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The Growth of *Escherichia coli* in Soil Layers Separating the Soil Surface from the Underground Water Table, in Central Africa: The Hierarchical Influence of the Soil Chemical Characteristics

[1]Moïse Nola, [1]Olive V. Noah Ewoti, [1]Mireille E. Nougang, [1]Luciane M. Moungang, [2]Nour-Eddine Chihib, [2]François Krier, [2]Jean-Pierre Hornez and [1]Thomas Njiné
[1]University of Yaounde 1, Faculty of Sciences, Laboratory of General Biology, Hydrobiology and Environmental Research Unit, P.O. Box 812 Yaounde, Cameroon
[2]Université des Sciences et Technologies de Lille 1, IUT « A », Département de Génie Biologique, Laboratoire Pro Bio GEM, Bd Paul Langevin-Cité Scientifique, B.P. 179-59 653 Villeneuve d'Ascq cedex, France

Abstract: The study was carried aimed at assessing the future of *E. coli* during its transfer through soil layers containing Biodegradable Organic Carbon (BOC) and to classify in order of hierarchy some soil chemical factors that may influence this process from soil surface to the underground water table. A circular hole was dug from the soil surface to the underground water table. This hole crossed 8 different soil layers which were named H_1, H_2, …, H_8 from the soil surface to the underground water table. Experiments were carried out with soil particles with or without BOC. In the absence of BOC, cell retention occurred in all soil layers used. In the presence of BOC, cell retention also, initially occurred and cell growth occurred with a delay which varied from one soil layer to another. Bacterial cell growth from soil layers closer to the surface to those in contact with the underground water table was 1.34, 1.23, 1.22, 1.01, 1.02, 0.86, 0.80 and 0.75/h, respectively. Using the sum of power law function, it was noted that the most 3 factors controlling the cell growth rate in most soil layers are Fe, K, Si, Al and N. The factor K influences in all soil layers. Si also influence in all soil layers with H_1 excepted. Fe mostly influences in the soil layers H_1, H_2, H_4 and H_6. The factor Al and the factor N mostly influence in the soil layers H_1 and H_3 and in the soil layers H_5, H_7 and H_8 respectively. During wastewater infiltration through soil layers, bacterial pollutants would be retained on the particles of soil. This retention may be offset by cell growth if the water contains BOC. The process is controlled by various chemicals of the soil, in different magnitude with respect to the soil layer.

Key words: BOC, chemical, dominant factor, *E. coli*, growth rate, retention, soil layer

INTRODUCTION

In many regions around the world, underground water remain one of the main source of drinking water, especially in rural than in urban areas. It has become evident during the past decades that protection of water resources must be a national priority in the future if continuous economic and technological development is expected. Since many effluents contain considerable quantities of bacteria, including some pathogenic species, it is indispensable to know their impact in the environment and their potentials to contaminate both surface and subsurface water supplies.

Its recharge can proceeds by infiltration of runoff or surface water. The risk of its chemical and biological pollution depend on the characteristics of surface runoff on one hand, and on the soil layers properties on the other hand. Its microbiological quality is also linked to the ability of soils to retain micro-organisms during water infiltration. This retention occurs in most cases by adsorption and proceeds by cell adhesion to the soil surface particles (Jucker *et al*., 1998). This process is sometimes reversible, and evolves at times due to cell activity and the modification of bacterium wall properties (Rijnaarts *et al*., 1995; Yee *et al*., 2000). The adsorption often increases with the bacterial wall hydrophobicity and decreases with its hydrophilicity (Van der Mei *et al*., 1998). Its relative stability depends on the number and properties of functional group sites on the bacterial surface, this number can vary with the chemical

Corresponding Author: Moïse Nola, University of Yaounde 1, Faculty of Sciences, Laboratory of General Biology, Hydrobiology and Environmental Research Unit, P.O. Box 812 Yaounde, Cameroon, Tel.: (+237) 99 43 26 39

conditions of the medium (Fein *et al*., 1997). This phenomenon is also influenced by the mobility of bacteria and solid particles (Camesano and Logan, 1998), chemical factors such as pH, ionic strength, dissolved minerals and the properties of bacterial species (Fowle and Fein, 2000; Yee *at al*., 2000).

Most studies have indicated the pollution of underground water resource by faecal bacteria such as *Escherichia coli* and *Streptococcus feacalis*, and by opportunistic bacteria such as *Aeromonas hydrophila* and *Pseudomonas aeruginosa* (Nola *et al*., 2002). The presence of such organisms is an indication of the existence of pathogenic bacteria such as *Salmonella typhi* and *Vibrio cholerae* which can cause health hazards.

In most African countries, wastewaters containing Biodegradable Organic Compound (BOC) are often discharged to the environment without any pre-treatment, and therefore, can potentially pollute soil and underground water. Numerous cases of infection due to underground water contamination by microorganisms are often reported around the world. Few data are available on the influence of individual soil layers in the transfer of bacteriological pollutants when water infiltration contains the BOC. Little is known on the result of bacteria-BOC-soil particles interactions. Little is also known on the magnitude of the influence of soil chemical properties in bacterial growth rate or inhibition rate during its transfer through the soil column. The main purpose of this study was to assess the future of *E. coli* (feacal coliform bacteria) during its transfer through soil layers containing BOC and to classify in order of hierarchy some soil chemical factors that may influence this process from soil surface to the underground water table.

MATERIALS AND METHODS

Soil collection: The Yaoundé region (Cameroon, Central Africa) is located at latitude 3º 52’ N, longitude 11º32’ E with an average altitude of 760 m. The climate is typically equatorial with 4 seasons (Suchel, 1988): a mild rainy season from March to May, a mild dry season from June to August, an intense rainy season from September to November and an intense dry season from December to February. Its soil is ferro-lateritic, acidic with pH values generally lower than 6 (Bachelier, 1959). The sand content sometimes reaches 73% near the soil surface and gradually decreases as soil depth increases. The soil porosity sometimes reach 71% with a density of 2.7. The vertical permeability of the soil attains 300 cm/h near the surface and its horizontal permeability undergoes spatial variation and reaches 40 cm/h (Suchel, 1988; Bachelier, 1959). Underground water in this region is contaminated by faecal bacteria and other opportunistic pathogens (Nola *et al*., 2001, 2002).

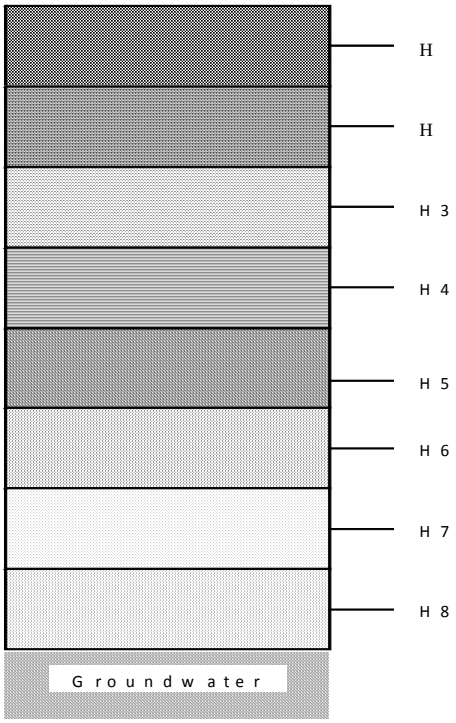

Fig. 1: Diagram showing soil layer sequences from the soil surface to the underground water table (the thickness of the different soil layers has not been considered in this diagram)

A circular hole of 180 cm of diameter on a site situated out of the urban area was dug until the appearance of the underground water table. This hole measured 914 cm deep and crossed 8 different soil layers. These layers were named from top (surface) to bottom (groundwater table) as H_1, H_2, H_3, …, H_8. Layer H_1 was in relation with the soil surface and layer H_8 was in relation with the water table. Figure 1 shows the sequence of different soil layers, from the soil surface to the underground water table. Two to three kg of samples from each of the soil layers were collected and dried in the laboratory at room temperature (23±2ºC) for 12 months.

Physical and chemical properties of soil layers: The thickness of soil layers were measured using a meter-tape. Some macroscopic characteristics of soil layers were determined. Soil colours were determined using Munsel code (Macbeth Division of Kollmorgen Corporation, 1975). The chemicals analysed include pH, Water Retention Capacity (WRC), organic carbon (C), total nitrogen (N), total phosphorus (P), potassium (K), silicon (Si), aluminum (Al) and iron (Fe) in each soil layer. After mineralization of soil samples according to triacid technique attack (Njopwouo and Orliac, 1979), chemical analyses were then carried out according to Demolon and Leroux (1952), Rodier (1996) and Beck *et al*. (2000).

Isolation of *E. coli:* The faecal bacterium *E. coli* was isolated from the Mfoundi stream (Cameroon). Physico-chemical properties of this stream were assessed using standard methods (APHA, 1998). The temperature was 22ºC and the pH 6.96. Concentrations in nitrogen ammonia, dissolved O_2, dissolved CO_2, total dissolved solids, total suspend solids were 1.6, 2.4, 98, 373 and 280 mg/L, respectively. The values of electrical conductivity, turbidity and biochemical oxygen demand in 5 days were 488 µS/cm, 370 FTU and 372 mg/L respectively.

E. coli was isolated using a membrane filtration technique on the Endo agar culture medium (Biokar) (Marchal *et al*., 1991; APHA, 1998). It was then identified by the usual biochemical criteria (Holt *et al*., 2000). Colony Forming Units (CFUs) were then obtained after culture on standard agar medium (Biokar).

Preparation of solutions, bacterial storage and inoculation: Glucose was used to mimic in a simplified manner the presence of the BOC, as often found in wastewater discharged without any treatment in the environment (Njine *et al*., 2000). A 99 mL of glucose solution (20 mg/L) was prepared in the glass flasks. The pH value was then adjusted at 7.0, and the flasks were sterilized.

For the preparation of stocks of bacteria, the CFUs from standard agar medium were inoculated into 100 mL of nutrient broth (Oxford) for 24 h at 37ºC. After, cells were harvested by centrifugation at 8000 rpm for 10 min at 10ºC and washed twice with NaCl (8.5 g/L) solution. The pellet was re-suspended in 25 mL of NaCl solution and then transferred into 300 µL tubes. The stocks were then stored in frozen conditions.

Prior to the experiments, the stock frozen vial containing *E. coli*, was thawed at room temperature. The culture (300 µL) was then transferred into 10 mL of nutrient broth (Oxford) and incubated at 37ºC for 24 h. After, 100 µL of suspension was added to 100 mL of the same nutrient broth and also incubated for 24 h at 37ºC. The cells were then harvested by centrifugation at 8000 rpm for 10 min at 10ºC and washed twice with sterile NaCl (8.5 g/L) solution. The pellet was then re-suspended in 10 mL of sterilized solution containing NaCl solution.

Assessment of the *E. coli* growth: During the preliminary analysis, investigation and isolation of *E. coli* in all considered soil layers using Endo agar medium were negative, showing that none of these did contain this species before the experiments. After serial dilutions using the sterile NaCl solutions, 1 mL of the bacterial suspension was added to 99 mL of the solutions containing BOC. Based on our preliminary studies, cells concentration was adjusted at 2.9×10^6 CFU/mL. A weight of 500 mg of soil from a specific layer dried as indicated above and crushed was added to this solution [soils were not sterilized because their exposure to a high temperature or a high pressure for sterilization can destroy the physicochemical properties of their mineral constituents (Nguetnkam, 1993)]. It was then incubated on a shaker GLF 3018 at a speed of 10 rpm for 5 h at room temperature. This speed was chosen to mimic in a simplified manner the water movement through the soil during percolation (Banton and Bangoy, 1997). The incubation duration was chosen based on the preliminary results which showed that it was sufficient to promote the saturation of surface sites on soil particles.

From the instant t_0, the bacteriological analysis to determine the concentration of planktonic cells was performed at 15 and 30 min, and then every 30 min until the end of the incubation period. Planktonic bacterial abundance in the solution was also determined using plate count method on Endo agar medium, and then incubated at 43±1ºC for 24 h. It was expressed as CFU/mL. The analyses were carried out in triplicates.

During the first part of these experiments just after the preliminary analysis, the same protocol as indicated above was carried out, in the presence of the same cell inoculums and soil particles, but in the absence of BOC. This method was adapted from the techniques proposed by other authors (Miller *et al*., 2001; Wang *et al*., 2002).

Data analysis: In each case, the experiments were carried out in triplicates. For each replicate, chemical analysis was performed on the sample of soil particles from each layer used. The variations in average values of chemical parameters with respect to the soil layers have been illustrated by histograms and curves. The standard deviations have not been mentioned on the graphs because some curves are too close.

The Cell Growth Rates (CGRs) were assessed during the periods corresponding to the increase in the bacterial cells abundance in the presence of BOC. The straight lines Ln(number of CFUs) against incubation durations were plotted during the period in which the increase in cells abundance was observed. Each straight line equation $Y = ax + b$ was calculated. The slope *a* of each regression line was considered as the CGR in each experimental condition. The variation of CGR values with respect to the soil layers was also illustrated.

Using the three values from triplicates, a ranking in order of hierarchy of the chemical parameters considered in their impact in changing the CGR in each soil layer *Hi* has been carried out. This was done using a sum of power-law function of the following form (Guerard, 1976; Lebart *et al*., 1982):

$$F\left(CGR, Hi\right) = \sum_{i=1}^{9} ai.xi^{ni}$$

Table 1: Some physical characteristics of moist soil layers

Soil layer		Characteristics
Code	Thickness (cm)	
H_1	33	Blackish brown colour, crumb structure, sandy-clay texture
H_2	113	Reddish brown colour, prismatic sub-angular to polygonal structure, plastic clayey texture
H_3	148	Vermicular brown-yellowish, patches plastic-clayey texture, polygonal structure
H_4	105	Great quantity of brown reddish nodules with purple fissures, clayey texture
H_5	160	Yellowish brown patches, clayey texture, low percentage of nodules
H_6	205	Brown nodules with blackish brown to purple red fissures, yellowish patch, red matrix with a clayey-silt texture and polygonal angular structure
H_7	115	Yellowish brown material, appearance of whitish patches cracked and translucent quartz grains, polyedric structure
H_8	35	Whitish to yellowish clayey silt patches, purple red to dark brown patches, quartz grains and quartzo-feldspathic beddings, little compact material, little or no preserved structure

Table 2: Expression of the power-law function indicating the exponential and proportionality coefficients of each considered chemical factor in the determination of CGR in each soil layer

Soil layer	Expression of the sum power-law function								
$F(CGR, H_1) =$	$0.294Fe^{1.293}$	$+0.798K^{1.184}$	$+0.065Al^{1.081}$	$+0.371Si^{0.817}$	$+0.554WRC^{-0.600}$	$-0.631N^{-1.665}$	$-2.143pH^{-4.026}$	$-10.029P^{-30.505}$	$-10.473C^{-32.225}$
$F(CGR, H_2) =$	$0.882K^{1.212}$	$+0.548Si^{1.149}$	$+0.100Fe^{1.075}$	$+0.315Al^{0.866}$	$+0.114N^{0.530}$	$-2.753pH^{-4.947}$	$-5.782C^{-13.759}$	$-15.776P^{-50.701}$	$-25.841WRC^{-94.337}$
$F(CGR, H_3) =$	$0.917Si^{1.093}$	$+0.978K^{1.056}$	$+0.677Al^{0.841}$	$-1.093Fe^{0.350}$	$+0.433N^{0.290}$	$-1.152C^{-1.932}$	$-1.777pH^{-3.314}$	$-14.479P^{-49.499}$	$-21.882WRC^{-82.597}$
$F(CGR, H_4) =$	$0.664Si^{1.145}$	$+1.033K^{1.004}$	$-0.075Fe^{0.871}$	$+0.433Al^{0.708}$	$+0.128N^{0.527}$	$-2.296pH^{-3.945}$	$-2.465C^{-4.372}$	$-18.221P^{-61.699}$	$-27.115WRC^{-101.402}$
$F(CGR, H_5) =$	$0.696K^{1.302}$	$+0.591Si^{1.289}$	$+0.431N^{1.209}$	$+0.137Fe^{1.073}$	$+0.043Al^{0.846}$	$-2.590pH^{-4.142}$	$-3.202C^{-5.677}$	$-17.699P^{-56.635}$	$-33.523WRC^{-126.574}$
$F(CGR, H_6) =$	$0.549Si^{1.244}$	$+0.899K^{1.207}$	$+0.022Fe^{1.090}$	$+0.333N^{1.077}$	$+0.103Al^{0.848}$	$-3.400C^{-3.786}$	$-2.748pH^{-4.627}$	$-20.804P^{-70.125}$	$-32.845WRC^{-124.284}$
$F(CGR, H_7) =$	$0.741K^{1.264}$	$-0.220Si^{1.214}$	$+0.933N^{1.158}$	$+0.269Fe^{1.110}$	$+0.151Al^{0.854}$	$-2.740C^{-4.282}$	$-3.042pH^{-5.018}$	$-14.877P^{-44.353}$	$-38.803WRC^{-149.277}$
$F(CGR, H_8) =$	$0.673K^{1.305}$	$+0.605Si^{1.291}$	$+0.837N^{1.266}$	$+0.033Fe^{1.103}$	$+0.060Al^{0.816}$	$-2.792C^{-4.531}$	$-3.033pH^{-5.131}$	$-22.033P^{-74.151}$	$-37.483WRC^{-144.310}$

In this Eq. (9) chemical factors were considered. *Xi* is the factor considered. *ni* is its power-law coefficient (scaling exponent or exponent coefficient), and *ai* is its coefficient of proportionality (or constant of proportionality). The calculations of the coefficients were performed by iterative method. This power-law function was performed using MATLAB 7.5.0 program.

According to the model used, the power coefficient (*n*) gives to the factor concerned more impact than that of the coefficient of proportionality (*a*). If *n* is greater than 1, the power-parameter is multiplied by itself *n* times on the changes in the CGR value. If it is equal to 1, it reflects a consistency in the behavior of the factor on the changes in the CGR value. If *n* is zero, the factor considered has no effect on the process studied. If it is less than 1 but greater than zero, the power of the chemical factor is the square root of the inverse of the value of *n*. If *n* is less than zero (i.e., negative), the effect of the chemical factor on the process studied is the inverse of its power multiplied by itself *n* times. The coefficient of proportionality (*a*) is a multiplication factor and its sign (negative or positive) indicates the right direction of the effect of the chemical factor.

RESULTS

Chemical characteristics of soil layers: Physical properties of the soil layers are summarized in Table 1. Their thicknesses varied from 33 to 205 cm. The highest thickness was recorded in layer H_6 and the lowest was recorded in H_1 (Table 1). All layers have an acid pH. Its value decreases from 5 in layer H_1 to 4.2 in layer H_5, then increases to 4.6 in layer H_8 (Fig. 2). The concentration in organic carbon was 18.10 mg/g of soil in layer H_1, 8.8 mg/g of soil in layer H_2, and 4.3 mg/g of soil in layer H_8 (Fig. 2). The concentration in carbon was relatively high in the layer H_1 and relatively low in the deeper layers (Fig. 2). The concentration of Fe was 0.35 mg/g of soil in layer H_1, 0.6 mg/g of soil in layer H_2, and 0.9 mg/g of soil

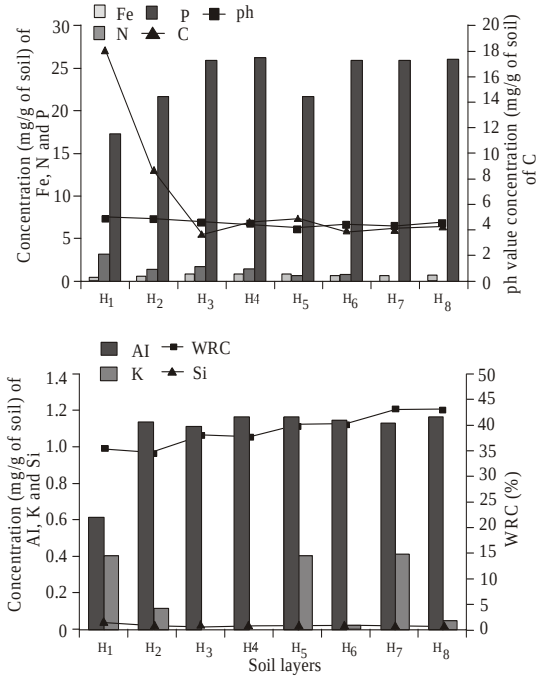

Fig. 2: Variation of pH value and concentrations of Fe, N and P (A), and WRC and the concentrations of Al, K and Si (B), with respect to soil layers

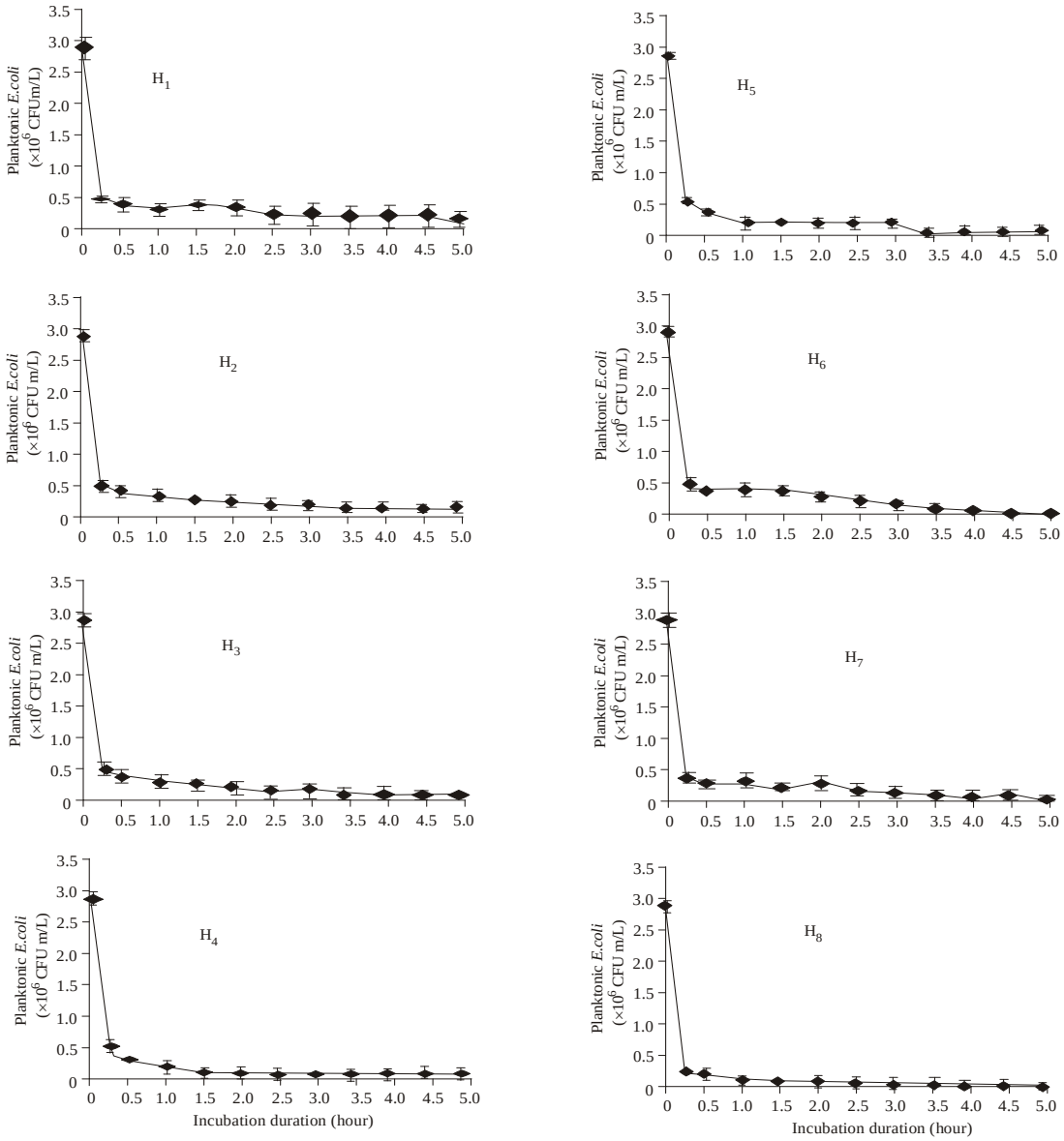

Fig. 3: Temporal variation of the mean values of the abundance of planktonic *E. coli* in the medium without BOC but containing the soil particles from layers H_1, H_2, H_3, H_4, H_5, H_6, H_7 and H_8

in layer H_3. From layers H_3 to H_8, it decreases gradually and attained 0.7 mg/g of soil (Fig. 2).

The concentration in phosphorus was 17.4 in the layer H_1, and 21.8 mg/g of soil in layer H_2. The highest value (26.1 mg/g of soil) was recorded in layers H_3, H_4, H_6 and H_8 (Fig. 2). The highest value of total nitrogen (3.2 mg/g of soil) was recorded in the soil layer H_1, and the lowest (0.1 mg/g of soil) in H_7 and H_8 (Fig. 2). It decreased from the layers nearer the soil surface to that in relation with the underground water table. The WRC increased from 34.88% in soil layers nearer the soil surface to 43.64% in that nearer the underground water table (Fig. 2). Concentration in aluminum was 0.62 mg/g of soil in layer H_1. From layers H_2 to H_8, it varied from 1.13 to 1.18 mg/g of soil. The highest concentration was recorded in H_4 and H_8. The potassium concentration varied from 0.01 mg/g to 0.41 mg/g of soil. The lowest values were recorded in layers H_3 and H_4, and the highest in layers H_1 and H_7 (Fig. 2). The concentrations of Si were 1.3 and 0.7 mg/g of soil in layers H_1 and H_2, respectively. These values were relatively constant from layers H_3 to H_8 (Fig. 2).

Cell retention and Cell Growth Rate (CGR): During the first part of the experiment (in the absence of BOC), the decrease in planktonic cells concentration was

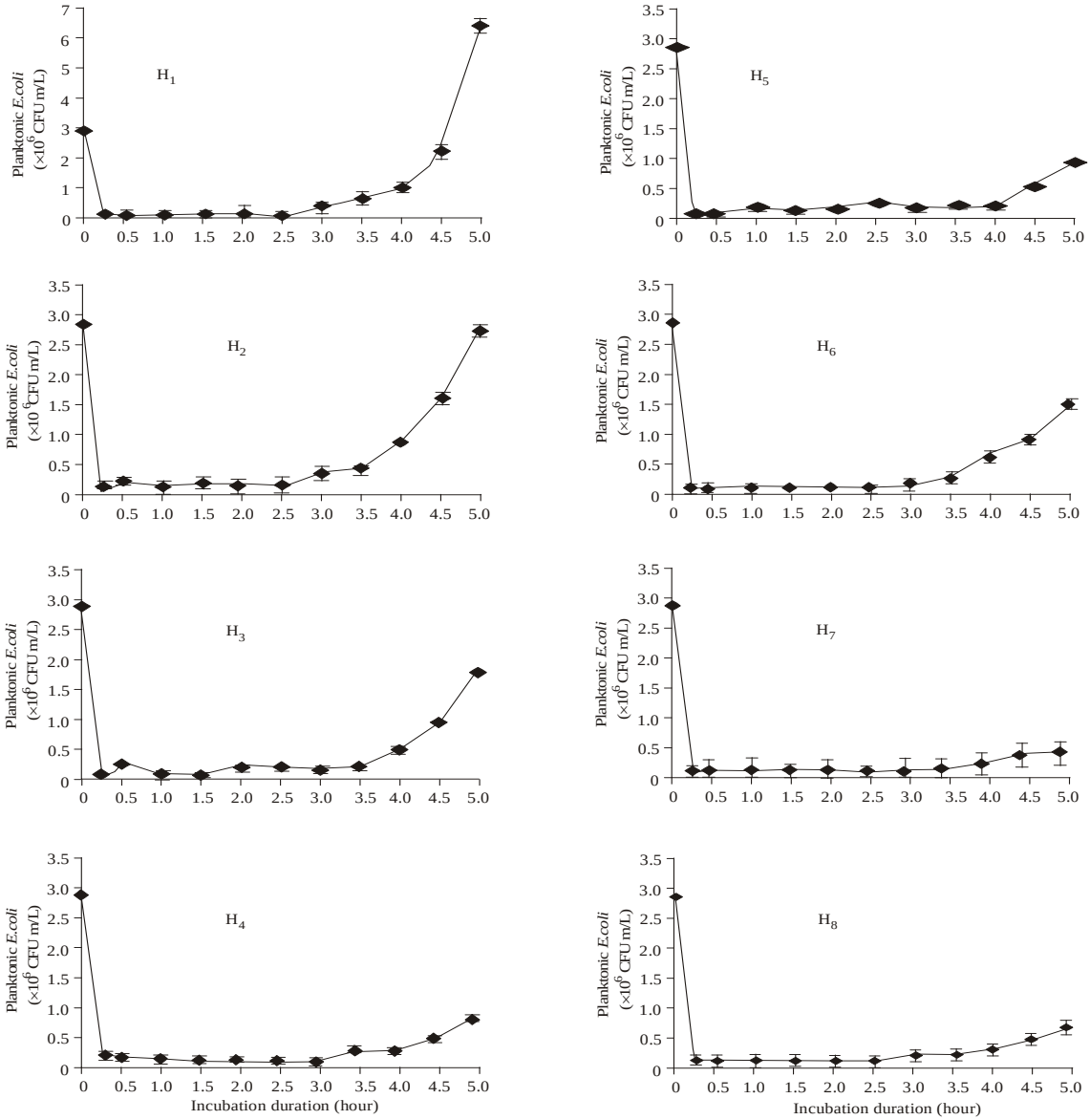

Fig. 4: Temporal variation of the mean values of the abundance of planktonic *E. coli* in the medium containing BOC and soil particles from the layers H_1, H_2, H_3, H_4, H_5, H_6, H_7 and H_8

observed (Fig. 3). This expresses cell sorption on soil particles. Adsorption of microbial cells on geological particles seems to swiftly take place during the 15 or 30 min that follow bacterial switch on soil particles in the aqueous phase (Fig. 3). This was noted from concentration of planktonic cells which decreases quickly during the first 30 min after incubation. After this period, concentration of non-attached bacteria decreases slowly, while undergoing in some cases fluctuations that are sometimes of important magnitude. Variation in the number of sorbed cells indicated the reversibility of bacterial process on soil particles. (During preliminary analysis, blank sorption experiments were performed in the absence of soils, and no variation occurred in bacterial concentration. It was concluded that all observed changes in bacterial concentration in the presence of soils could be attributed to sorption by soil particles).

In the second part of the experiment, cells were suspended in a solution containing the BOC at a concentration of 20 mg/L. It was noted in the first instance that, there was a rapid decrease in the planktonic cells concentration due to their retention on soil particles (Fig. 4) as noted in the first series of experiments. After, an increase in the number of planktonic bacteria was noted in all cases (Fig. 4).This increase often seems gradual and lead in some cases (layers H_1 and H_2) after 5 h of incubation to planktonic cells abundance greater than the cell concentration introduced at the beginning.

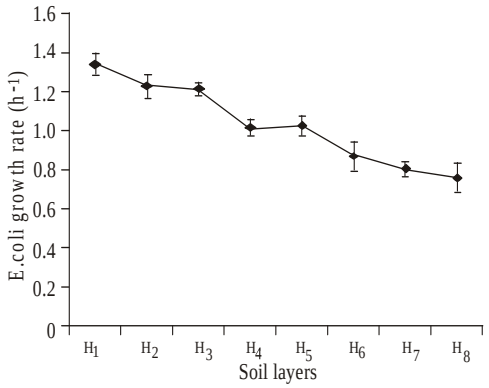

Fig. 5: Variation of the growth rate values with respect to the soil layers

The increase in the number of planktonic cells begins approximately after 2.5 h of incubation in the layers H_1, H_2 and H_6 (Fig. 4). In the soil layers H_3 and H_4, the increase began after 3h of incubation. It began after 3.5 h in layers H_5, H_7 and H_8. After 5h of incubation, the planktonic bacterial concentrations were 6.6×10^6, 2.8×10^6, 1.8×10^6, 0.8×10^6, 0.96×10^6, 1.52×10^6, 0.4×10^6 and 0.7×10^6 CFU/mL with soil particles from the layer H_1, H_2, H_3, H_4, H_5, H_6, H_7 and H_8, respectively (Fig. 4).

In the presence of BOC, the growth rates were assessed during the periods in which the increase in cells abundance was observed. The straight lines Ln(number of CFUs) against incubation duration were plotted during these periods. Each straight line equation was calculated. The slope of each regression line has been considered as the cell apparent growth rate (CGR) in each experimental condition. It was observed that growth rate values of *E. coli* varied from one soil layer to another (Fig. 5). The greatest value (1.343/h) was noted in soil layer H_1 and lowest value (0.755/h) was recorded in soil layer H_8 (Fig. 5). In soil layers H_2, H_3, H_4, H_5, H_6 and H_7, CGR values were 1.229, 1.217, 1.014, 1.024, 0.865 and 0.798/h, respectively. The CGR values decreased gradually from the soil layers in relation to the soil surfaces in contact with the underground water table (Fig.5).

Assessment of the influence of soil chemical properties: A ranking in the order of hierarchy of the considered chemical factors on their impact in the changes of CGR values in each soil layer was performed using the sum of power-law function. The power-law coefficient (n) and coefficient of proportionality (a) of each of the considered factors on each sorption parameter, in each soil layer are indicated in Table 2. It appeared that in the layer H_1, the CGR is mainly controlled firstly by Fe (n = 1.184, a = 0.294), secondly by K (n = 1.184, a = 0.798), thirdly by Al (n = 1.081, a = 0.065), and fourthly by Si (n = 0.817, a = 0.371). In layer H_2, it is mainly due to K (n = 1.212, a = 0.882), followed by Si (n = 1.149, a = 0.548), Fe (n = 1.075, a = 0.100), and finally Al (n = 0.866, a = 0.315). In H_3, CGR is mainly due to Si (n = 1.093, a = 0.917), followed by K (n = 1.056, a = 0.978), Al (n = 0.841, a = 0.677) and finally Fe (n = 0.350, a = 1.093). In the layer H_8 in contact with the water table, it is mainly controlled by K (n = 1.305, a = 0.673), followed by Si (n = 1.291, a = 0.605), N (n = 1.266, a = 0.837) and then by Fe (n = 1.103, a = 0.033). It is noted that for the 8 soil layers, the growth rate of *E. coli* is mainly controlled by iron, potassium and silicon. In the layers H_2, H_3 and H_4 on one hand and H_5, H_6, H_7 and H_8 on the other hand, the additional main factors are aluminum and nitrogen respectively. However, the power of each factor varies from one layer to another (Table 2).

In H_4, CGR of *E. coli* is predominantly influenced by Si (n = 1.145, a = 0.664), followed by K (n = 1.004, a = 1.033), Fe (n = 0.871, a = 0.075), and finally Al (n = 0.708, a = 0.433). In soil layer H_5, it is mainly due to K (n = 1.302, a = 0.696), followed by Si (n = 1.289, a = 0.591), N (n = 1.209, a = 0.431) and Fe (n = 1.073, a = 0.137). In layer H_6, CGR is dominantly controlled firstly by Si (n = 1.244, a = 0.549), secondly by K (n = 1.207, a = 0.899), thirdly by Fe (n = 1.090, a = 0.022), and fourthly by N (n = 1.077, a = 0.333). In H_7, it is mainly caused by K (n = 1.265, a = 0.741), followed by Si (n = 1.214, a = 0.220), N (n = 1.158, a = 0.933) and Fe (n = 1.110, a = 0.269) (Table 2).

DISCUSSION

During the first part of the experiments (in the absence of BOC), cell retention occurs during the full period of the experiments (Fig. 3). Cell sorption swiftly took place during the first 30 min following the introduction of bacteria in the aqueous medium containing soil particles. This swift sorption was also noted during the first 45 min of the experiments by Scholl and Harvey (1992). The partition of this process in swift initial phase and late slow phase would be related to the passivity of initial phase and the energy-dependence of late phase. According to Simoni *et al*. (1998), adsorption process is less energy-dependent indeed passive at the beginning, and turns to more energy-dependent phenomenon as incubation duration becomes longer, probably due to the restriction in the number of sites of bacterial sorption.

It has been indicated that bacterial sorption to surfaces is due to adhesin which is a hydrophobic protein, located at the cellular surface or in the cytoplasm (Nikolaev, 2000). Many interactions have been indicated to be among adhered bacteria and they can significantly influence their structure and their physiology (Millsap *et al*., 1998). These interactions which are sometimes proteins-mediated have also been observed between bacteria and yeasts. Yeasts adheres weakly to

particles due to their large sizes (Millsap *et al*., 1998). Interactions also exist between soil particles that are invariant and bacterial cell surfaces which depend on the bacterial physiological condition (Grasso *et al*., 1996). They often lead to the sorption reversibility (James *et al*., 1995), and would be as an origin to the irregular fluctuations of the number of sorbed *E. coli* sometimes observed during experiments (Fig. 3), changing from planktonic to the adhered state, and conversely exerting a regulation on some genes, although the nature of the signal sent to genes when a cell is attached is not clear (Brozel *et al*., 1995; O'toole *et al*., 2000).

In the presence of BOC, cell retention on soil particles was noted in the first instance, as seen in the first series of experiments. After, an increase in the number of planktonic bacteria was noted in all cases (Fig. 4). The growth rate value varied from one soil layer to another (Fig. 5). Many factors influence this cell growth process at different magnitudes. A relative variation of dominant factors with the changes in soil layer is observed along the soil column (Table 2). At an elementary level, the nutritional requirements of a bacterium such as *E. coli* are revealed by the composition of elements of the cell, which consists of C, N, P, K and Fe. Each element serves either a structural or functional role in the cells (Todar, 2010). For example, K serves as the main cellular inorganic cation and cofactor for certain enzymes and is present in the soil in mineral or organic form. The mineral sometime can be mobilized by two biological processes: the biological weathering such as silicates containing potassium and the shifting of the equilibrium between fixed and exchangeable forms (Dommergues and Mangenot, 1970). Phosphorous serves as a constituent of nucleic acids, nucleotides, phospholipids and teichoic acids. Nitrogen serves as constituent of amino acids, nucleic acids nucleotides, and coenzymes, carbon serves as the main constituent of cellular material.

In most soil layers, the main five factors which controls the growth rate are Si, K, Fe, Al and N (Table 2). Silicon compounds may stimulate the population of oligotrophic bacteria when added to soil, with the number increasing with increasing quantity of added silicon compounds (Al-Falih, 2003). Their presence in the environment may also increase the growth rate of most bacteria (Al-Falih, 2002). According to Wainwright *et al*. (2003), silicic acid can increase the numbers of both aerobic and facultative anaerobic bacteria under oligotrophic conditions.

Iron in the bacteria cell serves as a component of cytochromes and certain nonheme iron-proteins and a cofactor for some enzymatic reactions. Sritharan (2006) noted that the solution and reduction of iron by bacteria take place in the soil, and is the result of an increase in hydrogen ion concentration due to acid formation during the decomposition of organic matter and the removal of oxygen by metabolic processes. Under certain conditions, low iron levels have been shown to induce the expression of a number of bacterial toxins and virulence factors (Sritharan, 2006).

Soil contains aluminum in a variety of forms such as aluminosilicate minerals, and exchangeable or soluble A1. Aluminum ions are highly soluble in acidic environments. However, as soils become more acidic (due to natural weathering processes and anthropogenic factors), aluminum becomes more soluble and potentially toxic to microorganisms (Wood, 1995). This effect may increase with increasing environmental temperature (Illmer and Mutschlechner, 2004), and with a decrease in environmental pH (Rousk *et al*., 2009).

The pH is ranked at the 6^{th} or the 7^{th} dominant factor (Table 2). One of the most influential factors affecting the microbial community in soil is pH. It influences highly abiotic factors, such as carbon availability, nutrient availability, and the solubility of metals (Aciego Pietri and Brookes, 2008; Flis *et al*., 1993; Kemmitt *et al*., 2006). In addition, soil pH may control biotic factors, such as the biomass composition of fungi and bacteria (Fierer and Jackson, 2006). The effect of soil pH on bacteria activity, growth rate and biomass has been indicated. Bååth and Arnebrant (1994) noted in soils with pH varying from 3.9 to 7.0 that the cell growth rates increase with an increase pH value.

Carbon is the fundamental building component of all the organic compounds needed by living things, amongst which are nucleic acids, carbohydrates, proteins and fats. It is usually assumed to be the limiting factor for microbial growth in the soil (Smith and Paul, 1990), although nitrogen and phosphorus have also been reported as limiting factors in some soils (Duah-Yentumi *et al*., 1998). It is therefore probable that different substances serve as limiting factors in different soils and the degree could change with time. Soil organic matter is also a major source of nitrogen. The addition of nitrogen may also alter the soil pH, which could negatively affect bacterial activity (Bååth, 1996; Biikkila *et al*., 2000). In addition, it has been found that microorganisms decomposing straw with a high C/N ratio immobilize available nitrogen in soil (Ocio and Brookes, 1990).

In this study, the soil enzymes have not been taken into account. However, it is known that enzymes in the soil originated from microorganisms and plants, and their proportions vary with soil types. These enzymes may be in free form, or be adsorbed in soil colloids. Their concentration is influenced by factors such as pH, moisture content and texture and generally, it decreases with an increase in soil depth (Dommergues and Mangenot, 1970). In the soil layer H_1, the first 4 dominant factors were Fe, K, Al and Si. From the soil layers H_2 to H_8, the first 2 dominant factors were K and Si. The third or fourth dominant factors in soil layers H_2 to H_4, and H_5

to H_8 were Fe or Al, and Fe or N respectively (Table 2). The behavior of abiotic factors in the soil layer H_1 differs from that of other soil layers. This could be due to the interaction of other chemical factors, not considered in thi study. The soil layer H_1 is close to the soil surface and therefore, is more vulnerable to the impact of pollutants.

The introduction of bacteria in soil as well as their survivals is influenced by some stable abiotic factors such as the soil texture and granulometry, and by some dynamic abiotic factors such as temperature and various nutrients and other chemicals (Van Elsas and Heijen, 1990). In addition, some soils can sometimes contain various heavy metals at various concentrations (Stanier *et al*., 1990). Their inclusion as well as the presence of other bacterial species could modify the hierarchical order noted.

CONCLUSION

During wastewater infiltration through soil layers *E. coli* and other bacteria species would be retained on the particles of soil. This retention may be offset by cell growth if the water contains biodegradable organic matter. The process is controlled by various chemicals of the soil and the magnitude of their impact and their order of hierarchy relatively vary with respect to the soil layer. The wastewater discharged into the environment without any prior treatment may increase the risk of bacterial contamination of underground water.

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