4

ASSESSMENT OF FAECAL POLLUTION INDICATORS IN THE BRAZILIAN ANTARCTIC STATION WASTEWATER TREATMENT PLANT AND IN ENVIRONMENTAL SAMPLES AT ADMIRALTY BAY, ANTARCTIC PENINSULA

http://dx.doi.org/10.4322/apa.2014.073

Cristina Rossi Nakayama[2], Priscila Ikeda Ushimaru[1], Ana Carolina Vieira Araujo[1], André Rosch Rodrigues[1], Daniela Vilela Lima[1], Vivian Helena Pellizari[1,*]

[1]Laboratory of Microbial Ecology, Oceanographic Institute – IO, University of São Paulo – USP, Praça do Oceanográfico, 191, CEP 05580-120, São Paulo, SP, Brazil
[2]Institute of Environmental, Chemical and Pharmaceutical Sciences, Federal University of São Paulo – ICAQF/UNIFESP, Rua Professor Artur Riedel, 275, CEP 09972-270, Diadema, SP, Brazil
*e-mail: vivianp@usp.br

Abstract: *Assessment of faecal pollution indicators (total coliforms, Escherichia coli, Enterococcus sp., sulphite reducing clostridia and Clostridium perfringens) was carried out in water samples collected during the XXIX Brazilian Antarctic Expedition and sediment samples from the XXX Brazilian Antarctic Expedition. Wastewater at different stages in the sewage treatment plant were also analyzed for ammonia, total phosphorus, phosphates, chemical oxygen demand (COD) and faecal indicators, in order to characterize the wastewater and evaluate the system performance. Water and sediment analysis showed low populations of faecal coliforms and the presence of the indicators in all sites, with higher frequencies and concentrations at EACF and Ullman Point sediment, indicating that a possible human impact is of low magnitude and cannot be differentiated from interference caused by animal faeces. Assessment of the sewage treatment plant revealed that the wastewater produced at EACF has a typical faecal indicator composition and low contents of nutrients and COD, when compared to typical domestic sewage. Removal efficiency of COD was estimated in 20%, coliforms and enterococci removal varied from 84 to 98,7%, and no removal of nutrients was detected, indicating that the treatment process can be optimized.*

Keywords: faecal pollution, microbial indicators, sewage treatment, *E. coli, Clostridium sp., Enterococcus sp.*

Introduction

Faecal pollution indicators are groups of microorganisms used to study the impact caused by sewage discharge. Faecal coliforms and *Escherichia coli* are the most common indicators used in these assessments, but they do not survive long periods under stress in the environment. For that reason, other indicator groups of bacteria can be used as an auxiliary analysis, such as sulphite reducing clostridia (especially *Clostridium perfringens*), a group of spore forming bacteria usually considered as a remote contamination indicator due to their longer persistence in the environment, and *Enterococcus* sp., which has been more frequently used as indicator in marine environments due to their higher resistance to salinity when compared to coliforms and *E. coli* (CETESB, 1978; Ferguson *et al.*, 2005). Sewage treatment plants remove pollutants, nutrients and pathogenic microorganisms from wastewater through different stages: (1) preliminary, in which coarse solids are mechanically removed; (2) primary, which removes settable solids and part of the organic matter; (3) secondary, which consists biological removal of organic matter and eventually nutrients; (4) tertiary, aimed at removal of specific pollutants (toxic or non biodegradable), complementary removal of nutrients and disinfection. Sewage treatment plants configuration varies according to the characteristics of the

wastewater and required quality of the effluent based usually in legislation (von Sperling, 2005). In EACF secondary and tertiary stages are present, with the use of aerobic and anaerobic digesters and a UV light disinfection system.

In the present work, faecal pollution indicators were enumerated in sediment and water samples collected during Brazilian Antarctic Expeditions XXIX and XXX (BAE XXIX and BAR XXX). Wastewater samples at different stages of the EACF sewage treatment plant and soil in the effluent discharge area were also characterized for nutrients, chemical oxygen demand and faecal indicators in order to obtain information on sewage properties and treatment efficiency.

Materials and Methods

Sampling and sample processing

Samples were collected between December 2010 and March 2011 (Brazilian Antarctic Expedition XXIX) and December 2011 to February 2012 (Brazilian Antarctic Expedition XXX), and consisted of: (1) Water, collected in five stations (Table 1) along the water column (surface, middle and 1 m from the bottom, as determined by an ecobathymeter Vexilar mod LPS-1) and analyzed for coliforms (BAE XXIX) and enterococci (BAE XXX); (2) Sediment, collected in four stations (Table 1) at the 30 m isobaths (BAE XXX) and analyzed for coliforms, enterococci and clostridia; (3) Wastewater, sampled before treatment (affluent), after secondary treatment and before disinfection (secondary treatment effluent) and after disinfection with UV (effluent), analyzed for nutrients, chemical oxygen demand (COD), coliforms, enterococci and clostridia; and (4) Soil at the intertidal region in wastewater discharge area, analyzed for coliforms, enterococci and clostridia. All samples were stored at 4 °C until analysis, which took place in a maximum period of 24 hours after sampling.

Faecal pollution indicators enumeration

In BAE XXIX, coliforms and clostridia in water, wastewater and soil samples were enumerated by the Most Probable Number (MPN) technique (APHA, 2005) using Colilert® medium (IDEXX) for total coliforms and *E.coli* and DRCM (differential reinforced clostridial medium) for clostridia.

Table 1. Water and sediment sampling sites.

Water samples (Brazilian Antarctic Expedition XXIX)				
EACF (CF)	**Botany Point (BP)**	**Machu Picchu (MP)**	**Point Thomas (PT)**	**Arctowski AR)**
62º 05.217" S 58º23.017" W	62º 05.767" S 58º 19.967" W	62º 05.367" S 58º 28.183" W	62º 09.200" S 58º 29.100" W	62º 09.383" S 58º 27.933" W
Sediment samples (Brazilian Antarctic Expedition XXX)				
	EACF	**Botany Point**	**Ullman Point**	**Refuge 2**
Site 1	**CF1**	**BP1**	**PU1**	**RF1**
1	62º 05.131" S 58º 23.369" W	62º 05.701" S 58º 19.849" W	62º 05.015" S 58º 23.987" W	62º 04.21" S 58º 25.335" W
2	62º 05.142" S 58º 23. 370" W	62º 05.713" S 58º 19.844" W	62º 05.015" S 58º 23.987" W	62º 04.373" S 58º 25.335" W
3	62º05.130" S 58º23.370" W	62º 05.734" S 58º 19.919" W	62º 05.015" S 58º 23.987" W	62º 04.373" S 58º 25.335" W
Site 2	**CF2**	**BP2**	**PU2**	**RF2**
1	62º 05.050" S 58º 23.195" W	62º 05.181" S 58º 20.182" W	62º 05.038" S 58º 23.055" W	62º 04.1" S 58º 25.19" W
2	62º 05.049" S 58º 23.195" W	62º 05.48" S 58º 20.10" W	62º 05.133" S 58º 23.317" W	62º 04.18" S 58º 25.19" W
3	62º 05.130" S 58º 23.356" W	62º 05.48" S 58º 20.10" W	62º 05.133" S 58º 23.317" W	62º 04.1" S 58º 25.19" W

Presence of *C. perfringens* in DCRM cultures was confirmed by growth in Litmus Milk.

In BAE XXX, the membrane filtration technique (CETESB, 1978) was chosen to enumerate coliforms, enterococci and clostridia in water, sediment, wastewater and soil samples. The change was made in order to enhance sensitivity and precision, as larger volumes of samples can be analyzed by this method. Media and incubation conditions used for enumeration of coliforms and *E.coli*, enterococci and clostridia were, respectively: modified membrane-thermotolerant Escherichia coli Agar (Modified mTEC), incubated at 35 °C for 2 hours, followed by incubation at 44.5 °C waterbath for 22-24 hours; membrane-Enterococcus Indoxyl-β-D-Glucoside Agar (mEI), incubated at 41 °C for 24 hours; and mCP agar, incubated at 45 °C for 24 hours.

Nutrients and COD

Analysis of ammonia, total phosphorus, phosphates and chemical oxygen demand in wastewater samples were carried out using reagent kits and photometer from Hanna Instruments, Inc. from aliquots of wastewater affluent and effluent before and after disinfection with UV, diluted to factors of 1:10 and 1:20 when necessary.

Results

Water, sediment and soil samples

Water samples were analyzed for coliforms in BAE XXIX and for enterococci in BAE XXX (Figure 1). Analysis of coliforms, enterococci and clostridia in sediment at the 30 m isobaths were carried out in BAE XXX (Figure 2). Results of soil samples analysis in BAE XXIX showed *E.coli* and *C. perfringens* counts of 1.6×10^9 and 7.0×10^3 NMP/100 mL, respectively. In BAE XXX counts of E.coli, E. faecalis and C. perfringens were 1.0×10^6, 1.0×10^6, and 3.2×10^4 UFC/mL, respectively.

Wastewater samples

During BAE XXIX, analysis of sewage before treatment was carried out once (January 2011) and showed total coliforms and *E.coli* counts of 1.7×10^7 and 7.9×10^6 NMP/100 mL and sulphite reducing clostridia and C. perfringens counts of 1.7×10^5 and 1.1×10^3 NMP/100 mL, respectively. Analyses of wastewater at outfall pipe were carried out four times and results can be seen in Figure 3. During BAE XXX, faecal indicators and physical chemical analysis of wastewater were performed at different stages at the treatment system (Table 2 and Figure 3), and removal efficiencies calculated for microbial indicators and COD (Table 2).

Discussion

As observed in previous work, low concentrations and widespread distribution of faecal indicators were detected in Admiralty Bay (Figures 1 and 2). In sediment samples, higher values were found in EACF and Ullman Point sites, a pattern also observed in previous analysis (Nakayama *et al.*, 2010). In water samples, EACF site was the only one to

Table 2. Wastewater characterization at different treatment stages (data obtained from December 2011 to February 2012).

Parameter	Affluent	Effluent		Removal efficiency (%)
		Before UV disinfection	After UV disinfection	
Ammonia (mg/L)	4.91	5.32	8.26	-
Total phosphorus (mg/L)	0.7	0.9	1.8	-
Phosphate (mg/L)	2.1	2.8	5.5	-
DQO (mg/L)	114	97	91	20.18
Total coliforms (CFU/mL)	3.0×10^6-1.0×10^7	5.3×10^5-1.2×10^7	3.9×10^4-3.5×10^6	84.00-92.64
E. coli (CFU/mL)	1.0×10^6-2.7×10^7	5.3×10^5-2.0×10^7	3.9×10^4-2.8×10^6	85.00-92.64
Enterococcus sp. (CFU/mL)	2.5×10^6-2.9×10^7	4.0×10^3-1.7×10^6	1.7×10^4-2.3×10^6	92.61-98.72
Sulphite reducing clostridia (CFU/mL)	1.5×10^5-2.4×10^5	1.0×10^3-2.4×10^5	<2.2-1.0×10^5	33.33->99.78
Clostridium perfringens (CFU/mL)	3.7×10^4-2.4×10^5	1.0×10^3-2.3×10^5	<2.2-3.6×10^4	80.00->99.78

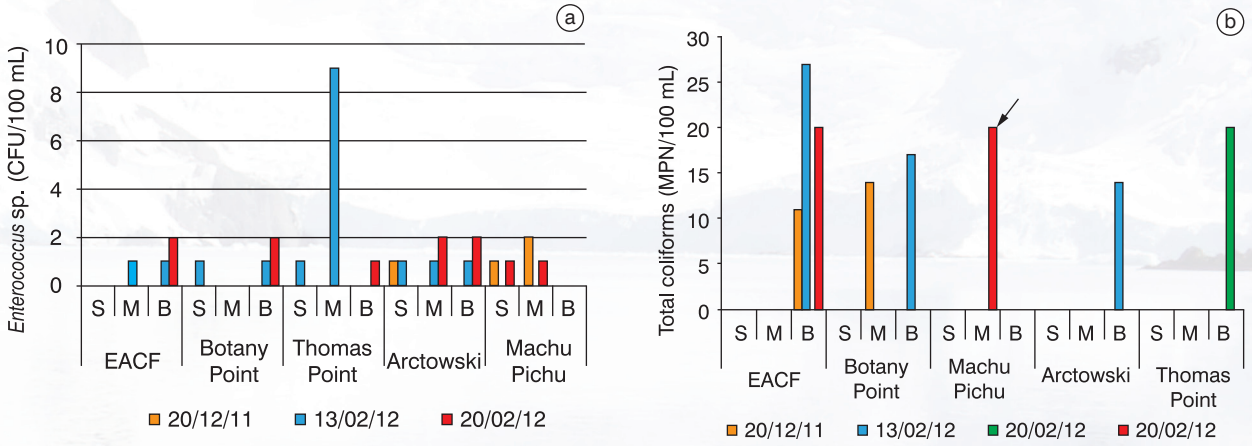

Figure 1. Membrane filtration counts of *Enterococcus* sp. (a) and Most Probable Number counts of total coliforms (b) in water samples from Admiralty Bay. S: surface; M: middle; B: bottom. The arrow indicates the only occurrence of *E.coli* in the analysed water samples.

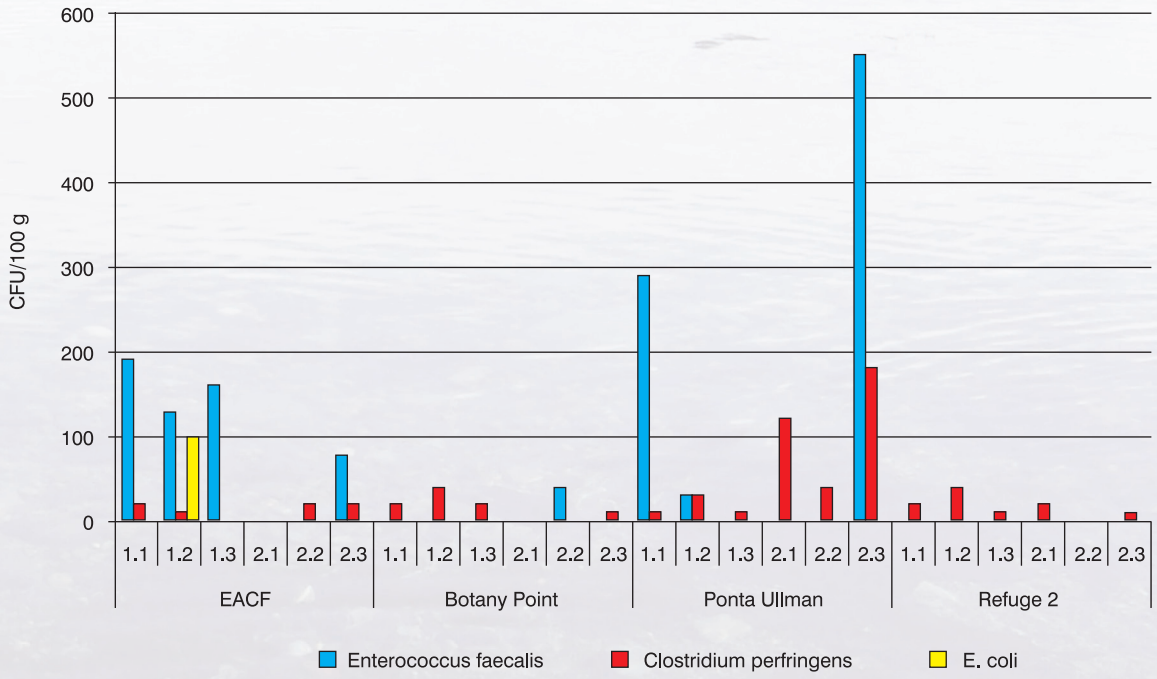

Figure 2. Membrane filtration counts of faecal pollution indicators in sediment samples from Admiralty Bay.

show total coliforms detection in three out of four analysis, but *E.coli* was detected only at Machu Picchu site. Similar results were found by Lisle *et al.* (2004) who also found low concentrations of faecal indicators in sites considered as control areas near McMurdo Station. Detection of microbial indicators in bottom water samples may be related to regional hydrodynamics but can also suggest some resuspension of cells from the sediment. Although survival for long time of indicators as coliforms and enterococci are not expected under adverse environmental conditions, Pote *et al.* (2009) observed that these groups of bacteria were able to maintain viability for at least 60 days in microcosms

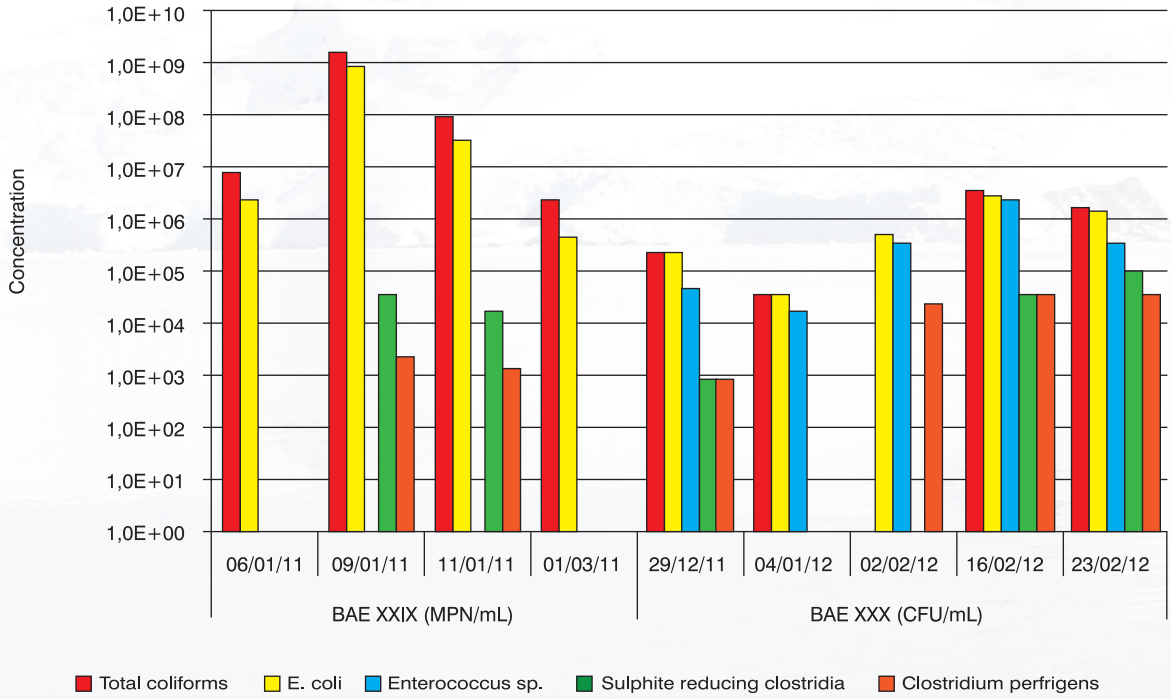

Figure 3. Most probable number and membrane filtering counts of faecal pollution indicators in wastewater samples at outfall pipe. BAE: Brazilian Antarctic Expedition.

containing sediment, water and sewage treatment plant effluent incubated at 10 °C. However the presence of indicators in the water and sediment samples studied can be derived not only from human but also from animal faeces. For instance, Lisle *et al.* (2004) confirmed the presence of *C. perfringens* in Weddel Sea scats and Wright *et al.* (2009) detected an average of 3.3×10^5 CFU/g enterococci in birds of a study recreational beach in Florida, USA. All this considered, the adaptation of molecular techniques for the detection of faecal contamination in Antarctic samples seems to be an alternative to be considered for future studies. Some examples include the use of PCR and qPCR techniques with genes related to coliform and enterococci groups as described by Bej *et al.* (1990) and Colford *et al.* (2012).

Even though the change in enumeration methods between BAEs XXIX and XXX may affect data comparisons, results of the analysis of the affluent wastewater did not show great differences during and between expeditions (Table 2). The populations of faecal indicators reflect the composition of the sewage produced in EACF, which is coherent to composition of domestic wastewater in general, with higher counts of coliforms and enterococci followed by clostridia (Leclerc *et al.*, 1977). Counts at the effluent of the sewage treatment plant were more variable (Table 2 and Figure 3), as expected, once they are related to the flow of sewage produced in the station. The highest counts of coliforms ($1,6 \times 10^9$ and $9,2 \times 10^8$ NMP/100 mL) were actually observed on 9 January 2011, when there were 150 people in the station, about twice the regular population at EACF.

Sewage secondary treatment is usually responsible for 60 to 99% coliforms removal (von Sperling, 2005). However, removal efficiencies calculated for the EACF plant (Table 2) showed that although the secondary treatment led to reduction of *Enterococcus* sp. populations (61,05 to 91,50%), no coliform removal occurred. Clostridia is a spore forming group of bacteria, which may explain the low removal values of about 30% observed in some analysis. The introduction of a tertiary treatment, represented by UV disinfection was determinant for removal of microorganisms from the effluent, especially the coliforms, but high amounts of

microorganisms are still discharged and can be detected in the soil near the sewage treatment plant outfall in concentrations in the order of 10^4 to 10^6 CFU/100 g. For that reason, improvements in the system should be made to enhance removal of microorganisms from the effluent.

Determination of nutrients and COD in effluent wastewater (Table 2) showed lower ammonia, total phosphorus and COD values than expected for regular domestic wastes (von Sperling, 2005). However, nutrient concentrations increased along wastewater treatment stages and COD removal efficiency was only 20.17%. Despite being preliminary data (determinations were carried out in only one sample), values obtained indicate that the process could be optimized to perform more efficiently. Enhancing treatment performance would also contribute to increase inactivation rates of microbial contaminants by the UV disinfection system, since the presence of suspended solids, chemical oxygen demand, color and organic compounds protect microorganisms by absorbing / scattering the UV light (Bitton, 1994).

Conclusions

Enumeration of faecal indicators in sediment and water samples revealed low populations with widespread distribution, as observed in previous work, indicating that influence of animal faeces cannot be discarded and for that reason impact of human activities in the studied area is not unequivocally detected. A first assessment of the EACF sewage treatment plant revealed that sewage produced in the research station has faecal indicators' densities compatible to domestic sewages but lower contents of nutrients and organic matter (as indicated by chemical oxygen demand) than typical domestic wastewater. According to preliminary data generated in the present work, the treatment plant removed about 20% COD, but no nutrient removal was observed. Removal efficiency of coliforms and enterococci varied from 84 to 98,7% after UV light disinfection, but discharged effluent still contained up to 10^6 indicators CFU/100 mL and 10^4 to 10^6 CFU/100 g were detected in soil samples near the discharge area. Although impact of wastewater discharge seems to remain restricted to the area of discharge, optimization of the process is desirable to attend to environmental protection goals for Antarctic ecosystems.

Acknowledgements

This work integrates the National Institute of Science and Technology Antarctic Environmental Research (INCT-APA) that receive scientific and financial supports of the National Council for Research and Development (CNPq process: n° 574018/2008-5) and Research Support Foundation of the State of Rio de Janeiro (FAPERJ n° E-16/170.023/2008). The authors also acknowledge the support of the Brazilian Ministries of Science, Technology and Innovation (MCTI), of Environment (MMA) and the Inter-Ministerial Commission for Resources of the Sea (CIRM).

References

Bej, A.K.; Steffan,R.J.; Dicesare, J.; Haff, L. & Atlas, R.M. (1990). Detection of coliform bacteria in water by Polymerase Chain Reaction and Gene Probes. Applied and Environmental Microbiology, 56(2): 307-314.

Bitton, G. (1994). Wastewater Microbiology, Nova lorque:Wiley-Liss, Inc. 478 p.

Companhia de Tecnologia de Saneamento Ambiental – CETESB (1978). NT-08 Análises microbiológicas de águas. São Paulo.

Colford, J.M.; Schiff, K.C.; Griffith, J.F.; Yau, V.; Arnold, B.F.; Wright, C.C.; Gruber, J.S.; Wade, T.J.; Burns, S.; Hayes, J.; McGee, C.; Gold, M.; Cao, Y.; Noble, R.T.; Haugland, R. & Weisberg, S.B. (2012). Using rapid indicators for Enterococcus to assess the risk of illness after exposure to urban runoff contaminated marine water. Water Research, 46: 2176-2186.

Ferguson, D.M.; Moore, D.F.; Getrich, M.A. & Zhowandai, M.H. (2005). Enumeration and speciation of enterococci found in marine and intertidal sediments and coastal water in Southern California. Journal of Applied Microbiology, 99(3): 598-608.

Leclerc, H.; Mossel, D. A.; Trinel. P. A. & Gavini, F. (1977). Microbiological monitoring: a new test for fecal contamination. In: Hohdley, A.W. & Dutka, B.J. Bacterial indicators / Health hazards associated with water. ASTM Special Technical Publication. vol. 635, 356 p.

Lisle, J.T.; Smith, J.J.; Edwards, D.D. & McFeters, G.A. (2004). Occurrence of Microbial Indicators and Clostridium perfringens in Wastewater, Water Column Samples, Sediments, Drinking Water, and Weddell Seal Feces Collected at McMurdo Station, Antarctica. Applied and Environmental Microbiology, 70(12): 7269-7276.

Nakayama, C.R.; Ushimaru, P.I.; Vilella, D. & Pellizari, V.H. (2010). Occurrence of microbial faecal pollution indicators in sediment and water samples at Admiralty Bay, King George Island, Antarctica. In: Annual activities report (2010) of National Institute of Science and Tecnology on Antarctic Environmental Research. São Carlos: Editora Cubo. p. 164-166.

Pote, J.; Haller L.; Kottelat R.; Sastre, V.; Arpagaus, P. & Wildi, W. (2009). Persistence and growth of faecal culturable bacterial indicators in water column and sediments of Vidy Bay, Lake Geneva, Switzerland. Journal of Environmental Sciences, 21: 62-69.

von Sperling, M. (2005). Introdução à qualidade das águas e ao tratamento de esgotos. 3. ed. Belo Horizonte: Editora UFMG. 452 p.

Wright, M.E.; Solo-Gabriele, H.M.; Elmir, S. & Fleming, L.E. (2009). Microbial load from animal feces at a recreational beach. Marine Pollution Bulletin, 58: 1649-1656.