

Water quality in a lagoon in the southeast pampa region of Argentina

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ABSTRACT

This work evaluates the chemical and bacteriological qualities of the recreational waters of the Sauce Grande lagoon (Argentina). Samples were taken between March 2002 and December 2003. Ninety-six samples from three sampling stations were analyzed in order to determine the density of aerobic heterotrophic microorganisms, the presence of sulphite-reducing clostridia, and the most probable number of total coliforms, *E. coli*, fecal enterococci and *P. aeruginosa*. The water pH, temperature and chemical composition (N-NO₃⁻, PO₄³⁻, Na⁺, Ca⁺⁺ +Mg⁺⁺, EC and SAR) were also determined. Statistical analysis shows an increase in the microbial parameters of fecal pollution and in the population of heterotrophic microorganisms during the warmest months, influenced by higher temperatures and the more intensive recreational use. Bacterial count indicated that fecal pollution was statistically lower at the recreational area monitoring station; however, *P. aeruginosa*, an opportunistic pathogen, was present in higher than permitted densities in all determinations. These results show that, from the physico-chemical point of view, anthropogenic activities do not significantly affect the quality of the resource.

Key words: coliforms, enterococci, *Escherichia coli*, indicator microorganisms, *Pseudomonas aeruginosa*, water quality.

RESUMEN

Calidad del agua en una laguna del sudeste pampeano (Argentina). En el presente trabajo se evaluó la calidad bacteriológica y química en aguas de la laguna Sauce Grande (Argentina). Los muestreos fueron realizados entre marzo de 2002 y diciembre de 2003. Se analizaron un total de 96 muestras provenientes de tres estaciones de monitoreo, determinando: densidad de microorganismos heterótrofos mesófilos, presencia de clostridios sulfito-reductores y número más probable de coliformes totales, *Escherichia coli*, enterococos fecales y *Pseudomonas aeruginosa*. También se efectuaron determinaciones de pH, temperatura del agua y composición química (N-NO₃⁻, PO₄³⁻, Na⁺, Ca⁺⁺ + Mg⁺⁺, CE y RAS). Se observó que en los meses más cálidos se produjo un aumento en los parámetros microbianos indicadores de contaminación fecal y en la población de microorganismos heterótrofos; dicho comportamiento estaría influenciado por el aumento de la temperatura y el mayor uso recreativo del recurso. El recuento de bacterias indicadoras de contaminación fecal fue menor en la estación de monitoreo donde se encuentra ubicado el balneario; no obstante, *P. aeruginosa*, patógeno oportunista, estuvo presente en todas las determinaciones con densidades mayores a los valores permitidos. Desde el punto de vista fisicoquímico, no hay un aporte antropogénico significativo de contaminantes que afecten la calidad del recurso.

Palabras clave: coliformes, enterococos, *Escherichia coli*, microorganismos indicadores, *Pseudomonas aeruginosa*, calidad de agua.

INTRODUCTION

Pollution of streams, rivers, and reservoirs occurs when undesirable microorganisms and/or chemical compounds become incorporated in the water, altering and destabilizing the system. Such pollutants may come from very different sources. However, water quality is defined not in absolute terms but rather in relation to the use that is made of the resource (drinking, watering, domestic recreational, etc.). A simple way of assessing water quality is to measure certain physical, chemical and bacteriological parameters that quantify the risk of disease (16).

Most of the microbial diseases associated with water are produced by pathogens such as viruses, bacteria and protozoa, which propagate by oral-fecal means, developing in the human intestine and subsequently becoming incorporated in the water via stools (3). Some agents of disease such as *Pseudomonas aeruginosa*, whose natural habitat is soil and fresh water, act as opportunistic pathogens (25).

Though indicator microorganisms are not pathogens themselves, their relation to the intestinal tract means that their presence in water is indicative of fecal pollution (13). Their counts vary according to the temporal and spatial

proximity of the sources of pollution and also according to the prevailing environmental conditions, since these can modify the survival rate of microorganisms in the waters (15).

The aim of the current work is to assess the chemical and bacteriological quality of water in the Sauce Grande lagoon, determining physico-chemical parameters and the following bacteria population: aerobic heterotrophic bacteria, total coliforms, *Escherichia coli*, *Pseudomonas aeruginosa*, fecal enterococci and sulphite-reducing bacteria. Variations in water quality over the year and the relationship with environmental variables are also analyzed.

MATERIALS AND METHODS

Description of the study area

The Sauce Grande lagoon (39° 50'S, 61° 24' O) is located in the southeast of the Province of Buenos Aires, Argentina (Figure 1). The original bed extends over an area of 22.9 km², with a volume of 23.7 hm³ and an average depth of 1.1m (11). Average annual temperature is 15 °C, June being the coldest month (7.7 °C) and February the warmest (21.7 °C). Average annual precipitation is 656.8 mm.

Among the many recreational activities carried out at the lagoon, one of the main attractions is mackerel fishing for sport.

Sampling

Three sampling stations were established: S1 in the Sauce Grande River before it flows into the lagoon; S2 in the lagoon, at a distance of 15 km from S1; and S3 in the river, before it flows into the sea, at a distance of 2 km from S2.

Eight samplings were taken between March 2002 and December 2003. Four samples were taken at a depth of 25 cm at each station. All the samples were collected in 250 ml-bottles, previously washed and sterilized, and transported to the laboratory in an iced cooler.

Plate count

The technique of decimal dilutions was employed. One ml of each dilution was inoculated in plate count agar (Oxoid L11,

Hampshire, England) following the poured agar technique. Four plates per dilution were used, two incubated at 37 °C for a period of 24 hours, and two at 22-25 °C for 48 hours.

Fecal bacteria and *Pseudomonas aeruginosa* count

The most probable number technique, using a combination of 3 - 3 - 3 tubes, was used for total coliform, *Escherichia coli*, fecal enterococci and *Pseudomonas aeruginosa* enumeration.

The Mac Conkey broth (Oxoid CM5, Hampshire, England) was used for total coliform, after incubation at 37 °C for 24-48 hours. The same medium was used for *Escherichia coli*, after incubation for 48 hours in a thermostated bath at 44-45 °C. Based on the number of positive tubes, and after confirmation with IMVIC, the MPN of *Escherichia coli* in 100 ml of water was calculated (24).

Glucose Azide broth (Merck 1590, Darmstadt, Germany) was used for fecal enterococci in the presumptive test. The Bromocresol Azide Purple broth (Merck 3032, Darmstadt, Germany) was used in the confirmation test. In both stages, incubation was at 37 °C for 48 hours. Asparagine broth (Mallinckrodt 9144, Los Angeles, U.S.A) was used for *Pseudomonas aeruginosa* in the presumptive phase, after incubation at 37 °C for 48 hours. The results were confirmed in Acetamide broth (Fluka 75434, Buchs, Switzerland) after incubation for 36 hours at 37 °C (4).

The quality standards for recreational waters were taken as those established by the U. S. Environmental Protection Agency (USEPA) of 126 *E. coli*/100 ml, and 33 enterococci/100 ml (7, 22, 23).

Presence-absence of sulphite-reducing clostridia

Three aliquots of 1 ml from each sample of water were sown in 10 ml of differential broth for clostridia DRCM (Merck 11699, Darmstadt, Germany), and incubated for 7-8 days at 30 °C under anaerobic conditions, using a Gas-Pak flask. (15).

Physical and chemical parameters

The water temperature was taken *in situ* by placing a thermometer at a depth of 10 cm. The pH was measured in the laboratory using a Broadley-James pHmeter.

A DR 2010 HACH spectrophotometer was used to measure N-NO₃⁻ and PO₄³⁻. The titolometer method of EDTA 3500 Ca.D was followed in the case of Ca⁺⁺, and the method of calculus 3500Mg.E was followed for Mg⁺⁺ (4).



Figure 1. Map of the study area (the Sauce Grande lagoon) showing location of sampling stations.

Electrical conductivity (EC) was measured with an Altronix conductivity meter. The sodium adsorption ratio (SAR) was calculated according to Allison *et al.* (1). All determinations were carried out in duplicate.

Statistical analysis

The two-factor analysis of variance was used to determine the influence of incubation temperature and sampling date on the count of heterotrophic microorganisms, and the one-factor analysis of variance to evaluate the effect of the sampling site and the sampling date on the density of indicator microorganisms and *P. aeruginosa*. The means of the different treatments were compared by applying the minimum significant difference (MSD, $p < 0.05$), with prior logarithmic transformation of the data (\log_{10}) to improve homocedasticity.

Simple linear regressions were also carried out between the different groups of bacteria and the environmental variable, water temperature (19).

RESULTS

A total of 96 water samples were collected in the area of the Sauce Grande lagoon between March 2002 and December 2003. Table 1 shows the physical and chemical values registered during the samplings.

In all the samplings pH values were higher than 8. Electrical conductivity (EC) oscillated between 0.64 dS/m and 2.77 dS/m at S1 and S3, respectively. EC values increased from S1 to S3, S3 being the downriver sampling station (closest to the sea).

Sodium adsorption ratio (SAR) fluctuated between 3.37, coinciding with periods of greater rainfall, and 24.6,

coinciding with periods of reduced rainfall, both characteristic of the Buenos Aires Province semi-arid area.

N-nitrate reached its maximum value in October 2002 at S3 (4 ppm). Phosphate levels showed their highest values in the samplings of March and May 2002.

Population of aerobic heterotrophic microorganisms

It was found that the number of viable heterotrophic bacteria was greater when the samples were incubated at 22-25 °C ($p < 0.05$). The results obtained with two-factor ANOVA are shown in Table 2.

Counts showed maximum values in March and December and minimum in July and August, with the exception of S2, with a minimum in March 2003 at 22-25 °C.

Microorganism indicators of fecal contamination and *Pseudomonas aeruginosa*

Total coliforms count remained high throughout the entire study period, with higher average densities at S1, followed by S3; S2 had the lowest average density ($p < 0.05$).

Figure 2 shows that the lowest mean concentration of coliform bacteria occurred in October 2002 at S2 (with a mean of 45.5 MPN/100 ml). The highest was in December 2003 at S3 (7800 MPN/100 ml on average).

Escherichia coli was detected throughout the study area, with a mean density ranging from 2.55 MPN/100 ml in May 2002 at S3, to 6250 MPN/100 ml in October 2003

Table 1. Values of physical and chemical parameters obtained in different samplings.

Date	Station	T °C	pH	EC dS/m	PO ₄ ³⁻ ppm	N-NO ₃ ⁻ ppm	Ca+Mg me/l	Na me/l	SAR
Mar-02	S1	18	8.3	1.27	3.07	0.50	12.63	8.67	3.37
	S2	20	8.7	2.16	3.07	1.07	21.53	14.70	4.40
	S3	17	8.3	2.77	4.03	0.47	27.57	18.13	4.83
May-02	S1	14	8.2	1.55	3.07	0.53	4.03	15.47	10.87
	S2	13	8.7	2.05	4.00	1.03	3.80	20.53	14.90
	S3	13	8.2	2.29	3.60	1.43	4.57	22.80	15.07
Oct-02	S1	19	8.0	1.61	1.50	2.70	5.22	20.93	12.93
	S2	23	8.5	2.26	2.30	3.63	5.63	32.47	19.33
	S3	19.5	8.3	2.43	2.77	4.00	5.89	34.97	20.37
Mar-03	S1	19	8.6	1.51	1.60	1.70	5.77	41.85	24.60
	S2	19.5	8.5	1.10	3.45	1.20	3.65	28.25	21.00
	S3	20.4	8.4	1.31	3.20	1.45	4.97	34.90	22.15
Aug-03	S1	10	8.2	1.64	0.67	0.63	5.88	25.63	14.93
	S2	9.5	8.3	1.67	0.30	0.57	5.64	25.27	15.07
	S3	10	8.2	1.84	0.47	0.37	6.57	30.57	16.87
Oct-03	S1	20	8.1	0.64	1.50	0.40	3.54	4.70	3.55
	S2	19	8.5	1.79	1.55	0.75	5.01	15.00	9.50
	S3	22	8.3	1.96	2.05	0.55	5.50	16.15	9.75
Dec-03	S1	18	8.1	1.51	1.40	1.20	5.47	19.27	11.67
	S2	20	8.4	1.85	2.63	0.30	5.32	24.33	14.93
	S3	19.5	8.3	1.99	2.33	0.13	5.67	25.13	14.93

Table 2. Heterotrophic plate count values at two incubation temperatures registered during monitoring.

Station	Incubation Temp.	Mar-02	May-02	Jul-02	Oct-02	Mar-03	Aug-03	Oct-03	Dec-03	Mean
S1	37 °C	3.30 ⁽¹⁾	2.51	2.17	2.51	3.41	2.78	2.94	3.24	2.86 a
	22-25 °C	3.68	3.16	3.41	3.56	3.79	2.85	3.50	3.78	3.47 b
Mean		3.49 a ⁽²⁾	2.84 bc	2.79 b	3.03 cd	3.60 a	2.82 bc	3.22 d	3.51 a	–
S2	37 °C	3.23	2.99	2.47	2.68	2.83	2.95	2.97	3.21	2.92 a
	22-25 °C	3.47	3.35	3.20	3.24	3.09	3.09	3.43	3.49	3.29 b
Mean		3.35 a	3.17 ab	2.84 c	2.96 c	2.96 c	3.02 bc	3.20 ab	3.35 a	–
S3	37 °C	3.53	2.92	2.46	3.35	3.28	3.03	2.97	3.48	3.13 a
	22-25 °C	4.25	3.49	3.20	3.75	3.61	3.16	3.37	4.01	3.61 b
Mean		3.89 a	3.21 bc	2.83 d	3.55 e	3.45 ce	3.09 bd	3.17 bc	3.74 ae	–

⁽¹⁾ Results are expressed as log₁₀ UFC/ml; each value is the mean of four repetitions.

⁽²⁾ Values with the same letters in the same row or column do not differ significantly. Values with different letters are significantly different (p<0.05).

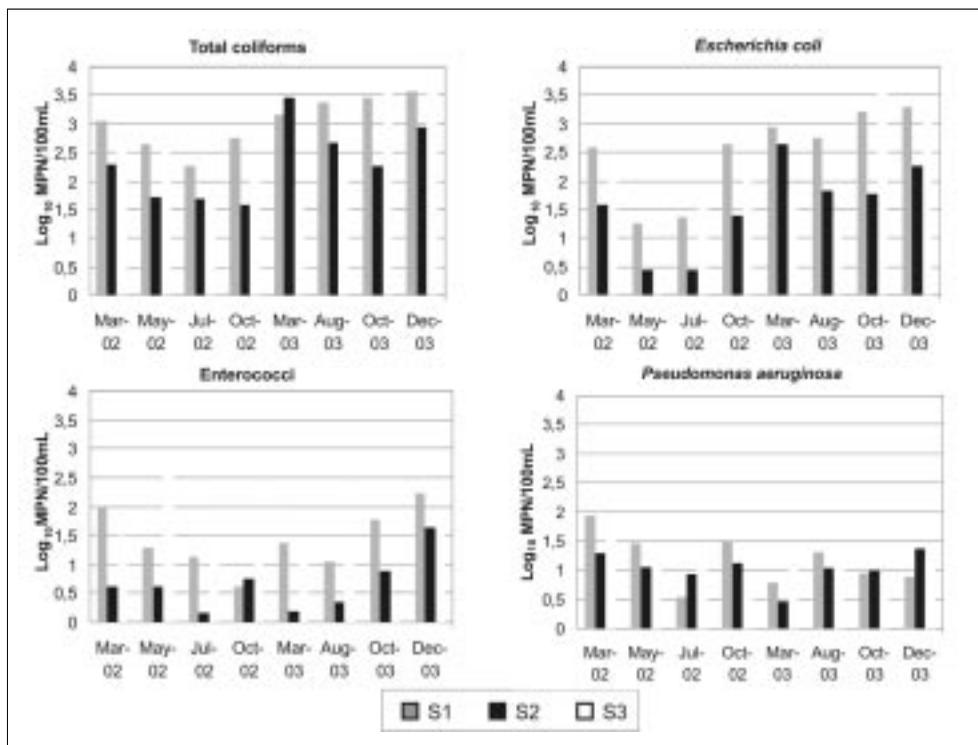


Figure 2. Variation in the concentration of bacteria in the three sampling stations between March 2002 and December 2003 (n=4).

at the same station (Figure 2). The density was higher at S1 and S3 than at S2 (p<0.05). Statistical analysis shows significant differences among sampling dates, with statistically higher values in October and December 2003, and lower in May and July 2002 (p<0.05). Fecal enterococci showed the lowest count in July 2002 at S2 with a mean of 1.5 MPN/100 ml, and the highest count in May 2002 at S3 (405 MPN/100 ml on average) (Figure 2). As in the case of total coliforms and *Escherichia coli*, S2 had a statistically lower density of bacteria than the other two stations (p<0.05).

The count of *Pseudomonas aeruginosa* reached a minimum in March 2003 at S2 and a maximum in March 2002 at S1, with means of 3.07 MPN/100 ml and 94.75 MPN/100 ml, respectively (Figure 2). The number of bacteria in March 2002 was higher than that for the other sampling dates (p<0.05), with no statistically significant differences among stations.

Measuring the values obtained in the present study against the USEPA standards for recreational waters (126 *E. coli*/100 ml, 33 enterococci/100 ml), *Escherichia coli* exceeded standard values at S1 in March and October

2002 and in March, August, October and December 2003; at S2 in March and December 2003 and at S3 in October 2002 and October and December 2003. In the case of enterococci, the count exceeded standard values at S1 in March 2002 and in March, October and December 2003; at S2 in December 2003 and at S3 in May 2002 and in March and December 2003.

The linear regression analysis shows statistically significant positive regressions ($p < 0.05$) among the variables: water temperature - \log_{10} MPN of total coliforms ($R^2 = 0.17$), water temperature - \log_{10} MPN of *E. coli* ($R^2 = 0.48$), water temperature - RHP at 37 °C ($R^2 = 0.31$), water temperature - RHP at 22/25 °C ($R^2 = 0.59$).

Presence-absence of sulphite-reducing clostridia.

Sulphite-reducing clostridia was present in 100% of the samples in July 2002 and March 2003, in 89% of the samples in October 2002 and in 67% of the samples of July and October 2003.

DISCUSSION

An increase in the population of aerobic heterotrophic microorganisms was registered in March and December, related to the rise in water temperature. Similar results were obtained by Fernández-Alvarez *et al.* (10). However, unlike that paper, the current study shows statistically significant differences in differential growth at 22-25 °C / 37 °C, which could be indicating an adaptation of the microbial flora in the Sauce Grande lagoon, since the water temperature never rises above 23 °C.

Although the amount of heterotrophic bacteria does not determine in itself the bacteriological quality of recreational waters, it is an additional parameter for assaying it and its variation over time (8).

It has been demonstrated that moderate changes in climatic conditions can influence the quality and quantity of hydric resources, indirectly affecting public health, since such changes give rise to alterations in the distribution, growth and survival of some pathogenic microorganisms (12).

The *E. coli* count was lower in May and July, probably as a result of the death of fecal coliforms caused by low temperatures (22); during the warmest months, on the other hand, the density was higher. Although regression analysis shows a statistically significant positive regression between water temperature and density of *E. coli*, the determination coefficient was low (below 50%). Similar results were obtained with heterotrophic bacteria and total coliforms.

The factors which determine the rise of *E. coli* in the warmest months could be: the increase in temperature, the greater number of tourists visiting the area, cattle in the nearby fields and the run-off originated as a vehicle for the input of fecal microorganisms.

Where the recreational site is located, sampling station S2, the density of microorganisms was lower than that at the other two sampling stations. These results coincide with the findings of Drovandi *et al.* (6) for the Carrizal Dam in Argentina, possibly reflecting the fact that the lagoon water is calm, thus favoring a self-cleaning process by means of sedimentation and/or the natural destruction of bacteria. This phenomenon has also been reported by Ruibal Conti (18) for the central area of the San Roque Dam in Argentina.

Though S2 shows a constant recuperation of water quality, the permanent presence of *P. aeruginosa* and the high counts of fecal pollution indicators, especially in December, constitute a potential health hazard to tourists, in particular those belonging to risk groups.

According to studies carried out by the World Health Organization, the indicators that best relate to the incidence of gastrointestinal disease caused by contact with recreational waters are fecal enterococci/streptococci and *E. coli* (2, 17). However, these indicators do not relate to other diseases, such as dermatitis or eye and ear infections caused by *Pseudomonas aeruginosa* (9). In fact, in our research this opportunistic pathogen was detected in all the samples and yet its presence had not relation with the density of fecal enterococci or *E. coli*.

For all this, though fecal indicators are very useful for determining water quality in microbiological terms, routine analyses to determine the quality of water used for recreational purposes should also include the search for *P. aeruginosa*. Falcao *et al.* (8), also consider that carrying out routine *Pseudomonas aeruginosa* counts could lead to a considerable reduction in the risk of skin infections and conjunctivitis.

The Sauce Grande River has a large number of tributaries that flow through fields used for farming and cattle breeding, thereby carrying pollution to the lentic systems (23). Further causes of bacterial pollution in the Sauce Grande lagoon are other anthropic influences and wild animals.

Though farming and cattle breeding may cause bacterial pollution (5), they do not have a negative impact on the physico-chemical aspects of the water in the Sauce Grande lagoon. The level of nitrate and phosphate nutrients found in the water is low, indicating that fertilizers are not a major cause of pollution. The same conclusion is reached by another study carried out in the area (14).

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