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Retention of *Escherichia coli* cells through a sand column during percolation of bacteria-contaminated water with a neutral pH: The potential role of the cell growth phase and sterilized or unsterilized nature of the sand grains

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ABSTRACT

The present study aims to assess the retention of Escherichia coli cells harvested from 3 different growth phases, contained in water pH 7 percolating through sterilized and unsterilized sand grains of 3 size ranges (0.1-0.3mm, 0.3-0.5mm, and 0.5-0.8mm). Water containing E. coli ($7x10^7$ CFU/mL) was prepared in an Erlenmeyer flask with the tap connected. The bacteriological analysis of water percolated was carried on the first drops, then after 2h, 4h, 6h and 8h. Results showed that through sterilized sand columns, cells abundances in water percolated varies from $5.2x10^4$ to $22.48x10^4$, from $2.43x10^4$ to $9.88x10^4$ and from $1x10^4$ to $4.26x20^4$ CFU/mL with cells harvested from the lag, and stationary phase, respectively. Through unsterilized sand columns, they varied from $2.02x10^4$ to $9.31x10^4$, from $0.5x10^4$ to $12.35x10^4$ and from $1.7x10^4$ to $5.04x10^4$ CFU/mL with cells from the lag, exponential and stationary phase, respectively. Overall, the cells retained percentages (CRP) seems smaller with sterilized sand compared to unsterilized sand grain when bacteria cells were harvested from lag and exponential phases. The CRP seems also varying with percolation duration considering each grain size and sand type, and when considering all 3 growth phases. The Mann-Whitney comparison test showed a significant difference ($P<0.05$) between sterilized and unsterilized sand in cells abundance contained in water percolated through sand size 0.1-0.3mm for each of the cells growth phase. It also shown significant difference in cells abundance contained in water percolated through sterilized sand when cells were harvested from different growth phases ($P<0.05$), but not through unsterilized sand columns.

Keywords: *water contaminated percolation, cells growth phase, E. coli retention, sterilized and unsterilized sand, Sand grain size, Percolated water collection-period*

1. Introduction

The migration, transfer or retention of bacteria in water through soil, from the soil surface to the top of the water table is a complex process due to several factors that often involved. Based on the dominating size of the particles within, soil is a complex which can be categorized into loam, chalk, peat, silt, clay and sand types. Sandy soils are often known as light soils due to their high proportion of sand and little clay [1]. Factors involved in the retention of bacteria in seepage water may be related to the soil, the seepage water, or the bacterial cells themselves. Soil-related properties include the physical, mineralogical and petrographic characteristics of the geological particles [2-4].

The water properties are mostly chemical. They are involved in interactions with the soil surface particles and can also contribute to the modification of ionic strength [5,6]. They can also concern water flow [7,8]. The properties related to the bacteria are both anatomical [9,10], as well as those whose result affects the characteristics of the wall surface [11,12].

Several authors have indicated the involvement of the properties of the bacterial wall surface in its retention on solid surfaces. This phenomenon can be reversible and involve interactions of different types between the macromolecules of the cell wall surface, the pili, and the flagella on the one hand,

and the surface of the solid particle on the other hand [13-15]. This phenomenon is often less energy-dependent at the beginning of the process, but becomes more energy-dependent as the contact time becomes longer, probably because the number of attachment sites has become very limited [14,15].

The envelope of some bacteria contains, among other things, glycocalyx and peptidoglycans. These compounds are involved in cell adhesion to solid surfaces as well as in genetic exchange [14-16]. It is indicated that the ratios of carbon, nitrogen, phosphorus and oxygen atoms on the bacterial surface vary depending on the cell species and its environment. The types of bonds between these atoms and the variability of these bonds confer particular characteristics to bacterial surfaces [14,15,17]. These bonds which closely depend on the ratios between the atoms and the isoelectric point (pH value at which positive and negative charges neutralize each other), influence cellular retention on the particles surface. The stability of this retention depends, among other things, on the degree of hydrophobicity and the chain length of the polymers on the bacterial surface [14,15,17].

It is indicated that the chemical properties of the cell surface undergo variations depending on its physiological state and growth phase [18-20]. Bacterial growth is the increase in the number of bacterial cells. Its growth curve consists of four different phases [21]. They includes : i) the lag phase during which the bacteria adapt to the new environment and prepares itself for reproduction, ii) the exponential phase during which cell number increases in a logarithmic fashion such that the cell constituent is maintained, iii) the stationary phase during which the cells bacteria growth rate is limited by the accumulation of toxic compounds and also depletion of nutrients in the media, iv) and the death phase during which the cells death rate is greater than the rate of formation of new cells [19-21].

Moreover, in natural environment, bacteria adhere on solid surface particles as sand grains, although this is affected by the cell size and shape, cell motility, electrophoretic mobility, zeta potential, hydrophobicity and its potential of interaction with the sand surface [22]. Virus also adhere to sand grains surface in this natural environment [3]. Completely removing viruses and other microorganisms from the sand grains' surface, without using chemical disinfectants, involves sterilization, including autoclaving. It is indicated that the autoclaving method can affects morphological and mineralogical properties of particles as well as their mechanical microstructure and compressive strength [23,24]. However, little data are available on potential changes in the adsorption properties of sand grains after sterilization by autoclaving. Little is also known about the similarities or differences between the cell retention capacities of sterilized and unsterilized sand grains.

The bacterial species *E. coli* is known for its importance in public health as well as in bioindication of food, tap and groundwater quality. Strains of this species can in fact be grouped into various pathotypes depending on the presence of specific virulence factors, mechanisms of infection, tissue tropism, interactions with host cells and clinical symptoms [25-27]. These pathotypes include: (i) an acute and prolonged diarrhea in infants associated to Enteropathogenic *E. coli*, (ii) hemorrhagic colitis and hemolytic uremic syndrome associated to Enterohemorrhagic *E. coli*, (iii) travelers' diarrhea associated to Enterotoxigenic *E. coli*, (iv) acute and chronic diarrhea associated to Enteroaggregative *E. coli*, (v) watery diarrhea in young children associated to Diffusely adherent *E. coli*, (vi) dysentery and watery diarrhea associated to Enteroinvasive *E. coli*, (vii) inflammatory bowel disease associated to Adherent-Invasive *E. coli*, (viii) urinary tract infections associated to Uropathogenic *E. coli*, (ix) neonatal meningitis associated to Neonatal meningitis *E. coli*, and (x) bacteremia and sepsis associated to Septicemiaassociated *E. coli* [25-27].

Depending on the nutritional conditions of a given environment as well as the residence time of the bacteria in it, the physiological state of the bacterial cell can vary. Little is known about the impact of each cell's growth phase on their retention on geological particles during their migration, transfer through a sand filter or through sandy soil. The present study aims to assess the retention of *E. coli* cells harvested from 3 growth phases (lag, exponential and stationary phases), contained in water percolating through sterilized and unsterilized sand grains of 3 different size ranges.

2. Materials and Methods

2.1. Sand and determining different grain sizes

According to Lees [28], sands are soils particles whose sizes ranged from 0.063 mm to 2mm. The sand used in this study was collected in a suburb of Yaounde (Cameroon, Central Africa). The sand underwent four successive sievings using sieves with mesh sizes of 0.8 mm, 0.5 mm, 0.3 mm, and 0.1 mm to obtain three different ranges. Range 1 contains sand with grain sizes from 0.1 to 0.3 mm, range 2 contains sand with sizes ranging from 0.3 to 0.5 mm, and range 3 contains sand with sizes ranging from 0.5 to 0.8 mm. The three ranges of sand resulting from the sieving were washed with sterile distilled water and dried at room temperature.

2.2. Isolation, identification of *E. coli* and strain cryopreservation

The bacteria *E. coli* was isolated from a stream in Yaoundé, using Endo agar culture medium (Difco) and the filter membrane method after 24h incubation at 44°C. It was then identified using standard biochemical methods [29,30]. Biochemical tests include Catalase, Indole production, Methyl Red, Voges-Proskauer, Nitrate reduction, Urease production, Citrate utilization, and the Glucose fermentation test. Each test was performed using standard microbiological procedures to evaluate the metabolic and enzymatic characteristics of the isolates [29,30]. Afterwards, cells were harvested by centrifugation at 8000 rpm for 10 min at 10°C and washed twice with physiological solution (8.5g/L, 1NaCl). The pellet was re-suspended in physiological solution (8.5g/L, NaCl) and then transferred to 500 µL tubes. The stocks were then freezer stored in glycerol vials [29,31].

2.3. Experimental design

Overall, three groups of non-biological materials were used: sterilized and unsterilized sands of different sizes, 36 fragments of polyvinyl chloride (PVC) pipes measuring 1050 mm in length and 33 mm in inner diameter, and 36 Erlenmeyer flasks of 2 liters, each with a tap fitted to its base. 1000 mL of sterile physiological saline solution was added to each Erlenmeyer flask. The content of each Erlenmeyer flasks was adjusted to pH 7 and the whole was then sterilized in an autoclave. The 36 Erlenmeyer flasks were then classified into 2 series A and B, with A for sterilized sand and B for unsterilized sand. Each series thus has 18 identical flasks. A sterile infuser was then connected to the tap of each Erlenmeyer flask.

At the same time, the inside of each of the 36 pipe fragments was washed with water treated with bleach and then rinsed with sterile physiological water.

These pipes were then classified into 2 series A and B as indicated above. Each series thus consists of 18 identical pipe fragments. The pipes from series A and B are intended for experiments on sterilized sand and unsterilized sand, respectively. Each series of 18 pipes was then classified into 3 groups I, II, and III of 6 pipes. Group I was intended for experiments with cells harvested from lag growth. Pipes of group II and III were intended for experiments with cells harvested from the exponential growth phase and stationary growth phases, respectively. Each group of 6 pipes was classified into 3 sub-groups 1, 2 and 3, each of them intended for experiments with a given sand grains size. Pipes were in duplicate for each of the 3 sand grain sizes (0.1-0.3mm, 0.3-0.5mm and 0.5-0.8mm).

Sands of given size were introduced into the pipes to a height of 1000mm. Sands of different diameters were then introduced into these pipes sub-groups 1, 2, and 3 in duplicate. Sand with sizes 0.1 to 0.3mm was introduced into sub-group pipes 1, sand with sizes 0.3 to 0.5mm into sub-groups pipes 2, and sand with sizes 0.5 to 0.8mm introduced into sub-groups pipes 3. The bottom of each pipe containing sand was double wrapped with sterilized wire mesh with a mesh size of less than 0.1mm. These pipes fragments, grouped in series and in duplicates were then arranged on supports on the supports in the laboratory. A sterile container was then placed below each pipe to collect the water that would percolate. Figure 1 shows the diagram of the experimental setup described above (a pipe placed on its support and the corresponding Erlenmeyer placed on the shelf).

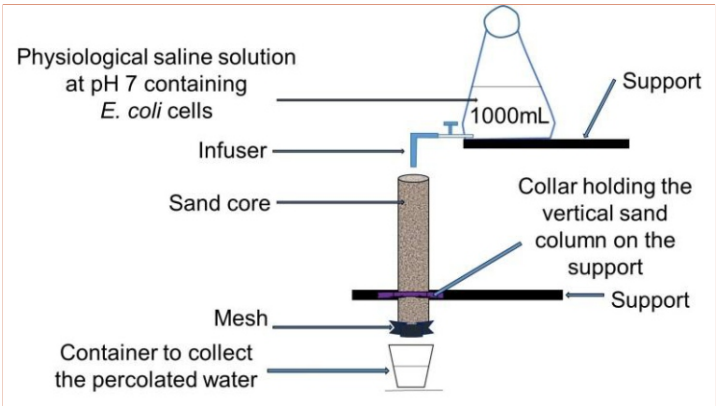

Figure 1: Experimental setup showing the sterile physiological saline solution containing E. coli cells, with the infuser connected, and the sand core for contaminated-water percolation and container below to collect the water percolated

2.4. Inoculation in physiological saline solution and percolation tests

Prior to experiments, *E. coli* growth curve was established. The total duration of the lag phase was 5h. The exponential growth phase started from the 5th hour to the 11th hour of incubation. And the stationary phase started from 11th to 23th hour of incubation. Before the experiments, stocks of frozen vials containing *E. coli* cells were thawed at room temperature. Then, 5mL of the culture were introduced into each of the 3 test tubes containing nutrient broth (Oxford) and incubated at 37 °C for 24 hours. Cells were then harvested from the first tube at 3th hour incubation for the lag phase, then from the second tube at 9th hour incubation for exponential phase, and 17th hour incubation for the stationary phase. The cells were harvested by centrifugation at 8,000 rpm for 10 minutes at 10 °C and washed twice with a sterile NaCl solution (8.5 g/L). After centrifugation and washing, pellets were then resuspended into 50 mL sterile NaCl solution (8.5 g/L).

After homogenization, the concentration of cell bacteria was adjusted to 7x10^{10} CFU/mL by reading the optical density 600 using a spectrophotometer, followed by culture on standard agar medium [32]. 1mL of the suspension was then added to 1000mL of sterilized NaCl solution (8.5 g/L) contained in an Erlenmeyer flask and the whole was then homogenized. The cells concentration in a 1000mL Erlenmeyer flask solution thus was 7x10^{7}CFU/mL.

The tap was then adjusted to a flow rate of 2mL per minute. This bacteria-contaminated water thus falls above the sand column. The moment at which the first percolated drops were obtained was considered as T0 and these first drops were analyzed. From this moment, the percolated water drops were accumulated in 2-hour increments and then analyzed. The bacteriological analysis of the water percolated thus carried out on the first drops of water percolated, then on water percolated after 2, 4, 6 and after 8 hours. This was done on Endo agar culture medium (Difco) as indicated using plate count method. Petri dishes were then incubated at 44°C for 24h. The results were expressed in CFU/mL.

2.5. Data analysis

The mean value of cells concentration in water percolated for each duplicate was calculated and illustrated. Comparisons amongst cells abundance were carried using the Kruskal-Wallis and Mann-Whitney, and SPSS 25.0 program. The cells retained percentages (CRP) contained in 1 mL of water percolated through the sand column and for each experimental condition have been calculated according to Gross et al [33]:

$$CRP = \frac{(Xi - Xp).100}{Xi}$$

In this formula, Xi = cell abundance in 1mL of water falling above the column of sand, and Xp = cell abundance in 1mL of water percolated

3. Results

3.1. Temporal variation of the cells abundance in percolated water with respect to the cells growth phase

The cells abundance in water percolates undergoes temporal variations depending on the bacterial cell growth phase and the sterilized or unsterilized nature of the sand used.

3.1.1. Water percolated through the sterilized sand column

The abundances of *E. coli* cells in water percolated through sterilized sand varied across different grain sizes, growth phases, and percolation times. Bacterial cells were present in the water percolated as soon as the first drops appeared. Overall, the lowest abundance (10^{4} CFU/ml) was recorded with cells harvested from the stationary phase on the first collected water drops, and the highest abundance (22.48x10^{4} CFU/ml) was observed with cells harvested from the lag phase after 2 hours of percolation (Figure 2).

With cells harvested from the lag phase sand sizes 0.1-0.3mm, the lowest abundance (5.2x10^{4} CFU/mL) was observed in the first drops of water percolated and the highest (15x10^{4} CFU/mL) was observed at 6 hours of percolation. With sand sizes 0.3-0.5mm, the lowest abundance (6.73x10^{4} CFU/mL) was observed in the first drops of percolated water and the highest (17.46x10^{4} CFU/mL) was observed after 2 hours of percolation. With sand sizes 0.5-0.8mm, the lowest abundance (14.1x10^{4} CFU/mL) was observed at 4 hours of percolation and the highest (22.48x10^{4} CFU/mL) was observed after 2 hours of percolation (Figure 2).

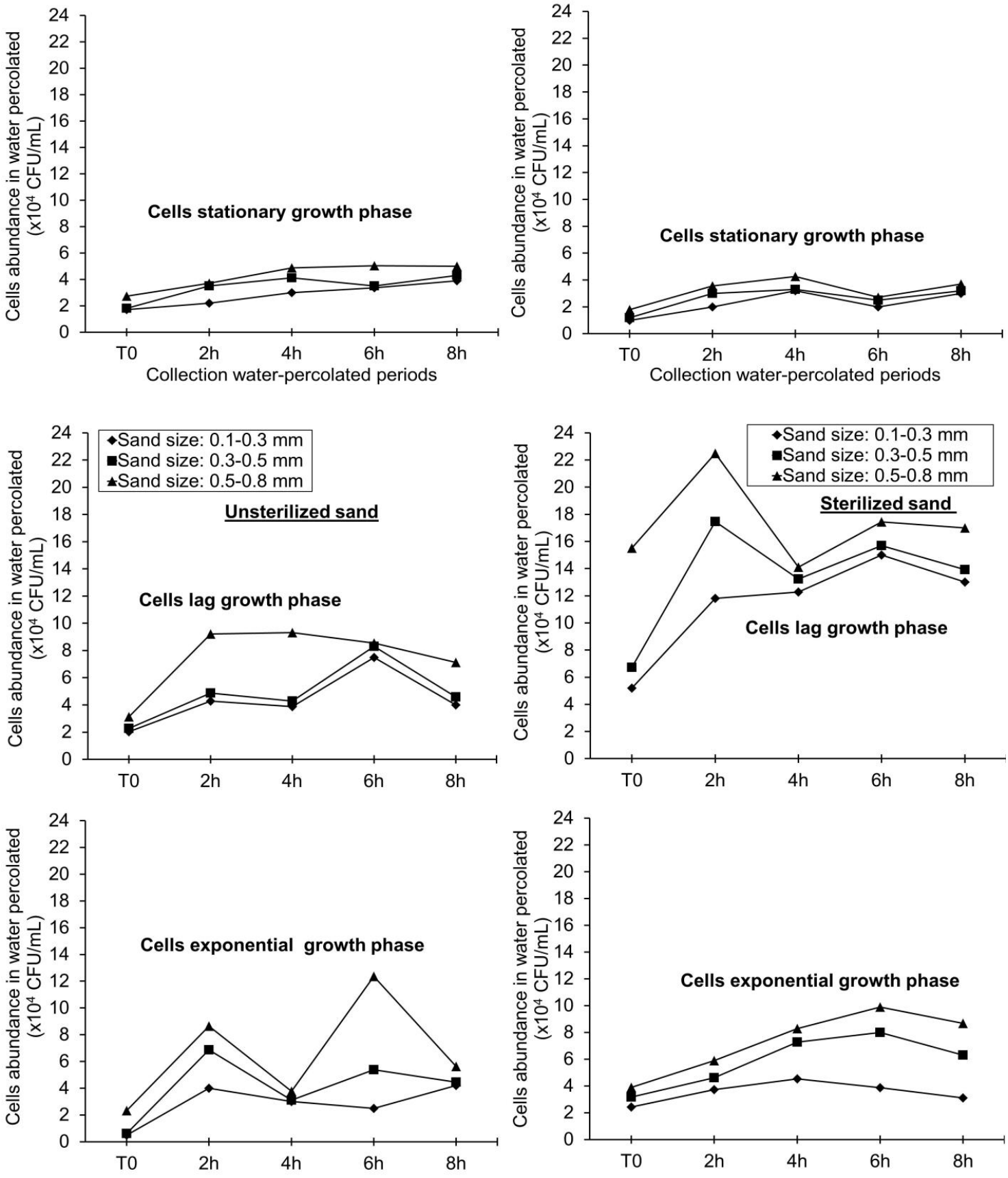

Figure 2: Temporal variation of the cells abundance in water percolated through sterilized sand and unsterilized sand columns, when cells were harvested from each of the 3 cells growth phases

With cells harvested from the exponential phase and with sand sizes 0.1-0.3mm, the lowest abundance ($2.43x10^4$ CFU/mL) was observed in the first drops and the highest ($4.53x10^4$ CFU/mL) after 4 hours of percolation. With sand sizes 0.3-0.5mm, cells abundances varied from $3.16x10^4$ CFU/mL (observed in the first drops of percolated water) to $8x10^4$ CFU/mL (registered after 6 hours of percolation). With sand sizes 0.5-0.8mm, they varied from $3.9x10^4$ CFU/mL (observed after 4 hours) to $9.88x10^4$ CFU/mL registered after 6 hours of percolation (Figure 2).

With cells harvested from the stationary phase, cells abundance in water percolated also underwent temporal variation depending on the sand grains sizes (Figure 2).

3.1.2. Water percolated through unsterilized sand column

Through unsterilized sand columns, cells abundance in water percolated also underwent temporal variation depending on cells growth phases on the one hand, and the sand sizes grains on the other hand.

The lowest abundance ($0.5x10^4$ CFU/mL) was recorded with cells harvested from exponential phase on the first drops of collected water percolated, and the highest ($12.35x10^4$CFU/mL) with cells from the same growth phase after 2 hours of percolation (Figure 2).

With cells harvested from the lag phase, the lowest cells abundance in water percolated through the sand columns size 0.1-0.3mm, 0.3-0.5mm and 0.5-0.8mm was $2.02x10^4$, $2.27x10^4$ and $3.13x10^4$ CFU/mL respectively. The highest was $7.5x10^4$, $8.3x10^4$ and $9.31x10^4$CFU/mL respectively.

With cells harvested from the exponential phase, the lowest cells abundance in water percolated through the sand columns size 0.1-0.3mm, 0.3-0.5mm and 0.5-0.8mm was $0.5x10^4$, $0.61x10^4$ and $2.33x10^4$ CFU/mL respectively. The highest was $4.2x10^4$, $6.87x10^4$ and $12.35x10^4$CFU/mL respectively.

With cells harvested from the stationary phase, cells abundance in water percolated also underwent temporal variation depending on the sand grains sizes (Figure 2).

3.2. Cells retained percentages (CRP) through the sand column

Cells retained percentages (CRP) through the sterilized sand column and unsterilized sand column were calculated for each sand grain size with cells harvested from each of the cells growth phases (Table 1). With cells harvested from lag phase, CRP varied from 99.78 to 99.97 with sand size 0.1-0.3mm, from 99.75 to 99.96 with sand size 0.3-0.5mm and from 99.67 to 99.97 with sand size 0.5-0.8 mm. The smallest CRP was registered with water percolating through sterilized sand with size 0.5-0.8mm and the highest (99.97%) was noted with water percolating through the both sterilized and unsterilized sand with size 0.1-0.3mm and 0.5-0.8mm (Table 1).

When cells harvested from exponential growth phase, the CRP varied from 99.93 to 99.99 with sand size 0.1-0.3mm grain size, from 99.88 to 99.99 with sand size 0.3-0.5mm, and from 99.82 to 99.96 with sand size 0.5-0.8mm. The smallest CRP as well as the highest was after different water percolated collection periods, and the both through unsterilized sand. The smallest was with size 0.5-0.8mm and the highest with sand size 0.1-0.3mm and 0.3-0.5mm (Table 1).

When cells harvested from stationary phase, the CRP also undergoes variations depending on sterilized and unsterilized nature of sand grain in the column and the sand grains size. Overall, the CRP seems smaller with sterilized sand compared to unsterilized sand grain when bacteria cells were harvested from lag and exponential phases. The CRP seems also varying percolation duration for each grain size and sand type and when considering all 3 growth phases (Table 1).

Table 1 : Cells retained percentages (CRP) through the sterilized sand column and unsterilized sand column

Cells growth phase and sand grains sizes		CRP for each of the water percolated collecting period and sand type											
		CRP at T0		CRP at 2h		CRP at 4h		CRP at 6h		CRP at 8h			
Cells growth phase	sand grains sizes (mm)	St. sand	Unst. sand	St. sand	Unst. sand	St. sand	Unst. sand	St. sand	Unst. sand	St. sand	Unst. sand		
Lag phase	0.1–0.3 mm	99.92	99.97	99.83	99.93	99.82	99.94	99.78	99.89	99.81	99.94		
	0.3–0.5 mm	99.90	99.96	99.75	99.93	99.81	99.93	99.77	99.88	99.80	99.93		
	0.5–0.8 mm	99.97	99.95	99.67	99.86	99.79	99.86	99.75	99.87	99.75	99.89		
Exponential phase	0.1–0.3 mm	99.96	99.99	99.94	99.94	99.93	99.95	99.94	99.96	99.95	99.94		
	0.3–0.5 mm	99.95	99.99	99.93	99.90	99.89	99.95	99.88	99.92	99.90	99.93		
	0.5–0.8 mm	99.94	99.96	99.91	99.87	99.88	99.94	99.85	99.82	99.87	99.91		
Stationary phase	0.1–0.3 mm	99.98	99.97	99.97	99.96	99.94	99.95	99.97	99.95	99.95	99.94		
	0.3–0.5 mm	99.98	99.97	99.95	99.95	99.95	99.94	99.96	99.95	99.95	99.93		
	0.5–0.8 mm	99.97	99.96	99.94	99.94	99.93	99.93	99.96	99.92	99.94	99.92		

St. sand : Sterilized sand ; Unst sand : Unsterilized sand

3.3. Comparisons of cells abundances contained in water percolated

3.3.1. Comparison between sterilized and unsterilized sand, in cells abundance in water percolated

Considering the cell abundances in percolated waters through all sand grain sizes, at all collection periods and for cells harvested from all 3 growth phases, a global comparison using the Kruskal-Wallis test showed the existence of a significant difference between sterilized and unsterilized sand of sizes 0.1-0.3mm.

The Mann-Whitney comparison test was then used to compare between sterilized and unsterilized sand in the cells abundance of cells in water percolated per sand grains size range and for each of the cells growth phase. The results are presented in Table 2. A significant difference is observed with the sand of sizes 0.1-0.3mm during the lag, exponential, and stationary phases ($P<0.05$). For sand sizes 0.3-0.5mm and 0.5-0.8mm, no significant difference was noted.

Table 2: P value of the Mann-Whitney comparison test of the cells abundance in water percolated between sterilized and unsterilized sand per sand grains size range and for each of the cells growth phase

Cells growth phase	Sand size ranges		
	0.1-0.3mm	0.3-0.5mm	0.5-0.8mm
Lag phase	**0.016***	0.602	0.293
Exponential phase	**0.016***	0.175	0.075
Stationary phase	**0.009***	0.465	0.076

3.3.2. Comparison amongst growth phases originating cells

An overall comparison by sand type using the Kruskal-Wallis test when considered the cell abundances in percolated waters through all sand grain sizes and at all water percolated collection periods also showed the existence of a significant difference amongst cell growth phases.

The Mann-Whitney comparison test was then carried out to compare the cells abundance in water percolated through sterilized and unsterilized sand per sand grains size range, between when cells were harvested from Lag and from Exponential phases, when they were harvested from Lag and from Stationary phases, and when they were harvested from Exponential and from Stationary phases. The results are presented in Table 3. Through the sterilized sand, there is a significant difference between the lag phase and the exponential, the lag and stationary phases, and between exponential and stationary phases with sand sizes 0.1-0.3mm, 0.3-0.5mm, and 0.5-0.8mm ($P<0.05$). In contrast, through the unsterilized sand, no significant difference was noted in cells originating from different growth phases (Table 3).

Table 3: P value of the Mann-Whitney comparison test between 2 cells harvested growth phases (Lag # Exponential, Lag # stationary, Exponential # stationary), of the cells abundance in water percolated through sterilized and unsterilized sand per sand grains size range phase

Cells growth phases and sand nature		Sand size ranges		
Compared cells growth phases	**Sand nature**	**0.1-0.3mm**	**0.3-0.5mm**	**0.5-0.8mm**
Lag # Exponential	Sterilized sand	**0.009***	**0.028***	**0.009***
	Unsterilized sand	0.295	0.754	0.602
Lag # stationary	Sterilized sand	**0.009***	**0.009***	**0.009***
	Unsterilized sand	0.117	0.116	0.076
Exponential # stationary	Sterilized sand	**0.047***	**0.028***	**0.016***
	Unsterilized sand	0.675	0.463	0.347

4. Discussion

A decrease in the concentration of *E. coli* was observed in the percolated water, compared to the bacteria-contaminated water introduced into the sand columns. This shows that some cells were retained in the sand column during the infiltration of the bacteria-contaminated water. This phenomenon of cell retention is often indicated as the result of the interactions occurring between the sand grains surface and the cell's surface [4,6,15]. It depends on several factors, some of which may be related to the properties of the bacterial cell, others to the properties of the geological particles, and still others to the abiotic properties of the infiltration water [4,6,15].

It has also been noted that cell concentrations in percolated water undergo temporal variations. The same is true for the percentages of retained cells. These variations would reflect phenomena of cellular retention and release occurring alternately. Several authors have indicated that this phenomenon, which is in reality cellular adhesion to the surface of solid materials, is reversible [10]. According to Vigeant et al [34], cells that become immobilized on the surface are affected by electrostatic interactions, and reversibly adhering cells are affected by DLVO-type forces (Derjaguin, Landau, Verwey, and Overbeek theory). For authors, initially bacteria reversibly attach to a surface through weak, transient interactions. Carniello et al [35] inidicated that it is four steps process : (i) bacterial mass transport towards a surface, (ii) reversible bacterial adhesion and (iii) transition to irreversible adhesion and (iv) cellwall deformation and associated emergent properties. The reversible attachment can transition to irreversible adhesion as bacteria produce extracellular polymeric substances (EPS) and utilize appendages like pili and flagella to strengthen their hold [14,15]. It is also less energy-dependent phenomenon at the beginning, but becomes more energy-dependent as the contact time becomes longer, probably because the number of attachment sites has become very limited [14,15].

The number of bacterial cells in the percolated water sometimes varied depending on the growth phase from which these cells originated. The characteristics of the cell surface would relatively vary depending on its physiology and growth phase.

It is indicated that cell surfaces, including cell walls and cell membranes, generally carry a net negative electrical charge. This negative charge arises from the presence of negatively charged molecules on the cell surface, such as phosphate groups, carboxyl groups, and certain proteins [36]. It is also known that peptidoglycan (murein) sacculus is a unique and essential structural element in the cell wall of most bacteria. Made of glycan strands cross-linked by short peptides, the sacculus forms a closed, bag-shaped structure surrounding the cytoplasmic membrane and its fine structure can vary with the cells growth conditions [37]. Its growth is regulated by central metabolism involving several enzymes, and coordinated with other cellular processes [18,38]. Do et al [39] noted that although the basic structure of peptidoglycan is highly conserved in young and old cells, consisting of long glycan strands that are cross-linked by short peptide chains, the mature cell wall is chemically diverse. In addition, the relative variation of the ratios of carbon, nitrogen, phosphorus and oxygen atoms on the bacterial surface concerning the cell growth phase [14,15,17], may impact its characteristics and its interactions with sand grains surfaces.

Several mechanisms have been described as allowing the bacterial cell to sense environment, to know when it is near a solid surface and to facilitate adhesion. They include Chemical signals, biological molecules and physical sensing [14]. In the present study, the main mechanism will be physical sensing involving surface appendages, bacterial cell wall deformation, envelope proteins, and secondary messenger [14].

Cell abundance in water percolated sometimes also varied depending on whether the sand grains were sterilized or not. Sterilization would result in substantial changes in the electrical, chemical and microstructural properties of the surface of sand grains. Autoclaving sand grains may affects morphological and mineralogical properties of their surface as well as their mechanical microstructure and compressive strength [23,24]. Some authors indicated that autoclaving geological particles induces a quantitative modification of their several minerals such as SiO_2, Al_2O_3, Fe_2O_3, CaO, MgO, Na_2O, K_2O and SO_3 inside and on their surfaces, and this modification can vary with the temperature, pressure and duration

considered for sterilization. It also induces a modification in the bonds between the atoms constituting these minerals [23,40,41]. Other authors noted that autoclave curing affects significantly the phases developed in hardened concrete, decrease the Ca/Si ratio, form crystallized calcium silicate hydrate and affects the interfacial transition zone and triggers the formation of crystalline C-S-H, for example [24]. All these microstructural and chemical modifications would induce a modification in the distribution of ionic forces on the surface of the sand grains. They would also affect the results of the interactions between the surfaces of the bacterial cells and the surfaces of the sand grains. This phenomenon would be complex when we integrate the potential variations in the chemical and electrical properties of cell surfaces depending on the growth phases of the bacteria.

5. Conclusion

Bacteria-contaminated water percolation through different types of soil (loam, chalk, peat, silt, clay and sand) and retention of the bacteria-contaminants may be complexe processes due to the physiological status of cells on the one hand, and characteristics of the porous material on the other hand. Through sand column, the cells retained percentages on geological particles undergoes temporal variations depending on the bacterial cell growth phase and the sterilized or unsterilized nature of the sand used. In this study, the cells retention percentages seems smaller with sterilized sand compared to unsterilized sand grain when bacteria cells were harvested from lag and exponential phases. Significant difference has been noted in cells abundance contained in water percolated through sterilized sand when cells were harvested from different growth phases ($P<0.05$), but not in water percolated through unsterilized sand columns. Future work should focus on the variability of transfer or retention of water bacteria-contaminants through other soil types.

Informed consent statement

No informed consent was required to conduct the study.

Conflict of interest

The authors declare no conflict of interest.

Authors' contribution

All authors contributed to conceptualization, data collection and analysis, and have critically reviewed the manuscript and agreed to submit final version.

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