (0 - 10 cm); three in the middle (20 - 30 cm) and three from the water table level (40 - 50cm) were collected immediately after application of the wastewater. Thereafter, three soil samples were taken per depth 24 hours after, and then once per week for four months per season, in the inoculation zone as well as in the control.

In order to guarantee the random and objective aspect of elementary sampling, two samples were collected per plot and per depth (Fig. 2). In order to establish the probable correlations between the microbiological content and other soil parameters during experiment, pH, electrical conductivity and the residual soil moisture were also determinated.The detection of *E. coli* was done as described above (section 2.2).

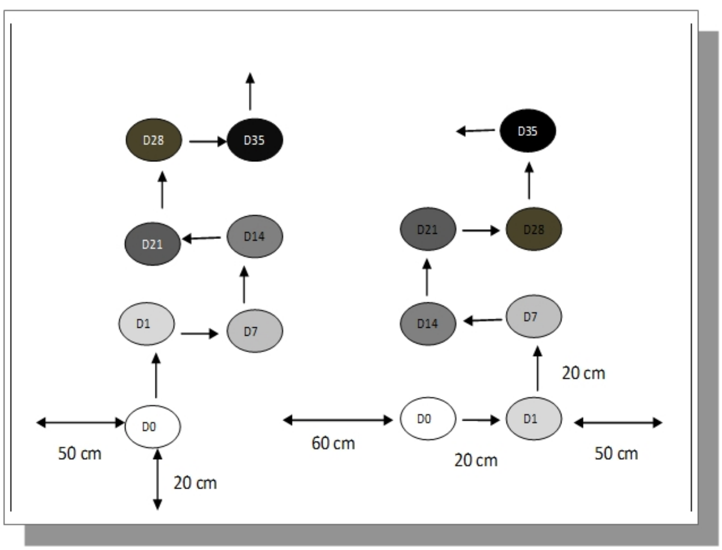

Fig. 2. Principle of displacement of the elementary sam pling, according to Vansteelant [15]

D0 = day zero, D1= day one, D7 = day seven, etc.

2.4 Statistical Analysis

Data were subjected to simple descriptive analyses and ANOVA. For that the softwares Excel and SPSS 17 for window were used. The correlation coefficients of Spearman were calculated, in order to establish the correlations which may exist between the variation of the number of *E. coli*, pH, conductivity and residual soil moisture.

3. RESULTS

3.1 Characteristics of the Soil Investigated

The hydromorphic soil profile is described in Table 1.

Table 1. Characteristics of soil horizons of the experimental site

Horizons	Characteristics
Litter	It is completely absent.
A1 horizon	It is a rich organic matter horizon, with argillaceous texture and, has a friable structure. The colour of this horizon is greyish-brown and very dull (2.5Y 3/2).
A/G horizon	This horizon can be described as transition horizon between horizon A and the pseudogley. In effect, with greyish-brown colour (2.5Y 5/2), it contains a larger quantity of clay than horizon A, with the presence of sand (sandy-clay texture). Its structure is of polyhedric fragmentary type. In the profile, one can distinguish deep greyish-blue spots, containing clay (argillaceous texture). Compared to the other horizons, clay is present but in a lesser quantity. Silt is present but in a smaller quantity. Its colour is dark greyish-green (4/10Y).
G horizon:	The grey colour, indicating the presence of iron in oxidized form (Fe^{2+}, ferrous iron), punctuated with spots of rusts (Fe^{3+}, ferric iron), and characterizes pseudogley. This horizon contains much clay, but not sand. It is very compacted. Its structure is of polyhedric fragmentary type. Its colour is greyish-green (6/5Gy).

As shown in Table 1, the soil profile is characterized as “Fairly organic hydromorphic soil” of French classification, (Humaquept of soil taxonomy, Humic Gleysol of FAO classification). The rusts spots, corresponds to iron in the oxidized state. The soil is saturated with water in the rainy season and the water level drops to a few centimetres in dry season.

Its pH is acid and it is very rich in nitrogen and organic matter (Table 2) due to the accumulated years of fallow. This soil is deficient in major mineral elements P, K, Ca, and Mg. Its content in cation exchangeable capacity (CEC) was average, and the rate of exchangeable bases was very low. The study site is in a wetland, and was protected during the fallow from surface rain waters by drainage, and is not a subject of floods. Also, during the fallow, the dominant plant on the plot was a prickly grass (*Mimosa indica*), which made the site inaccessible to the warm-blooded animals, and consequently, no *E. coli* was detected in the experimental surface area.

The initial characterization of the site soil showed no presence of *E. coli* in the site. The experimental site was closed, but some animal like small rodents could accessed. Then, each time that *E. coli* was detected in the control, the number of *E. coli* obtained from the control was subtracted from those obtained from the inoculated plots.

Table 2. Characteristics of the soil of the experimental site

Parameters	0-10 cm	20-30 cm	40-50 cm
Sand	17.31	32.34	30.15
Silt	31.96	17.96	13.35
Clay	50.73	49.70	56.50
pH water	5.33	6.54	6.71
pH KCl	4.46	6.40	6.42
Organic Carbon (%)	4.48	4.52	3.61
Total Nitrogen (%)	0.32	0.26	0.20
CEC ($cmol^+$/kg)	4.19	3.70	1.55
E. coli (CFU/g) (dry and rainy season)	not detected	not detected	not detected

3.2 Characteristics of Wastewater Applied on Experimental Plots

The wastewater used was carried from the primary treatment station in the University of Dschang. It contained levels of *E. coli* above 10^3 CFU/100 ml, is slightly basic and very rich in major fertilizing elements (Table 3). *E. coli* levels were around 8.5 log (CFU)/100ml. The content of nitrate (NO_3^-), phosphorus (PO_4^{2-}) and potassium (K^+) was 20, 238 and 69 mg/l in the rainy season, and 29, 137 and 53 mg/l in the dry season respectively. However, mineral nitrogen content of urban wastewater, after primary and secondary treatment, was generally between 20 and 60 mg/l, phosphorus between 6 and 15 mg/l and potassium between 10 and 40 mg [16]. COD is above 1000 mg/l at any season. Five days Biological Oxygen Demand (BOD_5) content was above 250 mg/l. Also, the phosphorus which is the nutrient generally present in smaller quantity in treated wastewater was the element most represented in this wastewater. This explains the malfunctioning nature of this primary treatment station.

Table 3. Characteristics of the wastewater applied on the plots (average ± standard deviation)

Parameters	Rainy season	Dry season
pH	7.90 ± 0.26	7.70 ± 0.39
NO_3^+ (mg/l)	20.00 ± 4.88	29.00 ± 7.07
PO_4^{2-}(mg/l)	238.00 ± 19.83	137.00 ± 35.48
K^+(mg/l)	69.00 ± 7.43	53.00 ± 9.11
SO_4^2 (mg/l)	48.00 ± 15.00	86.00 ± 22.24
COD (mg/l)	1005.00 ± 450.20	1369.00 ± 450.59
BOD_5 (mg/l)	316.00 ± 94.00	272.00 ± 73.80
E. coli log (CFU/100ml)	8.60 ± 0.37	8.80 ± 0.52

3.3 Survival and Vertical Migration of *E. coli*

3.3.1 Data quality

The analysis of variance (ANOVA) was used to compare the evolution of *E. coli* population in the soil. In dry season as well as in the rainy season, throughout the study period, the number of *E. coli* between 0 – 10 cm, 20 – 30 cm, and 40 - 50 cm of depth varied significantly (*P*< 0.01) with time. Throughout the study, the number of *E. coli* generally varied significantly (*P*< 0.01) with soil depth.

3.3.2 Levels of *E. coli* in the studied soil

Fig. 3 shows the levels of *E. coli* in the studied soil. The significant drop in number was observed just 24 hours after the application of wastewater into the soil. Approximately 8.8 log (CFU/100 ml) of *E. coli*, contained in the wastewater applied to the soil persisted on the surface and infiltrate progressively to reach the 20 – 30 cm depth by the end of the 14th day. *E. coli* then persisted in this band (0 – 30 cm) and by the 63rd day, it was no longer detected on the surface. *E. coli* was detected in the 40-50 cm band on the 84th day. From this moment, the number of *E. coli* decreased in the 20 - 30 cm band while it increased on the deeper depths. In the rainy season, the levels showed the same trend, except that, *E. coli* reached the greater depth (40 – 50 cm) a little earlier. Also in this season, it never disappeared on the surface and persisted in the 0 - 50 cm band of the soil. The estimated speed of migration of *E. coli* between 0 - 30 cm was around 18 mm/day. It was on the other hand slow between 30 - 50 cm (5 to 6 mm/day).

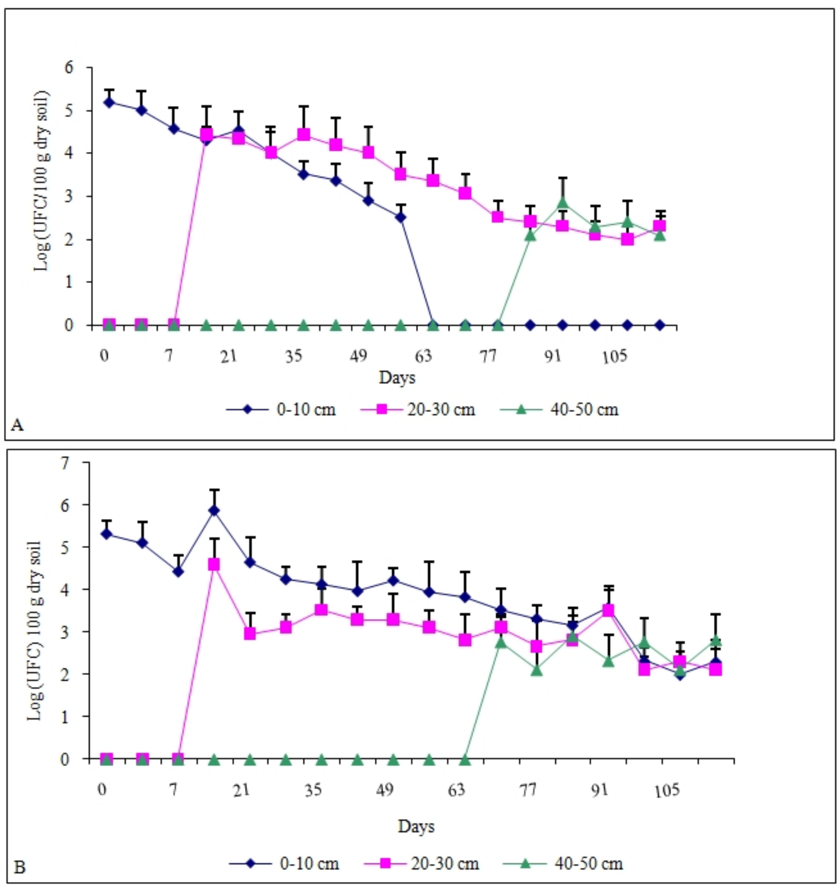

Fig. 3. Levels of E. coli as a function of time, depth during dry season (A) and during rainy season (B) (error bar = standard deviation)

3.3.3 Soil electrical conductivity, residual soil moisture and pH

In the rainy season, the electrical conductivity (EC) of the soil at 0 - 10 cm varied between 60.53 and 205.16 µS/cm; at 20 - 30 cm depth, EC was between 55.16 and 173.7 µS/cm; and at 40 - 50 cm, it varied between 57.16 and 199.33 µS/cm. In dry season in the same order, this parameter varied between 111.26 and 171.83 µS/cm; 110.23 and 176.23 µS/cm; 103.1 and 191.56 µS/cm respectively.

In rainy season, the residual soil moisture at 0 - 10 cm, 20 - 30 cm and 40 - 50 cm depths, varies respectively between 5.41 and 10.45%; 6.21 and 12.14%; 6.61 and 12.82%. In dry season in the same order, this parameter varies between 5.27 and 6.44%; 5.59 and 7.19%; 6.74 and 7.67%.

pH, in the rainy season, at 0 - 10 cm, 20 - 30 cm and 40 - 50 cm soil depths, varied respectively between 5.5 and 5.89; 4.41 and 5.82; 4, 22 and 6.08. In dry season in the same order, this parameter varied between 7.26 and 7.88; 7.22 and 7.89; 7.14 and 7.82.

3.3.4 Correlation between the number of *E. coli*, pH, electrical conductivity, and the residual soil moisture

In dry season, no correlation was observed between these parameters (Table 4). In rainy season on the other hand, a strong positive correlation was noted between the variation of the number of *E. coli* and electrical conductivity on the soil surface; a negative correlation between variation of the number of *E. coli* and pH at the 20 - 30 cm depth.

Table 4. Correlation "r" between the number of *E. coli*, pH, electrical conductivity, and the residual soil moisture, at different soil depths per season

Parameters	*E. coli* (Rainy season)			*E. coli* (Dry season)		
	0-10cm	20 – 30cm	40-50cm	0-10cm	20-30cm	40-50cm
pH	0.040	-0.492*	0.102	0.273	0.133	-0.402
Electrical conductivity	0.772**	0.126	-0.150	0.255	-0.015	0.428
Moisture	-0.300	-0.153	0.056	-0.179	-0.210	0.147

***Correlation is significant at P<0.01 (bilateral)*
**Correlation is significant at P<0.05 (bilateral)*

4. DISCUSSION

4.1 Persistence of *E. coli* in the Hydromorphic Soils

The water used for irrigation in Africa in general has contents of faecal bacteria higher than the threshold of 10^3 CFU/100 ml fixed by WHO [11, 17, 18]. *E. coli* of wastewater persisted for more than 16 weeks on the surface of the soil in rainy season and 9 weeks in dry season on a marshy soil in a wet tropical zone. According to Hartke *et al*. [19], the faecal bacteria have a survival capacity on the soil surface. But, the survival period of *E. coli* for the studied soil is much more extended. In effect, several studies showed much shorter durations (less than one month) of survival [15, 20, 21]. The long survival of *E. coli* observed here can be due to the clay texture nature of the soil, its high content in organic matter, its hydromorphic characteristic and also to the low temperature of medium. The optimal temperature of *E. coli* growth is close to 37°C. But in a rather paradoxical way, faecal bacteria survive better at low temperatures than at high temperatures [22]. In Dschang, the ambient temperature generally varies between 15 and 28°C. The soil temperature would be much lower (not analyzed here), and would have consequently also supported the survival of *E. coli*. The studied soil has more than 50 % - wt clay, conditions favourable to the survival of *E. coli.* Indeed, Cools *et al*. [23] and Ogden *et al*. [24] showed that clay contains many microhabitats, sufficient water and many protective sites. In addition to the organic matter brought by used wastewater, the studied soil has organic matter around 10 %-wt. This significant quantity of organic matter represents a significant source of nutrients for *E. coli* since it brings energy in the form of carbonaceous molecules [25]. Also, observations as those of Vansteelant [15] obtained on organic soil make it possible to think that the hydromorphic characteristics of the studied soil would have influenced the survival of *E. coli*. Indeed, the aerobic microbial activity increases with the soil water content, until water limits the diffusion and the availability of oxygen [26], returning the soil anaerobic. *E. coli* being facultative aerobic, is able to survive in flooded or saturated soils. In the rainy season, a negative correlation was established between the *E. coli* content and pH, a positive correlation between the *E. coli* and electric conductivity on the soil surface. As regards the pH, our observations are similar with those of many authors [27 - 29]. The optimal pH of *E. coli* growth lies between 6 and 7 [30], but *E. coli* are able to survive as well inalkaline pH up to 8.3, as in acidic pH (between 5.6 and 6.3) [31, 32].

4.2 *E. coli* Migrates Differently in Soil According to the Seasons

The hydromorphic soils are favourable to the survival and the vertical dissemination of bacteria in soil [33]. But, after the spread of wastewater, the *E. coli*, introduced into the soil by infiltration reaches the water table (40-50 cm) only from the 70th day, and changes a saw-teeth-like manner. Vantteland [15] obtained a shorter time: *E. coli* traverses more than 40 cm in 24 hours in a very argillaceous soil (more than 60% of clay). In dry season, one generally expects a fall at the level of the water table. This should facilitate the vertical transport of the bacteria. But *E. coli* was detected at the depth of 40 – 50 cm until the 84rd day. The slow migration observed in our case would be due to the absence of the preferential pathways of flow, caused by the roots and macroinvertebrates. In spite of the observations of Smith & Badawy [33], we think that the saturated character of this soil (observed on the field) would have prevented the vertical movement and consequently the vertical migration of *E. coli*. it is significant to note that our results are opposed to those of several authors [34-36] who obtained enhances reduction of *E. coli* in unsaturated condition. The problems of the migration of *E. coli* in the soil in general remain open, given that the observations in the field are very divergent. Indeed, according to Dorioz *et al.* [37] the vertical movement of bacteria can be carried out on profile entirety (higher than 5m); Palesse *et al*. [38] for example indicate an absence of migration of bacteria beyond 10 cm. Ndiaye [5] on the other hand detects the *E. coli* contained in up to 60 cm of depth in the dry season, and 180 cm in the rainy season in soudano-sahelianzone. Joergensen *et al*. [39] also discovered thermotolerant coliforms at 80 cm of grazed soil.

E. coli injected through the soil surface was detected at 20 – 30 cm from the 14th day and it persisted there until the end of the study, and one can pose many questions on the purification role of this argilaceous soil. Concerning the persistence in depth, the slightly anaerobic character, and the lack of nutrients in depth would have favoured this persistence. The similar observation was obtained by Oliver [40]. Also, many studies [41 - 43] have shown that the capacity of adaptation and survival of bacteria are better when the bacteria are deprived of food.

4.3 The Fate of *E. coli* in Hydromorphic Soils in Wet Tropical Climate

E. coli disappear more quickly on the surface in the dry season. This could probably be due to the bactericidal action of the sunrays, the increase in temperature at this period of the year, and also to the water deficit observed at the level of the first centimetres of the soil in the dry season, inspite of the watering frequency. Infact, in Dschang the mean sun shine from November to March is 242 hours/month, against 88 hours/month from June to September, with temperatures ranging between 13.4 - 27.5°C from November to March, and between 15 - 24.7 °C from June to September [39]. Generally, faecal bacteria survive better at low temperatures than at high temperatures [22]. Cools *et al.* [23] reported a better survival of *E. coli* in soils incubated at 5°C (68 days) versus 25°C (26 days) for the same soil. The rain through its water input increases not only survival, but also the replication of bacteria on the surface of the soil.

In the rainy season, the number of *E. coli* decreased with depth while in the dry season on the other hand, this number remained high in depth from the 42nd day. In depth, the bacteria are not only under shelter from the sun rays, but are also safe from predation. Protozoa are generally observed on the surface of the soil (10 - 15cm), and the elimination of bacteria was better in this zone of biological attachment. But this predation is equally possible in the rainy season. This observation enables us to affirm the bactericidal action of the sun rays, as well as the role of rainwater on the survival and replication on the bacteria.

The increase number of *E. coli* in the soil horizons is not accompanied by a significant drop in the bacteria population at the surface of the soil. The sum of the *E. coli* present on the surface of the soil and that pulled by percolation is generally higher than the number of bacteria introduced initially. This observation leads us to think that, the *E. coli* of wastewaters in hydromorphic soils, do not only survive but also multiply. These bacteria would have found favorable conditions for their multiplication, as moisture and especially organic matter are present.

5. CONCLUSIONS

The content of *E. coli* of urban wastewater used in this study varies between 8.6 and 8.8 log CFU/100 ml. This wastewater was used to irrigate a hydromorphic soil in the wet tropical zone of the west Cameroon. The test soil did not contain detectable levels of *E. coli.* After wastewater irrigation, in soil surface, *E. coli* survives more in the rainy season (around 16 weeks) than in the dry season (09 weeks). The purification role of hydromorphic soils is partial and *E. coli* is detected between 20 - 30 cm after 14 days, and at the level of water table, 10 weeks after the application of wastewater on the soil surface. These help to estimate the *E. coli* migration speed between 5 and 18 mm per day. This can lead to serious public health problems, given that the underground water constitutes the principal source of drinking water for many families. Also, plants can be contaminated by water splashes from the surface of the soil, or even in depth for the plants like carrots. The preliminary treatment of this water before their use for the irrigation of the cultures is the solution which is advised. Unfortunately African cities suffer from an insufficiency and dysfunction of the purification stations. Sensitization of consumers and of market-gardeners on the risks related to this practice, the prohibetion of uncook crops are the most suitable solutions.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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