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PROPOSED RANKING SCHEME AND BATTERY OF TESTS FOR EVALUATING HAZARDS IN CANADIAN WATERS AND SEDIMENTS

by

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ABSTRACT

The results of a four year, Canada-wide study of 178 water samples and 227 sediment samples, to evaluate a variety of microbiological, biochemical and toxicant screening tests to form the core group of a battery of tests, which could be used to prioritize samples and provide monitoring data, are described. The core group of tests were found to vary slightly depending on the type of sample or extracting procedure used. Using these battery of tests data and a ranking scheme based on the awarding of points for various positive values, lists were prepared of the 20 most hazardous water and sediment sampling sites.

RÉSUMÉ

Ce rapport présente les résultats d'une étude canadienne de 178 échantillons d'eau et de 227 échantillons de sédiments, étalée sur quatre ans, visant à évaluer une variété de tests microbiologiques, biochimiques et toxicologiques afin de former le noyau d'une batterie de tests dans le but de classer les échantillons par ordre d'importance et de fournir des données de surveillance. Le noyau de tests varie légèrement en fonction du type d'échantillon et de la méthode d'extraction utilisée. Des listes des 20 sites d'échantillonnage des sédiments et de l'eau les plus dangereux ont été préparées d'après ces données et un système de cotation dans lequel des points sont attribués pour différentes valeurs positives.

MANAGEMENT PERSPECTIVE

In 1985, the first of a series of studies were initiated to evaluate a variety of biochemical, microbiological and toxicological tests as potential candidates for the core group of a "battery of tests" approach to environmental studies. These core "battery of tests" data would permit a comparison to be made between Canada-wide sampling sites (water and/or sediment) thus, providing managers with a means or scheme for establishing areas of concern or for evaluating the efficiency of remedial programmes. In this paper, the results of these studies are detailed and three proposed core groups of tests for the battery of tests approach are described based on the type of sample or sediment extraction procedure used.

It was realized at the beginning of the programme that the provision of "battery of test" data alone, would be insufficient information on which to compare Canada-wide samples and areas. To this end, a point ranking scheme was developed based on values observed in pure chemical studies and in a variety of samples, and as new patterns of results were obtained from the Canada-wide studies, modifications were made and incorporated into the point ranking scheme. With the use of this point ranking scheme, we were able for the first time to produce a ranking of areas of concern in Canadian waters based on the "battery of tests" data.

Using the ranking scheme, the top 20 sampling sites with the greatest concerns due to their contained hazards were derived for each type of sample, water, Milli Q water-extracted sediments, and organically extracted sediments.

PERSPECTIVES-GESTION

En 1985, la première d'une série d'études a été amorcée pour évaluer divers tests biochimiques, microbiologiques et toxicologiques afin de constituer un noyau de tests susceptibles d'être utilisés dans les études environnementales. Ces données devraient permettre de comparer les sites d'échantillonnage (des sédiments et de l'eau) canadiens et de fournir aux gestionnaires un outil pour déterminer les secteurs de préoccupation et évaluer l'efficacité des programmes de remise en état. Dans ce rapport, les résultats de ces études sont détaillées et trois noyaux de tests proposés sont décrits en fonction de la méthode d'échantillonnage ou d'extraction des sédiments utilisée.

On a constaté, au début du programme, que les données sur la "batterie de tests" ne permettraient pas à elles seules de comparer les échantillons et les régions à l'échelle du Canada. À cette fin, un système de pointage a été mis au point d'après les valeurs observées dans les études chimiques et dans une variété d'échantillons, et à mesure que de nouveaux résultats étaient obtenus dans les études canadiennes, des modifications étaient apportées et intégrées au système de pointage. Grâce à ce système, les secteurs de préoccupation dans les eaux canadiennes ont pu être classés pour la première fois en fonction des données de la batterie de tests.

Avec le système de pointage, les 20 sites d'échantillonnage les plus dangereux ont été déterminés pour chaque type d'échantillon, d'eau, de sédiments extraits en milieu aqueux (Milli Q) et des sédiments extraits par des solvants organiques.

INTRODUCTION

A variety of tests, methods, and criteria have been developed internationally to assess the ecological impact of released chemicals and domestic and industrial effluents/discharges. However, with the increasing awareness of the long term effects of these contaminants discharged into aquatic systems, research efforts have been directed at short-term bioassay tests to alert monitoring agencies as well as dischargers of the presence of toxicants and other hazards in effluents and the aquatic ecosystem (Bulich and Green, 1979; Dutka and Kwan, 1981). Application of these short-term bioassays to environmental samples soon revealed that there was no single test which was responsive to all conditions. This realization led to the concept of using a battery of tests to ascertain the ecological impacts of effluents, discharges and emissions.

In 1985, we initiated the first of a series of studies to evaluate a variety of microbiological, biochemical and toxicological tests as potential candidates for the core group of a "battery of tests" which could be used by Canadian monitoring agencies. Once established, these core "battery of tests" data would then permit a comparison to be made between Canada-wide sampling sites or areas thus providing managers with a means or scheme for establishing priority areas of concern, or for evaluating the efficacy of remedial programs.

It was realized at the beginning of the program that the provision of "battery of test" data alone, would be insufficient information on which to compare samples/areas. To this end, a point ranking scheme was developed based on test values observed in pure chemical studies and in a variety of samples. As new patterns of test values were obtained from our Canada-wide studies (Dutka et al., 1987) modifications were incorporated. By the end of the data collection and analysis period, a modified point ranking scheme had evolved (Table 1) which now allowed for the unbiased comparison of "battery of tests" data from all parts of the country.

One of the interesting and important by-products of this "battery of tests" development study was the ability for the first time to produce a ranking of areas of concern in Canadian waters based on the "battery of tests" data.

In carrying out our Canada-wide studies, there was no plan to intensively sample all known areas of presently known concerns. Rather, samples were collected from areas which we believed would provide the greatest challenges to the testing procedures, from very polluted areas through to those we believed were not directly impacted by man's activities.

The results of these studies are presented and discussed.

METHODS

Sampling Sites

A total of 178 water and 227 sediment samples were collected from sites distributed between St. John Harbour, New Brunswick on the east to the Fraser River estuary in Vancouver on the west. Some of the estuarine samples were affected by salt water intrusion. Sample site latitudes and longitudes for the sites discussed are shown in Table 2.

Sample Collection

Sediments were collected with an Ekman dredge or shovel. Frequently it was necessary to Ekman many times before sufficient surface (1 to 3 cm) sediment was collected. At each site, the surface layers were pooled, well mixed into aliquots for each testing procedure and refrigerated.

Surface water samples (1 L) were collected for fecal coliform, fecal streptococci and coliphage tests. These tests were usually processed within eight hours of collection. Also at the same site another 1 L sample of water was collected and preserved at 4°C for toxicant screening tests, and another 1 L sample was collected and preserved with 1 mL concentrated H_2SO_4 and refrigerated at 4°C for coprostanol and cholesterol analyses.

Sample Processing

Sediments

After aliquots of homogenized sediment were removed for *Clostridium perfringens* testing, the sediment samples were each split into two portions.

One portion of sediment was sieved for size distribution following the procedure described by Dutka and Kwan (1988).

The second portion of sediment was extracted with Milli Q water 1:1 (100 g wet weight sediment:100 mL water) and the extract was used in toxicant screening tests (Dutka _et al_., 1986). At some sites, the 100 g portion of the water extracted sediment was freeze dried, then weighed on prefired aluminum foil (550°C overnight). The weighed, freeze dried sample was added along with 250 mL dichloromethane (DCM) into a 1 L Erlenmeyer flask, which was prerinsed twice with DCM and shaken for approximately 24 hr on a Burrell wrist action shaker at position #2. After settling overnight, the samples were filtered overnight through prewashed Na_2SO_4. To the filtrate 1.0 mL DMSO was added and the samples were evaporated in a rotary evaporator to 1 mL. The sample was transferred to a test tube with 2 mL DCM rinsings (twice) of the flask. The DCM was evaporated under N_2 in a water bath at 1.0 mL. This 1 mL of 100% DMSO contained sample, was used in all tests at the 1% level. A solvent blank was prepared for each test containing 250 mL DCM plus 1.0 mL DMSO evaporated to 1.0 mL DMSO. A method blank was also prepared as control and contained 250 mL DCM plus 1.0 mL DMSO, shaken, filtered and evaporated as per total sample procedure.

Water

With the exception of the _Daphnia magna_ test, all toxicant screening tests were performed on water samples concentrated 10X by flash evaporation at 45°C. Daphnia magna tests when performed, used natural water samples diluted with dechlorinated tap water.

Microorganism tests

Legionellaceae, fecal coliform (MF and MPN), fecal streptococci (MF), Clostridium perfringens and coliphage tests were performed as described by Dutka et al. (1987).

Biochemical tests

Coprostanol and cholesterol (fecal sterols) and dehydrogenase activity tests were performed as described by Dutka et al. (1986).

Toxicant screening tests

Microtox and Spirillum volutans screening tests were performed on water and DMSO extracts (1%) as detailed by Dutka et al. (1986). Genotoxicity tests on water and 1:1 water-sediment extracts and 1% DMSO extracts were performed as described by Xu et al. (1987) without S-9 addition. ATP-Tox System, a new toxicity screening test based on toxicant inhibition of bacterial growth and luciferase activity was applied to water and 1% DMSO and water extracts of sediment (Xu and Dutka, 1987). Daphnia magna tests as detailed in APHA (1985) and Algal-ATP tests as detailed by Dutka and Rao (1987) were performed only on sediment Milli Q water extracts and water. Algal growth inhibition, using the first phase of the Algal-ATP test was evaluated in microplates using 1% DMSO extracts. Results were recorded as positive or negative, based on visual differences between test samples and controls.

Data Analyses for Battery of Tests Reduction

To determine which tests provide the same type of results for the same locations, it was necessary to determine the extent of correlation between each pair of tests. If a correlation between two tests was high, then the two tests provide the same type of information about the sample and it is no longer necessary to measure both. If two measurements are not significantly correlated then the two tests appear to be providing different information about the samples being analyzed, and as such, both tests should continue to be performed.

Since data collected in all these studies are without replication, and since the distribution of points within any one study and test are not normally distributed, a non-parametric test, Spearman's Rank Correlation was used. It must be pointed out that some sensitivity is lost when non-parametric tests are used (compared to parametric tests), therefore a more robust level of significance (0.99) was chosen. This implies that only very significant correlations would be accepted as real.

The next step was to determine for each test, the number of other tests from which it was statistically independent. At one end of the spectrum, a test which is not correlated with any other test is considered to be providing unique information about the samples. At the other end of the spectrum, a test which was significantly correlated with all other tests is not providing any new information about these sites and may be dropped.

RESULTS AND DISCUSSIONS

During the four years of this study, tests were dropped, tests were added and the point ranking scheme was modified. As a result, greater variability in the study occurred than was envisaged at the start, four years earlier. This variability presented a challenge in data analyses and interpretation.

In the first study, six tests (microbiological, biochemical and toxicological) were performed on the water samples and six tests were also performed on the sediments and Milli Q water extracts of the sediments. In the final studies, there were 11 tests performed on the water samples, eight tests on the sediment and Milli Q water extracts, as well as five tests on 1% DMSO concentrate obtained from the organic extraction of the sediments.

The tests dropped early in the study were the Legionella enumeration procedure, dehydrogenase activity and coprostanol and cholesterol estimation in sediment. Added tests during the term of the study were the Daphnia magna, Spirillum volutans, ATP-TOX system and Algal ATP tests for toxicants.

Using the point ranking scheme (Table 1) which was developed over the course of the project (Dutka et al., 1988), all data were analyzed in an attempt to obtain a representative core group of tests from the "battery of tests".

In the development of the core group, it was believed important that hazards associated with or indicated by microbiological and toxicant pollution should be addressed. Therefore, the core group would have to contain some indicator microbiological tests.

Application of the Spearman's Rank Correlation test to the water data, indicated that the core group make up of the "battery of tests" should be: fecal coliform MF, coliphage, SOS chromotest (genotoxicity), algal ATP, and Daphnia magna. The Spirillum volutans test was rated as a questionable inclusion into this core battery. The surprise in this statistically proposed core group was the exclusion of the Microtox test. This exclusion was a result of analyses which indicated that it (Microtox) had a low independence from other tests and was not highly correlated with any one other test.

When the Spearman's Rank Correlation test was applied to the sediment data (sediment and Milli Q water extract), the following tests were suggested for the 'core group of the "battery of tests"'; A-1 MPN test for fecal coliforms, Clostridium perfringens, SOS chromatest, Algal ATP, and Daphnia magna. Similar to the water sample proposed core group, Spirillum volutans was rated as a questionable inclusion while the Microtox test again was not given support for core group membership. The Microtox test was found in the Milli Q water extracted sediments to have low or medium independence from other tests, was not highly correlated with any other test and was insensitive in three study areas and these factors suggested the non-inclusion of this in the core "battery of tests".

Analyses of the 1% DMSO sediment extract data, suggested the following composition for the core group of tests to indicate potential sampling sites of concern due to toxicological contamination; Microtox, algal ATP and SOS chromotest. Again, the Spirillum volutans test was rated as a questionable inclusion. The Microtox procedure in these samples received very strong support for inclusion into the core "battery of tests".

The proposed composition of the core "battery of tests" for water and Milli Q water extracted sediments was not unexpectedly similar and provides an indication of bacterial hazards, as well as genotoxic and toxicant pollutants in the aquatic ecosystem. The only difference between the water and sediment core group are the methods in obtaining fecal coliform populations and the use of the coliphage procedure in water and the Clostridium perfringens procedure in sediments. There is no doubt, had the A-1 broth MPN procedure been used with the water samples instead of the FC MF test, it would have been the selected procedure. The equivalence and/or superiority of the A-1 procedure to the FC MF procedure has been documented in many studies (Ratto et al., 1988). The coliphage procedure, a bacterial virus estimation procedure, has its value in providing an estimation of the efficiency of sewage treatment plant procedures to remove human pathogen viruses as they both have similar resistance to disinfection procedures. C. perfringens is an important test in the core battery as it can provide an estimation of past pollution (spore survival), as well as indicate present sewage contamination.

The SOS Chromotest in the core group of tests provides an indication of chemical pollution which may have genotoxic or mutagenic effects. Both the Daphnia magna and algal ATP tests provide an indication of the presence of an acute level of toxicants while the algal ATP test can also more subtlety provide an indication of lower toxicant levels which are indicated by slight growth inhibition.

The Spirillum volutans test which is a doubtful member of the core batteries, can also provide an indication of the presence of acute toxicants as well as presence of low levels if specific toxicants which can effect flagellar co-ordination (enzyme inhibition or neurotoxic effect).

In trying to rank the various sampling sites as to areas of greatest potential concern or hazard containment to those with the least, it was quickly realized that the ranking may be biased as some samples were subjected to different numbers of tests. Therefore, a series of comparisons were made using different test combination groups, and if a test was missing from that group it was the average score of the combination which counted, i.e., if seven tests were used the total point value obtained from Table 1 would be divided by seven to achieve the average point total; however, if only six of the seven tests were used on a specific sample, then the total point value would be divided by six for its average point total.

With water sample data, six different test combinations were evaluated, four combinations were evaluated for the sediments and Milli Q water extracted sediments, and two combinations were compared for the organically extracted sediments.

Table 3 presents in descending order, the 20 water samples which have been designated as being collected from areas of highest concern due to their high point rating. In developing this Table, several interesting features were noted. One of these observations was that 19 of the top 20 water bodies of concern were from the St. John River Basin in the province of New Brunswick. The other member of this top 20 list was Site #7 which is the Niagara River at Tonawanda, N.Y., U.S.A. Interestingly, the top 3 sites #49 (Little River at Bayside Drive, St. John), #52 (St. John Harbour slip next to container wharf) and #50 (St. John Harbour, near wharf by Market Square) never varied in their order no matter which combination of tests was used. Also, it should be noted that there were only 10 other sites (out of 178) which appeared in one to three of the test combinations, but not frequently enough to affect the overall ranking order. These 10 samples were divided amongst St. John River (5), Lake Ontario (3) and Detroit River (2). Another surprising feature was that no water sample sites west of Tonawanda, N.Y., appeared in the top 20 areas of concern.

In trying to establish the top 20 sediment sampling sites (Table 4) with greatest potential hazard (chemical and microbiological) and thus greatest potential concern, it was noted that there was much greater variability in ranking depending on the combination of tests used. However, there was greater stability within the group total as only three other samples, two from the Fraser River and one from the Port Hope area of Lake Ontario, were even found within the various combinations used to ascertain the 20 sediment sites with the greatest potential hazard concerns. Thus it would appear that no matter which combination of tests was used to designate the sediment collection site of greatest concern, the selected sites would be similar to that shown in Table 4.

In the final ranking (Table 4), it can be seen that this group was made up of 10 St. John River Basin samples, three Fraser River, four Detroit River, two Lake Erie, and one Niagara River sediment sample. Similar to the observation in the water samples, the top 2 sediment sites #10, St. John R., Florenceville and #36, Madawaska R. at headpond never varied in their pattern no matter which combination of tests were used, while the #3 rank #37 St. John R. at Ste. Basile appeared in 50% of the combinations in this spot. In Table 4 it can be seen that for the first time samples from Western Canada appear as areas of potential concern: in the No. 5 rank was sample #29, Quesnel, east side of Fraser River, B.C., in the No. 10 rank was sample #28, mouth of the Coquetlam River, B.C. and in the No. 12 rank was sample #30, Stoner on the Fraser River, B.C.

The use of the more stringent organic extraction procedures on some of the sediments produced a greater incidence of positive response in the battery of toxicant screening tests used. The validity of this increased toxicant response is debatable on three points. The first is that there are concerns that these more rigorous extraction procedures may only measure bound toxicants which would not normally return to the environment. Conversely, the other side of the coin is that these contaminants may be biomagnified by biota or biotransformed and become part of the food chain. The second point is that the solvent used as a carrier may react synergistically with its contents and produce a mangified response. The third point is based on the premise that efforts have been expended to develop simple, inexpensive, quick, toxicant screening tests which can be used to monitor and prioritize samples for the more expensive and time consuming chemical analyses. If greater time, diligence and expense are allocated to sample preparation and splitting in various fractions, the intent and goals of the screening tests may be defeated.

Notwithstanding the above, and using two combinations of screening tests, one containing all the tests used (4) and the other the same group minus the Microtox test, the top 20 sediment organic extracts with the greatest potential hazard were derived and shown in Table 5. This list of organically extracted sediments is comprised of 18 Lake Ontario Port Hope sediments and two Saskatchewan sediments (#42, N. Saskatchewan R. at Ft. Saskatchewan and #56, Qu'Appelle R. at

Lumsden) which ranked No. 2 and No. 20. The organically extracted sediment top 20 ranking was very stable in that no matter which combination of tests were used to derive the ranking, the same 20 sampling sites were included with only minor inner variation, however, the first two ranked sites never changed order.

If Tables 3, 4 and 5 are examined, it will be noted that sampling sites found in Table 5 did not occur on either Table 3 or 4. There are two explanations for this observation: (1) Table 5 data were the only ranking totally derived from toxicant screening tests and (2) Table 5 data were derived from bound chemicals which were only soluble in organic solutions and thus the rankings reflect a different class of hazardous compounds which remain in the sediments after the water soluble compounds have been removed.

The differences in degree of hazard to man and biota by these two different forms of chemicals have not yet been elucidated.

When Tables 3 and 4 are examined, it can be seen that there are seven sites common to both. Since Tables 3 and 4 reflect the hazards contained in the water and are soluble in water, they may be considered as the presently available ongoing hazards. Thus the seven sites common to both Tables may be truly reflective of those areas of greatest potential concern to man and biota due to their contained water transported hazards. Those seven common sites are in order of ranking:

#49 - Little River at Bayside Drive, St. John, New Brunswick

#52 - St. John Harbour, 1 km southwest of Courtenay Bay breakwater

#37 - St. John River at Ste. Basile

#40 - St. John River, 2 km below McCain's, Florenceville

#36 - Madawaska River at Headpond below mill, New Brunswick

#32 - Mill stream at Pond, St. Francois de Madawaska, New Brunswick

#7 - Niagara River at Tonawanda, N.Y., U.S.A.

Water and water-extracted sediment data deemed to have the least contained hazards following the criteria used to develop Tables 3, 4 and 5, are shown in Table 6. The rankings of the seven sites presented in this table are shown in descending order with the least hazardous site being at the bottom of the list. From this Table one is left with the impression that of the samples collected, Lake Ontario sediments have the least hazards, and the waters from the Western Canadian provinces of Manitoba, Saskatchewan and British Columbia are the least contaminated based on the criteria used in this study.

In reviewing the results of this four year study, it is difficult in many ways to comprehend the impact of the proposed "battery of tests" for ecological evaluation and the resulting classification of hazardous samples. There is no real structure available yet, to indicate how great or what type of hazards actually exist in or are implied by these sample data. Can these waters or sediments somehow cause infections, will they make man or biota more susceptible to infections due to some unknown chemical stresses, will there be a hastening of death in man or biota, or will they somehow cause chronic disability or future genetic or reproductive damage in man or biota living in or using these waters? All of these potential effects are unknown, and it is impossible to draw any of these inferences from this type of data. All that can be said is that, as we understand the present aquatic ecosystem, we are only measuring degrees of stresses on the ecosystem and various concentrations of substances originating from man's activities.

The test results are certainly biased by the point scheme shown in Table 1. However, no matter what formula is used to rank test results and the importance of one test result over another, a bias will appear.

One of the regretted failings of this study was the omission of any biological tests to measure chronic effects. In future studies of this nature, more stress is believed necessary, on tests which measure chronic effects and mutagenic effects, to provide an insight into long term subacute chemical stresses. Perhaps these new accumulated data from acute and chronic tests and microbial indicators will result in a better understanding of the implications of these test results.

The following statements summarize this study:

(a) three proposed core groups of tests for a battery of tests approach for testing environmental samples to establish priority studies and to evaluate potential hazards are described;

(b) the proposed core battery of tests for water samples and Milli Q water extracted sediments contained similar toxicological screening tests and only differed in their means of estimating microbial hazards;

(c) by the use of a point scoring scheme based on the degree of positiveness of various tests and by comparing various proposed core group of tests, it was possible to develop a ranking for water and sediment samples collected country-wide; and

(d) using the ranking scheme, the top 20 sampling sites with the greatest concerns due to their suspected contained hazards were derived for each type of sample, water, Milli Q water-extracted sediments, and organically extracted sediments suspended in 1% DMSO.

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Table 2. Sampling Site Location for Top 20 Areas of Potential Concern. Presented by Area not by Ranking

No.	Site #	Location	Latitude	Longitude
1	2	Detroit R. mouth, temporary dump site	42°00'13"	83°08'58"
2	7	Detroit R. mouth Turkey Creek	42°14'44"	83°06'24"
3	8	Detroit R. mouth of Little R. - downstream of WTP	42°20'26"	82°55'50"
4	9	Detroit R. mouth of Rouge River	42°16'30"	83°06'40"
5	13	Cedar Creek mouth	42°00'22"	82°46'28"
6	22	Port Burwell - offshore	42°38'43"	80°48'40"
7	78	Niagara R. Tonawanda USA mouth of Two Mile Creek	43°0'38"	78°54'26"
8	1	Inshore Lake Ontario east of Port Hope STP	43°57'06"	78°15'54"
9	2	Inshore Lake Ontario east of Port Hope STP	43°57'01"	78°15'10"
10	3	Inshore Lake Ontario opposite Port Hope sewage treatment plant (STP)	43°56'56"	78°16'24"
11	4	Inshore Lake Ontario west of Port Hope STP	43°56'52"	78°16'40"
12	5	Inshore Lake Ontario west of Port Hope STP at city limits	43°56'49"	78°16'54"
13	6	Inshore Lake Ontario east of Ganaraska R., Lake Ontario	43°56'39"	78°17'26"
14	7	Upstream Ganaraska R. Lake Ontario	43°56'49"	78°17'35.5"
15	8	Harbor Entrance, Port Hope, Lake Ontario	43°56'31"	78°17'31"
16	9	Breakwater Entrance to Port Hope, Lake Ontario	43°56'25"	17°17'27"
17	9A	Mouth of Turning Basin, Port Hope	43°56'39"	78°17'34.5"

Table 2. (con't)

No.	Site #	Location	Latitude	Longitude
18	10	1 km offshore, Port Hope breakwater entrance	43°56'21"	78°17'26.5"
19	12	100 m offshore west of Port Hope breakwater entrance	43°56'21"	78°17'36.5"
20	13	1 km offshore Lake Ontario opposite site 19	43°56'21"	78°17'48"
21	14	Inshore Lake Ontario west of Port Hope Harbour 500 m west of site 19	43°56'29"	78°17'46.5"
22	15	Inshore Lake Ontario west of Port Hope, Site #19	43°56'29"	78°17'55"
23	16	Inshore Lake Ontario west of Port Hope Site #22	43°56'30"	78°18'04"
24	17	Inshore Lake Ontario west of Port Hope Site #23	43°56'21"	78°18'12"
25	18	Inshore Lake Ontario, 100 m offshore Otty Point	43°55'57"	78°20'48"
26	42	N. Sasktachewan R. at Fort Sasktachewan Hwy 37 Bridge	53°42'22"	13°14'19"
27	56	Qu'Appelle R. at Lumsden	50°39'05"	104°52'15"
28	25	Mouth of Coquitlam R., B.C.	49°13'32"	122°48'13"
29	33	Quesnel E. side of Fraser R., B.C.	52°54'41"	122°28'40"
30	34	Stoner, Fraser River, downstream of Stone Creek, B.C.	53°52'0.5"	122°40'07"
31	1	Glasier Lake, 200 m from outfall at centre	47°13'05"	68°58'50"
32	2	Mill Stream at Pond, St. Francois de Madawaska	47°14'40"	68°42'08"
33	3	Lac Unique, 150 m from outfall	47°20'24"	68°44'55"
34	5	SJR[1] below Babin Brook at Fifth Island, near shore	47°18'08"	68°29'39"

[1]St. John River

Table 2. (con't)

No.	Site #	Location	Latitude	Longitude
35	6	Madawaska R. above mill	47°22'32"	68°20'58"
36	7	Madawaska R. at Headpond below mill behind Lynne Motel	47°22'01"	68°19'16"
37	9	SJR at St. Basile	47°21'18"	68°14'00"
38	12	SJR at Grand Falls, below falls near	47°02'37"	67°44'23"
39	13	Aroostook R. headpond at Canadian Border	46°47'42"	67°47'20"
40	15	SJR 2 km below McCains, Florenceville	46°26'29"	67°37'14"
41	21	SJR at Nackawic, 1 km below mill, 1/3 from left bank	45°59'11"	67°12'42"
42	25	Nashwaaksis Stream at mouth	45°58'12"	66°39'16"
43	26	Nashwaak R. at mouth upstream of hwy. bridge at left bank	45°57'11"	66°37'09"
44	27	SJR at Fredericton, 1 km below Princess Margaret Bridge, right bank	45°55'41"	66°37'03"
45	28	SJR, 0.5 km below confluence of Oromocto R., near right bank	45°51'23"	66°27'51"
46	30	SJR at Evandale Ferry, right bank	45°35'25"	66°01'55"
47	32	Grand Bay (SJR), 1 km from Saint John Marina	45°16'43"	66°09'00"
48	33	Grand Bay (SJR), i km from Boars Head, left bank	45°17'10"	66°08'15"
49	34	Little River at Bayside Drive	45°16'26"	66°01'36"
50	36	Saint John Harbour, near wharf by Market Square	45°16'19"	66°04'09"
51	37	Saint John Harbour, at slip next to container wharf	45°15'47"	66°03'54"

Table 2. (con't)

No.	Site #	Location	Latitude	Longitude
52	38	Saint John Harbour, 1 km southwest of Courtenay Bay Breakwater, N.B.	45°15'48"	66°02'00"
53	43	Entrance to Burlington Canal, Lake Ontario	43°18'08"	79°47'18"
54	41	Off Petro Canada Pier, Lake Ontario	43°22'26"	79°43'09"
55	8	Offshore Colburg, Lake Ontario	43°56'48"	78°08'54"
56	42	Offshore Spencer Smith Park, Burlington, Lake Ontario	43°19'12"	79°47'36"
57	44	Grimsby Beach, Lake Ontario	43°11'45"	79°32'01"
58	11	Offshore Newcastle, Lake Ontario	43°53'26"	78°35'20"
59	16	Offshore Oshawa, Lake Ontario	43°50'55"	78°50'00"
60	68	Split Lake, York Landing, Manitoba	56°04'42"	96°05'20"
61	67	Tobin Lake, off Carolls Cove, Saskatchewan	53°31'29"	103°47'00"
62	11	North Arm, N. Shore at Burnaby Bend, B.C.	49°10'52"	122°58'33"
63	5	Steveston Cannery Channel, Fraser River	49°07'09"	123°10'00"
64	75	Footprint Lake, Nelson House School Bay, Manitoba	55°47'10"	98°53'00"
65	73	Footprint Lake, Metis Beach, Manitoba	55°47'30"	98°51'00"
66	44	North Sasktachewan R. near Myron Hwy 881 Bridge, Saskatchewan	54°45'30"	108°19'00"

Table 3. The Twenty Most Hazardous Water Samples Based on the Battery of Tests Approach (20/178)

Rank	Sample No.	Site
1	49	Little River at Bay Side Drive, St. John, New Brunswick
2	52	St. John Harbour, Southwest of Courtenay Bay breakwater
3	50	St. John Harbour, near wharf by Market Square
4	51	St. John Harbour off slip next to container wharf
5	48	Grand Bay, St. John River, near Boars Head
6	33	Lac Unique, 150 m from outfall, New Brunswick
7	37	St. John River at St. Basile
8	47	Grand Bay, St. John River, 1 km from Saint John Marina
9	45	St. John River below confluence of Oromocto R.
10	40	St. John River, 2 km below McCain's at Florenceville
11	39	Aroostook R. headpond at Canadian Border, New Brunswick
12	42	Nashwaaksis Stream at mouth
13	7	Niagara River, mouth of Two Mile Creek, Tonawanda, N.Y., U.S.A.
14	35	Madawaska River above Mill, New Brunswick
15	46	St. John River at Evandale Ferry, right bank
16	36	Madawaska River at Headpond below mill, New Brunswick
17	31	Glasier Lake, 200 m from outfall, New Brunswick
18	43	Nashwaak R., upstream of Hwy bridge, New Brunswick
19	32	Mill stream at Pond, St. Francois de Madawaska, New Brunswick
20	44	St. John River at Fredericton, 1 km below Princess Margaret Bridge

Table 4. The Twenty Most Hazardous Sediments (Milli Q Water Extracted) Based on the Battery of Tests Approach (20/227)

Rank	Sample No.	Site
1	40	St. John River, 2 km below McCain's, Florenceville, New Brunswick
2	36	Madawaska R. at headpond below mill, New Brunswick
3	37	St. John River at Ste. Basile
4	32	Mill Stream at Pond, St. Francois de Madawaska, New Brunswick
5	29	Quesnel, east side of Fraser River, B.C.
6	52	St. John Harbour, 1 km southwest of Courtenay Bay breakwater
7	49	Little River at Bayside Drive, St. John, New Brunswick
8	1	Detroit River mouth temporary dump site, Ontario
9	38	St. John River at Grand Falls, below falls
10	28	Mouth of Coquitlam River, B.C.
11	34	St. John River below Babin Brook
12	30	Stoner, Fraser R. downstream of Stone Creek, B.C.
13	6	Port Burwell, Lake Erie, Ontario
14	5	Cedar Creek mouth, Lake Erie
15	3	Detroit River, mouth of Little C.R. downstream of WTP, Ontario
16	35	Madawaska River, above mill, New Brunswick
17	2	Detroit River, mouth of Turkey Creek, Ontario
18	4	Detroit River, mouth of Rouge River, Ontario
19	41	St. John River at Nackawic, 1 km below mill
20	7	Niagara River, mouth of Two Mile Creek, Tonawanda, N.Y., U.S.A.

Table 5. The Twenty Most Hazardous Sediments Organically Extracted and Suspended in 1% DMSO (20/44)

Rank	Sample No.	Site
1	11	Inshore Lake Ontario west of Port Hope sewage treatment plant (STP)
2	26	N. Saskatchewan R. at Fort Saskatchewan, Hwy 37 bridge
3	25	Inshore Lake Ontario, 100 m offshore, Otty Point
4	10	Inshore Lake Ontario, opposite Port Hope STP
5	14	Upstream Ganaraska R. at Port Hope
6	13	Inshore Lake Ontario, east of Ganaraska River
7	9	Inshore Lake Ontario, east of Port Hope STP
8	12	Inshore Lake Ontario west of Port Hope STP at city limits
9	16	Breakwater entrance to Port Hope Harbour
10	23	Inshore Lake Ontario west of Port Hope site #22
11	21	Inshore Lake Ontario west of Port Hope Harbour, 500 m west of site #19
12	20	1 km offshore, Lake Ontario opposite site #19
13	17	Mouth of turning basin, Port Hope Harbour
14	15	Harbour entrance to Port Hope
15	19	100 m offshore west of Port Hope breakwater entrance
16	18	1 km offshore Port Hope breakwater entrance
17	24	Inshore Lake Ontario, west of Port Hope site #23
18	8	Inshore Lake Ontario, east of Port Hope STP
19	22	Inshore Lake Ontario, west of Port Hope site #22
20	27	Qu'Appelle R. at Lumsden, Sasktachewan

Table 6. Sampling Sites with the Least Contained Hazards. Suspected Hazards Decrease Down the Column.

No.	Site #	and Site	Latitude	Longitude
		Sediment and Milli Q Water Sediment Extracts		
53	43	Entrance to Burlington Canal	43°18'08°	79°47'18"
54	41	Petro Canada Pier	42°14'44"	83°06'24"
55	8	Coburg	43°56'48"	78°08'54"
56	42	Spencer Smith Park	43°19'12"	79°47'36"
57	44	Grimsby Beach	43°11'45"	79°32'01"
58	11	Newcastle	43°53'26"	78°35'20"
59	16	Oshawa	43°50'55"	78°50'00"
		Water Samples		
64	75	Footprint Lake, Manitoba	55°47'10"	98°53'00"
65	73	Footprint Lake, Metis Beach	55°47'30"	98°51'00"
60	68	Split Lake, York Landing	56°04'42"	96°05'20"
61	67	Tobin Lake, Carolls Cove	49°10'52"	122°58'33"
62	11	North Arm Fraser River	49°07'09"	123°10'00"
63	5	Steveston, Fraser River	49°07'09"	123°10'00"
66	44	North Saskatchewan River	54°45'30"	108°19'00"

Table 1. Point Awarding Scheme for Sample Ranking, Based on Suspected Contained Hazards.

Fecal Coliform Fecal Streptococci Enterococci per 10 g/100 mL sediment /100 mL water	Coliphage per 100 mL water	*Clostridium perfringens* per 10 g/100 mL sediment	Points	Coprostanol ppb	SOS Chromotest Genotoxicity Induction Factor per mL 10x Water Sample 1:1 Milli Q Water Sediment Extract 1% DMSO Sediment Extract	Algal-ATP % Relative Light Units per mL 10x Water Sample 1:1 Milli Q Water Sediment Extract 1% DMSO Sediment Extract	Points	Cholesterol ppb	Points
1 - 100	5 - 24	1 - 25	1	<1.0	1.0 - 1.29	100 - 50	1	<2.0	1
101 - 500	25 - 100	26 - 100	2	1 - 3.0	1.30 - 1.50	49 - 20	3	2.1 - 4.0	2
501 - 2500	101 - 250	101 - 500	3	3.1 - 5.0	1.51 - 2.0	19 - 1.0	5	4.1 - 6.0	3
2501 - 16000	251 - 1000	501 - 2500	4	5.1 - 7.0	2.1 - 3.0	0.9 - 0.1	7	6.1 - 8.0	4
16001 - 160000	1001 - 5000	2501 - 10000	7	7.1+	3.1+	0.09+	10	8.1+	5
160000+	5001+	10000+	10						

ATP-TOX System % Inhibition per mL 10x Water Sample 1:1 Milli Q Water Sediment Extract	Microtox EC_{50} % per mL 10x Water Sample 1:1 Milli Q Water Sediment Extract	Points	*Daphnia magna* EC % per mL 1:1 Milli Q Water Sediment Extract	Points	*Daphnia magna* EC % per mL Water Sample	Points	*Spirillum volutans* Water Sample	Points	*Spirillum volutans* Sediment Extract and 10X Water	Points
1 - 30	40.0+	1	EC_{20} at 100%	1	EC_{20} at 100%	1	negative	0	negative	0
31 - 60	40.0 - 25.0	3	EC_{40} at 100%	2	EC_{40} at 100%	2	+	10	+	5
61 - 90	24.0 - 10.0	5	EC_{50} at 100%	4	EC_{50} at 100%	5				
91 - 99	9.0 - 1.0	7	at 75%	5	at 75%	7				
100	0.9+	10	at 50%	6	at 50%	8				
			at 25%	8	at 25%	10				
			at 10%	10						