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Sorption Kinetics of *Escherichia coli* and *Salmonella sp* on Two Soil Layers Associated with a Groundwater Table in Yaounde, Cameroon (Central Africa)

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Abstract: A laboratory study has been carried out on two soil layers (H_X and H_Y) located above a groundwater table in Yaounde, Cameroon (Central Africa). The main purpose of this study was to assess the retention potential or sorption kinetics of *Escherichia coli* and *Salmonella sp*. on these soil layers. For both soil layers, bacterial sorption on soil particles occurred rapidly during the first 30 minutes of incubation of bacteria and soil particles in aqueous media, and increased gradually with incubation time up to 300 min. In some cases, adsorption rates fluctuated after 30 min of incubation, probably due to bacterial cell sorption to and de-sorption from soil particles. Using Freundlich isotherms, it was noted that adsorption coefficient related to adsorption capacity varied from 19 to 4026 *E. coli*.mg^{-1} of soil, and from 506 to 847 *Salmonella sp*.mg^{-1} of soil. For both bacterial species, the adsorption coefficient of layer H_Y (located in close proximity of the water table) was greater than that of H_X (located above layer H_Y) and seemed to positively correlate with the pH values and N/P ratios, and to negatively correlate with the values of C/N and C/P ratios. The linearity coefficient related to adsorption intensity varied from 0.5841 to 1.0023 for *E. coli*, and from 0.7068 to 1.5236 for *Salmonella sp*. The physico-chemical characteristics of soil particles seemed to influence the sorption kinetics of bacteria on soil.

Key words: soil sorption kinetics, *Escherichia coli*, *Salmonella sp*.

Introduction

Bacterial movement in soil is important in the pollution follow-up of the surface and underground waters. In general, it requires the presence of water. Bacterial movements and transportation in soil and in underground water can occur by adsorption-desorption mechanisms, by filtration or by advection-scattering [1, 2]. This movement in underground water 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 [3-5]. Their persistence and survival in these underground media also are significantly influenced by the concentration of indigenous micro-organisms, their growth and decay rates, the concentration of available nutrients and the consumption kinetics, among others [2, 6].

Adsorption is the main process leading to a retarded bacterial transport in soil and underground water [2]. It is sometimes a reversible process that evolves in time, due to bacterial activity and variations of bacterial wall

properties [7-9]. Hydrophobicity is the main bacterial wall property which is involved in the cell adhesion on particles [10]. Stability of this adhesion depends on the number of sites and groups of functional sites properties on the bacterial surface, the sites number expected to vary with the chemical characteristics of the environment [11]. Bacterial exopolysaccharide matrix has been indicated as containing many chemically active sites involved in sorption process [12]. Bacterial sorption in aqueous medium is also impacted by the pH, ionic strength, chemicals, the nature of sorbent particle and its mobility coefficients [8, 9, 13].

In many regions of the world, groundwater is still the main source of drinking water supply. Most studies have indicated the pollution of this underground water resource by faecal bacteria such as *Escherichia coli* and *Streptococcus feacalis*, and by opportunistic bacteria such as *Aeromonas hydrophila* and *Pseudomonas aeruginosa*, distribution of bacteria being impacted by some chemical factors [14, 15]. The presence of such organisms is indicative of the existence of pathogenic bacteria such as *Salmonella typhi* and *Vibrio cholerae* which can cause health hazards. In most African countries, wastewaters containing faecal and non faecal bacteria are often discharged to the environment without any pre-treatment, and therefore, can potentially pollute soil and groundwater. Knowledge of the adsorption capacity and kinetics of bacterial retention in soil could contribute to the scientific understanding of the microbiological quality of groundwater in specific soil types. The main purpose of this research was to assess the sorption kinetics of *Escherichia coli* and *Salmonella sp*. on two soil layers collected above a groundwater table in Yaounde, Cameroon (Central Africa).

Materials and Methods

Soil Collection

The Yaounde region in Cameroon (Central Africa) is located at latitude 3°52' N, longitude 11°32' E, with average altitude of 760 m. Its soil is fero-lateritic [16]. A circular hole of 180 cm of diameter on a site out of the urban area was dug until the appearance of the groundwater table. This hole measured 914 cm depth and crossed 8 different soil layers. These layers were named from top (surface) to bottom (groundwater table) as H_1, H_2, H_3,…, H_8. In this paper, H_7 is referred to as H_X and H_8 as H_Y. Two to three kg samples from each of these two soil layers (H_X and of H_Y) were collected and dried at laboratory room temperature (23±1° C) for 12 months.

Physical and Chemical Properties of Soil Layers

Soil layer H_X is made up of yellowish brown material. There is appearance of whitish cracked patches and translucent quartz grains. It has polyedric structure. Soil horizon H_Y is made up of whitish to yellowish clay with silt patches. Purple red to dark brown patches, quartz grains and quartzo-feldspathic beddings and little compact material are observed. Soil colours were determined using Munsel code [17]. Analysed chemicals included pH, iron (Fe), organic carbon (C), total nitrogen (N) and total phosphorus (P). pH values were recorded according to Soil Analysis Handbook of Reference Methods [18]. Chemical analyses were carried out after mineralization of soil samples using triacid technique attack [19]. Fe, N and P were then analysed using spectrophotometer, and C was analysed using heat potassium dichromate oxidation method [20]. Each measure has been done in duplicate.

Bacteria Isolation and Identification

The faecal bacterium *E. coli* was isolated from waste water, using membrane filtration technique, on the Endo culture medium (Bio-Rad laboratories, France), and identified by usual biochemical criteria [21, 22]. After identification, a suspension has been done from Colony Forming Units (CFUs) cultivated by plate count method on standard agar medium (Diagnostics-Pasteur, France), in petri dish. From the suspension, culture of this strain has also been done, but on standard agar medium slant in test tube, and stored at 6-8° C for later use.

Salmonella sp was isolated from the Mingoa stream, a tributary of the Mfoundi hydrographic system in Yaounde. This isolation was performed using membrane filtration technique, on Wilson-Blair culture agar medium (Bio-Rad laboratories, France). It firstly necessitated a pre-enrichment in peptone water aiming in the recover of stressed bacteria, then an enrichment in the Müller-Kauffman culture medium (Bio-Rad laboratories, France), this medium being selective for *Salmonella*. Biochemical identification then was done using enzymatic tests [21]. From CFUs on standard agar medium in petri dishes, pure culture of this strain has also been done on standard agar medium slant in the test tube, as for *E. coli*. It has then been stored at 6-8° C, for later use.

Assessment of Soil Sorption Potential

It was not been possible to perform experiments with the same bacteria cell inoculums as great variations in concentrations of growing cells were observed when inoculums were stored at 6-8 °C. Each test was thus carried out with cells obtained from colony forming units on standard agar culture media. Separate experiments were carried out with *E. coli*, and *Salmonella sp*.

A bacterial colony from standard agar medium was introduced in a 250 ml flask containing 50 ml of sterile physiological solution (NaCl 0.85%), and the content was mixed by shaking. The bacterial concentration in this solution was determined using membrane filtration technique. The concentration at the initial instant ($t_{0'}$) in the absence of soil represented C_0. It was noted that bacterial colonies were of different cells quantities. A weight of crushed soil of a definite layer was then added in that solution. Soil samples were not sterilized as high temperature or pressure would destroy soil minerals and alter its physico-chemical properties. During preliminary analyses, neither *E. coli* nor *Salmonella sp* was found in the 2 studied soil layers. The samples were mixed with bacterial solutions in flasks and then incubated on a GLF 3018 Model shaker at 110 rpm for 300 minutes at

laboratory temperature (23±1 °C). For each of the two soil layers, experiments were carried out with 500 mg, 200 mg, 100 mg and 50 mg samples. (It has been noted during preliminary analyses that the number of bacterial cells per CFU vary significantly from one colony to another. For this reason, it has been concluded not necessary to replicate tests as initial concentration C_0 of planktonic cells will greatly differ from one test to another for the same soil weight sample). The test period of 300 minutes was chosen in according to results of preliminary analyses which showed that it was sufficient for the saturation of sites of soil particles surfaces.

For bacterial analyses, 1ml of solution contained in the flask was sampled. Dilutions and analyses to determine planktonic (non adsorbed on soil particles) bacterial concentrations were then performed using membrane filtration technique [22]. Endo culture agar medium was used to isolate planktonic *E. coli,* and Wilson-Blair culture agar medium was used to isolate *Salmonella sp*. Numbers of cells were determined and expressed as colony forming units (CFUs). From initial instant t_0, the bacterial analysis to determine planktonic cells was carried out at 15, 30 and after each 30 minutes, during the incubation period. The number of bacterial cells adsorbed on soil particles at each analysis was determined knowing cell concentration at initial instant t_0. This method was adapted from Wang *et al* [12] and Miller *et al* [23].

Data Analysis

Freundlich and Langmuir isotherms have been extensively used for evaluation of adsorption processes parameters [12, 23, 24]. Sorption data of *E. coli* and *Salmonella sp* on soil particles were analysed with Freundlich model to evaluate the kinetics parameters in the sorption process. Langmuir isotherm has not been used due to the variability of the number of adhesion sites groups on adsorbent particles [11], the mobility and the interactions generally noted amongst absorbed cells [9, 25] and considering the assumption of the heterogeneous nature of adsorbent particles on soil layers [12, 24].

Freundlich isotherm (non-linear) equation, according to Miller *et al* [23] and Wang *et al* [12], is described by equation:

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

where C_s is the amount of adsorbate adsorbed by adsorbent, C is the equilibrium concentration of non adsorbed, K_f is the Freundlich adsorption coefficient and it is related to adsorption capacity, *1/n* is the linearity exponent and *n* is related to adsorption intensity. Here, C_s is expressed as number of cells.mg^{-1} of soil and C as number of cells.ml^{-1}. When logC_s was plotted against logC, a straight line with slope *1/n* and intercepting logK_f was obtained.

Results and Discussion

Decrease in planktonic cells concentration expresses cells sorption on soil particles. Adsorption of microbial cells on geological particles seems to swiftly take place during the 15 or 30 minutes that follow bacterial switch on soil particles in aqueous phase (Figures 1-2). This was noted from concentration of planktonic cells which decreases quickly during the first 30 minutes that follow incubation. After this period, concentration of non-attached bacteria decreases slowly, while undergoing in some cases fluctuations that are sometimes of important magnitude. Variation in the number of sorbed cells indicated the reversibility of bacterial process on soil particles. (During preliminary analyses, blank sorption experiments were performed in the absence of soils, and no variation occurred in bacterial concentration. It was concluded that all observed changes in bacterial concentration in the presence of soils could be attributed to sorption by soil particles).

Equilibrium instant was considered as the moment at which the number of adsorbed *E. coli* or *Salmonella sp*, is the greatest (the concentration of planktonic cells is the lowest) during each experiment. Considering the absorbed bacterial number and the concentration of planktonic bacteria at equilibrium for each of performed tests, Freundlich isotherms have been established for each soil horizon (Figure 3). Isotherms parameters K_f and *1/n* have been obtained by linearly regressing data of the systems. Adsorption coefficient (K_f) of *E. coli* was 19.mg^{-1} and 4026.mg^{-1} of soil, for H_X and H_Y respectively; that of *Salmonella sp* was 506.mg^{-1} and 846.mg^{-1} for horizon H_X and H_Y respectively (Table 1). Linearity exponent (*1/n*) varied from 0.5841 to 1.5236. The highest value was for *Salmonella sp* on H_X and the lowest was for *E. coli* on horizon H_Y (Table 1). Lower linearity coefficient implies bacterial adsorption intensity relatively great and greater linearity coefficient implies adsorption intensity relatively low. Higher value of adsorption coefficients implies greater adsorption capacity and lower adsorption coefficient implies lower soil particles adsorption capacity. Variability in this soil adsorption potential would be due to the variability of the number of adhesion sites groups on adsorbent particles [11]. Some authors working on bacterial sorption on clean quartz sand yielded adsorption isotherms that linearity coefficients varied from 0.55 to 6.11, and adsorbed bacterial number at equilibrium of about 6.93x10^5 cells.mg^{-1} [26].

Table 1: Adsorption coefficient (K_f) and linearity coefficient (*1/n*) values

	E. coli		*Salmonella sp*	
Horizon	K_f (*E. Coli*.mg^{-1})	*1/n*	K_f (*Salmonella sp*.mg^{-1})	*1/n*
H_X	19	1.0023	506	1.5236
H_Y	4026	0.5841	847	0.7068

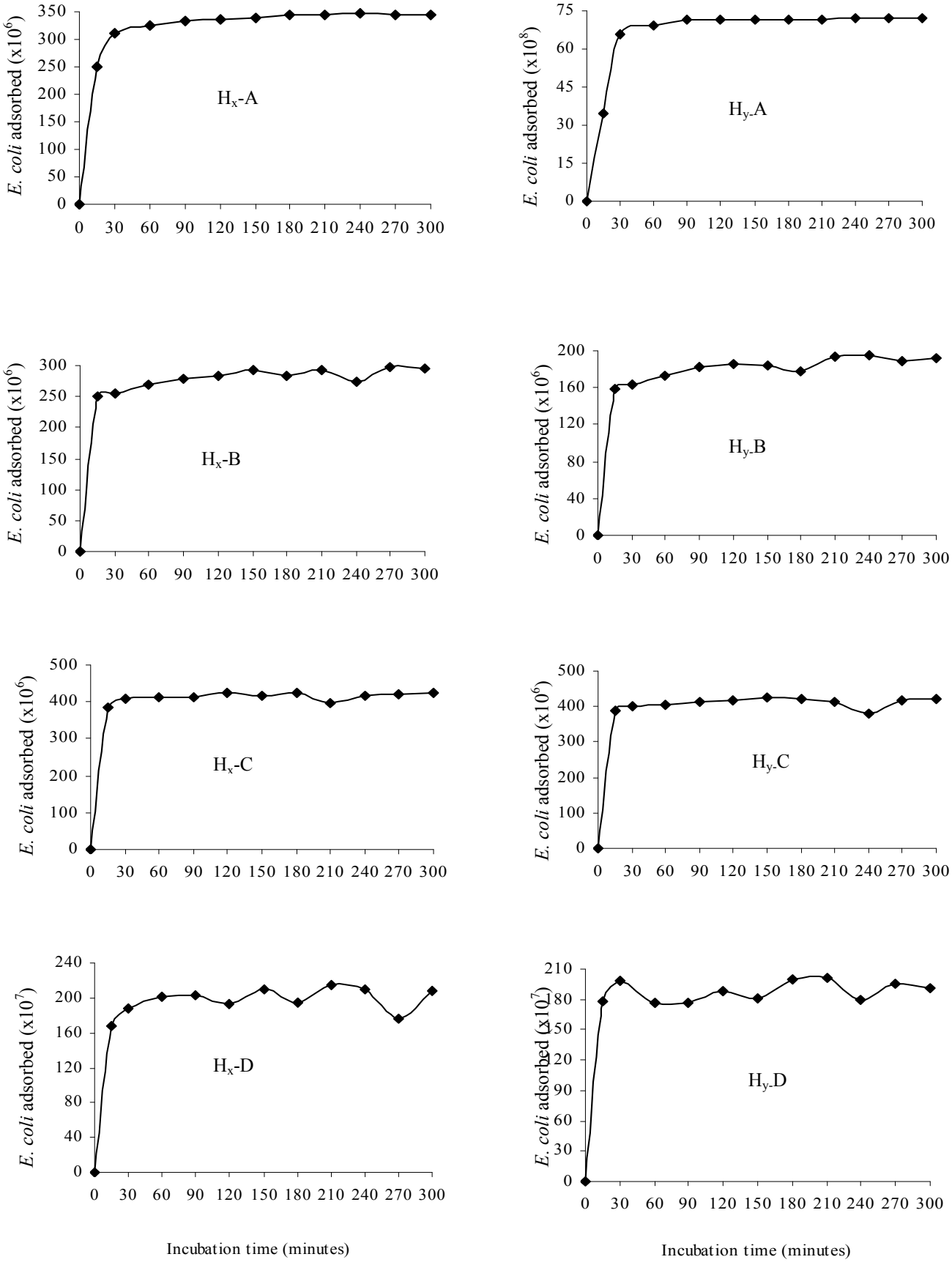

Figure 1: Temporal variations of adsorbed *E. coli*.mg-1 of soil particles, tests carried out with 500 mg (A), 200 mg (B), 100 mg (C) and 50 mg (D) of soil horizon H_X and H_Y.

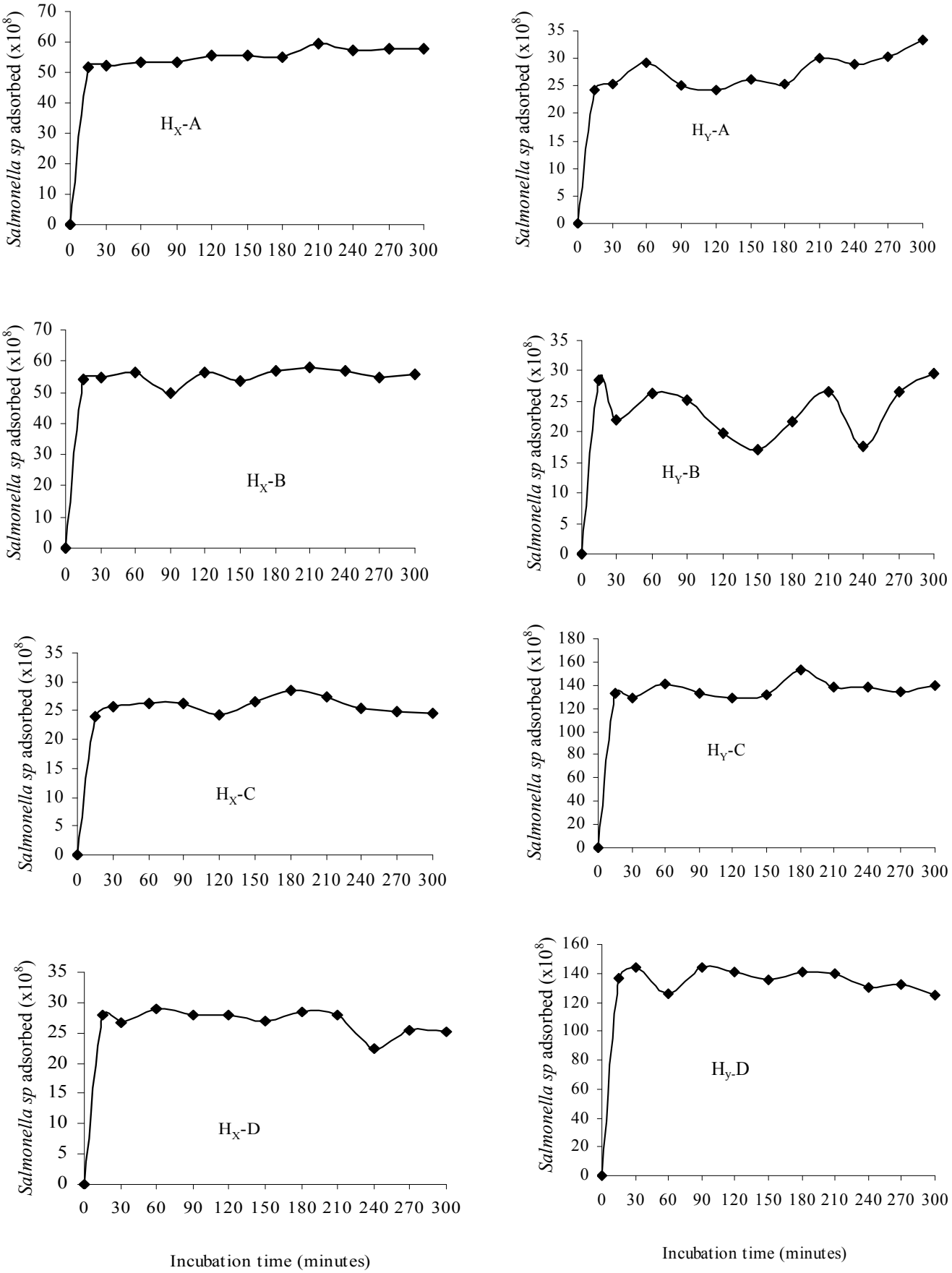

Figure 2: Temporal variations of adsorbed *Salmonella* sp.mg-1 of soil particles, tests carried out with 500 mg (A), 200 mg (B), 100 mg (C) and 50 mg (D) of soil horizon H_X and H_Y.

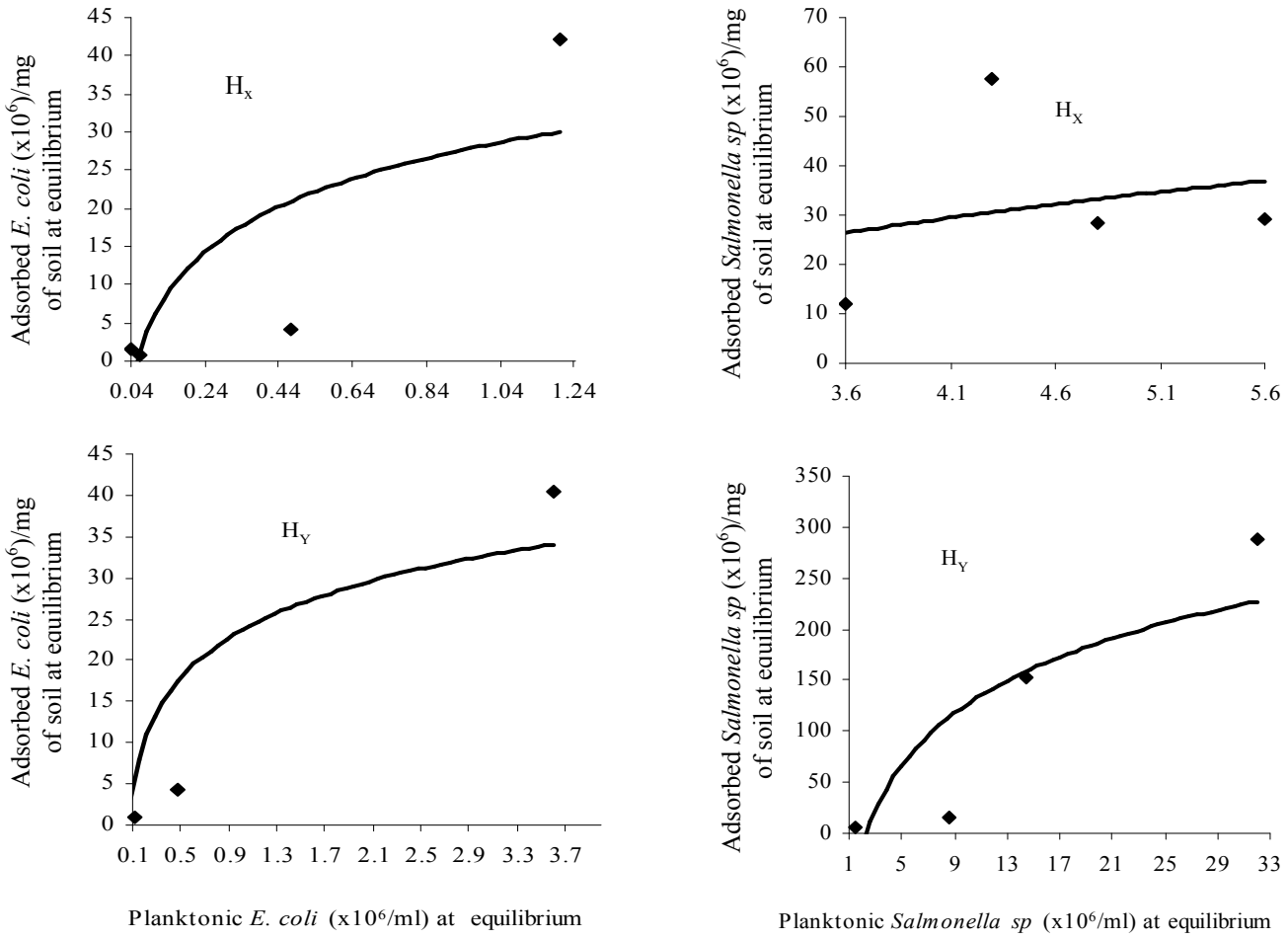

Figure 3: Adsorption isotherms of *E. coli* and *Salmonella sp* on soil horizons H_X and H_Y

Table 2: Mean (standard deviation) of values of pH and the concentrations (mg.g^{-1} of soil) in carbon (C), iron (Fe), total nitrogen (N) and total phosphorus (P) of soil horizon H_X and H_Y

Horizon	*pH*	*C*	*Fe*	*N*	*P*	*C/N*	*C/P*	*N/P*
H_X	4.435 (0.007)	4.140 (0.056)	0.749 (0.004)	0.100 (0.000)	17.425 (0.077)	41.400 (0.565)	0.237 (0.002)	0.005 (0.000)
H_Y	4.559 (0.012)	4.315 (0.091)	0.697 (0.024)	0.235 (0.021)	26.145 (0.091)	18.454 (2.056)	0.164 (0.002)	0.007 (0.000)

The two soil layers were of different chemical characteristics (Table 2). The concentration of C, N, and P, and the pH values increase from H_X to H_Y, while Fe concentration decreases from H_X to H_Y. The pH values of soil of this region have also been noted by Bachelier[16] and Yongue-Fouateu [27] as acid, varying from 4 to 5 with depending on the layer. According to Jewett *et al* [28], bacterial sorption to surfaces is not significantly affected at pH 5-9, although a perceptible decrease in this factor can sometimes increase the retention of *E. coli*, *Pseudomonas aeruginosa* and of *Salmonella infentis*, due to surface physico-chemical interactions between cells and soil particles [29]. Haas and Dichristina [30] working on *Shewanella putrefaciens* noted that sorption sites density on its wall varied according to the environmental pH and was 0.57 site per nm^2 in acid and 0.40 site per nm^2 in base conditions.

Cell sorption swiftly took place during the first 30 minutes following the introduction of bacteria in the aqueous medium containing soil particles (Figures 1-2). This swift sorption was also noted during the first 45 minutes of experiments by Scholl and Harvey [31]. It has been noted that 2 types of strengths exist in such medium: attractive forces lead the cells polymers

adhesion on particles surfaces, and repulsive strengths that compress cellular envelopes to a minimal energy resulting from Derjaguin-Landau-Verwey-Overbeek-AB (DLVO-AB) interactions [5]. The partition of this process in swift initial phase and late slow phase would be related to the passivity of initial phase and to the energy-dependence of late phase. According to Simoni *et al* [32], adsorption process is less energy-dependent indeed passive at the beginning, and turns to more energy-dependent phenomenon as incubation duration becomes longer, probably due to the restriction of bacterial sorption sites number.

It has been noted that bacterial sorption to surfaces is due to adhesin which is an hydrophobic protein of molecular weight greater than 10 kDa, located at the cellular surface or in the cytoplasm [33]. Its activity is inhibited in the presence of antiadhesin which is a hydrophobic non proteinic and heat-sensitive compound [34]. Many interactions have been indicated to be among adhered bacteria and they can significantly influence their structure and their physiology [35]. These interactions sometimes proteins-mediated, have also been observed between bacteria and yeasts, yeasts adhering weakly to particles due to their large size [35]. Interactions also exist between soil particles that are invariant and bacterial cell surfaces which depend on the bacterial physiological condition [4]. They often lead to the sorption reversibility [36] and would be one of the origins of the irregular fluctuations of the number of sorbed *E. coli* or *Salmonella sp* sometimes observed during experiments (Figures 1-2). Changing from planktonic to the adhered state, and conversely exert a regulation on some genes, although the nature of the signal sent to gene when cell is attached is not clear [37, 38]. It is noted from Figure 3 that except *Salmonella sp* on horizon H_X, adsorbed cell number increase swiftly at lower equilibrium planktonic cell concentrations. Adsorption isotherm of *Salmonella sp* on horizon H_X would express a relatively less complexity of systems [24], and would depend according to Sotelo *et al* [39] on the lower difference in interactions energies among equilibriums.

Layers H_X and H_Y are of different adsorption coefficients and linearity coefficients. Adsorption coefficients for each of bacterial species increase from H_X to H_Y (Table 1). Some authors have observed a considerable increase in polymers adhesion production by *E. coli* with increasing in environmental C/N ratios [40]. In this study, C/N and C/P ratios rather decrease from H_X to H_Y, and N/P ratios increase from H_X to H_Y (Table 2). Microbial adhesion on particles has also been indicated as related to the microbial surface thermodynamic theory which is impacted by environmental conditions, physiological status of microorganism and its surface structure [4, 41]. It is noted that linearity coefficient for both bacterial species decreases from H_X to H_Y (Table 1). It is also noted that concentration in Fe decreases from H_X to H_Y (Table 2). No relation has yet been established between iron concentration in soil horizon and linearity coefficient either for *E. coli* or *Salmonella sp*. Some authors have indicated that iron hydroxide reinforces ionic strength's action of bacilli sorption [26], but probably without significant impact on cocci bacterial sorption [42].

Surface thermodynamic properties of gram-negative bacteria as *E. coli* and *Salmonella sp* are influenced by moisture contents of surrounding environment as outer surface of the membrane is high in lipid contents and low in peptidoglycan contents [43]. Although chemicals-such as rhamnolipids that affect sorption reversibility by increasing negative electrical charges in the medium [44], and calcium that reinforces chemical links favouring specific and non specific interactions in the presence of protein and polysaccharide molecules of adhesion, and strengthening calcic bridges among cells [45, 46]-affect microbial adhesion to surfaces, no definite chemical influence has been clearly demonstrated in the present study. However, *E. coli* and *Salmonella sp* retention on soil layers could be highly influenced by the physico-chemical properties of soil particles.

Conclusions

This study demonstrated that two soil layers collected above a groundwater table in Yaounde, Cameroon, present significant differences in their physical properties and chemical characteristics. They also showed substantial differences in their sorption kinetics and retention potentials for both *Escherichia coli* and *Salmonella sp*. Sorption kinetics seemed to result from interactions between bacterial cells and soil particles, and to depend on both soil type and bacterial species. However, the correlation between bacterial sorption and soil chemistry remains to be elucidated.

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