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Assessment of historical fecal contamination in Curitiba, Brazil, in the last 400 years using fecal sterols

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HIGHLIGHTS

- Anthropogenic changes in Barigui River watershed are observed as from 1840.
- Fecal contamination started in 1820, high levels occurred only as from 1930.
- Sewage contamination strong related to demographic growth.

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ABSTRACT

A 400-year sedimentary record of the Barigui River was investigated using fecal biomarkers and nutrient distribution. The temporal variability in cholesterol, cholestanol, coprostanol, epicoprostanol, stigmastanol, stigmasterol, stigmastenol, sitosterol, and campesterol between 1600 and 2011 was assessed. Anthropogenic influences, such as deforestation and fecal contamination from humans and livestock, were observed from 1840. The sterol ratios exhibit evidence of hens, horses, cows, and an unknown herbivore, which may be a capybara (*Hydrochoerus hydrochaeris*), from 1820 and has been observed more markedly from 1970 onward. Human fecal contamination was detected from 1840 and was observed more markedly from 1930 due to population growth. Thus, the sanitation conditions and demographic growth of Curitiba seemed to be the main factors of human sewage pollution, as the coprostanol concentration over time was strongly correlated with the population growth ($r = 0.71$, $p < 0.001$) although diagenetic processes have also been observed.[1]

1. Introduction

Since the first human settlements, the discharge of sewage, especially untreated sewage, has been primarily responsible for the degradation of surface waters and aquatic environments, as well as of groundwater (Martins, 2007). The use of micro-organisms (e.g., *Escherichia coli*) to study and confirm domestic sewage contamination in water samples and sediments is limited due to a lack of specificity, light sensitivity (Chevremont et al., 2012), short lifetime, and little resistance to temperature variation (Bebianno and Mudge, 1997; Martins, 2007), making it impossible to study past contaminations.

Biomarkers can be used to assess the presence of sewage, both recent and past. They are organic molecules of biological origin, and are useful since their complex structure reveals information about the organisms from which they originated (Peters et al., 2005). Fecal sterols are a typical example, and are considered the "fingerprints" of fecal pollution due to their distribution in human and animal feces (Walker et al., 1982). In addition, due to their hydrophobic nature, sterols are easily associated with particulate material and sediment (Fattore et al., 1996). Some sterols, such as dinosterol, cholesterol, campesterol, β-sitosterol, β-sitostanol, cholestanol, and stigmasterol, among others, may originate from natural sources, while coprostanol, cholestanol, and epicoprostanol are exclusively of fecal origin (Bebianno and Mudge, 1997; Evershed et al., 2002). Previous studies have revealed that cats, pigs, cows, horses, sheep, and humans can transform cholesterol into coprostanol (Sherwin et al., 1993; Standley et al., 2000; Ali and Mudge, 2005). Nevertheless, the sterol profiles of herbivores such as sheep or cows are dominated by specific sterols, including sitosterol, stigmasterol, and campesterol, but contain very little coprostanol, while the sterol profiles of humans and pigs are dominated by coprostanol (Leeming et al., 1996). According to Meyers and Ishiwatari (1993), sterols can be used to examine (a) historical contributions by sewage to a water body and (b) the distribution and transportation of sewage into the environment and the age of the effluent.

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[1] Capsule: Record of fecal contamination in the Barigui River over time with an indication of potential sources and the relationship with demographic growth.

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0048-9697/

Several biological factors in the gut during metabolism control their distribution. This specificity results from the combination of three factors: i) animal's diet, ii) biosynthesis of endogenous sterols (when some sterols are not ingested or there is less sterol in the diet), and iii) biohydrogenation of sterols to stanols of various isomeric configurations by anaerobic bacteria in the tract of warm-blooded animals (Leeming et al., 1996). The combination of these three factors allows for the use of fecal sterols as specific biomarkers of animal feces in the sediment core, which can be used to indicate the main sources of fecal contamination. In addition, several studies have developed indices or proportions between the fecal sterols to discern the possible source: human, animal, sewage treatment plant, etc. (Leeming et al., 1996; Hudson et al., 2001; Grimalt et al., 1990). These indices have been used in different types of water (lakes, oceans, rivers, water treatment and sewage plants) (Furtula et al., 2012) and different matrices worldwide (Froehner and Sanez, 2013).

One factor to take into account is the propensity of sterols to undergo diagenetic processes, coupled with their ubiquitous occurrence, which makes the usefulness of such measurements questionable in the majority of fecal pollution studies. Diagenesis affects the organic matter in the sediment and takes place prior to deposition and during the early stages of burial under conditions of relatively low temperature and pressure, leading to microbiological, chemical, and physical processes of transformation (Killops and Killops, 1993; Ali and Mudge, 2005). Therefore, some sterols, such as coprostanol and cholesterol, under anoxic conditions are converted into other sterols, such as epicoprostanol and cholestanol, respectively. This production in-situ makes sterols less reliable as indicators of fecal contamination.

The main objective of this study was to assess the historical pollution in Curitiba, Southern Brazil, over the last 400 years by using fecal sterols in a sediment core of the Barigui River. Some sterol ratios were investigated in order to differentiate between human and animal origins. In addition, some specific sterol ratios, encompassing nutrient chemical parameters, were investigated in order to observe the effect of diagenesis over time. This study also aimed to contribute to better environmental management using fecal steroids to restore past and natural conditions.

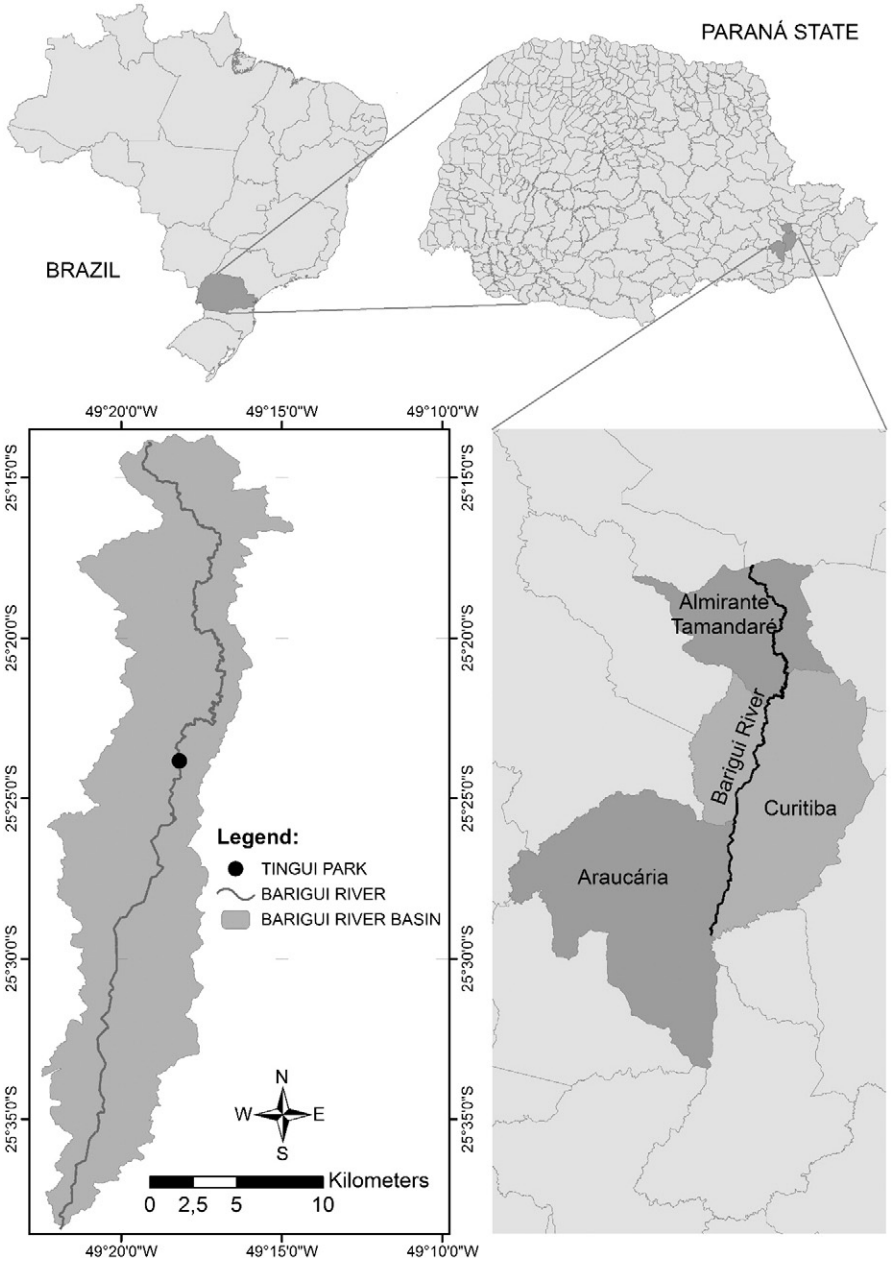

Fig. 1. Map of the Barigui Basin and sampling site.

2. Materials and methods

2.1. Study area and sampling

The Barigui River watershed is located between 25°13′24″ and 25°38′23″ South and 49°15′00″ West, oriented in the north–south direction toward the cities of Almirante Tamandare, Curitiba, and Araucaria (Fig. 1). The Barigui River is 67 km long, draining a watershed of 279 km^2 with 120 km^2 of drainage located in the municipality of Almirante Tamandare, 144 km^2 in the municipality of Curitiba, and 15 km^2 in the municipality of Araucaria.

The sampling station is located inside Tingui Park (latitude 25°23′ 55.81″ and longitude 37°35′33.84″). This site was selected based on previous monitoring campaigns that defined this area as suitable for collection, since this area has been strongly influenced by urbanization over time (Froehner and Martins, 2008; Dombroski et al., 2012; Machado et al., 2014).

A PVC tube with 6 cm internal diameter (i.d.) was used for collection of the core in November 2011. The sediment core was sectioned every 2 cm from the sediment–water interface to a depth of 40 cm, every 4 cm between depths of 40 cm and 60 cm, and every 10 cm between depths of 60 and 100 cm. The subsamples were frozen at −20 °C and lyophilized prior to analysis. Total organic carbon, phosphorus and nitrogen contents, dating, and sterol contents were analyzed for each fraction.

2.2. Dating of the sedimentary core

Details of the dating analyses are found elsewhere (Machado et al., 2014), and are herein described just briefly. In order to determine the date of each sediment core segment, radionuclides ^{210}Pb, ^{226}Ra, and ^{137}Cs counting were analyzed in an EG&G ORTEC® low-background gamma spectrometer (hyper-pure Ge, model GMX25190P) following the method described by Figueira (2000). Three certified reference materials: IAEA-326 (soil), IAEA-327 (soil), and IAEA-385 (marine sediment) were used to evaluate the precision and accuracy of the methodology. Sedimentation rates were calculated by CIC (constant initial concentration) and CRS (constant rate of supply) models for unsupported ^{210}Pb. The CIC model assumes that there is a continuous sediment input to the system, and it results in a time-integrated sedimentation rate, i.e., a mean sedimentation rate for the core. Meanwhile, the CRS model is intended for dating sediment profiles, but can be used to assess time-variable sedimentation rates. The age of sample deposition (I) was established, considering the sample depth in the core (z) and the time of core collection (A_0), and sedimentation rate (v), according to $I = A_0 - (v/z)$, and considering the time of core collection, year 2011. The ages of the samples until 1760 present the accuracy of the method. The ages of samples before 1760 are attributed to historic dates, such as the foundation of Curitiba and previous features of the area reported by historians. The sedimentation rates and age dating results are presented in vertical profiles graphs, Fig. S1 in the Supplementary materials.

2.3. Phosphorus, nitrogen, and total organic carbon analysis

A phosphorus analysis was carried out based on methods described by Mater et al. (2004). For each sample, two subsamples of 0.5 g dried sediment were used for the phosphorus analyses. One subsample was initially placed in a furnace for 1 h at 500 °C, after which both of the subsamples were shaken with 10 mL 1.0 M HCl solution for 1 h. After centrifugation (6000 rpm, 10 min), the supernatants were digested for 4 h with 1.6 mL potassium persulfate and 4.5 M sulphuric acid solution at 80 °C. The extracted phosphorus from the calcinated fraction was considered total phosphorus (TP), while the extracted phosphorus without calcination was considered inorganic phosphorus (IP). After extraction, both the TP and IP contents were quantified as orthophosphate using the molybdate ascorbic acid method (Koroleff, 1983). The organic fraction contents of phosphorous (OP) were obtained by the difference between the TP and IP contents.

Total organic carbon (TOC) and total nitrogen (TN) contents were measured using a vario Micro cube. The TOC contents were determined after acid digestion of the carbonate fraction with hydrochloric acid 16% v/v (Holtvoeth et al., 2010).

2.4. Extraction, separation, and analysis of sterols

The procedure that is described by Holtvoeth et al. (2010) was followed with minor modifications. The bulk sediment (5 g) was extracted using an accelerated solvent extractor (ASE, Dionex). Activated copper was added directly after the extraction in order to remove elemental sulfur. The sterol fractions were isolated using 7 mm i.d. columns filled with silica gel, eluted with hexane (3 × 4 mL — Fraction 1), then with DCM (3 × 4 mL — Fraction 2) and finally with methanol (3 × 4 mL — Fraction 3). Internal standard, 5α(H)-cholestane, was added to the last fraction and dried under a gentle flow of nitrogen. The extract was derivatized using N,O-bis-(trimethylsilyl)-trifluoroacetamide (+1% trimethylchlorosilane) at 65 °C for 30 min. After derivatization, it was dried and dissolved in 100 μL of DCM prior to analysis. Recovery tests were greater than 70% for all sterols. Gas chromatographic and mass spectrometric analyses (GC/MS) of the derivatized sterols were performed using a Trace 2000 Series gas chromatograph (GC) fitted with a J&W Scientific D-5MS capillary column (60 m, 0.25 mm i.d.; 5% phenyl/95% methylpolysiloxane, 0.1 μm film thickness). The carrier gas was helium at 1.6 $mL \cdot min^{-1}$. The oven temperature was programmed to increase from 60 °C to 170 °C at 6 $°C \cdot min^{-1}$, and after 1 min the temperature increased to 315 °C at 2.5 $°C \cdot min^{-1}$ and held for 10 min. The column was fed directly into a ThermoQuest Finnigan TSQ 7000 mass spectrometer (MS). The operating conditions were ionization potential 70 eV, source temperature 215 °C, and trap current 300 μA. The mass data were collected at a resolution of 600, cycling every second from 50 to 600 Thompsons. The sterols were identified by their mass spectra, from their relative retention times, and by comparison with authentic standards. The sterols were then quantified by relating their peak area to the peak area of the internal standard. Limit of quantification was 10 $ng \cdot g^{-1}$ of sediment.

3. Results and discussion

Analyzed inorganic parameters and sterol contents are presented. In addition, some important ratios used to elucidate potential sources of pollution and human activities were calculated. Those results and the calculated ratios were plotted against the data obtained as described in Section 2.2 in order to observe their temporal variability. In general, sterol content is presented in ng per g of dried sediment, with exceptions mentioned.

3.1. Dating results

The vertical profiles of ^{210}Pb and ^{137}Cs in Bq kg^{-1} and sedimentation rates in the subsamples from the Barigui River are presented in Fig. S1 in the Supplementary materials. The location of the ^{137}Cs peak of 1963, reported in Machado et al. (2014), relative to the global maximum fallout from a past nuclear explosion was used to compare and verify the dating results that are presented in this study.

3.2. Temporal variability of nutrients

The TP, IP, OP, TOC, and TN contents were assessed to detect variation in the nutrient load to the Barigui River. The results, in percentage related to dried mass of sediment, are presented in Table S1 in the Supplementary materials. Those parameters are impacted directly by human activities, such as deforestation and untreated sewage

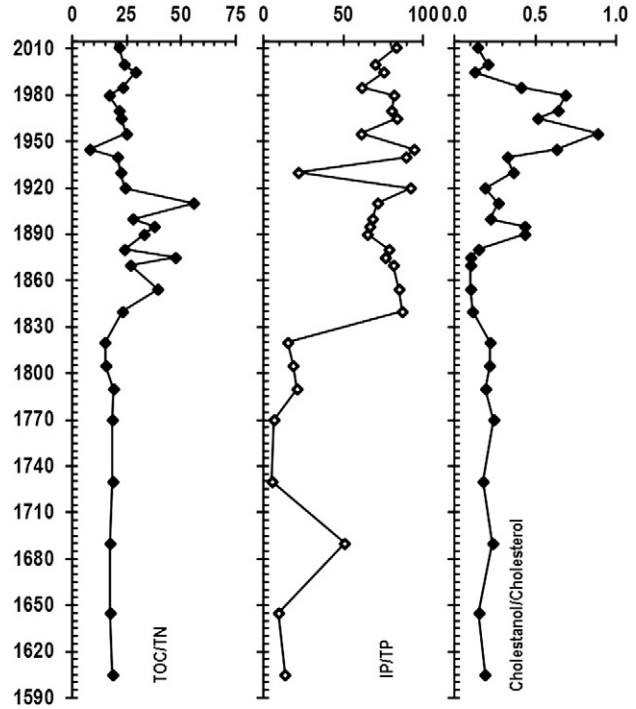

Fig. 2. Temporal variability of TOC/TN, IP/TP, and cholestanol/cholesterol ratios in the sediment core of the Barigui River.

discharges. The contents of TOC ranged from 0.73 to 6.4%, those of TN from 0.03 to 0.33%, and those of TP from 0.25 to 1.9% (Table S1).

TOC/TN ratios greater than 20 indicate terrestrial source predominance, including fecal contamination (Hahn et al., 2013), whereas values between 5 and 10 are attributed to phytoplankton and primary productivity (Meyers and Ishiwatari, 1993). In this study, the TOC/TN ratios ranged from 7.9 to 55.5 (Fig. 2). In the period from 1605 to 1820, these ratios are consistently less than 20, while from the period 1840 to 1920 they suddenly increased, and a peak was observed in 1910 (Fig. 2). Among the terrestrial sources of organic matter, detritus from deforestation are the main causes of nutrient input into lakes and rivers because plants are removed and entrained throughout the watershed, and soil is exposed, contributing to erosion in the catchment area. In the same area, Machado et al. (2014) have demonstrated the impact of grass and wood combustion due to PAH distribution. Therefore, the increase of the TOC/TN ratios in the period 1840 to 1920 as observed in the present study seems to be related to the removal of vegetation, whether by deforestation or bush fire.

The anthropogenic influence observed in the TOC/TN ratios is also detected with the phosphorus content. Considering sediments without sewage contamination, the IP contents represent approximately 60% of the TP (Mater et al., 2004). In the sediment core, values greater than 60% are observed as far back as 1840 (Fig. 2), consistent with the changes that were observed in the TOC/TN ratios (Fig. 2).

Thus, according to the phosphorus analysis and TOC/TN ratios, the evidence suggests that since 1840, anthropogenic interferences have changed the studied area. Deforestation and sewage contamination may be the result of urbanization during the expansion of the city, as in 1850 the population of Curitiba was almost 40% higher than the population in 1800 (IPPUC, 2009).

3.3. Temporal variability of sterols

Nine sterols, including cholesterol, cholestanol, coprostanol, epicoprostanol, stigmastanol, stigmasterol, sitosterol, campesterol, and stigmastenol, were identified in the core sediment representing the period from 1605 to 2011. The temporal variability of those sterols is presented in Fig. 3.

Sitosterol was the most prominent sterol (125–1417 $ng \cdot g^{-1}$), is typical of vascular plants (Meyer, 1997) and is present in the feces of herbivores (Leeming et al., 1996). Two other sterols typically detected together with sitosterol in sediments are stigmasterol (17–653 $ng \cdot g^{-1}$) and campesterol (9.0–151 $ng \cdot g^{-1}$). Concentrations of stigmastanol and stigmastenol ranged from 59.7 to 373 $ng \cdot g^{-1}$ and from 4.4 to 303 $ng \cdot g^{-1}$, respectively (Fig. 3). Both are found in higher plants (Hudson et al., 2001) and consequently in the feces of herbivores (Leeming et al., 1996).

Cholesterol was the second most prominent sterol (50–710 $ng \cdot g^{-1}$) and can be traced from the human and animal feces and carcasses of fish and zooplankton (Ali and Mudge, 2005). Cholesterol, when reduced under anoxic conditions by microorganisms in the guts of mammals, produce stanols, which later under microbial reduction produce cholestanol, or in the case of intensive microbial reworking, into their epimer, epicoprostanol (Evershed et al., 2002). In this study, cholestanol and epicoprostanol were detected at concentrations of 11.7–333 $ng \cdot g^{-1}$ and 2.6–203 $ng \cdot g^{-1}$, respectively (Fig. 3). Cholestanol does not have specific sources, but it is present in both vegetation and animals (Biache and Philp, 2013), while epicoprostanol is present in relatively higher amounts in the feces of nonhuman mammals and in treated sewage (Mudge et al., 1999; Standley et al., 2000).

In the Barigui River core, concentrations of coprostanol ranged from 13.6 to 447 $ng \cdot g^{-1}$ (Fig. 3). Coprostanol is one of the main sterols used as an environmental biomarker to assess fecal contamination in fresh waters and that undergo reduction via the bacterial hydrogenation of cholesterol in the gut of warm-blooded animals (Fernandes et al., 1999).

In general, temporal sterol variability presented three distinct trends, most likely associated with the sources. Stigmastanol, campesterol, stigmastenol, and cholestanol showed high concentrations from 1840 and peaked in 1955. These sterols are found in terrestrial plants and herbivore feces, among other sources (Biache and Philp, 2013; Hudson et al., 2001). Meanwhile, despite similar sources, sitosterol, stigmasterol, and cholesterol showed a different trend. Higher concentrations of these sterols were observed from 1730 followed by fluctuations that have persisted, whereas coprostanol and epicoprostanol demonstrated fewer fluctuations and higher concentrations from 1930 (Fig. 3).

Sterols are hydrophobic compounds and are easily trapped by OM, which may affect their transport and preservation. Taking that into account, the temporal sterol variability relative to the TOC contents was calculated and is shown in Fig. S2 in the Supplementary materials. Significant differences were observed between the vertical sterol profiles per gram of sediment and the sterols' relative TOC contents. In general, fewer fluctuations and more defined peaks were observed with epicoprostanol, cholesterol, campesterol, and stigmasterol relative to the TOC contents. Cholestanol was unique in that it did not present significant differences in its temporal variability relative to the organic carbon content, suggesting that it is less influenced by the OM content of the sediment (Fig. S2).

3.4. Sterols and diagenetic process

The cholestanol/cholesterol ratio can be used to assess the level of diagenetic transformation in the sedimentary environment (Wakeham and Canuel, 2006). In this study, the cholestanol/cholesterol ratio was calculated, and its temporal variability is shown in Fig. 2. Values close to and greater than 0.5 were observed between 1890 and 1980, indicating a significant influence of diagenetic processes during this period (Carreira et al., 2001). This increase in the diagenetic process may be related to an early increase in the TOC/TN ratio between 1840 and 1920 (Fig. 2), when terrestrial OM input, with microorganisms and nutrients in the soils, could have affected the primary productivity and redox conditions at the water/sediment interface (Ali and Mudge, 2005). In the other periods, the cholestanol/cholesterol ratio was

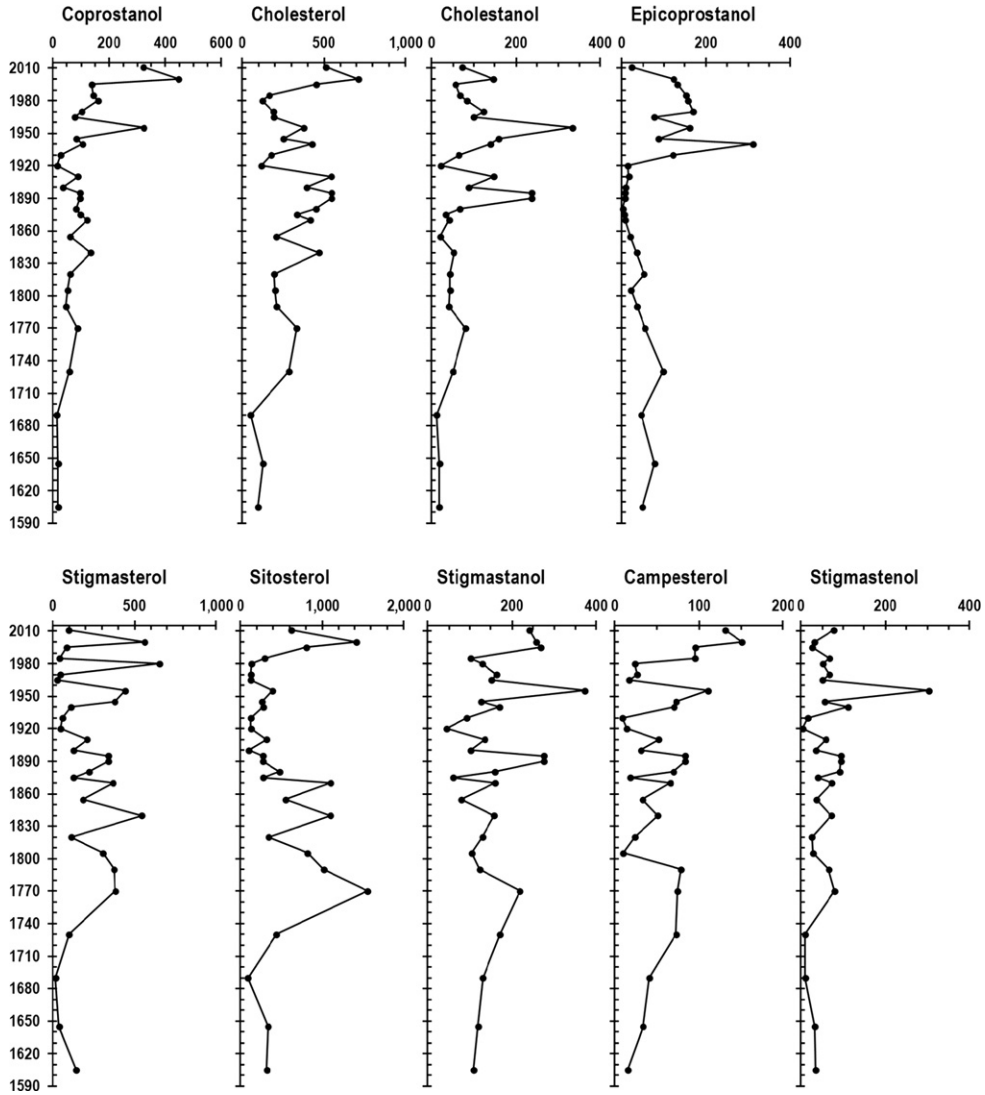

Fig. 3. Temporal variability of nine sterols (ng·g^{-1} of dry sediment) in the sediment core of the Barigui River. Limit of quantification = 10 ng/g-dry sediment.

between 0.1 and 0.5, indicative of recently fresh OM produced by autotrophic processes, (Araujo et al., 2011).

3.5. Sterol ratios for identifying human and animal fecal pollution

Diverse sterol ratios have been proposed to identify human fecal contamination or to discriminate between several types of animal and human fecal sources, since those animals can convert cholesterol into coprostanol and based on qualitative and quantitative differences in the production of sterols by humans and animals.

Coprostanol/coprostanol + cholestanol (Ratio 1) ratios greater than 0.7 indicate fecal contamination (Grimalt et al., 1990; Araujo et al., 2011; Furtula et al., 2012). In this study, values close to and greater than 0.7 were observed in two periods: from 1840 until 1875 and after 1980 (Fig. 4). Diagenetic processes seem to have affected the values of this ratio from 1890 to 1980, when these values decreased, and this period also experienced higher cholestanol/cholesterol ratios (>0.5). Therefore, evidence of fecal contamination has been observed as far back as 1840 and is consistent with the anthropogenic influence that was observed in the phosphorus distribution (Table S1 in the Supplementary materials) and the change in the TOC/TN ratio, both from 1840 (Fig. 2).

In addition to the coprostanol/coprostanol + cholestanol ratio, four others were used (Table 1): coprostanol/cholestanol + cholesterol ratio (Ratio 2) (Chan et al., 1998; Fattore et al., 1996); stigmasterol + sitosterol + stigmastanol + stigmastenol + campesterol/coprostanol + colestanol + colesterol + epicoprostanol stanol ratio (Ratio 3); campesterol + sitosterol/cholesterol ratio (C_{28} + C_{29}/C_{27}, Ratio 4) (Jarde et al., 2007a,b); and campesterol + sitosterol + stigmastenol/cholesterol + coprostanol ratio (Ratio 5) (Jarde et al., 2007a). These ratios were used because they can discriminate between omnivore feces and herbivore feces sources (Leeming et al., 1996). Table 1 presents specific values of these ratios to distinguish among human, hen, cow, sheep, horse, and pig sources.

These ratios were applied in the Barigui sediment core in the period from 1822 to 2011, span where the fecal contamination was more evident, and its temporal variability can be observed in Fig. 4. Observing the profile of the ratios in this span, the core sediment can be categorized into three periods. Period 1: from 1820 to 1870 with an increase in values, most likely due to the beginning of the fecal contamination; Period 2: between 1875 and 1970, when the ratios decreased due to diagenesis processes; and Period 3: from 1980 to 2011, when the ratio values increased slightly.

Ratio 2 indicates human fecal contamination. Values greater than 0.2 are observed as far back as 1820 (Fig. 4), indicating the presence of human feces according to the classification of Chan et al. (1998) and Fattore et al. (1996). However, compared with the values in Table 1 for human feces (9.5), the results of Ratio 2 seem to be strongly affected by diagenesis or by another fecal source that decreases the values of Ratio 2 in the Barigui core.

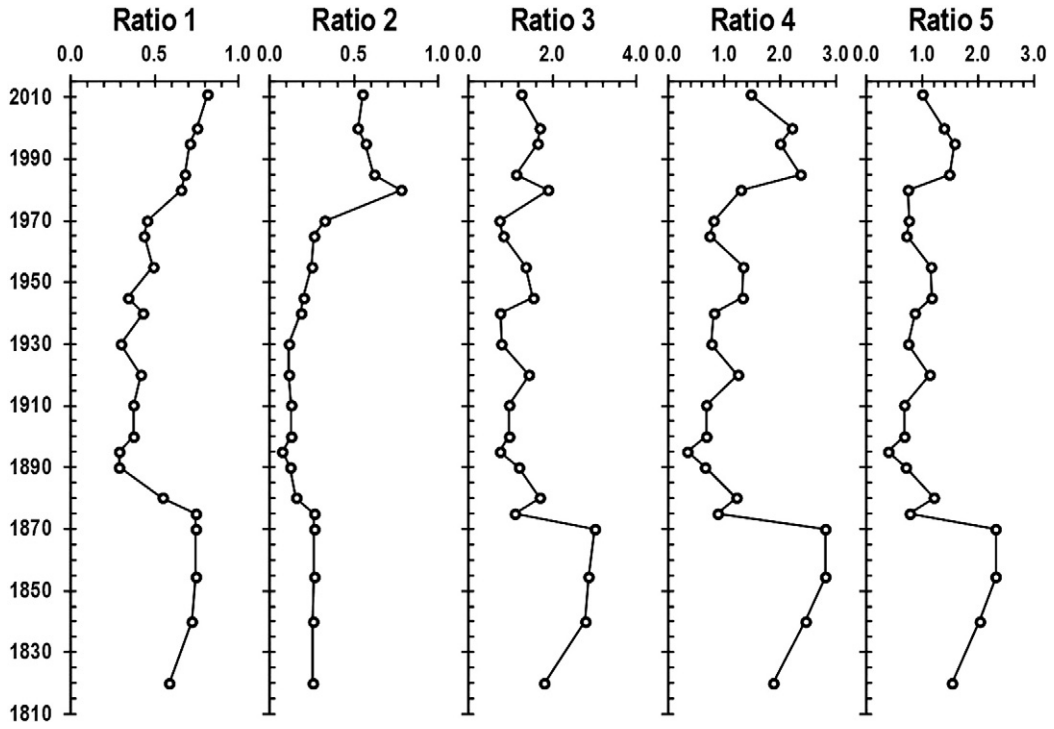

Fig. 4. Temporal variability of sterol ratios applied for assessment of fecal source contamination. Ratio 1 = coprostanol/coprostanol + cholestanol; Ratio 2 = coprostanol/coprostanol + cholesterol; Ratio 3 = C_{28} + C_{29}/C_{27}; Ratio 4 = campesterol + sitosterol/cholesterol; ratio 5 = campesterol + sitosterol + stigmastenol/cholesterol + coprostanol.

Values ranging from 1.5 to 3 were observed for Ratios 3, 4, and 5 (Fig. 4) in Period 1. These values suggest animal feces sources when compared with reference values in Table 1. Using Ratio 3, these values indicate hen, sheep, and horse feces sources (Table 1). The same animals except for horse are indicated by the values of Ratio 4, and only hen is indicated by the values of Ratio 5 (Table 1).

During Period 2, Ratio 2 decreased to 0.2 and Ratios 3, 4, and 5 to approximately 1.0 (Fig. 4). This decrease may be related to the diagenetic influence and to the increase in the human fecal contamination, as human feces presents small values in Ratios 3, 4, and 5 (0.05–0.46, Table 1). This decrease is also coincident with the slight increase in the coprostanol concentration from 1870, as observed in Figs. 3 and S2. Herbivore sources such as cow and sheep also must be considered, as values of approximately 1.0 are observed for herbivore feces in Ratios 3 and 4. During Period 3, the ratios increased again from 0.5 to 0.8 in Ratio 2 and from 1 to 2.5 in Ratios 3, 4, and 5, suggesting a decrease in the diagenetic processes (early in this period) and herbivore (sheep) fecal contribution.

Thus, evidence of hen, sheep, and possibly horse were observed during the period from 1820 to 1870. Herbivores such as cow and sheep are more strongly evident from 1975, while sheep evidence is observed even currently. The contribution of human feces is evident as far back as 1820 and persists, despite being affected by other fecal sources and diagenetic processes.

Although the presence of sheep was evident in Ratios 3, 4, and 5, the local history does not confirm this information. Cows were very common in the studied area in the past, but sheep were not (Wachowitcz, 1995). In contrast, the capybara (*Hydrochoerus hydrochaeris*), a giant rodent herbivore native to Brazil, with a high reproduction rate, can be easily found in several lakes in Curitiba, where there is a large food availability and no natural predators are around (Almeida et al., 2013). The sterol distribution in capybara feces is unknown. Thus, considering the same diet and a similar sterol composition among herbivores (Leeming et al., 1996), Ratios 3, 4, and 5, which suggested the presence of sheep, might instead suggest the presence of capybara. Then, their presence could explain the strong evidence of herbivore feces contamination from 1870, when the city began the urbanization process (IPPUC, 2009; Wachowitcz, 1995).

Table 1
Reference values obtained from the application of omnivore and herbivore feces results reported by Leeming et al. (1996) in ratios previously selected.

Ratio	Human	Hen	Cow	Sheep	Horse	Pig
Ratio 2*	9.53	0.05	0.79	1.06	0.67	2.05
Ratio 3**	0.05	1.87	1.35	1.96	3.00	0.31
Ratio 4**	0.46	1.81	1.13	2.18	6.3	1.09
Ratio 5	0.05	1.78	0.50	1.10	3.70	0.35

Ratio 2 = coprostanol/coprostanol + cholesterol; Ratio 3 = C_{28} + C_{29}/C_{27}; Ratio 4 = campesterol + sitosterol + stigmastenol/cholesterol + coprostanol.
Note: *Chan et al. (1998) and Fattore et al. (1996); **Jarde et al. (2007a,b).

3.6. Sanitation conditions and relationship with demographic growth

In addition to human fecal influence identification, sterol ratios have also been used as chemical markers for detecting treated sewage effluents. The coprostanol + epicoprostanol/cholesterol ratio reported by Standley et al. (2000) for sediments suggests that values lower than 0.1 are observed in sediment with wastewater from sewage treatment plants, meanwhile for the same ratio values greater than 0.5 indicate untreated wastewaters (Furtula et al., 2012). Sherwin et al. (1993) reported that the coprostanol + epicoprostanol/Σtotal sterols ratio can be used to identify untreated sewage contamination when values are greater than 0.2. These two ratios were calculated and are presented in Fig. S3, in the Supplementary materials. Based on the coprostanol + epicoprostanol/cholesterol ratio values higher than 0.1, which indicate no sewage treatment plant influence and is consistent with the characteristics of the area, this area has no collection system. However, a significant increase in values greater than 0.5 were observed from 1930, indicating an increased human influence. This observation is supported by a significant increase in the coprostanol and epicoprostanol concentrations from 1930 (Figs. 3 and S2). The same pattern is observed for the coprostanol + epicoprostanol/Σtotal sterol ratio, indicating the influence of untreated wastewater from 1930, when the ratio was greater than 0.20. Furthermore, the coprostanol + epicoprostanol/cholesterol ratio is significantly correlated with the total phosphorus content ($r = 0.54$,

$p < 0.002$), showing that this nutrient, these sterols, and these ratios present a similar trend.

The history of Curitiba is most likely related to the abovementioned results. Sanitation began with 2632 sewage connections in 1917, when the population included approximately 71,000 inhabitants (IPPUC, 2009; Wachowitcz, 1995). Only 62 years later, in 1979, when the population included almost 1 million inhabitants, the first sewage treatment plant was built, and the sanitation conditions improved (IPPUC, 2009; Wachowitcz, 1995). During this period, the city suffered from urban disorder and obvious sewage pollution. Sewage under basic conditions or any treatment is often stored in soil or driven to rivers. Thus, climatic events such as floods may have contributed to the contamination of rivers by untreated sewage. Geissler and Loch (2004) reported floods in the area in 1932, 1968, 1975, 1976, and 1983, which may be responsible for the peaks in the sterol concentrations in Figs. 3 and S2.

The amount and distribution of sterols are influenced by demographic growth and sanitation conditions. A significant correlation between demographic growth and sterols was found ($r = 0.71$, $p < 0.001$), most likely due to the influence of animal feces as sources of sterols and the high amounts of coprostanol in human feces. The demographic indices and historical records of sedimentary coprostanol are illustrated in Fig. 5. The fast population growth from 1930 was reflected in peaks of coprostanol, primarily in 1935, 1955, 1980, and 2000 (Fig. 5). This relationship demonstrates that, although coprostanol has limitations as a biomarker due to diagenetic processes, it still presents great potential to represent human fecal contamination over time.

4. Conclusions

Fecal biomarkers combined with nutrient measurements of the Barigui River sediment record were used to assess the evolution and increase in fecal contamination over the last 400 years in Curitiba. Anthropogenic changes as far back as 1840 are reflected by phosphorus analysis and the TOC/TN and coprostanol/coprostanol + cholesterol ratios. Between 1840 and 1920, the TOC/TN ratio showed an increase in nutrient input, most likely related to the deforestation of the area during this period. In response to the nutrient input and soil microorganisms, significant diagenetic processes were triggered in the Barigui River sediment between 1890 and 1980. Despite the diagenesis of OM, fecal contamination from humans and animals is observed. The presence of hens, horses, cows, and possibly capybaras is evident as far back as 1820, while significant human fecal contamination seems to have begun in 1870 though higher levels of pollution are observed from 1930 due to the increased population. Demographic growth associated with the sanitation conditions seems to be the main factor in human sewage contamination in the Barigui River, which is reflected by the distribution of coprostanol over time.

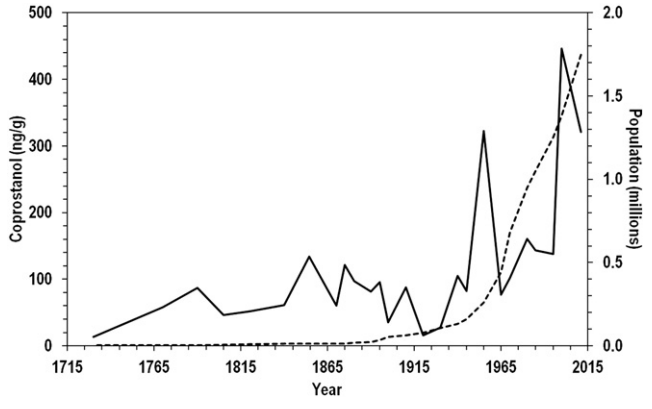

Fig. 5. Chronological profile of Curitiba demographic growth and the content of coprostanol in the sediment core ($ng \cdot g^{-1}$ dry sediment) from the Barigui River.

Overall, this work could help to elucidate the use of biomarkers in determining past pollution scenarios and how detailed information can be gained through the sediment record.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2014.06.104.

References

Ali MM, Mudge SM. Lipid geochemistry in a sediment core from Conwy Estuary, North Wales. Sains Malaysiana 2005;34:23–33.
Almeida ARA, Arzua M, Trindade PWS, Silva Junior A. Capybaras (*Hydrochoerus hydrochaeris*, Linnaeus, 1766) (Mammalia: Rodentia) in green areas of Curitiba (PR). Biol Stud 2013;35:9–16.
Araujo MP, Costa TLF, Carreira RS. Sterols as indicators of accumulation of domestic sewage in sediments of a tropical system lagoon-estuarine (Mundaú — Manguaba, AL). Quim Nova 2011;34:64–70.
Bebianno MJ, Mudge SM. Sewage contamination fallowing an accidental spillage in the Ria Formosa, Portugal. Mar Pollut Bull 1997;34:163–70.
Biache C, Philp RP. The use of sterol distributions combined with compound specific isotope analyses as a tool to identify the origin of fecal contamination in rivers. Water Res 2013;47:1201–8.
Carreira R, Wagener ALR, Fillman T, Readman JW. Distribution of coprostanol (5β(H) cholestan-3β-ol) in surface sediments of Guanabara Basin: indicator of recent pollution by domestic sewage. Quim Nova 2001;24:37–42.
Chan KH, Lam MHW, Poon KF, Yeung HY, Chiu TKT. Application of sedimentary fecal stanols and sterols in tracing sewage pollution in coastal waters. Water Res 1998;32:225–35.
Chevremont AC, Farnet AM, Coulomb B, Boudenne JL. Effect of coupled UV-A and UV-C LEDs on both microbiological and chemical pollution of urban wastewaters. Sci Total Environ 2012;426:304–10.
Dombroski LF, Fernandes CVS, Siqueira M. Monitoring the behavior and distribution of particle size, organic carbon, nutrients and metals in bottom sediments of the Iguassu River: implication for sediment quality data. X ENES — Brazilian Meeting on Sediments, 1st ed., 1. Porto Alegre: ABRH; 2012. p. 314–30.
Evershed RP, Bull ID, Lockheart MJ, Elhmmali MM, Roberts DJ. The origin of faeces by means of biomarker detection. Environ Int 2002;27:647–54.
Fattore E, Benfenati E, Marelli R, Cools E, Ranelli R. Sterols in sediment samples from Venice Lagoon, Italy. Chemosphere 1996;33:2383–93.
Fernandes MB, Sicre M-A, Cardoso JN, Macedo SJ. Sedimentary 4-desmethyl sterols and n-alkanols in an eutrophic urban estuary, Capibaribe River, Brazil. J Sci Total Environ 1999;231:1–16.
Figueira RLC. Inventory of artificial radionuclides in seawater and sediments of the southern coast of Brazil [PhD thesis] Brazil: University of Sao Paulo; 2000.
Froehner S, Martins RF. Evaluation of the chemical composition of sediments from Rio Barigüi Metropolitan region of Curitiba. Quim Nova 2008;31:2020–46.
Froehner S, Sanez J. Evaluation of potential sewage contamination by fecal sterol biomarkers adsorbed in natural biofilms. Environ Sci Process Impacts 2013;15:2080–6.
Furtula V, Liu J, Chambers P, Osachoff H, Kennedy C, Harkness J. Sewage treatment plants efficiencies in removal of sterols and sterol ratios as indicators of fecal contamination sources. Water Air Soil Pollut 2012;223:1017–31.
Geissler HJ, Loch REN. Analysis of historical floods in Curitiba-PR: proposed measures and observed consequences. Brazilian Symposium on Natural Disaster. 1, 2004. Florianópois: GEDN/UFSC; 2004. p. 507–23. [(CD-ROM), in Brazilian Portuguese].
Grimalt JO, Fernandez P, Bayona JM, Albaiges J. Assessment of fecal sterols and ketones as indicators of urban sewage inputs to coastal. Water Sci Technol 1990;24:357–62.
Hahn A, Kliem P, Ohlendorf C, Zolitschka B, Rosén P. Climate induced changes as registered in inorganic and organic sediment components from Laguna Potrok Aike (Argentina) during the past 51 ka. Quat Sci Rev 2013;2013(71):154–66.
Holtvoeth J, Vogel H, Wagner B, Wolff G. Lipid biomarkers in Holocene and glacial sediments from ancient Lake Ohrid (Macedonia, Albania). Biogeosciences 2010;7:3473–89.

Hudson ED, Parrish CC, Helleur RJ. Biogeochemistry of sterols in plankton, settling particles and recent sediments in a cold ocean ecosystem Trinity Bay, Newfoundland. Mar Chem 2001;76:253–70.
IPPUC (Institute for Urban Panning of Curitiba). Curitiba in data — indicators of social and economic development of Curitiba. Data of Tabulation, 22ª Ed., Curitiba — Brazil; 2009.
Jarde E, Gruau G, Mansuy-Huault L. Detection of manure-derived organic compounds in rivers draining agricultural areas of intensive manure spreading. Appl Geochem 2007a;22:1814–24.
Jarde E, Gruau G, Mansuy-Huault L. Using sterols to detect pig slurry contribution to soil organic matter. Water Air Soil Pollut 2007b;178:169–78.
Killops S, Killops VIntroduction to organic geochemistry1st ed. Australia: Blackwell Publishing; 1993.
Koroleff FIn: Grasshoff K, Ehrhardt M, Kremling K, editors. Methods of seawater analysis. 2nd ed. Weinheim: Verlag Chemie; 1983. p. 125.
Leeming R, Ball A, Ashbolt N, Nichols P. Using faecal sterols humans and animals to distinguish faecal pollution in receiving waters. Water Res 1996;30:2893–900.
Machado KS, Figueira RCL, Cocco LC, Froehner S, Fernandes CVS, Ferreira PAL. Sedimentary record of PAHs in the Barigui River and its relation to the socioeconomic development of Curitiba, Brazil. Sci Total Environ 2014;482–483:42–52.
Martins C. Natural and anthropogenic sterols inputs in surface sediments of Patos Lagoon, Brazil. J Braz Chem Soc 2007;18:106–15.
Mater L, Alexandre MR, Hansel FA, Madureira LAS. Assessment of lipid compounds and phosphorus in mangrove sediments of Santa Catarina Island, SC, Brazil. J Braz Chem Soc 2004;15:725–34.
Meyer PA. Organic geochemical proxies of paleoceanographic, paleolimnologic, and paleoclimatic process. Org Geochem 1997;27:213–50.
Meyers PA, Ishiwatari R. Lacustrine organic geochemistry: overview of indicators of organic matter sources and diagenesis in lake sediments. Org Geochem 1993;20:867–900.
Mudge SM, Bebbiano MJF, East JA, Barreira LA. Sterols in the Ria Formosa Lagoon, Portugal. Water Resour 1999;33:1038–49.
Peters KE, Walters CC, Moldowan JMThe biomarker guide, vol. 1. Biomarkers and isotopes in the environment and human history; 2005. [Ed. Cambridge, vol. I].
Sherwin MR, Van Vleet ES, Fossato VU, Dolci F. Coprostanol (5β-cholestan-3β-ol) in lagoonal sediments and mussels of Venice, Italy. Mar Pollut Bull 1993;26:501–7.
Standley LJ, Kaplan L, Smith D. Molecular tracers of organic matter sources to surface water resources. Environ Sci Technol 2000;34:3124–30.
Wachowitcz RCHistory of Paraná1st ed. Curitiba: Editora Gráfica Vicentina; 1995. p. 32.
Wakeham SG, Canuel EAIn: Volkman JK, editor. Handbook of environmental chemistry. Reactions and processes 2 (N)Berlin: Springer; 2006. [cap. 6].
Walker R, Wun C, Litsky W. Coprostanol as indicator of faecal pollution. Crit Rev Environ Control 1982;24:208–14.