BACKGROUND VALUES AND ASSESSMENT OF FECAL STEROIDS DISCHARGED INTO TWO INLETS (MACKELAR AND EZCURRA) IN ADMIRALTY BAY, KING GEORGE ISLAND, ANTARCTICA

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Abstract: Steroids are efficient geochemical markers of natural and anthropogenic environmental events, because they present stability and resistance to the degradation process, keeping a record of their signature origin, allowing interpretations about the organic matter sources. The Antarctic region is considered one of the best preserved environments in the world; however human activities have resulted in changes in this pristine location. Sampling was collected during the 2006/07 austral summer at three points: Refuge II (REF) (Mackellar Inlet), Thomas Point (PTH) and Barrel Point (BAR) (Ezcurra Inlet). After Soxhlet extraction, clean up using adsorption column and derivatization, steroids concentrations were determined by gas chromatography with flame ionization detector (GC-FID). Concentrations of fecal sterols (coprostanol and epicoprostanol) in all locations studied were $<0.10 \mu\text{g}\cdot\text{g}^{-1}$, suggesting natural contributions of these compounds. This statement is corroborated by values found for the coprostanol/epicoprostanol ratio for all cores (<2.50). Based on these results, background values were established for the analyzed compounds in the two studied inlets. The values for REF, PTH and BAR were, respectively, (0.06 ± 0.02) , (0.04 ± 0.03) and (0.03 ± 0.01) for coprostanol + epicoprostanol.

Keywords: fecal sterols, coprostanone, background values, Admiralty Bay

Introduction

The Antarctic region is considered one of the best-preserved environments in the world. However, there are approximately 79 centers of research in Antarctica located mainly in coastal areas, discarding their sewage, directly into the open sea (Hughes & Thompson, 2004). A study realized by Hughes (2004) showed that the influence of untreated sewage discharged by Rothera Research Station – UK (Marguerite Bay), reached approximately 570 m to the North and more than 800 m east of this station. In 2004, this influence was not detected in locations with more than 50 m, due the implantation of sewage treatment facilities.

These results showed the importance of monitoring inputs and to treat waste, preventing environmental contamination. To study the extent of the sewage contribution around research stations, research involving organic markers has been developed (Green & Nichols, 1995; Martins *et al.*, 2002), due to specific source, resistance to degradation processes and chemical stability (Colombo *et al.*, 1989). Martins *et al.* (2005), using organic geochemical markers such as sterols, verified that the presence of sewage from Comandante Ferraz station had reached up to 400 m distant from the initial sewage discharge. As the Admiralty

Bay environment is subjected to little human influence and fecal steroids can be associated to marine mammals, the determination of background values of these organic markers in different Antarctic regions are important for future studies, especially in places where scientific stations are established (Montone *et al.*, 2010). Thus, background values of fecal steroids, based on the concentration of these markers, were obtained from three short sedimentary columns, which were sampled at Mackellar and Ezcurra Inlet.

Material and Methods

Admiralty Bay is situated in King George Island, in the South Shetland Islands. Human occupation in the Bay is represented by the presence of three main stations and some refuges. Martel Inlet is the location of Brazilian Ferraz Station and two refuges; Mackellar Inlet is the base of the Peruvian Macchu Picchu station, located near Crepin Point, while the Polish Henryk Arctowski station is located at Ezcurra Inlet, on the western side of the Admiralty Bay.

Two cores were collected in Ezcurra Inlet, near the Thomas Point and Barrel Point; both at 30 m deep, and the third core was collected on the opposite side of Macchu Pichu station, near the Brazilian refuge, at 20 m deep, in Mackellar Inlet. The sampling was undertaken using a box corer between December 2006 and January 2007. 25 mm diameter wide aluminum tubes were introduced into the box, and cores of approximately 20 cm were sampled and sectioned at 1 cm intervals.

Steroid analysis was based on a method described by Kawakami and Montone (2002). More than 20 g of sediment from each site were extracted using a Soxhlet system for 8 hours with 70 mL of ethanol. The surrogate, 5 α -cholestane, was added before each extraction. The ethanol extract was reduced to c. 2 mL by rotoevaporation. The concentrated ethanol extract was submitted to a clean up with column chromatography using 2 g of 5% deactivated alumina and elution with 15 mL of ethanol. The extracts were evaporated to dryness and derivatized to form trimethylsilyl ethers using BSTFA (bis(trimethylsilyl)trifluoroacetamide) with 1% TMCS (trimethylchlorosilane) for 90 minutes at 65 °C. The mixture of TMS-sterols derivatives was determined by

the injection of 2 μ L into a gas chromatograph equipped with a flame ionization detector (GC-FID). Instrumental details are described by Montone *et al.* (2010).

Results and Discussion

The sedimentation rates used in this work to determine the time scale indicated by the cores were calculated by Martins *et al.* (2010). BAR presented 0.13 cm.y⁻¹. For other cores (REF and PTH), we used a mean value between several sites in Admiralty Bay (0.22 cm.y⁻¹).

Among the most widely used sterols, coprostanol and epicoprostanol (named fecal sterols) are found in sediments contaminated by sewage (Venkatesan & Kaplan, 1990; Venkatesan & Santiago, 1989), because they are associated primarily with human feces (Grimalt *et al.*, 1990). However, feces of marine animals, such as some species of whales, seals, sea lions, contributes with large quantities of these compounds to the Antarctic environment (Venkatesan *et al.*, 1986; Venkatesan & Santiago, 1989) and it is assumed as “natural contribution”. The same sources can be assigned to ketone coprostanone (Martins *et al.*, 2002; Venkatesan & Kaplan, 1990). González-Oreja and Saiz-Salinas (1998) proposed limits to coprostanol in order to define natural input or sewage contribution, where values below 0.50 μ g.g⁻¹ may be related pristine environments.

In REF, the coprostanol presented values between 0.01 and 0.06 μ g.g⁻¹ and the epicoprostanol between 0.01 and 0.04 μ g.g⁻¹ (Table 1). These values are below the established limit, suggesting an introduction related to marine mammals, despite of this point being located on the opposite side to a research station (Macchu Pichu). To the PTH, the values of coprostanol and epicoprostanol were also below the limit, between 0.01 and 0.04 μ g.g⁻¹ and 0.01 and 0.09 μ g.g⁻¹, respectively (Table 1). As well as in REF, the maximum values for each fecal sterol are lower than the limit established by González-Oreja and Saiz-Salinas (1998). These values suggest that although variations occur throughout the profile, the introduction of these compounds seems to be from natural sources, despite the proximity of these sites to human activities. As verified in other points, the coprostanol and epicoprostanol were also low in BAR

Table 1. Concentration (in $\mu\text{g.g}^{-1}$) of fecal sterols and ketone coprostanone in cores collected at REF, PTH and BAR. Mean value and standard deviation (SD) were calculated for all sections of each core.

Refuge II															
Section	0-1 cm	1-2 cm	2-3 cm	3-4 cm	4-5 cm	6-7 cm	8-9 cm	10-11 cm	11-12 cm	12-13 cm	13-14 cm	14-15 cm	Mean	SD	
Date	2002-2007	1998-2002	1993-1998	1989-1993	1984-1989	1975-1980	1966-1971	1957-1962	1952-1957	1948-1952	1943-1948	1939-1943			
Coprostanol	0.04	0.05	0.03	0.04	0.04	0.05	0.06	0.05	0.03	0.01	0.03	0.03	0.04	0.01	
Epicoprostanol	0.04	0.02	0.04	0.02	0.04	0.03	<LD	0.02	0.01	0.01	0.02	0.02	0.02	0.01	
Coprostanone	3.62	1.37	0.69	0.49	0.46	0.46	0.17	0.47	0.11	0.04	0.21	0.23	0.69	0.94	
cop/e-cop	1.00	2.50	0.75	2.00	1.00	1.67	nc	2.50	3.00	1.00	1.50	1.50	1.67	0.71	
Thomas Point															
Section	0-1 cm	1-2 cm	2-3 cm	3-4 cm	4-5 cm	5-6 cm	7-8 cm	8-9 cm	9-10 cm	10-11 cm					
Date	2002-2007	1998-2002	1993-1998	1989-1993	1984-1989	1980-1984	1971-1975	1966-1971	1962-1966	1957-1962	Mean	SD			
Coprostanol	0.04	0.03	0.04	0.02	0.01	0.01	0.01	0.02	0.01	0.02	0.02	0.01	0.02	0.01	
Epicoprostanol	0.04	0.02	0.09	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.02	0.02	0.02	0.02	
Coprostanone	0.32	0.15	0.52	0.12	0.04	0.03	0.06	0.08	0.01	0.06	0.14	0.15	0.14	0.15	
cop/e-cop	1.00	1.50	0.44	2.00	1.00	1.00	0.50	2.00	1.00	2.00	1.24	0.57	1.24	0.57	
Barrel Point															
Section	0-1 cm	1-2 cm	2-4 cm	4-5 cm	5-6 cm	6-7 cm	7-8 cm	8-9 cm	10-11 cm	11-12 cm	12-13 cm	13-14 cm	14-16 cm		
Date	1999-2007	1992-1999	1976-1992	1969-1976	1961-1969	1953-1961	1945-1953	1938-1945	1922-1930	1915-1922	1907-1915	1899-1907	1884-1899	Mean	SD
Coprostanol	0.02	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.01	0.01	0.005
Epicoprostanol	0.01	0.02	0.03	0.02	0.02	0.01	0.01	0.02	0.01	0.02	0.02	0.02	0.01	0.02	0.01
Coprostanone	0.02	0.02	0.03	0.02	0.02	0.01	0.01	0.01	0.60	<LD	<LD	<LD	<LD	0.02	0.01
cop/e-cop	2.00	0.50	0.67	0.50	0.50	1.00	1.00	0.50	1.00	0.50	1.00	1.00	1.00	0.86	0.40

and associated to natural contribution. Values observed for these compounds are 0.01 to 0.02 $\mu\text{g.g}^{-1}$ (coprostanol) and 0.01 to 0.03 $\mu\text{g.g}^{-1}$ (epicoprostanol) (Table 1).

Concentrations of coprostanone also can be attributed to natural contribution, since it is assigned to marine mammal feces, such as fecal sterols. Higher values ($>0.50 \mu\text{g.g}^{-1}$) in the most recent layers are found at REF (0.69 – 3.62 $\mu\text{g.g}^{-1}$) and PTH (0.32 – 0.52 $\mu\text{g.g}^{-1}$). Coprostanone from sewage input in these sites was not expected as none of the stations located in the two inlets discharge sewage in the region (Martins *et al.*, 2002).

In order to minimize the ambiguity of sources for these compounds, the use of numerical ratio involving fecal sterols are important tools in the differentiation of the sources of fecal organic matter. Venkatesan and Santiago (1989) have proposed specific indexes as the ratio between the concentration of coprostanol and epicoprostanol, as a way to distinguish the places studied in relation to the contribution of fecal sterols from human or marine mammals, specifically to the Antarctic environment. Values below 2.50 may indicate natural contribution, while values above 2.50 are strongly related to sewage. REF did not show values exceeding 2.50 in any section (1.67 ± 0.71), suggesting that the main source of sterols are marine mammals. The same results occurred to the sediment cores from Ezcurra Inlet (1.24 ± 0.57 – PTH; and 0.86 ± 0.40 – BAR) (Table 1).

The results of this ratio showed that the source of sedimentary fecal steroids is from natural contributions. At which point, background values can be determined, which are useful to evaluate a hypothetical sewage discharge in these regions. In Martel Inlet, the background values related to natural sources of fecal sterols (coprostanol+epicoprostanol) have been established as $0.19 \mu\text{g.g}^{-1}$ (Montone *et al.*, 2010). Considering the mean value obtained for each three cores, the background values to the sum of coprostanol and epicoprostanol, the purpose of this study are: (0.06 ± 0.02) $\mu\text{g.g}^{-1}$ (REF), (0.04 ± 0.03) $\mu\text{g.g}^{-1}$ (PTH) and

(0.03 ± 0.01) $\mu\text{g.g}^{-1}$ (BAR). Coprostanone did not present a regular distribution according to the depth, showing increased concentrations in the top layers and low levels in the bottom cores, in that it is difficult to establish background values to this compound. In this case, it is necessary to consider the degradation processes in top sections and unusual inputs in specific layers of REF and PTH.

Conclusions

Concentrations of fecal sterols (coprostanol and epicoprostanol) in all locations studied were $<0.10 \mu\text{g.g}^{-1}$, suggesting natural contributions of these compounds and corroborated by values found for the coprostanol/epicoprostanol ratio for all cores (<2.50). The background values related to natural sources of fecal sterols (coprostanol + epicoprostanol) to Mackellar and Ezcurra inlet were lower than Martel Inlet and this information can be used for future evaluations about sewage discharges from stations established in the inlets studied.

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References

- Colombo, J.C.; Pelletier, E.; Brochu, C.; Khall, M. & Catoggio, J.A. (1989) Determination of Hydrocarbon Sources Using *n*-Alkane and Polyaromatic Hydrocarbon Distribution Indexes. Case Study: Rio de La Plata Estuary, Argentina. *Environmental Science & Technology*, 23(7): 888-94.
- González-Oreja, J.A. & Saiz-Salinas, J.I. (1998). Short-term spatio-temporal changes in urban pollution by means of faecal sterols analysis. *Marine Pollution Bulletin*, 36(11): 868-75.
- Green, G. & Nichols, P.D. (1995). Hydrocarbons and sterols in marine sediments and soils at Davis Station, Antarctica: a survey for human-derived contaminants. *Antarctic Science*, 7(2): 137-44.
- Grimalt, J.O.; Fernandez, P.; Bayona, J.M. & Albaiges, J. (1990). Assessment of fecal sterols and ketones as indicator of urban sewage inputs to coastal waters. *Environmental Science & Technology*, 24(3): 357-63.
- Hughes, K.A. (2004). Reducing sewage pollution in the Antarctic marine environment using a sewage treatment plant. *Marine Pollution Bulletin*, 49(9-10): 850-53.
- Hughes, K.A. & Thompson, A. (2004). Distribution of sewage pollution around a maritime Antarctic research station indicated by faecal coliforms, *Clostridium perfringens* and faecal sterol markers. *Environmental Pollution*, 127(3): 315-21.
- Kawakami, S.K. & Montone, R.C. (2002) An efficient ethanol-based analytical protocol to quality fecal steroids in marine sediments. *Journal of the Brazilian Chemical Society*, 13(2): 226-32.
- Martins, C.C.; Venkatesan, M.I. & Montone, R.C. (2002). Sterols and linear alkylbenzenes in marine sediments from Admiralty Bay, King George Island, South Shetland Islands. *Antarctic Science*, 14(3): 244-52.
- Martins, C.C.; Montone, R.C.; Gamba, R.C. & Pellizari, V.H. (2005). Sterols and fecal indicator microorganisms in sediments from Admiralty Bay, Antarctica. *Brazilian Journal of Oceanography*, 53(1/2): 1-12.
- Martins, C.C.; Bícigo, M.C.; Rose, N.L.; Taniguchi, S.; Lourenço, R.A.; Figueira, R.C.L.; Mahiques, M.M. & Montone, R.C. (2010). Historical record of polycyclic aromatic hydrocarbons (PAHs) and spheroidal carbonaceous particles (SCPs) in marine sediment cores from Admiralty Bay, King George Island, Antarctica. *Environment Pollution*, 158(1): 192-200.
- Montone, R.C.; Martins, C.C.; Bícigo, M.C.; Taniguchi, S.; Silva, D.A.M.; Campos, L.S. & Weber, R.R. (2010). Distribution of sewage input in marine sediments around a maritime Antarctic research station indicated by molecular geochemical indicators. *Science of the Total Environment*, 408(20): 4665-71.
- Venkatesan, M.I.; Ruth, E. & Kaplan, I.R. (1986). Coprostanols in Antarctic marine sediments: A biomarker for marine mammals and not human pollution. *Marine Pollution Bulletin*, 17(12): 554-7.
- Venkatesan, M.I. & Santiago C.A. (1989). Sterols in oceans sediments: novel tracers to examine habitats of cetaceans, pinnipeds, penguins and humans. *Marine Biology*, 102: 431-7.
- Venkatesan, M.I. & Kaplan, I.R. (1990). Sedimentary Coprostanol as an index of sewage addition in Santa Monica Basin, southern California. *Environmental Science & Technology*, 24(2): 208-14.