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INFLUENCE OF PHYSICAL AND CHEMICAL SOIL PROPERTIES ON THE ADSORPTION OF *Escherichia coli* IN MOLLISOLS AND ALFISOLS OF ARGENTINA

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Key words: water pollution, grazing, bacterial adsorption, Argiudolls, Natraqualfs

ABSTRACT

Bacterial adsorption on soils and sediments is one of the main factors that control bacterial transport to water bodies. In this work, 32 soil samples representative of the most important arable areas of the Rolling Pampa region (Argiudolls) and bottomlands devoted to livestock production (Natraqualfs) were analyzed in order to evaluate bacterial-soil adsorption. The first axis of a principal component analysis explained 45% of the total variation among soils in 11 physical and chemical properties, and was strongly and positively related to bacterial adsorption (r^2=0.67). Soil bacterial adsorption presented a large range of values (25-73%), being those for Argiudolls significantly higher than those for Natraqualfs. For both soils, cation exchange capacity (CEC) (r^2=0.67) and clay content (r^2=0.55) were positively associated with bacterial adsorption; whereas exchangeable sodium percentage (ESP) showed a negative tendency (r^2=0.42). It is concluded that in the basin studied, granulometry, CEC and ESP proved to be important properties to discriminate bacterial-soil adsorption, and the following equation to estimate mean soil bacterial adsorption in these soils is proposed: $y=1.73\times CEC - 0.05\times sand(50\text{-}250\ \mu m)[g\ kg^{-1}]-0.54\times ESP$ ($R^2 adjust=0.77$). These results would help to monitor water quality of surface water bodies by the development of bacterial transport models using standard soil data.

Palabras clave: contaminación hídrica, pastoreo, adsorción bacteriana, Argiudoles, Natracualfes

RESUMEN

La adsorción bacteriana a suelos y sedimentos es uno de los principales factores que controlan el transporte bacteriano hacia los cuerpos de agua. En este trabajo se analizó la adsorción bacteriana en 32 muestras de suelos representativas del área agrícola más importante de la región de la Pampa Ondulada (Argiudoles) y de las áreas bajas asociadas con uso ganadero (Natracualfes). En el análisis de componentes principales el primer eje explicó en 45% de la variación total de los suelos en 11 propiedades físicas y químicas y se relacionó de forma positiva con la adsorción bacteriana (r^2=0.67). Esta adsorción bacteriana presentó un importante rango de valores (25-73%), encontrándose para los Argiudoles valores significativamente más altos que para los Natracualfes. Para ambos tipos de suelos, la adsorción bacteriana se correlacionó positivamente con la capacidad de intercambio catiónico (CIC) (r^2=0.67) y el contenido de arcilla (r^2=0.55); mientras que el porcentaje de sodio intercambiable (PSI) presentó una tendencia negativa (r^2=0.42). Se concluyó que en los suelos de la cuenca bajo estudio la granulometría, la CIC y el PSI son importantes propiedades para evaluar los procesos de adsorción bacteriana en el suelos y se propone la siguiente ecuación para su predicción en estos tipos de suelos: $y=1.73\times CIC - 0.05\times arena\ (50\text{-}250\ \mu m)\ [g\ kg^{-1}] - 0.54\times PSI$ ($R^2 ajust=0.77$). Estos resultados proporcionan elementos para el modelado de la calidad de los cuerpos superficiales de agua utilizando datos estándares de suelos.

INTRODUCTION

The understanding of bacterial transport through soils is essential for the modeling of biological contamination in soils and waters. Several authors have developed both mechanistic and empirical models of bacterial transport (Moore *et al.* 1989, Tian *et al.* 2002, Walker *et al.* 1990), aiming to predict the movement of pathogens from farm systems into lentic and lotic waters. However, there are important information gaps regarding the factors that affect bacterial transport and exchange between ecosystems. In this sense, the association between soils, sediments, and microorganisms has not been sufficiently addressed in agricultural basins. Knowledge on the factors affecting this association is essential for the understanding of the microbial ecology in these subsystems and would have direct applications in livestock and manure management and contaminated soils treatment (Ling *et al.* 2002) and in the generation of early alert systems of biological contamination, among others.

The most common indices used to characterize the association of bacteria on solid surfaces are the adsorption percentage and distribution coefficient (Reddy 1981, Ling *et al.* 2002). Although numerous works on these indices have been published in the last decade, the information about faecal bacteria in the soil is still limited. These contaminants can be transported by surface run-off and underground percolation in the form of free cells or in association with particles (Tyrrel *et al.* 2003, Jamieson *et al.* 2004). Drozd and Schwartzbrod (1996) reported that the adsorption of pathogens to the soil cannot be attributed to only one factor since there are different forces that interact in this process. The mechanisms proposed include Van der Waal forces, protonation phenomena, and cationic bridges, among others. These mechanisms depend on the physical and chemical properties of each soil type (Schijven *et al.* 2002). Some soil components and properties have been relatively more studied than others, such as clay content (Weaver *et al.* 1978, Ling *et al.* 2002) and clay mineralogy (Jian *et al.* 2007), pH (Reddy *et al.* 1981), ionic strength (Fontes *et al.* 1991, Stevik *et al.* 1999), CEC (Stotzky 1985) and the content of multivalent cations (Marshall 1980). Along with these soil properties, bacteria cell wall properties (Gannon *et al.* 1991) and extracellular polymeric substances (Cao 2011, Wei *et al.* 2011) could also interact with this adsorption process.

Soils constitute complex microhabitats that present highly variable components and structural organization (Marshall 1985). Soil surfaces present environments with different behaviours, which result from being covered by minerals of clay, oxides and organic matter, and different charges, which depend on pH and are subject to the fluctuations of the concentration of electrolytes. The aggregation of particles with organic matter or clay minerals can modify this microhabitat, and thus affect bacterial transport, sedimentation and survival (Labelle and Gerba, 1979). It is also important to consider the type of clays, which may modify the physicochemical status of the microhabitat and thus the microbiological balance of the site (Marshall 1975, Stozky 1985). The heterogeneity of these microhabitats restricts the direct application of many mechanistic concepts of adhesion such as the DVLO theory or the critical surface tension, since those theories assume homogeneous and clean surfaces (Tadros 1980 in Stoztky 1985). This complexity would explain the scarce information available on the form and place where these bacterial adsorption phenomena occur (Stotzky 1985). All this has impaired the characterization of the variables mentioned in unaltered soil samples with wide ranges of variability and thus hinder the transfer of information towards monitoring activities or the construction of models of contamination.

The aim of this work was to evaluate the effects of soil characteristics on *Escherichia coli* adsorption in soils from a representative basin of the Rolling Pampas (Argentina). Previous studies on biological contamination in the basin of the Tala´s creek in the Rolling Pampas of Argentina have demonstrated that soil erosion promoted by rainfall and contamination were closely related (Dorner *et al.* 2006, Chagas 2007). Soils from this region are prone to sealing and crusting processes due to their large silt content. High intensity rainfall events and large slope lengths have caused erosion processes in these fragile soils both the arable Argiudolls from the uplands and the non arable Natraqualfs located in the bottomlands (Bujan *et al.* 2000, 2003). Long term soil erosion was promoted mainly by intensive annual cropping activities in the arable soils as well as cattle overgrazing both in arable and non arable soils. The resulting sediments with adsorption capacity of both chemical and biological contaminants have been transported by surface runoff water and then deposited on topsoils along the slopes (Chagas *et al.* 2007). Thus, the adsorption processes can take place firstly in higher areas due to crop residue grazing by cattle and then in lower areas devoted to rangeland before reaching main water courses.

Our specific aims were to: a) describe some important soil properties, their spatial variability, and the interrelationship of soils from the Tala´s creek basin, b) evaluate the adsorption of *E. coli* in this system and correlate this with soil properties, c) analyze the elements that are necessary to obtain a simple predictive model of bacterial adsorption, as well as obtaining local adsorption values, that allow comparison with data from other soils.

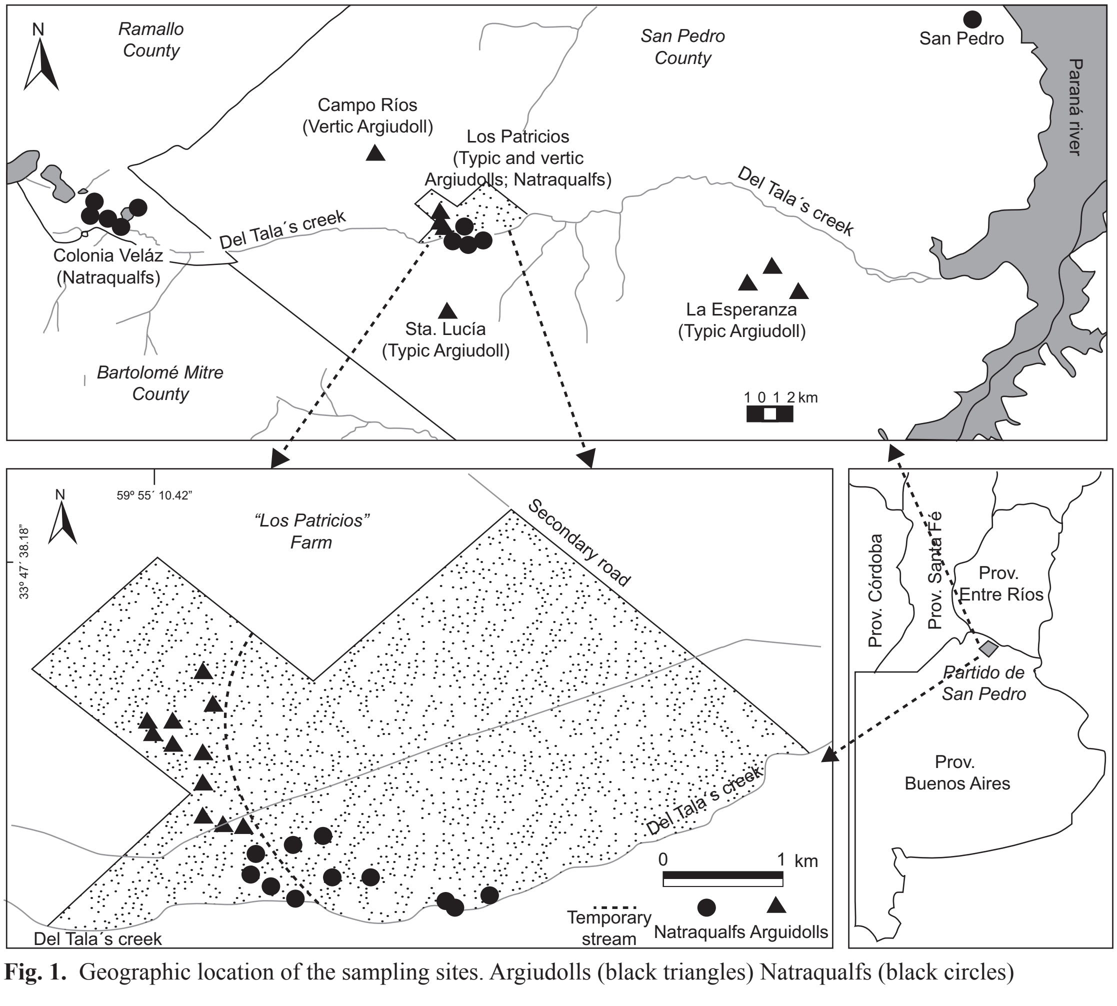

Fig. 1. Geographic location of the sampling sites. Argiudolls (black triangles) Natraqualfs (black circles)

MATERIALS AND METHODS

Site characterization and sampling

A total of 32 sites were sampled along the middle and high sectors of the Tala´s creek basin, in the Rolling Pampa, Province of Buenos Aires, Argentina (**Fig. 1**). Further description of the Tala´s creek soils and physiography is reported by Chagas (2007) and INTA (1973). Sites were distinguished by their physiography (high, middle, and low land), soil type, predominant human activities (agriculture, livestock breeding), and presence of either permanent or transitory stream, to assess the soil properties variability in these sectors of the basin.

Twenty two samples consisting of three 0-50 mm deep subsamples were collected in the agricultural-livestock farm "Los Patricios" (central Tala´s basin), where most of the regional environmental variability can be found (INTA, 1973), and ten additional samples were collected in the surrounding area along the Tala´s creek (**Fig. 1**).

Each sample was sieved through a 2-mm mesh and analyzed for pH (1:2.5 solid:water) using a potentiometer; organic carbon (OC; Walkley and Black 1934), electric conductivity (with a conductivity meter in either saturation or double saturation extract as appropriate), and moisture equivalent (ME; Mizuno *et al.* 1978). Ions (Na^+ and K^+) in the soil solution (aqueous extract 1/10) and in the interchange complex of the soil (extraction with ammonium acetate 1N) were measured by flame photometry, and Mg^{2+} and Ca^{2+} were measured by atomic absorption. The percentage of Ca^{2+} and Na^+ in the soil solution Ca^{2+}-sol and Na^+-sol were calculated as the percentage of those cations in relation to the total sum of soil bases. The anions in the soil solution (HCO_3^-, Cl^-, SO_4^-) were measured by titrations with standard methodology (Klute 1986). The ionic strength (IS) of the solution of each soil was calculated from these ions. The cation exchange capacity (CEC) was measured by extraction with potassium chloride (Klute 1986). The specific surface area (SS; Lombardi *et al.* 2001) and the particle size distribution (Robinson's pipette method; Soil Conservation Service 1972) were also measured. The mineralogy of the clay fraction in oriented homoionic samples glycolated and heated at 520 ºC was determined by X-ray diffractometry with a Philips PANanalytical X'Pert PRO equipment and a semi-quantitative method was used to obtain the relative abundance of clay minerals (Holtzappfel 1985). Soil aggregates of the sieved fraction (<2 mm) were described and photographed using a Wild MZ8 Leica photomicroscope.

Quantification of microbial adsorption on solid particles

Before any biological assay, soil samples were sterilized with a minimum and uniform dose of ionizing radiation equivalent to 25 kGy h^{-1} (McLaughlin *et al.* 1989) to prevent changes in organic matter and soil aggregates. Bacteria for experiments, *Escherichia coli* ATCC 8739, were grown on Trypticase soy broth, at 35 ºC for 24 h, resuspended, centrifuged, and washed twice with sterile saline solution (0.85 NaCl). The pellet (corresponding to $\sim 1\times10^7$ CFU mL^{-1}; Colony Forming Units), was equivalent to that used by numerous researchers (Guber *et al.* 2005, Oliver *et al.* 2007). Measurements of adsorption of *E. coli* on the solid soils fraction followed Ling *et al.* (2002) method, with some modifications. A suspension of 6 ml of *E. coli* solution (1×10^7 CFU mL^{-1}) was added to 6 g of sieved (<2 mm) soil in a 50 mL sterile conical tube. The slurry was manually shaken for 1 min and then left to rest for 5 min. The separation between bacteria and soil was done by centrifugation, establishing a diameter of 1 µm as the limit between them. The energy for such separation was of 50 G for 6 minutes according INRA (1986). This value was adjusted experimentally for the specific conditions of the present work. The purity of the separated fractions was corroborated by optical microscopy (photomicroscope Wild MZ8 Leica). The supernatant of each sample was incubated at 35 ºC for 24 h in VRB-Agar (Biokar Diagnostics) for plate counting (APHA 1998). The determinations were carried out in triplicate on the thirty two samples analyzed. The proportion of bacteria adhered to the soil (percentage of adsorption) was calculated as: Ads (%) = (Nt – Ns) / Nt × 100, where Nt = total number of bacteria added to the soil (CFU mL^{-1}) and Ns = total number of growing colonies from the supernatant (CFU mL^{-1}).

Statistical analysis

Principal component analysis (PCA) was carried out to summarize the physical and chemical characteristics of the soils and to evaluate the association among the measured soil variables, including pH, organic carbon (OC), exchangeable sodium percentage (ESP), moisture equivalent (ME), clay, sand, cation exchange capacity (CEC), specific surface area (SSA), ionic strength (IS), sodium and calcium percentage in soil solution (Na^+-sol, Ca^{2+}-sol). The PCA was performed on the correlation matrix (for further details see Balzani *et al.* 2008). Two-way analysis of variance (ANOVA) was perform to evaluate the effects of site location (*SL*) (two levels: inside and outside "Los Patricios"), soil type (*ST*) (two levels:

Argiudoll or Natraqualf), and their interaction on the scores of the principal component 1 (PCA1) or the principal component 2 (PCA2) as dependent variable (Table 3). To study the influence of soil type and site location on bacterial adsorption (%) the same two-way ANOVA was performed. Linear regression analyses with category variables (soil type: Argiudoll or Natraqualf) were carried out to study the influence of soil properties on bacterial adsorption. PCA1, PCA2, pH, OC, ESP, ME, clay, sand, CEC, SSA, IS, Na^+ -sol and Ca^{2+} -sol were evaluated as independent variables each one in separate models, being bacterial adsorption always the dependent variable. Differences in the slopes and intercepts between soil types were tested by means of ANOVA (Snedecor and Cochran 1980). Finally, multiple linear regression analysis was carried out to select the best prediction model of the bacterial adsorption. The criterion used to select the variables was the minimization of the mean square of the error (MSE), the maximization of adjusted R^2 and statistical significance (*P* entrance and exit: 0.15). All the statistical analyses were performed with Infostat/P v1.1, 2002. ANOVA assumptions were met in all cases.

RESULTS

Physical and chemical properties of the soils

Clay mineralogy was quite similar for all surface soils, consisting of 2:1 clays, mainly illites with a

TABLE I. PHYSICAL AND CHEMICAL PROPERTIES OF THE SOILS EVALUATED; SOIL TYPE NATRAQUALF (Natr), ARGIUDOLL (Arg), ORGANIC CARBON (OC), CATIONIC EXCHANGE CAPACITY (CEC), ELECTRIC CONDUCTIVITY (EC), EXCHANGEABLE SODIUM PERCENTAGE (ESP), IONIC STRENGTH (IS), PERCENTAGE OF Na+ AND Ca+2 IN SOIL SOLUTION (Na+ -sol / Ca2+ -sol), CLAY, SILT AND SAND PERCENTAGES, SPECIFIC SURFACE AREA (SS) AND MOISTURE EQUIVALENT (ME).

Site location†	Soil type	pH	OC	CEC	EC	ESP	IS	Na^+ -sol	Ca^{2+} -sol	Clay (<2 µm)	Silt (2 - 50 µm)	Sand (> 50 µm)	SS	ME	Bacterial Adsorption‡
		(1-2,5)	(%)	cmol$_c$/kg	dS/m	(%)	(M)	(%)	(%)		(%)		g m^{-2}	(%)	(%)
Los Patricios_1	Natr	7.7	0.2	26.7	0.89	2.8	0.0063	54.8	28.2	22.0	48.0	30.0	211	19.0	52.5 (1.6)
Los Patricios_2	Natr	8.5	0.6	13.8	0.91	10.4	0.0059	42.5	5.8	24.0	57.5	18.5	107	18.3	25.2 (1.1)
Los Patricios_3	Natr	6.3	3.7	23.8	0.73	3.5	0.0067	35.4	5.9	39.0	53.0	8.0	262	26.6	64.9 (7.2)
Los Patricios_4	Natr	6.1	3.7	17.9	1.48	3.2	0.0096	17.4	25.4	30.0	61.0	9.0	149	25.1	49.4 (3.4)
Los Patricios_5	Natr	5.6	3.1	16.8	0.65	4.5	0.0046	11.2	18.7	32.0	59.5	8.5	146	25.3	57.6 (5.1)
Los Patricios_6	Natr	8.5	1.8	18.4	1.00	13.0	0.0104	65.3	11.9	25.5	56.5	18.0	170	23.8	35.4 (1.9)
Los Patricios_7	Natr	9.3	0.8	16.5	1.35	23.2	0.0134	66.0	17.4	28.5	55.5	16.0	181	22.7	38.1 (2.3)
Los Patricios_8	Natr	8.5	1.5	18.1	1.63	13.9	0.0099	53.4	21.9	34.0	52.0	14.0	146	19.2	52.7 (5.1)
Los Patricios_9	Natr	7.3	0.9	24.2	1.29	2.5	0.0097	33.8	28.2	43.0	45.0	12.0	314	21.5	65.4 (5.9)
Los Patricios_10	Natr	7.9	2.0	16.5	1.60	16.9	0.0116	38.7	25.2	29.0	56.5	14.5	132	17.8	30.5 (5.0)
Los Patricios_11	Natr	6.0	3.1	16.6	0.60	2.4	0.0036	28.3	6.3	35.5	56.5	8.0	153	22.4	49.5 (5.0)
Colonia Velaz_12	Natr	6.7	3.6	19.5	3.46	4.5	0.0204	15.5	12.1	23.0	59.0	18.0	136	25.4	53.4 (1.1)
Colonia Velaz_13	Natr	7.2	3.4	18.7	2.20	4.9	0.0126	47.7	17.6	28.0	57.0	15.0	191	25.2	55.2 (1.0)
Colonia Velaz_14	Natr	7.3	1.0	14.2	3.84	11.3	0.0243	51.6	7.9	24.5	58.5	17.0	163	18.3	34.5 (2.6)
Colonia Velaz_15	Natr	7.2	2.4	17.5	2.28	7.7	0.0140	25.5	24.0	31.0	56.0	13.0	183	19.5	48.3 (5.2)
Colonia Velaz_16	Natr	8.8	1.8	30	2.20	10.7	0.0142	29.2	26.6	36.0	57.0	7.0	201	29.7	71.9 (5.0)
Los Patricios_17	Arg	6.5	1.0	26	0.42	1.8	0.0027	45.1	21.7	51.0	44.0	5.0	271	22.9	68.9 (0.9)
Los Patricios_18	Arg	6.4	0.9	24.5	0.56	0.5	0.0036	4.7	6.2	52.5	39.0	8.5	264	23.6	70.7 (1.8)
Los Patricios_19	Arg	6.2	0.9	22.3	0.80	0.6	0.0045	28.0	28.0	43.5	44.5	12.0	224	22.0	63.5 (5.2)
Los Patricios_20	Arg	5.7	3.8	27.6	0.89	1.6	0.0056	30.6	44.2	44.0	52.5	3.5	224	26.5	63.7 (2.6)
Los Patricios_21	Arg	6.2	2.8	26.1	0.96	0.5	0.0045	6.3	19.8	37.5	57.0	5.5	236	24.5	58.8 (2.2)
Los Patricios_22	Arg	5.3	3.1	24.7	0.63	0.7	0.0040	31.2	39.0	38.5	59.5	2.0	200	25.5	70.4 (3.3)
Los Patricios_23	Arg	5.5	2.4	26.7	0.40	1.7	0.0026	41.2	39.8	44.0	53.0	3.0	194	25.9	73.0 (1.5)
Los Patricios_24	Arg	5.6	2.8	20.8	0.78	2.1	0.0056	10.1	25.1	38.0	54.0	8.0	186	24.3	46.9 (4.4)
Los Patricios_25	Arg	8.1	5.1	22.6	0.87	6.7	0.0072	38.3	27.3	33.0	45.5	21.5	206	19.6	60.6 (4.0)
Los Patricios_26	Arg	5.4	3.7	29.4	0.96	1.8	0.0070	10.7	33.1	40.0	58.0	2.0	223	29.3	73.3 (1.0)
Los Patricios_27	Arg	5.4	3.4	18.2	1.94	0.7	0.0072	8.1	28.8	31.0	59.0	10.0	182	21.0	54.5 (5.7)
La Esperanza_28	Arg	5.8	3.6	27.7	0.30	1.8	0.0030	45.9	23.9	42.5	48.5	9.0	287	26.7	68.0 (4.5)
La Esperanza_29	Arg	5.8	3.7	18.6	1.21	2.6	0.0074	25.6	38.2	29.0	57.0	14.0	150	24.7	42.4 (3.0)
La Esperanza_30	Arg	6.2	1.6	27.8	0.54	1.3	0.0052	10.4	22.0	49.0	45.0	6.0	326	25.9	66.0 (5.1)
Campo Rios_31	Arg	5.6	4.2	22.1	1.49	2.0	0.0076	20.1	40.0	36.2	59.5	4.3	193	27.4	69.5 (7.6)
Sta. Lucia_32	Arg	5.8	3.1	20.5	0.61	2.8	0.0042	9.3	17.7	34.0	61.0	5.0	194	19.8	62.6 (4.6)

† Numbers indicate geographic location in **Fig. 1**

‡ Values between parentheses indicate standard errors

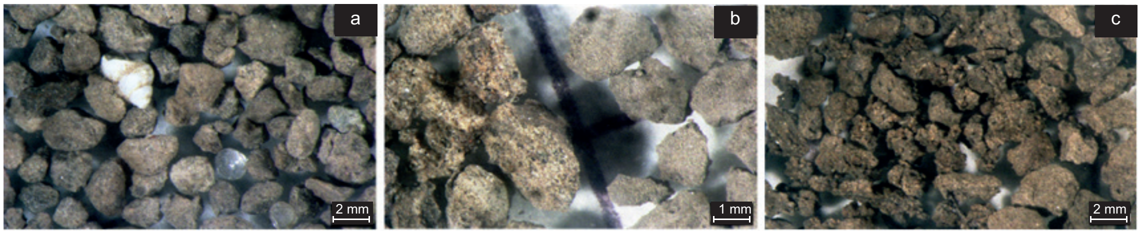

Fig. 2. Morphology of the aggregates <2 mm. Photograph a and b (left) correspond to samples from the cattle-breeding soils Los Patricios 1 and Los Patricios 2, respectively. Photograph b (right) and c correspond to samples from agricultural soils (Los Patricios 15 and 14 respectively)

small proportion of interstratified illite-smectite minerals, and traces of kaolinite. Semi quantitative analysis of clay minerals did not find any differences between soils sites. On the contrary, a considerable variability regarding other physical and chemical properties of the soil surface horizons was found (**Table I**). The content of OC varied between 0.2 and 5.1%, whereas the clay content ranged between 220 and 525 g kg^{-1}; the group of natric soils evidenced a higher content of sand in comparison to the Argiudolls. The pH varied between 5.3 and 9.3, the CEC between 13.8 and 30.0 cmol$_c$ kg^{-1} and the ESP between 0.5 and 23.2 %. The other measured soil properties also showed considerable differences among the studied sites (**Table I**).

Regarding the morphology and surface characteristics of soil aggregates <2 mm, a considerable heterogeneity was also observed (**Fig. 2**). The samples evidence different morphology and color distributions at their surface, indicating differences in the composition and arrangement of their inorganic and organic fractions. This color heterogeneity is found predominantly in soil aggregates from the livestock sites (**Fig. 2a** and **b** left) while color becomes more homogenous in aggregates from agricultural fields (**Fig. 2b** right). The aggregates from the former situations also show more spherical shapes with more rounded faces, as for example in **figure 2a**. Inversely aggregates from agricultural sites are predominantly very fine angular and subangular blocks (**Fig. 2c**). Plant debris under decomposition and a higher macroporosity of peds was also observed in agricultural soils (**Fig. 2c**). Because of the proximity to water courses, and coincidently with their higher sand content, natric soils also show coarser particles equivalent in size to the small peds, and specific biological features as snails also appear (**Fig. 2a**).

In agreement with the important soil heterogeneity mentioned, the values of bacterial adsorption found in this work oscillated between 25.3 and 73.3 % (**Table I**).

The principal components analyses of chemical and physical parameters show that the first two components were responsible for 62 % of the total variability. The first component (PCA1) explained 45 % of the variability due mainly to high variance of clay content, ESP, sand content, and, to a lesser extent, pH (**Table II**), whereas the second component (PCA2), explained 16% of the variability and OC, SSA and the Na$^+$ -sol attained the highest variance in this component. **Figure 3** shows an important grouping of soil properties such as ESP, pH and Na$^+$ -sol, indicating environments typical of sodic and saline soils. **Figure 3** also shows the clustering of other soils properties such as clay content, CEC, SSA, and variables associated with the presence of fine particles. This clustering is represented by the sites evaluated on Argiudolls. Moreover, other expected relations were observed such as the diametrically opposite position occupied by sand content and ME on one hand and the relation between Ca^{2+} -sol with ESP on the other.

Natraqualf soils showed on average lower PCA1 scores than Argiudolls, whereas no differences were

TABLE II. PCA LOADING VALUES. THE SIGNIFICANCE CORRESPONDS TO PEARSON'S CORRELATION ANALYSES BETWEEN THE PRINCIPAL COMPONENTS AND EACH VARIABLE.

Variables	PC1	PC2
pH	–0.33***	–0.31***
OC	0.16*	0.51***
CEC	0.31***	–0.23
EC	–0.27***	0.34**
ESP	–0.35***	–0.14
IS	–0.31***	0.22
Na$^+$ -sol	–0.25***	–0.35***
Ca^{2+} -sol	0.20**	0.12
Clay	0.35***	–0.28*
Sand	–0.33***	–0.13
SS	0.27***	–0.38***
ME	0.27***	–0.17

*P<0.05
**P<0.01
***P<0.001

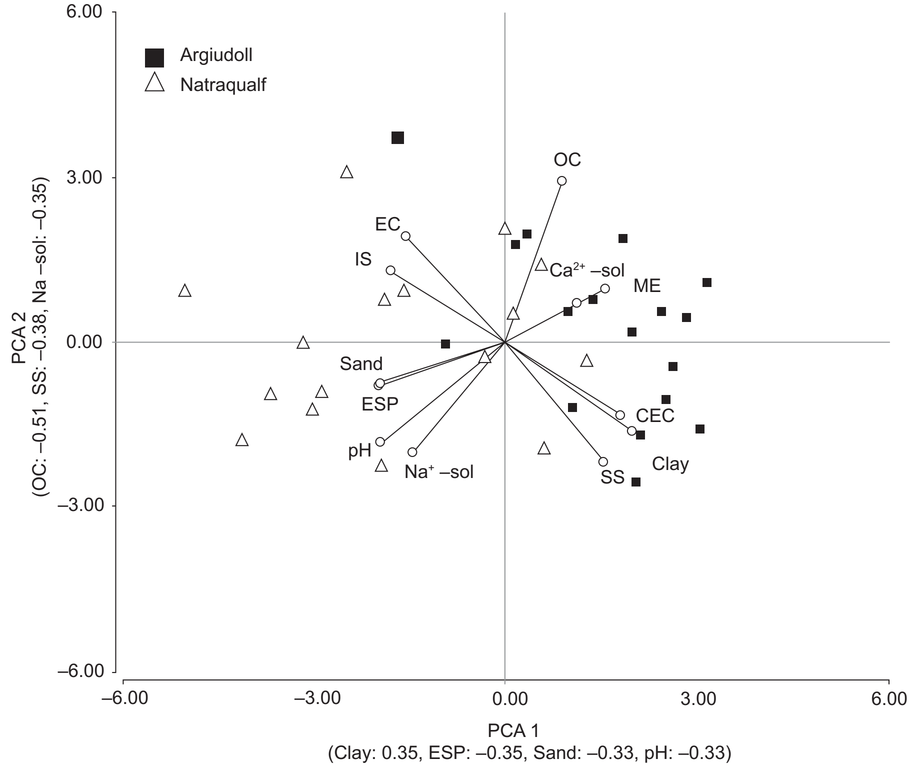

Fig. 3. Scores on the first (PCA1) and second axes (PCA2) of the principal component analysis. The length of the vectors represents the magnitude of the representation of each variable for each component and the angles between the variables indicate the correlation between them. Angles of 90º between two variables indicate that they are not correlated. Below both axes principal autovectors are presented in parenthesis

found in PCA2 scores (**Table III**, **Fig. 3**). Consistently, Natraqualf soils showed higher pH, ESP and Na^{+} -sol values and lower Ca^{2+} -sol values than Argiudoll soils (**Fig. 3**). On the other hand, differences between site locations in soil properties were evident in the scores of the PCA2, whereas no differences were observed for PCA1 scores (**Table III**). In addition, no interaction between soil type and site location was found on PCA1 or PCA2 scores.

Bacterial adsorption was significantly associated with the soil type, the highest values of bacterial adsorption being related to Argiudolls and the lowest ones to Natraqualfs (**Fig. 4**, **Table III**).

TABLE III. ANALYSIS OF VARIANCE FOR THE EFFECTS OF SOIL TYPE AND SITE LOCATION ON THE SCORES OF THE FIRST (PCA1) AND SECOND (PCA2) AXES OF THE PRINCIPAL COMPONENT ANALYSIS, AND THE PERCENTAGE OF BACTERIAL ADSORPTION. VALUES ARE *F* STATISTICS FOR EACH MODEL TERM

	PCA1	PCA2	Bacterial adsorption (%)
Soil type	37.7***	0.01	12.31**
Site location	1.05	6.36*	0.06
Soil type × site location	0.07	0.3	0.95

*P<0.05
**P<0.01
***P<0.001

Association between bacterial adsorption and soil properties

Bacterial adsorption increased consistently with higher PCA1 scores ($R^2 = 0.67$; $P < 0.0001$; **Fig. 4**). When this regression was analyzed by means of categorical variables (Argiudoll and Natraqualf), neither the intercept nor the slope showed effects of these soil types or *SL* ($P>0.05$). Soil properties analyzed individually presented no significant differences due to the *ST* or *SL* ($P<0.05$). These properties explained up to 67 % of the variation of the bacterial adsorption. The highest values of determination coefficient were those of the parameters with a positive tendency in relation with the bacterial adsorption, such as CEC (R^2= 0.67), clay content (R^2= 0.55) and SSA (R^2= 0.45) (**Fig. 5**). In contrast, ESP (R^2=0.42), sand content (R^2= 0.38) and pH (R^2= 0.25) were the variables

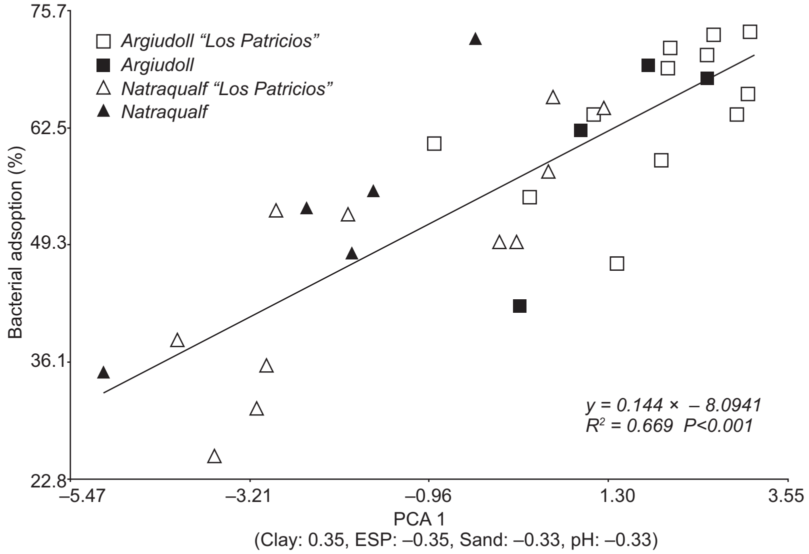

Fig. 4. Linear regression of bacterial adsorption on the scores of the first axis from the principal component analysis (PCA1). Autovectors are in parenthesis

that best explained the negative tendency to the adsorption (**Fig. 5**). However, other important soil properties in PCA2 presented differences in the behavior in the bacterial adsorption due to the soil type, although such properties did not present important determination coefficients or significant differences. **Figure 6a** shows that OC presented a slightly negative behavior in Argiudolls and a positive one in Natraqualfs. **Figure 6b** shows that the Ca^{2+} -sol did not present a marked tendency in Argiudolls but presented a positive tendency in Natraqualfs. In turn, IS presented an R^2 of 0.2 ($P<0.05$), whereas EC and Na^{+} -sol presented an R^2 of 0.14 ($P<0.05$) and 0.15 ($P<0.05$) respectively, evidencing different slopes between soils ($P<0.05$) with negative tendencies.

Prediction of soil bacterial adsorption

The soil properties included in the bacterial adsorption model (minimum square error) were: clay content, OC, CEC, EC and IS, and the model presented a R^2 of 0.79 (R^2_{adjust}: 0.77). The same soil properties and determination coefficient were found when maximizing the R^2 methodology was performed. Then stepwise regression was carried out to evaluate the behavior of the model evaluated by the selection of significant properties (P entrance and exit= 0.15). The model adjusted with this methodology was the following: $y = 1.73\,CEC - 0.05\,sand(50\text{–}250\,\mu m) - 0.54\,ESP$, which also presented a determination coefficient of 0.79 with an adjusted R^2 of 0.77. This simple model allowed us to explain the high percentage of variability of bacterial adsorption present in the studied area.

DISCUSSION

Soils characterization

The wide ranges of values of the physical and chemical properties present in the Tala´s creek basin (**Table I**) provide an adequate frame to study the soil variability and its influence on bacterial adsorption. The measured value even exceeds the application ranges of the prediction formulae of bacterial adsorption such as that proposed by Ling *et al.* (2002). It is to be mentioned that all of the topsoils evaluated present evidences of erosion and redeposition sharing similarities to sediments on which bacteria would be attached. In this sense, it is important to point out the morphological variability evidenced by the aggregates <2 mm which reflects the compositional diversity of the soil materials studied. The clay content, the interaction with other organic compounds, the variations in pH, and the presence of cations are important factors in the determination of the degree of bacterial adsorption. Field clays are not dispersed but rather form aggregates, domains, cutans and can be complexed in part with organic matter or oxides (Stotzky 1985). For example, bacterial adsorption is highly increased by the presence of oxide cover layers in the quartz grains relative to that in the pure grains (Mills *et al.* 1994). Also, Hoek and Agarwal (2006) found that the surface roughness causes an important modification in the repulsive or attractive forces. Then, surface heterogeneity creates a greater distance between the particle and the substrate, causing a reduction in the interaction energy (Jacobs *et al.* 2007). Consequently, the wide ranges of

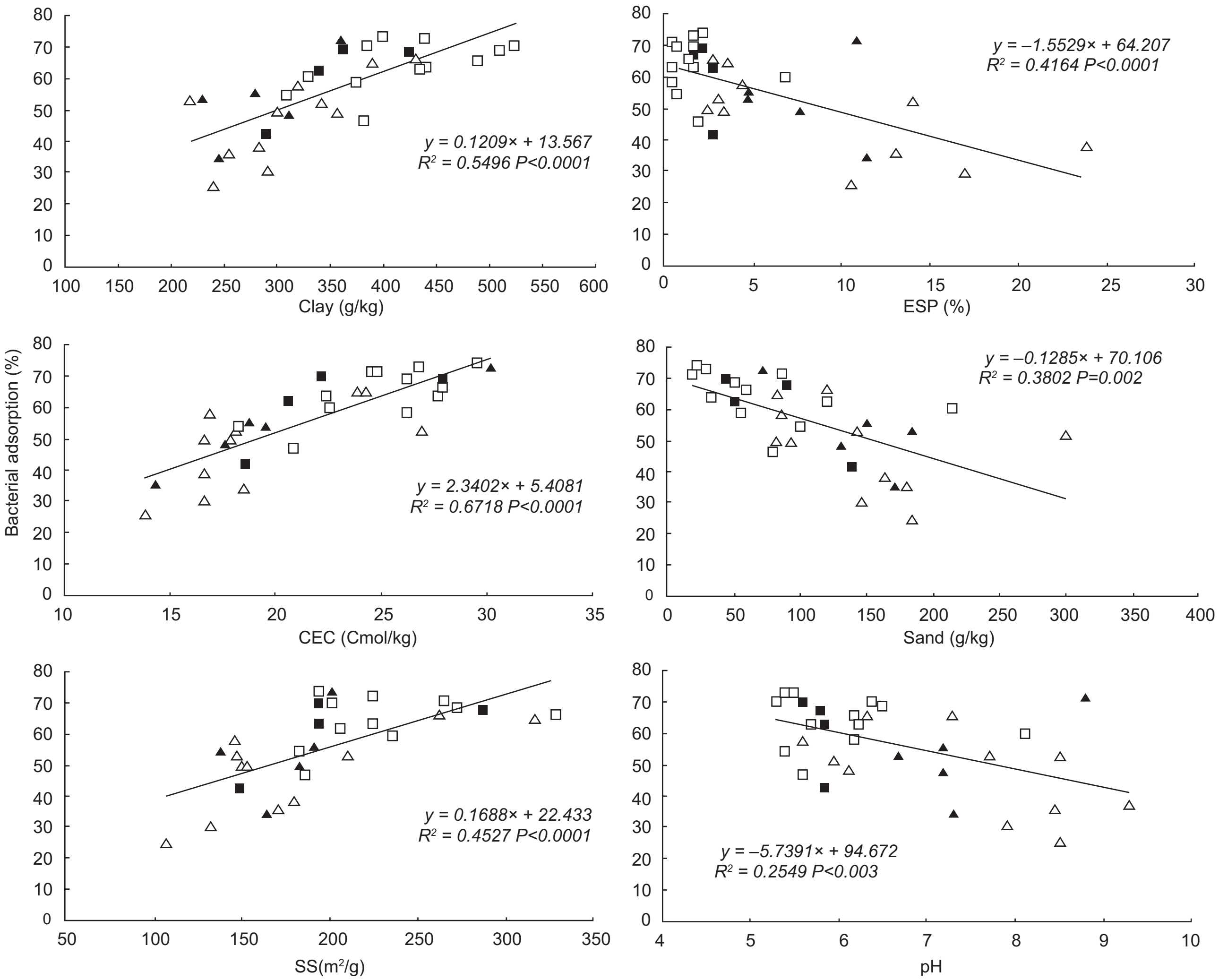

Fig. 5. Regression of the variables with positive tendency, clay content (%), CEC (Cmol Kg^{-1}) and specific surface area (g m^{-2}) –left panel– and those with negative tendency, ESP (%), sand content (%) and pH –right panel–. Squares correspond to Argiudolls, whereas triangles correspond to Natraqualfs. Empty symbols show the soils sampled within "Los Patricios", whereas filled symbols show samples collected outside "Los Patricios"

bacterial adsorption values measured in the present work should be understood as a conjunction of the soil complexity.

Association between bacterial adsorption and soil properties

Although the bacterial adsorption (25.3 -73.3 %, **Table I**) obtained presented a wide range, they were relatively low compared with Ling *et al.* (2002) that found bacterial adsorption values of up to 99 % in silty soils, whereas Oliver *et al.* (2007) found 35% of association in a clay loam soil. Furthermore, Characklis *et al.* (2005) found 20 to 35 % of *E. coli* associated with the sedimentable aggregates measured by means of centrifugation techniques.

When analyzing the relationship between PCA1 and bacterial adsorption (**Fig. 3**), the soil type did not present any effect, indicating that the general behavior of the environment expressed by PCA1 in relation with the bacterial adsorption can be represented by means of only one equation with an important degree of adjustment (R^2=0.67), which includes the properties of the two dominant soils (Argiudoll and Natraqualf). By means of linear regressions, such properties were the ones that best described the behavior of bacterial adsorption. These are cited as properties that can affect bacterial adsorption either positively or negatively.

Positive tendencies

Within this group, clay content is usually cited as the main factor in the regulation of adsorption and Kd

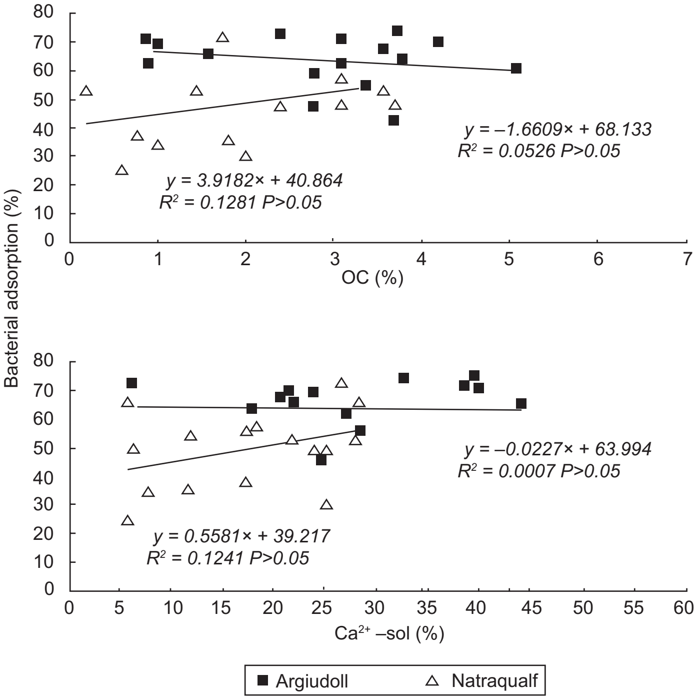

Fig. 6. Linear regression of bacterial adsorption on organic carbon (a) or Ca^{2+} -sol (b) for different soil types. Argiudolls (white squares); Natraqualfs (white triangles)

(Hagedorn *et al.* 1978, Bengtsson 1989). For example, Weaver et al. (1978) studied this variable in a water-soil solution and observed that the adsorption of *E. coli* varied between 7 % in loamy sandy soils (10 % clay) to 90 % and more in clayey soils. Using centrifugation techniques, Ling *et al.* (2002) found values of 24.5 % and 99.2 of adsorption of *E. coli*, for soils with 14 % and 35 % clay content respectively. These findings match the relations found in this work, although samples with the higher clay contents (51 and 52 %) did not reach adsorption values as high as mentioned above. For the soils evaluated, the adjustment of the regression model for such relation (**Fig. 5**) reached a determination coefficient of 0.55. This value, although not so high in relation with the other properties evaluated, did not evidence all the expected predictive potential of the clay content as an estimator of adsorption. A possible explanation of this fact is the high degree of aggregation and different organic content presented by the samples analyzed (**Fig. 2**). Also, in a previous work with the same soils (Kraemer *et al.* 2011), clay content together with <3 µm particles showed better correlations confirming also the importance of very fine silt in the adsorption process.

As regards the specific surface area, a property closely related to clays, a high degree of adjustment in relation with bacterial adsorption was expected. However, this adjustment was very similar to that of clay content. Since most samples were similar in their clay mineralogy, showing equivalent proportions of different clay minerals (illite, interstratified I-S and kaolinite), this variable did not improve the explanation of bacterial adsorption.

On the other hand, the CEC, a property closely related to the content and type of clays and organic matter, allowed the adjustment of the regression model with the maximum determination coefficient of the assay, which reflects the importance of electrostatic balances in reversal adsorption processes. In studies with reoviruses, Lipson and Stozky (1983) also concluded that the CEC was one of the properties involved in the adsorption. In 2:1 clay minerals, 80% of the negative charge depends on the isomorphic substitutions, presumably being this the reason why CEC was the variable that best explained the behavior of bacterial adsorption in the studied soils. Furthermore, not only clays size particles could present CEC, Morrás (1995) found important values of this parameter in fine silts, fraction size dominant in all samples studied.

Surface interactions between biological entities and clays are usually greater when the valence and concentration of the exchangeable cations is higher. According to the DVLO theory (Derjaguin and Landau 1941, Verwey and Overbeek 1948), this results, in part, from the reduction of the extension of the double diffuse layer of clays, which allows the latter and the biological entities to approach each other. The tendency found with the Ca^{2+} -sol seems to support the role of cations in the importance of the CEC in the increase in bacterial adsorption, mainly in Natraqualfs, where the concentration of this cation is low (**Fig. 6b**). This would be due to the double diffuse layer, as explained before, which would also involve the partial or total flocculation of clays and aggregates, altering the electrostatic charges balance. Besides, bivalent cations such as Ca^{2+} reduce the expansibility and dispersibility of 2:1 clays. Thus, clay flocculation seems to indirectly decrease the expression of negative charges in 2:1 clays increasing the effect of bacterial adsorption on soils. In the same sense, with adsorption of inorganic cations (cationic polyelectrolites), the surface charges would be neutralized, and, if the adsorption continues, the net charge turns positive. Marshall (1980) reported that the appearance of this type of flocculation may be a mechanism of approach of the bacterial cells to the soil particles so that Van der Waals forces can then act.

Negative tendencies

According to the above-mentioned mechanisms, the high determination coefficient in the regression adjustment between the ESP and bacterial adsorption (**Fig. 5**) seems to be reflecting the environment of aggregate dispersal generated when the expression of negative charges increases. This fact would affect the increase in the repulsion of bacteria. Similarly, the negative tendency found with the Na^{+} -sol confirms this phenomenon.

As regards pH, Jiang *et al.* (2007) found a decrease in the adsorption of *Pseudomonas putida* in minerals when the pH increased from 3 to 10. Scholl and Harvey (1992) found that a large number of colonies of *Arthrobacter* sp. were associated with quartz at pH 5.0, but that the number of colonies decreased at pH 7.5. In summary, there seems to be a close relationship between bacterial adsorption and the pH of the medium confirming the role of electrostatic forces in the bacterial adsorption to minerals. Higher pH generates an increase on the electronegativity of soil colloids and also results in the increase in the electronegativity of the surfaces of the bacterial membranes, a phenomenon that would potentiate the repulsion between bacteria and mineral surfaces. It can be observed that pH, which in the PCA discriminated the variability of the soils evaluated to a large extent, did not have the same preponderance as an individual predictive variable of bacterial adsorption. Although this relation showed a high significance ($P<0.003$), its R^2 was lower than 0.25. This could be due, in part, to the preponderance of 2:1 minerals in the samples analyzed, since their negative charge, responsible for the bacterial adsorption is barely dependent on pH. In this sense, the variation of the negative charges dependent on pH would be mainly due to the soil organic matter and, in a very low proportion, to kaolinite. The organic matter is a component that can affect the adsorption phenomenon since it modifies the surface of inorganic fractions, modifying its properties. Aislabie *et al.* (2001) and Marshall (1971) reported that organic matter is one of the main soil components affecting bacterial adsorption. However, in the present work, divergent relations according to the soil type were found (**Fig. 6a**). Organic matter can either increase or decrease bacterial adsorption depending on its quality, solubility and size of the fraction affected. Gray *et al.* (1968) and Guber *et al.* (2005) found that organic carbon had a greater importance in soils with sandy texture. If the behavior of bacterial adsorption is analyzed relating OC to soil texture, a positive trend is evidenced between the organic fraction and bacteria adsorption in the sites with a larger content of sands (Natraqualfs), and, in contrast, a negative trend is evidenced in the sites with lower content of sands (Argiudolls) (data not shown). In this last case, the organic compounds in the soil may affect the bacterial association to the minerals.

Regarding ionic strength (IS), numerous authors have recognized that an increase in the concentration of electrolytes increases the bacterial association to solids (Marshall 1980, Sharma *et al.* 1985, Fontes *et al.* 1991, Mills *et al.* 1994). In this sense, Huub et al. (1995) used eight different bacterial strains in several liquid media with ionic strengths that varied between 0.0001 and 1 M, whereas in another study of adsorption in columns, Stevik *et al.* (1999) applied distilled water as medium and two solutions of 0.00725 and 0.097 M. In both works, the bacterial adsorption was increased with higher ionic strengths. Here, negative tendencies of this property in relation with adsorption were found. However, it should be considered that this variable takes Na^{+} into account, a cation present in numerous samples that increased ionic strength but dispersed at the same time soil aggregates.

Agronomic implications; erosion and contamination

The quantity and quality of the sediments generated in a watershed, and therefore their contaminating power, depend on several factors such as the magnitude and type of the erosive process, the geomorphology, and the type and management and of soils. Watersheds that present slopes with moderate length and scarce gradient, such as the one of the present study, suffer processes of erosion mainly of laminar type. This is a common feature in the Rolling Pampa. Such process is characterized by the generation of "fine" sediments enriched in organic matter and clays with considerable capacity of cation exchange. Therefore, the sediments generated by laminar erosion in the Rolling Pampa would have a high capacity to adsorb and transport diverse chemical and/or contaminants such as pesticides as well as bacteria and viruses associated with human activity (Chagas 2007). It is because of this, that is important to highlight that CEC and clay content were the properties that best explained the bacterial adsorption both in Argiudolls and in Natraqualfs.

Prediction of the bacterial adsorption

The prediction model of bacterial adsorption based on multiple regressions was consistent with the results of the principal components analysis and with the individual soil parameters analyses. Sand and clay content, ESP and CEC were important variables in the principal component analysis and in the multiple regressions, allowing to predict bacterial adsorption in both types of soils (Argiudolls and Natraqualfs). All of the parameters selected are relatively easy to measure, integrate some of relevant physical and chemical characteristics of soils, and are relatively stable along time. To validate these soils parameters in order to elaborate a bacterial adsorption prediction model other bacterial strains should be used. Regarding clay content, Kraemer *et al. (*2011) using a laboratory (same strain of this work) and a wild strain found similar correlations with clay vs. bacterial adsorption that the ones measured by Ling *et al.* 2002. While the wild strain presented almost a perfect correlation, the laboratory strain showed lower adsorption values but the same behavior along clay contents.

CONCLUSIONS

The soils of the Tala´s creek basin presented a wide range of bacterial adsorption capacity (25% to 73.3%), which allowed discriminating between different environments: the bacterial adsorption capacity of Argiudolls was significantly higher than that of Natraqualfs.

Principal components analysis demonstrated that the main properties that explained the variation of adsorption in both soil types are the same, although some properties such as organic carbon or the Ca^{2+}-sol presented different behaviors according to the soil type. In the present study, the importance of properties such as texture, CEC and ESP were corroborated as tools to differentiate environments of bacterial adsorptions, even in very heterogeneous environments such as the one evaluated. In that sense, the following equation: $y=1.73\times CEC - 0.05\times sand(50\text{–}250\ \mu m)[g\ kg^{-1}]-0.54\times ESP$ ($R^2 adjust=0.77$) is proposed. The results obtained would be useful for the development of bacterial transport models from standard soil data in environments characterized by fine materials of illitic mineralogy. In order to confirm the predictive value of these properties it would be useful to evaluate such variables in environments with different textures and mineralogy since sandy soils did not integrate the data set and the mineralogy was relatively homogeneous in the surface horizons of the soils studied.

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REFERENCES

Aislabie J., Smith J.J., Frase R. and McLeod M. (2001). Leaching of bacterial indicators of faecal contamination through four New Zealand soils. Aust. J. Soil Res. 39, 1397-1406.

APHA (1998). Standard methods for the examination of water and wastewater. 20th ed. American Public Health Association. Washington, USA. 1325 pp.

Balzarini M.G., Gonzalez L., Tablada M., Casanoves F., Di Rienzo J.A. and Robledo C.W. (2008). Infostat. Manual del Usuario, Editorial Brujas, Córdoba, Argentina.

Bengtsson G. (1989). Growth and metabolic flexibility in groundwater bacteria. Microb. Ecol. 8, 235-248.

Buján A., Santanatoglia O.J., Chagas C., Massobrio M., Castiglioni M., Yáñez M.S., Ciallella H. and Fernández J. (2000). Preliminary study on the use of the 137Cs technique for soil erosion investigation, in the Pampean Region of Argentina. Acta Geológica Hispánica 35, 271-277.

Buján A., Santanatoglia O.J., Chagas C., Massobrio M., Castiglioni M., Yáñez M.S., Ciallella H. and Fernández J. (2003). Soil erosion in a small basin through the use of 137Cs technique. Soil Tillage Res. 69, 127-137.

Chagas C.I. (2007). Calidad y contaminación del agua superficial como recurso para bebida animal en una cuenca representativa de Pampa Ondulada. Tesis Doctoral (Área Toxicología). Facultad de Ciencias Veterinarias, Universidad de Buenos Aires. Buenos Aires, 9 de agosto de 2007. 144 pag.

Chagas C.I., Santanatoglia O.J., Moretton J., Paz M. and Behrends Kraemer F. (2010). Movimiento superficial de contaminantes biológicos de origen ganadero en la red de drenaje de una cuenca de Pampa Ondulada. Cienc. Suelo 28, 23-31.

Characklis G.W., Dilts M.J., Simmons III O.D., Likirdopulos C.A., Krometis L-A.H. and Sobsey M.D. (2005). Microbial partitioning to settleable particles in stormwater. Water Res. 39, 1773-1782.

Cao Y., Wei X., Cai P., Huang Q., Rong X. and Liang W. (2011). Preferential adsorption of extracellular polymeric substances from bacteria on clay minerals and iron oxide. Colloid Surface B. 83, 122-7.

Derjaguin B. and Landau, L.D. (1941). Theory of the stability of strongly charged lyophobic sols and of the adhesion of strongly charged particles in solutions of electrolytes. Acta Physicochim. U.R.S.S 14, 633-662.

Di Rienzo J.A., Casanoves F., Balzarini M.G., Gonzalez L., Tablada M. and Robledo C.W. InfoStat versión 2012. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. URL http://www.infostat.com.ar

Dorner S.M., Anderson W.B., Slawson R.M., Kouwen N. and Huck P.M. (2006). Hydrologic modeling of pathogen fate and transport. Environ. Sci. Technol. 40, 4746-4753.

Drozd C. and Schwartzbrod J. (1996). Hydrophobic and electrostatic cell surface properties of *Cryptosporidium parvum*. Appl. Environ. Microbiol. 62, 1227-1232.

Fontes D.E., Mills E.L., Hornberger G.M. and Herman J.S. (1991). Physical and chemical factors influencing transport of microorganisms through porous media. Appl. Environ. Microbiol. 57, 2473-2481.

Gray T.R.G., Baxby P., Hill L.R. y Goodfellow M. (1968). Direct observation of bacteria in soil. In: *The ecology of soil bacteria.* (T.R.G. Gray and D. Parkinson, Eds.) University of Toronto Press, Toronto, Canada, pp. 171-197.

Guber A.K., Shelton D.R. and Pachepsky Y.A. (2005). Effect of manure on *Escherichia coli* attachment to soil. J. Environ. Qual. 34, 2086-2090.

Hagedorn C., Hansen D.T. and Simonson G.H. (1978). Survival and movement of fecal indicator bacteria in soil under conditions of saturated flow. J. Environ. Qual. 7, 55-59.

Hoek E.M.V. and Agarwal, G.K. (2006). Extended DLVO interactions between spherical particles and rough surfaces. J. Colloid Interface Sci. 298, 0-58.

Holtzapffel T. (1985). Les minéraux argileux: préparation, analyse diffractométrique et détermination. Société Géologique du Nord, publication n°12, 136 p.

Huub H.M.R., Norde W., Lyklema J. and Zehnder A.J.B. (1995). The isoelectric point of bacteria as an indicator for the presence of cell surface polymers that inhibit adhesion. Colloid. Surface. B. 4, 191-197.

Infostat / P v1.1, (2002). InfoStat, v. 1.1. Grupo InfoStat, FCA. Universidad Nacional de Córdoba. Argentina.

INTA (1973). Carta de Suelos. Hoja 3360-33. Pérez Millán. E=1:50.000. Instituto Nacional de Tecnología Agropecuaria. Buenos Aires, Argentina, 45 pp.

INRA (1986). Note Technique nº 1.0. Séparations granulométriques des particules par sédimentation. Science du Sol –Versailles, equipe Matieres Organiques des Sols. Institut National de la Recherche Agronomique, France.

Jacobs A., Lafolie F., Herry J.M. and Debroux M. (2007). Kinetic adhesion of bacterial cells to sand: Cell surface properties and adhesion rate. Colloid Surfaces B. 59, 35-45.

Jamieson R., Gordon R., Joy D. and Lee H. (2004). Assessing microbial pollution of rural surface waters: a review of current watershed scale modeling approaches. Agr. Water Manage. 70, 1-17.

Jiang D., Huanga Q., Caib P., Rong X. and Chena W. (2007). Adsorption of *Pseudomonas putida* on clay minerals and iron oxide. Colloid .Surface. B. 54, 217-221.

Klute A. (1986). Water retention: Laboratory methods. In: *Methods of soil análisis* (A. Klute, Ed.). 2nd.ed. ASA y ASSA. Madison, WI, USA, pp V.1,pp. 1.188 .

Kraemer F.B., Chagas C.I., Moretton J. and Paz M. (2008). Capacidad de resuspensión de patógenos en sedimentos de la cuenca del Tala. Efecto del medio líquido. Memorias. XXI Congreso Argentino de la Ciencia del Suelo. Potrero de los Funes, SL, Argentina. 13 al 16 de mayo, 2008. CD-ROM.

Kraemer F.B., Chagas C.I., Cosentino D.J., Paz M. and Moretton J.A. (2011). La textura del suelo como factor regulador de la adsorción de *Escherichia coli* en una cuenca de la Pampa Ondulada (Argentina). Rev. Argent. Microbiol. 43, 87-93.

LaBelle R.B. and Gerba, C.P. (1979). Influence of pH, salinity, and organic matter on the adsorption of enteric viruses to estuarine sediment. Appl. Environ. Microbiol. 38, 93-101.

Ling T.Y., Achberger E.C., Drapcho C.M. and Bengtson R.L. (2002). Quantifying adsorption of an indicador bacteria in a soil–water system. T ASAE. 45, 669-674.

Lipson S.M. and G. Stotzky. (1983). Adsorption of reovirus to clay minerals: Effects of cation- exchange capacity, cation saturation, and surface area. Appl. Environ. Microbiol. 46, 673-682.

Lombardi B., Dapino M.A., Montardit P.R. and Torres Sánchez R.M. (2001). Aproximación al valor de superficie especifica por un método manual y simple. Jornadas SAM - CONAMET - AAS 2001, Septiembre de 2001. 251-256.

Marshall K.C. (1975). Clay mineralogy in relation to survival of soil bacteria. Annu. Rev. Phytopathol. 13, 357-373.

Marshall K.C. (1980). Adsorption of microorganisms to soils and sediments. In: *Adsorption of Microorganisms to Surfaces (*G. Bitton and K.C. Marshall, Eds.) Wiley, NewYork, pp. 317-330.

Marshall K.C. (1985). Mechanisms of bacterial adhesion at solid-water interfaces. In: *Bacterial adhesion: Mechanisms and physiological significance* (D.C. Savage and M.H. Fletcher, Eds.). Plenum Publishing, New York, N.Y, pp.133-161.

McLaughlin W.L., Boyd A.W., Chadwick K.H., McDonald J.C. and Miller A. (1989). Dosimetry for radiation processing, Taylor and Francis, London.

Mills A.L., Herman J.S., Hornberge G.M. and DeJesus T.H. (1994). Effect of solution ionic strength and iron coating on mineral grains on the sorption of bacterial cells to quartz sand. Appl. Environ. Microbiol. 60, 3300-3306.

Mizuno I.M. Arrigo N. and Svartz H.A. (1978). Método rápido para la determinación de la humedad equivalente. Reunión Argentina de la Ciencia del Suelo, Buenos Aires (Argentina). 25-30 Sept. Resúmenes de trabajos y comunicaciones p. 4.

Moore J., Smyth J., Baker E., Miner J. and Moffitt D. (1989). Modeling bacteria movement in livestock manure systems. T ASAE 32, 1049-1053.

Morrás H.J.M. (1995). Mineralogy and cation exchange capacity of the fine silt fraction in two soils from the Chaco region (Argentina). Geoderma 64, 281-95.

Oliver D.M., Clegg C.D., Heathwaite A.L. and Haygarth P.M. (2007). Preferential attachment of Escherichia coli to different particle size fractions of an agricultural grassland soil. Water Air Soil Pollut.185, 369-375.

Reddy K.R., Khaleel R. and Overcash M.R. (1981). Behavior and transport of microbial pathogens and indicator organisms in soils treated with organic wastes. J. Environ. Qual 10, 255-266.

Schijven J.F., Hassanizadeh S.M. and Bruin H.A.M. (2002). Column experiments to study nonlinear removal of bacteriophages by passage through saturated dune sand. J. Contam. Hydrol. 58, 243-259.

Sharma M.M., Chang Y.I. and Yen T.F. (1985). Reversible and irreversible surface charge modification of bacteria facilitating transport through porous media. Colloid. Surface. 16, 193-206.

Scholl M.A. and Harvey R.W. (1992). Laboratory investigations on the role of sediment surface and groundwater chemistry in transport of bacteria through a contaminated sandy aquifer. Environ. Sci. Technol. 26, 1410-1417.

Snedecor G.W. and Cochran W.G. (1980). Statistical methods, 7th Ed. Iowa University Press.

Stevik T.K., Geir A., Hanssen J.F. and Jenssen P.D. (1999). The influence of physical and chemical factors on the transport of *E. Coli* through biological filters for wastewater purification. Water Res. 33, 3701-3706.

Stotzky G. (1985). Mechanisms of adhesion to clays, with referente to soil systems. In: *Bacterial adhesion: Mechanisms and physiological significance* (Savage D.C. and Fletcher M.H., Eds.). Plenum Publishing, New York, N.Y, pp.195-253.

Tian Y., Gong P., Radke J. and Scarboroug J.H. (2002). Spatial and temporal modeling of microbial contaminants on grazing farmland. J. Environ. Qual. 31, 860-869.

Tyrrel S.F. and Quinton J.N. (2003). Overland flow transport of pathogens from agricultural land receiving faecal wastes. J. Appl. Microb. 94, 87-93.

Verwey E.J.W. and Overbeek J.Th.G. (1948). Theory of the stability of lyophobic colloids, Elsevier, Amsterdam.

Walker S., Mostaghimi S., Dillaha T. and Woeste F. (1990). Modeling animal waste management practices: Impacts on bacteria levels in runoff from agricultural lands. T ASAE 33, 807-817.

Walkley A. and Black I.A. (1934). An examination of the Degjareff method for determining soil organic matter and a proposed modification of the cromi acid titration method. J. Amer. Soc. Agr. 24, 256-275.

Weaver R.W., Dronen N.O., Foster B.G., Heck F.C. and Fehrmann R.C. (1978). Sewage disposal on agricultural soils: Chemical and microbiological implications. Vol. II: Microbiological implications. Ada, Okla. USEPA.

Wei X., Fang L., Cai P., Huang Q., Chen H., Liang W. and Rong X. (2011). Influence of extracellular polymeric substances (EPS) on Cd adsorption by bacteria. Environ Pollut. 159, 1369-74.