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Research Paper

Abundance of heterotrophic aerobe bacteria (HAB) adsorbed on Granite, Basalt and Migmatite rock fragments immersed in wells in Central Africa: Temporal variation and assessment of the hierarchical influence of some abiotic factors

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Abstract

This study aimed at assessing the daily abundance of heterotrophic aerobe bacteria (HAB) adsorbed on fragments of Granite, Basalt and Migmatite immersed in two wells W1 and W2 on the one hand and the contribution of some abiotic factors on this process on the other hand. The incubation durations of rock fragments immersed were 4, 8 and 12h. In W1, the abundance of bacteria adhered on the Basalt varied between $2x10^2$ and $2.3x10^4$ CFU/cm^2 after 4h, between $4x10^2$ and $1.3x10^4$ CFU/cm^2 after 8h, and between $1.7x10^3$ and $1x10^5$ CFU/cm^2 after 12h. On the Migmatite, it ranged from $9x10^2$ to $2.7x10^4$ CFU/cm^2 after 4h, from $3.6x10^3$ and $2.4x10^4$ CFU/cm^2 after 8h and from $4.4x10^3$ and $8.8x10^5$ CFU/cm^2 after 12h. On the Granite, the abundance after 4, 8 and 12h varied between $4x10^2$ and $4.1x10^4$, between $2x10^2$ and $1.3x10^5$ and between $4.2x10^3$ and $7.3x10^4$ CFU/cm^2 respectively. In W2, the abundance of cells adsorbed after 4h ranged from $1.1x10^3$ to $1.3x10^5$ CFU/cm^2 on the Basalt, from $8x10^2$ to $2.2x10^4$ CFU/cm^2 on Migmatite and from $9x10^2$ to $6.2x10^4$ CFU/cm^2 on the Granite. After 8h, it ranged from $5x10^2$ to $1.2x10^4$ CFU/cm^2 on the Basalt, from $4x10^4$ to $2.5x10^4$ CFU/cm^2 on the Migmatite, and from $9x10^2$ to $3.9x10^4$ CFU/cm^2 on the Granite. After 12h, it fluctuated between $6x10^2$ and $9.8x10^4$ CFU/cm^2 on the Basalt, $5x10^3$ and $1.9x10^5$ CFU/cm^2 on the Migmatite, and $1.3x10^2$ and $1.5x10^5$ CFU/cm^2 on the Granite. Using the power function law, it was noted that the process was mainly controlled by depth of water column (DWC), suspended solids, electrical conductivity (EC), and the amount of bacteria adhered after the first 4h incubation on Basalt, the color and turbidity on Granite, and by the DWC and EC on Migmatite. The dominant factors varied from one rock to another, and the hierarchy order undergoes temporal variation.

Keywords: Well water, HAB, adhesion, rock fragment, abiotic parameter, power factor

Introduction

It has long been recognized that colloid-sized particles, including microorganisms, are transported through porous media. This implication is of obvious importance when pathogens are involved, due to public health concerns. The importance of microbial attachment to solid surfaces is recognized in a wide variety of environments. Although many different materials have been used as substrates for the study of microbial attachment, most were manmade. The use of untreated or treated glass, plastics, and various metals, with experimental coatings, is appropriate for questions related to biofilm formation. However, when studying the role of attached bacteria in a natural ecosystem, it is more relevant to use local native substrates (e.g. rocks) found in that environment. Bacteria attached to rocks are termed *epilithic* [1].

In some region of the world, the daily use of water is increasing with increase in populations and wealth, and water scarcity is being further exacerbated by climate change [2]. In other regions, the real problem is that of microbial quality of water, because groundwater which is most consumed is polluted. The use of polluted groundwater by people is caused by poverty on one hand and by unfavorable natural conditions in the other hand.

Bacteria origin in various ground waters is still debated. Ghiorse and Wilson (1988) [3] assumed that, microbial distribution in surface aquifers may be controlled by infiltration from the unconfined zones above, while new populations of microorganisms may colonize an aquifer by vertical or lateral migration. Mayer et al. (1997) [4] and Dzeda et al. (1998) [5] postulated that, although the origin of some microorganisms in underground water is not clear, most bacteria in aquifers would come from propagation or transportation by seeping contaminated water. According to Fenchel (2001) [6], autochthonous bacterial ancestors in underground aquatic ecosystem originated from anoxigenic microorganisms, which have undergone mutations. The methods aims at reducing the abundance of planktonic bacteria in groundwater are still in progress.

Rocks found in subsurface are of various petrography and mineralogical properties. They can thus be metamorphic, sedimentary and volcanic, among others. Their difference can potentially affect bacteria cell retention on their surfaces. Little is known on the influence of their properties on microbial adsorption on their surfaces during groundwater movements. Furthermore, no data is available relative to the temporal evolution of bacterial abundance retained on rock surfaces in groundwater. The main purpose of this study was to assess the daily temporal variation of heterotrophic bacterial abundance adsorbed on Granite, Basalt and Migmatite immersed in wells, and the contribution of some biotic and abiotic parameters of the medium on this process.

Materials and Methods

Description of study area

Well water points are numerous in the Yaounde area (Cameroon, Central Africa) (latitude 3° 52’ N, longitude 11° 32’ E, with average altitude of 760 m), and are widely used by the population. The climate is typically equatorial, with 4 unequal seasons [7]. A mild rainy season from April to June, a mild dry season from July to August, a peak rainy season from September to November and a peak dry season from December to March of the next year. The soil is ferro-lateritic, acidic with pH values sometimes lower than 5. The sand abundance sometimes reached 73% near the soil surface, and decreases gradually as soil depth increases. The soil porosity sometimes reached 71%, and its density is 2.7. The vertical permeability of the soil reaches 300 cm/h near the surface and its horizontal permeability undergoes spatial variation and reaches 40 cm/h [7, 8]. In this region, groundwater is contaminated by faecal bacteria and other opportunistic pathogens [9, 10]. Two wells were selected among those used by the population, in two different districts. They were coded W1 and W2 and were covered by a sheet of aluminum. They were of depths 6 m and 6.5 m respectively and 180 cm diameter.

Description of sorbent substrates, their immersion and removal from wells

Three different rocks were used: basalt (volcanic rock), granite (igneous rock) and migmatite (metamorphic rock). Three fragments A, B and C of rectangular shaped (0.9 cm wide, 3.24 cm length

and 0.9cm in height) of the same surface structure were obtained from each type of rock in triplicate. The total surface of each fragment was 13.28 cm^2.

Both well water points were not used by the population during the experiments days. Each of the triplicate fragments A, B, C was fixed first on 3 small clips. Each clip holds 0.025 cm^2 on each of the 2 sides of chip. The clip was then attached to a wire of 0.25 mm in diameter. The coupon with the clip and wire were then sterilized using autoclave. Before the immersion of the fragments, the depth of water column (DWC) was measured using a sterile wire at a 6 am at each well water point on the sampling day. Then two water samples were collected, one in a sterile glass bottle of 250 mL for bacteriological analysis and the other in a clean polyethylene bottle of 500 mL for the physico-chemical analysis.

Each fragment connected to the wire was then introduced in the mid-depth of water column, and then incubated *in situ*. The incubation durations were 4, 8 and 12h respectively for the triplicate fragments A, B and C, as the study aimed at assessing the daily abundance of the heterotrophic bacterial adsorbed. The removing instant was 10 am for the fragments A, 2 pm for fragments B, and 6 pm for fragments C. Each rock-coupon removed from the water was immediately placed in a sterile Falcon tube containing 20 mL of a sterile NaCl solution (8.5 g/L). This was then transported to the laboratory in refrigerated conditions (7 ± 1°C) to harvest the bacteria which had adhered on them.

Harvest of adhered bacteria from the fragment surface

Each fragment was washed four times in 20mL sterile NaCl solution (8.5 g/L), by shaking for 10 seconds using a Vortex at 1000-1200 rpm. According to Dukam et al. (1995) [11], variation in rotation speed maximizes desorption of cells. The solution containing the suspended bacteria was transferred into a sterile flask glass of 250mL. Another 20mL of NaCl solution was then introduced into the Falcon tube and the whole was immersed in a sonicator for 20 second at 10 °C and then immediately agitated for 10 seconds and transferred into the glass flask of 250mL. The final volume of the bacterial suspension after the bacterial harvest was 100mL.

Water samples analysis

The physico-chemical parameters considered were the temperature, pH, electrical conductivity, total suspended solids (TSS), color, turbidity and Biochemical Oxygen Demand during 5 days (BOD5). The physico-chemical analyses were carried out after homogenization of each sample, using standard techniques according to Rodier (1996) [12] and APHA (1998) [13]. These analyses were done out in triplicate.The bacteriological analyses were performed on water sampled before the immersion of fragments, and on samples containing cells collected from coupons surfaces after desorption processes. The heterotrophic aerobe bacteria (HAB) were considered. The standard agar medium was used for cell cultures (Biokar) [14, 15]. The plate count and membrane filtration methods were used [13]. The experiments were carried out each 2 weeks from February to November 2010.

Data analysis

The amounts of cells adhered per cm^2 of the rock surface were assessed in each sampling well and after 4, 8 and 12 hours of incubation. Spearman correlation coefficients between the monthly values of physico-chemical factors and those of the abundance of bacteria adsorbed on Basalt, Migmatite and Granite surface in each well water point were assessed after each incubation period. Using the values of biotic and abiotic parameters recorded, a ranking in hierarchical order of the physic-chemical parameters in their impact in changes the abundance of bacteria adhered on rock fragment during each incubation period *ti* has been carried out. This was done using a sum of power-law function of the following form [16, 17]:

$$\text{Adhered} - \text{Bacteria}(ti, Rock) = \sum_{i=1}^{y} ai \,.\, Xi^{ni}$$

In this equation, *y* is the number of considered factors. After 4h incubation, the amount of cell adhered was supposed to be one of the factors which may influence the instantaneous changes of the cell abundance on substrate surface. Therefore, the number of factors considered for incubated samples increased by 1. *Xi* is the considered factor and *ni* is its power-law coefficient (scaling exponent or

exponent coefficient), and *ai* is its proportionality coefficient**.** The calculation of the coefficients was performed by iterative method. This power-law function was performed using MATLAB 7.5.0 program.

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

Results and Discussion

Physico-chemical and bacteriological properties of wells

The pH ranged between 5.5 to 5.6 in W1 and between 4.6 and 5.3 in W2. The TSS content ranged from 1.5 to 6 mg/L in W1 and from 1 and 5 mg/L in W2. The electrical conductivity varied from 445 to 525 µS/cm in W1 and from 246 and 286 µS/cm in W2. In W1, the values of water color fluctuated between 27 and 33 Pt.Co. Those of turbidity ranged between 1 .7 and 7.8 FTU. The water column depth fluctuated between 100 and 214 cm. In W2, the values of water color varied from 1.5 and 5.8 FTU; those of turbidity ranged from 27 and 32 Pt.Co. The water column thickness fluctuated between 120 and 210 cm (Figure 1). The standard deviations have not been mentioned on the graphs because most of the curves are too close. The average value of water temperature was 20°C and the BOD5 values were lower than 0.01mg/L during all the sampling campaigns.

The concentration of the planktonic bacteria before immersion of the rock-coupons fluctuated between $8.8x10^2$ and $2.3x10^5$ CFU/mL in W1; it fluctuated between $9.1x10^2$ and $1x10^5$ CFU/mL in W2 during all sampling campaigns. The highest value was registered during August in the two well points (Figure 2).

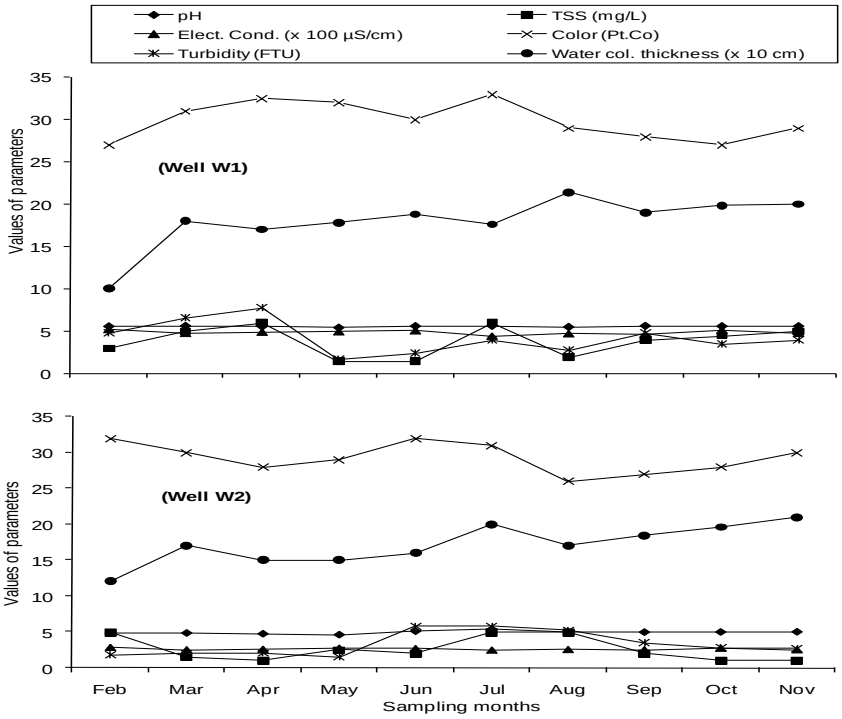

Figure 1: Mean values of physico-chemical parameters in water samples from the wells W1 and W2 at 6 am on the day of each sampling month (the standard deviations have not been mentioned on the graphs because most of the curves are too close)

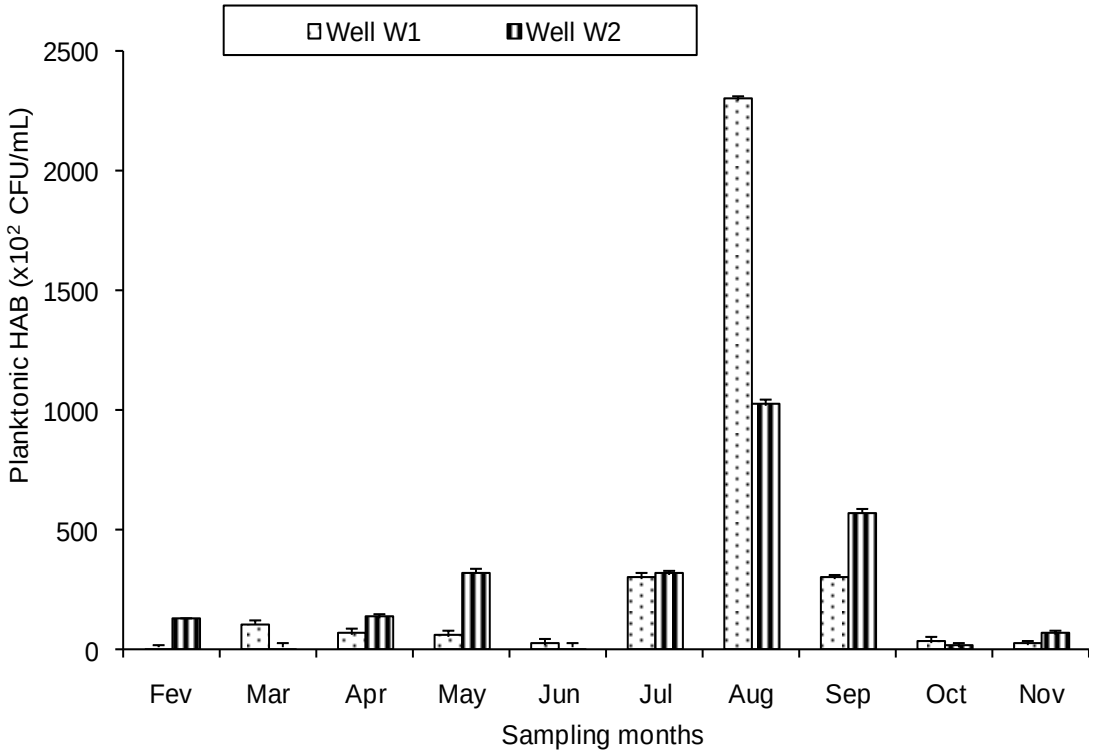

Figure 2: Variation of the average values of the abundance of planktonic bacteria in the well W1 and W2 at 6 am on the sampling day, during each month

Temporal variation of the abundances of bacteria adhered on fragment surfaces during immersion period

For all sampling campaigns in W1, the abundance of bacteria adhered on the Basalt varied between $2x10^2$ and $2.3x10^4$ CFU/cm^2 after 4h of incubation, between $4x10^2$ and $1.3x10^4$ CFU/cm^2 after 8h of incubation, and between $1.7x10^3$ and $1x10^5$ CFU/cm^2 after 12h of incubation (Figure 3). On the Migmatite, it ranged from $9x10^2$ to $2.7x10^4$ CFU/cm^2 after 4h incubation, from $3.6x10^3$ and $2.4x10^4$ CFU/cm^2 after 8h, and from $4.4x10^3$ and $8.8x10^5$ CFU/cm^2 after 12h of incubation. On the Granite, the abundance of bacteria adhered after 4, 8 and 12h of incubation varied between $4x10^2$ and $4.1x10^4$, between $2x10^2$ and $1.3x10^5$ and between $4.2x10^3$ and $7.3x10^4$ CFU/cm^2 respectively (Figure 3). The lowest abundance of HAB adhered per unit area was recorded after 4 h of incubation, during February, on the basalt. The highest was registered on the migmatite after 12h of incubation during October (Figure 3).

In W2, the number of cells adsorbed after 4h of immersion ranged from $1.1x10^3$ to $1.3x10^5$ CFU/cm^2 on the Basalt, from $8x10^2$ to $2.2x10^4$ CFU/cm^2 on Migmatite and from $9x10^2$ to $6.2x10^4$ CFU/cm^2 on the Granite (Figure 3). After 8h of incubation, it ranged from $5x10^2$ to $1.2x10^4$ CFU/cm^2 on the Basalt, from $4x10^4$ to $2.5x10^4$ CFU/cm^2 on the Migmatite, and from $9x10^2$ to $3.9x10^4$ CFU/cm^2 on the Granite. After 12h of immersion, the concentration of the adhered bacteria fluctuated between $6x10^2$ and $9.8x10^4$ CFU/cm^2 on the Basalt, between $5x10^3$ and $1.9x10^5$ CFU/cm^2 on the Migmatite, and between $1.3x10^2$ and $1.5x10^5$ CFU/cm^2 on the Granite (Figure 3. The lowest abundance $1.3x10^2$ CFU/cm^2 was noted on the granite after 12h of incubation during April. The highest $1.9x10^5$ CFU/cm^2 was registered after 12h of incubation on the migmatite during September (Figure 3).

For all the campaigns in both wells (W1 and W2), a relative temporal variations in the abundances of cells adhered on each fragment surface was noted, but these variations were not significant except on the Basalt surface in W1 ($F= 4.861$, $P= 0.016$). A comparison of the abundances of cells adhered was performed among rock-coupons after each incubation period and the differences observed from one rock to another was not significant ($P<0.05$).

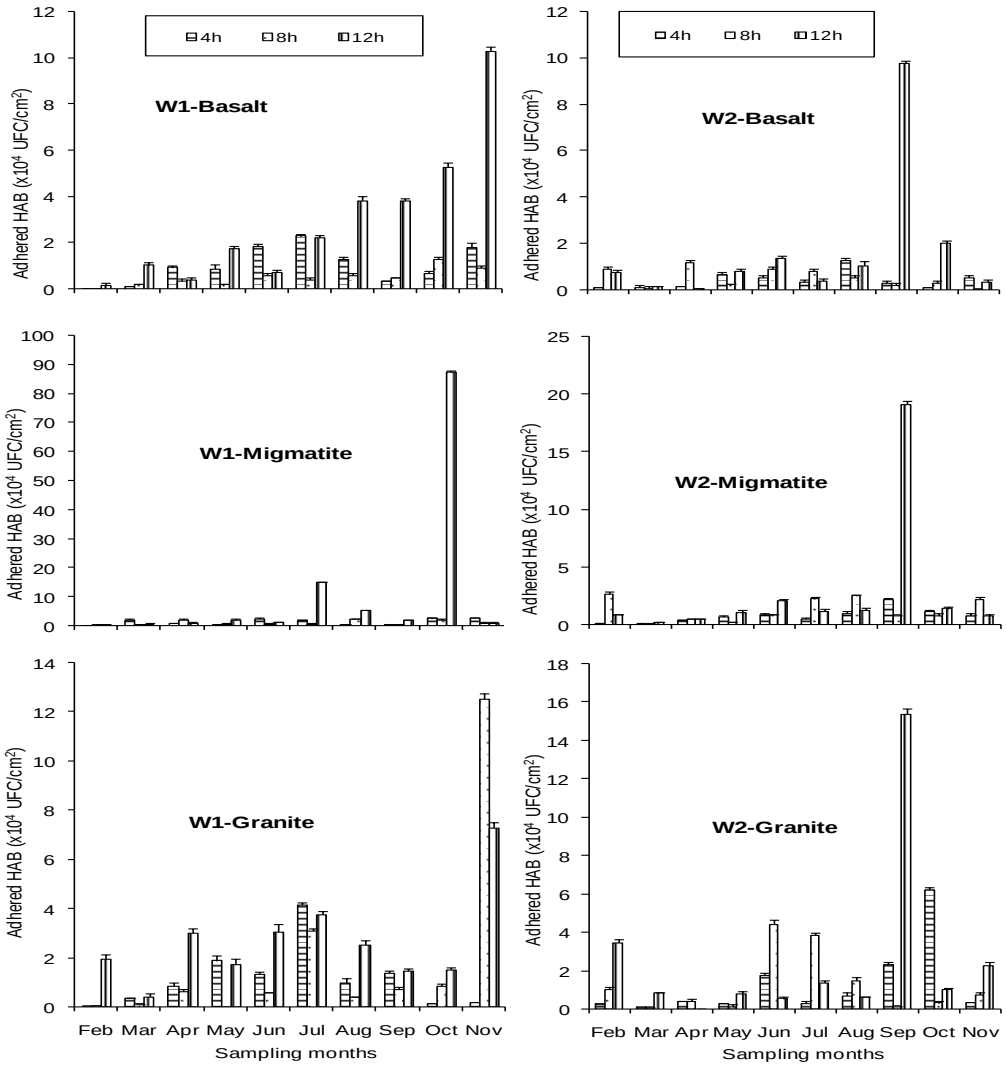

Figure 3: Variation with respect to sampling months of the averages of the abundance of bacteria adhered per cm^2 on Basalt, Migmatite and Granite surface after 4, 8 and 12 hours of incubation respectively in the well W1 and W2

Assessment of the impact magnitude of considered factors in the bacterial adsorption process

The correlation test showed that apart from W2 in which increase in concentration of planktonic bacteria significantly promotes the abundance of bacteria adhered after 4h of incubation ($P<0.05$), the fluctuation of the abundance of cell adhered to the coupons surfaces seems to be of no great relationship with that of planktonic bacteria (Table 1). The increase in pH promotes significantly ($P<0.05$) the retention of bacteria on the Granite surface after 8h of incubation (Table 1). The increase in concentration of TSS in W1 is concomitant ($P<0.05$) to a decrease of the number of cells adhered on Migmatite in W1. The increase in water turbidity occurs concomitantly ($P<0.05$) with an increased in the abundance of bacteria adhered on the Granite surface after 8h, and on the Migmatite after 12h of incubation in W2. The increase in the water column thickness in W1 and that of the amount of cells adhered on each fragment surface occur at the same time. Overall, the degree of relationship between biotic and abiotic properties of the water before the immersion of fragment and the abundance of bacteria adhered after each incubation period varies relatively (Table 1).

The low values of correlation coefficients noted in most cases between the abundances of cells adhered and the biotic and abiotic parameters considered, would signify that the observed process results from the interactions of several factors which would be dominant or not. A hierarchy order of environmental factors that could influence the variation of the abundance of bacteria adhered on each

Table 1: Spearman correlation coefficients between the monthly values of physico-chemical factors and those of the abundance of bacteria adsorbed on Basalt, Migmatite and Granite surface in the wells W1 and W2 after 4, 8 and 12 hours of incubation respectively

Biotic and abiotic factors, and incubation duration		Wells and rock species					
		Well W1			Well W2		
		Basalt	Migmatite	Granite	Basalt	Migmatite	Granite
Planktonic cells at initial moments	4h	0.142	-0.337	0.065	0.748*	0.421	-0.156
	8h	0.039	0.489	-0.150	-0.039	0.360	-0.028
	12h	0.091	-0.098	-0.048	0.339	0.347	0.293
pH	4h	0.085	0.565	0.182	0.286	0.365	0.243
	8h	0.140	0.274	0.657*	-0.036	0.559	0.736*
	12h	0.207	0.413	0.590	0.231	0.438	0.140
TSS	4h	0.116	-0.661*	0.399	0.287	-0.100	-0.337
	8h	-0.037	-0.287	-0.430	0.299	0.586	0.411
	12h	0.116	0.137	0.056	0.206	0.249	0.156
Electrical conductivity	4h	-0.345	-0.438	-0.328	-0.176	0.036	0.176
	8h	-0.030	-0.176	-0.596	0.438	0.006	-0.049
	12h	-0.382	-0.103	-0.243	0.328	0.152	-0.170
Color	4h	0.530	0.128	-0.116	-0.220	-0.544	-0.532
	8h	-0.293	-0.477	-0.092	0.232	0.232	0.391
	12h	-0.280	-0.416	0.361	-0.275	0.220	0.122
Turbidity	4h	-0.433	0.409	0.396	0.341	0.524	0.470
	8h	-0.427	0.329	0.561	0.098	0.396	0.659*
	12h	-0.280	0.524	0.378	0.402	0.640*	-0.040
Depth of Water column	4h	0.236	0.677*	0.104	0.116	0.427	0.250
	8h	0.782**	0.299	0.848**	-0.591	0.098	0.067
	12h	0.806**	0.506	0.238	0.116	0.207	0.390

N= 10 observations ; *: P<0.05 ; **: P<0.01

substrate surface in each well after each incubation duration has been performed using a sum of power-law function. The power-law coefficient and proportionality coefficient of each of the considered factors at each instant are indicated in the decreasing order in Tables 2 and 3. The main four dominant factors are identified at each instant. It appeared that during the first 4h incubation in W1, the depth of water column (DWC) is the main factor controlling the bacterial adsorption on Basalt surface (*n*= 3.86, *a*= 0.00), Granite surface (*n*= 1.68, *a*= 0.24), and Migmatite surface (*n*= 1.53, *a*= 1.08). The DWC is followed on Basalt and Granite surface first by the total suspended solids (TSS), second by the turbidity (Turb), and thirdly by the pH (Table 2). On the Migmatite surface, it is followed first by the electrical conductivity (EC), second by the TSS, and the pH (Table 2).

After 8h of incubation, it is noted that the adsorption process is attributed to the DWC on the Basalt surface (*n*= 3.36, *a*= 0.00) and Migmatite surface (*n*= 1.39, *a*= 1.07). On the Basalt surface, the DWC is followed first by the impact of the bacteria adhered during the first 4h of incubation ($Bact_{ad\text{-}4h}$), second by the water turbidity, and thirdly by the TSS. On the Migmatite surface, the DWC is followed first by the influence of the electrical conductivity, second by the pH, the TSS, and the turbidity. On the granite surface, it is noted that the water color (Col) (*n*= 1.66, *a*= 1.17) seems to dominate in controlling the adsorption process after 8h incubation. It is followed first by the turbidity, second by the TSS, and thirdly by the pH (Table 2). After 12h incubation, the DWC remains the dominate parameter controlling the bacterial adsorption on the Basalt surface (*n*= 1.49, *a*= 1.08) and the Migmatite surface (*n*= 1.77, *a*= 1.03). It is then followed on the Basalt surface in decreasing power by the electrical conductivity, pH, the TSS and the turbidity. On the Migmatite surface, it is followed in decreasing power by the pH, the TSS and the turbidity. The bacterial sorption process on the granite surface after

12h of incubation seems to be mainly attributed to the electrical conductivity (*n*= 1.35, *a*= 1.01). It follows a decreasing power by the pH, the TSS and the turbidity (Table 2).

Table 2: Power functions F showing in decreasing hierarchical order the power-law and proportionality coefficients values of each factor on the abundance of bacteria adhered on Basalt, Migmatite and Granite surface in the well W1 after 4, 8 and 12 hours of incubation

Proportional and power-law coefficients
$F(4h, Basalt) = 0.00DWC^{3.86} -0.63TSS^{1.48} -0.20Turb^{0.41} -0.19pH^{-0.69} +4.15PBact_{im}^{-2.04} +1.84EC^{-3.45} - 2.01Col^{-8.34}$
$F(4h, Granite) = 0.24DWC^{1.68} +0.99TSS^{1.01} +0.96Turb^{0.95} +0.96pH^{0.93} +0.81Col^{0.38} +0.92PBact_{im}^{0.20} +0.749EC^{-0.47}$
$F(4h, Migmatite) = 1.08DWC^{1.53} +1.02EC^{1.05} +1.00pH^{1.00} +1.00TSS^{1.00} +1.00Turb^{1.00} +1.00Col^{0.99} + 0.95PBact_{im}^{0.20}$
$F(8h, Basalt) = 0.00DWC^{3.36} +0.04Bact_{ad-4h}^{1.08} -0.26Turb^{0.49} +0.38TSS^{0.48} +0.88PBact_{im}^{-0.43} +-0.47pH^{-1.18} +0.32EC^{-3.10} -0.52Col^{-3.16}$
$F(8h, Granite) = 1.17Col^{1.66} +1.03Turb^{1.07} + 1.03TSS^{1.05} +1.00pH^{1.01} 0.99EC^{0.95} +0.93DWC^{0.55} +0.18B_{ad-4h}^{-6.99} -162.26PBact_{im}^{-2674.22}$
$F(8h, Migmatite) = 1.07DWC^{1.39} 1.05EC^{1.29} +1.00pH^{1.00} +1.00TSS^{1.00} +1.00Turb^{1.00} +1.00Col^{0.98} +1.05Bact_{ad-4h}^{0.76} -5.44PBact_{im}^{-101.40}$
$F(12h, Basalt) = 1.08DWC^{1.49} +1.02EC^{1.07} +1.00pH^{1.00} +1.00TSS^{1.00} +1.00Turb^{1.00} +1.00Col^{0.99} +1.00Bact_{ad-4h}^{0.88} -3.43PBact_{im}^{-71.88}$
$F(12h, Granite) = 1.01EC^{1.35} +1.00pH^{1.00} +1.00TSS^{1.00} +1.00Turb^{0.99} +0.87Bact_{ad-4h}^{0.99} +0.99Col^{0.95} +0.96DWC^{0.76} +0.95PBact_{im}^{0.20}$
$F(12h, Migmatite) = 1.03DWC^{1.77}+1.00pH^{1.00} +1.00TSS^{1.00} +0.99Turb^{0.98} +1.12EC^{0.88} +0.97Col^{0.87} +1.48Bact_{ad-4h}^{0.33} -2.14PBact_{im}^{-50.2}$

$PBact_{im}$: planktonic bacteria at the initial moment; $Bact_{ad-4h}$: bacteria adhered after the first 4h incubation; TSS: total suspend solids; EC: electrical conductivity; Col: Water color; Turb: Water turbidity; DWC: depth of water column.

In W2, the bacterial adsorption process during the first 4h of incubation seems to be dominated by the DWC on the Basalt (*n*= 1.30, *a*= 1.05) and Migmatite (*n*= 1.24, *a*= 1.04). It follows on both surfaces a decreasing power: electrical conductivity, water color, and turbidity. Meanwhile on the Granite surface, the process is mostly attributed during this incubation period to the electrical conductivity (*n*= 1.33, *a*= 1.00). It follows a decreasing power by: water color, the TSS and water turbidity (Table 3). After 8h of incubation, the electrical conductivity seems to be the dominant factor controlling the bacterial adsorption process on the Basalt surface (*n*= 1.19, *a*= 1.03) and Granite surface (*n*= 1.21, *a*= 1.03). In the meantime, this process on the Migmatite surface seems most attributed to the water color (*n*= 1.45, *a*= 1.37). Bacteria adsorbed during the first 4h of incubation (*n*= 0.97, *a*= 0.57) are among the main four factors controlling the sorption process on the Migmatite surface (Table 3).

After 12h of incubation, the water color seems to be the predominant parameter which has impact on bacterial sorption on the Basalt surface (*n*= 1.07, *a*= 1.01) and the Granite surface (*n*= 1.97, *a*= 1.25). In the meantime, this process on the Migmatite surface is mainly due to the electrical conductivity (*n*= 1.39, *a*= 1.01). It is also noted that adsorbed bacteria during the first 4h of incubation are also among the four main factors which influence the bacteria sorption on the Basalt surface (*n*= 1.01, *a*= 0.60) and the Migmatite surface (*n*= 1.04, *a*= 0.75) (Table 3). The initial planktonic bacterial abundance ($PBact_{im}$) is not among the main four factors which have high impact on bacterial sorption on Basalt, Granite and Migmatite surfaces during incubation in the two wells (Tables 2 and 3).

Table 3: Power functions F showing in decreasing hierarchical order the power-law and proportionality coefficients values of each factor on the abundance of bacteria adhered on Basalt, Migmatite and Granite surface in the well W2 after 4, 8 and 12 hours of incubation

Proportional and power-law coefficients
$F(4h, Basalt) = 1.05DWC^{1.30} +1.04EC^{1.16} +1.01Col^{1.03} +1.00Turb^{1.01} +1.00pH^{1.00} +1.00TSS^{1.00} +0.95PBact_{im}^{0.20}$
$F(4h, Granite) = 1.00EC^{1.33} +1.08Col^{1.29} +1.01TSS^{1.02} +1.01Turb^{1.02} +1.00pH^{1.00} +0.95DWC^{0.76} +0.94PBact_{im}^{0.20}$
$F(4h, Migmatite) = 1.04DWC^{1.24}+1.02EC^{1.16} +1.02Col^{1.08} +1.00Turb^{1.01} +1.00pH^{1.00} +1.00TSS^{1.00} +0.94PBact_{im}^{0.20}$
$F(8h, Basalt) = 1.03EC^{1.19} + 1.02Col^{1.07} +1.00TSS^{1.01} +1.00pH^{1.00} +1.00Turb^{1.00} +0.975DWC^{0.806} +0.94Bact_{ad-4h}^{0.42} -25.60PBact_{im}^{-408.60}$
$F(8h, Granite) = 1.03EC^{1.21} +1.01Col^{1.03} +1.01DWC^{1.02} +1.00pH^{1.00} +1.00TSS^{1.00} +1.00Turb^{1.00} +0.97Bact_{ad-4h}^{0.66} -12.31PBact_{im}^{-203.80}$
$F(8h, Migmatite) = 1.37Col^{2.45} +1.04TSS^{1.06} +1.01Turb^{1.06} +0.57Bact_{ad-4h}^{0.97} +0.94pH^{0.91} +0.71EC^{-0.49} +0.65DWC^{-0.66} -4.04PBact_{im}^{-74.82}$
$F(12h, Basalt) = 1.01Col^{1.07} +0.60Bact_{ad-4h}^{1.01} +1.00TSS^{1.00} +1.00Turb^{1.00} +0.99pH^{0.99} +0.89DWC^{0.48} +0.87EC^{0.30} -8.99PBact_{im}^{-143.74}$
$F(12h, Granite) = 1.25Col^{1.97} + 1.03Turb^{1.07} + 1.04TSS^{1.05} +0.97DWC^{1.03} +1.00pH^{1.00} +0.87EC^{0.34} +0.32Bact_{ad-4h}^{-4.67} -230.39PBact_{im}^{-375.80}$
$F(12h, Migmatite) = 1.01EC^{1.39} +1.08Col^{1.30} +0.75Bact_{ad-4h}^{1.04} +1.01TSS^{1.02} +1.00Turb^{1.01} +1.00pH^{1.00} +0.95PBact_{im}^{0.20} +0.80DWC^{-0.21}$

$PBact_{im}$: planktonic bacteria at the initial moment; $Bact_{ad-4h}$: bacteria adhered after the first 4h incubation; TSS: total suspend solids; EC: electrical conductivity; Col: Water color; Turb: Water turbidity; DWC: depth of water column

The abundance of planktonic HAB relatively varied from one month to another. The peak of this abundance was noted during the August (Figure 2). This period corresponds to the ending of a mild dry season [7].The higher abundance of planktonic HAB could result in the less infiltration of antibacterial substances to the underground water as at this period, the water dilution is relatively minimized and the concentration of the indigenous nutrients can become relatively higher. In general, the temporal variation noted in the abundance of planktonic HAB can be related to many factors. It is known that groundwater often harbors numerous organisms of different phylum and sizes and it is always in movement [18].It is also known that selective and non-selective grazing of groundwater bacteria by nanoflagellates can occur during incubation, and the uptake rate depends on the concentration of bacteria and predators [19]. In addition, Van Elsas and Heijnen [20] and Alden and co-workers [21] indicated that the introduction of bacteria in soil is usually followed by an initial decrease in biomass of the introduced cells, due to the environmental influences on the cell survival and the stability of abiotic factors such as texture, soil particle size, and dynamic abiotic factors such as temperature, water content, pH, nutrients and other chemicals.

The results above revealed that the abundance of adhered bacteria on each coupon surface undergoes temporal variation (Figure 3). It could be due to the sorption and desorption of some cells. It is known that bacterial adsorption is sometimes a reversible process that evolves with time, due to bacterial activity and variations of bacterial wall properties [22, 23, 24]. Stability of this adhesion depends on the number of sites and groups of functional sites properties on the bacterial surface; the site number is expected to vary with the chemical characteristics of the environment [25]. It is less energy-dependent or passive at the beginning of the process, but more energy-depend when the contact duration period becomes longer, probably because the number of binding sites became very limited [26].It involves non-specific interactions of different types (Van der Walls, electrostatic and

hydrophobic) between bacteria and solid particles on the one hand, and the specific interactions between macromolecules of bacterial wall, pili, flagella and solid surface on the other hand [27, 28].

In addition, HAB considered in this study are of various species and could be in different physiological states. The bacterial growth in natural aquatic medium is always in different steps among which the exponential growth phase and stationary phase are sometimes of most importance for the adsorption process. According Hamadouche Nora [29], the exponential phase results in a high cell activity while the stationary phase exhibits a slowdown of this activity, causing chemical changes to the surface of the cell. The bacterial adhesion to substrates is relatively of lower rate in stationary phase than exponential growth phase [29].

In addition, the water movements in natural conditions sometimes significantly impact the adhesion mechanism. They involve convection, diffusion and mobility [30, 18]. The planktonic HAB in water can also be transported to the surface of substrates immersed using flagella movements. Many interactions amongst many bacteria on one hand, and between the rocks surface properties, the physico-chemical properties of water (these two properties undergoing temporal variations) and surfaces of bacterial cell can lead to temporal variation in the abundance of cells adsorbed to solid surfaces as observed in (Figure 3).

The abundance of cell adhered after each incubation period varies relatively among fragments. This could be related to a relative difference among chemical characteristics of the rock. The basalt contains mostly quartz, alkali feldspar mainly orthoclase, sodium plagioclase, calcic plagioclase and olivines, pyroxene and amphibole minerals which are ferromagnesian, Granite is composed mainly of quartz, micas, orthoclase and plagioclase and migmatite consists mainly of quartz, biotite, cordierite and aluminum silicates. Davis and Luttge (2005) [31] when working on the *Shewanella oneidensis* attachment on calcite, dolomite and magnesite surface noted that attached cell densities after 8h of incubation were $2.1x10^3$, $4.3x10^3$ and $3.5x10^3$ cells/cm^2 respectively. Attached cell density increases with increasing incubation duration. Jennifer (2009) [32] noted that anorthoclase and basalt were the most heavily colonized due to the presence of both P, as apatite, and Fe as iron oxides. He noted a strong correlation between the bacterial colonization and P-content of the silicates, and the presence of ferric iron appears to intensify the degree of colonization.

Some physico-chemical and hydrological properties of the well relatively influence the bacterial sorption on the rock surface despite the absence of significant correlation (Table 1). The degree of contribution of each considered factor in the cell adhering process undergoes temporal variation (Tables 2 and 3). Chemical properties of water including pH, dissolved organic carbon; ionic strength of the medium and nutrients are known to affect microbial attachment behavior [33, 23, 24]. Researchers have found that the attachment behavior of *Pseudomonas fluorescens* decreases between pH 5.5 to 7 to silica beads and with decreasing ionic strength [34]. In addition, reactive mineral surface in groundwater can be altered [35]. Phoenix and Konhauser (2008) [36] suggested that biomineralization offers many advantages to bacteria. Some biominerals may act as a source of essential nutrients [37].

Adhesion of bacteria could depend on the water properties. Lehman et al. (2001), [38] when working on bacterial communities in acidic and crystalline rock aquifer, noted that attached and unattached heterotrophs were observed throughout the depth profile. In contrast, chemolithotrophs were not found attached to the rock but were commonly observed in the underground water.

The variation in DWC is one of the most important factors which control the bacterial sorption on the rock surface (Tables 2 and 3). Bacteria can be transported by water movement through aquifer sediments [3]. This movement is influenced by the hydrodynamics and hydro-mechanical coefficients of bacterial scattering, water diffusion coefficient, the coefficient of active mobility of bacteria, the gradient of bacterial concentration, the velocity of underground water movement and retardation factor, magnitude of each parameter varying according to geological conditions and physiological and anatomical status of bacterial cells [39,40].

Conclusion

The rock-fragments used in this study retained bacterial cells on their surfaces. The abundance of cells adsorbed varies relatively with time, and the impact of intrinsic properties of water matrix in this process undergoes a relative temporal evolution. Each environmental factor influences this process at

a different magnitude. If the time required to saturate the adsorption sites on the surface of immersed rock fragments is estimated, and if these fragments are changed regularly, this process can be exploited in rural area to reduce the flux of planktonic microorganisms in wells.

This study showed that bacteria adhere on the surface of rock fragments immersed in wells. The rate of this process undergoes a relatively daily variation. Although man can raise public awareness in using this method in large scale, to reduce the flux of planktonic bacterial contaminant in wells he use, it seems necessary to assess the potential of other rock types (petrography and mineralogical properties) in this process, and compare the potentiality of cell retention of each immersed substrate during night and day period. Other study could also be focused in the assessment of the species diversity of bacteria adhered and on the temporal variation of this diversity with respect to different seasons.

References

1. Mills A.L. and Maubrey R., Effect of mineral composition on bacterial attachment to submerged rock surfaces. Microbial Ecology 7, 315-322, **(1981).**

2. Pittocka J. and Lankford B.A., Environmental water requirements: demand management in an era of water scarcity. Journal of Integrative Environmental Sciences. 7, 75-93, **(2010).**

3. Ghiorse W.C. and Wilson J.T., Microbial ecology of the terrestrial subsurface. Advances in Applied Microbiology. 33, 107-172, **(1988).**

4. Mayer A.S., Carrière P.P.E., Gallo C., Pennel K.D., Taylor T.P., Williams G.A. and Zhong L., Groundwater quality. Water and Environmental Research. 69, 778-844, **(1997).**

5. Dzeda B., Kaiser M. and Mach S., Bacteria and groundwater. Soil and groundwater pollution. http://www.cee.vt.edu/program-areas/environmental/teach/gwprimer/bacteria.html (accessed May 2004), **(1998).**

6. Fenchel T., Micoorganisms (microbes), role of. Encyclopedia of Biodiversiy 4, 207-219, **(2001).**

7. Succhel B., La répartition des pluies et des régions pluviométriques au Cameroun. Travaux et document de géographie tropicale (C.E.G.CNRS) 5,1-288, **(1988).**

8. Bachelier G., Etude pédologique des sols de Yaoundé. Contribution à l'étude de la pédogenèse des sols ferralitiques. Agronomie Tropicale 19, 279-305, **(1959).**

9. Nola M., Njine T., Monkiedje A., Sikati Foko V., Djuikom E. and Tailliez R., Qualité bactériologique des eaux des sources et des puits de Yaoundé (Cameroun). Cahiers Santé 8: 330-336, (1998).

10. Nola M., Njine T., Djuikom E. and Sikati Foko V., Faecal coliforms and faecal streptococci community in the underground water in an equatorial area in Cameroon (Central Africa): The importance of some environmental chemical factors. Water Research 36, 3289-3297, **(2002).**

11. Dukam S., Pirion P. and Levi Y., Modélisation du développement des biomasses bactériennes libres et fixées en réseau de distribution d'eau potable. In : Adhésion des microorganismes aux surfaces. Bellon-Fontaine, M.N., and J. Fourniat (eds), Paris, **(1995).**

12. Rodier J., L'analyse de l'eau. Dunod, Paris, **(1996).**

13. APHA, Standard Methods for the Examination of Water and Waste Water. 20th edition. American Public Health Association/AmericanWater Works Association/Water Environment Federation, Washington, DC, **(1998).**

14. Marchal N., Bourdon J.L. and Richard Cl., Les milieux de culture pour l'isolement et l'identification biochimique des bactéries. Doin, Paris, **(1991).**

15. Holt J.G., Krieg N.R., Sneath P.H.A., Staley J.T. and Williams S.T., Bergey's manual of determinative bacteriology. Williams and Wilkins, Philadelphia, **(2000).**

16. Guerard Cl., Programmation linéaire. Eyrolles ed., Paris, **(1976).**

17. Lebart L., Morineau A. and Ferrelon J.-P., Traitements des données statistiques, méthodes et programmes. Dunod ed, Paris, **(1982).**

18. Banton O. and Bangoy LM., Hydrogéologie, multiscience environnementale des eaux souterraines. PUQ/AUPELF, Sainte-Foy, Québec, 460, **(1997).**

19. Kinner N.E., Harvey R.W., Blakeslee K., Novarino G. and Mekeer I.D., Size-selective predation on groundwater bacteria by nanoflagellates in an organic-contaminated aquifer. Appl. Environ. Microbiol. 64(2), 618-625, **(1998).**

20. Van Elsas J.D. and Heijen C.E., Methods for the introduction of bacterial into soil: A review. Biolology and Fertility of Soils. 10, 127-133, **(1990).**

21. Alden L., Demoling F. and Baath E., Rapid method of determining factors limiting bacterial growth in soil. Appl. Environ. Microbiol. 67, 1830-1838, **(2001).**

22. Rijnaarts H.H.M., Norde W., Bouwer E.J., Lyklema J. and Zehnder A.J.B., Reversibility and mechanism of bacterial adhesion. Colloids and Surfaces B: Biointerfaces. 4, 5-22, **(1995).**

23. Fowle D.A. and Fein J.B., Experimental measurements of the reversibility of metal-bacteria adsorption reactions. Chemical Geology 168, 27-36, **(2000).**

24. Yee N., Fein J.B. and Daughney C.J., Experimental study of pH, ionic strength, and reversibility behaviour of bacterial-mineral adsorption. Geochimica and Cosmochimica Acta. 64, 609-617, **(2000).**

25. Fein J.B., Daughney C.J., Yee N. and Davis T.A., A chemical equilibrium model for metal adsorption onto bacterial surfaces. Geochimica and Cosmochimica Acta**.** 61, 3319-3328, **(1998).**

26. Simoni S.F., Bosma T.N., Harms H. and Zehnder A.J.B., Bivalent cations increase both the subpopulation of adhering bacteria and their adhesion efficiency in sand columns. Environmental Science and Technology. 34, 1011-1017, **(2000).**

27. Rijnaarts N.H., Norte W., Lyklema J. and Zehnder A.J., DLVO and sterics contributions to bacterial deposition in media of different ionic strengths colloids surface. Biointerfaces, 14, 179-195, **(1999).**

28. Camesano T.A. and Logan B.E., Influence of fluid velocity and cell concentration on the transport of motile and non mobile bacteria in porous media. Environmental Science and Technology. 32, 1699-1708, **(1998).**

29. Nora Hamadouche, Interactions des bactéries marines responsables de la formation des Biossalissures avec les matériaux biospécifiques. Thèse Institut Galilée-Université Paris, XIII. 1-235, **(2003).**

30. Humbel F.X. et Pelletier J.L., Porosité, densité et perméabilité des sols ferralitiques rouge et jaune près de Yaoundé. Yaoundé-ORSTOM : 28 pages. **(1996).**

31. Davis K.J. and Luttge A., Quantifying the relationship between microbial attachment and mineral surface dynamics using vertical scanning interferometry (VSI). American Journal of Science. 305,727–751, **(2005).**

32. Jennifer R.R., Why do Bacteria Colonize Aquifer Surfaces? Geochemical and Nutrient Controls of Bacterial Colonization of Silicate Surfaces. http://water.usgs.gov/ogw/pubs/ofr0289/jrr_whydobacteria.htm (accessed May 2009), **(2009).**

33. Mueller R.F., Bacterial transport and colonization in low nutrient environments. Water Research. 30, 2681-2690, **(1996).**

34. Deshpande P.A. and Shonnard D.R., Modeling the effects of systematic variation in ionic strength on the attachment kinetics of Pseudomonas fluorescens UPER-1 in saturated sand columns. Water Ressources Research. 35, 1619-1627, **(1999).**

35. Herman J.S., Mills A.L. and Cozzarelli I.M., Alteration of reactive mineral surfaces by ground water. In “Monitoring oxidation-reduction processes for groundwater restoration”. Wilkin, R.T.,

R.D. Ludwig, and R.G. Ford (eds). Proceedings of the workshop Dallas, Texas, April 25-27, 2000, Cincinnati, OH, US Environmental Protection Agency, EPA/600/R-02/002, 39-42, **(2000).**

36. Phoenix V.R. and Konhauser K.O., Benefits of bacterial biomineralization. Geobiology. 6, 303–308, **(2008).**

37. Bennett P.C., Rogers J.R., Choi W.J. and Hiebert F.K., Silicates, silicate weathering, and microbial ecology. Geomicrobiology Journal. 18, 3-19, **(2001).**

38. Lehman R.M., Roberto F.F., Earley D., Bruhn D.F., Brink S., O'Connell S.P., Delwiche M.E. and. Colwell F.S, Attached and unattached bacterial communities in a 120-meter corehole in an acidic, crystalline rock aquifer. Applied and Environmental Microbiology. 67, 2095–2106, **(2001).**

39. Grasso D., Smets B.F., Strevett K.A., Machinist B.D., Van Oss C.J., Giese R.F. and Wu W., Impact of physiological state on surface thermodynamics and adhesion of Pseudomonas aeruginosa. Environmental Science and Technology. 30, 3604-3608, **(1996).**

40. Jucker B.A., Zehnder A.J.B. and Harms H., Quantification of polymer interactions in bacterial adhesion. Environmental Science and Technology. 32, 2909-2915, **(1998).**