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Research Article

Modification of The Bacterial Abundance Properties of Water by Immersed Non-Activated Charcoal

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Abstract:

The present studies aim at reducing the planktonic bacterial load in drinking water using non-activated charcoal. The evaluation is dependent on the incubation time and the different physiological states of the bacterial cells. Three bacteria (*Vibrio cholerae, Escherichia coli* and *Staphylococcus aureus*) and two charcoal substrates (Okan and Tali) were used for the experimentation. The Bacteria adhered to charcoal fragments in varying degrees. Overall, the concentrations of fixed *Vibrio cholerae* reached $53.4x10^7$ CFU/cm^2 and $39.6x10^7$ CFU/cm^2 after 9 hours of contact in exponential phase on the Okan and Tali respectively. Those of fixed *Staphylococcus aureus* reached $5.8x10^6$ CFU/cm^2 after 3 hours of contact in exponential phase and $3.4x10^6$ CFU/cm^2 after 3 and 6 hours of contact in the same phase, on the Okan and the Tali, respectively. The highest abundances of *Escherichia coli* adhered to the charcoal fragments were $50.4x10^7$ CFU/cm² on Okan and $53.2x10^7$ CFU/cm² on Tali after 9 hours of contact in the exponential phase. The highest adsorption coefficient (639.06 adhered cells/cm^2) was noted on Okan in the exponential phase with *Vibrio cholerae* cells; and the lowest (1.02 adhered cells/cm^2) on Tali in stationary phase with *Staphylococcus aureus.* The incubation time significantly ($P<0.01$) influenced the adhesion of bacterial cells to charcoal substrates. Although the adsorption capacity and intensity of Okan were relatively higher, the comparison of the adsorption potential of the two substrates considered did not reveal any significant difference ($P>0.05$), reflecting the absence of the influence of the physical properties of these substrates on cell retention.

Key words: **Adhesion, bacteria, non-activated charcoal, contact duration, growth phases.**

Introduction

Water makes up two-thirds of body fluids in humans and plays an important role. However, due to its quality, it can harm human health (WHO, 2004). However, out of a population of nearly 24 million inhabitants, around 8 million Cameroonians do not have access to a drinking water service (Guepi, 2019). Only 52.3% of the population nationally uses an improved (modern) sanitation facility. Furthermore, when water is available, its quality is not always guaranteed. Due to the low availability of drinking water in urban, peri-urban and rural areas in certain African countries, populations are forced to obtain water whose potability is very doubtful (ACA, 2019). These are most often wells (35.9%), undeveloped sources (5.6%) and developed sources (46.6%) (ACA, 2019). The quality of this water is strongly influenced by pollution which can be physical, chemical or biological (Vilaginès, 2003). Biological pollution of water is due to the presence in it of pathogenic microorganisms such as protozoa, fungi, viruses and bacteria (WHO, 2004). An essential resource for life, water paradoxically constitutes a potential source of disease transmission in developing countries (Hounsou *et al*., 2010). As a result, several chemical and physical biological water treatment processes have been recommended. However, in addition to the disinfection by-products formed, they have a preferential action on one type of microorganisms. Furthermore, if they are not applied in conditions where their lethal activity can be expressed, they promote the selection and even the proliferation of resistant species and also can alter the organoleptic properties of water (Bouacherine, 2013). Thus, research has focused on treatment processes using natural materials (plant charcoals, clays, sands, rocks and certain macromolecular resins, among others) due to their availability for all and their low cost (Bouacherine, 2013). This is how the adhesion of bacteria-pollutants to submerged supports in aquatic environments such as basalt, sandstone, migmatite, granite and micaschist has been proposed by certain authors as contributing to the reduction in the density of bacteria planktonic (Noah *et al*., 2011; Moungang, 2015). The application of vegetable charcoal in the field of water treatment began to develop after the Second World War. During the first

half of the century, they were used to discolor water, while coal was used for its adsorbent properties (Ismahane *et al*., 2013). However, there is little data on the adsorption of microorganisms on non-activated charcoal. Until now, very few studies have been carried out on the influence of contact duration, physiological state and motility of bacterial cells in the selective or non-selective retention of vegetable charcoal immersed in an aquatic environment. Likewise, the quantitative potential of each species of charcoal to retain bacterial microorganisms has so far been little addressed.

The present study therefore aims to evaluate the potential of non-activated charcoal to retain bacteria-pollutants in aquatic microcosms, depending on the incubation time and the different physiological states of the bacteria.

Material and Methods

Making coal fragments

The non-activated charcoal used as adhesion supports were obtained from two (2) wood species: *Cylicodiscus gabunensis* commonly called "Okan or Adoum". It is a heavy wood from the Fabaceae family widely used for construction because its antimicrobial properties make it an effective shield against bacterial, fungal or parasitic attacks. It is also used for its therapeutic virtues because on the digestive level, it facilitates intestinal transit and serve as effective anti-spasmodic (Ayarkwa and Owusu, 2008). *Erythrophleum ivorense* commonly called "Tali" belongs to the Caesalpiniaceae family. It is very popular for carpentry, parquetry and shipbuilding (Lemmens *et al*., 2008). The bark extract is used as an emetic and laxative and externally it is used to soothe pain. In Ivory Coast the young branches bark is used to treat smallpox (Nga, 2016).

The specimens were cut using a metal saw into rectangular parallelepipeds of 22 cm^2. The substrates made were sterilized in an autoclave at 121°C for 15 minutes before carrying out the adhesion tests.

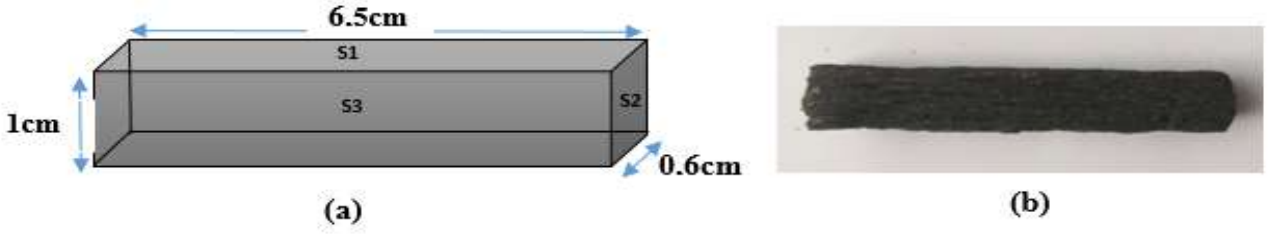

Figure 1: (a) - Graphical representation of the substrate; (b) - Photograph of the support.

Let A be the total surface area of these supports; S1, S2 and S3 are the different surfaces respectively:

A= 2(S1+S2+S3)

The pieces were placed on standard counting agar poured into 15 flat Petri dishes of 90 mm in diameter. These boxes were then separated into 3 groups of 5 boxes each. The first group was incubated at 42°C for 2 days; the second, at 37°C for 3 days and the third at laboratory temperature (23±2°C) for 5 days. The absence of any colony-forming unit after the various incubations assures effective sterility of the stored charcoal fragments.

Isolation and identification of bacterial strains

Three bacterial species, namely *Vibrio cholerae, Staphylococcus aureus* and *Escherichia coli* were used base on their importance in hygiene and public health (Medema *et al*., 2003; Rodier *et al.*, 2009). *E. coli* is a commensal bacteria which can sometimes be pathogenic, its presence in drinking water indicates the presence of other pathogenic germs as well. Staphylococci are among the most difficult pore-forming microorganisms to eliminate. Vibrionacae constitute approximately 10% of the total load of cultured marine bacteria (Eilers *et al.*, 2000). The bacteria were isolated from surface water in Yaoundé-Cameroon (Central Africa) using the spreading technique until exhaustion on the surface of agar poured into Petri dishes. The agar culture media used were TCBS (Thiosulfate Citrate Bile Saccharose), ENDO and Chapman mannitol, respectively for *V. cholerae, E. coli* and *S. aureus.* Typical colonies on the agar media were identified using conventional biochemical tests (Holt *et al.*, 2000; Gyung Yoon *et al.*, 2012; Su *et al.*, 2018).

Preparation of bacterial suspensions

For each bacterial species, a pre-culture was prepared by bacterial seeding on ordinary agar sloped into test tubes, followed by incubation for 24 hours at 37°C. Since the study also focused on the different physiological states of the bacteria, a second culture was prepared from this first culture by inoculating 1 mL of the bacterial suspension in 10 mL of peptone water. The incubation periods of the latter varied depending on the physiological state sought. At the end of each incubation period, this peptone water was centrifuged for 5 minutes. A bacterial suspension was prepared at an optical density (OD) 600 nm between 0.08 and 0.1 (corresponding to Mac Farland). An OD of (0.08 – 0.1) corresponds to 10^8 CFU/mL (Haddouchi *et al.*, 2009). The tests were carried out with bacterial cells blocked in the exponential phase and the stationary phase of growth. The protocol for measuring the growth of bacterial species allowed us to have the different incubation times necessary to maintain the cells in non-renewed peptone liquid medium at the desired growth phases. The time frame was 5-11h and 11-23h respectively for the exponential and stationary phases of *E. coli* (Tamsa, 2017); 3-22 h and 22-24h respectively, for the exponential and stationary phases of *V. cholerae* (Rafael *et al.*, 1996); and 2-4h and 4-8h respectively for the exponential and stationary phases of *S. aureus* (Beck *et al.*, 2001).

Inoculation of bacterial suspensions and immersion of charcoal fragments in the flasks

The densities of the microorganisms in each stock solution were adjusted to an initial concentration (T0) of 10^8 CFU/mL corresponding to Mac Farland. A volume of 1 mL of bacterial suspension blocked at the different growth phases was taken and introduced using a sterile pipette into sterile glass bottles containing 100 mL of sterile physiological water (NaCl 0.85%). The vials were shaken to make the suspension homogeneous. The previously sterilized charcoal fragments were introduced in horizontal contact into the vials containing the bacterial suspensions blocked at the different growth phases. The coal immersion times were 3 hours, 6 hours and 9 hours, respectively.

Removal of substrates and unhooking of attached bacterial cells

Removal of substrates from the bacterial suspension

At the end of each incubation period, each charcoal fragment was removed from the vial under sterile conditions created by means of the blue Bunsen burner flame, using sterile forceps and introduced into test tubes containing 10 mL of sterile physiological water.

Release of bacterial cells attached to the supports

The bacterial cells attached to the different substrates were removed by stirring the latter in 10 mL of sterile physiological water (NaCl 0.85%), using a BUNSEN brand AGT-9 tube shaker, operated at increasing speeds of 30 to 50 revolutions per minute for approximately 20 seconds. Variations in dropout speed led to obtaining the maximum number of dropped cells (Dukam *et al.*, 1995). The substrates removed from each bottle was done by a series of 6 agitations each time in 10 mL of sterile physiological water. The six suspensions resulting from the dropout were sterilely transferred into a sterile 100 mL glass bottle. The final volume of the bacterial suspension resulting from the dropout was 60 mL. This suspension was used to determine the number of cells that adhered to each substrate.

Analysis of suspensions obtained from dropouts

The dropped cells were isolated and counted using standard techniques (Holt *et al.*, 2000). These analyzes were carried out on selective agar media. The culture media used were TCBS agar, ENDO agar and CHAPMAN mannitol agar for *V. cholerae*, *E. coli* and *S. aureus* respectively. Incubation temperatures were 37°C for *V. cholerae* and *S. aureus* and 44°C for *E. coli* for 24 to 48h. The results were expressed in Colony Forming Unit per unit volume of the suspension of stall water, then reported per cm^2 of the surface of the submerged charcoal substrate (CFU/cm^2). The experiment was thus carried out six times in a row and the average results obtained during these six campaigns were analyzed using the appropriate software.

Evaluation of the abundance of bacterial cells that remained planktonic

The adhesion of the microorganisms to the immersed solid supports was carried out in sterile physiological water contained in the Erlenmeyer flasks. Followed by evaluating the abundance of cells that remained planktonic at time T0 (initial moment) and after 3 hours, 6 hours and 9 hours of incubation. The isolation and counting of the cells that remained planktonic were carried out after the removal of each charcoal fragment from each Erlenmeyer flask, by culture on specific medium followed by incubation of the petri dishes at 37°C for *Vibrio cholerae* and *Staphylococcus aureus* and at 44°C for *Escherichia coli* for 24 to 48 hours.

Data analyses

Temporal variations in bacterial abundances expressed in CFU/cm^2 were represented by histograms. The degrees of connection between the abundances of adhered cells and the incubation times, between adhered bacterial cells and those remaining planktonic were evaluated by Spearman's "r" correlation tests at each experimental condition. Comparisons of data means were made using the Kruskal-Wallis "H" test. All these analyzes were carried out using SPSS software version 16.0.

Calculation of the speed of cell movements

The speeds of movement of the microorganisms on the non-activated charcoal substrates were evaluated using Excel software with the equation of the regression line of the cells adhered to each incubation period of 3 hours in the form $\boldsymbol{y = ax+b}$. In this equation, "**y**" represents in CFU, the number of adhered cells per incubation period and "**x**" represents each incubation time expressed in hours (3 hours). The slope ratio "**a**" of the line over three (03) hours gave the apparent speed of cell movements per hour. This slope was then equated to the cell attachment speed when it was positive and to the cell detachment speed when it was negative. The results are expressed as number of cells adhered or detached per cm^2 and per hour.

Expression of adsorption parameters

In the present study, the adsorption data were analysed using the Freundlich model described by the equation:

$$C_s = K_f C^{1/n}$$

With **Cs**: quantity of bacteria adsorbed by the adsorbent

C: concentration of planktonic cells at equilibrium

$\mathbf{K_f}$: Freundlich adsorption coefficient

1/n: linearity coefficient; "n" being the adsorption intensity.

Results

Evaluation of the density of bacterial cells that remained planktonic

The densities of the microorganisms in each stock solution (T0) were adjusted to an initial concentration of 10^8 CFU/mL after a series of dilutions. The abundances of cells that remained planktonic after 3, 6 and 9 hours of incubation were evaluated as shown below in Figure 2 (A', B', C').

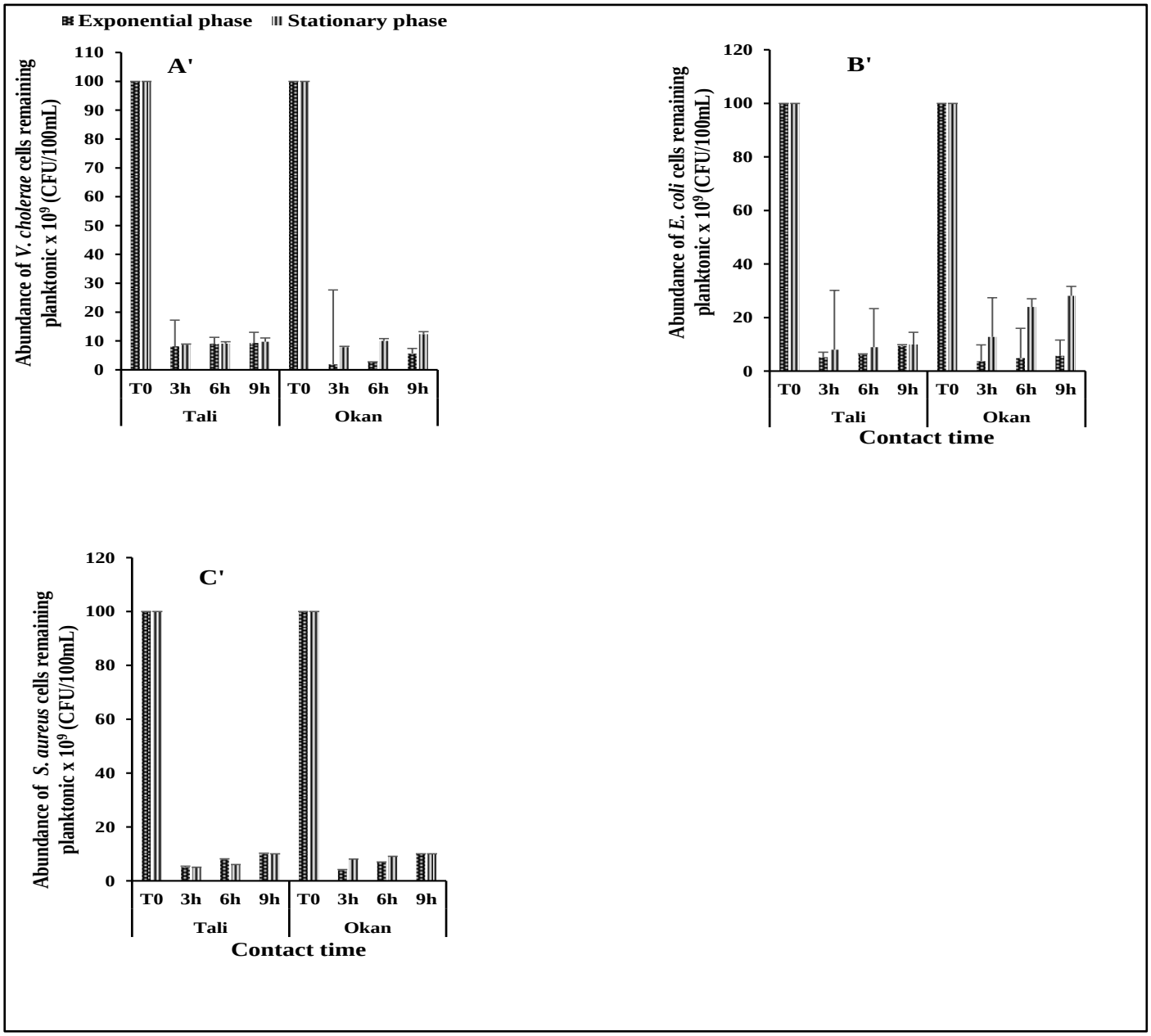

Figure 2: Temporal variation of the mean abundances and standard deviations from the mean of bacterial cells (A': *Vibrio cholerae*, B': *Escherichia coli* and C': *Staphylococcus aureus*) which remained planktonic at each growth phase.

Density of *Vibrio cholerae* cells remaining planktonic

The evolution of the average abundances of *Vibrio cholerae* cells which remained planktonic, after 3, 6 and 9 hours of incubation are illustrated by histograms as presented in Figure 2A'. Overall, the average abundances of *V. cholerae* cells that remained planktonic in stationary phase oscillated between $9x10^9$ CFU/100 mL and $100x10^9$ CFU/100 mL in the presence of Tali and between $8x10^9$ CFU/100 mL and $100x10^9$ CFU/100 mL in the presence of the Okan. They varied from $8x10^9$ CFU/100 mL to $100x10^9$ CFU/100 mL in the presence of Tali and from $2x10^9$ CFU/100 mL to $100x10^9$ CFU/100 mL in the presence of Okan at exponential phase. The minimum abundance ($2x10^9$ CFU/100 mL) of *V. cholerae* cells that remained planktonic was observed in exponential phase in the presence of Okan, after 3 hours of immersion of the charcoal fragments (Figure 2A').

Density of *Escherichia coli* cells that remained planktonic

The abundances of *E. coli* cells, which remained planktonic, fluctuated from $8x10^9$ CFU/100 mL to $100x10^9$ CFU/100 mL in the presence of Tali and from $13x10^9$ CFU/100 mL to $100x10^9$ CFU/100 mL in the presence of Okan in the stationary phase. They oscillated between $5x10^9$ CFU/100 mL and $100x10^9$ CFU/100 mL and between $4x10^9$ CFU/100 mL and $100x10^9$ CFU/100 mL, in the exponential phase in the presence of Tali and Okan, respectively. The minimum abundance ($4x10^9$ CFU/100 mL) of *E. coli* remained planktonic, was observed in the exponential phase in the presence of the Okan, after 3 hours of immersion of the charcoal fragments (Figure 2B').

Density of *Staphylococcus aureus* cells that remained planktonic

The cell densities of *S. aureus*, which remained planktonic in the stationary phase, varied between $5x10^9$ CFU/100 mL and

100x10^9 CFU/100 mL in the presence of Tali; and between 8x10^9 CFU/100 mL and 100x10^9 CFU/100 mL in the presence of Okan. They fluctuated from 5x10^9 CFU/100 mL to 100x10^9 CFU/100 mL and from 4x10^9 CFU/100 mL to 100x10^9 CFU/100 mL in the exponential phase in the presence of Tali and Okan, respectively. The lowest density (4x10^9 CFU/100 mL) of *S. aureus* cells, which remained planktonic, was observed in stationary phase in the presence of Okan after 3 hours of immersion of the charcoal substrates (Figure 2C').

Evaluation of the abundances of adhered bacteria

The temporal variations in the abundances of bacteria adhered to the charcoal substrates are illustrated by histograms in order to better appreciate the distribution of cell densities (Figure 3).

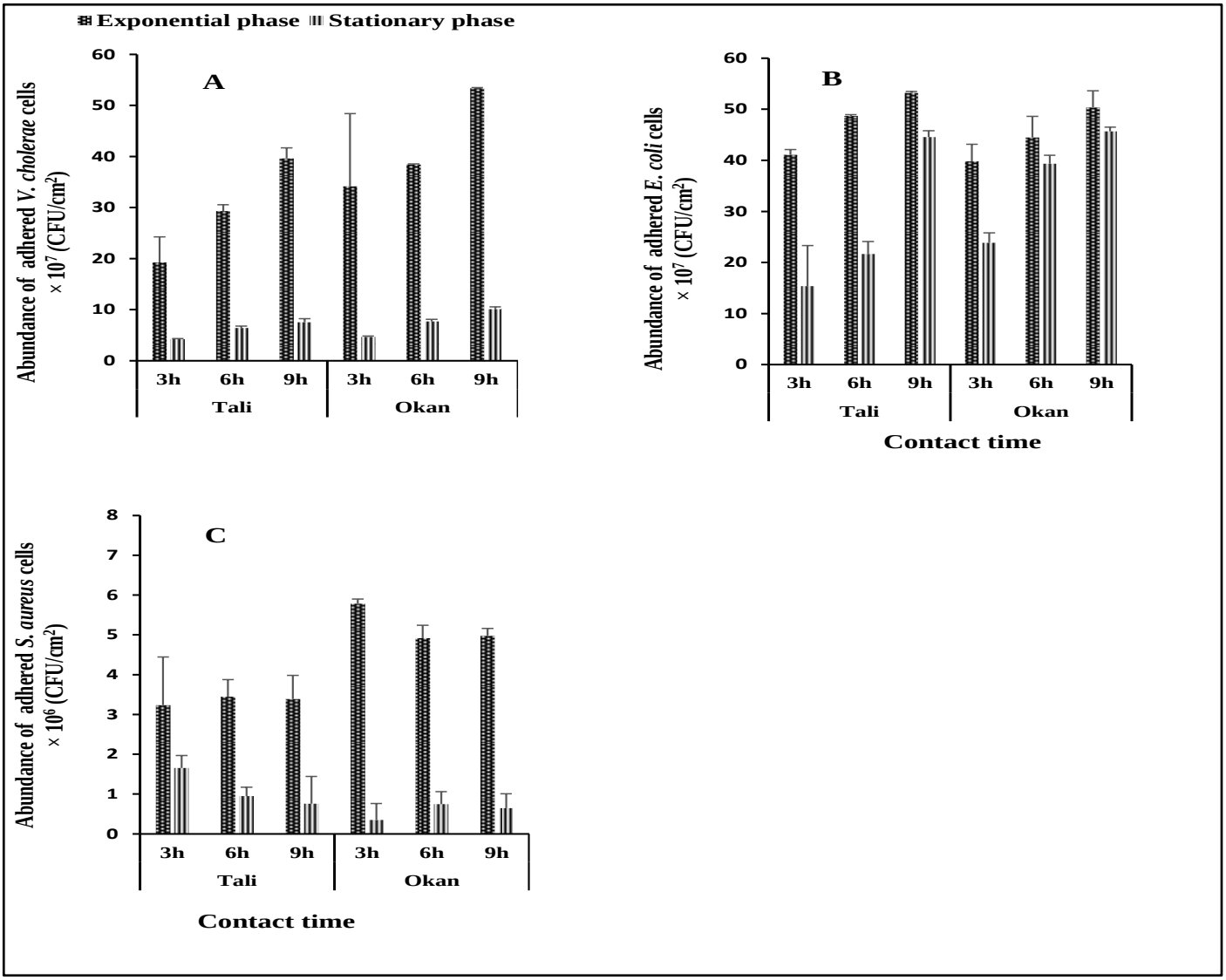

Figure 3: Temporal variation of mean abundances and standard deviation from mean of adhered bacteria/cm^2 on the Tali and Okan (A : cells of *Vibrio cholerae*, B : cells of *Escherichia coli* and C : cells of *Staphylococcus aureus*) at each growth phase.

Abundances of adhered *Vibrio cholerae* cells

The evolution of the average abundances of *V. cholerae* cells adhered to the surface of the charcoal substrates, after 3, 6 and 9 hours of immersion of the substrates are illustrated by histograms as presented in Figure 3A. Overall, the average abundances of adhered *V. cholerae* cells fluctuated from 1x10^7 CFU/cm^2 to 2x10^7 CFU/cm^2 on both Tali and Okan in stationary phase. They reached 40x10^7 CFU/cm^2 and 53x10^7 CFU/cm^2, respectively in the Tali and Okan at the exponential phase. The minimum abundances of adhered *V. cholerae* cells (1x10^7 CFU/cm^2) were observed in stationary phase on the two charcoal substrates, after 3 hours of incubation. The maximum abundance of adhered *V. cholerae* cells (53x10^7 CFU/cm^2) was observed in exponential phase on the Okan, after 9 hours of immersion of the charcoal fragments (Figure 3A).

Abundances of adhered *Escherichia coli* cells

The abundances of *E. coli* cells adhered to charcoal substrates reached 53x10^7 and 50x10^7 CFU/cm^2 respectively on Tali and Okan in the exponential phase. They varied from 15x10^7 CFU/cm^2 to 45x10^7 CFU/cm^2 on the Tali; and from 24x10^7 CFU/cm^2 to 46x10^7 CFU/cm^2 on the Okan in stationary phase. The lower abundance of *E. coli* adsorbed (15x10^7 CFU/cm^2) was observed in stationary phase on the Tali, after 3 hours of immersion of the carbon fragments. The maximum cell density of *E. coli* adhered (53x10^7 CFU/cm^2) was observed in the exponential phase on the Tali, after 9 hours of immersion of the

charcoal fragments (Figure 3B).

Abundances of adhered *Staphylococcus aureus* cells

The cell densities of adhered *S. aureus* reached $3x10^6$ CFU/cm^2 and $6x10^6$ CFU/cm^2 respectively on Tali and Okan in exponential phase. In the stationary phase, they reached $2x10^6$ CFU/cm^2 on the Tali and $1x10^6$ CFU/cm^2 on the Okan. The lowest abundance of adhered *S. aureus* cells was observed in stationary phase on the Okan after 3 hours of immersion of the charcoal fragments. The maximum density of these adhered cells ($6x10^6$ CFU/cm^2) was observed in the exponential phase on the Okan after 3 hours of immersion of the substrates (Figure 3C).

Adsorption isotherms of bacteria on carbon substrates

Considering the number of adsorbed bacterial cells and the concentration of planktonic (non-adsorbed) bacteria, the Freundlich isotherms were constructed for each charcoal substrate and for each growth phase.

Adsorption isotherms of *Vibrio cholerae* cells

The adsorption coefficient (**Kf**) varied from 12 to 192 adhered cells/cm^2 on Tali and from 131 to 639 adhered cells/cm^2 on Okan. The lowest value (12 adhered cells/cm^2) was recorded in stationary phase on the Tali and the highest value (639 adhered cells/cm^2) in the exponential phase on the Okan (Table I).
The linearity coefficient (***1/n***) varied from 0.77 to 1.23 on the Tali and from 0.54 to 0.75 on the Okan. Low values of this parameter reflect relatively strong adsorption intensities and high values, relatively low adsorption intensities. The lowest value (0.54) was recorded in the exponential phase on the Okan and the highest (1.23) was noted in the exponential phase on the Tali (Table I).

Table I: Values of the adsorption coefficient (Kf) and the linearity coefficient (*1/n*) depending on the charcoal substrates and the growth phases of the *Vibrio cholerae* bacteria

Growth phase	Charcoal substrates	Adsorption coefficient(Kf) (adhered cells/cm^2)	linearity coefficient (*1/n*)
Exponential phase	Tali	192	1.23
	Okan	639	0.54
Stationary phase	Tali	12	0.77
	Okan	131	0.75

Adsorption isotherms of *Escherichia coli* cells

The parameters **Kf** and ***1/n*** of the isotherms obtained by linear regression of the data varied at each growth phase depending on the carbonaceous substrates. The linearity coefficient (*1/n)* varied from 1 to 1.19 on the Tali and from 0.69 to 1.78 on the Okan. The lowest value (0.69) was recorded in the exponential phase on the Okan and the highest (1.78) was noted in the stationary phase on the same species of wood (Table II).

The adsorption coefficient (Kf) varied between 1 and 2 adhered cells/cm^2 and between 1 and 7 adhered cells/cm^2 respectively on Tali and Okan. The lowest value (1 adhered cell/cm^2) was recorded in stationary phase with the Tali and the highest value (7 adhered cells/cm^2) was observed in exponential phase on the Okan (Table II).

Table II: Values of the adsorption coefficient (Kf) and the linearity coefficient (*1/n*) depending on the charcoal substrates and the growth phases of the *Escherichia coli* bacteria

Growth phase	Charcoal substrates	Adsorption coefficient(Kf) (adhered cells/cm^2)	linearity coefficient (*1/n*)
Exponential phase	Tali	2	1
	Okan	7	0.69
Stationary phase	Tali	1	1.19
	Okan	1	1.78

Adsorption isotherms of *Staphylococcus aureus* cells

The adsorption coefficient (**Kf**) varied from 1 to 2 adhered cells/cm^2 on both Tali and Okan. The lowest value (1 adhered cells/cm^2) was recorded in the stationary phase for both charcoal substrates, while the highest (2 adhered cells/cm^2) was recorded at the exponential phase on both charcoal (Table III).
The linearity coefficient (***1/n***) varied from 1.87 to 1.94 on the Tali and from 1.86 to 1.90 on the Okan. The smallest value (1.86) was recorded in the exponential phase with the Okan and the highest (1.94) in stationary phase for Tali (Table III).

Table III: Values of the adsorption coefficient (Kf) and the linearity coefficient (*1/n*) depending on the charcoal substrates and the growth phases of the *Staphyloccocus aureus* bacteria

Growth phase	Charcoal substrates	Adsorption coefficient (Kf) (adhered cells/cm^2)	linearity coefficient (*1/n*)
Exponential phase	Tali	2	1.87
	Okan	2	1.86
Stationary phase	Tali	1	1.94
	Okan	1	1.90

Correlations between average densities of adsorbed bacteria and contact times with charcoal substrates

It appears from this analysis that increasing the incubation time leads to a significant increase ($P<0.05$) in *E. coli* adhered to the Okan. In the majority of cases, the incubation time does not significantly influence the adhesion of bacterial cells attached to charcoal substrates (Table IV).

Table IV: Spearman's "r" correlation coefficient between the average abundances of adsorbed bacteria and the contact times with the substrates.

	Tali			Okan		
	E. coli	***V. cholerae***	***S. aureus***	***E. coli***	***V. cholerae***	***S. aureus***
Incubation time	0.7	0.5	-0.1	0.84*	0.5	0.0001

Correlation between the densities of adhered bacterial cells and those of cells that remained planktonic

Spearman's "r" correlation test showed no significant connection between the average abundances of adsorbed bacteria and those remaining planktonic, for all incubation times and growth phases.

Comparison between the average abundances of bacterial cells adhered to charcoal substrates

The Kruskal Wallis "H" comparison test revealed no significant difference between the average abundances of *Vibrio cholerae, Escherichia coli and Staphylococcus aureus* cells adhered to the Tali and those adhered to the Okan (P>0.05).

Discussion

The variations in the abundance of cells that remained planktonic revealed that certain microorganisms adhered to the charcoal fragments following reversible and irreversible adhesion mechanisms. According to Pouneh (2009), microbial adhesion on supports takes place in two main stages: adhesion and adhesion in itself which correspond respectively to reversible adhesion and irreversible fixation of germs. Reversible adhesion is generally non-specific and short-lived (5 to 10 hours) (Kendall *et al*., 2011; Samandoulgou, 2015). Irreversible adhesion is a slower stage than the previous one. The irreversibility of adhesion is due to the fact that the microorganism secretes a matrix of exopolymers forming around it an envelope called glycocalyx, as well as other specific molecules (adhesins) which allow it to consolidate its adhesion to the support (Fridjonsson *et al.*, 2011; Kone, 2012). The incubation time is the time the bacteria come into contact with the submerged carbon supports. The study shows temporal variations in the abundances of cells adhered to charcoal fragments. These abundances of cells adhered to charcoal substrates differed depending on the duration of incubation. The abundances of adhered bacteria undergo temporal variations. According to Vance (2002), the different stages of the process involves: initial adsorption which is reversible, and final adsorption which is irreversible. During reversible adsorption, Van Der Walls forces and electrostatic repulsion forces intervene, it also involves acid-base bonds of the Lewis type, and receptor-ligand types among others (Branger *et al.*, 2007). Irreversible adhesion occurs through the cellular production of exopolymers which anchor the cells to the surface of the solids (Boutaleb, 2007). During incubation times, adsorptions and desorption of cells often occur on the surfaces of solid substrates (Nola *et al.*, 2004). This process would be partly responsible for the temporal variation in the abundances of cells adhered to the surface of the charcoal fragments, from one incubation period to another.

The adsorption graphs indicate temporal variations in bacterial abundances. These irregular variations in the number of adsorbed cells show the reversibility of the process and reflect the phenomenon of adsorption-desorption-readsorption. This process, which can involve 30 to 67% of the adsorbed microbial biomass (Grasso *et al.*, 2002), has been indicated as sometimes linked to the humidity of the substrate. According to Hamadi *et al.* (2013), water molecules disrupt the configuration of the surfaces of the cell and the substrates in contact, especially when the adsorption is long-lasting. As already noted by Vernhet (2005), the processes of adsorption, desorption and re-adsorption occur with different rates from one substrate to another. The presence of air can also induce the reversibility of adsorption, the synergy of the actions of oxygen and the duration of incubation often leading to the reduction of cellular adsorption to solid particles (Nikolaev, 2000). Numerous interactions exist between adsorbed cells and can significantly modify their structure and physiology (Da Silva *et al.*, 2011). These interactions, sometimes in the form of protein signals, have also been noted between bacteria and yeasts, the yeasts adhering more weakly to the particles because of their large size (Da Silva *et al.*, 2011).

The variation in the adsorption potential of carbon substrates could be linked to that of the properties of the adhesion sites located on the surface of these substrates, these surfaces containing groups of attachment sites susceptible to variation (Kim *et al.*, 2011), although the exopolysaccharide molecules of bacteria have chemically active sites involved in the adhesion process (Wang *et al.*, 2002). The synthesis of these exopolysaccharide polymers is stimulated by the initial adhesion of the bacterial cell, and its concentration increases considerably as soon as the attachment of the bacteria to the charcoal fragment is effective (Vance, 2002). These physicochemical properties of the sites determine the strength and capacity of adsorption, as well as the specificities of interactions between the two surfaces (Levy *et al.*, 2012). According to Wang *et al.* (2004), bacterial adsorption processes are often energetic, the interaction energy between the two surfaces can vary depending on the properties of the bacteria wall. The variation in adsorption potential observed would also be linked to the characteristics of the bacterial wall, characteristics often described as dynamic (Van Der Mei *et al.*, 2000). This dynamic character would be linked to the chemical composition which is, at any moment, rapidly modified in response to the physicochemical environment (Hamadi *et al.*,

2013).

Low linearity coefficients express a relatively strong intensity of adsorption while high values express the relatively weak intensity of adsorption of bacteria to substrates. Due to the reversibility of the adsorption process of these bacteria to carbonaceous substrates, the values of the adsorption and linearity coefficients would be unstable. Variations in the values of these coefficients could also result from the various physical properties of these different charcoal fragments, which can significantly and selectively influence cellular adsorption to substrates (Jeljaszewicz *et al.*, 2000).

Conclusion

This work consisted of investigating on the one hand, the abundances of adhered cells and the remaining planktonic, on the other hand, the parameters linked to charcoal substrates likely to influence the cell adhesion process studied using *V. cholerae*, *E. coli* and *S. aureus*. The results obtained shows that the abundances of *V. cholerae*, *E. coli* and *S. aureus* undergo temporal variations. The densities of adhered bacterial species were higher in the exponential phase than in stationary phase. Freundlich isotherms demonstrated that the absorption capacity and intensity of microorganisms varied depending on the type of coal used. The highest value of the adsorption coefficient (Kf) and the lowest value of the linearity coefficient (1/n) were recorded on the Okan. The latter presented a relatively high cellular adsorption potential compared to Tali, although no statistically significant difference was noted between the two substrates.

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