Control No. AM295

PRIORITY SITE SELECTION FOR DEGRADED AREAS BASED ON MICROBIAL AND TOXICANT SCREENING TESTS. 1. LAKE ONTARIO, CANADIAN INSHORE AREAS

by

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December 1985
NWRI Contribution #86-87

ABSTRACT

This study is part of a larger ongoing study to identify degraded or degrading water bodies by using a variety of microbiological, biochemical and bioassay tests. These tests are being evaluated as potential candidates for a battery of test procedures which can be used naturally to prioritize water bodies and sediments or selected areas within water bodies for remedial action. For this study 51 inshore sampling sites were selected along the north shore of Lake Ontario from Kingston to Niagara River. Results of the tests are given and discussed.

Sélection des emplacements prioritaires dégradés au moyen des essais de dépistage microbien de la toxicité. 1. Lac Ontario, régions littorales du côté canadien. B.J. Dutka, K. Walsh, K. Kwan, A. El-Shaarawi, D. Liu et K. Thompson

SOMMAIRE

Cette étude fait partie d'une étude plus vaste qu'on mène à l'heure actuelle, en effectuant diverses épreuves biochimiques et microbiologiques ainsi que des essais biologiques dans le but de repérer les étendues d'eau dégradées ou en voie de dégradation. On évalue ces méthodes analytiques pour déterminer si on peut les incorporer dans une batterie d'épreuves qui seraient utilisées dans tout le pays pour établir l'ordre dans lequel il faut apporter des correctifs aux cours d'eau et aux sédiments ou aux parties de certaines étendues d'eau. Pour cette étude on a choisi 51 emplacements pour le prélèvement d'échantillons le long de la rive nord du lac Ontario entre Kingston et la rivière Niagara. Dans le rapport, on présente les résultats et la discussion des essais précités.

EXECUTIVE SUMMARY

The goal of this ongoing series of studies is to identify degraded or degrading water bodies by using a variety of microbiological, biochemical and bioassay tests. These tests, fecal coliforms, E. coli, Legionella, coliphage, coprostanol, cholesterol, dehydrogenase activity, genotoxicity test and Microtox test, are being evaluated as potential candidates for a battery of test procedures which can be used nationally to prioritize water bodies and sediments or selected areas within water bodies for remedial action or further investigations. The battery approach should make it possible to establish "hot spots", areas for immediate concern which were not previously suspected due to inappropriate or one-dimensional testing procedures. Tests which can be performed on refrigerated or frozen samples, 24-96 hours after collection, will be given priority when the selection of the final recommended battery of microbiological, biochemical and bioassay tests is made. The coliphage test, one of the parameters being investigated for the test battery, is of particular importance as it provides information on the potential presence of indicator organisms and bacterial and viral pathogens. The coliphage data from these studies will be related to data from an eight-country, three continent study (S.E. Asia, South America and Northern Africa) monitored by B.J. Dutka through the sponsorship of the International Development Research Centre (IDRC), Ottawa, Canada.

In this study, 51 inshore sampling sites were selected along the north shore of Lake Ontario from Kingston to the Niagara River. Both sediment and water samples were examined.

An arbitrary rating scheme was developed for the results obtained from the various tests. Based on this point rating scheme, there were four areas where sediment and water data were both in the top ten point totals: sewage treatment plant outfall, Humber River area; mouth of Mimico Creek; mouth of Credit River; and near Belleville sewage treatment plant outfall in the Bay of Quinte.

Additions to and deletions from the present test battery are planned for the next series of studies.

SOMMAIRE ADMINISTRATIF

Cette série d'études a pour but de repérer les étendues d'eau dégradées ou en voie de dégradation en effectuant diverses épreuves biochimiques, microbiologiques et biologiques. On est en train d'évaluer les épreuves portant sur les coliformes fécaux, E. coli, Legionella, les coliphages, le coprostanol, le cholestérol, la déhydrogénase, la génotoxicité et le test Microtox, pour voir si on peut les incorporer dans une batterie d'épreuves qui pourrait être utilisée partout au Canada pour déterminer les étendues d'eau ou les parties de celles-ci et les sédiments nécessitant une intervention ou une étude plus approfondie. À l'aide de la batterie d'épreuves, on sera à même de repérer des zones critiques auxquelles il faut apporter des correctifs immédiats alors que celles-ci ont jusqu'à maintenant échappé au dépistage parce que les méthodes analytiques appliquées ne contrôlaient qu'un paramètre ou n'étaient pas appropriées. Pour ce qui est du choix des épreuves microbiologiques, biochimiques et biologiques faisant partie de la batterie qu'on recommandera en définitive, on accordera la priorité aux épreuves qui peuvent être effectuées sur des échantillons réfrigérés ou congelés et conservés ainsi entre 24 et 96 heures après avoir été prélevés. L'épreuve des coliphages, l'un des essais qu'on envisage d'intégrer à la batterie d'épreuves, revêt une importance particulière dans la mesure où elle permet de déceler la présence possible d'indices organiques ainsi que de bactéries et de virus pathogènes. Les données sur les coliphages issues de ces études seront comparées avec celles d'une étude à laquelle participent huit pays répartis sur trois continents (Asie du Sud-Est, Amérique du Sud et Afrique du Nord), sous la surveillance de B.J. Dutka, parrainée par le Centre de recherches pour le développement international (CRDI), à Ottawa, Canada.

Dans la présente étude, on a choisi 51 emplacements le long de la rive nord du lac Ontario entre Kingston et la rivière Niagara dans lesquels on a prélevé des échantillons d'eau et de sédiments.

On a élaboré une échelle arbitraire pour pondérer les résultats des diverses épreuves. À la lumière des cotes numériques ainsi attribuées, on a pu isoler quatre régions pour lesquelles la pondération de l'eau et celle des sédiments atteignait le pointage maximum de 10 : les émissaires des usines de traitement des eaux d'égout, la région de la rivière Humber, l'embouchure du ruisseau Mimico, l'embouchure de la rivière Credit et les environs de l'émissaire de l'usine de traitement des eaux d'égout de Belleville dans la baie de Quinte.

Au cours de la prochaine série d'études, on prévoit retrancher certaines épreuves de la batterie et en ajouter d'autres.

INTRODUCTION

In the investigation and monitoring of waters and sediments, a variety of procedures and techniques have been used to designate waterbodies or sediments that are degraded, or are being degraded, or will be degraded. When used in the above manner, degraded covers a variety of conditions such as unacceptable levels of chemicals, unacceptable responses to bioassay tests, unacceptable levels of health indicator bacterial populations and pathogenic microorganisms, presence of algal blooms, presence of macrophytes, aesthetically deteriorated waters and sediments due to floating debris, garbage, silt and untreated or minimally-treated sewage effluents, etc.

From this and similar future studies, it is intended to identify degraded or degrading water bodies by using a variety of microbiological, biochemical and bioassay tests. These tests are being evaluated as potential candidates for a battery of test procedures which can be used nationally to prioritize water bodies and sediments or selected areas within waterbodies for remedial action or further investigations. The battery approach should make it possible to establish "hot spots", areas for immediate concern, which were not previously suspected due to inappropriate or one-dimensional testing procedures.

Tests which can be performed on refrigerated or frozen samples 24-96 hours after collection will be given priority when the

selection of the recommended battery of microbiological, biochemical and bioassay tests is made.

The waters chosen for this first study were the inshore waters of the Canadian side of Lake Ontario from Kingston on the east to the Niagara River on the west.

1. METHODS

1.1 Sampling Sites

A total of 51 inshore sampling sites were selected along the north shore of Lake Ontario from Kingston on the east to the Niagara River on the western end of Lake Ontario. The sampling sites were chosen to reflect river and stream inputs into Lake Ontario, industrial and domestic sewage outfalls and for background information some unpolluted areas (Figure 1, Table 1).

2. SAMPLE COLLECTION

2.1 Sediment Samples

All sediments were collected in mid June (1985) with an Ekman-dredge and the whole dredge sample was split between various containers. Where little sediment was available several casts were made and the samples pooled.

Approximately 500 grams of sediment were placed in large whirl-pak bags and refrigerated for toxicity screening tests; 100 grams were placed in smaller whirl-pak bags and refrigerated for dehydrogenase activity and fecal coliform (A1 broth) tests; and 100 grams of sediment were also placed in screw-capped glass jars and frozen for future sterol analysis. The fecal coliform tests were

usually completed within ten hours of sample collection. Sediments were collected from every station, although all of the tests were not performed on every sediment collected.

2.2 Water Samples

One litre surface water samples were collected from selected sites for fecal coliform, E. coli and coliphage tests. These samples were refrigerated and processed within 12 hours of collection. A 500 mL surface water sample was collected at every station for toxicity tests and the sample was kept at 4°C until processed. Also at selected stations a one litre water sample was collected and preserved with 5 mL H_2SO_4 for fecal sterol analysis and a two-litre sample for Legionella enumeration studies was collected and refrigerated at 4°C until the sample could be processed, usually within ten days.

3. MICROORGANISM TESTS

3.1 Legionella

The detailed procedure used to isolate and enumerate Legionella organisms is described in a 1984 report prepared by Dutka and Walsh. The minimum number of organisms detectable by the membrane

filter procedure used in these studies is 33 Legionella per litre of sample, based on the finding of one Legionella organism in 1 mL of acidified and neutralized membrane filter sample (Dutka et al. 1983).

3.2 Fecal Coliforms

Fecal coliform densities were estimated by the membrane filtration technique and the most-probable-number technique. The membrane filtration procedure used, is detailed in the Department of Environment Inland Waters Directorate Manual "Methods for Microbiological Analysis of Waters, Wastewaters and Sediments" (1978), and the medium used was FC agar. The sensitivity of this MF technique is such that it is theoretically possible to enumerate one fecal coliform in 100 mL of water sample. However, studies by Dutka, Kuchma and Kwan (1979) have shown that this procedure only measures 6 to 26% of the potential population present.

A five-tube, three to four decimal series (10, 1.0, 0.1 and 0.01) most-probable-number (MPN) procedure using Al broth (Dutka 1978) was used to estimate fecal coliform population in sediments. Studies by Dutka, Kuchma and Kwan (1979) have shown that this medium is fairly specific for E. coli with over 90% of the enumerated population being E. coli. The sensitivity of the technique is such that, if a healthy fresh population is estimated, the count estimated is between the 95% confidence limits (APHA 1985), however, with enviromentally stressed

populations subjected directly to stressful incubation temperature of 44.5°C, as few as 40% of the true population are enumerated (Dutka, Kuchma and Kwan 1979).

3.3 E. coli

E. coli densities were estimated by the membrane filtration technique using MTEC agar and with membranes being transferred to pads saturated with urea substrate as detailed by Dufour, Strickland and Cabelli (1981). In fresh waters, it has been reported that 87-91% of the population enumerated (Dufour, Strickland and Cabelli, 1975, 1981) by this procedure were E. coli. The sensitivity of this MF technique is such that it is theoretically possible to isolate one E. coli from 100 mL of water sample. However, studies by Dutka, Kuchma and Kwan (1979) have shown that in reality MF procedures measure 6-90% of the potential population depending on the stress state of the organisms being enumerated.

3.4 Coliphage Test

Coliphage are bacterial viruses (bacteriophage) which infect and replicate in lactose fermenting Enterobacteriaceae (coliform and fecal coliforms). Since coliphages replicate only in coliform and fecal coliform organisms, the presence of coliphage in waters also

indicates the probable presence of these indicators. The procedure used in these studies to estimate coliphage concentrations is that found in Section 919 C of 16th edition APHA Standard Methods (1985). This procedure can theoretically detect 1 coliphage in 100 mL of water sample, where water turbidity is not in excess of 25 NTU. Under normal test conditions, the minimum sensitivity of the test procedure is 5 coliphage/100 mL. Waters with turbidity in excess of 25 NTU may show coliphage counts reduced up to 90% due to the ready adsorption of phage to particles and thus being concentrated and/or precipitated.

4. BIOCHEMICAL TESTS AND TOXICITY SCREENING TESTS

4.1 Fecal Sterols

Both coprostanol (5β-cholestan-3β-ol) and cholesterol are present in mamallian feces and have been found in domestic sewage and receiving waters and have been considered for use as molecular markers of domestic sewage contamination (Dutka and El Shaarawi 1975). Coprostanol is one of the major fecal sterols, found only in feces, and is readily formed in the gut by the conversion of cholesterol to coprostanol by intestinal bacteria. This conversion has been unequivocally substantiated by both in vivo and in vitro studies. Both coprostanol and cholesterol are readily removed by activated sludge and secondary sewage treatment with coprostanol being almost

totally removed in well-operated sewage treatment systems. Thus the finding of any traces of coprostanol and perhaps cholesterol in raw waters would be indicative of the presence of fecal materials and therefore potential health hazards.

Fecal sterol analyses were performed on water by the IWD laboratories using procedures described by Dutka, Chau and Coburn 1974. This procedure provided for 91-97% recoveries of coprostanol and 74-96% for cholesterol.

The IWD Laboratory's procedure for analysis of fecal sterols in sediments is described below in detail.

4.1.1 Extraction

1. Weigh 20.0 g sediment sample in a 250 mL stainless steel beaker.
2. Add 100 mL hexane to the sample.
3. Extract the sample by using the sonifer at full power, 70% duty cycle for three minutes. Remove sample and let settle for one minute, then carefully decant the hexane extract through 6 cm of wash celite. Suck the solvent to a 1 L separatory funnel. Repeat the extraction with 2x100 mL hexane.
4. Wash the hexane extract with 2x50 mL 70% ethanol.
5. Wash the hexane extract with 2x50 mL acetonitrile.
6. Dry the washed hexane through anhydrous Na_2SO_4 to a 500 mL round bottomed flask.
7. Rota-evaporate the hexane extract to about 2 mL.

4.1.2 Cleanup

1. Prepare 3% deactivated neutral alumina by adding 3% XAD-2 purified water into neutral alumina (Woelm) and mix it for two hours.
2. Pack a column 2 cm x 50 cm with 30.0 g 3% deactivated neutral alumina and top the column with 5 mm anhydrous Na_2SO_4.
3. Wash the column with 100 mL P. ether; then transfer the hexane extract to the column. Wash the flask with 2x2 mL P. ether and add all the washings to the column. Elute the column with 100 mL P. ether and discard this fraction.
4. Elute the column with 50 mL ethanol and collect this fraction in a 300 mL round bottomed flask.
5. Rota-evaporate the ethanol fraction to about 2 mL? then transfer it to a 15 mL centrifuge tube. Wash the flask with 3x2 mL toluene and combine all the washings to the tube. Adjust it to 10 mL with toluene.

   Then it is ready for GC-F10 analysis.

4.1.3 Detection limit set up for fecal sterols in sediment samples

1. The detection limit (D.L.) setup is based on the old concept of definition, which is twice the noise level of the baseline. For example, if the noise level of sample x+y is 0.1 cm, set the

detection limit at 2x0.1 cm = 0.2 cm. The ppm of that would be as follows:

0.2 cm in the chromatogram is equal to 0.5 ng/μL (coprostanol), the final volume is 1.0 mL, the sample weight is 10.0 g, therefore, ppm D.L. is

$$0.5 \text{ ng}/\mu\text{L} \times \frac{1.0 \text{ mL}}{10 \text{ g}} = 0.1 \text{ ppm}$$

4.2 Dehydrogenase Activity

Under varying conditions, studies on the measurement of toxicant effects at cellular (enzyme) levels are more sensitive than traditional population toxicant effect tests such as those using LC_{50} or EC_{50} end points. As dehydrogenases are involved in the vital anabolic and catabolic reactions of organisms, their use in toxicity screening tests holds much promise.

Sediments were extracted with glass distilled water, 5 gm wet weight with 5 mL H_2O. This mixture was vigorously shaken for three minutes, centrifuged at 5000 rpm and the supernatant tested for dehydrogenase inhibition activity.

In this study, the resazurin reduction procedure (Liu 1981) using Bacillus cereus and 5 mg resazurin tablets was followed. The resazurin test for dehydrogenase activity is very stable having a very good reproducibility (average r.s.d. 3%) (Liu, Thompson and Kaiser 1982) and can be performed within a two hour period.

4.3 Genotoxicity Test

This test was performed using an SOS Chromotest Kit produced by Organics Ltd. of Israel. Genotoxic agents cause lesions in the DNA of bacterial cells. Immediately after damage the cell tries to restore the DNA to its original native condition by activating a repair system called SOS. The results of SOS repair efforts will determine the future of the cell. In a successful complete repair, the cell will resume its normal cycle and activities. In the case of an impossible repair, the damage will be too extensive and the cell will die. An incomplete repair will cause permanent changes in the genetic structure of the cell and may result in transmissible mutation or cancerous tranformation of the cell (Fish et al. 1985).

The bacterial strain used in the test has been restructured by genetic engineering methodology. An unrelated enzyme gene, β galatosidase, normally absent from the bacteria, was linked to an SOS operator gene. When the SOS system is activated by genotoxic assault, the enzyme is produced and detected by a colour reaction. In the SOS Chromotest, the activity of the β-galactosidase is the result of genotoxic assault. Even cells that do not divide (and thus do not produce colonies) are reported to give positive result in the SOS Chromotest.

The test consists of colorimetric assays of enzymatic activities after incubating the tester strain (E. coli K12-PQ37) in

the presence of various amounts of compound. An exponential-phase culture of E. coli K12-PQ37 is introduced into the cells of a microtitration plate containing samples and controls. After two-hour incubation at 37°C, a chromogenic substrate is introduced which lyses the bacteria and the colour develops after a short incubation. The results can be analysed visually. For more precise analysis, the SOS Chromotest plate can be read in a microtitration plate (Elisa) photometer.

The sensitivity of the SOS Chromotest (lowest amount detected) is equal to that of the mutatest and generally 4-40 times higher than the phage induction assay (inductest) (Quillardet et al., 1982). Environmental sample data and information on the sensitivity of the test of the environmental samples are not yet available. For the genotoxicity test, sediments were extracted with glass distilled water, 5 gm wet wt with 5 mL H_2O. The mixture was vigorously shaken for three minutes, centrifuged for 10 minutes at 4°C at 5000 rpm and the supernatant tested for genotoxicity activity with and without addition of S-9 mix (Fish et al. 1985).

4.4 Microtox Test

Beckman Instruments Inc. have developed a rapid test for acute water toxicity in which a specialized strain of luminescent bacteria is used as the bioassay organism. This test is functional because the metabolism of the luminescent bacteria is influenced by

low levels of toxicants, which in turn affects the intensity of the organisms' light output. By sensing these changes in light output, the presence and relative concentration of toxicants can be detected. (Beckman 1982).

For the test the Microtox reagent is prepared by reconstituting a vial of lyophylized luminescent bacteria and allowing the vial to stabilize for 2-3 minutes in a cooling block. A testing vial is then placed in a light tight turret and exposed to a photomultiplier tube. Total light output is read from the digital panel meter on an accessory chart recorder. The sample to be tested is injected or pipetted (10 to 100 µL) into the vial, and the light output after a 5-15 minute incubation period is checked. Toxicity is reported as a percent decrease in light output and is calculated as:

$$\frac{\text{base light level} - \text{final light level}}{\text{base light level}} \times 100$$

In keeping with toxicology convention, a given sample of toxicant is characterized in terms of effective concentration (EC) causing a stated percent decrease in light output, thus EC_{50} is that concentration of toxicant (or dilution of unknown) causing a 50% reduction in light from the base level.

The Microtox test is very stable. When seven different lots of reagent were tested against 2.5 mg/L Malathion and 25 mg/L phenol, the standard deviations were 1.8 and 2.4 and the coefficients of

variation were 4.3% and 6.4%. The sensitivity of the test is such that it can detect 0.0005 milligrams per litre of napthalene and 0.05 milligrams per litre of $HgCl_2$.

For the Microtox test on sediments, the sediments were extracted with glass distilled water, 1 gm wet wt sediment with 1 mL H_2O. This mixture was vigorously shaken for three minutes, centrifuged at 4°C for ten minutes at 5000 rpm and the supernatant tested for toxicant activity. Water samples were tested neat and concentrated 10 x by flash evaporation at 45°C.

5. RESULTS AND DISCUSSION

A brief characterization of the 56 sediments collected from the 51 stations (Table 1) is presented in Table 2. The scheme used to rank each sample is shown in Table 3. This scheme is biased toward toxicant presence and the direct presence of hazardous microorganisms. Samples with the most number of points by this scheme are deemed to contain the greatest potential hazard, to man and the living organisms found in the aquatic ecosystem. High toxicant levels may have reduced microbial levels/activity in some sediment samples, however, the cause and effect relationships were not investigated.

Table 4 present the results of the surface-water analyses. Both Microtox and Legionella parameters were found to be negative at all stations tested by the test volumes and concentrations used, a

finding not totally unexpected. From previous studies (Dutka and Walsh, 1984), it was realized that the stressful procedures used in Legionella isolation, recover less than 10% of the potential population. Also, the background growth in many instances tends to mask the Legionella colonies. Thus the finding of Legionella organisms in minimally polluted waters, i.e. waters with 500-1000 bacteria per mL, is like trying to find a needle in a haystack, due to the low natural levels of Legionella. A certain degree of intuitive skill is also required to differentiate possible Legionella colonies from the hundreds of background colonies on the agar medium. Thus the recovery of Legionella organisms from natural mesotrophic and eutrophic water samples are a rarity.

Previous experience in screening for toxicant activity in Canadian natural waters has shown that the receiving water sample must be concentrated by flash evaporation 10-100 times before a positive Microtox test is recorded. This is due to the low amount of chemicals in the water sample volume usually tested (i.e. 0.5 mL in the Microtox test). Recent studies using continuous flow centrifugation have shown that approximately 150 litres of water must be concentrated and these concentrates extracted (usually 5 volumes H_2O to 5 grams concentrate) before a positive toxicant screening test is found. Effluents or waters directly affected by effluents will, of course, require much less concentration to show a toxicant activity. Therefore, the lack of positive results in the Microtox tested samples is due to the low

is due to the low concentration of chemicals in these waters. Undoubtedly, if the samples were concentrated 100 or 1000 times, some of the samples would have yielded a positive toxicity screening test. In our studies, we have limited ourselves to testing 1x and 10x (flash evaporation) samples in order to obtain reasonable baseline data on toxicant distribution.

The finding of cholesterol concentrations in all of the 26 water samples tested (Table 4) and coprostanol concentrations in only five samples was totally unexpected and confusing. The pattern one expects to find is higher coprostanol concentrations and low or nondetectable cholesterol levels (Dutka et al., 1974) an indication that cholesterol is more readily degraded by water bacteria or that cholesterol inputs into the water system are at lower levels than coprostanol. The ready biodegradation of cholesterol by water bacteria has been noted by Marshock et al., 1972 and Switzer-House and Dutka, 1978. Switzer-House and Dutka (1978) reported that, in controlled studies, natural water bacteria degraded cholesterol slightly faster than coprostanol and in natural water samples, 90% of fecal sterols (coprostanol and cholesterol) can be degraded within two weeks by indigenous microbial populations.

Cholesterol is always found in vertebrates, often in invertebrates, but rarely in plants. It has also been found in bacteria and blue-green algae. Bunch and Tabak (1973) noted the following: "cholesterol is not normally excreted to any extent in the urine. Most excreted cholesterol is via the intestine in the feces.

Because the gastrointestinal tract provides conditions favourable for microbial transformations, the unabsorbed cholesterol excreted into the gut does undergo several transformations, one of which is the sterol coprostanol (5β-cholostan-3β-ol) which is formed by bacterial action in the lower intestine. Coprostanol is one of the principal sterols in feces of man and higher animals. Mammalian feces are believed to be the only source of this compound. Thus, the finding of coprostanol in water or sediments would indicate excreta from either domestic wastes or runoff from pastures or barnyards." The presence of cholesterol in surface waters could come not only from excreta, but also from eggs, milk, lard, wool grease and bacteria, and blue-green algae. The latter two sources may be the sources of most of the cholesterol found in this study. Murtaugh and Bunch, in 1967, suggested that the persistent finding of cholesterol in natural waters may be due to a high-level resistance of cholesterol to biodegradation, production of cholesterol by microbiological forms of aquatic life and leaching of cholesterol from bottom sediments. The lack of detectable levels of cholesterol in the majority of sediments in this study strongly negates the assumption that production of cholesterol by microbiological forms of aquatic life, is the prime source of cholesterol in the water samples. Smith and Gouron (1969) also noted that cholesterol may be detected in most marine waters. The conclusion drawn from these data and contradictory research studies is that the use of cholesterol concentrations should be

further evaluated in a variety of waters before a final decision is made on the applicability of this parameter in the proposed "battery of tests".

To assess the association between coliphage, fecal coliforms, E. coli and fecal sterols, the observations on these parameters were transferred to logarithms prior to analysis. The logarithm transformations provide a suitable scale for the analysis of bacteriological data, since the variance of bacterial counts increases with the observed count. Also in these analyses, due to the fact that some values were not observed completely but recorded as less than or greater than, these values were replaced by their cutoff point (i.e.) a value of <5 is used as 5.

Let X_1 = ln cholesterol, and X_2 = ln E. coli, X_3 = ln coliphage, X_4 = ln cholesterol and X_5 = ln coprostanol. The association between the fire water quality indicators is given in the following correlation matrix.

	X_1	X_2	X_3	X_4	X_5
X_1	1.00	0.93**	.60**	.01	.17
X_2		1.00	.47*	.01	.14
X_3			1.00	.04	.65**
X_4				1.00	.49**
X_5					1.00

This matrix gives the correlation between each pair of log parameters. For example, in the first row and second column, we have 0.93 which is the correlation coefficient between log fecal coliform and log E. coli. Values marked by * and ** are significant at the 5% and 1% levels, respectively. Fecal coliform densities show significant correlations with E. coli and coliphage densities. Coliphage is also correlated with E. coli and coprostanol. Cholesterol and coprostanol are highly correlated in these samples.

In order to study the total variation in the surface waters to determine which of the five water quality parameters have the largest contribution, principal component analysis was used to divide the total variation into five uncorrelated components. The results showed that the first two components contain 80.7% of the total variation. The percentage explained variation is given below:

	Principal Components				
%	1	2	3	4	5
Explained Variation	51.4	29.3	15.2	3.3	0.8

The first principal component is dominated by fecal coliform, E. coli and coliphage. The second is dominated by coprostanol and cholesterol, while the third is dominated by coliphage and cholesterol. The first three components are respectively;

$$PC_1 = 0.55\ \ln x_1 + 0.52\ \ln x_2 + 0.52\ \ln x_3 + 0.14\ \ln x_4 + 0.37\ \ln x_5$$

$$PC_2 = 0.33\ \ln x_1 - 0.35\ \ln x_2 + 0.11\ \ln x_3 + 0.63\ \ln x_4 + 0.60\ \ln x_5$$

$$PC_3 = 0.22\ \ln x_1 - 0.33\ \ln x_2 + 0.54\ \ln x_3 - 0.67\ \ln x_4 + 0.29\ \ln x_5$$

where PC_2 denotes the ith principal component, ln = natural log and x_1, x_2, x_3, x_4, x_5 denote fecal coliforms, E. coli, coliphage, cholesterol and coprostanol, respectively.

Finally, stepwise regression was used to model the ln coliphage using the other four parameters. The results indicate that coliphage can be modelled as a function of Fecal coliforms and E. coli. The model is

$$\ln x_3 = 1.6582 + 0.6512\ \ln x_1 - 0.3305\ \ln x_2$$

with x_1 being the first parameter to enter the regression equation. Thus, it would appear that coliphage counts provide similar indications of fecal pollution as do fecal coliform and E. coli counts.

Based on the point scheme developed in Table 3, the ten areas of greatest potential concern are:

1. Rouge River mouth; 2. Toronto area around sewage treatment plant outfall; 3. Cataraqui River, middle of channel; 4. Mimico Creek mouth; 5. inside of bay at Port Dalhousie; 6. Etobicoke Creek mouth; 7. Credit River mouth; 8. opposite Sunnyside Beach in Toronto; 9. Humber River mouth; 10. Bay of Quinte near Belleville sewage treatment plant outfall (Table 4).

Fecal coliform/E. coli population estimates, by the Al Broth MPN Procedure, in the sediment samples are shown in Table 5. Based on previous studies by Dutka, Kuchma and Kwan (1979), the specificity of the Al Broth Procedure for E. coli is very high, i.e. over 90% of the fecal coliforms were shown to be E. coli. Also, on selected sediments from the Great Lakes Basin, it was confirmed that over 90% of fecal coliforms in the sediments were E. coli (indol positive and acid and gas production at 44.5°C). Therefore, in this report we use, for the Al Broth Procedure, the terms fecal coliforms and E. coli interchangeably.

In the study, fecal coliform/E. coli estimates varied from 5 to >16000 per 10 gm (wet wt) of sediment. Sixteen sediments were found to have E. coli counts greater than 1600 per 10 gm sediment, a very strong indication of contamination by fecal material. Of those 16 sediments, #5, #9A and #31, which ranked, 9, 1 and 2, respectively, also showed the presence of coprostanol and cholesterol.

Contrary to the water sample findings, where cholesterol was found in every sample tested, cholesterol was only found in sediments which also contained detectable levels of coprostanol (fecal sterols). These findings present an interesting problem. If the presence of cholesterol in surface water samples is due to one or more of these possibilities: a) microbial and blue-green algal excretion, b) widespread minute contamination by egg, milk or lard products or wool grease, or c) the relatively greater resistance of cholesterol to biodegradation and thus persistence so that we are observing and tracing diluted sewage throughout inshore Lake Ontario waters, then one would also expect the sediments to contain detectible cholesterol levels, which was not the case. Instead, we found what we had expected to find, i.e. in samples which are obviously contaminated by fecal material (presence of coprostanol), concentrations of both coprostanol and cholesterol, and in samples not containing coprostanol, no cholesterol.

The question then arises, could it be possible that sediment bacterial flora biodegrade coprostanol and cholesterol faster than bacteria in the aqueous phase? If this hypothesis is true, then it is probable that the sediment samples containing coprostanol and cholesterol are those which continue to receive large consistent influxes of diluted sewage at a rate faster than the bacterial flora can handle? Is it also possible that microbes and blue-green algae do not play any detectable role in the presence of cholesterol in surface waters? Surely, if they did actively excrete cholesterol in their

living process, cholesterol would be as easily detected in the sediments as in the water column. The results of the cholesterol analyses present an interesting dilemma and contradictory evidence. In the water column, cholesterol appears to be ubiquitous, while in sediments, it is found only in places where it is positively known there is a continuous input of fecal material (sewage). Is it possible to dismiss the presence of cholesterol in the water column as an artifact, or does the presence of cholesterol in all of the water samples indicate that sewage pollution is more widespread than we suspected? This aspect will require further investigations.

The procedures followed in applying the SOS Chromotest (genotoxicity) to the sediment extracts requires a 30 minute reaction time for colour development. Experimental studies have shown that the procedure becomes more sensitive with increased reaction time. However, in this study, the manufacturers' guidelines were adhered to with the result that only a few sediments were found to have genotoxic activity. This activity was only noted in those wells (microtitration plate) which had the highest concentration of sediment extract; which in turn was equivalent to the colour reaction to the lowest or second lowest concentration of positive control. Thus, a dose response curve could not be established and the concentrations of toxicant reported were in terms of concentration of positive control which had the same colour reaction.

Four samples were positive #9T, 18, 18A and 23, i.e. four samples contained small amounts of mutagens/carcinogens which were

soluble in 10% DMSO in H_2O. Only samples 9T and 18 were in the top ten of the most contaminated sediments, and they also had positive Microtox tests for toxicants.

Because of the the ease with which this test can be performed and with an increased colour reaction time, there is great promise for this test as a relatively inexpensive screen for mutagenic/carcinogenic activity in sediments. Further evaluation of the genotoxicity test is definitely warranted.

Fifteen of the sediment water extracts were found to have toxic activity as measured by the Microtox test. Samples which contained the greatest toxicant levels were in decreasing order of concentrations: #7, 0.181 gm wet wt; #32, 0.188 gm; #14, 0.188 gm; #35, 0.216 gm; #9A, 0.222 gm; #9T, 0.228 gm; #17, 0.281 gm; #1, 0.313 gm; #15, 0.381 gm, #9H, 0.400 gm; #3, 0.425 gm; #2, 0.425 gm, #18, 0.488 gm, #11, 0.500 gm; and #8, 0.500 gm. From the above, it can be seen that three of the Port Hope inner harbour sediment samples had toxicant activity with #9T which is ranked #3 also had mutagen/carcinogen activity. Two unexpected results were the high concentration of toxicant in sediments at Ruby Head and at the mouth of the Moira River.

In the ranking of sediment samples, based on Table 3, seven of the top ten sampling sites had EC_{50} Microtox levels with less than 0.3 gm (wet wt) of sediment. As in previous studies by Microbiology Laboratories Section staff, the Microtox test is proving to be a

valuable test in screening of samples for potential chemical toxicants.

All dehydrogenase activity tests were negative. It is possible that the water extraction procedure was not effective in extracting all the chemicals which would give a positive response, or the levels of chemicals found in the sediments were not sufficiently high enough to give a positive response. As it is planned to continue with the water extraction of sediments, the potential of the dehydrogenase test for testing sediments is not very promising and will not be persued in future studies.

Based on the point ranking scheme developed in Table 3, the top ten sediments for priority concern (Table 5) would be:
1, sample 9A, Port Hope Harbour; 2, sample 31, sewage treatment plant outfall, Humber River area; 3, sample 9T, Port Hope Harbour; 4, sample 32, mouth of Mimico Creek; 5, sample 7, mouth of Trent River; 6, sample 14, Ruby Head; 7, sample 35, mouth of Credit River; 8, sample 17, mouth of Corbett Creek at Whitby; 9, sample 5, Bay of Quinte near Belleville sewage treatment plant outfall; and 10, sample 18, inner harbour of Whitby.

Comparing the top ten areas of concern from Table 4, water samples and Table 5, sediment samples, there are four common stations, which are listed below:

Table 4 Water Sample Rank	Table 5 Sediment Sample Rank	Sample Site
1	2	#31 Humber, STP outfall
4	4	#32 Mimico Creek mouth
7	7	#35 Credit River mouth
10	9	#5 Bay of Quinte, near Belleville STP outfall

Thus, based on this study, the areas of highest priority concern would be sample sites #5, #31, #32 and #35.

From the data presented in Tables 4 and 5, it is obvious that microbial populations or toxicant/mutagen screening tests performed independently are not sufficient to provide realistic estimates of priority concern areas and that the battery approach is required.

Refining of the battery of tests to establish priority concern areas will continue. In the next study, the dehydrogenase and Legionella tests will be discarded and an ATP test for toxicant activity and a test for the presence of Clostridum welchii (sediments) will be added.

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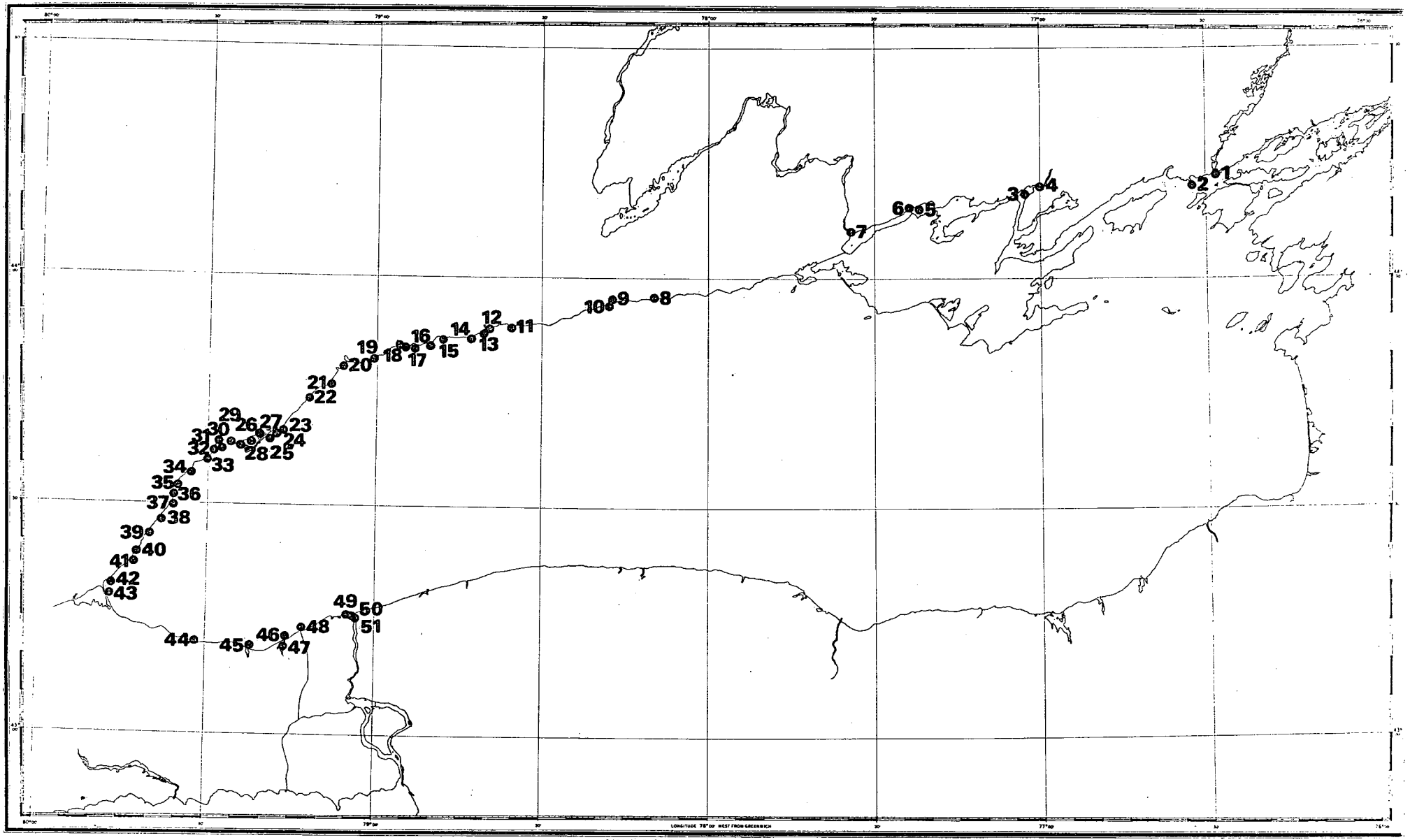
Figure 1 Site of Sediment and Water Collection for Priority Concern Site Selection Study, 1985



Published by the Canadian Hydrographic Service, Marine Sciences Branch,
DEPARTMENT OF MINES AND TECHNICAL SURVEYS, OTTAWA

Ontario
LAKE ONTARIO

1050

TABLE 1 Station Positions, 1985 Lake Ontario Toxicant and Microbial Indicator Study.

Station Number	Latitude N.	Longitude W.
1	44° 13' 27"	76° 28' 25"
2	44° 12' 06"	76° 32' 50"
3	44° 11' 15"	77° 03' 04"
4	44° 11' 54"	77° 01' 00"
5	44° 09' 14"	77° 22' 12"
6	44° 09' 12"	77° 22' 54"
7	44° 05' 54"	77° 34' 15"
8	43° 56' 48"	78° 08' 54"
9 (A,D,H,J.M,T)	43° 56' 45"	78° 17' 40"
10	43° 56' 30"	78° 17' 43"
11	43° 53' 26"	78° 35' 20"
12	43° 53' 07"	78° 39' 48"
13	43° 52' 58"	78° 40' 12"
14	43° 51' 48"	78° 42' 54"
15	43° 52' 05"	78° 49' 40"
16	43° 50' 55"	78° 50' 00"
17	43° 51' 06"	78° 53' 12"
18	43° 51' 00"	78° 55' 36"
18A	43° 50' 50"	78° 54' 52"
19	43° 48' 58"	79° 02' 08"
20	43° 47' 36"	79° 07' 00"
21	43° 46' 01"	79° 08' 41"
22	43° 44' 30"	79° 11' 36"
23	43° 40' 08"	79° 17' 00"
24	43° 39' 42"	79° 18' 00"
25	43° 38' 56"	79° 18' 50"
26	43° 38' 04"	79° 22' 04"
27	43° 38' 51"	79° 21' 16"
28	43° 37' 36"	79° 24' 52"
29	43° 38' 06"	79° 27' 14"
30	43° 37' 35"	79° 28' 05"
31	43° 37' 53"	79° 28' 16"
32	43° 37' 05"	79° 28' 27"
33	43° 34' 00"	79° 32' 31"
34	43° 35' 01"	79° 32' 24"

TABLE 1 Station Positions, 1985 Lake Ontario Toxicant and Microbial Indicator Study. cont'd.

Station Number	Latitude N.	Longitude W.
35	43° 32' 48"	79° 34' 49"
37	43° 29' 45"	79° 36' 24"
38	43° 27' 45"	79° 38' 06"
39	43° 26' 21"	79° 40' 00"
40	43° 23' 31"	79° 42' 20"
41	43° 22' 26"	79° 43' 09"
42	43° 19' 12"	79° 47' 36"
43	43° 18' 08"	79° 47' 18"
44	43° 11' 45"	79° 32' 01"
45	43° 11' 12"	79° 22' 16"
46	43° 12' 42"	79° 15' 56"
47	43° 12' 06"	79° 15' 54"
48	43° 13' 55"	79° 13' 00"
49	43° 15' 50"	79° 04' 48"
50	43° 15' 44"	79° 04' 35"
51	43° 15' 34"	79° 15' 44"

Technical Operations Division
July 31, 1985

TABLE 2 Description of Lake Ontario Ekman Dredge Sediment Samples.

1.	Mud	23.	Sand
2.	Mud	24.	Sand
3.	Mud	25.	Sand
4.	Sand	26.	Mud
5.	Mud	27.	Mud
6.	Sand	28.	Sand
7.	Sand & Shells	29.	Fine Sand
8.	Fine Sand	30.	Sand
9A.	Sandy Mud	31.	Sandy Mud
9D.	Fine Sand	32.	Mud
9H.	Fine Sand	33.	Mud
9J.	Fine Sand	34.	Sand
9M.	Fine Sand	35.	Sandy Mud
9T.	Fine Sand	37.	Sand
10.	Sand	38.	- No Sample -
11.	Sand	39.	Sand
12.	Sand	40.	Brown Sand
13.	Fine Sand	41.	Sand
14.	Sand	42.	Sand
15.	Mud	43.	Sand
16.	Sand and Small Stones	44.	Sand
17.	Sand	45.	Sand
18.	Mud	46.	Mud
18A.	Sand	47.	Sand
19.	Sand	48.	Mud
20.	Sand	49.	Sand
21.	Sand	50.	Sand
22.	Sand	51.	Sand

TABLE 3. Point Awarding Scheme Used to Rank Samples, Based on Suspected Contained Hazards.

Fecal Coliform/*E. coli* Sediment 10/100 mL MPN Water /100 mL	Pts	Coliphage /100 mL	Pts	Legionella /L	Pts	Coprostanol Sediment ppm Water ppb	Pts
<100	1	5 - 25	1	1 - 33	2	<1.0	1
100 - 500	2	25 - 100	2	34 - 100	4	1 - 3	3
500 - 2,500	3	100 - 250	3	100 - 500	6	3 - 5	5
2,500 - 10,000	4	250 - 1000	4	500 - 1000	8	5 - 7	7
10,000+	5	1000+	5	1000+	10	7+	10

Cholesterol Sediment ppm Water ppb	Pts	Dehydrogenase IC_{50}/gm wet wt sediment	Pts	Microtox EC_{50}/gm wet wt or/mL	Pts	Genotoxicity Equivalent to ng/mL 4NQO*	Pts
<2.0	1	<0.1	10	<0.1	10	<200	2
2 - 4	2	.1 - .2	8	.1 - .2	8	200 - 400	4
4 - 6	3	.2 - .3	6	.2 - .3	6	400 - 600	6
6 - 8	4	.3 - .4	4	.3 - .4	4	600 - 800	8
8+	5	.4+	2	.4+	2	800+	10

*4 Nitro Quinoline Oxide

TABLE 4 Rsults of Surface Water Analyses, Lake Ontario, 1985.

Sample No. and Site	Fecal Coliform MF-MFC /100 mL	*E. coli* MF-MTEC /100 mL	Coliphage /100 mL	Fecal Sterol		Legionella	Microtox EC_{50}/mL		Points	Rank
				Cholesterol ppb	Coprostanol ppb	/Litre	1x	10x		
1 Cataraqui R. Kingston	7500	>5000	5	2.2	<0.1	neg	neg	neg	11	3
2 Carruthers Point Kingston							neg	neg		
4 Napanee R.						neg	neg	neg		
5 Sewage Outfall Area Belleville	210	120	5	4.2	<0.1	neg	neg	neg	8	10
6 Moira River						neg	neg	neg		
7 Trent River						neg	neg	neg		
8 Colburg	<2	<2	<5	2.5	<0.1	neg	neg	neg	2	23
9A Harbour - Port Hope							neg	neg		
9D Harbour - Port Hope							neg	neg		
9H Harbour - Port Hope							neg	neg		
9J Harbour - Port Hope							neg	neg		
9M Harbour - Port Hope							neg	neg		
9T Harbour - Port Hope	10	<2	<5	1.5	<0.1		neg	neg	2	22
10 Breakwall - Port Hope						neg	neg	neg		
13 Bowmanville Creek							neg	neg		
14 Ruby Head							neg	neg		
15 Marina, Oshawa							neg	neg		
17 Corbett Creek Whitby							neg	neg		
18 Harbour Whitby							neg	neg		
19 Duffin Creek							neg	neg		
20 Rouge River	<1	<1	<5	1.2	<0.1	neg	neg	neg	1	25
21 Highland Creek	6	2	<5	3.2	<0.1	neg	neg	neg	4	20
22 Scarborough	2	3	<5	2.2	<0.1	neg	neg	neg	4	21
23 Industries Area Toronto							neg	neg		
24 Between Toronto Islands							neg	neg		
25 STP Toronto	10	6	120	5.5	5.3	neg	neg	neg	15	2

TABLE 4 **Results of Surface Water Analyses, Lake Ontario, 1985.** cont'd.

Sample No. and Site	Fecal Coliform MF-MFC /100 mL	E. coli MF-MTEC /100 mL	Coliphage /100 mL	Fecal Sterol		Legionella	Microtox EC_{50}/mL		Points	Rank
				Cholesterol ppb	Coprostanol ppb	/Litre	1x	10x		
26 Harbour, Toronto	68	39	10	3.3	<0.1	neg	neg	neg	5	14
27 Cherry St., Toronto							neg	neg		
28 Ontario Place, Toronto	55	35	10	1.2	<0.1	neg	neg	neg	4	17
29 Sunnyside Beach, Toronto	360	230	60	0.8	0.4	neg	neg	neg	8	8
30 Humber River, Toronto	530	330	125	1.5	0.3	neg	neg	neg	8	9
31 STP, Humber River	430	110	520	6.7	8.3		neg	neg	22	1
32 Mimico Creek	190	64	55	2.0	1.1	neg	neg	neg	10	4
33 Etobicoke Creek	1500	900	145	1.3	<0.1	neg	neg		10	6
34 Lakeview Generator							neg	neg		
35 Mouth of Credit R.	1000	390	170	0.9	<0.1	neg	neg	neg	9	7
37 Gulf Oil							neg	neg		
38 Ford plant							neg	neg		
39 16 Mile Creek							neg	neg		
40 Bronte Creek	270	130	20	0.8	<0.1	neg	neg		6	11
41 Petro Canada Pier							neg	neg		
42 Spencer Smith Park	2	3	20	0.7	<0.1	neg	neg		4	16
43 Entrance to Burlington Canal							neg	neg		
44 Grimsby Beach	<1	<1	<5	0.8	<0.1	neg	neg		1	26
45 Jordan Harbour	<1	<1	<5	1.3	<0.1		neg		1	24
46 Entrance Port Dalhousie	94	2	35	1.5	<0.1	neg	neg		5	13
47 Inside of Bay Port Dalhousie	4400	700	50	1.8	<0.1		neg		10	5
48 Port Weller	100	15	30	0.8	<0.1	neg	neg		5	12
49 Mouth of Niagara R.	3	1	<5	1.3	<0.1	neg	neg		4	19
50 Mouth of Niagara R.	1	<1	5	5.0	<0.1		neg	neg	5	15
51 Mouth of Niagara R.	31	7	10	1.4	<0.1	neg	neg	neg	4	18

TABLE 5 Results of Sediment Analyses, Lake Ontario, 1985.

Sample No. and Site	Fecal Coliform/ *E. coli* Al Broth 10 g/100L MPN	Fecal Sterol		Microtox EC_{50}/gm wet wt sediment	Genotoxicity Equivalent to ng/mL 4NQO*	Dehydrogenase IC_{50}/gm wet wt sediment	Points	Rank
		Coprostanol ppm	Cholesterol ppm					
1 Cataraqui R. Kingston	350	<.1	<.1	0.313	neg	neg	6	13
2 Carruthers Point Kingston	<2	<.1	<.1	0.425	neg	neg	2	23
3 Deseronto	240	<.1	<.1	0.425			6	14
4 Napanee R.	920	<.1	<.1	neg	neg	neg	3	22
5 Outfall Area Belleville STP	>1600	2.3	2.1	neg	neg	neg	8	9 ✓
6 Moira River	540	<.1	<.1	neg	neg	neg	3	22
7 Trent River	110	<.1	<.1	0.181	neg	neg	10	5
8 Colburg	23	<.1	<.1	0.500	neg	neg	3	22
9A Harbour - Port Hope	>1600	3.3	2.8	0.222	neg	neg	16	1 ✓
9D Harbour - Port Hope	110	<.1	<.1	neg	neg	neg	2	23
9H Harbour - Port Hope	>1600	<.1	<.1	0.400	neg	neg	7	12
9J Harbour - Port Hope	350	<.1	<.1	neg	neg	neg	2	23
9M Harbour - Port Hope	>1600	<.1	<.1	neg	neg	neg	3	22
9T Harbour - Port Hope	46	<.1	<.1	0.228	312	neg	11	3 ✓
10 Breakwall - Port Hope	>1600	<.1	<.1	neg	neg	neg	3	22
11 Newcastle	70	<.1	<.1	neg	neg	neg	1	24
12 Bowmanville	>1600	<.1	<.1	0.500	neg	neg	5	15
13 Bowmanville Creek	>1600	<.1	<.1	neg	neg	neg	3	22
14 Ruby Head	49	<.1	<.1	0.188	neg	neg	9	6 ✓
15 Marina Oshawa	>1600	<.1	<.1	0.381	neg	neg	7	11
16 Oshawa	23	<.1	<.1	neg	neg	neg	1	24
17 Corbett Creek Whitby	540	<.1	<.1	0.281	neg	neg	9	8 ✓
18 Harbour Whitby	23	<.1	<.1	0.488	312	neg	7	10
18A Lasco Steel	8	<.1	<.1	neg	234			22
19 Duffin Creek	790	<.1	<.1	neg	neg	neg	3	22
20 Rouge River	140	<.1	<.1	neg	neg	neg	3	23
21 Highland Creek	790	<.1	<.1	neg	neg	neg	2	22
22 Scarborough		<.1	<.1	neg	neg	neg	3	22

TABLE 5 **Results of Sediment Analyses, Lake Ontario, 1985.** cont'd.

Sample No. and Site	Fecal Coliform/ E. coli Al Broth 10 g/100L MPN	Fecal Sterol		Microtox EC_{50}/gm wet wt sediment	Genotoxicity Equivalent to ng/mL 4NQO*	Dehydrogenase IC_{50}/gm wet wt sediment	Points	Rank
		Coprostanol	Cholesterol					
		ppm	ppm					
22 Scarborough	790	<.1	<.1	neg	neg	neg	3	22
23 Industries Area, Toronto	46	<.1	<.1	neg	312	neg	5	16
24 Between Toronto Islands	110	<.1	<.1	neg	neg	neg	2	23
25 STP, Toronto	2800	<.1	<.1	neg	neg	neg	4	21
26 Harbour, Toronto	130	<.1	<.1	neg	neg	neg	2	23
27 Cherry St., Toronto	>16000	<.1	<.1	neg	neg	neg	5	17
28 Ontario Place Toronto	350	<.1	<.1	neg	neg	neg	2	23
29 Sunnyside Beach, Toronto	1300	<.1	<.1	neg	neg	neg	3	22
30 Humber River, Toronto	1400	<.1	<.1	neg	neg	neg	3	22
31 STP Outfall Humber River	9200	3.9	6.5	neg	neg	neg	13	2 ✓
32 Mimico Creek	2400	<.1	<.1	0.188	neg	neg	11	4 ✓
33 Etobicoke Creek	>16000	<.1	<.1	neg	neg	neg	5	18
34 Lakeview Generator	110	<.1	<.1	neg	neg	neg	2	23
35 Mouth of Credit R.	2400	<.1	<.1	0.216	neg	neg	9	7 ✓
37 Opposite Gulf Oil Plant	490	<.1	<.1	neg	neg	neg	2	23
39 16 Mile Creek	490	<.1	<.1	neg	neg	neg	2	23
40 Bronte Creek	49	<.1	<.1	neg	neg	neg	1	24
41 Petro Canada Pier	33	<.1	<.1	neg	neg	neg	1	24
42 Spencer Smith Park	23	<.1	<.1	neg	neg	neg	1	24
43 Entrance to Burlington Canal	11	<.1	<.1	neg	neg	neg	1	24
44 Grimsby Beach	5	<.1	<.1	neg	neg	neg	1	24
45 Jordan Harbour	5	<.1	<.1	neg	neg	neg	1	24
46 Mouth of Port Dalhousie	>16000	<.1	<.1	neg	neg	neg	5	19
47 Inside of Bay Port Dalhousie	>16000	<.1	<.1	neg	neg	neg	5	20

TABLE 5 Results of Sediment Analyses, Lake Ontario, 1985. con't.

Sample No. and Site	Fecal Coliform/ E. coli Al Broth 10 g/100L MPN	Fecal Sterol		Microtox EC_{50}/gm wet wt sediment	Genotoxicity Equivalent to ng/mL 4NQO*	Dehydrogenase IC_{50}/gm wet wt sediment	Points	Rank
		Coprostanol ppm	Cholesterol ppm					
48 Port Weller	790	<.1	<.1	neg	neg	neg	3	22
49 Mouth of Niagara R.	110	<.1	<.1	neg	neg	neg	2	23
50 Mouth of Niagara R.	110	<.1	<.1	neg	neg	neg	2	23
51 Mouth of Niagara R.	170	<.1	<.1	neg	neg	neg	2	23

*4 Nitro Quinoline Oxide