

Adsorption and affinity of *Escherichia coli* to different aggregate sizes of a silty clay soil

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Abstract

The sediments produced by water erosion are the main source of pollution of agricultural origin of surface water bodies. These sediments may be associated to bacteria, compromising the quality of nearby water bodies. Therefore, to understand this biological contamination, it is necessary to find out the adsorption capacity and bacterial affinity to aggregate sizes that may result in a differential sedimentation. To this end, in the present work, the distribution, adsorption capacity and affinity to different aggregate sizes of two strains of *Escherichia coli* in two liquid media of contrasting ionic strength were evaluated in a silty clay soil. The <2 μm fraction showed a higher proportion of bacteria than the other aggregate sizes (48%), whereas among the fractions >2 μm, the 20–50 μm fraction was the one that showed the highest bacterial adsorption in both liquid media (37.9%). On the other hand, the highest values of bacterial affinity were found in the 20 to 50 μm fraction (coarse silt) in the low ionic strength media and 20–50 and >50 μm in the high ionic strength media. However, the bacterial strains used revealed only some trends in the modification of these variables. This work contributes to the development and implementation of strategies to mitigate pollution, such as control of sediment generation and its subsequent capture in filter strips.

Key Words: Biological contamination, Surface transport, Water quality

1 Introduction

The sediments produced by water erosion and transported by runoff are the main source of pollution of agricultural origin of surface water bodies. Depending on the degree of chemical and biological contamination, the quality of these water bodies can be significantly reduced (Chagas, 2007). The concentration of microorganisms in the sediments is governed both by their degree of association and survival in solid particles (Guan et al., 2003; Chagas, 2007; Ling et al., 2002) and by the intrinsic characteristics of the sediment itself (Burton et al., 1987). The quantification of these factors is key to the modeling of the process of biological contamination. When heavy rains occur on soils with high concentrations of microorganisms in the upper horizon, the runoff that takes place from higher lands to lower lands will carry large amounts of microorganisms to watercourses (Oliver et al., 2005; Signor et al., 2005). The particles of clay, silt and sand also reach the watercourses, which, as a result, decrease their quality (Chagas, 2007). However, it is unclear whether microorganisms are mobilized independently of soil particles, or whether the soil particles act as their means of transport (Kay et al., 2007; Jamieson et al., 2004; Tyrrel and Quinton, 2003). If microorganisms such as bacteria are associated to soil particles, they may undergo sedimentation processes that depend on the properties of the solid to which they are associated, such as density and size of the particle (Schillinger and Gannon, 1985). Thus, to understand the dynamics of bacterial transport, it is important to know not only the amount of sediment produced, but also its particle size distribution (Sutherland, 1983). The importance of the association between bacteria and soil particles is also given by the impact on the survival of these microorganisms in the liquid medium. Some studies point out that certain pathogens can survive longer in surface waters when they are associated to sediments that are either

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suspended or deposited at the bottom of watercourses (Sherer et al., 1992; Edwards et al., 1997). Although several works have found significant quantities of sediment-associated pathogens, the distribution of microorganisms according to the sizes of particles is still uncertain. Schillinger and Gannon (1985) reported that about 15–20% of the fecal coliform bacteria present in storm water were adsorbed to soil aggregates, mostly with diameters >30 µm. In contrast, Muirhead et al. (2005) emphasized that, in field tests, the bacteria were predominantly associated to small particles; however, they also stated that it is possible that the bacteria associated with large particles had been quickly removed, leaving the free bacteria or those associated with small particles in the water current. Oliver et al. (2007) reported the existence of a preferential association of bacteria from fecal matter (e.g. *E. coli*) to different sizes of soil aggregates. They found that, in silty clay soils, 35% of the *E. coli* cells were associated with aggregates >2 µm and that most of this 35% were linked to fractions between 4 and 15 µm. Finding out the bacterial adsorption capacity and affinity is of great value for the development and implementation of strategies to mitigate pollution, such as the control of the generation of sediments and their subsequent capture in filter strips. The microbial adsorption and affinity to soil particles is influenced by a large number of factors, including the electrostatic attraction (Marshall, 1975), the cell-surface hydrophobicity (Strenstrom, 1989), the type of clay present (Stotzky et al., 1985), and the ionic environment of the water-soil mixture and its interface (Gannon et al., 1991). The latter, in particular, can be modified during a rainfall event. The heterogeneous characteristics of the liquid medium from the rainfall to the runoff deposition and the different status of the bacteria involved in this process allow establishing diverse environments where bacterial association occurs.

Fontes et al. (1991) studied the movement of bacteria in soil columns with contrasting textures and ionic media and found that bacterial retention was almost total in the soil with fine texture and high ionic strength and of only 10% in the soil with coarse texture and low ionic strength. However, few studies have assessed these factors by discriminating by soil particle or aggregate size. The study of the bacteria-sediment association in natural situations will allow a better understanding of the dynamics of bacterial transport. The use of indicators of biological contamination such as the presence of *E. coli* has become widespread both in pollution monitoring activities and in research. However, the bacterial strain used can also vary the association between the microorganisms and the soil. The features of the biological cell present (e.g. size, shape, presence of polysaccharides, etc) may change the balance of electrostatic forces and the force of collision, among other mechanisms, thus varying the resulting adsorption and affinity. In this aspect, there is very little quantitative information regarding the use of different strains associated with the soil and even less information of this association discriminated by aggregate size.

Therefore, the aim of this study was to quantify bacterial adsorption and affinity in various aggregate sizes of a silty soil in a medium with low ionic strength (distilled water) and another one with high ionic strength (small pond water), with two bacterial strains (a laboratory strain and wild strain).

2 Materials and methods

2.1 Description of the soil and water under study

Soil from the middle third of the watershed of the Tala stream in the department of San Pedro, Buenos Aires, Argentina (33°48'36.62''S, 59°54'54.28''W), was collected to perform the experiment in the laboratory. Samples from 0–5 cm depth were collected from a high and tilted environment, with a slope between 1% and 2%. The soils in these areas consist of high erosion phases and typical vertic Argiudolls (INTA, 1973). The land is used mainly for annual extensive crops and, to a lesser extent, for cattle wintering.

The soil presented the features characteristic of an eroded and later sedimented phase, superficially enriched with organic carbon and clay. The mineralogy consisted of 2:1 clays, mainly illites, with a small proportion of kaolinite and interstratified illite/smectite (Kraemer et al., 2011a). Samples were air-dried and sieved through 2 mm for further analysis. The following parameters were determined: pH (1:2.5 solid: water), organic carbon (OC) (Walkley and Black, 1934), electrical conductivity in saturated paste (EC), exchangeable cations (ammonium acetate 1N, pH 7), cation exchange capacity (CEC) (Klute, 1986), and specific surface area (SSA) (Lombardi et al., 2001). Particle size distribution was analyzed by the pipette method of Robinson (Soil Conservation Service, 1972) (Table 1a).

For the assays with bacteria in the laboratory, we used two different liquid media. A low ionic strength water (LIS) -distilled water- and a high ionic strength water (HIS) collected in a small pond near the area where the soil of this study was sampled. Both liquid media were chosen in order to simulate the ion concentrations in rainfall and runoff respectively. LIS and HIS water were analyzed for pH, cations and anions (in solution) and electrical conductivity with the methodologies presented above (Table 1b). The determination of the ionic strength of both liquids media took into account the ions present in the soil solution, reaching values of 0.0021 and 0.024 for LIS and HIS respectively.

Prior to the start of the experiments, the soil and water were sterilized in the Centro Atómico Ezeiza (CNEA) by applying the equivalent of 25 kGy h⁻¹ of ionizing radiation, a level that does not cause changes in the structure of the soil particles. This allowed avoiding the use of chemical reactive that could have left harmful remains for the microorganisms that were later inoculated (Chagas, 2007). The effectiveness of this technique has been confirmed in earlier works (Kraemer et al., 2008; Kraemer et al., 2011b).

Table 1 a) Physical and chemical properties of the soil tested; OC: organic carbon; CEC: cation exchange capacity; SSA: specific surface area; and EC: electrical conductivity

pH	OC (%)	CEC (cmol kg ⁻¹)	SSA (m ² g ⁻¹)	EC (dS m ⁻¹)	Na ⁺	K ⁺	Ca ²⁺	Mg ²⁺	Clay (<2µm)	Silt (2–50 µm) (g kg ⁻¹)	Sand (>50 µm)
					(mg kg ⁻¹)						
5.7	3.8	27.6	224	0.89	6	14	233	128	440	525	35

Table 1 b) Chemical characteristics of the High ionic strength water (HIS) and Low ionic strength water used in the biological assays

Liquid medium	pH	Cations				Anions				Ionic [†] Strength M
		Na ⁺	K ⁺	Ca ²⁺	Mg ²⁺	CaCO ₃	HCO ₃ ⁻	Cl ⁻	SO ₄ ²⁻	
		(mg l ⁻¹)				(mg l ⁻¹)				
HIS	7.4	33.8	2.7	186	111.6	0	122	72.8	traces	0.024
LIS	5.9	–	–	–	–	–	–	–	–	0.0021

[†] (Ionic strength of liquid medium + ionic strength of soil solution)

2.2 Assays of bacterial affinity and adsorption to different aggregate sizes

2.2.1 Bacterial adsorption

The bacterial adsorption assay was based on the protocol proposed by Oliver and Clegg (2007), which consists of four stages:

1) Preparation of suspensions

Suspensions of 1 g soil and 10 ml of high and low ionic strength water were prepared. The suspensions were shaken for 15 h with an end-to-end shaker at 500 rpm. The energy allowed obtaining an aggregate size distribution (ASD) similar to that found in situ after the effect of a representative rainfall over the basin under study. The ASD values obtained by this procedure were similar to those found by simulating rain with intermediate intensities (60 mm h⁻¹) (Kraemer, 2011b).

2) Inoculation

LIS and HIS water-soil suspensions were inoculated with two strains of *E. coli*: a laboratory strain (ATCC 8739) and a wild strain. The wild strain was isolated from bovine feces collected where the soil and water samples were collected. The strain was isolated and identified by the use of differential selective media (CHROMOBRIIT, Britania) and by morphological (shape, size, gram test and metallic green) (Kraemer et al., 2011c) and biochemistry tests (Brock and Madigan, 1993). These strains were grown on Trypticase soy broth, at 35 °C for 24 h, resuspended, centrifuged, and washed twice with sterile saline solution (0.85 NaCl). After shaking the soil suspensions, inoculation was carried out to achieve a final concentration of 1×10⁷ CFU ml⁻¹. To achieve this concentration a curve of absorbance (600 nm) / bacterial concentration was built. The inoculum concentration chosen has been previously used by many researchers to represent a maximum concentration of microorganisms in runoff on fecal matter (Guber et al., 2005, Oliver et al., 2007). After inoculation, the solution was taken back to the shaker for 2 h at 500 rpm in order to achieve an optimal relationship between the microorganisms and the soil. It has been previously determined that 2 h are sufficient to achieve a balance between the microorganisms in suspension and those associated to the soil fractions (Ling et al., 2002, Gannon et al., 1991).

3) Sedimentation

The inoculated suspensions were transferred to a 100 ml flask and diluted with 50 ml of sterile HIS and LIS as corresponded. The dilution facilitates the separation of the different aggregate sizes during sedimentation (Oliver et al., 2007). Then, we proceeded to separate different size fractions by taking 0.1 ml aliquots at 10 mm depth in the times established according to the law of Stokes for sizes of 50, 20, 3 and 2 µm.

4) CFU count

Aliquots of the different aggregate sizes obtained by sedimentation were seeded in triplicate in VBR Agar (Biokar Diagnostics) and incubated in an oven at 36 °C (± 1 °C) for 24 h. Finally, we proceeded to count the CFU ml⁻¹ in all treatments (APHA, 1996).

The experiment was conducted under sterile conditions at a constant temperature of 20 °C.

CFU values ml⁻¹ of each aggregate size (bacterial distribution) were obtained by the difference between the CFU ml⁻¹ of the fraction considered and the following fraction of a smaller size. This difference is attributed to the joint sedimentation of *E. coli* and the soil aggregates. Changes in the size of the populations of *E. coli* by regrowth and cell death in the suspensions during the sedimentation were considered insignificant due to the very short time of sedimentation (40 minutes).

The relative bacterial adsorption was calculated from the sum of the bacterial proportion of all the fractions in relation to the bacterial proportion found in the <2 µm fraction, in which it is considered that there are no adsorbed bacteria. It can not be stated that in this fraction the bacteria found are attached, since they may be either adsorbed or in the form of

"free and independent cells." Thus, the bacterial adsorption obtained is relative and not real because the adsorption that may occur in particles <2 µm is not computed.

2.2.2 Bacterial affinity

The bacterial affinity to the different aggregate sizes was calculated as the ratio between the CFU ml⁻¹ of each fraction and the respective specific surface area (SSA) (Table 2).

To simulate the ASD and be able to calculate the specific surface area, 20 g of soil were shaken (with an end-to-end shaker) for 15 h (in triplicate). Since the energy used in this procedure was similar to that used in the preparation of the soil suspensions to be inoculated, the two methodologies are comparable. This analysis was carried out by the method of Robinson's pipette with distilled water. The ASD was grouped in the same ranges of sizes used for the bacterial assay (>50 µm, 20–50 µm, 3–20 µm, 2–3 µm and <2 µm). We considered a particle density of 2.65 g cm⁻³ and the volume of particles was calculated from the radius of the mid point of each size fraction except for >50 µm, where 200 µm was used as the upper limit to weigh particles in smaller fractions. Results are shown per milliliter to reflect the 1 ml pipette sample (Oliver et al., 2007).

Table 2 Calculation of the specific surface area by aggregate size for the sample evaluated

Size of the particle (diameter, µm)	Equivalent radius used in the calculations (µm)	Total mass (g ml ⁻¹)	% of soil	Spheric volume of the particle (cm ³)	Number of particles in 1 ml	Spheric surface of the particle (mm ²)	Surface by particle size in 1 ml (mm ²)
>50	37.5	0.198	19.8	1.24×10^{-7}	4.14×10^3	1.77×10^{-2}	7.32×10^1
20 - 50	17.0	0.128	12.9	1.15×10^{-8}	2.89×10^4	3.63×10^{-3}	1.05×10^2
3 - 20	6.0	0.474	47.4	5.08×10^{-10}	2.42×10^6	4.52×10^{-4}	1.09×10^3
2 - 3	1.3	0.050	5.0	5.17×10^{-12}	2.51×10^7	2.12×10^{-5}	5.33×10^2
<2	–	–	15.0	–	–	–	–

2.3 Statistical analysis

The effects of the treatments on bacterial distribution, absorption and affinity were evaluated using general linear models in the R program (lm function, R Development Core Team, 2011). These models included three factors: liquid medium (HIS vs. LIS), aggregate size (>50, 20–50, 3–20, 2–3 µm, and only in the case of bacterial distribution, the <2 µm category), bacterial type (laboratory vs. wild) and their interactions. The significance was assessed by analysis of variance (ANOVA) followed by Tukey HSD test. Bacterial affinity was log10 transformed to meet ANOVA assumptions.

3 Results

The aggregate size was the treatment with most impact on all the biological variables measured, with a significant interaction with the liquid medium for all variables (Table 3). The bacterial strain was not significantly associated with any of the variables analyzed, showing only some positive trends in the relative bacterial adsorption and the bacterial affinity for the wild strain.

Table 3 Analysis of deviance for the effects of aggregate size, liquid medium and type of bacteria on bacterial distribution, adsorption and affinity. The F statistic is presented for each term in the model

Aggregate size	Bacterial distribution (%)	Relative bacterial absorption (%)	Bacterial affinity (CFU mm ⁻²)
Liquid medium	46.7***	6.9**	38.6***
Bacterial strain	<0.1	<0.1	1.1
Aggregate size × liquid medium	<0.1	<0.1	2.1
Aggregate size × bacterial strain	4.5**	3.9*	3.6*
liquid medium × bacterial strain	1.4	2.4	1.8
Aggregate size × liquid medium × bacterial strain	<0.1	<0.1	0.1
	2.0	3.1*	0.7

***P<0.001, **P<0.01, *P<0.05.

3.1 Bacterial distribution

The bacterial distribution showed differences according to the different aggregate sizes but there was no marked effect of the liquid medium used (Fig. 1) or of the different strains used. The <2 µm fraction showed a much greater proportion of bacteria than the other aggregate sizes, with an average of 48% (both strains and liquid media). The 3–20 µm and 2–3 µm fractions showed a low proportion of bacteria, with no significant differences between the two liquid media (P <0.05), with averages of 12.62% and 9.23% respectively.

The 20–50 µm fraction showed an average bacterial proportion of 19.36% for both liquid media. The only size

fraction whose bacterial distribution differed significantly between the liquid media used was that greater than 50 μm , corresponding 3.8% for the treatment with LIS and 13.3% for that with HIS.

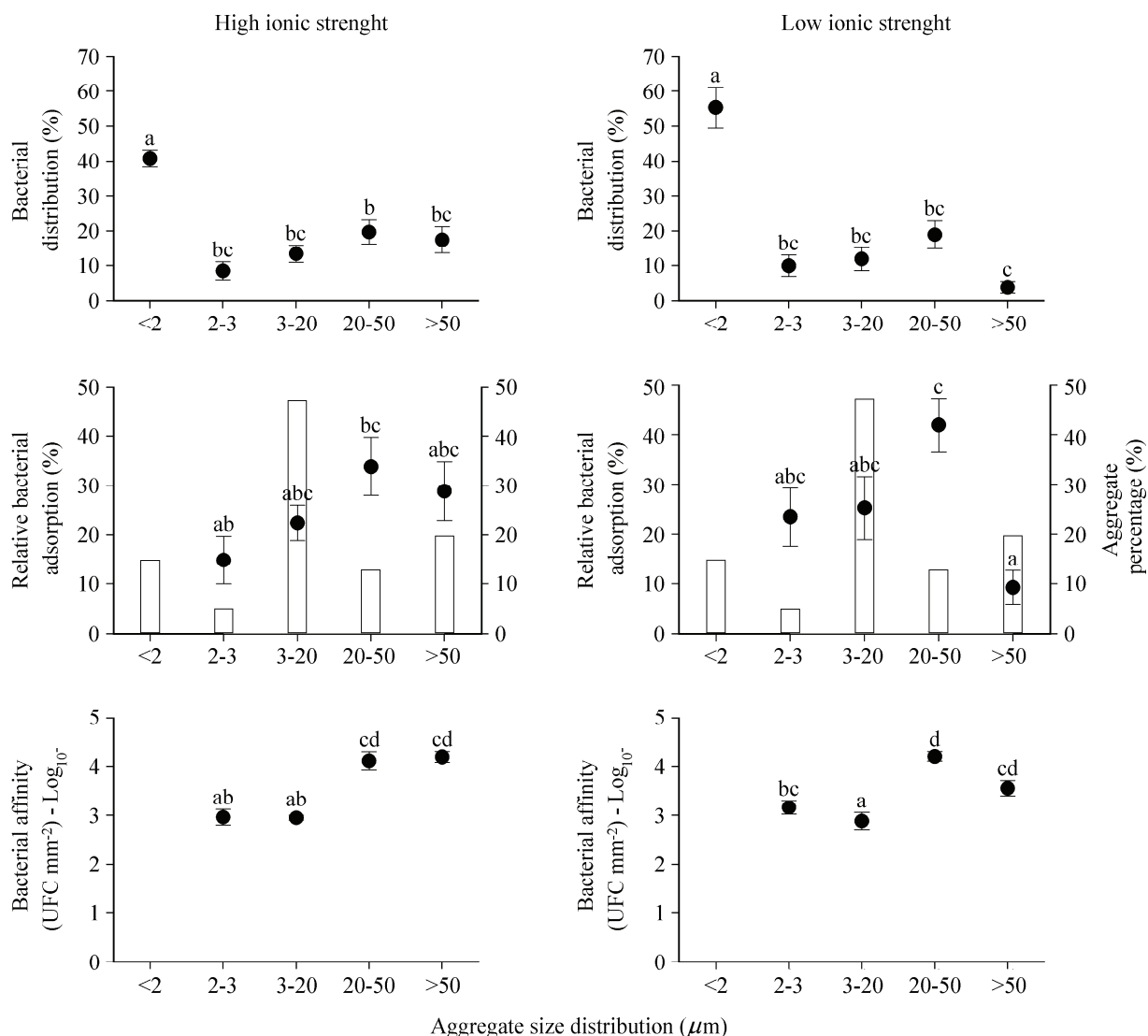


Fig. 1 Bacterial distribution, relative bacterial adsorption and bacterial affinity for different aggregate sizes and liquid media (high and low ionic strength). The values correspond to the average of both bacterial strains. Horizontal lines represent ± 1 standard error. Different letters indicate significant differences $P < 0.05$

3.2 Bacterial adsorption

Bacterial adsorption did not show the same trend as the aggregate size distribution (Fig. 1). While the higher percentage of aggregates was observed in the 3–20 μm fraction, for the fractions $>2 \mu\text{m}$, the highest proportion of bacteria adsorbed was found in the 20–50 μm fraction (coarse silt).

In the fractions with aggregate sizes $>2 \mu\text{m}$ (where there are no free bacteria), the 20–50 μm fraction was the one which showed highest average bacterial adsorption (37.9%) for both liquid media (Fig. 1). The remaining fractions showed average percentages for both liquid media of 19.2 and 23.9% for the 2–3 and 3–20 μm fractions respectively. The $>50 \mu\text{m}$ fraction showed an average microbial adsorption of 19%. This fraction showed an adsorption of 28.8% for the HIS treatment, whereas in the LIS $>50 \mu\text{m}$ fraction presented the lowest adsorption for all the fractions and the two liquid media (9.24%).

As a general trend, from the total absorption of each treatment (sum of the percentage of all fractions of bacteria associated with particles $> 2 \mu\text{m}$), we found relative bacterial adsorption values for the laboratory strain of 40.5% and 41.03% for the treatments LIS and HIS respectively, whereas for the wild strain the microbial adsorption was 49.3% for the LIS treatment and 56.6% for the HIS.

Although the effect of bacterial strains was not significant, these strains showed a trend of bacterial adsorption with an average difference in favor of the wild strain of 23%, with differences in the liquid medium of 17.7 and 27.5% for the

LIS treatment and HIS treatment, respectively. This means that the highest values of bacterial adsorption were found in the HIS treatment and wild strain and that the lowest values were found in the LIS treatment and laboratory strain.

3.3 Bacterial affinity

When normalizing the concentrations of microorganisms in the specific surface area of each aggregate size (bacterial affinity) (Table 2), significant differences of this variable in relation to aggregate size were observed (Table 3). At the same time, there was a significant interaction between aggregate size and the liquid medium but not with the strains used. As shown in Fig. 1, the highest values of bacterial affinity were found in the 20–50 μm fraction, in LIS and 20–50 and >50 μm in HIS. The 3–20 and 2–3 μm fractions showed the lower affinity values, regardless of the two ionic strength treatments.

4 Discussion

The results show that the aggregate size and the liquid medium used had a significant impact on the distribution of the bacterial proportion, adsorption and affinity to the solid medium. This highlights the importance of including both factors in the study of the transport of biological contaminants in liquid media.

4.1 Bacterial association by aggregate size

Considering all treatments, we obtained an average bacterial adsorption of 52%, associated to aggregates >2 μm . These values are high as compared with those found by other authors. Oliver et al. (2007) found 35% of bacterial association in similar soils, whereas, using centrifugation techniques, Characklis et al. (2005) found an adsorption of 20–35% of *E. coli* associated to sedimentable particles. On the other hand, using significantly less diluted water/soil suspensions, Ling et al. (2002) found 99% adsorption in loamy soils.

In previous works, using the method of Ling et al. (2002) and the same soil sample as that used in this study, we found 63.8% of bacterial adsorption (Kraemer et al., 2010). Therefore, it is possible that the real adsorption was underestimated because bacteria associated to aggregates <2 μm not taken into account by the methodology used in this work.

With respect to this size fraction of clay and its influence on water pollution, we may hypothesize that there would be no differences in the hydrological behavior between free organisms and those absorbed to so small particles. However, the latter would present higher survival in the water than the former. For the above reasons, the impact of this solid fraction in the process of biological contamination of surface waters should not be underestimated.

In the >2 μm fractions, the size range that presented the highest bacterial proportion was coarse silt (20–50 μm). This is not in agreement with that found by Oliver et al. (2007), who, using the same methodology, found the highest proportion in the 15–4 μm range (followed by that of 30–16 μm) or with that observed by Auer and Niehaus (1993), who found an association of more than 90% between fecal coliform and particles in the range of 0.4 to 10 μm . Borst and Selvakumar (2003), on the other hand, found no relationship between coliforms and fecal streptococci and particle size.

Considering the size range of aggregates of 2–50 μm (total silt), the soil had a relative adsorption of 79.5% and 11.2% for the sand fraction, whereas, as mentioned above, the calculation of the fraction associated to the clay size was not taken into account. In experiments carried out with *E. coli* in storm runoff, Jeng et al. (2005) found very similar values of approximately 80%, 18% and 2% associated to the aggregate size of silt, clay and sand respectively. In previous works carried out in the same soil under simulated field rain, we found that 98% of the microorganisms were associated to silt-sized aggregates and that only 2% were associated to sands (Kraemer et al., 2011b).

4.2 Liquid medium and bacterial strain

It has been shown that the use of HIS water induces higher adsorption than LIS. Many authors have recognized that an increase in electrolyte concentration results in an increase in bacterial association to solids. Huub et al. (1995), using eight bacterial strains in liquid media with ionic strengths between 0.0001 and 1 M, and Stevik et al. (1999), using distilled water and two solutions of 0.00725 and 0.097 M, concluded that bacterial adsorption increases as ionic strength increases.

Regarding the cation type present, we have previously found that calcium concentration was the water chemical parameter that best explained the adsorption of *E. coli* in liquid media (Kraemer et al., 2008). In the present study we found an important concentration of this divalent cation (Table 1b). In bacterial transport studies, Powelson and Mills (2001) found similar results, where the inclusion of a solution dominated by Ca^{+2} resulted in a lower transport of microorganisms in the water, indicating an increase in the adsorption to the solid fraction.

Both the ionic strength of the solution and the type of dominant cation influence the balance of electrostatic charges, which would explain the reversible adsorption of the microorganism to the solid. According to the DVLO theory (Derjaguin and Landau, 1941, Verwey and Overbeek, 1948), the ionic strength of the liquid will affect the thickness of the double layer and therefore the charge balance and the adsorption capacity of microorganisms to the particles.

The ionic strength of the liquid affects the charge balance in small aggregates, where there is a higher proportion of

colloids. Nevertheless, the $>50\ \mu\text{m}$ fraction was the one which showed a greater impact in HIS treatment on the increase in bacterial proportion. Conversely, a higher proportion of bacteria was found in the $<2\ \mu\text{m}$ fraction in the LIS treatment than in the HIS treatment.

This difference could be a result of the higher ionic strength and the presence of Ca^{+2} in the HIS treatment, which increases the adsorption of aggregates $>2\ \mu\text{m}$, whereas the increase in bacterial proportion in the $<2\ \mu\text{m}$ fraction in the LIS treatment could be due to an increase in the proportion of microorganisms associated to soil aggregates.

In this sense, it is possible LIS causes a greater dispersion of the soil than HIS and because of that there would be a lower concentration of aggregates in the $>50\ \mu\text{m}$ fraction than that previously calculated, and thus fewer bacteria. To test this hypothesis, we analyzed the ions present in the HIS treatment and found a significant concentration of calcium but also of bicarbonates, which would precipitate this cation (Table 1b). Given that the sodium concentration and the SAR (Sodium Adsorption Ratio) are not negligible, the maintenance of soil aggregation by the HIS water is also limited, since it behaves similarly to the LIS treatment.

Regarding the behavior between the two strains used, there were no significant effects of the strain used on any of the variables tested. However, the wild strain tended to present higher values of bacterial adsorption (an average increase of 23% in both treatments). Working on four silt and clay-loam soils and using the same wild strain, we also previously found a higher adsorption with the wild strain than with the laboratory bacteria, in all the cases with an average increase of 48%, roughly equal to the percentage found in HIS treatment (Kraemer et al., 2011c).

More marked differences have been reported by Muirhead et al. (2006) in laboratory studies where the wild strain showed an adsorption of 81%, while the laboratory strain showed an adsorption of only 24%. A possible cause of this differential adsorption is the morphological differences between species and microbial strains, which could modify the balance and distribution of the electrostatic charges on the bacterial surface.

The laboratory and wild bacteria used in this experiment presented morphologies of coccus and coccobacillus respectively (Kraemer et al., 2011a). Similarly, other properties such as differences in extracellular substances, physiological state, hydrophobicity and motility could have influenced their behavior (Camper et al. 1993). The highest adsorption trends found in the wild strain imply a greater potential for contamination because it is very likely that the bacteria have a higher survival rate and thus greater opportunities to reach watercourses.

4.3 Bacterial affinity

The chemical, physical and structural differences of each fraction could be the cause of the different bacterial affinities in each aggregate size range. The high bacterial affinity found in the $20\text{--}50\ \mu\text{m}$ aggregate size is consistent with the results by Oliver et al. (2007), who, using the same methodology, found that its maximum value corresponded to the $16\text{--}30\ \mu\text{m}$ fraction (almost completely included in the $20\text{ to }50\ \mu\text{m}$ fraction). This affinity would explain the higher bacterial concentration found in this fraction, even when this was not the one with the largest relative proportion in weight. Palmateer et al. (1993), quoted by Oliver et al. (2007), who also normalized their data using the specific surface area, found a high colonization of fecal bacteria at a concentration of $6.5 \times 10^5\ \text{CFU mm}^{-2}$ in suspended sediments coming from agricultural drainage. This value is just above the overall average found in the present work: $1.78 \times 10^4\ \text{CFU mm}^{-2}$. The bacterial affinity for the coarse silt could be explained by the high percentage of low density porous mineral that exists in the region where samples were taken (Cosentino and Pecorari, 2002). These silt-sized minerals may have physical, chemical and structural properties that may favor microbial association. In addition, working with soils from the same region, Morrás et al. (1995) showed that silt-sized particles have electrostatic phenomena evidenced by the existence of ion exchange sites.

To advance in the understanding of the causes of the existence of different affinities, the chemical and physical analysis of each size fraction should be carried out separately in order to isolate the factors involved and to improve the understanding of the processes that control bacterial affinity.

4.4 Implications for control of water pollution

The particle size distribution of the soil analyzed, together with the high bacterial affinity found in the silt size fraction, especially in coarse silt, suggests a potential risk of these particles in the dynamics of bacterial transport. This corroborates the hypothesis by Chagas (2007), who stated that the sediments generated by sheet erosion in the Rolling Pampa may have a high ability to absorb and transport various chemical and/or biological agents such as pesticides, bacteria and viruses downhill.

Muirhead et al. (2006) estimated that bacteria need to be associated with soil particles $>63\ \mu\text{m}$ in diameter so as to sediment in the runoff and to particles $>500\ \mu\text{m}$ in order to be filtered by the pasture. The data presented here suggest a low effectiveness of filter strips. On the other hand, the study area has very low slopes (0.5%) and very long slope lengths (1000 m), where there is a greater possibility of selective sedimentation and where vegetation can act as a filter.

Coyne et al. (1998) found that filter strips reduced 99% of the sediments, with a bacterial removal rate of 74 and 34% in different slopes, indicating that each situation should be analyzed specifically, separating the effect of the filtering strips in the infiltration/runoff relationship and the specific features of each situation, such as slope, soil type, and vegetation type used. On the other hand, it has been verified that there is significant bacterial association to particles

larger than 2 μm that can not be controlled by filter strips, although they can indeed promote an increase in bacterial survival (Sherer et al., 1992; Jamieson et al., 2004).

Various computer programs such as SWAT (Soil and Water Assessment Tool) simulate bacterial transport according to the microbial adsorption (relative to the soil/water partition coefficient) and survival (by modifying the mortality rate). A critical mass of experiments aimed at studying bacterial adsorption to different soil sizes could allow an important adjustment in these models, improving the bacterial dynamics in the environment. The use of different water/soil relations and soil qualities is needed to corroborate the results presented.

4 Conclusions

The soil evaluated presented a different distribution, adsorption and affinity to *E. coli* for each aggregate size. The <2 μm aggregate size showed a higher proportion of bacteria than the rest of the aggregate sizes, with an average bacterial proportion of 48%. In the >2 μm fractions (where there are no free bacteria), the 20–50 μm fraction (coarse silt) was the one which presented highest average bacterial adsorption for both liquid media of 37.9%. On the other hand, the highest values of bacterial affinity were found in the 20 to 50 μm fraction (coarse silt) in the low ionic strength media and 20–50 and >50 μm in the high ionic strength media. However, the bacterial strains used revealed only some trends in the modification of these variables.

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