Journal of Soil Science and Environmental Management Vol. 2(11), pp. 341-353, 29 November, 2011
Available online at http://www.academicjournals.org/JSSEM
ISSN 2141-2391 ©2011 Academic Journals

Full Length Research Paper

Bacterial retention in three soils of the Rolling Pampa, Argentina, under simulated rainfall

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Accepted 13 October, 2011

Bacterial retention by soils is a key factor in predicting bacterial transport through surface runoff into water bodies. The objective was to evaluate biological, soil and hydrologic factors that affect bacterial retention in three soil types of the Rolling Pampa, Argentina. Simulated rainfall was applied on field plots previously inoculated with *Escherichia coli* and simultaneously biological variables such as bacterial adsorption and distribution coefficient were measured at laboratory. Soil variables, particularly pH, exchangeable sodium percentage and organic carbon as well as biological variables proved to be important properties in the regulation of bacterial retention processes. There were no significant differences between the biological variables measured in soils and in the sediments. Most of the microorganisms in the runoff from all sites were either free of (in the < 2 µm sediment fraction) or associated with small soil particles (2 to 50 µm), therefore management practices, such as filter strips, should be regarded with caution when implemented.

Key words: Fecal contamination, bacterial transport, bacterial adsorption, distribution coefficient, runoff.

INTRODUCTION

Agriculture and livestock breeding are important sources of water pollution as they provide significant amounts of sediments through water erosion, as well as of nutrients, pesticides and pathogens (Ongley, 1997; Coote and Gregorich, 2000). If contaminated surface water is used as a source of drinking water, recreation, irrigation, etc, it can act as a factor of disease transmission, and thus compromise human health. According to the USEPA (1996), agriculture and livestock breeding continue to be recognized as the main factors responsible for the lack of compliance with water quality standards based on microorganisms that act as indicators of biological contamination. Surface water contamination by those activities depends largely on the climatic, geomorphological and soil characteristics, as well as on the use of the watershed land where these activities are carried out, being surface runoff the axis that allows fitting these variables together (Chagas et al., 2007). In this sense, Stainer et al. (1979) showed that surface runoff is the main route by which fecal contaminants from agriculture and livestock breeding reach watercourses. Some studies have shown that the runoff generated by rainfall in livestock breeding areas may lead to significant transfers of microorganisms that are indicators of fecal contamination of surface water (Rodgers et al., 2003; Signor et al., 2005; Piazza, 2006; Chagas et al., 2007).

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Abbreviations: CEC, Cation exchange capacity; **CFU,** colony forming unit; **ESP,** exchangeable sodium percentage; **FIR,** final infiltration rate; K_d, distribution coefficient; **OC,** organic carbon; **SSA,** specific surface area; θ_{FC}, water content at field capacity.

It is therefore necessary to quantify the efficiency of bacterial retention of soils and to gain knowledge on the factors affecting this efficiency, given that there are currently few studies that address this issue. Some of these factors are the surface runoff, the duration and intensity of rainfall, the source of contamination and the degree of adsorption of contaminants to soil particles (Walker et al*.,* 1990).

Jamieson et al. (2004) have highlighted the need to investigate the relationship between the rates and volumes of runoff and infiltration and the transport of sediments and bacteria. According to these authors, the proportion of bacteria that enters the soil during a runoff event is probably controlled by the rate of soil infiltration and the vegetation status. On the other hand, although it is still unclear whether bacteria and sediments are transported independently in watercourses, an increasing number of works are confirming this issue (Vinten et al., 2004; Oliver et al., 2007). In field trials, Abu-Ashour and Lee (2000) distributed a strain of *Escherichia coli* (*E. coli)* resistant to nalidixic acid on small plots in order to monitor its movement, but could not identify whether the tracer moved as a free colloid or was transported in association with eroded particles. In experiments with rainfall simulation and manure applied on the surface of plots either with or without vegetation and a 20% gradient, Roodsari et al. (2005) observed that about 20% of the mobilized bacteria in the plots without vegetation were found in the suspended solids, and that the values in the vegetated plots were negligible.

The association between bacteria and sediments makes it difficult to predict bacterial survival and transport in runoff, because numerous and complex interactions between abiotic particles and the mobilized biological organisms are established (Jamieson et al., 2004). The variables that commonly describe this association are adsorption percentage and distribution coefficient (K_d) (Ling et al., 2002). Both the soil type and the characteristics of the sediment may affect bacterial transfer due to differences in the adsorption properties related to the soil colloidal material (Schijven et al., 2002). Ling et al. (2002) have highlighted the complexity of the dynamics of bacterial adsorption to soils. This process is regulated by numerous factors. Some of these factors are the electrostatic attraction (Marshall, 1975), in which the pH and content and type of clays play an important role, and the ionic environment of the soil/water mixture and interface (Gannon et al., 1991), which is also governed by pH and the relative concentration of cations and anions, among others. Once the bacteria associated with particles are mobilized by runoff, they may suffer successive processes of sedimentation and retransport that depend not only on the above-mentioned properties, but also on the density and size of the particles transported (Schillinger and Gannon, 1985). Thus, to predict the dynamics of bacterial surface movement it is not only important to study the hydrological and biological variables, but also the quantity, quality and size of the sediments mobilized. This is important when implementing management practices aimed to reduce water erosion and biological contamination, such as filter strips or others (Sutherland, 1991; Vinten et al., 2004).

Studies in the Rolling Pampa have shown that runoff is one of the most important variables in the regulation of the dynamics of water and regional erosion associated with heavy rainfalls, gentle slopes and soils with slow permeability (Chagas, 2007). In this region, it is expected that the dynamics of surface transport of biological contaminants is controlled both by the parameters of runoff and infiltration and by the physical and chemical properties of the soil. The overall aim of this study was to assess the dynamics of bacterial transport in different soils and managements of the Rolling Pampa, through the analysis of surface runoff. Our specific aims were to: a) quantify bacterial retention related to soil, biological and hydrological properties in three soils types; b) to compare the values of bacterial adsorption and K_d evaluated in sediments (field) and in soils (laboratory); c) to analyze the removal of microorganisms from the system, according to the size of the aggregates resulting from rainfall simulations.

MATERIALS AND METHODS

Bacterial retention was assessed through tests of simulated rainfall applied to runoff plots in soils previously inoculated with *E. coli.* This variable was calculated as the difference between the inoculum applied and the total removal of bacteria measured in the runoff. Three groups of variables were analyzed in each of the sites studied: a) soil variables, b) hydrological variables (runoff and final infiltration rate -FIR-), and c) biological variables (bacterial adsorption and K_d). The latter were measured both in the runoff sediments (that is, in the field) and in the soil (laboratory). To further characterize the runoff, we also measured some of its physical and chemical properties, such as the distribution of bacteria associated with different aggregate sizes of the sediments.

Bacterial strain

To determine the watershed factors in the fate and transport of pathogen, a wild strain of *E. coli was* chosen because its predicted numbers were reported to be clearly linked to hydrologic processes (Dorner et al., 2006). The *E. coli* used had been previously isolated from the study site and later identified using differential selective media (CHROMOBRIT, BRITANIA). Bacteria grown in trypticase soy agar (Biokar Diagnostics) at 35°C for 24 h were resuspended in saline solution, centrifuged and washed twice. The inoculum concentration was 1.10^7 CFU ml^{-1}, a similar value used by many researchers to represent a standard maximum concentration of microorganisms in runoff (Guber et al., 2005; Oliver et al., 2007). In order to obtain this value, a curve of absorbance vs. known concentrations of microorganisms (CFU ml^{-1}) was used (absorbance at 600 nm).

Field experiment and hydrological variables

The field experiment took place in the farm “Los Patricios”, which belongs to the University of Buenos Aires and is located in the middle basin of the Tala´s creek, Buenos Aires Province (Figure 1).

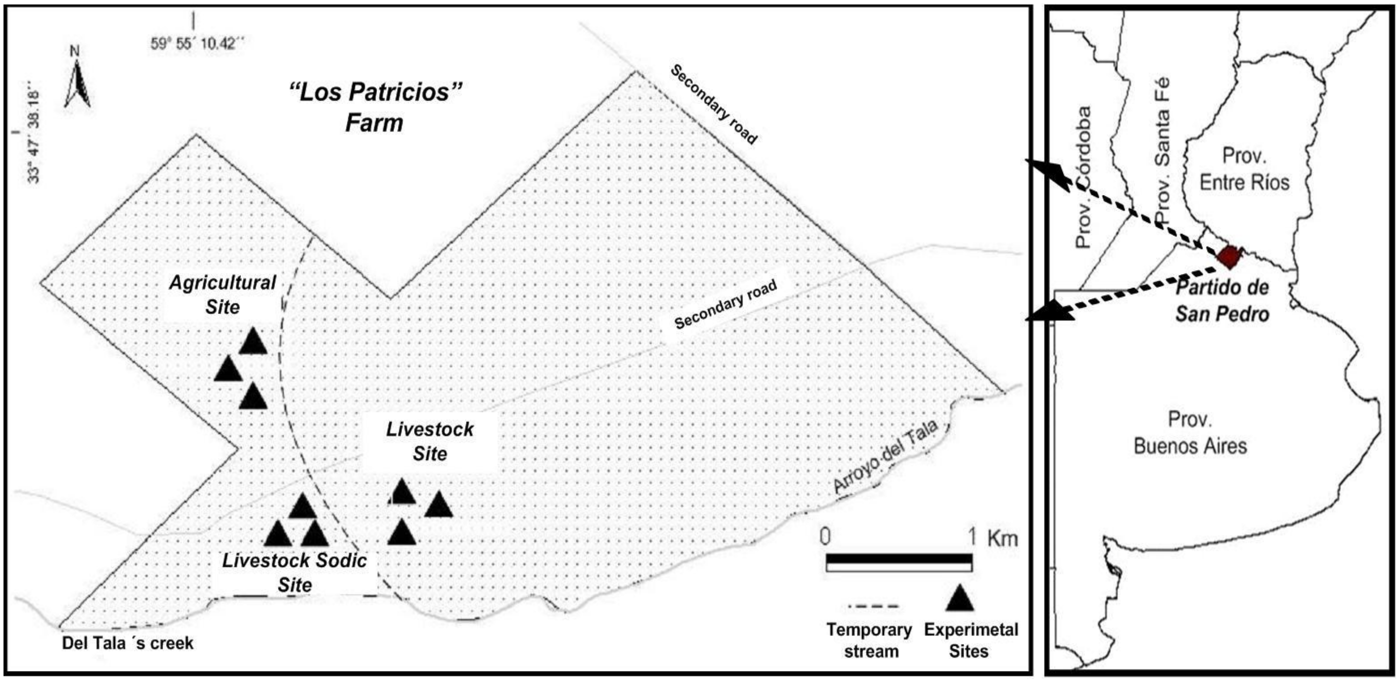

Figure 1. Location of the sites (and three plots within each site) where the rainfall simulation experiments were carried out. Sites were called "Agricultural", "Livestock" and "Livestock sodic", according to the main activity carried out and the soils characteristics

We applied simulated rainfall on three plots in each of three sites: an agricultural site -*Vertic Argiudoll*- near a watercourse used for annual crops (hereafter referred as "Agricultural"), and two sites typical of low lands -*Natraqualfs*-, also near a watercourse, used for livestock breeding and with different contents of soil organic carbon, pH, erosion degree and sodium level (hereafter referred as "Livestock" and "Livestock sodic", Figure 1). It must be noted that the experiments constrained to three sites and three sub replicas for each site. Hence, our experiment was pseudoreplicated for inferring the behavior of bacterial retention and subsequent transport at a catena level (Hurlbert, 1984).

To assess bacterial dynamics, we used a rain microsimulator that applied distilled water as 4.7 mm diameter drops falling from a height of 150 cm on plots framed by a square metal frame whose sides measured 25 cm. The drippers totalized 49 units and were arranged in a grid on an acrylic plate (Kamphorst, 1987; Irurtia and Mon, 2004). Prior to the simulation of rains, each plot was fitted as follows: a) the aerial part of the living vegetation and surface residues were carefully hand-removed in order to isolate the effects of vegetation; b) the top 1cm of soil was aerated lightly with a knife and then smoothed so as to standardize the surface and thereby eliminate microdepressions; c) 100 ml of the *E. coli* inoculum was uniformly applied using a manual sprinkler; this was done five minutes before the rains started to enable the association between the bacteria and the soil. After these three steps, the simulated rainfall was applied for 1 h with an average intensity of 60 mm h^{-1}, equivalent to the maximum rainfall expected in the region, with a return period of 10 years. The energy applied was 15 kJ $m^{-1}m^{2}$, an intensity equivalent to that used by Roodsari et al. (2005). To calculate the infiltration rate of soil water, we collected and measured runoffs obtained at 5 min intervals throughout the experiment. The final infiltration rate (FIR) was calculated from the average of the last three readings of the simulation. In all three soils, the infiltration rates stabilized at half an hour from the beginning of the rainfall simulations.

Physical and chemical soil variables

Samples from the topsoil (0 to 5 cm) were collected from a zone next to each simulation assay to determine bulk density by the cylinder method (Blake and Hartge, 1986). In addition, composed samples (3 subsamples) (0 to 5 cm) were used for the physical and chemical characterization of the soils under study. The latter were air-dried and sieved through a 2 mm sieve. The values of soil pH were measured at a solid to liquid ratio of 1: 2.5 and organic carbon (OC) by the method of Walkley and Black (1934). We also determined electrical conductivity (saturation paste), cation exchange capacity (CEC) through extraction with potassium chloride (Klute, 1986) and specific surface area (SSA) (Lombardi et al., 2001). The water content at field capacity (θ_{FC}) was measured at 0.33 atm. using a pressure membrane (Dane and Hopmans, 2002) and the particle size distribution was analyzed by the pipette method of Robinson (Soil Conservation Service, 1972). We also measured pH, electrical conductivity and sediment concentration (mg l^{-1}) in the runoff fractions, using the same methodology already mentioned.

Bacterial analyses

Field bacterial analyses

Chemical and physical quality of the runoff at beginning and at the end of the rain simulation could vary greatly (De la Vega, 2004). In that sense bacterial adsorption and distribution coefficient (K_d) of the sediments were measured on the runoff at the beginning (first 15 min) and at the end (last 5 min) of the simulated rainfall. The choice of 15 min at the beginning of the simulation was made in order to avoid variability in the initiation of the runnoff events and also to level the amount of runoff samples between the three sites. One intermediate measurement was done in the half of the simulation period. This measure was used to calculate the total bacterial removed from the plot, to obtain the soil bacterial retention more

more accurable.

The runoff was collected in sterile containers and kept at 8°C until processing in the laboratory, which was performed the day after collecting the samples. Once in the lab, the liquid obtained was left to sediment for 12 h at 8°C, after which the bacteria in the supernatant were counted. The liquid was then subjected to an intense manual agitation (3 min), after which the total concentration of microorganisms in the entire sample was measured. The adsorption percentage and K_d were calculated as follows:

Ads (%) = 100. $(N_t – N_s) / N_t$, (Ling et al., 2002)

where N_t = total number of bacteria in the runoff (CFU ml^{-1}) and Ns = total number of bacteria in the runoff supernatant (CFU ml^{-1}).

$K_d = C_s / C_l$, (Ling et al., 2002)

where K_d = distribution coefficient (ml g^{-1}) and C_s and C_l, the concentrations in the solid (CFU g^{-1}) and liquid (CFU ml^{-1}), respectively.

Parallel field simulations were carried out without previous inoculation, which allowed us to obtain the basal bacterial concentration of the soil under study. We found bacterial counts lower than 2% compared to the inoculum concentration used in the rainfall simulation experiments, thus the indigenous microbial population was considered negligible.

Bacterial adsorption on different fraction sizes present in the runoff

The runoff obtained was also analyzed to determine the proportion of bacteria associated with the different sizes of sediments. Aliquots corresponding to the following soil fraction sizes: > 50, 20, 3 and 2 µm were obtained by the pipette method (Soil Conservation Service, 1972) according to the Stokes' law. A subaliquot of each fraction was heated in an oven at 105°C in order to obtain the values of aggregate size distribution. It should be noted that this determination did not use any dispersant that may affect the bacterial association to the aggregates. Another subaliquot (0.1 ml) was diluted and plated for the bacterial count (APHA, 1998). Since the sedimentation of *E. coli* takes place together with the soil particles, the latter value was obtained by the difference between the CFU in the fraction considered and the CFU in the following fraction. Changes in the size of the *E. coli* population in the water-soil solution by regrowth or cell death were considered negligible due to the short period of sedimentation studied. By combining information from both subaliquots, we were able to determine the concentration of CFU for each sediment size/aggregate considered.

Laboratory bacterial analyses

At the same time, we measured the soils bacterial adsorption and K_d at the laboratory in the three sites. Both variables were quantified by slow centrifugation technique similar as described by Ling et al. (2002). A suspension of 6 ml of bacterial solution was prepared in 6 g of soil. The bacterial strain and inoculum concentration corresponds to those of the field experiments. Soil samples were sterilized in the reactor of the Comisión Nacional de Energía Atómica (CNEA) by applying a minimum highly uniform dose of ionizing radiation equivalent to 25 kGy, with the aim to prevent changes in the structure of the particles. The efectiveness of this sterilization technique has been verified in previous works (Chagas, 2007; Kraemer et al., 2008). After its inoculation in the soil, the suspension was manually shaken for 1 min and then left to rest for 5 min. This resting period was enough to lead to a bacteria-soil reversible association (Ling et al., 2002). In order to separate both phases we used a horizontal centrifuge. The energy calculated for such separation was of 50 G for 6 min (INRA, 1986). This energy allowed unassociated bacteria to remain in the supernatant while bacteria attached to soil particles within the range of 1 to 2 µm remain in the precipitated sediment. This value was adjusted experimentally for the specific conditions of the present work. The purity of the separated fractions was corroborated by optical microscopy. The supernatant of each sample was incubated at 36°C for 24 h in violet red bile agar (Biokar Diagnostics) for the plate counting of colonies (APHA, 1998). All determinations were carried out in triplicate and the mean temperature through all laboratory experiments was 20°C.

Statistical analyses

In order to describe the sites evaluated, a one-way ANOVA (three levels: agricultural, livestock and livestock sodic sites) was performed on their hydrological and physical variables obtained from the rainfall simulation experiments. Tukey HSD at 0.05 confidence level was used to compare means between treatments. To describe the behavior of the biological properties of rainfall during the simulation, we evaluated the variation in bacterial adsorption and K_d at the beginning and at the end of the simulations using a Student't test (bilateral 0.95 confidence level). To evaluate bacterial retention, we adjusted simple linear regression models between this dependent variable and the soil, biological and hydrological properties. Finally, to compare the bacterial adsorption and K_d obtained in soils and in sediments, linear regression models were adjusted with categorical variables (type of experiment) for all sites tested. Statistical analysis was performed using Infostat software (v1.1, 2002).

RESULTS

Soil and hydrological characterization of the sites

The plots of the "Agricultural" site showed a relatively important surface microroughness degree, associated with the appropriate aggregation that characterized the soil. The "Livestock" site presented the same features, although the origin of this roughness could be given by the abundant remains of vegetation found at the subsurface, especially of roots and stolons. The "Livestock sodic" site presented little roughness and a relatively powdered surface.

The three sites showed the same textural class, silty clay loam, and hence the CEC and the SSA showed a narrow range of variation and a tendency toward higher values in the "Agricultural" site (Table 1). The "Agricultural" and "Livestock" sites showed similar values for pH, OC and ESP, whereas "Livestock sodic" showed higher values of ESP and lower alkalinity and OC content than the previous two, associated with its status of soil degraded by water erosion. Although bulk density did not differ between treatments, the chemical and physical properties showed a large gradient that allowed analyzing changes in the bacterial adsorption processes (Table 1). The sites showed a different hydrological response to the simulated rainfall (Table 2). The values of runoff and generation of sediments in the "Livestock sodic" site were higher, whereas FIR was lower, than in the other two

Table 1. Physical and chemical properties of evaluated sites in the Rolling Pampa (Average values).

Physical and chemicals properties	Site		
	Agricultural	Livestock	Livestock sodic
Clay [< 2 µm] (g kg^{-1})	310	355	290
Silt [2 - 50 µm] (g kg^{-1})	590	565	565
Sand [>50 µm] (g kg^{-1})	100	80	145
BD (g cm^{-3})	1.0	1.1	1.1
Tot. Por. (%)	58	51	50
θ_{FC} -0.33 atm- (%)	32.5	35.4	33.7
SSA (m^2 g^{-1})	182.5	153.0	132.4
OC (%)	3.4	3.1	2.0
pH -1: 2.5 in H_20-	5.4	6	7.9
EC (dS m^{-1})	1.90	0.60	1.60
CEC (cmol kg^{-1})	18.2	16.6	16.5
ESP (%)	0.7	2.4	16.9

BD = Bulk density, Tot. Por. = total porosity, θ_{FC} = water content at field capacity, SSA = specific surface area, OC = organic carbon, EC = electric conductivity, CEC = cation exchange capacity, ESP = exchangeable sodium percentage.

Table 2. Hydrological results obtained after applying 1 h of simulated rainfall (intensity 60 mm h^{-1}) on the three sites in the Rolling Pampa.

Site	Runoff (%)	FIR (mm h^{-1})	Sediment yield (g)	Sediment concentration (g l^{-1})
Agricultural	45.6 (2.7)^a	25.4 (2.4)^a	6.7 (1.2)^a	4.9 (0.4)^a
Livestock	33.8 (0.4)^b	26.2 (4.0)^a	4.0 (1.9)^a	3.3 (1.5)^a
Livestock sodic	84.2 (2.4)^c	0.96 (0.1)^b	21.8 (2.4)^b	7.4 (1.1)^a

Means followed by same letter do not differ significantly (p > 0.05). Means followed by same letter do not differ significantly (p > 0.05). Values in parentheses are standard errors. FIR = final infiltration rate.

sites (Table 2). In contrast, the values of sediment concentration did not differ significantly ($p > 0.05$) between the three sites.

Characterization of the runoff

Bacterial adsorption and K_d obtained at the beginning and at the end of the rain simulation showed a slight decrease, although not significant ($p > 0.05$) (Table 3). Therefore, average values of bacterial adsorption and K_d were taken to describe bacterial retention (BR). The adsorption values calculated in the field for “Agricultural” and “Livestock” were similar and their mean (including the intermediate measurement) were 73.4 and 70.2%, respectively, whereas those for “Livestock sodic” were much lower, with an average adsorption (including one intermediate measurement) of 35.9%. The K_d values for “Agricultural”, “Livestock” and “Livestock sodic” were 4.82, 12.4 and 1.66 ml g^{-1}, respectively. Both the EC and the sediment concentration in the runoff also decreased towards the end of the experiment ($p < 0.05$). The pH in the runoff of the “Agricultural” site did not vary, but it increased slightly in both livestock sites, being the value in “Livestock sodic” similar to that of the topsoil ($p < 0.1$).

As shown in Figure 2, the aggregate size distribution followed almost the same trend in all three sites analyzed. The main size fraction for all simulations was fine silt (20 to 3 µm) in accordance with the silty clay loam texture of the soils. Given that no chemical dispersants were used in this study, this fraction might be composed both of elementary particles and microaggregates. In contrast, there were relatively few particles or aggregates larger than 50 µm. With regard to biological entities, the bacterial concentration associated with the soil fraction smaller than 2 µm was maximal for the three sites analyzed. Within the categories larger than 2 µm, maximum bacterial concentration was associated with a particle size of 20 to 3 and 3 to 2 µm for the “Agricultural” site and with 3 to 2 µm for “Livestock sodic”, while for the “Livestock” site, the maximum value corresponded to the fraction > 50 µm (Figure 2).

Bacterial adsorption and K_d in the soils and in sediments

Bacterial adsorption in soils yielded average values of70.8, 64.5 and 39.7% (Figure 3A) for “Agricultural”, “Livestock” and “Livestock sodic” sites, respectively,

Table 3. Biological, physical and chemical variables at the beginning (first 15 min of the rain fall simulation) and at the end of runoff (last five min) for the three sites in the Rolling Pampa.

Site	Time of sampling	Biological variables		Physical and chemical properties		
		Adsorption (%)	Kd (ml g^{-1})	pH	EC(dS m^{-1})	Sediment concentration (mg l^{-1})
Agricultural	beginning	73.9 (4.4)	4.96 (0.83)	6.6 (0.03)	0.110 (0.025)	5.9 (0.9)
	end	72.8 (6.2)	4.62 (1.00)	6.6 (0.02)	0.024 (0.0006)	5.3 (1.3)
Livestock	beginning	80.4 (8.1)	20.08 (4.50)	6.4 (0.04)	0.068 (0.008)	5.0 (1.1)
	end	77.7 (8.4)	14.71 (3.90)	6.6 (0.02)	0.026 (0.001)	1.3 (2.6)
Livestock sodic	beginning	36.5 (3.9)	1.61 (0.08)	7.6 (0.04)	0.035 (0.004)	9.4 (1.9)
	end	29.3 (5.9)	1.51 (0.15)	7.9 (0.02)	0.019 (0.002)	5.9 (1.8)

K_d = distribution coefficient, EC = electrical conductivity. Values in parentheses are standard errors.

which were very similar to those obtained in the sediments (p = 0.9). As regards K_d, we found no significant differences (p = 0.19) between the values obtained in soils and in sediments (Figure 3B), although a greater dispersal of the results was found, the values being 2.9, 5.3 and 1.2 ml g^{-1} soil for the "Agricultural", "Livestock" and "Livestock sodic" sites, respectively.

Bacterial retention

This property, calculated from the rainfall simulation tests in the field, resulted in an average of 81 and 75% for the "Agricultural" and "Livestock" sites, respectively, showing a tendency towards differentiation from the value found for "Livestock sodic" (p < 0.10), which reached 52% (Figure 3C). Both bacterial adsorption and K_d measured in soils explained about 50% of the variability in bacterial retention (BR) observed in the three soils (Table 4). The same properties measured in sediments showed lower coefficients of determination. However, there were no differences between the intercept and slope of the linear equation estimated from sediment observations and those estimated from soils for bacterial adsorption (F – int = 1.07 P = 0.31, F – slope = 1.23, P = 0.28) and K_d (F – int = 0.93 P = 0.35, F–slope = 2.9 P = 0.11). For this reason, Figure 4 shows the regressions obtained from the sediments and soil data grouped together. With regard to soil properties (Table 4), we found that the pH, ESP, OC content, and SSA and sand content (to a lesser extent) were the variables that best explained BR (p < 0.05). Finally, the relationship between hydrological variables and BR was poor and showed no significant effect.

DISCUSSION

Properties of the system analyzed

According to the results, the "Livestock sodic" site seems to have the greatest capacity for physical, chemical and biological contamination of the neighboring watercourses. This is due to its vulnerability to water erosion that presented the higher runoff and sediment generation, together to its location adjacent to the main watercourse of the region. In addition, it is important to highlight that the "Livestock" site is also a hydromorphic soil with natric origin, although with adequate soil properties in the surface horizon. Both the FIR and sediment yield of this site responded similarly to those of the "Agricultural" site (which corresponded to a well-drained Mollisol soil). These results are associated with the adequate content of humidified organic matter in both soils, but especially with the presence of traces of stems and roots in the "Livestock" site. It is noteworthy that a slight difference was found in sediment concentration between the three sites analyzed. The adsorption percentage and K_d are variables sensitive to the concentration of solids in the liquid. Thus, the slight difference found between these sites allows a correct interpretation of these biological variables according to the intrinsic characteristics of the soil.Bacterial adsorption and K_d measured both in soils and sediments generally showed similar values. Taking into account the significant differences involved in the two methodologies used in this study, these

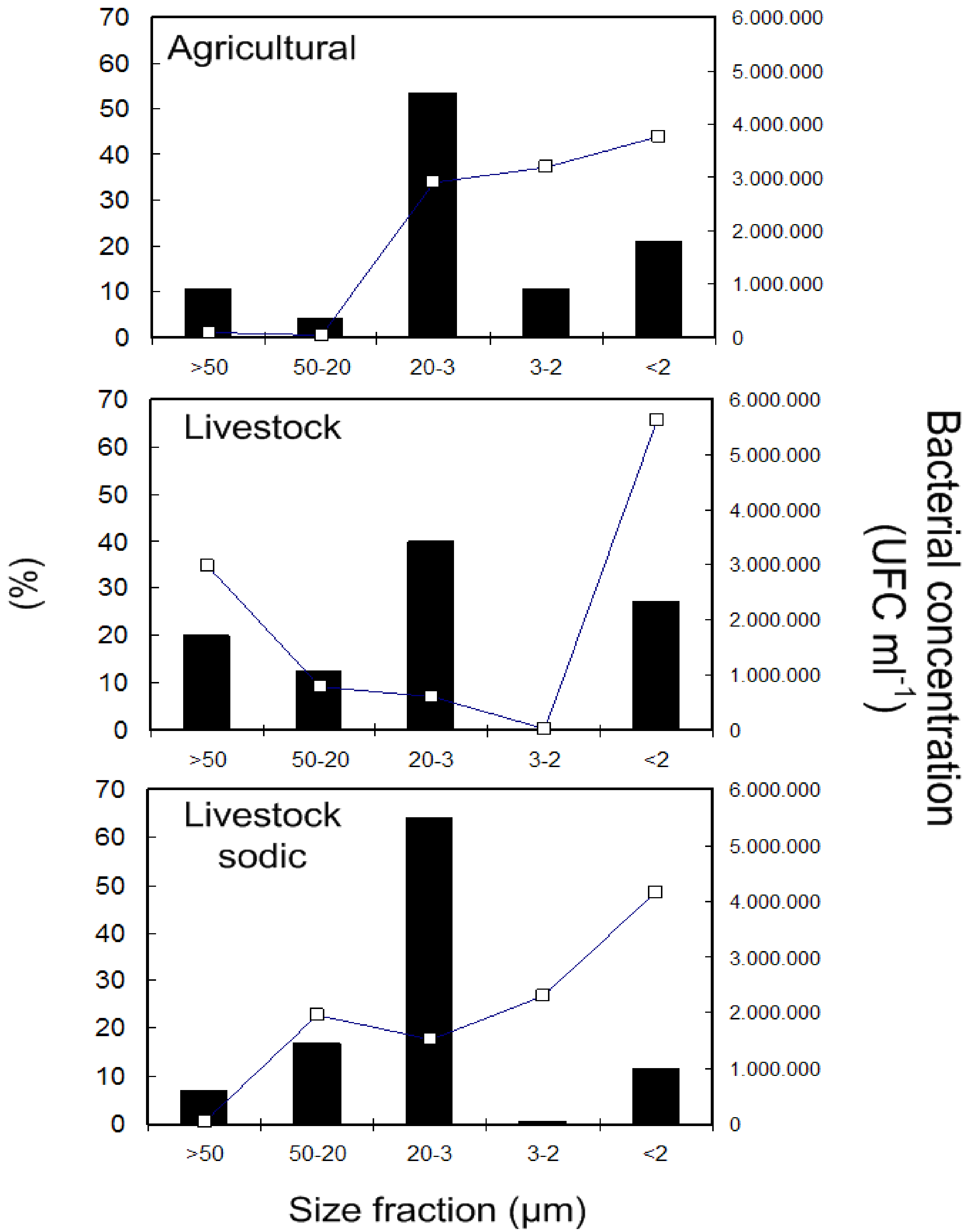

Figure 2. Aggregate size distribution (%) (black bars) and bacterial concentration (CFU ml^{-1}) (squares) for each soil size fraction (µm) for the three sites evaluated.

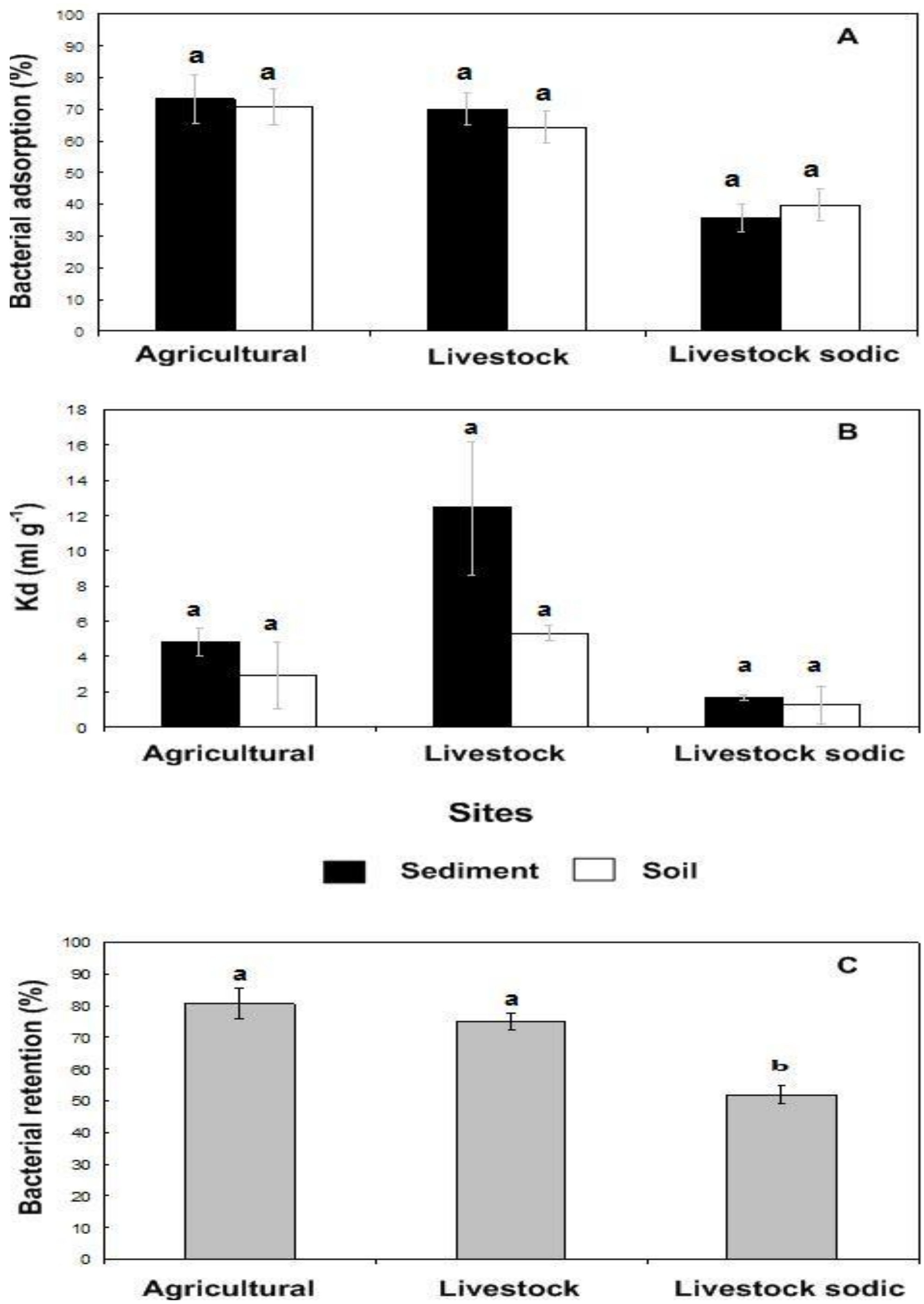

Figure 3. Bacterial adsorption (panel A) and distribution coefficient (K_d; panel B) obtained in sediments (black columns) and in soils (white columns). Bacterial retention obtained in the field (panel C). Means followed by same letter do not differ significantly ($p > 0.05$). Vertical bars indicate standard error.

results are remarkable. A possible explanation for this is that the adsorption time of 5 min used in both sediment and soils measurements the field experiments may have been sufficient to achieve bacterial adsorption and that the large volume of runoff, together with the subsequent resting period in cold (12 h) used in the field experiment (sediment), may not have significantly altered that association. In addition, those parameters measured in the sediments did not presented significant variations at the beginning and at the end of the simulation test. This

Table 4. Linear regressions between bacterial retention after 1 h rainfall simulation with an intensity of 60 mm h^{-1} and biological, soil and hydrological variables at three sites in the Rolling Pampa (n = 9 plots) and the bacterial adsorption and Kd obtained in soil.

Bacterial retention	R^2	Slope	Intercept	P value†
Biological variable				
Adsorption (sediment)	0.43	0.87	-7.48	0.019
Adsorption (soil)	0.56	0.60	16.90	0.013
K_d (sediment)	0.27	0.20	-7.41	0.200
K_d (soil)	0.44	0.07	-1.44	0.037
Soil variable				
Sand	0.43	-1.11	182.90	0.041
Silt	0.20	0.32	550.10	0.195
Clay	0.20	0.79	266.90	0.192
Tot. Por.	0.27	0.11	45.00	0.126
θ_{FC}	<0.01	-0.002	34.12	0.957
SSA	0.44	0.81	99.00	0.036
CEC	0.23	0.02	15.50	0.160
ESP	0.55	-0.33	29.03	0.013
pH	0.56	-0.05	9.71	0.013
EC	0.01	-3.83	1569.00	0.770
OC	0.56	0.03	0.91	0.013
Hydrological variable				
Runoff	0.16	-0.57	81.80	0.248
FIR	0.27	0.75	57.70	0.120

† $p < 0.05$ in bold. Tot. Por. (total porosity, θ_{FC} = water content at field capacity, SSA = specific surface area, CEC = cation exchange capacity, ESP = exchangeable sodium percentage, EC = electric conductivity, OC = organic carbon, FIR = final infiltration rate.

evidences that both properties would be relatively stable during the high-intensity rainfall. The K_d and adsorption values obtained in the soils and in the sediments were comparable to those found by several authors. For example, Guber et al. (2005) found K_d values of 9.4, 46.3 and 513.2 ml g^{-1} for two silty soils and one silty clay loam soil, respectively, while Ling et al. (2002) obtained values of 0.33 g ml^{-1} in loamy soils and 127 ml g^{-1} in clay loam soils. Therefore, the values obtained are intermediate to those reported by these authors. In a previous study (Kraemer, 2010), we found values very similar to those obtained here, confirming the repeatability of the information obtained for these soils. Regarding adsorption, Ling et al. (2002) measured more than 90% of this property in all the treatments analyzed.

Oliver et al. (2007) working in loamy soils found values of 35% of adsorption with centrifugation techniques, Characklis et al. (2005) found between 20 and 35% of association of *E. coli* with sedimentable particles. These data show the disparity of values reported for the property analyzed. Local data were also intermediate to those reported by the above-mentioned authors. Regarding the size of the sediments, silt and clay (< 50 µm) dominated in the runoff. This fact was expected taking into account the impact of the droplets with high kinetic energy and the limited transport capacity that characterizes the sheet erosion (Nearing et al., 1990). In this regard, it is highly probable that the low proportion of aggregates > 50 µm observed was because they were disaggregated to smaller sizes and/or were not removed from the plot. Since most of the microorganisms tested were free (in the < 2 µm fraction) or associated with small particles (50 to 2 µm) (Figure 3), they may have a movement analogous to that of runoff. Muirhead et al. (2006) have reported that the technique to control water pollution known as “filter strips” is effective in retaining sediments greater than 500 µm and that the sedimentation during runoff is affected in particles larger than 63 µm. For these reasons, the implementation of these strips would be of little use in sectors of the Rolling Pampa having short slopes with scarce gradient in which laminar erosion would be dominant over other types of erosion.

It is important to point out the differences found in the bacterial concentration for the different aggregate sizes larger than 2 µm in the three sites analyzed. Like that found for the “Agricultural” site, Oliver et al. (2007) found high bacterial concentration in the 20 to 3 µm fraction, whereas in studies and in the laboratory with the same soil they found a higher bacterial concentration in the 50 to 20 µm fraction, the second in significance being the 20 to 3 µm fraction (Kraemer, 2010). In contrast, Borst and Selvakumar (2003) found no relationship between fecal coliforms, fecal streptococci and particle size. These results highlight the need to explore the heterogeneity in the composition and properties of different sediments to interpret their association with the biological fraction.

Bacterial retention and its relation with the biological, physical and chemical properties of the soil

The significant differences found in bacterial retention in the three soils studied seem to indicate a possible differential regulation by those sites with respect to the dynamics of bacterial transport. Among the properties considered, the biological ones and some soil properties explained a great part of this dynamics. With regard to the former, and as reported by Jamieson et al. (2004) and Marshall (1980), bacterial adsorption was of fundamental importance in the transport dynamics of these organisms. Although we found a similar correlation between the results obtained in soils and sediments, the lowest coefficients of determination and significance in the properties measured in sediments (Table 4) may reflect a lower control of the experimental conditions in this kind of evaluation. It should be noted that applying the laboratory method (Ling et al., 2002), the soil/water relation was 1/1, while in the sediment measurement the relation was lower and also relatively variable as already mentioned in a previous point. It is possible that under laboratory conditions the environment of adsorption was more accurately reproduced and is therefore possible to consider that these parameters are more sensitive to describe the bacterial movement.

The effects of the physical and chemical properties of the soil on the bacterial retention were due to the configuration and modification of the mechanisms of adsorption as well as to their influence on the hydrological dynamics. In this sense, pH, OC, ESP and content of sand would act as key variables in the modification of the bacterial adsorption environment and as determinant factors of the hydrological results of the simulations. This is in agreement with the results by Marshall (1980), who reported that the environment of bacterial adsorption is affected mainly by pH and the presence of organic and inorganic compounds which alter the surface loads. Several authors point out pH as an important variable that modifies the load balance of the clays, increasing or decreasing the adsorption (Stotzky, 1985). In this study, the relation between bacterial retention and pH showed a negative slope. In agreement, Scholl and Harvey (1992) found that a large number of colonies of *Arthrobacter sp.* associate to quartz at pH 5.0 and then decrease at 7.5. Kinoshita et al. (1993) observed a decrease in the adsorption of *P. fluorescens* to small silicon balls as a result of increasing the solution pH from 5.5 to 7. The behavior of pH is partly associated with ESP, a property that determines the dispersal of the aggregates by increasing their negative charges, thus decreasing the adsorption phenomena. Besides, this dispersal causes negative effects on the infiltration since it decreases the structural stability of aggregates as this favors the collapse and subsequent occlusion of macropores. On the other hand, the adjusted linear regression between bacterial retention and OC presented a positive slope. Although organic compounds are often identified as important in the adsorption phenomena, there is little information supporting this claim. According to Labelle and Gerba (1979), the aggregation of particles with organic matter or clay minerals plays an important role both in bacterial transport and bacterial sedimentation and survival. In this experiment, the effect of OC would not be explained only by this variable, but also by the indirect effects of this component on the hydrological dynamics of simulations. With regard to the physical properties tested, the sand content showed a negative slope explained by the low interaction of this fraction with water (transport vehicle of microorganisms) and microorganisms. Also the SSA explained 44% of the variation in bacterial retention, probably by its effect on bacterial adsorption. This can be attributed to a difference in the areas available for adsorption (Oliver et al., 2007; Stotzky, 1985).

In contrast to that expected, the hydrological variables were not significantly associated with bacterial retention. The even distribution of the inoculum in all plots on dry, removed soil with lack of vegetation cover may have led the soil properties to be the ones governing the bacterial dynamics at the expense of the hydrological parameters. In this context, the intrinsic properties of the soil, together with their moisture and structure, greatly affected the determination of bacterial adsorption and thus regulated the subsequent bacterial movement. In experiments with gradual bacterial release, associated with previously wet soil, bacteria may have less contact time with the soil and this interaction would in turn occur in a more watery medium, thus preventing the mechanisms of adsorption from acting as significantly as they did in this experiment.

Conclusion

Bacterial retention, which was significantly lower in the “Livestock sodic” site than in the “Agricultural” and “Livestock” sites, was adequately explained by the biological variables (adsorption and K_d), especially those measured in the soils. However, even when taking into consideration the large methodological differences between the soil and sediment measurements, we found no significant differences between them. Therefore, this work suggests the possibility of transferring the values of these biological variables measured in soil to the field. On the

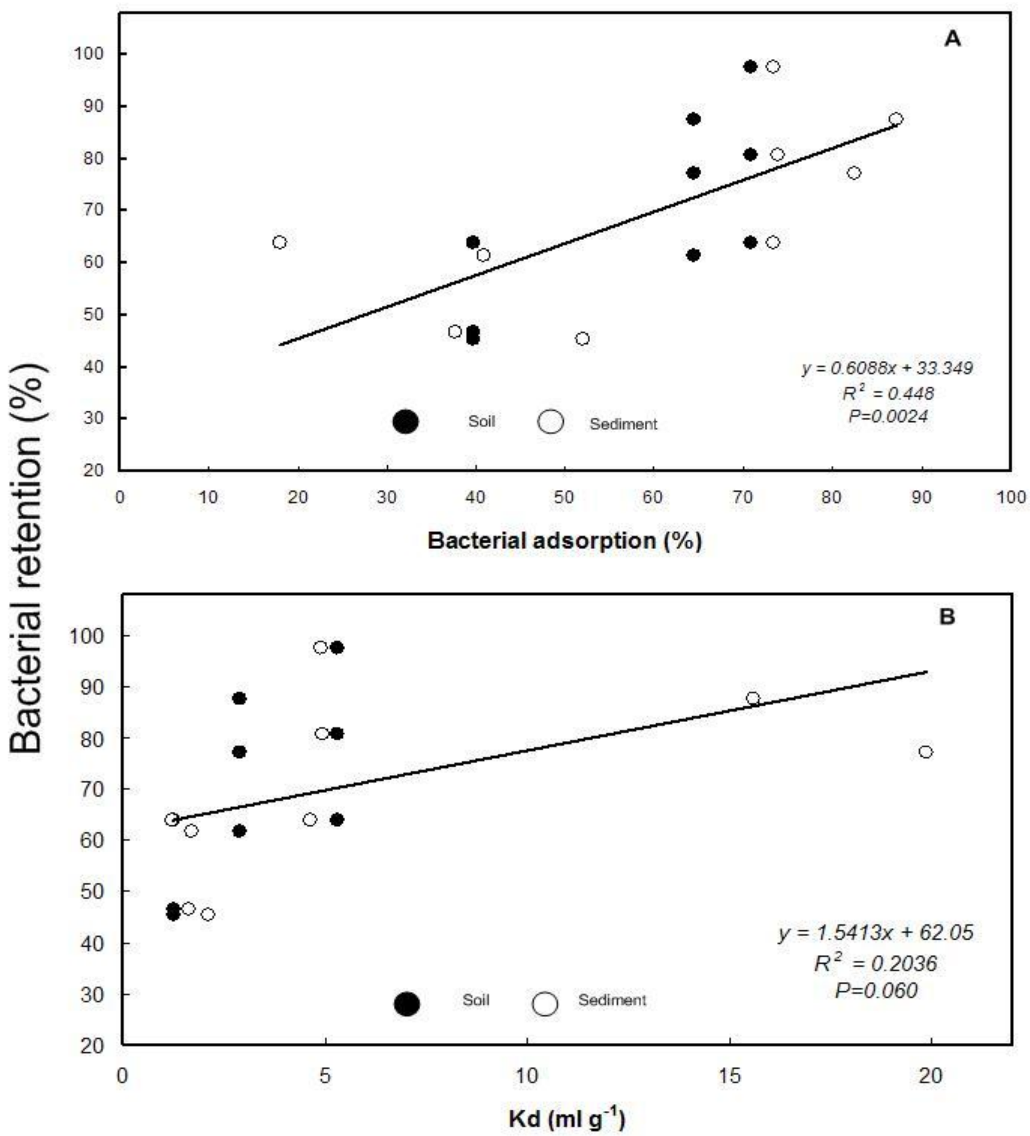

Figure 4. Bacterial retention related to the bacterial adsorption (panel A) and bacterial K_d (panel B) measured in soil (filled circles) and in sediments after rainfall simulations (empty circles).

other hand, the soil variables, particularly pH, ESP and OC proved to be the important properties in the process of bacterial retention. In analyzing the distribution of microorganisms in the runoff caused by the simulated rain, we observed that high bacterial concentration was associated to aggregates < 2 μm or small aggregates (50 to 2 μm), which have a movement analogous to that of runoff. This suggests that management practices used in other parts of the world to control biological contamination of water courses, such as filter strips, should be regarded with caution if to be implemented at the local level.

At local level, the high values of runoff and sediment production observed in the “Livestock sodic” site, together with its low bacterial adsorption capacity, lead to a significant risk of pollution for nearby watercourses, as indicated by the low value of bacterial retention found in this site. This situation is aggravated due to the proximity of these soils to the main watercourses in the region. In contrast, the adequate infiltration rate found in the “Agricultural” and “Livestock” sites, together with their high bacterial adsorption value and distance to watercourses, would conclude that, with rains of moderate intensity, the biological pollutants associated to these soils would take longer time to reach watercourses, and their total amount would be significantly lower than that of “Livestock sodic” site.

ACKNOWLEDGEMENTS

This work was supported by the Project UBACYT G045.

We thank Dr. Alfonso Buján and Dr. Eva Pawlac from the CNEA (Comisión Nacional de Energía Atómica) for their contribution in the sterilization of soil samples.

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