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Microbiological Contamination of Araucanian and Nordpatagonic Lakes. Xth Region. Chile

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ABSTRACT. The microbiological distribution in two lakes from the Xth Región of Chile is studied. In the Lake Ranco (Araucanian), the viable counts were high and ranged from 20 to 1050 CFU/ml. The numbers of total coliforms were high, with densities between 9 and 842 CFU/100 ml. The predominant bacterial flora in the water were Enterobacteriaceae (34.0 %). In the sediment the bacterial densities were always higher. Sulphate-reducing bacteria were detected in the sediments. The Lake Yelcho (Nordpatagonic), presented a very high counts in relation with its oligotrophic state. In some places there were fecal coliforms. The predominant bacterial flora in the water samples corresponded to *Flavobacterium-Flexibacter* (26.4 %) and *Pseudomonas* (22,0 %). In the sediment the higher viable counts and total coliforms were obtained in sites adjacent to a salmon farm; the MPN of sulphate-reducing bacteria was 29500 bacteria/100g.

Key Words: Microbiological Contamination, Chile.

RESUMEN. Se estudió la distribución microbiológica en dos lagos de la X región de Chile. En el Lago Ranco (Araucano), los recuentos de bacterias heterotróficas viables fueron altos y fluctuaron entre 20 y 1050 UFC/ml. Los recuentos de coliformes totales fueron altos, con densidades entre 9 y 842 UFC/100 ml. La flora bacteriana predominante en el agua fueron Enterobacteriaceae (34,0 %). En el sedimento siempre fueron mayores las densidades bacterianas. Bacterias reductoras de sulfato se detectaron en todas las muestras analizadas. El Lago Yelcho presentó elevados recuentos bacterianos en relación a su estado oligotrófico. En algunos lugares se encontró coliformes fecales. La flora bacteriana predominante en las muestras de agua correspondió a *Flavobacterium-Flexibacter* (26,4 %) y *Pseudomonas* (22,0 %). En el sedimento, los más altos recuentos de bacterias heterotróficas viables y de coliformes totales se obtuvieron en sitios próximos a salmoniculturas; el NMP de bacterias reductoras de sulfato fue de 29500 bacterias/100 g.

Palabras Clave: Contaminación Microbiológica, Chile.

INTRODUCTION

The most of the lakes in the South of Chile, have traditionally been oligotrophic, because they contain low amounts of nutrients, low primary productivity and minor biological pollution.[7] The steady economic and population growth in this area of the country, particularly in the Xth Region, has produced an increase in the demand for the use of natural resources, a demand that has not been absent in lake ecosystems. A wide variety of organic and inorganic waste products, from riverside industries, agricultural activities and human populations are being dumped into these systems.[18] The organic load from the increasing salmon farming activities must also be added.[1]

Water pollution may be measured using different approaches; some of the best means to evaluate impact are: population dynamics and variations in microbial populations. Changes in the organic matter flows modify bacterial communities within a lake, thus becoming a sensitive bioindicator of pollution processes. The evaluation of continental water quality is important. This is performed by the quantification of the indicator microorganisms such as, coliform bacteria, enterococcus, yeast-like molds and enteroviruses.[9,15,19,21,23,27] The analysis must consider water columns,- which may experience rapid variations, as well as bottom sediments where microorganisms achieve higher survival rates and may establish permanent populations.[4]

There are very few studies regarding microbiological pollution of freshwater environments in Chile.[5,11,17] The most relevant ones in the south- austral regions, are mainly refered to the pollution of the rivers Valdivia[26] and Rahue.[10] On the other hand, the chemical, physical and primary productivity aspects of the lakes in this region have been systematically studied,[6,7] leaving the study of their microbiological pollution aside.

In this study, two lakes of the Xth region have been chosen as models. They represent two distinct realities: one is an Araucanian lake, the Ranco and the other is a North-

patagonic lake, the Yelcho. The former one has experienced an important increase in it's trophic state due to the use of it's water course by agricultural, salmon farming and industrial activities;[6,7] the latter one, is oligotrophic, with minor intervention, scarce surrounding populations and basic agricultural and aquaculture activities.[28] For determining the increase of the bacteriological contamination, indicator bacterial populations in water columns and bottom sediments are quantified and identified in both lakes. Besides, it was tested the presence of *Salmonella* as direct indicator of the occur of pathogens in the water.

MATERIAL AND METHODS

Selection of the stations. In lakes Ranco (40°13'S, 72°23'W) and Yelcho (43°18'S, 72°19'W) five stationary sites were chosen based on four parameters: proximity to villages, affluents and effluents, salmon farm and center of the lake (Fig. 1). Lake Ranco: Station A1, Futrono; A2, Quimán Bay; A3, center of the lake; A4, Llifén; A5, Calcurrupe river. Lake Yelcho: Station B1, Port Piedra; B2; center of the lake; B3, proximity to salmon farm; B4, Port Cárdenas; B5, Yelcho river.

Sample collection. In both lakes, samplings were carried out in December and May (1994-1995). In order to obtain information on vertical distribution, the sampling design considered a range from 0.3 m. to 45 m. For the water column a Zobell (J-Z) bacteriological sampler was used. Samples of bottom sediment were collected using an Eckman dredge. They were all stored in containers at 4°C and transported to the laboratory for their processing.

Physico-chemical parameters. Water transparence was assessed *in situ* using a standard Secchi disk; temperature and pH were measured with portable digital instruments (Cole Parmer). Weight of the sediment was determined on a fraction of the sample after it's drying at 110°C for 24 h.

Bacteriological examination of the water.

Viable aerobic heterotrophic bacteria. It were enumerated by spreading onto Plate Count agar (Difco). The dilutions were made with sterilized filtered lake water (0,2 µm membrane filters). The plates were incubated at 25°C for 72 h. and colony-forming units (CFU) were counted.[11] All determinations were performed in triplicate.

Total Coliforms. It were determined by the membrane filter technique.[2] 10, 50 and 100 ml of each sample were filtered through cellulose nitrate membranes (0.45 µm pore) (Sartorius). The filters were then placed on plates with m-Endo Agar LES (Difco) and were incubated at 37° C for 24 h.

Fecal Coliforms. The same volumes as for total coliforms were used. The samples were filtered and placed on M-FC Agar plates (Difco) and incubated in a water bath at 44.5°C for 24 h. Blue colonies were considered to be presumptive fecal coliforms. For their confirmation, they were

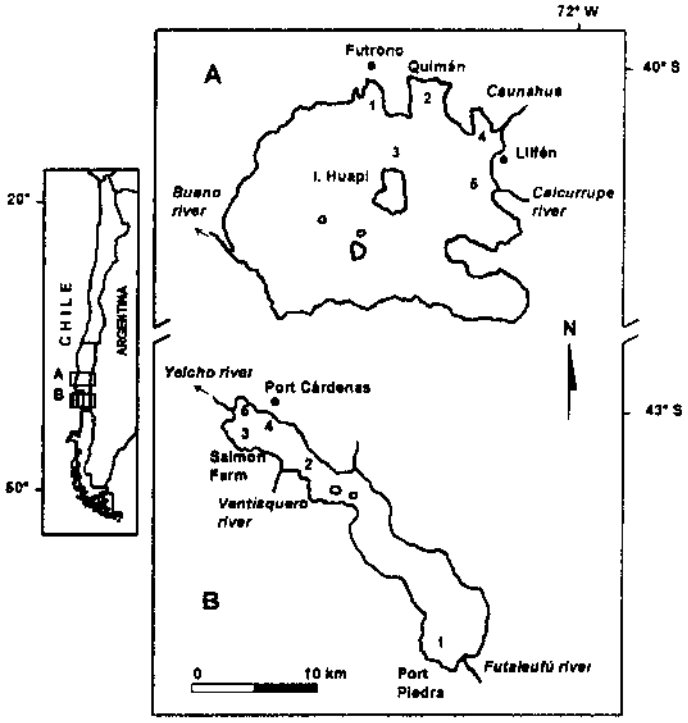

Figure 1. Location of sampling stations. A) Lake Ranco. B) Lake Yelcho.

inoculated in tubes containing EC (Difco) broth with a Durham tube, and were incubated at 44.5°C for 24 h in a water bath.[2]

Sediment analysis. 10 g of sample were homogenized with 90 ml of sterile lake water; after a vigorous shake they were left to stand for 5 min and then, the same protocols as for water tests were used. For the detection of viable heterotrophic aerobic bacteria, total and fecal coliforms, 10, 50 and 100 ml of the sediment supernatant were used.

Sulphate-reducing bacteria. Enumeration was carried out by the Most Probable Number method (MPN), using medium API[20]. The tubes were incubated at 25°C for up to 28 days. Were considered positive the tubes that presented smell to H_2S and exhibited blackening, as result of Fe_2S precipitation.

Detection of Salmonella. 500 ml of the sample were filtered. The filteres were folded and submerged in 100 ml of selenite enrichment broth (MERK) and incubated at 37°C for 18 h. Inoculum were streaked in duplicates on Salmonella-Shigella Agar plates and incubated at 37°C for 24 h. Suspicious colonies were replicated in tubes with nutritive agar and then were subjected to biochemical and serological confirmation.[17]

Identification of heterotrophic flora. From each Plate Count Agar plates, 6 colonies were randomly chosen and purified by streak sowing on peptone agar (PA). Pure cultures were stored in PA slants at 4°C. The isolations were

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Table 1. Bacterial counts of viable heterotrophic bacteria, total and fecal coliforms in the Lake Ranco.

Sites	Depth (m)	Viable counts (UFC/ml)		Total coliforms (UFC/100 ml)		Fecal coliforms (UFC/100 ml)	
		Dec	May	Dec	May	Dec	May
A1	0	110	20	154	10	0	0
	20	160	230	60	418	0	0
A2	0	*	190	*	193	*	4
	5	*	100	*	126	*	5
A3	0	20	30	150	9	6	0
	5	440	170	761	842	0	0
	15	40	100	650	278	0	0
	25	100	190	168	470	0	0
	45	100	422	422	456	1	0
A4	0	1050	290	130	376	9	61
A5	0	20	50	76	74	0	3
	15	120	90	216	24	0	0

* No sampling due to turbulence.

identified up to genera using the schemes of HOLT *et al.*[12] and WARD *et al.*[32] The major test used for the identification of the isolates were as follow: Gram stain, cell morphology, motility by hanging drop, OF-glucose, catalase, oxidase, gelatin liquefaction, nitrate-nitrite reduction and production of pigments.

RESULTS

Physico-chemical characterization of lakes Ranco and Yelcho. Temperature and pH averages in spring and autumn were recorded at every sampling site of both lakes, at different depths. No notorious horizontal or seasonal thermal differences were registered. In Lake Ranco, the maximum superficial temperature registered was 19.8°C, with an average of 18.4°C, 7°C higher than Lake Yelcho. pH was stable, almost neutral, no important spacial variations were seen and it kept within the ranges described for araucanian lakes. Water transparency of Lake Ranco was high, up to 18 m in station A3. However, in Lake Yelcho, the visibility records of the disk did not go beyond 7 m.

Distribution of heterotrophic bacteria in the water column. In tables 1 and 2, the viable heterotrophic bacterial counts for five sites of both lakes are shown. In Lake Ranco, the viable counts ranged from 20 to 1050 CFU/100 ml, while in lake Yelcho the variations were between 20 and 970 CFU/ml. The concentration is lower in the superficial part of the water column and it increased with depth on may. In Lake Ranco, the highest counts were obtained in sites close to villages, while in Lake Yelcho the counts progresively increased towards the outlet of this lake, near a limniculture station (B3) and the village of Puerto Cardenas (B4).

Total and Fecal coliforms in the water column. In tables 1 and 2, coliform counts are presented, with a maximum of 842 CFU/100 ml in the Ranco and 309 CFU/100 ml in Yelcho. The horizontal variation was similar to that observed with heterotrophic bacteria. Fecal pollution was low in both lakes, with a maximum of 61 ufc/ 100 ml at A4 station in Lake Ranco. The detection of fecal coliforms in the center of Lake Yelcho was unexpected because there are no pollution focuses in that area. *Salmonella* was not detected in samples from either lakes.

Bacteriological analysis of the sediment. The viable heterotrophic bacterial counts were high, specially in stations of Lake Yelcho, where the counts were as high as 36,058 UFC/g (Table 3). These values were two magnitude

Table 2. Bacterial counts of viable heterotrophic bacteria, total and fecal coliforms in the Lake Yelcho.

Sites	Depht (m)	Viable counts (UFC/ml)		Total coliforms (UFC/100 ml)		Fecal coliforms (UFC/100 ml)	
		Dec	May	Dec	May	Dec	May
B1	0	420	30	42	14	0	0
	5	*	70	*	49	*	0
	15	250	30	100	29	0	0
B2	0	260	80	228	48	0	10
	5	320	*	302	*	0	*
	15	110	160	309	68	0	5
	25	665	*	152	*	2	*
B3	0	20	110	204	68	1	0
	5	*	170	*	79	*	2
	15	50	360	200	256	0	4
B4	0	710	700	290	270	4	42
	5	970	*	84	*	3	*
B5	0	640	130	190	86	0	0

* No sampling due to turbulence.

Table 3. Bacterial counts of viable heterotrophic bacteria, total and fecal coliforms and sulphate-reducing bacteria in the sediment of the lakes Ranco and Yelcho.

	Lake Ranco		Lake Yelcho	
	Max.	Min.	Max.	Min.
Viable counts (cfu/g)[1]	18213	1905	32593	32058
Total coliforms (cfu/100 g)	23000	1000	25300	400
Fecal coliforms (cfu/100 g)	0	0	0	0
Sulphate-reducing bacteria (NMP/100 g)	1100	100	29500	200

[1] cfu [dry weight]

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Table 4. Percentaje distribution of viable heterotrophic microorganisms in the lakes Ranco and Yelcho.

Microorganism	Percentaje distribution			
	Lake Ranco		Lake Yelcho	
	Water	Sediment	Water	Sediment
Gram negative				
Pseudomonas sp.	9,7	26,7	22,0	20,8
Alcaligenes sp.	4,9	3,3	3,8	0,0
Acinetobacter sp.	6,8	0,0	2,2	4,2
Runella sp.	0,5	0,0	0,0	0,0
Flavobacterium-Flexibacter	10,7	10,0	26,4	14,6
Aeromonas sp.	11,2	3,3	4,9	8,3
Chromobacterium sp.	0,0	0,0	0,0	4,2
Enterobacteriaceae	34,0	30,0	14,8	8,3
Gram positive				
Bacillus sp.	0,5	16,7	1,1	8,3
Corineforms	4,4	0,0	2,7	2,1
Micrococcus sp.	1,0	0,0	4,9	4,2
Staphylococcus sp.	4,9	0,0	1,6	0,0
Other				
Yeast	0,0	0,0	2,7	14,6
Unidentified	11,7	10,0	12,6	10,4

Ranco. A similar situation was found in lake Yelcho despite the oligotrophy reported by Soto *et al.*[28]. High bacterial counts, the presence of total coliforms and Enterobacteriaceae suggest that the watershed are being affected by the input of allochthonous materials, mainly plants and animal waste and washing of soil.

Although no *Salmonella* were detected, it does not mean that the recreational use of this water or it's use as a source of drinking water for humans and domestic animals is sanitary risk free, due to the presence of fecal coliforms in some areas.

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REFERENCES

1. Alvial, A. 1993. Una aproximación al impacto ambiental de la acuicultura. Aquanoticias Int. 5:20-27.
2. American Public Health Association. 1992. Standard methods for the examination of water and wastewater. 18th ed. American Public Health Association. Washington, D.C.
3. Buchanan, J. M., P. M. Wallis and A. G. Buchanan. 1986.Enumeration and identification of heterotrophic bacteria in groundwater and in a mountain stream. Can. J. Bot. 32:93-98.
4. Burton, G. A., D. GUNNISON and G.R. LANZA. 1987. Survival of pathogenic bacteria in various freshwater sediments. Appl. Environ. Microbiol. 53:633-638.
5. Campos, H., W. Steffen, R. Zimmermann, O. Parra, L. Zuñiga, C. Bertran, G. Agüero, J. Navarro and A.

Brown. 1990. Estudio del efecto producido sobre el lago Ranco por el cultivo de especies salmonídeas con el sistema de balsa jaula. EICOSAL-UACH, 281 pp.
6. Campos, H., G. Agüero and O. Parra. 1992a. Evaluación de la carga de fósforo y nitrógeno en los lagos Ranco y Puyehue. Subsecretaría de Pesca-UACH.
7. Campos, H., W. Steffen, G. Aguero, O. Parra and L. Zuñiga. 1992b. Limnology of lake Ranco (Chile). Limnologica 22:337-353.
8. Erkenbrecher, C. W. 1981. Sediment bacterial indicators in an urban shellfishing subestuary of the lower Chesapeake Bay. Appl. Environ. Microbiol. 42:484-492.
9. Esterby, S. R. and A. H. El-Shaarawi. 1984. Coliform concentrations in Lake Erie-1966 to 1970. Hydrobiologia 111:133-146.
10. Gebauer, M. T. and T. G. Donoso. 1988. Contaminación bacteriológica de los ríos Rahue y Damas, Osorno, Chile. Bol. ISP 27: 65-71.
11. González, G., M. Domínguez, L. Vergara and R. Zemelman. 1993. Bacilos Gram negativos heterotróficos aerobios del sistema fluvial Itata, Chile. Rev. Lat.-Amer. Microbiol. 35:289-296.
12. Holt, J. G., N. R. Krieg, P. H. A. Sneath, J. T. Staley and S. T. Williams (ed). 1994. Bergey's Manual of Determinative Bacteriology. 9th ed. Williams and Wilkins Co. Baltimore.
13. Instituto Nacional de Normalización (INN). 1978. Norma Oficial Chilena N° 1333: Requisitos de calidad de agua para diferentes usos. Santiago. Chile.
14. Leduc, L. G. and G. D. Ferroni. 1979. Quantitative ecology of psychrophilic bacteria in an aquatic environment and characterization of heterotrophic bacteria from permanently cold sediments. Can. J. Microbiol. 25:1433-1442.
15. Lokk, S. and V. Kisand. 1996. Microbiological characteristics and sanitary status of Lake Peipsi-Pihkva and its inflows in 1980s. Hydrobiologia 338:133-138.
16. López Fernández, A. and E. Anchia Vilda. 1988. Ecology of some species of Thiobacillus and sulfate-reducing bacteria in the middle course of the River Guadalquivir (Spain). Int. Rev. Gesamten Hydrobiol. 73:309-318.
17. Martínez, M., T. Maugeri, M. A. Mondaca, H. Abarzúa, H. Urrutia and K. Paredes. 1993. Características bacteriológicas del río Biobío, VIII Región de Chile: bacterias aeróbicas heterotróficas, biomasa y productividad bacteriana. Serie de Monografías Científicas EULA 12:279-291.
18. Méndez, R. 1992. Lagos del sur llaman a ineludible reflexión. Aquanoticias Int. 4:4-13.
19. Moorhead, D. L., W. S. Davis and C. F. Wolf. 1998. Coliform densities in urban waters of West Texas. J. Environ. Health 60:14-18.
20. Pankhurst, E. S. 1971. The enumeration and isolation of sulphate-reducing bacteria. p. 223-240. In:. SHAPTON, D.A. and R.G. BOARD (ed.) Isolation of anaerobes. Academic Press. London.
21. Petrovicova, A. 1989. Detection of coliphages and enteroviruses in drinking water and its sources. Arch. Hydrobiol. Beih. Ergebn. Limnol. 33:285-287.
22. Pillay, T. V. R. 1992. Aquaculture and the environment. Fishing News Books. Oxford.
23. Poikolainen, M. L., J. S. Niemi, R. M. Niemi and V. Malin. 1995. Fecal contamination of Finnish fresh waters in 1962-1984. Water, Air Soil Pollut. 81:37-47.
24. Quevedo-Sarmiento, J., A. Ramos-Cormezana and J. González-López. 1986. Isolation and characterization of aerobic heterotrophic bacteria from natural spring waters in the Lajaron area (Spain). J. Appl. Bacteriol. 61:365-372.
25. Schoebitz, R. 1983. Flora microbiana en aguas superficiales del río Valdivia (Chile). Zbl. Vet. Med. B. 30: 775-784.
26. Schoebitz, R. and L. Montes. 1984. Indicadores de contaminación bacteriológica y presencia de Salmonella en aguas del río Valdivia. Arch. Med. Vet.. 16: 83-92.
27. Slavikova, E., R. Vadkertiova and A. Kockova-Kratochvilova. 1992. Yeasts isolated from artificial lake waters. Can. J. Microbiol. 38:1206-1209.
28. Soto, D., H. Campos, W. Steffen and R. Palma. 1994. Situación ambiental actual del lago Yelcho y proyecciones para el uso de sus aguas. En: Soto, D. Evaluación de las potencialidades económicas del lago Yelcho en un marco ambiental aceptable. Informe Intendencia Región de Los Lagos. Pp. 1-50.
29. Stuart, S. A., G. A. McFeters, J. E. Schillinger and D.G. Stuart. 1976. Aquatic indicator bacteria in the high alpine zone. Appl. Environ. Microbiol. 31:163-167.
30. Thomason, B. M., J. W. Biddle and W. B. Cherry. 1975. Detection of Salmonellae in the environment. Appl. Environ. Microbiol. 30:764-767.
31. Velázquez Del Valle, M. and T. Gutiérrez-Castrejón. 1984. Variación estacional de las poblaciones bacterianas en el embalse Huapango. Estado de México. Rev. Lat.-Amer. Microbiol. 26: 223-230.
32. Ward, N. R., R. L. Wolfe, C. A. Justice and B. H. Olson. 1986. The identification of Gram negative nonfermentative bacteria from water, problems and alternative approaches to identification. Adv. Appl. Microbiol. 31:293-365.