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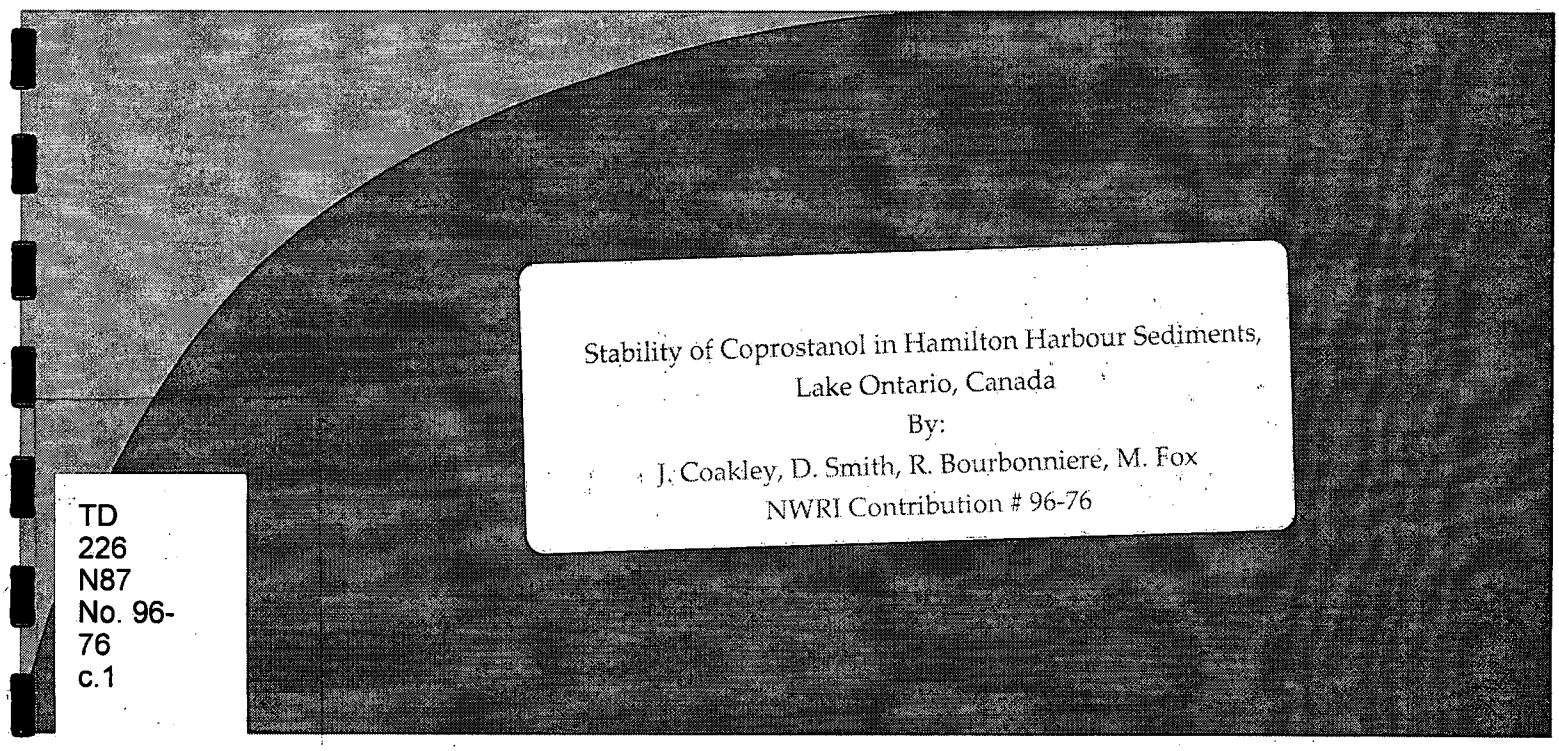

Stability of Coprostanol in Hamilton Harbour Sediments, Lake Ontario, Canada

By:

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STABILITY OF COPROSTANOL IN HAMILTON HARBOUR SEDIMENTS, LAKE ONTARIO, CANADA.

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ABSTRACT

Coprostanol (5β-cholestan-3β-ol) has been used extensively as a tracer of sewage-contaminated sediments, and in studying historical trends in sewage-related input to urban water-bodies. However, more needs to be known about the stability of coprostanol in sediment environments. Laboratory studies of the stability of coprostanol under conditions prevalent in fresh-water sediments indicate that over a 50-day period, coprostanol levels remained uniform in natural sediments exposed to microbial processes. For tests over longer periods, a ^{210}Pb-dated sediment core from Hamilton Harbour was used to compare vertical changes in the coprostanol profile to the known sewage input history. The core, located 1.5 km away from the Burlington Skyway Sewage Treatment Plant (STP) outfall, had relatively low surface concentrations (3 μg/g or less), but showed a sharp decline to background levels below a depth of 10 cm. The dating results indicated that the drop to background levels occurred around 1984, almost 20 years after initial STP discharges. The delay in sewage impact at this far-field location is probably due to efficient mixing and dilution of the effluent at initial discharge levels. The rise in coprostanol at the top of the core is linked to the 7-fold increase in STP effluent discharge beginning in the late 1970's in response to outfall modification and increased population in Burlington. It is still a challenge to recreate in the laboratory, over periods longer than 50 days, the occasional resuspension and variably-oxidized conditions that characterize the surface layers of the sediments. Nevertheless, the results show that this is necessary before stability under those conditions can be confirmed definitely. On the other hand, the study demonstrates that once buried, coprostanol is stable in sediments at time scales of decades.

Management perspective

Title: STABILITY OF COPROSTANOL IN HAMILTON HARBOUR SEDIMENTS, LAKE ONTARIO, CANADA.

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Issue:

Remedial Action Plans for Hamilton Harbour requires quantification of the net dispersal patterns for contaminated sediments associated with the Burlington Skyway Sewage Treatment Plant (STP) outfall in the northeastern part of the Harbour. STP outfalls are major sources of particulates containing a variety of priority contaminants. The most efficient way to study net long-term transport of contaminated sediments is by sediment tracers. Coprostanol (5β-cholestan-3β-ol), a faecal steroid uniquely related to human wastes, has been used extensively as a natural tracer of sewage-contaminated sediments, and for identifying historical trends in sediment cores. However, its stability in sediments is poorly understood, and this has hampered interpretation of previous studies.

Current Status:

This paper demonstrates, using both laboratory and field studies (Hamilton Harbour cores), that coprostanol is stable in sedimentary environments over periods of decades. Therefore, it is suitable as a long-term tracer of contaminated sediment plumes related to STP outfalls.

Next steps:

The coprostanol tracer technique will be applied in other areas to determine the extent and trajectory of sewage plant outfall plumes.

INTRODUCTION

Coprostanol (5β-cholestan-3β-ol) belongs to a large family of lipid compounds, sterols, which are characterized by carbon numbers of 22 to 29 (Figure 1). Because it is believed to be uniquely produced by microbial reduction of cholesterol in the gut of higher mammals, including humans and chickens (Midtvedt et al. 1993), coprostanol (C27) has been used extensively as a tracer for sewage-related pollution, both in water and in sediment. However, because of its low solubility in water and its affinity for sediment particles, it is most used as a sediment tracer. The use of coprostanol for this purpose is based primarily on its specific origin, i.e. a digestion by-product, so its presence in the aqueous environment could therefore only be due to discharge into such waters of human wastes, usually through the sewage treatment system. An important assumption in the use of coprostanol as a tracer of long-term sediment transport patterns is that it is a conservative compound whose concentration distribution in the environment is due primarily to dilution away from a point source. In other words, this compound is generally assumed to be resistant to biodegradation in surface sediments. The published literature on the subject, though reasonably large, is often conflicting, and sheds no clear light on this subject.

In this paper, we seek to enhance the basis of the above assumption by investigating the stability of coprostanol in sediments. The investigation will be built around:

- a thorough review of the literature on coprostanol use and its stability under varying environmental conditions;
- a laboratory study of coprostanol persistence in the presence of vigorous bacterial action under aerobic, room-temperature conditions over a period of 50 days;
- an examination of coprostanol concentration profiles in dated cores collected near the outfall of the Burlington Sewage Treatment Plant (STP) in Hamilton Harbour, Lake Ontario.

Hamilton Harbour background

Hamilton Harbour (Figure 2) is a 21.5 km^2 embayment at the western end of Lake Ontario. It is separated from the lake by a natural sand bar through which an artificially created ship canal passes. The harbour receives large quantities of treated sanitary effluent from sewage treatment plants (STPs) serving the cities of Hamilton (~400,000 inhabitants) and Burlington (~100,000). The harbour also receives untreated stormwater runoff and combined sewer overflow (CSO) from the urbanized areas in its watershed.

The Burlington Skyway STP, around whose outfall the core samples studied here were taken, began operations in 1964, when the population of Burlington was approximately 50,000. Effluent discharges at the time averaged around 3 million gallons/day (14,000 m^3/day). Around 1977, the STP was upgraded; it now discharges around 21 million gallons / day (95,000 m^3/day).

Literature summary of coprostanol sources and stability

The earliest references to the use of coprostanol as an indicator of sewage-related pollution was by Murtaugh and Bunch (1967). Kirchmer (1971) first documented the utility of coprostanol as a tracer in a comprehensive way. Since these early studies, more than 25 references on coprostanol use as a sewage indicator and sediment tracer and on its chemical stability have been published (see attached bibliography). Studies have been performed in many marine environments; for example, Antarctica (Green et al. 1992, and Venkatesan et al. 1992), New York Bight (Hatcher et al. 1979) and Venice, Italy (Sherwin et al. 1993). Coprostanol has also been applied as a sewage indicator in fresh water environments, i.e., Lake Constance in southern Germany (Müller et al. 1979), in Finnish lakes (Düreth et al., 1986), and in the Great Lakes (Coakley, et al., 1992).

Background studies on coprostanol stability. Coprostanol owes its origin to the microbial reduction of cholesterol in the gut of higher mammals. The degradation of coprostanol, in turn, results in a variety of stenols, stanols and stanones; some of these are also supplied by aquatic organisms, such

LABORATORY STUDIES OF COPROSTANOL STABILITY, HAMILTON HARBOUR

The stability of coprostanol with respect to a mixed culture of four randomly selected bacteria under aerobic conditions was investigated in the laboratory. Seven other compounds were added to provide control in assessing coprostanol behaviour; these are listed in Table 1.

Experimental method

The experiment is described in detail elsewhere (Smith et al. 1996) so only a summary will be repeated here. A fine-grained inorganic sediment was dosed with a bacterial flora and with known amounts of the organic compounds listed in Table 1. The sediment used in these experiments consisted of very fine grained glaciolacustrine clay (ca. 12 000 years old), from a borehole near Lake Erie. The sediment contained no detectable coprostanol. The experiment was replicated in three flask reactors.

Ten grams of sediment were placed in three different Erlenmyer flasks and 500 mL of MilliQ water was added to create a slurry. The flasks were then placed on a shaking table for 24 hours to allow equilibration to occur before anything was added.

Organic spike. A spike solution containing the compounds to be added to the sediment was prepared in methanol/toluene (1:1). The entire list of compounds selected and their concentrations in the stock solution is presented in Table 1.

One mL of the spike was injected into the sediment slurry and allowed to equilibrate 24 hrs. before the bacteria were added. The original sediment concentration was about 20 μg/g for all compounds except coprostanol which was 100 μg/g of dry sediment. Concentrations of the compounds were monitored over 50 days, along with the viability of the bacteria used. The experiment was

performed at room temperature and under aerobic conditions. All glassware used was pre-rinsed with methanol followed by dichloromethane prior to use.

Bacteria. A 2L sample of Burlington Sewage Treatment Plant effluent was collected from the outflow canal in a glass jar. Agar plates were used to culture the bacteria present in this sample. For logistical reasons, only four of the more than 30 species identified were selected for the experiment. These species were then cultured in four separate nutrient broths. The four separate broths were combined to produce a mixed culture of bacteria for injection into each flask. The bacteria concentration in the flasks was originally 10^7 organisms/mL.

The bacteria were quantified and qualified after 1081 hours by using serial dilutions and agar plates. Also at 1081 hours, a nutrient additive (Lab Lemco Broth from Oxoid Ltd.) was added to rejuvenate the culture.

Sampling. The first sampling, after the sediment and water had mixed for 24 hrs., served as a blank. The organic spike was then added and the slurry equilibrated for 24 hrs. before the bacteria were added. The second sample was taken a day after the bacteria were added . Subsequent samples were taken at increasing intervals of time. The value for time-zero was obtained in a separate experiment using identical methodology as the first, but with smaller amounts.

The sampling procedure involved pipetting out 25 mL sample from each of the slurries while it was being homogenized by a magnetic stir bar; the bar was later removed. A preliminary experiment in which the sample was not homogenized with a magnetic stir bar during sampling showed that coprostanol had a marked affinity for the finer sediment particles.

Compound Extraction. First, the sample was centrifuged and the supernatant water decanted off into another tube. This water was then acidified (pH < 2) to flocculate the very fine materials. After being again centrifuged, these fines were combined with sediment from the first tube and extracted using a 1:1 mixture of methanol/dichloromethane as the solvent. 15 mL of the solvent were added to the sediment plug in the centrifuge tube and shaken vigorously for two minutes. An extraction efficiency test showed only <0.4% of the total coprostanol remained in the aqueous fraction. After

removing water by treating with anhydrous sodium sulphate, the combined extract was filtered. The extract was then evaporated to dryness and silylated with 100 μL of N-methyl-N-trimethylsilyl-trifluoroacetimide (MSTFA) for 20 minutes at 130 °C. After cooling, 1 mL of heptane was added and the sample transferred to a vial for gas chromatographic (GC) determination.

Chromatography. The compound concentrations were determined using a Hewlett-Packard S890 GC with on-column injection onto a DB-5 fused silica column programmed from 70 to 300 ° C. Detection was by Flame Ionization Detector (FID) and responses were corrected using response factors relative to the C23 alkane internal standard.

Results

Viability of bacteria. Initially the combined population of the four different bacteria was 10^7 organisms/mL in the slurry. After 1081 hours only two species had survived; one was still 10^7 organisms/mL and the other was 10^5 organisms/mL. In addition to these species, 8 other species < 10^5 organisms/mL were found throughout the three replicate slurries. After addition of the nutrient broth at 1081 hours, only the two dominant bacteria types survived and remained at > 10^7 organisms/mL, for the remainder of the experiment.

Sampling. Figure 2 shows the sample mass for all the samples taken. Three points lie outside two standard deviations about the mean; the results from these outlier points were not included in the final analysis.

Stability of compound concentrations. The concentrations with time for the four selected compounds are summarized in Figure 4. The concentrations are plotted as fractions of the original load versus time. The main reason why the concentration ratios are consistently less than one is the tendency of the injection standard method to underestimate the concentrations. Furthermore, the quick extraction technique is not 100% effective for all compounds. Standard deviations (shown as bars around each plotted value) were found to vary between 5 - 10 %.

The only compounds that were seen to have definitely decreased were the alkane and the alkene compounds. It is possible that benzo (a)anthracene also decreased, but the T94 (final) measurement seems anomalous. The values for coprostanol do not decrease; in fact, almost without exception they plot between the mean and the standard deviation lines (Figure 4) and show no increasing or decreasing trend. Coprostanol concentrations remained virtually unchanged after the nutrient broth was added and the bacteria culture became more vigorous. Even after an additional 900 hours, coprostanol does not show any statistically meaningful decrease.

Conclusions

In the 50-day laboratory experiment, coprostanol concentrations remained steady within a narrow range of variation. This suggests that it is highly resistant to biodegradation even under aerobic conditions and at room-temperature. Other compounds, such as the C20 alkene, showed definite decreases in concentration. Nevertheless, the 20% drop noted in coprostanol concentrations at the beginning of the monitoring period is unexpected, and could be explained by:

1. consistent, but incomplete, extraction of the compounds from the sediments. The simplified extraction technique involved only shaking the sample in the solvent with repeated decantation;

2. incomplete sampling by the pipette of all sediment fractions, especially the finest particles that carry a disproportionately high proportion of the adsorbed compounds. This possibility is negated somewhat by the consistency of the sample masses extracted throughout the experiment (Fig. 2).

3. uncontrolled adsorption of these hydrophobic compounds to the glass walls of the experiment vessels.

The stability experiment was successful in demonstrating the stability of coprostanol over a period of 50 days. It also demonstrates that it remains a challenge to recreate in the laboratory, over periods

longer than 50 days, the occasional resuspension and variably-oxidized conditions that characterize the surface layers of the sediments. Such experiments are necessary in order to confirm definitely the longer-term stability of coprostanol under such conditions.

COPROSTANOL PROFILES IN DATED CORES FROM HAMILTON HARBOUR

The area in northeastern Hamilton Harbour around the outfall of the Burlington STP was the site for sediment tracer studies by Bachtiar et al. (1996) using coprostanol as a sewage tracer (Figure 5). Cores were taken over a radial grid centred on the STP outfall and the top 2 cm of sediment analyzed. As part of the present study on coprostanol stability, two of the tracer sites sampled were reoccupied and cored by divers to minimize disturbance of the upper layers. The locations of the cores are shown in Figure 5. Core D1, within 10 m of the outfall, consisted of a black organic mud with much visible organic matter down to its base of 41 cm. Diver coring was impossible below this level due to much greater penetration resistance; gravity coring revealed that the mud rested disconformably on a dense fine grey sand unit. Core D4, about 1.5 km to the southwest, consisted of dark-brown mud throughout its 1 m length.

Analysis of core sediments

The two cores were extruded, sub-sampled at 2 cm intervals, and freeze-dried prior to storage. The following procedures were carried out:

1. Determination of down-core profiles of coprostanol and related steroid isomers;
2. Dating of the cores using Pb^{210};
3. Determination of organic carbon profile for the core successfully dated.

Coprostanol and isomer determination. The sediment samples were extracted using a Supercritical Fluid Extraction technique (SFE). The details of the technique are described in DeLuca and Fox (1995). The extracts were then run through a FID-equipped GC for determination of coprostanol and related steroid isomers. The isomers determined were coprostanol (5β-cholestan-3β-

ol), coprostanone (5β-cholestan-3β-one), cholestanol (5α-cholestan-3β-ol), and cholestanone (5α-cholestan-3β-one). Although resolved in the GC analysis, concentrations of epicoprostanol (5β cholestan-3α-ol) and other related epimers have not yet been quantified. Total organic carbon (T.O.C.) profiles were obtained on both cores using a LECO-12 analyzer. The results of the determinations are listed in Table 2.

Instrument precision for the GC / FID analysis was determined by repeated injections at < 1%. Accuracy was maintained by introduction of standards into the sample flow at regular intervals. Determination of analytical precision carried out on one sample was calculated at 4%. Detection limits were in the range of 1 μg/g. Results obtained previously on identical samples using a Soxhlet extraction technique were in satisfactory agreement (Table 2).

Results

Coprostanol Coprostanol concentrations are shown plotted against depth in cores D-1 and D-4 in Figures 6A and 7A, respectively. As expected, surface values near the STP outfall (D-1) are extremely high compared with those further away at D-4. In addition, the downcore profile at D-1 shows a wide variability, oscillating between high (400 μg/g) and low (<10 μg/g) values. Even at the base D-1 (-40 cm), coprostanol levels were very high. D-4, on the other hand, showed a rapid drop in coprostanol (below -10 cm) to what appear to be background levels.

Total organic carbon (T.O.C.) As expected, surface values near the STP outfall (D-1) were found to be extremely high compared with those further away at D-4. The profile for D-1 shows a wide variability downcore (<2 to 14.5%), while in D-4, the profile is virtually uniform with concentrations between 3 and 5%.

The ratio of coprostanol to total organic carbon has been used by others (Hatcher and McGillivary, 1979; Writer et al., 1995) to reduce variations due to grain size and to detect source differences. Normalization of coprostanol with respect to T.O.C. in cores D-1 and D-4 is shown in Figures 6B and 7B, respectively. The results for core D-1 show that with the exception of the data

point at the base of the core, the coprostanol / T.O.C. ratio was reasonably constant, indicating similar sources for both the organic carbon and the coprostanol. This is not surprising as the core was located less than 10 m from the STP outfall, the presumed dominant source. The anomalously high coprostanol / T.O.C. ratio at the base of the core remains difficult to explain. Core D-4 shows a completely different picture, with little change between the raw and normalized data plots. This indicates that the organic carbon and coprostanol sources at this site are different; the organic carbon levels here are consistent with dispersed sources in Hamilton Harbour.

Steroid isomers Normalization with other steroid isomers (coprostanol / coprostanol + cholestanol) was carried out to remove the effect of naturally-occurring coprostanol isomers as recommended by Grimalt et al.(1990). This ratio was used by Grimalt et al. as a guide to the degree of sewage impact; values above 0.3 indicate significant sewage impact. In core D-1 (Figure 6C), the ratio was always above the guideline for sewage impact, as could be expected on the basis of its location adjacent to the STP outfall. Core D-4, however, showed ratios consistently below 0.3, even at the surface. This reflects its far-field relationship with the outfall, and the effectiveness of hydrodynamic mixing and sediment dilution at this distance. Like the case of T.O.C., normalization with the sum of the 5α and β isomers had a significant effect on the profile of core D-1. Unlike the T.O.C. case, the ratio was apparently not constant, and the curve changed shape dramatically. This suggests no consistent relationships between inputs of the two epimers. The isomer normalization profile for core D-4 is similar to that for raw coprostanol T.O.C.; this suggests a uniform input of the 5α isomer, most likely from natural sources. When the epicoprostanol data are available, this relationship can be investigated further.

^{210}Pb dating of the Hamilton Harbour cores. The dating of the two cores was carried out at the National Water Research Institute. The methodology and comprehensive results are reported in (Turner, 1994; 1995).

Core D-1, located within 10 m of the outfall, could not be dated because of extremely low ^{210}Pb activity throughout the core (upper core values were up to 20 times less than measured elsewhere in Lake Ontario). Turner (1994) speculates that this result might be due regular resuspension and mixing

of surface sediments by wave action or to anomalous adsorptive properties of the STP organic effluent. It is possible that the entire 40 cm of soft sediment above the sand has been deposited fairly recently, consistent with the above suggestion that this site is subject to periodic massive sediment removal, followed by rapid deposition. Similar low ^{210}Pb activity was noted near STP outfalls in the Toronto area (J.P. Coakley, unpublished data).

Core D-4, located 1.5 km away (Figure 5) had higher surface ^{210}Pb activity and could be dated (Turner, 1995). The profile of ^{210}Pb age vs. depth is plotted in Figure 8. The profile indicated a uniform sedimentation with no obvious breaks. The ^{210}Pb-based average sedimentation rate was found to be between 7 and 9 mm/y.

DISCUSSION

History of coprostanol input

In using dated cores for testing the stability of a compound, it is most important to interpret properly the time-dependent concentration profile of the compound. Given that the compound is added only at the sediment surface, it is assumed that after burial, no further inputs occur. If the compound is input to the sediments at a fairly constant rate, and is characterized by a certain systematic decay or degradation rate, then there should be a consistent decrease downcore. The difference in concentration at any depth, compared with that at the surface, and the age of that sediment horizon could then be used to estimate the half-life of the compound in the sediments. In the model used here, one would expect the initial rise from background levels to serve as a good time marker for 1964, the start-up year of the Burlington STP. Depending on the stability of the compound, then it is expected that the upper part of the profile would show a consistent upward increase over the 30-year time frame. ^{210}Pb dating of the core would assist in clarifying the history of sewage discharges in the area and changes in coprostanol input or sedimentation rate.

As mentioned earlier, the coprostanol profile in the core nearest to the outfall (D-1) shows very high surface concentrations with no overall decrease over its 40 cm length. No drop to presumed background, or pre-STP, levels are noted. This lack of a background level can only mean that the

entire 40 cm length of the core was deposited subsequent to 1964; indicating a sedimentation rate of more than 1.3 cm/y. Because this core could not be ^{210}Pb-dated, no further resolution of input history is possible. However, the fact that high values of coprostanol even at the base of the core, compared with present values, are strong evidence of degradation resistance over many years.

The coprostanol profile in the dated core (D-4) located 1.5 km away shows a drop to background levels below the 10 cm level. These very low levels are close to the analytical detection limit. The profile can nevertheless be interpreted as indicating that input of STP-related coprostanol began at the time corresponding to around the 10 cm depth. By superimposing the ^{210}Pb chronology for this core onto the coprostanol profile (Figure 8), this depth in the core is seen to correspond to approximately 1984, i.e. about 20 years after STP discharge initiation or about 11 years before core collection.

The above 20-year delay between the STP opening and the initiation of storage of coprostanol in the sediments at the D-4 site can best be explained as follows:

1. The capacity (and discharge) level of the Burlington STP was raised around 1976-78 to 21 mill. gallons/day (95,000 m^3 / day) from 3 mill. gallons/day, i.e. a seven- fold increase.
2. Because hydrodynamic mixing is very effective in this area due to its position down-fetch of the prevailing wind direction, it is assumed that the coprostanol discharges at the earlier rate were diluted to background values before they reached the D-4 site. Expansion of the STP to present levels in the late 1970's apparently expanded the zone of incomplete mixing, thus enabling coprostanol to reach and to accumulate in the sediments at D-4 soon after that time (i.e. early 1980's).

Stability of coprostanol in sediments

The occurrence of coprostanol in sediments dating back at least 11 years is evidence of its persistence over decades. Such a conclusion is compatible with that drawn by others, concerning both freshwater (Müller et al., 1979) and marine environments (Hatcher and McGillivary, 1979). It is

unfortunate that we were unable to date the near-field core (D-1) containing sediments and coprostanol associated with the initiation of the Burlington STP outfall around 1964. The extent and rate of degradation losses over this 30 year period, as evidenced in the coprostanol concentration profile, would have been a valuable addition.

Core D-1 profiles (Figures 6 B and 6C) show that, excluding the anomalous data points at the base, ratios of coprostanol, both to its epimers and to T.O.C., decline with depth . Assuming that for sewage discharges such ratios are conservative (Grimalt et al., 1990), this suggests that over the time interval involved, either the relative concentration of coprostanol has declined slightly over time, or there has been an increasing addition of T.O.C. and sterol epimers from other sources with time. It is known that sources for T.O.C. other than the STP exist in Hamilton Harbour, and the 5α epimer can be formed by natural reduction processes of Δ^5 sterols found in algae, for example. If it is assumed that T.O.C. is sufficiently stable to be used as a reference, then the profile suggests that there is a slight degradation of coprostanol over the presumed 30-year time period involved. Although there is a definite need for dated profiles close to the outfall, it appears that coprostanol is stable in sediments over periods of decades. Such a level of stability is more than adequate to validate its use as a surface sediment tracer, and, to a lesser extent, as a tracer of historical changes in sewage-related contaminant inputs.

CONCLUSIONS

Laboratory and field studies of the stability of coprostanol under conditions prevalent in fresh-water sediments were carried out in Hamilton Harbour. The laboratory results indicate that over a 50-day period, coprostanol levels remained uniform in natural sediments exposed to microbial processes. Compared to reference compounds, such as various PAH's (benzo (a) anthracene), n-alkanes, and stigmasterol, coprostanol demonstrated its stability under these conditions. For tests over longer periods, it was necessary to use natural sediment cores, dated by means of ^{210}Pb to examine vertical changes in the coprostanol profile that could be interpreted against input history. Cores taken near the outfall of the Burlington STP outfall in Hamilton Harbour showed a marked variability in profile type.

Core D-1, within 10 m of the outfall showed essentially unchanged and high levels of coprostanol over its 40 cm length. Unfortunately this core could not be dated. Coprostanol was correlated with organic carbon except for the basal samples in the core. Core D-4, located some 1.5 km away showed relatively low levels, but the change from high surface to background levels below was visible in the coprostanol concentration profile. Dates obtained for this core indicated that the drop to background levels dated at about 1984, almost 20 years after the STP began discharging effluent in 1964. It is believed that this discrepancy can best be related to the delayed impact of sewage discharges at this distance from the outfall. Such a change could correlate to the 7-fold increase in STP effluent discharge beginning in the late 1970's in response to outfall modification and increased population in Burlington.

Despite some difficulties in setting up realistic natural conditions in the laboratory, and controlled, predictable conditions in the field, we believe that the study demonstrates that coprostanol is stable in sediments at time scales of decades. It was not possible to calculate a half-life for this compound but the results indicate that the stability of this compound should not be a problem in its use as a sediment tracer.

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LIST OF FIGURES AND TABLES

Figure 7 Plot of similar coprostanol profiles in core D-4.

Figure 8 Plot of ^{210}Pb date profile for core D-4, showing times of historical changes in STP operation.

Figure 9 ^{210}Pb chronology superimposed on the coprostanol profile in core D-4, Hamilton Harbour.

TABLE 1: List of organic compounds added in coprostanol stability experiment

Compound	Concentration µg/mL)
n-C20-alkene	222
n-C21-alkane	200
Benzo (a)anthracene	201
Erucic Acid	243
Tricosanoic Acid	203
n-C26-alcohol	200
Coprostanol	1000
Stigmasterol	180

Table 2. Results of SFE / GC / FID analysis of sediment core material for coprostanol and related steroids, Hamilton Harbour cores

		5B-ol	5B-one	5A-ol	5A-one			
Sample Number	Depth (cm)	Coprostanol (ug/g)	Coprostanone (ug/g)	Cholestanol (ug/g)	Cholestanone (ug/g)	T.O.C %	Pb-210 Date	yrs. before 1994
94JPC-D4-1	1.00	3.00	7.00	8.00	3.00	4.10	1993	1.00
94JPC-D4-3	6.00	2.00	8.00	13.00	4.00	3.97	1988	6.00
94JPC-D4-5	11.00	0.50	2.00	5.00	0.10	4.56	1983	11.00
94JPC-D4-7	16.00	0.50	2.00	4.00	0.10	4.04	1976	18.00
94JPC-D4-9	21.00	0.10	2.00	4.00	0.10	4.13	1968	26.00
94JPC-D4-13	31.00	0.50	2.00	4.00	0.10	3.52	1950	44.00
94JPC-D4-15	36.00	0.10	1.00	2.00	0.00	3.08	1939	55.00
94JPC-D4-19	46.00	0.50	2.00	5.00	0.10	3.04	1914	80.00
94JPC-D4-25	61.00	0.50	1.00	3.00	0.00	3.56	1860	134.00
94JPC-D4-29	71.00	0.50	2.00	10.00	0.75	2.88	1820	174.00
94JPC-D4-33	81.00	0.00	1.00	2.00	0.00	2.07		

94JPC-D4-35	86.00	0.10	1.00	5.00	0.10	3.10
94JPC-D4-39	96.00	0.00	1.00	4.00	0.00	4.05
JPC93-STP-D1-1	1.00	270.00	309.00	53.00	44.00	13.12
JPC93-STP-D1-10	10.00	80.00	66.00	43.00	18.00	2.90
(D1-10 Soxhlet)	10.00	63.00	43.00	41.00	15.00	2.90
JPC93-STP-D1-20	20.00	313.00	304.00	158.00	89.00	14.53
JPC93-STP-D1-30	30.00	9.00	6.00	7.00	0.75	1.63
JPC93-STP-D1-33	33.00	3.00	3.00	3.00	0.75	1.63
JPC93-STP-D1-40	40.00	304.00	251.00	70.00	23.00	4.62
(D1-40 Soxhlet)	40.00	396.00	355.00	95.00	37.00	4.62

(Determinations <1 shown as 0.5; "trace" shown as 0.1; and "N.D." shown as 0.00)

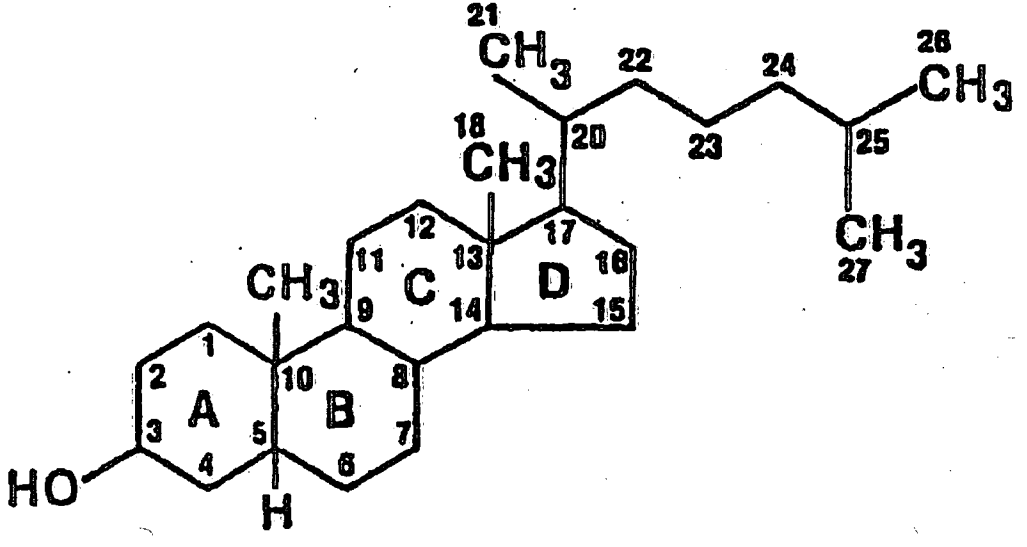

21
CH3
22
24
26
CH3
18
CH3
20
23
25
CH3
27
12
17
11
13
16
CH3
C
D
9
14
15
1
2
10
8
A
B
3
5
7
4
6
HO
H

Figure 1

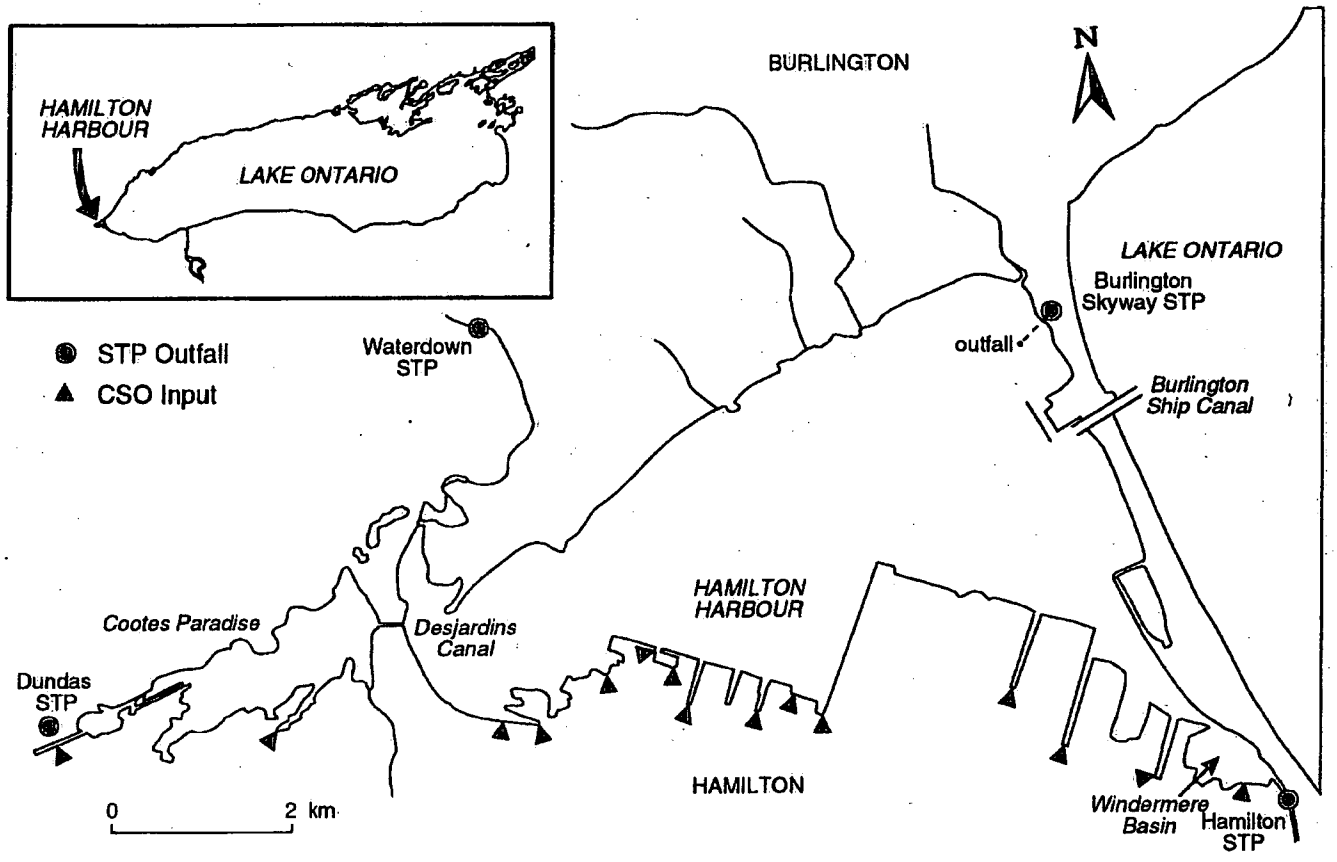

HAMILTON HARBOUR
LAKE ONTARIO
BURLINGTON
N
LAKE ONTARIO
Burlington Skyway STP
outfall
Burlington Ship Canal
STP Outfall
CSO Input
Waterdown STP
Cootes Paradise
Desjardins Canal
HAMILTON HARBOUR
Dundas STP
HAMILTON
Windermere Basin
Hamilton STP
0
2 km

Figure 2

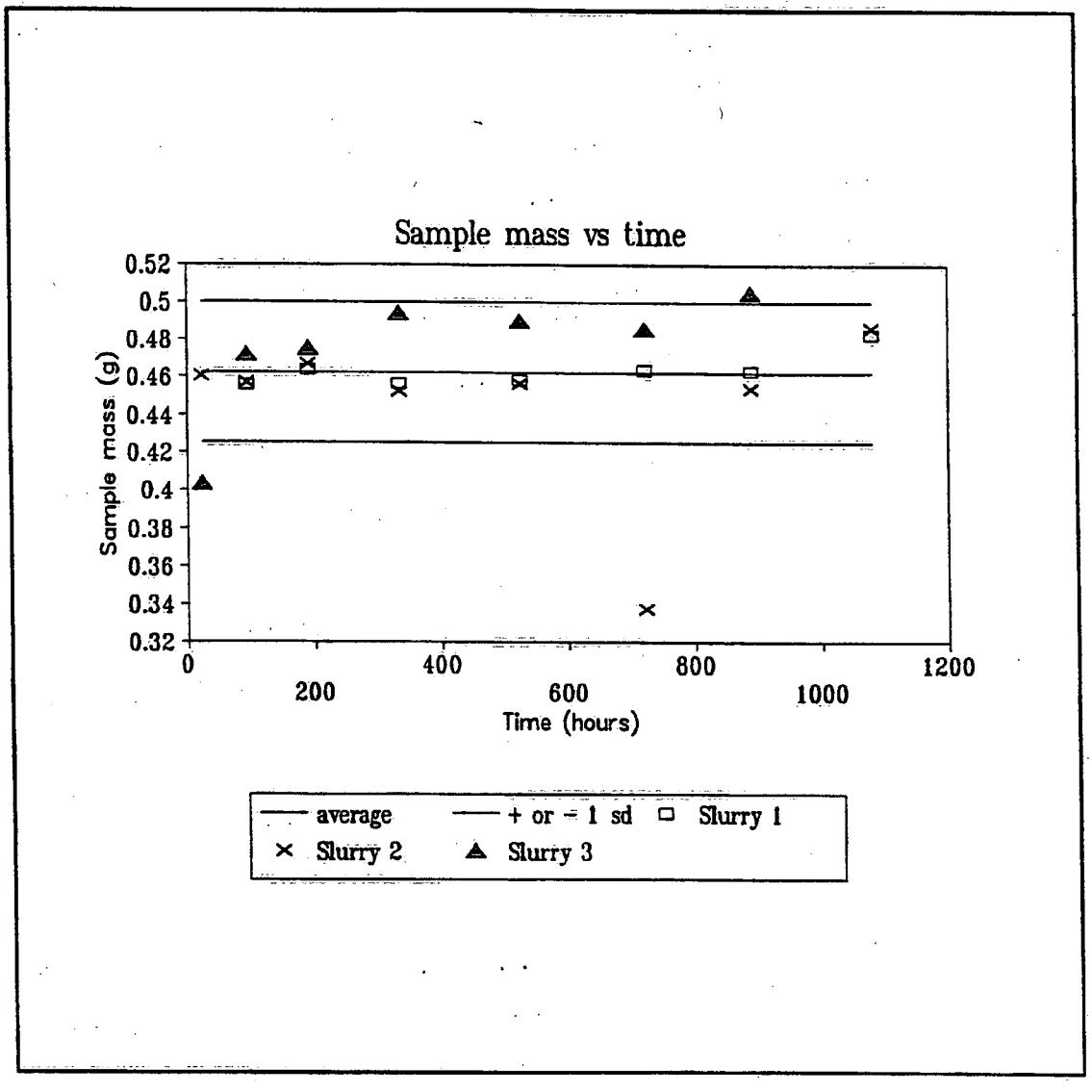
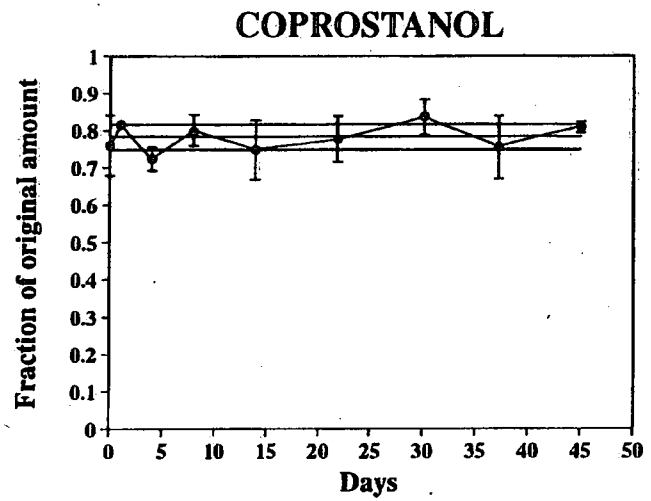
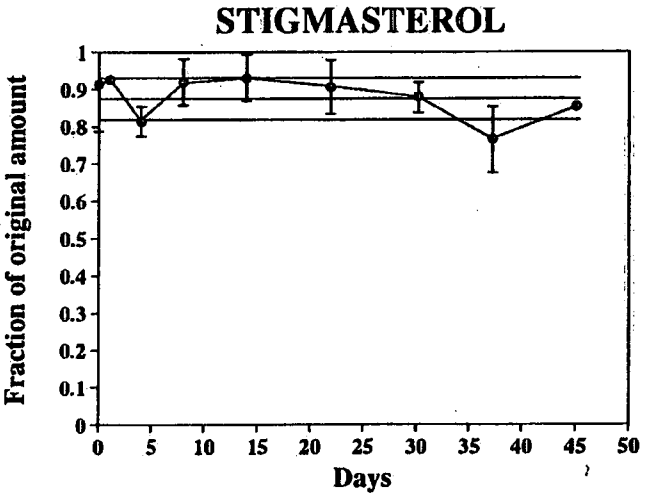
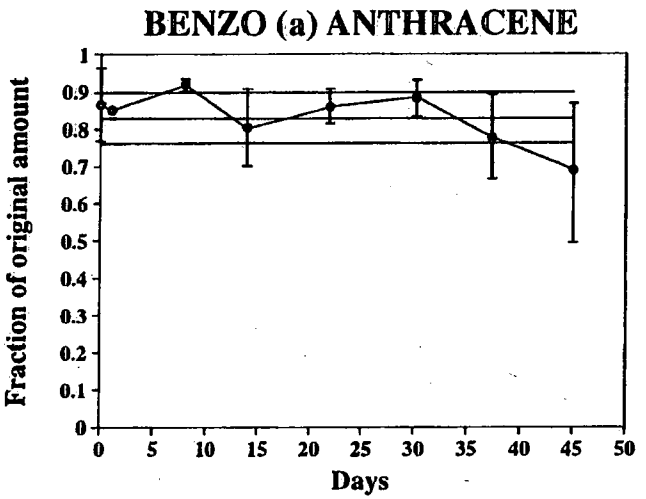

Sample mass vs time
Sample mass (g)
0.52
0.5
0.48
0.46
0.44
0.42
0.4
0.38
0.36
0.34
0.32
0
200
400
600
800
1000
1200
Time (hours)
average
+ or - 1 sd
Slurry 1
Slurry 2
Slurry 3

Figure 3


COPROSTANOL
Fraction of original amount
Days


STIGMASTEROL
Fraction of original amount
Days


BENZO (a) ANTHRACENE
Fraction of original amount
Days

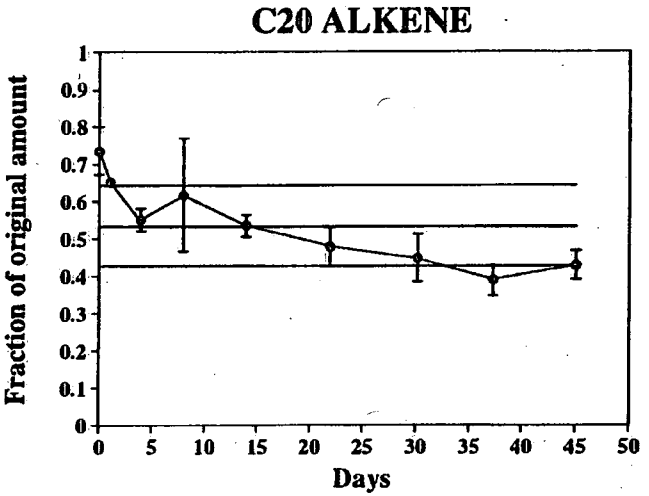

C20 ALKENE
Fraction of original amount
Days

Figure 4

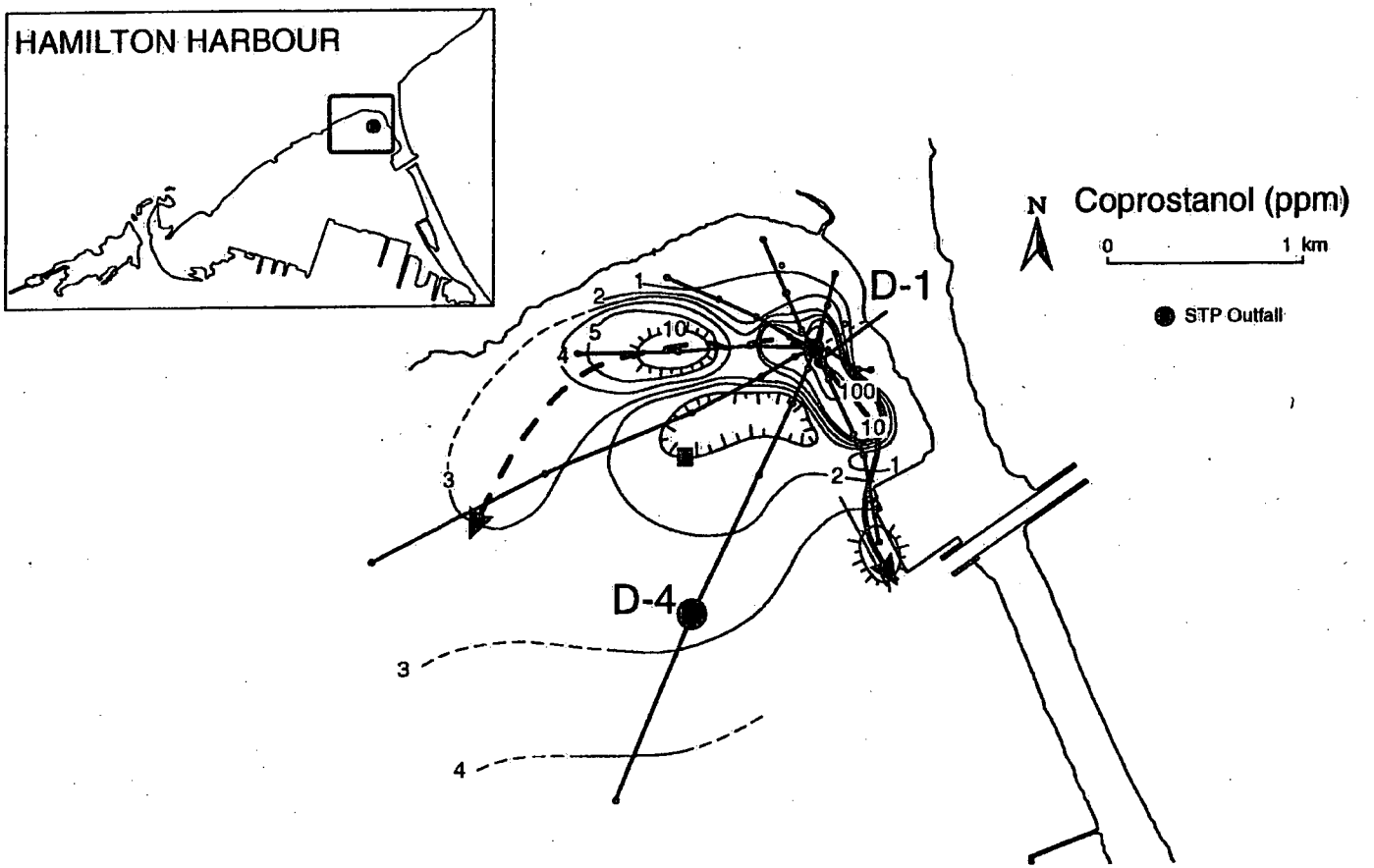
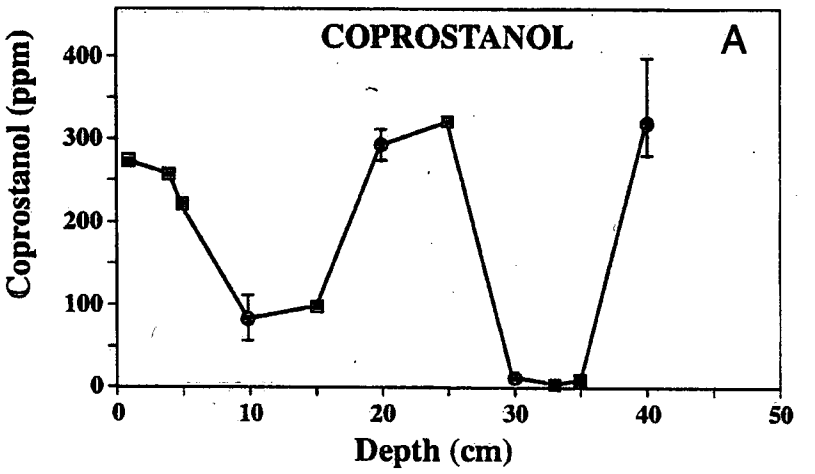
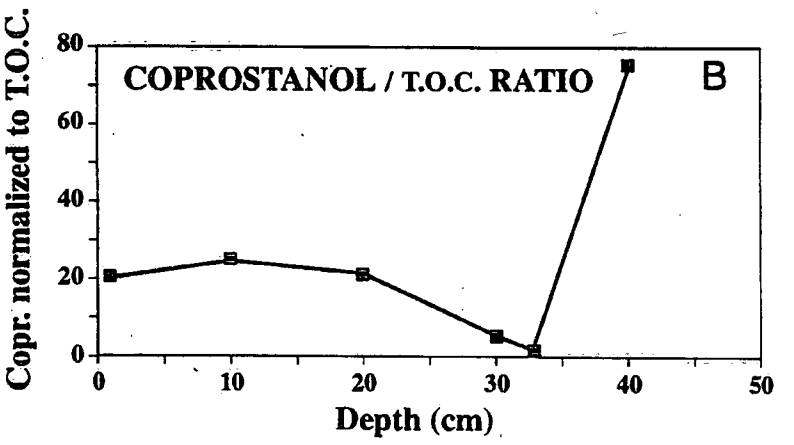

HAMILTON HARBOUR
N
Coprostanol (ppm)
0
1 km
STP Outfall
D-1
D-4
1
2
3
4
5
10
100

Figure 5

D1


COPROSTANOL
A
Coprostanol (ppm)
400
300
200
100
0
0
10
20
30
40
50
Depth (cm)


COPROSTANOL / T.O.C. RATIO
B
Copr. normalized to T.O.C.
80
60
40
20
0
0
10
20
30
40
50
Depth (cm)

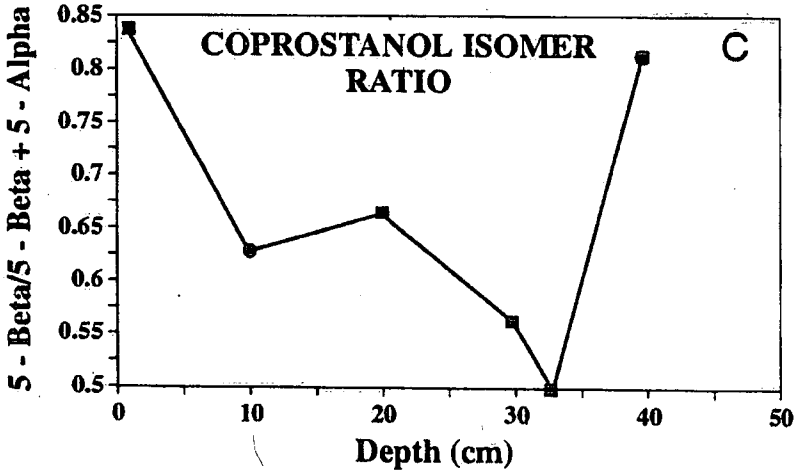
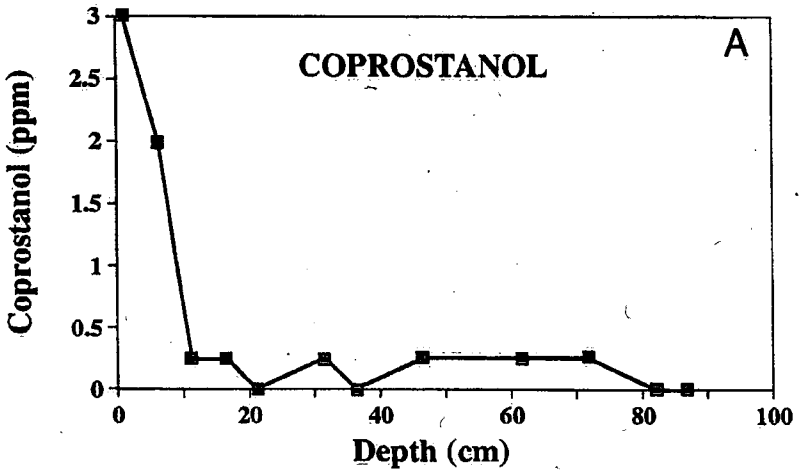
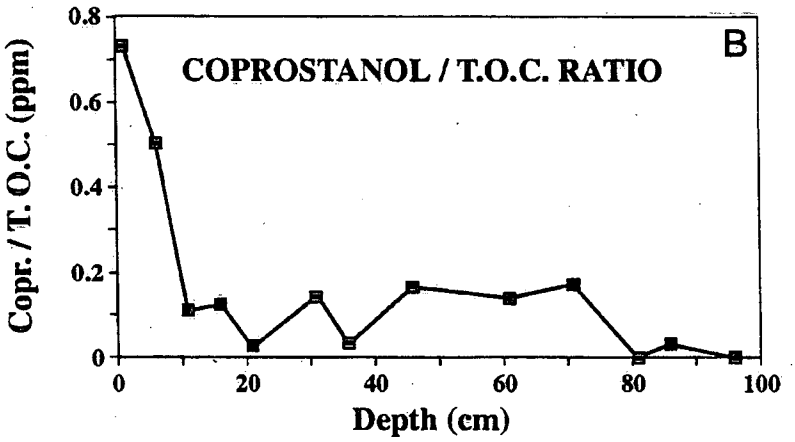

COPROSTANOL ISOMER RATIO
C
5 - Beta/5 - Beta + 5 - Alpha
0.85
0.8
0.75
0.7
0.65
0.6
0.55
0.5
0
10
20
30
40
50
Depth (cm)

Figure 6


A
COPROSTANOL
Coprostanol (ppm)
3
2.5
2
1.5
1
0.5
0
0
20
40
60
80
100
Depth (cm)


B
COPROSTANOL / T.O.C. RATIO
Copr. / T. O.C. (ppm)
0.8
0.6
0.4
0.2
0
0
20
40
60
80
100
Depth (cm)

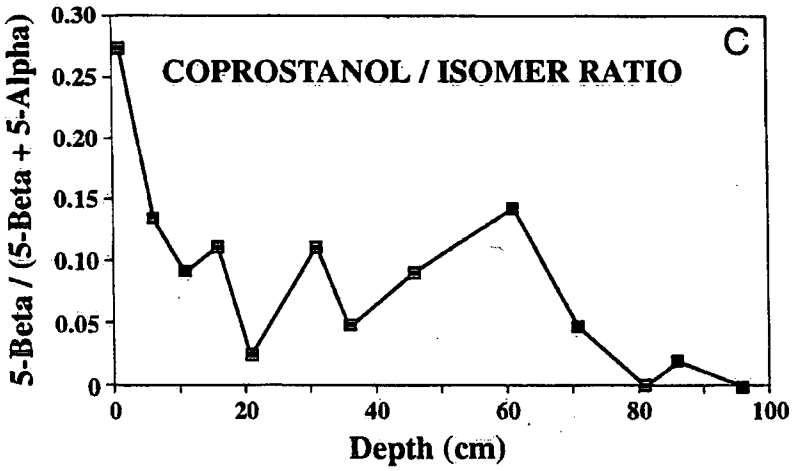

C
COPROSTANOL / ISOMER RATIO
5-Beta / (5-Beta + 5-Alpha)
0.30
0.25
0.20
0.15
0.10
0.05
0
0
20
40
60
80
100
Depth (cm)

Figure 7

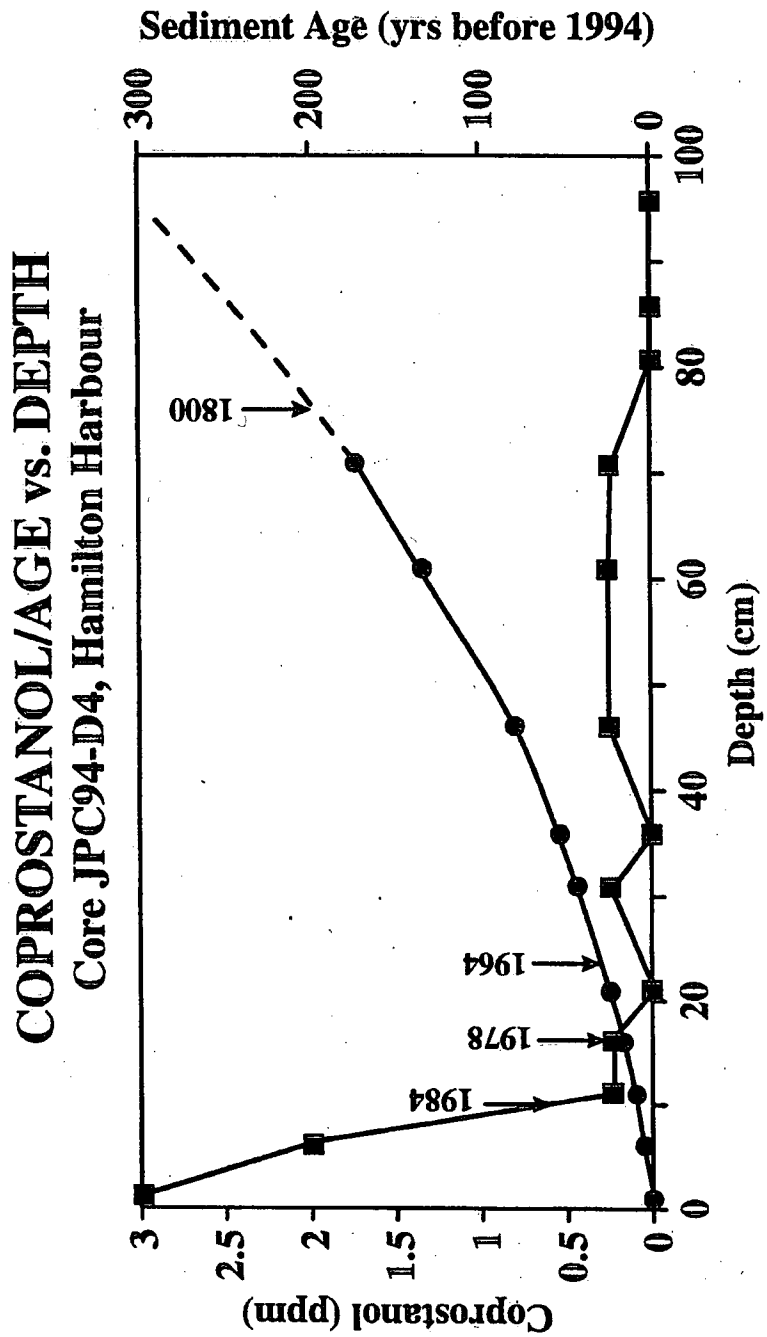

COPROSTANOL/AGE vs. DEPTH
Core JPC94-D4, Hamilton Harbour
Sediment Age (yrs before 1994)
300
200
100
0
1800
1964
1978
1984
Coprostanol (ppm)
3
2.5
2
1.5
1
0.5
0
Depth (cm)
0
20
40
60
80
100

Figure 9

Environment Canada Environnement Canada

Canada

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