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Adhesion of *Escherichia coli* and *Pseudomonas aeruginosa* on Rock Surface in Aquatic Microcosm: Assessment of the Influence of Dissolved Magnesium Sulfate and Monosodium Phosphate

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Abstract: This study aims at assessing the aquatic microcosm conditions, the influence of $MgSO_4$ and NaH_2PO_4 on the adhesion of *P. aeruginosa* and *E. coli* on the basalt, micaschist, granite and sandstone fragments during 180 to 1440 min. Results showed that in the presence of $MgSO_4$ at concentrations of 0.02, 0.04, 0.06 and 0.08 mol/L the abundances of *P. aeruginosa* adhered on the fragments rock expressed as CFU/cm^2 which varies from 2.8×10^5 to 6.2×10^8 at 0.02 mol/L, 2.5×10^4 to 4×10^6 at 0.04 mol/L, 1.2×10^4 to 1.8×10^8 at 0.06 mol/L, and from 2.5×10^5 to 1.9×10^7 at 0.08 mol/L. *E. coli* also varies from 2.1×10^4 to 7.3×10^4 at 0.02 mol/L, 2.7×10^4 to 4.5×10^5 at 0.04 mol/L, 2.3×10^4 to 4×10^5 at 0.06 mol/L, and from 10^4 to 1.2×10^5 at 0.08 mol/L. In the presence of NaH_2PO_4, the degree of *P. aeruginosa* adherence varies from 3.8×10^4 to 4.6×10^7 at 0.02 mol/L, 1.3×10^5 to 3.4×10^7 at 0.04 mol/L, 9×10^4 to 1.9×10^7 at 0.06 mol/L, and from 5.3×10^3 to 1.4×10^7 at 0.08 mol/L. *E. coli* equally, varies from 2.8×10^4 to 1.9×10^7 at 0.02 mol/L, 7.6×10^3 to 1.6×10^7 at 0.04 mol/L, 2.2×10^4 to 2×10^7 at 0.06 mol/L, and from 1.2×10^4 to 1.7×10^5 at 0.08 mol/L. Cells adherence was significantly influenced by the mineralogical properties of rocks and the concentration of dissolved ions in the solution ($p<0.05$). The presence of *P. aeruginosa* and those of *E. coli* adherence also differed significantly ($p<0.01$). This would be as a result of the insertion of flagellum on their surface.

Key words: Adhesion, aquatic microcosm, dissolved salts, *E. coli, P. aeruginosa*, rock

INTRODUCTION

In natural environments, bacteria transported in run-off contain a diverse composition of some Gram-positive and negative bacteria of various cells. They may be spherical, curved, spiral or rod, and their surfaces may or may not have a capsule as well as flagella (Banton and Bangoy, 1997; Mayer *et al*., 1997; Nola *et al*., 1998). The cilia when present may be polar as in *Pseudomonas aeruginosa* or peritrichous as in *Escherichia coli* (Le Minor and Veron, 1989; Holt *et al*., 2000, Leclerc, 2003).

Bacterial behavior in soil particle during infiltration is the sum of a number of various parameters. Some chemical elements such as carbon, nitrogen, phosphorus, potassium, silicon, aluminum and iron often found in the environment have physiological functions in the bacterial cell. They can also intervene in the elementary composition of the microbial cells, vitamins or coenzymes. Physiological functions of bacterial cells are always regulated by the internal and external pH values (Stanier *et al*., 1990).

One of the important properties of the soil in the protection of the microbial quality of underground water is its capacity to retain bacteria and viral particles. This retention takes place in most cases by absorption. This process of absorption of wall polymers to the solid surface (Jucker *et al*., 1998) is a reversible process and this reversibility undergoes evolution in time due to the biological activity and the variation of the properties of the bacteria wall (Rijnaarts *et al*., 1995; Fowle and Fein, 2000; Yee *et al*., 2000). Bacterial introduction into the soil and its movements are influenced by various static and dynamic abiotic properties of soil layers (Van Elsas and Heijen, 1990). Their transfer in this medium as well as in groundwater is controlled by soil particles properties (Zelenev *et al*., 2000; Alden *et al*., 2001).

It was reported that the initial step of a Bacteria cell attachment to a soil particle involves generating movement that can approach the surface to be colonized (O'Toole and Kolter, 1998). This process involves interactions of non-specific types Van Der Waals, electrostatic and hydrophobic between bacteria and solid

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particles on one hand, and on the other handy specific interactions between molecules on the surface of bacterial cell wall, pili, flagella and surface materials of the medium, on the other (Camesano and Logan, 1998; Rijnaarts *et al*., 1999). This phenomenon, although less energy-dependent at the beginning of the process can be more energy-dependent when the contact time becomes longer. This is because the number of binding sites are very limited (Kolter and Losick, 1998; Simoni *et al*., 1998).

The groundwater supply is the result of a process of vertical and horizontal infiltration of rain and surface water through layers of soil and rock fissures, and the bacteriological properties of groundwater will depend amongst others on the nature of the rocks stratum (Banton and Bangoy, 1997). The rocks nature can be of petrographic and mineralogical types. They can be metamorphic, sedimentary, and magmatic. Petrographic and mineralogical differences potentially have impacts on the retention of bacterial cells on their surfaces. Similarly, the cell retention depends on the chemical properties of water infiltration through rocks joints.

Little studies have been made in assessing the influence of dissolved salts on the retention of bacteria on rocks surfaces. We know little about the impact of the mineralogical properties rocks on the potential retention of microorganisms on its surface. Very little data is available on the importance of the mode of insertion of the flagellum on the retention of bacteria on rock surfaces. This study aims to evaluate in the laboratory under static conditions, the impact of dissolved $MgSO_4$ and NaH_2PO_4 on the retention of *Pseudomonas aeruginosa* and *Escherichia coli* to the surfaces of 4 rocks: Granite, Basalt, Micashist and Sandstone. *P. aeruginosa* is an opportunistic pathogen bacterium belonging to the family *Pseudomonadaceae* and has a polar flagellum (Holt *et al*., 2000). *E. coli* is an *Enterobacteriaceae*, with peritrichous flagella, which is often used in evaluating the bacteriological quality of drinkable water (Holt *et al*., 2000).

MATERIALS AND METHODS

Collection of used rocks: The used rocks were collected in four different regions of Cameroon (Central Africa). The climate of those regions is equatorial. The used rocks were basalt, granite, micaschist and sandstone respectively. They are respectively magmatic volcanic plutonic igneous, metamorphic and sedimentary rocks. The basalt used was taken from the massive Mbep between 5º32′25″ latitude North and 10º35′10″ East longitude. The granite was taken from the Adamawa region located between 7º38′8″ North latitude and 12º40′13″ East longitude. The micaschist is derived from the Yaounde region, located between 3º52′ North latitude and 11º32′ East longitude. The sandstone was collected from the Douala region located 4º1′ North latitude and 9º45′ East longitude.

Rock characterisation and manufacture of the plates used: Each rock was fragmented into four pieces A, B, C and D, using a hammer. Each piece was then subjected to mineralogical analysis by X-ray diffraction and geochemical analysis by XRF. Then the plates of rectangular parallelepiped rocks have been manufactured at the Institute of Geological and Mining Research of Cameroon. The total area of each plate was mapped at 13.28 cm^2. The dimension of this area was chosen to allow its subsequent introduction into the test tube during the process of stall cell, following testing of adherence in the water. Before the experiment, each of the 4 plates A, B, C and D of each rock type was attached to a wire diameter of 0.01 mm and was then introduced into a glass vial. The assembly was then autoclaved.

Collection and Identification of bacteria used: The bacteria used were *E. coli* and *P. aeruginosa*. *E. coli* was isolated from the groundwater in the Douala region and *P. aeruginosa* from groundwater in the Yaounde region. They were isolated on Pyocyanosel agar culture medium (Bio-Rad) and Endo (Bio-Rad) respectively, using membrane filtration technique (Marchal *et al*., 1991; Rompré *et al*., 2002). Their identification was made according to standard techniques (Holt *et al*., 2000). For the preparation of bacterial stocks, a colony forming unit (CFU) of each strain from standard agar medium was inoculated into 100 mL of nutrient broth (Oxford) for 24 h at 37ºC. After cells harvested by centrifugation were at 8000 rev/min for 10 min at 10ºC and washed twice with NaCl (8.5 g/L) solution. Each pellet was re-suspended in 50 mL of NaCl solution and 500 mL in sterile tubes was transferred to be stocked. The stocks were then stored frozen in a glycerol-NaCl.

Concentrations of dissolved salts and ions considered: Magnesium sulphate ($MgSO_4$) and monosodium phosphate (NaH_2PO_4) were used. $MgSO_4$ in solution liberates Mg^{2+} and SO_4^{2-}. NaH_2PO_4 solution contains the ions Na^+, PO_4^{3-} and H_3O^+. The final concentrations in the solutions used were 0.02, 0.04, 0.06 and 0.08 mol/L. These concentrations of salts and ions in solution are presented in Table 1. For each concentration of salt, 200 mL of solution were prepared in a 500 mL Erlenmeyer flask, and then sterilized in an autoclave.

Experimental protocol (experimental design): This laboratory study was conducted at the Faculty of Sciences of the University of Yaounde 1 (Cameroon, Central Africa) from February to September 2010. Prior to the experiment, each of the frozen stock vial containing *P. aeruginosa* or *E. coli*, were thawed at room temperature. Then 100 μL of the culture was transferred

Table 1: Concentrations of ions in each solution

Concentration of salt in solution (mol/L)	Salt and concentration of ions in solution				
	$MgSO_4$		NaH_2PO_4		
	Mg^{2+}(mol/L)	SO_4^{2-} (mol/L)	Na^+ (mol/L)	H_3O^+ (mol/L)	PO_4^{3-} (mol/L)
0.02	0.10	0.03	0.11	0.05	0.03
0.04	0.21	0.05	0.22	0.10	0.05
0.06	0.31	0.08	0.33	0.16	0.08
0.08	0.41	0.10	0.43	0.21	0.11

into 10 ml of nutrient broth (Oxford) in tube and incubated at 37ºC. After 24 h, 100 µL of the suspension was added to 100 mL of the same nutrient broth, incubated also for 24 h at 37ºC. Cells were then harvested by centrifugation at 8000 rev/min for 10 min at 10ºC and washed twice with sterile NaCl solution (8.5 g/L). The pellets were then re-suspended in 50 mL of sterilized solution containing either $MgSO_4$ or NaH_2PO_4. After serial dilutions, 1 mL of the suspension was added to 199 mL of each sterilized salt solution as indicated above. Based on our preliminary study, cell concentration was adjusted at 2×10^7 CFU/mL. For all the experiments, only one bacteria species was added to each solution in the flask.

For the actual experiment, each sterilized plate rock is submerged until mid-height of the solution in the flask. The Erlenmeyer is then covered with aluminum foil and incubated under static conditions on a bench in the laboratory at a temperature of (23±2ºC). The incubation period was 180, 360, 540 and 1440 min for the plate A, B, C and D, respectively. The experiments were conducted in static water conditions to promote the adhesion of bacterial cells to the surfaces of the plates (Déziel *et al*., 2001). Each test was performed in triplicate.

Removal of rock slides and adherent cells stall for bacteriological analysis: After each incubation time, each plate is removed from the solution, then suspended in sterile air for one minute, then introduced into a Falcon tube containing 20 mL sterile NaCl solution (8.5 g/L). The suspension of the plate in air for 1 min allows you to drain the plate. Each fragment is washed four times in 20 mL sterile NaCl solution (8.5 g/L), by shaking for 10 sec using a vortex at 1000-1200 rpm. According to Dukam *et al*. (1995), variation in rotation speed maximize desorption of cells. The solution containing the suspended bacteria was transferred into a sterile glass flask of 250 mL. Another 20 mL of NaCl solution (8.5 g/L) was then introduces into the Falcon tube and the both were immersed in a sonicator for 20 sec at 10ºC, and immediately agitated for 10 sec and transferred into the glass flask of 250 mL. The final volume of the bacterial suspension after the bacterial harvest was 100 mL.

Bacteriological analysis was carried out on the suspension. It concerned the enumeration of *P. aeruginosa* and *E. coli*, using the membrane filtration technique and plate count techniques respectively, on Pyocyanosel and Endo agar culture medium. The results were then expressed as the number of Colony Forming Units (CFU) of bacteria adhered/cm^2.

Data analysis: Temporal variations of the abundances of bacteria adhered to each bedrock were illustrated on histograms. The relationship between cell abundance and concentrations of salts and ions in solution on one hand, and between these abundances and the chemical properties of rocks on the other hand, were assessed by Spearman correlation tests. Comparism of the abundances of bacteria adhered between the rocks have been carried out using the H-test of Kruskal-Wallis and U-test of Mann-Whitney.

RESULTS

Mineralogical characteristics of rocks used: The rocks used contain silicon oxide, aluminum, manganese, sodium, magnesium, potassium, phosphorus, and iron. The average proportion of each mineral is shown in Fig. 1. The silica is relatively high for 4 types of rock (43-75%). The highest proportion was recorded in the granite. Proportions of manganese oxides were generally very low for the four rocks. This mineral is only a trace in the granite (Fig. 1).

Time evolution of the abundances of cells adhered to bedrock:

In the presence of $MgSO_4$: Considering all types of rocks, it was noted that the average abundances of adherent cells in the presence of 0.02 mol/L of $MgSO_4$ is between 2.8×10^5 and 6.2×10^8 CFU/cm^2 for *P. aeruginosa*, and between 2.1×10^4 and 7.3×10^4 CFU/cm^2 for *E. coli* (Fig. 2). At concentrations 0.04, 0.06 and 0.08 mol/L $MgSO_4$, considering all the submerged bedrock, the average abundances of adherent cells ranges respectively from 2.5×10^4 to 4×10^6, 1.2×10^4 to 1.8×10^8, and 2.5×10^5 to 1.9×10^7 CFU/cm^2 for *P. aeruginosa*, respectively, from 2.7×10^4 to 4.5×10^5, 2.3×10^4 to 4×10^5, and 10^4 to 1.2×10^5 CFU/cm^2 for *E. coli* (Fig. 2).

The highest abundances of *P. aeruginosa* adhered in the presence of 0.02 mol/L of $MgSO_4$ was recorded after 540 min in all rocks (Fig. 2). At the concentration 0.04 mol/L, this was registered after that period on the micaschist and granite. On sandstone and basalt, which were registered after 1440 and 360 min respectively. At

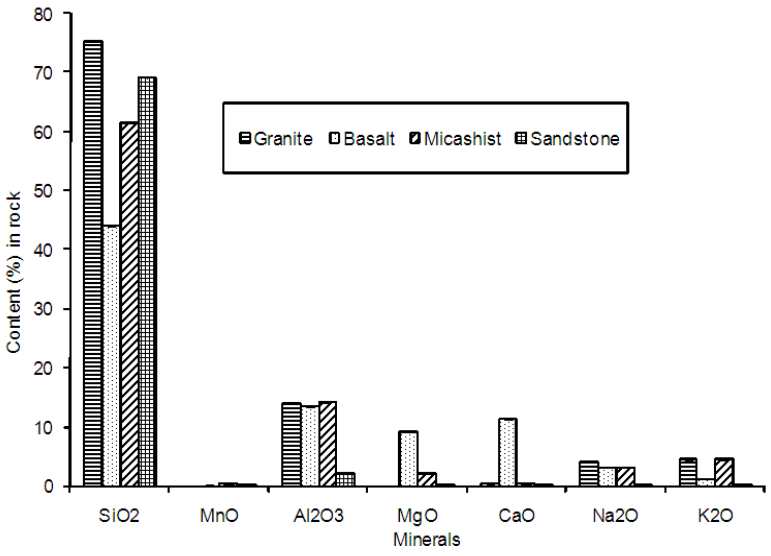

Fig. 1: Mineral properties of rocks used

the concentration 0.06 mol/L $MgSO_4$, the highest abundances of cells adhered to all the rocks were registered after 1440 min. At 0.08 mol/L, they are observed after 540 min on the sandstone. On other substrates, they are registered after 1440 min. Generally, it was note that the abundances of *P. aeruginosa* adhered are relatively low before 540 min of incubation, after this period or when the concentration of $MgSO_4$ in the medium is 0.02 mol/L (Fig. 2). Variation is observed when this concentration is 0.04 mol/L. At 0.06 mol/L, the highest abundances of adherent cells were recorded after 1440 min of incubation. At 0.08 mol/L, almost the same observation is made, except on the sandstone (Fig. 2).

At concentration of 0.02 mol/L, the abundances of *E. coli* adhered on the micaschist decrease gradually as the incubation time increases (Fig. 2). Similar observation was made on the basalt. On granite and sandstone, the highest abundances of *E. coli* adhered were recorded after 1440 and 360 min of incubation respectively. At the while concentration of 0.04 mol/L, the abundances of *E. coli* adhered to the highest micaschist and basalt were recorded after 360 min of incubation. On sandstone and granite, it was recorded after 180 and 1440 min (Fig. 2). At 0.06 mol/L, the abundances of *E. coli* adhered highest were recorded after 180 min of incubation on the granite and sandstone, and after 360 min on the micaschist and basalt. At the concentration of 0.08 mol/L, the relative variability is observed. The highest abundance of *E. coli* was observed after 1440 min on granite and basalt, 540 min on the micaschist and after 180 min on the sandstone (Fig. 2). Overall, there is a moment at which the highest abundance of adherent cells varies with the concentration of $MgSO_4$ in the medium, and the nature of the bedrock (Fig. 2).

In the presence of NaH_2PO_4: In the presence of NaH_2PO_4 and considering all types of bedrock, it was noted that the abundances of *E. coli* adherence is between 2.8×10^4 and 1.9×10^7 CFU/cm^2 to 0.02 mol/L NaH_2PO_4, between 7.6×10^3 and 1.6×10^7 CFU/cm^2 to 0.04 mol/L, between 2.2×10^4 and 2×10^7 CFU/cm^2 to 0.06 mol/L, and between 1.2×10^4 and 1.7×10^5 CFU/cm^2 to 0.08 mol/L NaH_2PO_4 solution. Abundances of *P. aeruginosa* adhered ranges from 3.8×10^4 to 4.6×10^7, from 1.3×10^5 to 3.4×10^7, 9×10^4 to 1.9×10^7, and from 5.3×10^3 to 1.4×10^7 CFU/cm^2 when tests were performed respectively with 0.02, 0.04, 0.06 and 0.08 mol/L NaH_2PO_4 dissolved (Fig. 3).

At the concentration 0.02 mol/L of NaH_2PO_4 and in the presence of *P. aeruginosa*, the abundance of adherent cells was the highest observed after 360 min on the micaschist, and after 540 min of incubation on all other substrates (Fig. 3). At the concentration 0.04 mol/L, it was observed after 540 min on all bedrock. At concentrations 0.06 and 0.08 mol/L, the abundances of *P. aeruginosa* adhered at most levels were observed after 540 min incubation on granite, sandstone and basalt, and after 1440 min of incubation on the micaschist. Overall concentrations 0.04 to 0.08 mol/L NaH_2PO_4, showed high level of *P. aeruginosa* adhered before 540 min of incubation, or after this period are relatively low (Fig. 3).

As for *E. coli*, the abundance of cells adhered to the highest concentration of 0.02 mol/L was observed after 180 min of incubation on the micaschist, granite and sandstone. On basalt, it was registered after 360 min of incubation (Fig. 3). At the concentration 0.04 mol/L, it was recorded after 180 min of incubation on the micaschist and basalt, after 360 min on the sandstone, and after 1440 min of incubation on the granite. At 0.06 mol/L, it was recorded after 540 min on the micaschist,

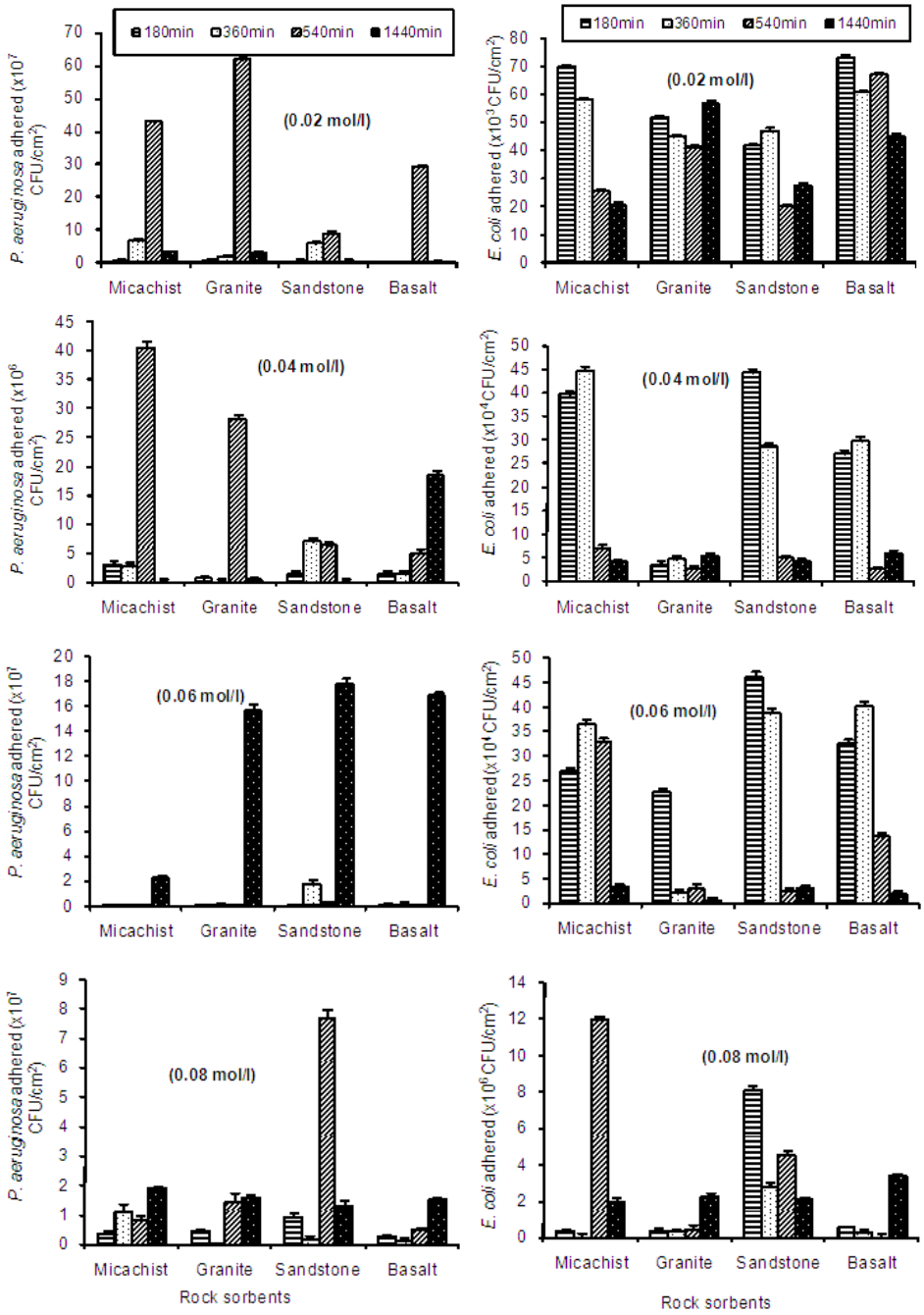

Fig. 2: Variation of the means of abundance of cells adhered with respect to rock species and concentration of $MgSO_4$ in the medium

sandstone and basalt, and after 1440 min of incubation on the granite. When the concentration of NaH_2PO_4 is dissolved 0.08 mol/L, the abundance of *E. coli* the highest was recorded after 360 min on the sandstone, and after 1440 min of incubation on the micaschist, granite and basalt. Overall, we note that the abundances of *E. coli* adhered on the micaschist; granite and basalt are relatively low in concentration 0.02 mol/L of NaH_2PO_4. Similarly at the concentrations 0.04 and 0.06 mol/L, these abundances are relatively low on granite, sandstone and basalt (Fig. 3).

Relationship between adherent bacteria and composition of the rocks used: The correlations between the abundances of adherent cells and the chemical composition of substrates immersed in each incubation

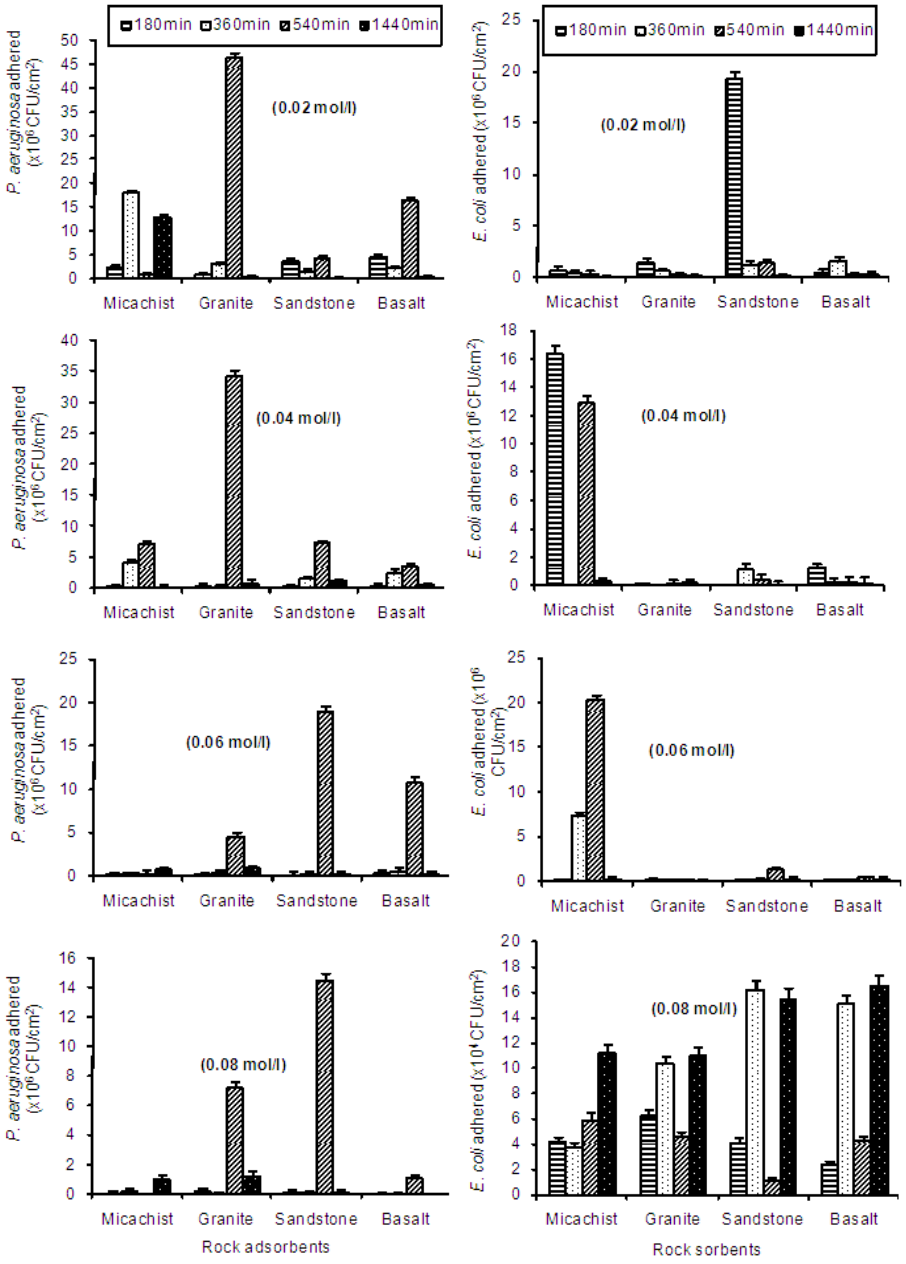

Fig. 3: Variation of the means of abundance of cells adhered with respect to rock species and concentration of NaH_2PO_4 in the medium

time and each experience condition were evaluated. The results are presented in Table 2.

In the presence of $MgSO_4$: When the medium contains $MgSO_4$, it was notes that the mineralogical properties of the rocks do not influence significantly the adhesion of *P. aeruginosa* in solution, except on the micaschist and sandstone, after 1440 and after 540 min, respectively. During these two incubation periods, an increase in these two rocks were noticed Na_2O is concomitant with a significant decrease in cells of *P. aeruginosa* adhered. By cons, when the solution containing the cells of *E. coli*, we observed during most periods of incubation, the increase in the mineral micaschist is significantly concomitant with decreases in abundances of adherent cells, with the exception of the period 540 min.

During this incubation period, the mineral micaschist in this seems to play no significant impact in the adhesion

Table 2: Correlation between mineral content in rock (micaschist, granite, sandstone and basalt) and the abundance of *P. aeruginosa* and *E. coli* adhered during each incubation period with respect to the salt in solution

		Rock used, bacteria adhered and salt in solution															
		Micaschist				Granite				Sandstone				Basalt			
		$MgSO_4$		NaH_2PO_4		$MgSO_4$		NaH_2PO_4		$MgSO_4$		NaH_2PO_4		$MgSO_4$		NaH_2PO_4	
Mineral and incubation period (min)		*P. aer*	*E. col*	*P. aer*	*E. col*	*P. aer*	*E. col*	*P. aer*	*E. col*	*P. aer*	*E. col*	*P. aer*	*E. col*	*P. aer*	*E. col*	*P. aer*	*E. col*
SiO_2	180	NS	*(-)	*(-)	NS	NS	NS	NS	NS	NS	NS	*(-)	NS	NS	NS	NS	NS
	360	NS	*(-)	NS	NS	NS	*(-)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	540	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*(+)
	1440	NS	*(-)	NS	NS	NS	NS	*(+)	NS	NS	*(-)	NS	NS	NS	NS	NS	NS
MnO	180	NS	*(-)	*(-)	NS	NS	NS	NS	NS	NS	NS	*(-)	NS	NS	NS	NS	NS
	360	NS	*(-)	NS	NS	NS	*(-)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	540	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*(+)
	1440	NS	*(-)	NS	NS	NS	NS	*(+)	NS	NS	*(-)	NS	NS	NS	NS	NS	Ns
Al_2O_3	180	NS	*(-)	*(-)	NS	NS	NS	NS	NS	NS	NS	*(-)	NS	NS	NS	NS	NS
	360	NS	*(-)	NS	NS	NS	*(-)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	540	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*(+)
	1440	NS	*(-)	NS	NS	NS	NS	*(+)	NS	NS	*(-)	NS	NS	NS	NS	NS	NS
MgO	180	NS	*(-)	*(-)	NS	NS	Ns	NS	NS	NS	NS	*(-)	NS	NS	NS	NS	NS
	360	NS	*(-)	NS	NS	NS	*(-)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	540	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*(+)
	1440	NS	*(-)	NS	NS	NS	NS	*(+)	NS	NS	*(-)	NS	NS	NS	NS	NS	NS
CaO	180	NS	*(-)	*(-)	NS	NS	NS	NS	NS	NS	NS	*(-)	NS	NS	NS	NS	NS
	360	NS	*(-)	NS	NS	NS	*(-)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	540	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*(+)
	1440	NS	*(-)	NS	NS	NS	NS	*(+)	NS	NS	*(-)	NS	NS	NS	NS	NS	NS
Na_2O	180	NS	*(-)	*(-)	NS	NS	*(-)	NS	NS	NS	NS	*(-)	NS	NS	*(-)	*(-)	NS
	360	NS	*(-)	NS	NS	NS	*(-)	*(-)	NS	NS	*(-)	*(-)	NS	NS	*(-)	*(-)	NS
	540	NS	NS	*(-)	NS	NS	*(-)	NS	NS	*(-)	NS	NS	*(-)	NS	*(-)	*(-)	*(+)
	1440	*(-)	*(-)	NS	*(+)	NS	NS	*(+)	NS	NS	*(-)	NS	*(+)	NS	NS	*(-)	NS
K_2O	180	NS	*(-)	*(-)	NS	NS	NS	NS	NS	NS	NS	*(-)	NS	NS	NS	NS	NS
	360	NS	*(-)	NS	NS	NS	*(-)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	540	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*(+)
	1440	NS	*(-)	NS	NS	NS	NS	*(+)	NS	NS	*(-)	NS	NS	NS	NS	NS	NS

P. aer: *P. aeruginosa* ; *E. col*: *E. coli*; NS: Non significant correlation; *: Significant correlation ($p<0.01$); (-): Negative correlation; (+): Positive correlation

of these cells to the surface of this rock. Increasing proportions of SiO_2, MnO, Al_2O_3, MgO, CaO and K_2O in the granite decreased significantly ($p<0.01$) adherence of *E. coli* on the surface during the first 360 min of incubation (Table 2). Increasing the amount of Na_2O is concomitant with a decrease in these cells after 180, 360 and 540 min. When the rock is submerged sandstone, it is notes in most cases, a significant negative influence of minerals on the accession of *E. coli* after 1440 min of incubation. Considering the basalt, the only mineral that has a negative influence on adherence of *E. coli* is Na_2O. This influence can be observed after 180, 360 and 540 min incubation (Table 2).

In the presence of NaH_2PO_4: When in water the salt is dissolved in the NaH_2PO_4, there is in most cases, a reversal of the behaviour of the surface rocks from the presence of $MgSO_4$. The influence of minerals in the micaschist, granite and sandstone seems more significant side by side the cells of *P. aeruginosa* (Table 2). On the basalt and in the presence of $MgSO_4$ dissolved Na_2O significantly influence the adhesion of *E. coli* after 180, 360 and 540 min of incubation, and has no impact on adherence of *P. aeruginosa* on this rock. By cons in the presence of NaH_2PO_4 dissolved, the impact of this mineral is significant ($p<0.01$) on adherence of *P. aeruginosa* during all periods of incubation. It is significant adherence of *E. coli* after 540 min of incubation. It was also after this period of incubation that we observed a significant impact on adherence of *E. coli*, on other minerals (Table 2).

In the presence of Na^+, H_3O^+ and PO_4^{3-} released NaH_2PO_4, increases in levels of SiO_2, MnO, Al_2O_3, MgO, CaO, K_2O and Na_2O after 540 min of incubation ($p<0.01$) increased significantly promote accession of *E. coli* on basalt. Increasing proportions of Na_2O will significantly increase the cell adhesion of *E. coli* after 1440 min of incubation on the micaschist and sandstone. It was notes that after this period of incubation in the presence of NaH_2PO_4, higher levels of minerals in granite supports ($p<0.01$) cell adhesion of *P. aeruginosa*. But there is no significant correlation between the chemical composition of granite and abundance of *E. coli* adhered on this rock submerged in the solution containing the Na^+, PO_4^{3-} and H_3O^+ ($p<0.01$) (Table 2).

Relation between the abundances of adherent cells and concentrations of ions in solution: Spearman correlations between the abundances of adherent cells on each rock, and concentrations of each ion in solution at each incubation time were evaluated. The results are presented in Table 3. It shows that increasing the concentration of Na^+ and PO_4^{3-}, H_3O^+ significantly promotes cell adhesion of basalt *E. coli* after 540 min of incubation ($p<0.01$). These ions affect negatively the adhesion of *P. aeruginosa* on the micaschist and sandstone during the first 180 min of incubation, but positively on the granite after 1440 min of incubation. These ions have little influence on the adhesion of *E. coli* to granite, to sandstone and micaschist. Furthermore, increased levels of Mg^{2+} and SO_4^{2-} is significantly concomitant with a decrease in cell number of *E. coli*

Table 3: Correlation between the concentration of ions in solution and the abundance of *P. aeruginosa* and *E. coli* adhered on each rock fragment, after each incubation period

		Salt in solution									
		$MgSO_4$				NaH_2PO_4					
		P. aeruginosa		*E. coli*		*P. aeruginosa*			*E. coli*		
Rock used and incubation duration (min)		Mg^{2+}	SO_4^{2-}	Mg^{2+}	SO_4^{2-}	Na^+	H_3O^+	PO_4^{3-}	Na^+	H_3O^+	PO_4^{3-}
Micaschist	180	NS	NS	*(-)	*(-)	*(-)	*(-)	*(-)	NS	NS	NS
	360	NS	NS	*(-)	*(-)	NS	NS	NS	NS	NS	NS
	540	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	1440	NS	NS	*(-)	*(-)	NS	NS	NS	NS	NS	NS
Granite	180	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	360	NS	NS	*(-)	*(-)	NS	NS	NS	NS	NS	NS
	540	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	1440	NS	NS	NS	NS	*(+)	*(+)	*(+)	NS	NS	NS
Sandstone	180	NS	NS	NS	NS	*(-)	*(-)	*(-)	NS	NS	NS
	360	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	540	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	1440	NS	NS	*(-)	*(-)	NS	NS	NS	NS	NS	NS
Basalt	180	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	360	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	540	NS	NS	NS	NS	NS	NS	NS	*(+)	*(+)	*(+)
	1440	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

*: Significant correlation ($p<0.01$); (-): Negative correlation; (+): Positive correlation; NS: Non significant correlation

Table 4: Risk values of probability related to the comparison between the abundance of *P. aeruginosa* and *E. coli* adhered on each rock fragment, in each experimental condition and after each incubation period

		P. aeruginosa and *E. coli* adhered			
Salt used and incubation duration (min)		Micaschist	Granite	Sandstone	Basalt
$MgSO_4$	180	P = 0.149	P = 0.149	P = 0.021*	P = 0.043*
	360	P = 0.149	P = 0.043*	P = 0.083	P = 0.043*
	540	P = 1.000	P = 1.000	P = 0.773	P = 0.564
	1440	P = 0.110	P = 0.564	P = 0.149	P = 0.564
NaH_2PO_4	180	P = 0.248	P = 1.000	P = 1.000	P = 0.561
	360	P = 0.772	P = 0.773	P = 1.000	P = 1.000
	540	P = 0.149	P = 0.043*	P = 0.021*	P = 0.083
	1440	P = 0.386	P = 0.386	P = 0.386	P = 0.386

*: Significant difference ($p<0.05$)

adhered on the micaschist (180, 360 and 1440 min), on granite (360 min), and sandstone (1440 min), the threshold $p<0.01$. Changes in concentrations of Mg^{2+} and SO_4^{2-} in solution do not significantly affect the abundance of *P. aeruginosa* cells adhered to substrates as show by Table 3.

Comparisons of the concentration of cells of *P. aeruginosa* and *E. coli* adhered to substrates: A comparison of the abundances of cells of *P. aeruginosa* and *E. coli* adhered on each rock was made using the Mann-Whitney and Kruskal-Wallis, after each incubation time and condition in each experiment. The risk values of probability are presented on Table 4. It shows that when the medium contains $MgSO_4$, the abundances of cells of *P. aeruginosa* adhered to the granite after 360, 180 min on the sandstone, basalt and after 180 and 360 min of incubation. This process differ significantly from those of *E. coli* ($p<0.05$). When the medium containing NaH_2PO_4 is dissolved, the abundances of adherent cells of 2 species do differ significantly between them at surfaces of granite and sandstone, after 540 min of incubation. No significant differences were found between the abundances of the two cell species which adhered to micaschist, when the medium contains NaH_2PO_4 or $MgSO_4$ (Table 4).

DISCUSSION

This present study shows that *P. aeruginosa* and *E. coli* adhere to bedrock in different degrees. This adhesion varies with bacterial species, the frequency of incubation of the rock, the mineralogical composition of this rock, the nature and concentration of dissolved ions. The abundances of bacteria adhered to undergo temporal variations according to Vance (2002), at different stages: the initial absorption is reversible and irreversible adhesion. Van Der Waals forces and repulsive electrostatic forces are known to intervene during reversible absorption (Branger *et al*., 2007), while irreversible adhesion occurs through cellular production

of exopolymers that anchor the cells to the surface of solids. It also involves acid-base links and receptor-ligand types amongst others (Branger *et al*., 2007). During incubation, absorption and desorption of cells occurr on the surface of rock substrates (Callow and Fletcher, 1994; Nola *et al*., 2004). This may be responsible for the temporal variation of the abundances of adhered cells of *P. aeruginosa* and *E. coli* from one incubation period to another (Fig. 2-3).

The colonization of rock surfaces by *P. aeruginosa* and *E. coli* can be significantly influenced by the chemical constituents of rocks ($p<0.05$). In most cases, this influence is negative. In fact, the chemical properties of the surfaces of inert materials can cause changes see the limitation of bacterial adhesion by increasing the hydrophilic character of the rocks (Boulangé-Petermann *et al.*, 1993; Rubio, 2002; Hamadouche Nora, 2003).

The abundance of adherent cells may differ significantly ($p<0.05$) from one bedrock to another, and depending on the nature of dissolved salt (Table 2). The absorption of bacteria to substrates is dependent on the composition of each substrate and bacteria structure (Nola *et al*., 2010). The chemical compounds of each substrate give them a surface pH. It was noted that the adherence of molecules and microorganisms on substrates is optimal at pH neutral or slightly alkaline, such as coal and basalt (Michael *et al*., 1991). The interactions between water and rock surface can lead to substantial corrosion of the latter. It can then lead to a substantial modification of the chemical properties of the medium, and subsequently affect the accession process observed. The relative differences between the mineralogical properties of bedrock used could partly explain the variations of the abundances of adherent cells when the medium contains the same salt dissolved and a single species of bacteria. Moreover, the magnitude of interaction between water and the substrate surface, and corrosion of the latter vary with the rock. Corrosion and erosion are amongst the factors promoting cell adhesion on the geological rock (Ginet and Decou, 1977).

In addition, the accession of the two bacterial species is sometimes influenced significantly ($p<0.05$) by the ions in the solution (Table 3). Inorganic elements in water absorbs on the materials, changing their surface properties and thus influencing the bioadhesive behaviour (Fletcher, 1996). Similarly, dissolved ionic species by their nature and their concentrations, significantly affect the bioadhesion of micro-organisms for the media (Bayoudh *et al*., 2006). They play an important role both in their growth by providing the elements essential to their metabolism on the surface properties.

The presence of ionic species may also modify the surface properties, whether that of organisms or solid carriers (Boutaleb, 2007). By classifying the effects of anions on absorption in aquatic environments, Michael *et al.* (1991) believes that anions such as PO_4^{3-} can inhibit or increase the adsorption of organic compounds and microorganisms by changing the pH related to the dissolution of these anions in the medium. Moreover, it was reported that high concentrations of NaCl and $MgSO_4$ medium promote the development of halophilic bacteria such *Staphylococcus*, but slowly, however the growth of several non-halophilic bacteria as *Pseudomonas* (Membre and Burlot, 1994). Also, the ionic strength also influences the mechanism of bacterial adhesion to the substrate. Bivalent ions such as Mg^{2+}, Ca^{2+}, Pb^{2+} and Cu^{2+} and cations such as Na^+ and K^+ increase the adhesion of bacteria to particles of aquatic geological environments, thus by reducing the electrostatic repulsion between mineral surfaces and bacteria (Scholl and Harvey, 1992; Simoni *et al*., 2000).

Whether we consider *P. aeruginosa* or *E. coli*, cells adhere well to the surfaces of micaschist, granite, sandstone and basalt. In fact, according to Holt *et al.* (2000), *P. aeruginosa* and *E. coli* are mobile and possess polar monotriche and peritrichous flagellation respectively. The initial step of attachment of micro-organisms to substrates involves generating motion appendages that allow them to approach the surface to colonize (O'Toole and Kolter, 1998). This is a flagellum and pili at the origin of specific interactions between bacterial surface molecules parietal and surface substrates (Camesano and Logan, 1998; Rijnaarts *et al*., 1999). The presences of these appendages and their normal functioning have been identified as important factors for the approximation of micro-organisms for the carriers (Vallet *et al*., 2001). These flagella are also responsible for chemical communication between microorganisms adhered on the one hand, and between these micro-organisms and supports the other, sometimes under the control of genes involved in virulence (Filloux and Valet, 2003).

It was noted that the abundances of *P. aeruginosa* adhered sometimes differ significantly ($p<0.05$) from those of *E. coli* (Table 4). Cell adhesion was significantly influenced by the mobility, more or less of the bacteria and the absorbs particles (Camesano and Logan, 1998). The relatively high abundances of *P. aeruginosa* cells adhered may be caused by a relatively high mobility of the bacteria, linked to the presence of polar flagella (O'Toole and Kolter, 1998; Holt *et al*., 2000; Filloux and Valet, 2003). Compared to the peritrichous flagella observed in *E. coli*, the polar flagellum in *P. aeruginosa* is driven by sodium motive force and may then propel the bacterium at relatively high speeds to the bedrock. *E. coli*, cells propelled by numerous lateral flagella proton-powered, can move in highly viscous environments,

colonise surfaces and form communities that sometimes have a highly unstable multicellular architecture (McCarter, 1999; Nola *et al*., 2010).

CONCLUSION

The bacteriopolluants *P. aeruginosa* and *E. coli* in water, adhere at varying degrees to the surfaces of bedrocks such as granite, basalt, micaschist and sandstone, immersed in water. This adhesion evolves or increases with the incubation time of submerged rocks, the bacterial species and type of rock under consideration, also depends on the nature and concentrations of dissolved minerals. It would be interesting to model the process of adhesion of bacterial cells in water, taking into account amongst other things, the nature and concentration of dissolved chemical elements, pH and properties of the solid support and the physical characteristics of the medium.

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