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Wastewater contamination in Antarctic melt-water streams evidenced by virological and organic molecular markers

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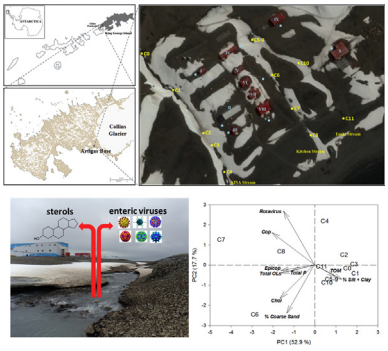
HIGHLIGHTS

- Wastewater contamination was assessed in three streams in the Antarctic continent.
- Viruses and sterols were analyzed in surface waters and sediments, respectively.
- Rotaviruses and high sterols values evidenced a wastewater contamination.
- Complementation of these biomarkers improves the assessment of wastewater pollution.

GRAPHICAL ABSTRACT



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ABSTRACT

Human activities in the Antarctica including tourism and scientific research have been raised substantially in the last century with the concomitant impact on the Antarctic ecosystems through the release of wastewater mainly from different scientific stations activities. The aim of this study was to assess the wastewater contamination of surface waters and sediments of three melt-water streams (11 sites) by leaking septic tanks located in the vicinity of the Uruguayan Scientific Station in the Fildes Peninsula, King George Island, Antarctica, during summer 2015. For this purpose, we combined the analysis of fecal steroids in sediments by using gas chromatography and six enteric viruses in surface waters by quantitative and qualitative PCR. Coprostanol concentrations (from 0.03 to 3.31 μg g^{-1}) and fecal steroids diagnostic ratios indicated that stations C7 and C8 located in the kitchen stream presented sewage contamination. Rotavirus was the only enteric virus detected in five sites with concentration ranging from 1.2×10^5 gc L^{-1} to 5.1×10^5 gc L^{-1} being three of them located downstream from the leaking AINA and Kitchen septic tanks. This study shows for the first time the presence of both virological and molecular biomarkers of wastewater pollution in surface waters and sediments of three melt-water streams in the vicinity of a scientific station in the Antarctica. These results highlight the importance of the complementation of these biomarkers in two different matrices (surface waters and sediments) to assess wastewater pollution in an Antarctic environment related to anthropogenic activities in the area.

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1. Introduction

Human activities in the Antarctic continent date back to 18th century, however, from the 1960s to present days these activities have continued to increase together with the production of wastes and interaction with the landscapes, flora and fauna. According to the Council of Managers of National Antarctic Programs, 53 research stations are located in the Antarctica where near 4000 people live and work at its peak each summer. Regular activities of the scientific stations have caused several environmental impacts that affect the air, water (marine and freshwater) and soil quality (including ice and snow) mainly due to the land or marine traffic, waste and sewage management and disposal and fuel oil contamination (Braun et al., 2012, 2014; Tin et al., 2009, 2014).

Domestic wastewater discharge in the Antarctic environment has been evidenced in several research stations mainly through the analysis of organic molecular indicators (Delille and Delille, 2000; Howington et al., 1992; Martins et al., 2005, 2014; Montone et al., 2010). Fecal steroids (e.g. coprostanol and coprostanone) are organic compounds used as wastewater molecular markers since they are resistant to environmental degradation and specific to their origin. These compounds have been used as tracers of domestic wastewater contamination in the Antarctic region (Hughes and Thompson, 2004; Leeming et al., 2015; Martins et al., 2005, 2012; Venkatesan and Mirsadeghi, 1992). The major human fecal sterol is coprostanol that is widely used because it represents near 60% of total fecal sterols in human feces (Leeming et al., 1998). Coprostanol is associated with particulate material and sediments, is very resistant to anaerobic degradation and together with cholesterol and cholestanol are consistent markers of human fecal contamination (Leeming et al., 1998; Martins et al., 2005). In addition, epicoprostanol is used to evaluate the treatment of wastewaters since it is present in sewage sludge and produced during extensive anaerobic sewage treatment (Leeming et al., 2015; Martins et al., 2012; Mudge and Seguel, 1999). Moreover, fecal sterols are insoluble in water and tend to accumulate in sediments where they are highly resistant to degradation and the presence of these sterols in sediments represents the concentration at the time of deposition. Depending on local sedimentation rates, the time of deposition for surface sediments is generally of the order of years (Martins et al., 2005; Muller et al., 1979).

Wastewater discharge can also be assessed by viral indicators of fecal contamination like Human Adenovirus (HAdV), which is an enteric virus presenting environmental dissemination via contaminated feces from an infected person. Other enteric viruses like Group A Rotavirus (RVA), Norovirus (NoV), Human Astrovirus (HAstV) and Enterovirus (EV) are frequently present in domestic wastewater evidencing their spread from infected people to the environment (Bosch et al., 2008).

The combination of fecal sterols and enteric viruses as markers of fecal contamination to assess wastewater pollution is an important approach, but has been performed only in a few studies worldwide, nevertheless, the complementation of these two indicators have never been used in the Antarctic continent in order to determine the impact of the anthropogenic activities in this pristine environment (Rusiñol et al., 2016; Souza et al., 2012). The aim of this study was to evaluate the presence of sewage on melt-water streams by leaking septic tanks (confirmed by visual observation) in the vicinity of the Artigas Antarctic Scientific Base (BCAA) by using virological and molecular organic markers (fecal sterols) as indicators of fecal contamination in surface waters and sediments, respectively. Also, we aimed to evaluate if both matrices (surface waters and sediments), can be used in a complementary manner to obtain a more reliable assessment of sewage contamination in these aquatic environments since the presence of enteric viruses in surface running waters suggests a relatively recent fecal contamination when compared with high concentrations of sterols in sediment which indicate a prolonged presence of fecal contamination (Martins et al., 2005).

2. Material and methods

2.1. Study area and sample collection

The Fildes peninsula, located in the South of King George Island, present a high biodiversity and six permanent scientific stations are located in this peninsula, representing important logistic centers in the area (Braun et al., 2012). The BCAA, located in the Fildes Peninsula (62°11′4″S and 58° 51′7″W) comprised 11 buildings with several septic tanks (Fig. 1). During February 2015 (summer) a sampling survey was performed in 11 sites located along the three melt-water streams nearby the BCAA (AINA melt-water stream: C0 to C4, Kitchen melt-water stream: C5-9 to C8 and Tanks melt-water stream: C10 and C11). These melt-water streams flow into the sea in approximately 100 m. From the 11 sampling sites, seven were located close to the septic tanks (C1, C2, C3, C5-9, C6, C7, C10), three downstream (C4, C8, C11) and one as a control site (C0: upstream the AINA stream and away from the buildings area) (Fig. 1). During the sampling survey, approximately 60 persons were present in the BCAA although nine are the residents along the year. These people tend to congregate mainly in the AINA building (number II in Fig. 1) where the rest rooms, offices and the laboratory are located and in the kitchen (number VIII in Fig. 1) where people feed and perform recreation activities.

It is worth mentioning that current protocols at BCAA establish the treatment of organic garbage by incineration and the removal from the wastewater of the solid phase from BCAA to the continent, however, the liquid phase of this wastewater remains in the septic tanks where occasionally leaks to the streams.

Physicochemical water parameters (temperature and conductivity) were measured in situ with an YSI *Pro Plus* multi parameter. Surface sediment samples for granulometric and chemical analysis were collected using a stainless steel corer and were maintained frozen (−20 °C) in pre-combusted (450 °C, 4 h) aluminum containers until laboratory analysis.

Sediment samples were kept frozen during transport until laboratory analysis (maximum of 10 days after collection).

Surface water samples were collected in 500 mL bottles and viral concentration was performed at the laboratory of the BCAA. Concentrated samples were kept frozen until their transfer to the laboratory in Salto city, Uruguay, for virological analysis.

2.2. Determination of grain size, total organic matter and phosphorous

Grain size was determined by sieving according to Suguio (1973) and total organic matter (TOM) content by calcination and weight difference (Byers et al., 1978). Total phosphorus (TP) determination was performed by partial digestion (USEPA 3050B (USEPA, 1996)) and quantified by optical emission spectrometry with inductively coupled plasma.

2.3. Extraction, purification and analysis of sterols

The analysis of steroids was based on the method described by Kawakami and Montone (2002). Prior to extraction, sediments were freeze-dried and homogenized in a porcelain mortar. About 10 g of dried sediment were Soxhlet extracted for 8 h with a mixture of (1:1; v:v) n-hexane/dichloromethane. The 5α-androstan-3β-ol (Sigma®) was added as surrogate, before blank, reference material and sample extraction. Concentrated extracts were fractionated and purified by column chromatography using 5% deactivated silica and alumina with 15 mL of methanol and evaporated to dryness. All solvents were analytical grade. Steroids were derivatized to form trimethylsilyl ethers using BSTFA (bis(trimethylsilyl) trifluoroacetamide) with 1% TMCS (trimethylchlorosilane) (Supelco) for 90 min at 65 °C. The 5α-cholestane was used as internal standard for quantification. Steroids were analyzed using an Agilent 6890 gas chromatograph with a flame