

Original Article



Transfer/Retention of *Escherichia coli* Cells from Exponential Phase in Acidic and Alkaline Percolating Water Through Sterilized/Unsterilized Sandy Soil

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Abstract

Introduction: Little is known about *E. coli* transfer and retention in acidic or alkaline water through sandy soils, and the role of sand sterilization. This study evaluated the transfer and retention of exponential-phase *E. coli* cells in acidic or alkaline water seeping through sterilized or unsterilized sand.

Methods: Sterilized and unsterilized sand of three size ranges was poured into pipe fragments. Cells from the exponential phase were inoculated into sterile NaCl solution (8.5 g/L) contained in Erlenmeyer flasks (concentration 7×10^7 CFU/mL) at a given pH. Water percolation tests were then carried out by allowing bacteria-contaminated water to fall above the sand column. Bacteriological analysis of the percolated water was subsequently performed.

Results: Cell abundance in percolated water was lower than influent, with temporal variations depending on sand treatment and grain size. Smallest grains retained cells most efficiently. Few differences in retention efficiency occurred among sterilized sand sizes, but several differences occurred among unsterilized sizes. For pH 5 percolates, cell abundances differed significantly from pH 6, 8, and 9 ($P < 0.05$) for both sand types, but no differences were observed among pH > 5.

Conclusion: Cells are more retained in acidic influent than in alkaline influent, and also more retained through unsterilized sand grains than through sterilized sand. For a given pH value, the cell retention percentage increases relatively as sand grain size decreases.

Keywords: Bacteria-contaminated water, Acidic/alkaline pH, Percolation, Sterilized/unsterilized sand, Cells transfer/retention, Percolated water

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Introduction

In humans, the bacterium *Escherichia coli* is categorized into various pathotypes based on specific virulence factors, infection mechanisms, tissue tropism, interactions with host cells, and clinical symptoms.¹ The pathotypes include Enteropathogenic *E. coli*, Enterohemorrhagic *E. coli*, Enterotoxigenic *E. coli*, Enteroaggregative *E. coli*, Diffusely Adherent *E. coli*, Enteroinvasive *E. coli*, Adherent-Invasive *E. coli*, Uropathogenic *E. coli*, Neonatal Meningitis *E. coli*, Septicemia-associated *E. coli*, and Avian

Pathogenic *E. coli*.¹⁻³

E. coli is commonly used as an indicator of sanitary quality of foods and water. Tap water, swimming pool water, beach water, and groundwater are among the most impacted water sources. Concerning groundwater in particular, its properties often depend on the nature of the soils crossed. pH is one of the most important parameters of soil because of its implications in microbio-geochemical interactions. While soils in tropical regions are predominantly acidic,⁴ those in temperate regions exhibit a pH range from acidic



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to alkaline⁵ Groundwater properties are also influenced by the chemical composition of the infiltrating water, along with the type and physiological characteristics of the microorganisms present.^{6,7}

As bacteria-contaminated water moves downward from soil surface toward the groundwater, microorganisms present may reach the water table. Alternatively, they may become attached to the surfaces of geological particles, whose composition varies with soil type. Based on the predominant particle size, soils are classified as loam, chalk, peat, silt, clay, or sand. Due to their high sand content and low clay content, sandy soils are frequently referred to as light soils.⁸ Bacterial transfer or retention during water percolation through soil is influenced by a complex interplay of factors including soil properties, bacterial characteristics, and water flow dynamics.⁹ For example the attachment and thus survival of bacteria to soil surfaces (considering their cell hydrophobicity and surface macromolecules such as flagella) from very dry to moist conditions, can intricately influence subsequent transport and retention.^{9,10}

Bacterial attachment involves the surfaces of both the bacterial cell and the solid material, and therefore depends on the properties of each.¹¹ The initial step in this process is the interaction of cells with a surface, a process governed by long-range forces, primarily van der Waals and electrostatic interactions.¹² Bacterial cells are protected externally by a wall which is regulated and whose properties undergoes temporal variation. During growth and propagation, a bacterial cell enlarges and subsequently divides its peptidoglycan (PG) sacculus.¹³ Cytosolic scaffolding proteins connect PG synthases with cytoskeletal elements, and protein phosphorylation regulates cell wall growth in Gram-positive species. PG-active enzymes engage in multiple protein-protein interactions within peptidoglycan synthesis multienzyme complexes, and some of the interactions modulate activities. PG synthesis is also regulated by central metabolism, and by PG maturation through the action of PG hydrolytic enzymes.¹³

Some authors indicated that sterilizing soil particles induces changes in their electrical, chemical and microstructural surface properties and could thus significantly impact cell retention on their surfaces.^{14,15} Others indicated that environmental pH impacts the bacterial membrane electrical potential. It is a source of free energy which enables cells to do chemical and mechanical work. It is dynamic and regulates a wide range of bacterial physiology and behaviors, for example, pH homeostasis, membrane transport, motility, antibiotic resistance, cell division, electrical communication, and environmental sensing.¹⁶⁻¹⁸

The physiological characteristics of bacteria are sometimes linked to their growth phases. Bacterial growth is the increase in the number of bacterial cells. Its growth curve consists of four different phases,¹⁹ including (i) the lag phase, during which the bacteria adapt to the new environment and prepare for reproduction; (ii) the

exponential phase, during which cell number increases in a logarithmic fashion such that cell constituents are maintained; (iii) the stationary phase, during which the bacterial growth rate is limited by the accumulation of toxic compounds and the depletion of nutrients in the medium; and (iv) the death phase, during which the cell death rate exceeds the rate of new cell formation.¹⁹⁻²¹

During the exponential (log) phase in particular, bacterial cells exhibit high metabolic activity, primarily focused on synthesizing the components necessary for cell division and rapid growth. Key metabolic processes include DNA replication, RNA and protein synthesis, cell wall synthesis.²² During this growth phase, the functions of activated genes include synthesis of amino acids (alanine and arginine), energy metabolism (glycolysis/gluconeogenesis and nitrogen metabolism), and translation, all of which promote active cell division.²³

The physiological status of a bacterial cell in seepage water depends on a set of previous environmental conditions as well as current factors. Little is known about the similarities or differences in the fate of bacterial cell behavior in acidic versus alkaline seepage water, as well as in naturally sterilized versus unsterilized geological particles. The present study aimed to evaluate the transfer or retention of *E. coli* bacterial cells harvested from the exponential growth phase and contained in acidic or alkaline waters infiltrating through sterilized/unsterilized soils or sand columns.

Materials and Methods

Obtaining and Determining Different Grain Sizes

Sandy soils are defined as those whose particle sizes ranged from 0.063 mm to 2 mm.²⁴ The sand used in this study was collected from a suburb of Yaounde (Cameroon, Central Africa). The sand was sieved four times using mesh sizes of 0.8 mm, 0.5 mm, 0.3 mm, and 0.1 mm, successively, to obtain three different size ranges. Three sand size ranges were thus obtained. Range 1 contains sand with grain sizes ranging from 0.1 to 0.3 mm, range 2 contains sand with grain sizes ranging from 0.3 to 0.5 mm, and range 3 contains sand with grain sizes ranging from 0.5 to 0.8 mm. The three ranges of sand resulting from the sieving were washed with sterile distilled water to eliminate native microbial community contamination and biodegradable organic matter. The complete elimination of bacterial cells was confirmed by an absence of bacteria by culture in peptone water. The sands were then dried at room temperature for one month. The sand grains were separated in two parts. Unsterilized sand was kept. The other part was placed in 5-litre steel jars and then sterilized in an autoclave for 15 min at 121 °C. Before the experiment, the presence of *E. coli* in unsterilized sand was tested by percolating 500 mL of sterile NaCl solution (8.5 g/L) contained in an Erlenmeyer flask. The percolated water was analyzed on Endo agar culture medium (Difco) using the plate count method. Petri dishes were then incubated at 44 °C for 24 h. The results showed 0 CFU/mL of *E. coli* in the unsterilized sand.

Isolation, Identification of *E. coli* Strain and Cryopreservation

Escherichia coli was isolated from a stream in Yaoundé using Endo agar culture medium (Difco) and the filter membrane method after 24 h of incubation at 44 °C. It was then identified using standard biochemical methods.^{25,26} Biochemical tests included catalase production, indole production, methyl red, Voges-Proskauer, nitrate reduction, urease production, citrate utilization, and glucose fermentation. Each test was performed using standard microbiological procedures to evaluate the metabolic and enzymatic characteristics of the isolates.^{25,26} Afterward, the cells were harvested by centrifugation at 8000 rpm for 10 min at 10 °C and washed twice with physiological saline solution (8.5 g/L NaCl). The pellet was resuspended in physiological saline solution and then transferred to 500- μ L tubes. The stocks were then frozen and stored in glycerol vials.^{25–27}

Experimental Design

Overall, three groups of non-biological materials were used: sterilized and unsterilized sands of different sizes; 48 fragments of polyvinyl chloride (PVC) pipes, each 1050 mm in length and 33 mm in inner diameter; and 48 2-litre Erlenmeyer flasks, each with a tap fitted at its base. A volume of 1000 mL of the sterile physiological saline solution were added to each Erlenmeyer flask. The contents of the flasks were adjusted to pH 5, pH 6, pH 8, and pH 9, respectively, using 0.01 M NaOH/HCl, and the flasks were then sterilized in an autoclave at 121 °C for 15 min. The 48 Erlenmeyer flasks were then divided into two series, A and B: series A for sterilized sand, series B for unsterilized sand. Each series thus contained 24 flasks. A sterile infuser was then connected to the tap of each Erlenmeyer flask. At the same time, the inside of each of the 48 pipe fragments was washed with bleach-treated water and then rinsed with sterile physiological saline solution. These pipes were also divided into series A and B as described above. Each series thus consisted of 24 identical pipe fragments. Pipes from series A and B were intended for experiments with sterilized sand and unsterilized sand, respectively. Each series of 24 pipes was further divided into 4 groups (I, II, III, and IV) of 6 pipes each. Group I was intended for experiments at pH 5; groups II, III, and IV for experiments at pH 6, pH 8, and pH 9, respectively. Each group of 6 pipes was divided into 3 subgroups (1, 2, and 3), each subgroup intended for experiments with a given sand grain size. Pipes were set up in duplicate for each of the three sand grain sizes (0.1–0.3 mm, 0.3–0.5 mm, and 0.5–0.8 mm). Sands of a given size were introduced into the pipes to a height of 1000 mm. Sands of different diameters were then introduced into subgroups 1, 2, and 3 in duplicate: sand of size 0.1–0.3 mm into subgroup 1 pipes, sand of size 0.3–0.5 mm into subgroup 2 pipes, and sand of size 0.5–0.8 mm into subgroup 3 pipes. The bottom of each pipe containing sand was double-wrapped with sterilized wire mesh having a mesh size of less than 0.1 mm. These pipe

fragments, grouped in series and in duplicate, were then arranged on supports in the laboratory. A sterile container was placed below each pipe to collect the percolated water. Figure 1 shows a diagram of the experimental setup described above (a pipe placed on its support and the corresponding Erlenmeyer flask on a shelf).

Inoculation in Physiological Saline Solution and Percolation Tests

Prior to the experiments, *E. coli* growth curve was established. The exponential growth phase lasted from the 5th h to the 11th hour of incubation. Before the experiments, the frozen stock vials containing *E. coli* cells were thawed at room temperature. Then, 5 mL of the culture were introduced into a test tube containing nutrient broth (Oxford) and incubated at 37 °C for 24 h. The cells were harvested at the 9th h of incubation (exponential phase). The cells were harvested by centrifugation at 8,000 rpm for 10 min at 10 °C and washed twice with sterile NaCl solution (8.5 g/L). After centrifugation and washing, the pellets were resuspended in 50 mL of sterile NaCl solution (8.5 g/L). During preliminary experiments, bacterial suspensions were prepared. Cell concentrations in suspensions were assessed by turbidity expressed as optical density using a spectrophotometer at a wavelength of 600 nm.²⁸ During these preliminary experiments, the optical densities of cell suspensions were measured, and cell concentrations were determined using the culture method on agar medium. Following this phase, the optical density of the bacterial suspension was adjusted by dilution, and the cell concentration was adjusted to 7×10^{10} CFU/mL. From this, 1 mL of the suspension was then added to 1000 mL of sterile NaCl solution (8.5 g/L) contained in an Erlenmeyer flask, and the whole was homogenized. The final cell concentration in the 1000-mL Erlenmeyer flask solution was thus 7×10^7 CFU/mL. The tap was then adjusted to a flow rate of 2 mL per minute. The bacteria-contaminated water thus fell onto the sand column. The moment at which the first percolated drops were obtained was considered T_0 , and these first drops

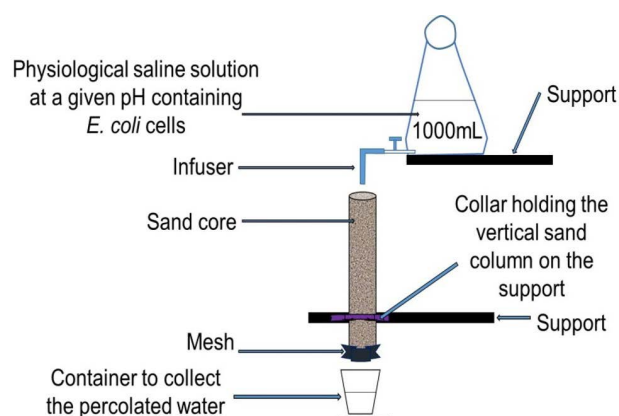


Figure 1. Experimental Setup For Percolation of *E. coli*-contaminated Water Through Sand Columns. The System Consists of an Erlenmeyer flask Containing Sterile Physiological Saline Solution with *E. coli* cells (exponential growth phase), connected via an infuser to a PVC pipe packed with sand. A sterile container placed below the pipe collects the percolated water.

were analyzed. From this moment, the percolated water drops were collected in 2-hour increments and then analyzed. Bacteriological analysis of the percolated water was carried out on the first drops (T_0) and then on water percolated after 2, 4, 6, and 8 h. This was done using Endo agar culture medium (Difco) as described, employing the plate count method. Petri dishes were then incubated at 44 °C for 24 h. The results were expressed in CFU/mL.

Data Analysis

The mean cell concentration in the percolated water was calculated for each duplicate and illustrated. Comparisons of cell abundance among groups were performed using the Kruskal-Wallis and Mann-Whitney tests with the SPSS 25.0 software. The percentage of cells retained (CRP) in 1 mL of water percolated through the sand column for each experimental condition was calculated according to other authors²⁹ as shown in Equation 1:

$$CRP = \frac{(X_i - X_p) \cdot 100}{X_i} \quad (1)$$

Where, X_i = cell abundance in 1 mL of water falling above the column of sand, and X_p = cell abundance in 1 mL of water percolated.

Results and Discussion

Temporal Variation of the Cells Abundance in Percolated Water with Respect to the Water pH

With sand sizes 0.1–0.3 mm, the abundances of *E. coli* cells in water percolated through sterilized and unsterilized sand grains varied with respect to pH and collection period. Bacterial cells were present in the first drops of water percolated. Through sterilized sand, the lowest abundance (0.025×10^4 CFU/mL) in water percolated was recorded on the first collected water drops at pH 5, and the highest abundance (7.1×10^4 CFU/mL) was registered at pH 8 after 2 h of percolation (Figure 2).

Through unsterilized sand, the lowest abundance (0.013×10^4 CFU/mL) in water percolated was also recorded on the first collected water drops at pH 5, and the highest abundance (5.1×10^4 CFU/mL) was also registered at pH 8 after 2 h of percolation. Overall, *E. coli* cells abundance in percolated water appears relatively lower with acidic water percolates than alkaline water percolates at the same collection periods, through both sterilized and unsterilized sand grains. Moreover, *E. coli* cells abundance in water percolated seems relatively higher with water percolation through sterilized sand than unsterilized sand grains whatever the water pH and percolated water collection period (Figure 2). For sand grain sizes of 0.3–0.5 mm and 0.5–0.8 mm, the abundances of *E. coli* cells in water percolated through sterilized and unsterilized sand also changed depending on water pH and percolated water collection period. Through both sterilized and unsterilized sand, the lowest cell abundances (0.021×10^4 CFU/mL) were again recorded in the first drops of percolated water at pH 5. Through sterilized sand of 0.3–0.5 mm, the highest

abundance (8.5×10^4 CFU/mL) was noted at pH 9 after 6 h of percolation; through unsterilized sand of the same size, the highest abundance was 7.8×10^4 CFU/mL at pH 8 after 2 h of percolation (Figure 2). With sand sizes of 0.5–0.8 mm, the highest cell abundances were 9.6×10^4 CFU/mL (pH 9 after 6 h) and 9.8×10^4 CFU/mL (pH 9 after 8 h of percolation) through sterilized and unsterilized sand grains, respectively (Figure 2). As noted above, *E. coli* cell abundance in percolated water also appears relatively lower with acidic percolates than with alkaline percolates through both sterilized and unsterilized sand.

Bacterial cell surfaces have a negative charge at neutral pH due to the deprotonation of carboxyl and phosphate groups. This cell surface charge mediates the adhesion of cells to minerals and other surfaces, potentiates the binding of metal ions to the cell surface, and influences the aggregation of multiple cells into clusters.³⁰ Acidic conditions can alter the composition and fluidity of the bacterial cell membrane.³¹ Changes in membrane properties can influence the interaction of the bacterium with surfaces, including the functional groups present on the cell surface.³² Under acidic conditions, these groups become protonated, making the cell surface more positively charged and reducing electrostatic repulsion with other positively charged species, or increasing attraction to negatively charged surfaces.^{33,34}

In addition, some authors have indicated that aerobic bacteria may survive as a function of the electrochemical gradient for protons across their plasma membrane (i.e., the proton motive force) and maintain it relatively constant by adjustment of the transmembrane potential to compensate for changes in the transmembrane pH gradient.³⁵ Variation in environmental pH causes major rearrangements of extracellular polymeric substances (EPS) structure on the bacterial surface, although EPS structure is also impacted by ionic strength.³⁶ EPS appears more compact as environmental pH decreases and less compact as pH increases.³⁶ Cell surface protein synthesis varies with environmental pH. This chemical parameter also induces changes to the bacterial transcriptome, affects the viscosity of the bacterial membrane and interactions between microbial EPS, and alters the biophysicochemical characteristics of the cell surface; this appears to be the primary driver of changes in cell adhesion to solid particles.^{37,38} EPS generation is also impacted by environmental pH and temperature, although optimal production conditions depend on the bacterial species.³⁹ Environmental pH also impacts the bacterial membrane electrical potential and regulates bacterial physiology and behavior.^{16–18} It changes energy yields both directly and indirectly. The direct effect applies to reactions that consume or produce protons, whereas the indirect effect—which applies to all redox reactions—comes from the regulation of chemical speciation by pH. Low pH conditions promote the diffusion of short-chain fatty acids across the membrane, which dissipates the proton motive force across the membrane. Low pH would also help dissolve ferric and ferrous minerals in sands, making

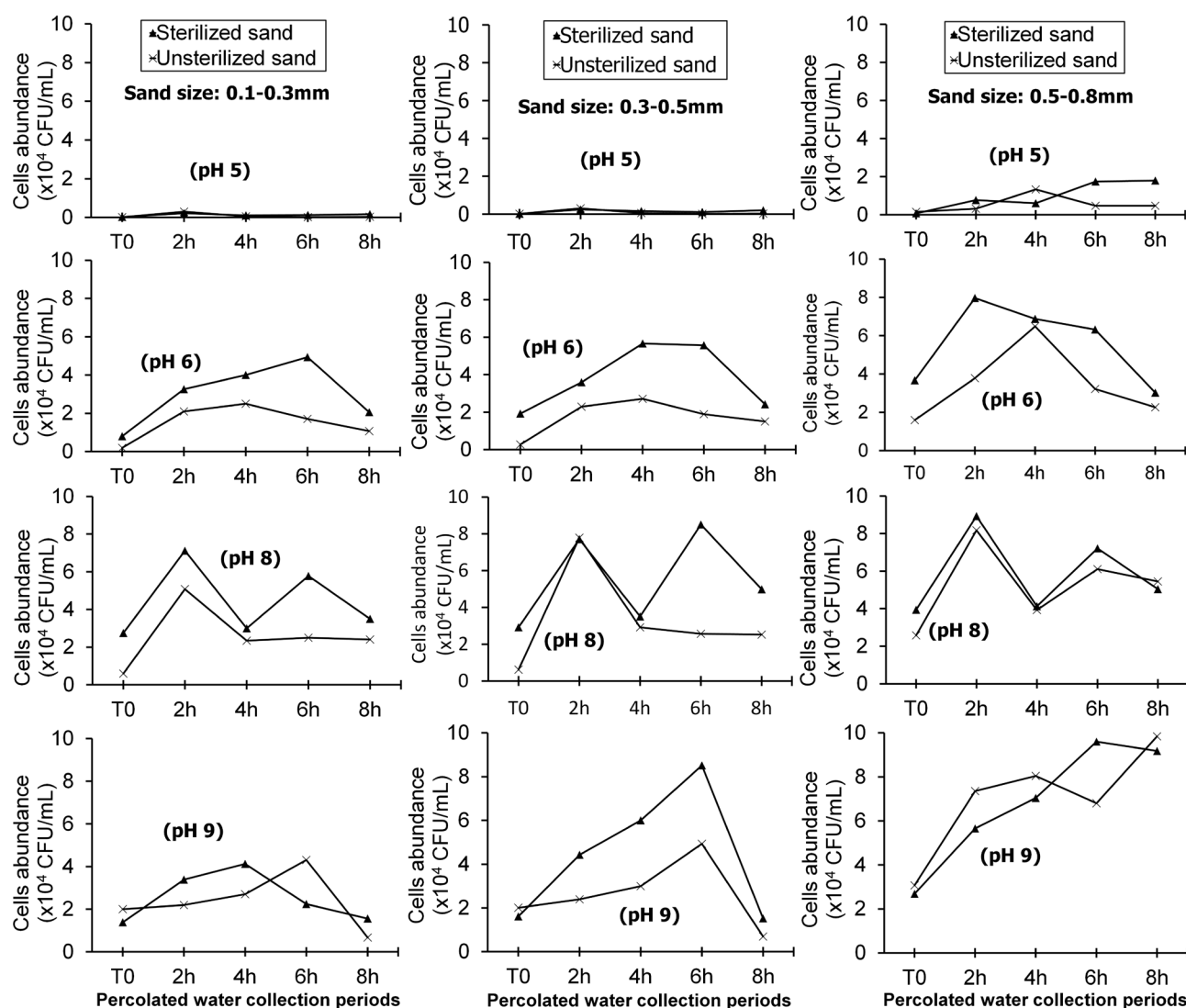


Figure 2. Cells Abundance in Water Percolated Through Sterilized and Unsterilized Sand Size 0.1–0.3 mm, Sand Size 0.3–0.5 mm and Sand Size 0.5–0.8 mm

ferric iron available to iron reducers and ferrous iron to iron oxidizers, thereby promoting the biogeochemical cycling of iron.⁴⁰

Cells Retained Percentages (CRP) Through the Sand Column

(CRPs) through the sterilized and unsterilized sand columns were calculated for each sand grain size at each pH (Table 1). For water percolates at pH 5, CRPs were 99.99% through sterilized and unsterilized sand grain sizes of 0.1–0.3 mm and 0.3–0.5 mm, and varied from 99.97% to 99.99% through sterilized and from 99.98% to 99.99% through unsterilized sand of grain size 0.5–0.8 mm (Table 1). For water percolates at pH 6 through sterilized sand grain ranges of 0.1–0.3 mm, 0.3–0.5 mm, and 0.5–0.8 mm, CRPs varied from 99.92% to 99.98%, 99.91% to 99.97%, and 99.88% to 99.95%, respectively. Through unsterilized sand, they varied from 99.96% to 99.99%, 99.96% to 99.99%, and 99.90% to 99.97%, respectively (Table 1). For water percolates at pH 8 through sterilized sand grain size ranges of 0.1–0.3 mm, 0.3–0.5 mm, and 0.5–0.8 mm, CRPs varied from 99.89% to 99.96%, 99.87%

to 99.95%, and 99.87% to 99.94%, respectively. Through unsterilized sand, they varied from 99.92% to 99.99%, 99.88% to 99.99%, and 99.88% to 99.96%, respectively (Table 1). For water percolates at pH 9 through sterilized sand grain size ranges of 0.1–0.3 mm, 0.3–0.5 mm, and 0.5–0.8 mm, CRPs varied from 99.94% to 99.98%, 99.87% to 99.97%, and 99.86% to 99.96%, respectively. Through unsterilized sand, they varied from 99.93% to 99.99%, 99.92% to 99.99%, and 99.85% to 99.95%, respectively (Table 1). Overall, CRPs appear relatively higher with acidic percolates than with alkaline percolates through both sterilized and unsterilized sand grains.

It is known that the exponential growth phase is the period during which bacteria express to the maximum all their metabolic potential for growth. Substances produced at that moment include EPS, which are high-molecular-weight polymers that microorganisms secrete into their extracellular environment.^{41,42} EPS are mainly composed of polysaccharides, proteins, nucleic acids, and lipids, which mediate cell adhesion to surfaces and form a cohesive, three-dimensional polymer network for mechanical stability.^{41,43} EPS facilitates microbial

Table 1. Cells Retained Percentages (CRP) Through The Sterilized and Unsterilized Sand Column for Each Water pH Percolates

Cells growth phase and sand grains sizes		CRP for each of the water percolated collecting period and sand type									
		CRP at T0		CRP at 2 h		CRP at 4 h		CRP at 6 h		CRP at 8 h	
Percolating water pH	sand grains sizes (mm)	St. sand	Unst. sand	St. sand	Unst. sand	St. sand	Unst. sand	St. sand	Unst. sand	St. sand	Unst. sand
pH 5	0.1–0.3 mm	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	0.3–0.5 mm	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	0.5–0.8 mm	99.99	99.99	99.98	99.99	99.99	99.98	99.97	99.99	99.97	99.99
pH 6	0.1–0.3 mm	99.98	99.99	99.95	99.97	99.94	99.96	99.92	99.97	99.97	99.98
	0.3–0.5 mm	99.97	99.99	99.94	99.96	99.91	99.96	99.92	99.97	99.96	99.97
	0.5–0.8 mm	99.94	99.97	99.88	99.94	99.90	99.90	99.90	99.95	99.95	99.96
pH 8	0.1–0.3 mm	99.96	99.99	99.89	99.92	99.95	99.96	99.91	99.96	99.95	99.96
	0.3–0.5 mm	99.95	99.99	99.88	99.88	99.95	99.95	99.87	99.96	99.92	99.96
	0.5–0.8 mm	99.94	99.96	99.87	99.88	99.94	99.94	99.89	99.91	99.92	99.92
pH 9	0.1–0.3 mm	99.98	99.97	99.95	99.96	99.94	99.96	99.96	99.93	99.97	99.99
	0.3–0.5 mm	99.97	99.97	99.93	99.96	99.91	99.95	99.87	99.92	99.97	99.99
	0.5–0.8 mm	99.96	99.95	99.91	99.89	99.89	99.88	99.86	99.90	99.86	99.85

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

attachment, embedding, and immobilization, and also crucially influences the properties of microbial aggregates, such as adsorption ability, stability, and formation capacity, thereby determining the physicochemical properties and functional complexity. EPS creates an ideal environment for interfacial reactions while also acting as a buffer zone against environmental stresses.^{42,43} EPS concentration varies depending on environmental characteristics as well as the properties of the surfaces of the solid particles present.⁴⁴ The decrease in cell concentration in the percolated water compared to that in the water introduced into the sand column would be a consequence of the retention of these cells on the surfaces of the sand grains, retention mediated by cellular EPS. In addition, bacterial cells are externally protected by a wall containing scaffolding proteins that connect peptidoglycan synthases; this wall is regulated and its properties vary with respect to environmental factors, and it is known to be involved in cell adhesion to solid particles.²⁵ It has been noted that cell abundance in percolated water sometimes undergoes temporal variations. 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.^{45–47} This phenomenon is often less energy-dependent at the beginning of the process but becomes more energy-dependent as the contact time lengthens, probably because the number of attachment sites becomes very limited.^{46–49}

Comparisons Amongst Cells Abundances Contained in Water Percolated of Various pH Values

When considering all sterilized and unsterilized sand grain sizes and all percolated water collection periods, an overall comparison using the Kruskal-Wallis test carried

out among cell abundances in percolated water of various pH values showed a significant difference ($P < 0.05$). The Mann-Whitney comparison test was then performed to compare cell abundances in percolated water between two different water percolates (by pH) for each sterilized and unsterilized sand grain size range. The results are presented in Table 2. It can be noted that through sterilized and unsterilized sand grain sizes of 0.1–0.3 mm, 0.3–0.5 mm, and 0.5–0.8 mm, there were significant differences between water percolates at pH 5 and pH 6, between pH 5 and pH 8, and between pH 5 and pH 9 ($P < 0.05$). There was also a significant difference between water percolates at pH 6 and pH 9 through unsterilized sand grain size 0.5–0.8 mm ($P < 0.05$) (Table 2).

Some researchers have noted that cell abundance and consumption rates in sandy sediments are influenced by the surface area available for microbial colonization and by the exposure of these surfaces to the solute-supplying porewater flow.⁵⁰ Cell densities on individual sand grains depend more on grain sphericity than on grain size. Transport mechanisms in water filtration include straining, sedimentation, interception, diffusion, and hydrodynamic effects, and microbial retention is all the more effective the smaller the grains constituting the porous material.⁵¹ Sand size retention efficiency is sometimes modulated by environmental temperature, and this may vary with bacterial species and sand particle properties.⁵² In addition, concerning the particles sometimes encountered in water seepage, other authors have indicated that their size distribution influences water-retention capacity by affecting the pore size distribution of contaminants; the volume of small pores increases with the surge of fine-grain content, while the maximum pore size contracts with increasing fine-grain content.⁵³

Comparisons Between Sterilized and Unsterilized Sand

Table 2. *P* value of the Mann-Whitney Comparison Test Between the Cells Abundance in Water Percolated From Two Different Water pH Percolates Through Each Sand Grain Sizes Range, with Sterilized and Unsterilized Sands

Compared pH values	Sand size ranges and sterilized/unsterilized nature of sand grains					
	0.1-0.3 mm		0.3-0.5 mm		0.5-0.8 mm	
	St. sand	Unst. sand	St. sand	Unst. sand	St. sand	Unst. sand
pH 5 # pH 6	0.009*	0.016*	0.009*	0.016*	0.009*	0.009*
pH 5 # pH 8	0.009*	0.009*	0.009*	0.009*	0.009*	0.009*
pH 5 # pH 9	0.009*	0.009*	0.009*	0.009*	0.009*	0.009*
pH 6 # pH 8	0.347	0.209	0.347	0.175	0.602	0.175
pH 6 # pH 9	0.754	0.251	0.917	0.251	0.465	0.047*
pH 8 # pH 9	0.117	0.754	0.602	0.754	0.465	0.251

St. sand: sterilized sand grains; Unst. Sand: unsterilized sand grains; *: Significant difference ($P < 0.05$).

Grains of Cells Abundances in Water Percolated

Considering all three sand grain sizes and the five periods of percolated water collection, the abundances of cells percolated through sterilized sand were compared to those percolated through unsterilized sand. The data are presented in Table 3. It appears that at pH values of 6 and 8, cell abundances in water percolated through sterilized sand differ significantly from those recorded in water percolated through unsterilized sand ($P < 0.05$). At these pH values, the cell retention process appears to be significantly different on the surfaces of the two types of sand.

Cell abundance in percolated water differs in some cases depending on the sterilized or unsterilized nature of the sand. Sterilization would result in substantial changes in the electrical, chemical, and microstructural properties of the surface of sand grains. Autoclaving sand grains may affect the morphological and mineralogical properties of their surface as well as their mechanical microstructure and compressive strength.^{14,15} Some authors have indicated that autoclaving geological particles induces a quantitative modification of several of their minerals—such as SiO_2 , Al_2O_3 , Fe_2O_3 , CaO , MgO , Na_2O , K_2O , and SO_3 —both inside and on their surfaces, and this modification can vary with the temperature, pressure, and duration of sterilization. It also induces a modification in the bonds between the atoms constituting these minerals.^{14,48,49} Other authors have noted that autoclave curing significantly affects the phases developed in hardened concrete, decreasing the Ca/Si ratio, forming crystallized calcium silicate hydrate, affecting the interfacial transition zone, and triggering the formation of crystalline C-S-H, for example.¹⁵ All these microstructural and chemical modifications would induce a change in the distribution of ionic forces on the surface of the sand grains. They would also affect the outcomes of the interactions between the surfaces of the bacterial cells and the surfaces of the sand grains.

Conclusion

Cell abundance in water seeping through sandy soils decreases due to retention of cells on sand particles. This retention undergoes temporal variation. Sterilized sand appears less efficient at retaining cells, probably due to substantial changes in its electrical, chemical, and microstructural surface properties. Cells in more

Table 3. *P* value of the Mann-Whitney U Comparison Test Between of Cells Abundances in Water Percolated Between Sterilized and Unsterilized Sand

Parameters	pH values			
	pH 5	pH 6	pH 8	pH 9
Sterilized sand #	0.213	0.007**	0.031*	$P = 0.663$
Unsterilized sand				

*: Significant difference ($P < 0.05$); **: Significant difference ($P < 0.01$).

acidic water percolates seem to be more retained than those in alkaline or less acidic water. The relevance of sand grain size involvement appears to vary depending on whether the sand is sterilized or not, as well as on the pH of the infiltrating water. Future studies will need to clarify the potential role of mineralogical, chemical, and petrographic characteristics of sand grains.

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Competing Interests

The authors declare no conflict of interest.

Ethical Approval

Not applicable.

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Informed Consent Statement

No informed consent was required to conduct the study.

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