Coprostanol levels and organic enrichment in sediments of the Bilbao estuary (north of Spain)

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Abstract – An assessment was made of the distribution of the faecal sterol coprostanol (COP) in sediments in the Bilbao estuary. Concentrations of COP ranged from 135.15 to 10.77 μg g^{-1} dry weight, from the inner to the open estuary, with a mean value of 51.43 μg g^{-1}; the highest concentrations were found near the mouths of several tributaries flowing into the estuary. Comparisons with other published values indicate that sediments from the Bilbao estuary are affected by sewage pollution in a similar degree to that reported for other estuaries in the world. Organic enrichment and anaerobic sediment conditions prevail in three-fourths of its watercourse bed. Since the spatial distribution of several organic sedimentary parameters could be clearly correlated with COP levels, we can conclude the existence of major pollution by the addition of untreated sewage, coming from mainly domestic sources. The implementation of a sewerage treatment plan by the local water authority needs the monitoring of reliable sewage tracers, such as faecal sterols, to assess the effectiveness of the plan in reducing the effects of pollution. © Elsevier, Paris

Bilbao estuary / coprostanol / sediment / organic enrichment / sewage pollution

Résumé – Teneurs en coprostanol et enrichissement organique dans les sédiments de l'estuaire de Bilbao (nord de l'Espagne). La répartition du stérol fécal coprostanol (COP) a été évaluée dans les sédiments de l'estuaire de Bilbao. Les concentrations en COP décroissent de 135,15 à 10,77 μg g^{-1} de poids sec, de l'intérieur vers le large, avec une valeur moyenne de 51,43 μg g^{-1}, les valeurs les plus élevées étant trouvées à l'embouchure de plusieurs affluents. La comparaison avec d'autres résultats publiés indique que les sédiments de l'estuaire de Bilbao sont atteints par la pollution des égoûts à un niveau comparable à celui d'autres estuaires dans le monde. L'enrichissement organique et les conditions anaérobiques règnent sur les 3/4 du lit du cours d'eau. Comme la répartition spatiale de plusieurs paramètres de la sédimentation organique est corrélée aux niveaux de COP, on peut conclure à une pollution majeure par des rejets non traités, provenant principalement de sources domestiques. La réalisation d'un plan de traitement des rejets par l'"Autorité de l'Eau" locale exige le suivi de traceurs pertinents, comme les stérols fécaux, pour apprécier l'efficacité du plan sur la réduction de la pollution. © Elsevier, Paris

estuaire de Bilbao / coprostanol / sédiment / enrichissement organique / pollution

1. INTRODUCTION

Recently, there has been great interest in the use of biological and chemical markers to quantify urban and sewage-derived pollution in estuarine and marine environments and to control the sanitary quality of waters. One of these chemical markers, coprostanol (5ß-cholestan-3ß-ol or COP), which is produced in the intestines of man and higher vertebrates by the microbial reduction of cholesterol [25], offers a wide range of advantages over the classical microbiological techniques that use several classes of bacteria, particularly those of the coliform group, to trace faecal-derived inputs in the environment [14, 24, 25].

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The physical environment of the Bilbao estuary has been extremely modified since the end of the nineteenth century mainly through the construction of docks, canalisation, and harbour dredgings to improve its navigation. Although a sewerage plan is now underway, large amounts of untreated domestic sewage from about one million people together with wastes from industries and harbour activities are continually discharged daily into a 14-km long estuary with a limited freshwater inflow of 25 $m^3 \cdot s^{-1}$. The biological effects of these man-induced activities lead to a severe oxygen deficiency in the waters of the middle and upper reaches of the estuary, affecting aerobic life in subtidal sediments to such an extent that macro-infauna is absent in almost three-fourths of its watercourse bed [19].

The object of this study is to use the faecal sterol COP as a sewage tracer for evaluating contamination of the sediments of the Bilbao estuary by sewage addition. In the future, these results could provide background data in the assessment of the effectiveness of the previously mentioned sewerage plan in reducing pollution effects.

2. MATERIALS AND METHODS

2.1. Study area

A total of eight stations were selected along a longitudinal gradient of salinity dilution from the mouth of the estuary (St. 2: Benedicta Dock) to the upper reaches, where the city of Bilbao lies (St. 9: La Salve); a 'control' or reference station (RS) was established in a nearby and relatively unpolluted estuary (St. 1: Plentzia; *figure 1*). A single tide level was sampled at each station (approx. 1 m above Spanish Ordnance Datum).

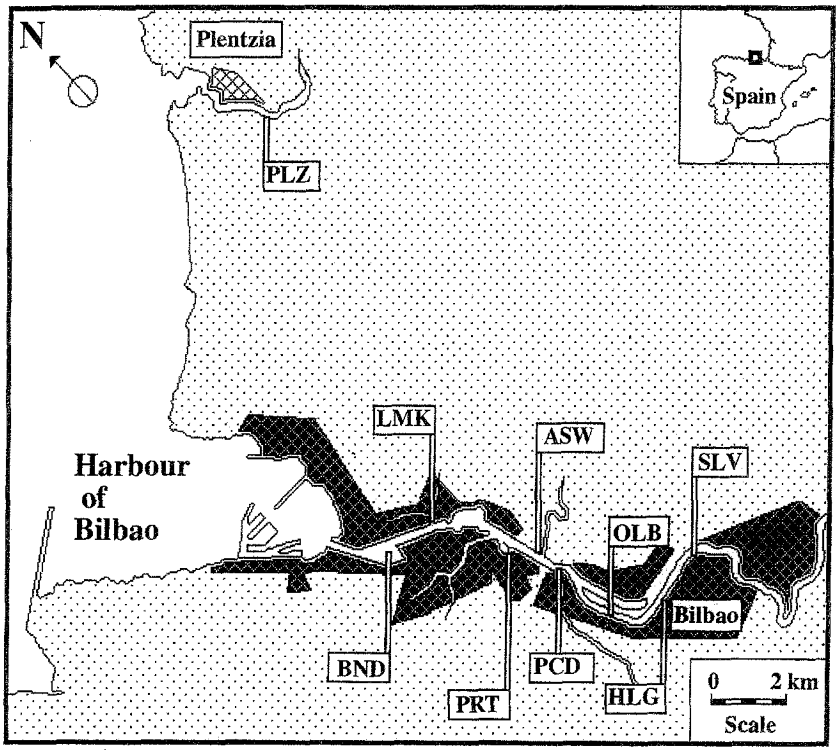

Figure 1. Diagram of the Bilbao and Plentzia estuaries, with the location of the sampling sites used in this study. The populated zones are shaded. At the time of the sampling, the real population living in the Bilbao estuary was approximately 950 000 habitants, and the served population was about 375 000 habitants [4]. The inset shows the position of the investigated area in Spain.
Station acronyms and names: PLZ: Plentzia ('reference site'); BND: Benedicta Dock; LMK: Lamiako; PRT: Portu Dock; ASW: Asúa; PCD: El Puntal; OLB: Olabeaga; HLG: Helguera; SLV: La Salve. The HLG site, Helguera, was the foremost sewage discharge source into the estuary at the time of the sampling.

2.2. Sample collection and analysis

Redox potential (Eh) profiles in sediment columns were obtained in situ by using ORP (Hanna HI 3130B or HI 3131B) electrodes coupled to a Hanna model HI 9025c mV-meter. Readings of Eh were taken after an arbitrary 60 s period [15]. A standard redox solution (Hanna HI 7020) was used as reference in the checking routine. The upper 10 cm of sediment columns were centrifuged for 10 min at 4 °C, and the interstitial water (IW) collected for salinity measures with a Hanna FC 300 sodium ion electrode connected to a Hanna HI 9190 sodium chloride analyser. A reference sodium solution Hanna HI 7081 was used to evaluate the measuring accuracy. During low tides, surficial sediment samples (top 0.5 cm layer) from an area of approximately 1 m^2 were scraped off with a polyethylene spatula and transferred to labelled polyethylene vials. Once in the laboratory, sediment samples were frozen until analysis (–25 °C), focusing on the surficial layer due to both its relation to more recent material inputs and its higher biological signification. Particle size distribution was performed by standard sieve and pipette (settling rate) methods of a sediment column of 10 cm deep, following the technique recommended by Buchanan [2]. Organic content (loss-on-ignition or LOI and reported as ash-free dry weight) of surficial sediments was estimated by ashing at 520 °C for 6 h and re-weighing [12]. Analysis of total and organic carbon and nitrogen content in the surficial sediment were determined with a Perkin-Elmer model 240 CHN-analyser calibrated with acetanilide, utilising the difference on ignition (DOI) method proposed by Kristensen and Andersen [9].

2.3. Coprostanol measures

The faecal sterol COP was analysed after the EEC method no. 2568/91 for sterols. Weighed samples of dry sediments were Soxhlet extracted for 24 h with a 2/1 (v/v) mixture of dichloromethane-methanol as solvent, and then the extract was saponified by adding 50 mL of 2 N KOH in ethanol. Unsaponified fractions were dried over anhydrous sodium sulphate and evaporated under vacuum to approximately 2 mL. An internal standard (stigmasterol, 1 000 µg g^{-1}) was added to all extracts prior to chromatography analysis. Extracts were applied to thin layer chromatography plates (silica gel, activated at 100 °C for 1 h; hexane-ethyl acetate 85/15 (v/v) as mobile phase). 2,7-Dichlorofluorescein was sprayed over the plate, and the sterol fraction was removed after visualisation with ultraviolet light and eluted with chloroform. Derivatisation into trimethylsilyl (TMS) ethers was prior to gas chromatography analysis. Silylation reactive (1 mL) was a mixture of 1:10^{-5}:3 (v/v/v) of N,O-*bis*-(trimethylsilyl)-acetamide:trimethylchlrosilane: pyridine, 15 min being the reaction time.

2.4. Gas chromatography

The TMS-sterol fraction thus extracted was analysed on a Hewlett-Packard Model 5890 Series II. A 30 m × 0.25 mm i.d., 0.25 µm film thickness, fused silica capillary column coated with poly(5 %-diphenyl-95 %-dimethylsiloxane) was used for analysis. A three-ramp temperature program was employed. Helium was the carrier gas, and the eluate was detected with a flame ionisation detector (FID). Coprostanol was identified by comparing relative retention times to previously analysed standards, and quantified by establishing the relative response of the FID for the standard added to each samples prior to thin layer chromatography analysis of the sample COP.

2.5. Data analysis

Statistical analysis and data transformations were conducted using SPSS for Macintosh. Data sets were analysed for normality using the Kolmogorov-Smirnov test. If this analysis failed to meet the assumptions for parametric statistics, arcsine transformation was applied to the data, and the transformed data set was then re-evaluated for normality. Correlation analysis (Pearson's Product-Moment Correlation) was used to test for the strength and direction of associations between all the variables. Statistical significance was reported at $P < 0.05$ [27].

3. RESULTS

IW salinities showed a longitudinal gradient that increased toward the mouth from the upper reaches of the estuary. Salinity was 10.9 g L^{-1} at upstream sites but increased progressively downstream to a maximum of 22.2 g L^{-1} close to the mouth. No longitudinal patterns were apparent for redox potential, particle size and organic content of depositional sediments, although stations in the middle reaches of the estuary had usually finer particle sizes and higher organic content. Sediments from the Bilbao estuary were anaerobic. Redox potentials at the sediment surface ranged from –64 to –385 mV and averaged –265 mV. Sediment parameters showed no longitudinal trends, with sedimentary organic matter content dropping from 57.2 to 6.6 %, whereas the organic carbon ranged from 2.03 to 15.97 % and total nitrogen varied from 0.2 to 0.86 % on a dry weight basis. Measures of these sediment parameters at the RS were lower than the Bilbao estuary averages, although within the ranges found for this polluted estuary.

Coprostanol concentrations in surficial sediments from the Bilbao estuary ranged between 10.77 and 135.15 μg g^{-1} of dry sediment ($\bar{x}$ = 51.43; standard deviation = 45.56), and were generally similar throughout the study area, except at the Helguera site, which was located close to the major sewage outfall in the estuary (*figure 1*), and the Portu Dock. The RS exhibited a concentration of COP of 2.24 μg g^{-1} of dry sediment (*table I*).

Coprostanol values showed highly significant correlations ($P < 0.01$) with four organic content parameters of the sediment: surficial ($r = 0.802$) and total organic matter ($r = 0.810$), organic carbon ($r = 0.876$) and total nitrogen ($r = 0.844$).

4. DISCUSSION

Theoretically, unpolluted sediments do not contain COP [6] and certainly contain levels below the limit of detection of the technique [13]. There is no consensus, however, on whether COP clearly indicates sewage-derived pollution. On the one hand, several researchers concluded that quantitation of COP provides, per se, a clear measure of sewage contribution to marine environments [13]. Indeed, Pierce and Brown [16] stated that the extent to which COP is distributed through an estuary provides an indication on the distribution of other sewage-derived pollutants [21]. On the other hand, other researchers needed more information about environmental stability and source specificity for its general acceptance as a sewage indicator, since COP occurrence in coastal areas cannot be unambiguously attributed to urban sewage pollution [3, 5, 17].

Although COP has been used by numerous researchers to evaluate this pollution, no specific level has been legislated defining excessive contamination [26]. Nichols and Leeming [13] stated that sediments considered to be grossly contaminated with sewage contained 9 μg COP per gram sediment; however, there is great heterogeneity among all COP concentration values previously reported, ranging within several orders of magnitude. Some values for sediment concentration of COP, registered in both open and closed systems, are those of Hatcher and McGillivary [6]: 56–5 200 ng g^{-1} in the New York Bight; Brown and Wade [1]: 19–454 ng g^{-1} (mean 142) in the Chesapeake Bay; Grimalt et al. [5]: 0.41–3.5 μg g^{-1} in several cities mainly from the Spanish Mediterranean seashore; Venkatesan and Kaplan [23]: 1.1–5.1 μg g^{-1} in Santa Monica Basin; Sherwin et al. [20]: 0.2–41 μg g^{-1} in Venice; and Jeng and Han [8]: 0.58–230 μg g^{-1} (mean 20.8) in Kaohsiung Harbour and the Tan-Shui estuary. The sediments of the Bilbao estuary are located in the middle of this scale, the mean value (51.43 μg g^{-1}) being

Table I. Coprostanol concentrations, redox potential at the surface, salinity (interstitial water) values, organic matter content, and particle size fractions in sediments from all the stations considered in this study.

				Organic matter content							Particle size	
Station name	**Coprostanol** (μg·g^{-1})	**Redox** (m·V)	**Salinity** (g·L^{-1})	**SOM** (%)	**TOM** (%)	**TC** (%)	**OC** (%)	**TN** (%)	**ON** (%)	**C/N** (%)	**> 100 μm** (%)	**< 63 μm** (%)
PLZ: Plentzia	2.24	168	22.2	7.3	3.9	2.79	2.27	0.23	0.2	9.7	8.29	78.31
BND: Benedicta Dock	20.98	–300	20	14.2	7.5	7.16	5.52	0.37	0.06	11.2	10.4	77.1
LMK: Lamiako	37.35	–283.5	17	7.2	5.2	2.29	2.03	0.14	0.12	15	40.3	27
PRT: Portu Dock	112.14	–385	18.4	21.6	14.1	10.97	9.08	0.57	0.52	14.9	20.9	54
ASW: Asúa	58.88	–160	15.2	9.2	7.7	5.09	4.9	0.44	0.43	9.9	7.9	78.6
PCD: El Puntal	10.77	–64	17.5	6.6	11.1	4.04	2.76	0.25	0.24	10	23.3	52.1
OLB: Olabeaga	11.48	–335.3	10.9	14.7	5.5	6.87	5.62	0.43	0.43	11.2	22.3	77.6
HLG: Helguera	135.15	–285	12.2	57.2	16.2	19.98	15.97	0.86	0.8	17.1	26.8	58.3
SLV: La Salve	24.71	–340	17.4	8.5	2.4	3.64	3.19	0.2	0.17	15.8	26.5	49.6

SOM: surficial organic matter; TOM: total organic matter; TC: total carbon; OC: organic carbon; TN: total nitrogen; ON: organic nitrogen; C/N: carbon to nitrogen ratio (molar fraction). Units are indicated in parentheses.

certainly higher than that of the reference by Nichols and Leeming [13]. Is it a clear indication of urban pollution?

Several researchers have demonstrated a positive correlation between COP concentrations and organic carbon in sediments [1, 6, 20, 23, 26]. Our results agree with this correlation. Applying the criteria developed for classifying sediments according to their content in organic matter [7], the more degraded zones in the Bilbao estuary are the upstream sites, i.e. Helguera, La Salve, and also the area around the Portu Dock, with organic matter contents over 12 %. Furthermore, the carbon/nitrogen (C/N) ratio provides a way of knowing the origin of this organic matter. Thus, a low C/N ratio reflects particulates derived from a predominantly marine system, while a high C/N ratio generally reflects terrestrial inputs [11], i.e. a C/N > 12 identifies a terrestrial origin, while a C/N between 6 and 9 generally detects marine origin [22]. Values obtained in this study ranged from 9.7 to 17.1, 12.3 being the mean value (2.83 the standard deviation), which is a clear indication of a terrestrial origin. Waste waters enhance both carbon and nitrogen inputs into the sediment. However, while a fraction of carbon is easily degraded via bacterial metabolism, other fractions remain in the system as refractarial carbon, which in turn enhance the C/N ratio. Several tributaries flow into the Bilbao estuary (Gobelas at the Lamiako site, Galindo close to the Portu Dock, and Helguera), where the C/N ratios are the highest. The same is true for the Portu Dock and La Salve, tracing important, presumably sewage-derived organic matter inputs in the estuary.

Organic enrichment and anaerobic sediment conditions appear to be the dominant anthropogenic influences in the Bilbao estuary. Sediments are completely anoxic up to the water interface, with redox potential values as low as –498 mV (at 10 cm depth [19]). At these low redox values, the organic matter undergoes breakdown by anaerobic processes, with liberation of noxious gas bubbles at the air/water interface, a circumstance which prevails in many parts of the estuary during low tides. A sewerage scheme involving a network of interceptor and collector sewers with major wastewater treatment plants (WWTP) is now under construction. A progressive improvement of the estuarine water quality in several stages is expected [4]. The usefulness of COP as an indicator of treatment plant efficiency was proposed by Le Blanc et al. [10] and Quéméneur and Marty [18], since it is degraded under conditions of aerobic digestion during the secondary WWTPs. Thus, the quantification of COP and related organic chemical parameters in sediments, as they have been used here, are especially valuable in helping to assess the efficiency of the sewerage plan promoted by the local water authority in the forthcoming years.

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