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USE OF MICROBIAL AND TOXICANT SCREENING TESTS FOR PRIORITY SITE SELECTION OF DEGRADED AREAS IN WATER BODIES

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MANAGEMENT PERSPECTIVE

The goal of this study is to identify degraded or degrading water bodies so that managers will have a strong data base on which decisions can be made. This information is provided by using the "battery of tests" approach. Another goal of this study is to evaluate a variety of microbiological, biochemical and bioassay tests for their potential of becoming the core group of tests in the "battery of tests" approach. This core group of tests can and will be used nationally to prioritize water bodies and sediments or selected areas within water bodies for remedial action, further investigations or to monitor the effects of remedial actions.

The "battery of tests" approach should make it possible to establish "hot spots" areas of 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, 48-96 hours after collection, or later, 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 enteric 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.

PERSPECTIVE-GESTION

Cette étude a pour but d'identifier les cours d'eau qui se sont détériorés ou qui sont en train de se détériorer de façon à fournir aux gestionnaires une base de données solide pouvant leur servir à prendre des décisions. Ces données seront fournies par une "batterie d'épreuves". L'étude a également pour but de choisir parmi diverses épreuves microbiologiques et chimiques et divers dosages biologiques, ceux qui pourraient former le noyau des épreuves utilisées dans le cadre de la "batterie d'épreuves". Ce noyau d'épreuves servira au niveau national à établir les priorités concernant les cours d'eau et les sédiments ou certaines régions de cours d'eau qui pourraient bénéficier de mesures correctives, de plus amples recherches ou pour surveiller les effets des mesures prises.

Cette "batterie d'épreuves" devrait permettre d'établir les "points chauds", c'est-à-dire les régions qui nécessitent une attention immédiate et qui n'ont pas été signalées auparavant par suite de méthodes inadéquates ou de l'utilisation d'une seule épreuve.

Les épreuves qui peuvent être effectuées sur des échantillons réfrigérés ou congelés, 48 à 96 heures après le prélèvement, ou plus tard, se verront accorder la priorité lors du choix final de la batterie recommandée d'épreuves microbiologiques ou biochimiques et de dosages biologiques. L'épreuve des coliphages, l'un des paramètres qui feront l'objet de l'étude dans la batterie d'épreuves, est tout particulièrement importante car elle procure des données sur

la présence potentielle d'organismes indicateurs et d'agents pathogènes entériques bactériens et viraux. Les données de l'épreuve des coliphages provenant de ces études seront comparées aux données d'une étude portant sur huit pays dans trois continents (Asie du sud-est, Amérique du Sud, Afrique du Nord). Travaux effectués par B.J. Dutka et parrainés par le Centre de recherche pour le développement international (CRDI), Ottawa (Canada).

ABSTRACT

In this study, a new approach has been taken to evaluate Saint John River water and sediment conditions. A battery of biochemical, microbiological and bioassay tests were used to identify degraded or degrading sediments and waters. Data were obtained from waters and sediments at 38 sites within the Saint John River Basin. The data suggested that the following four sites had the highest priority concern: Little River #34, Grand Bay, Saint John River near Boars Head #33, Madawaska River below mill #7 and St. Francois-de-Madawaska, mill stream #2. The data also indicate that microbial population, biochemical or bioassay tests performed independently do not provide realistic estimates of priority concern areas and that the "battery of tests" approach is necessary to provide management with information on which decisions can be made.

RÉSUMÉ

Au cours de cette étude, une nouvelle approche a été choisie pour déterminer les conditions de l'eau et des sédiments dans la rivière St-Jean. Une batterie d'épreuves biochimiques, microbiologiques et de dosages biologiques ont été utilisés pour identifier les sédiments et les eaux qui se sont détériorés ou qui sont en train de se détériorer. Les données provenaient d'eaux et de sédiments prélevés à 38 bassins de la rivière St-Jean. D'après les résultats obtenus, les quatre endroits suivants doivent être traités en priorité : Little River n° 34, Grande Baie, la rivière St-Jean près de Boars Head n° 33, la rivière Madawaska en aval de l'usine n° 7 et le canal d'évacuation de l'usine de Saint-François-de-Madawaska n° 2. Les résultats indiquent également que la population microbienne, les épreuves biochimiques où les dosages biologiques exécutés indépendamment ne permettent pas d'effectuer une estimation réaliste de l'état des régions prioritaires et qu'une "batterie d'épreuves" doit être utilisée pour fournir les données qui serviront à prendre les décisions.

INTRODUCTION

World wide there has been a dramatic increase in industrialization and population concentration over the past three decades. With these increased population and industry centres, both the developed and developing nations face increasing ecological and toxicological problems from the release of domestic wastes and contaminants into the environment. In response to these expanding stresses on the environment and in the belief that there is no single criterion to adequately judge the potential hazard (either to man or the environment) of an effluent or substance, (Draggan and Giddings, 1978), a multitude of biological and biochemical procedures have been and are being developed and used to assess these biological and toxicant impacts (Kohn, 1980; Bringmann and Kuhn, 1980).

Within the last two to three decades there has been an increasing awareness of the multitude of new chemicals being produced and eventually discharged to the environment. There has also been a slower but increasing realization that chemical analysis of all suspected effluents, emissions, waters and sediments is impractical and impossible. Therefore quick, inexpensive, simple screening tests must be developed to prioritize samples, water bodies or sediments for chemical analyses.

Many enzyme, bacterial and algal tests have been developed for the monitoring or screening of toxicant/genotoxciant effects in effluents, waters and sediments (Bitton and Dutka, 1986). The

majority of these tests are rapid, simple, relatively reproducible and inexpensive and require little space and time as compared to fish and Cladocern tests (Bitton and Dutka, 1986a; Dutka and Bitton, 1986). However, little information is available on comparative studies of short-term microbial assays for estimating the impact of toxicants on the aquatic environment. Such studies could give valuable information on reproducibility, sensitivity, cost and rapidity of the various tests.

Also, due to escalating costs and transportation problems, traditional and newer proposed microbiological tests for water and sediments must be re-evaluated. In this re-evaluation process, bias should be given to those tests which are amenable to short-term refrigeration/preservation (48-72 hr), are easy to perform and do not require excessively sophisticated equipment and specialized staff, and are cost effective.

In this paper, we continue to evaluate the suitability of a variety of microbiological, biochemical and toxicant screening tests to become part of a "battery of tests" approach. The final goal of these evaluation studies is to develop a "battery of tests" containing two or three toxicant/genotoxicant screening tests and two or three microbiological hazard screening tests which can be used internationally to designate and prioritize specific water bodies and sediments that are degraded or are being degraded for further investigation or remedial action.

In these Phase III studies, water and sediment from the Saint John River (New Brunswick, Canada) and rivers and lakes within the Saint John River Basin, as well as inshore marine waters influenced by the Saint John River, were used to evaluate the testing procedures.

Data from this Phase III study are presented and results discussed.

METHODS

Sampling Sites

A total of 38 sites were sampled in this study during late October 1986. Twenty-two of the samples were from the Saint John River, four of which were affected by salt water intrusion. Three of the samples were marine samples in Saint John Harbour and are influenced by the Saint John River. Nine samples were from tributaries of the Saint John River and four samples were from lakes within the drainage basin (Table 1, Fig. 1). Most of the Saint John River sites could be considered to be under various anthropogenic influences such as sewage, sewage treatment plant discharges, pulp and paper mill discharges, and food processing plant discharges.

Sample Collection

Sediments were collected with an Ekman dredge or shovel. Usually several drops or shovel loads were required to obtain sufficient

surface layer sediment (2-3 cm). At each site the surface layers were pooled, well mixed and aliquots dispensed for each testing procedure and refrigerated. Prior to performing toxicant screening tests, sediments were extracted with Milli Q water (4 cartridge system - A, Super C carbon cartridge, B, Ion-Ex^{tm}, C, Ion-Ex^{tm}, D, Organet-Q^{r} and E. Milli-Stak^{tm} filter; with a glass distilled water feed) by mixing sediment and Milli Q water in a 1:1 ratio, shaking vigorously by hand for 2 minutes, then centrifuging at 10,000 rpm in a refrigerated centrifuge for 20 minutes. The supernatant was used in toxicity screening tests.

Surface water samples were collected at each site, a 500 ml sample for fecal coliform, fecal streptococci and coliphage tests which were usually processed within 8 hours of collection, and another 500 ml sample which was preserved at 4°C for toxicant screening tests. Samples for toxicant screening tests were tested after being concentrated 10x by flash evaporation at 45°C.

Also at all sites a one litre surface water sample was collected and preserved with 1 ml H_2SO_4 for coprostanol and cholesterol analyses.

Microorganism Tests

Fecal coliform, fecal streptococci, E. coli and coliphage tests were performed as described by Dutka et al. (1986). Clostridium perfringens MPN enumeration techniques were performed as described by Bonde (1963) and Dutka et al. (1986b).

Lactose Fermenting Isolates

A total of 393 isolates were collected and identified from positive A-1 Broth tubes (MPN fecal coliforms in sediments) as well as from typical fecal coliform colonies on MF-mFC plates. Identification procedures included lactose fermentation, oxidase reaction, IMViC Tests, motility, H_2S production, inositol and sorbitol fermentation. Isolates were collected to ascertain the sensitivity of the two techniques for selecting and enumerating E. coli in these waters and sediments.

Biochemical and Toxicity Screening Tests

Coprostanol and cholesterol analyses were performed on water samples and the Microtox tests were performed on water and sediment extracts as detailed by Dutka et al. (1986). Genotoxicity tests on water and sediment 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 extracts (Xu and Dutka, 1987). Spirillum volutans, a large aquatic bacterium with a rotating fascicle of flagella at each pole was also used to test samples for toxicity following procedures described by Dutka and Kwan (1982).

The Algal-ATP toxicant screening test is based on the inhibition of ATP production in cultures of the green algae Selenastrum capricornutum (Blaise et al., 1984). The ATP content of the stressed

Selenastrum capricornutum was measured by the procedure described in the Turner Luminescence Review (1983). The results are reported as a percentage of Relative Light Output (RLU) of the non-stressed controls which is 100%.

Results - Discussion

In Table 1, a brief visual description of the 38 sediments is presented for comparison along with site description and the latitude and longitude of each sampling site. The format used to award points for specific data values in order to rank the waters and sediments from areas of most concern to least concern, is presented in Table 2. The point allocation scheme is biased, and not scientifically defensible, but it reflects the authors' experience with various concentration levels of toxicant activity and health related bacteria in Canadian waters and sediments. The present rating scheme is a viable entity which will change with increased inputs and when greater experience is gained.

Samples with the most points are deemed to contain the greatest potential hazard to man and organisms found in the aquatic ecosystem. High toxicant levels may have reduced microbial levels/activity in some sediment samples; however, cause and effect relationships were not investigated.

Table 3 presents the results of the sediment analyses. Fecal coliform densities were found to vary in the drainage basin from <2

(Glasier Lake #1 to >1,600,000 (Little River #34) per 10 gram wet weight sediment. The fecal coliform data suggest that there is a continuing large input of fecal material into the Saint John River from Babin Brook to Florenceville and also into the Saint John Harbour. Clostridium perfringens levels shown in Table 3 were the highest encountered in this series of studies (Dutka et al., 1986a) with densities varying from a low of 21 (Lac Unique #3) to a high of >160,000 (Madawaska River below paper mill #7). To illustrate the exceedingly high C. perfringens densities found in the Saint John River sediment, a study covering inshore Lake Erie and river and stream mouths entering this lake and the lower Detroit River and upper Niagara River, the maximum C. perfringens density found was 34/10 gram sediment. At some of the sampling sites, which are known recreational areas with mainly summer usage (Environ. New Brunswick, 1977), the data suggest, based on C. perfringens and fecal coliform densities that historical fecal pollution had occurred and has not continued to the same degree e.g. Glasier Lake #1, fecal coliforms <2, C. perfringens 1700; Lac Baker #4, fecal coliforms 8, C. perfringens 2,200; Longs Creek #22, fecal coliforms 13, C. perfringens 13,000; and Mactaquac head pond #23, fecal coliforms 2, C. perfringens 12,000.

A total of 140 positive A-1 broth tubes were subcultured onto MacConkey's agar for isolate identification and confirmation for the presence of fecal coliforms. A total of 36% of the tubes confirmed as having E. coli and 18% as having Klebsiella sp. These fecal coliform

estimates by A-1 broth, produced the lowest E. coli confirmation rate that we have ever encountered (usually 90%+, Dutka et al., 1986). This relatively low E. coli presence in the sediments may be an indication of the variety of the organic pollution reaching the Saint John River, e.g. piggery wastes, pulp and paper mill wastes, light industrial effluents, food processing wastes, and domestic sewage.

In Table 3, it can be seen that only six sites, #11, #13, #25, #26, #27 and #35 were completely negative for any toxicant and/or genotoxicant activity. Also sites #1 and #9 were only positive in the Spirillum volutans tests, an unexpected finding as the S. volutans test was usually found to be the least sensitive of the various toxicant screening tests studied (Dutka and Kwan, 1982; Dutka et al., 1983). Only three sites, #3, #7 and #15 were positive in all the toxicant screening tests, with #15 also showing a slight response in the genotoxicity test. A total of 11 sediment sites were positive for toxicants by the Microtox test, 13 sites by the algal-ATP test, 11 sites by the S. volutans test and 26 sites by the ATP-TOX System. These data suggest ATP-TOX System is the most sensitive screening test for indicating the possible presence of toxicants within the Saint John River basin. However, it must be pointed out that the points used to assess toxicant effects are different for each test, e.g. ATP-TOX System is recorded as a positive when there is only a 1% inhibition of ATP and growth while the Microtox test results are based on the concentration of toxicants that produce a 50% inhibition of light output (EC_{50}). In trying to relate ATP-TOX System positive sites to sites positive by the other tests, it can be seen that there

were nine sites positive by both the Microtox and ATP-TOX System, twelve sites by the algal-ATP and ATP-TOX System, and eight sites by the S. volutans and ATP-TOX System. These findings accentuate the need for the "battery of screening tests" approach to examine and prioritize environmental samples.

The SOS Chromotest kits used on these samples produced very low values and it is suspected from the positive controls that the test organisms were not operating at maximum efficiency. Nevertheless, site #29 sedimemt extract was found to have a substantive positive induction effect with four other sites, #4, #12, #15 and #19, showing weak inducing ability.

Based on the point scheme developed in Table 2 the ten sediments of the greatest potential concern are: 1, Saint John R. at Florenceville #15; 2, Madawaska R. below mill #7; 3, Grand Bay, Saint John R., 1 km from Boars Head #33; 4, Little R. #34; 5, Mill Stream pond, St. Francois-de-Madawaska #2; 6, Saint John R. below Babin Brook #5; 7, Saint John R. at Longs Creek #22; 8, Madawaska R. above mill site #6; 9, Saint John R., near Grand Falls S.T.P. outfall #12; and 10, Saint John R. at Nackwick below mill #21.

Table 4 displays the data obtained from the 38 water samples by the various techniques used. The microbiological data indicate tht the first definite signs of fecal pollution start at site #5, Saint John R. below Babin Brook at Fifth Island. This fecal pollution is shown in both the water and sediment sample. With the exception of sites #25 and #27, and down river from site #19 (Saint John R., .5 km below Pokiok R. mouth) to site #31 (Saint John R. at Westfield ferry)

the Saint John R. appears to be only slightly affected by microbiological pollution. However, within the Saint John River basin, waters at 24 of the 38 sampling sites produced elevated fecal coliform counts and fecal coliform; fecal streptococci ratios of 4:1 and greater. Based on Geldreich's hypothesis (1972) that 4:1 FC:FS ratios are indicative of human fecal pollution, data from these sites strongly suggest that the source of these elevated health related bacterial populations are human fecal material and sewage treatment plant discharges.

The highest water and sediment fecal coliform densities were found at site #34, Little River, with sediment fecal coliform concentrations of >1,600,000 and water column concentrations of >5,000,000 and only ten coliphage. Fecal coliform MF and MPN isolates collected from these samples were found to be 100% Enterobacter sp from the MF plate (11 isolates) and 80% Enterobacter sp and 20% Citrobacter sp from the MPN test (10 tubes).

Coliphage counts from the 38 water sample sites were inconsistent and showed no pattern or relation to fecal coliform concentrations in the water column or sediments. This may be a reflection of the lower E. coli concentrations in these samples as indicated by isolate identification from the A-1 broth MPN, 36% E. coli, and the membrane filter mFC agar, 77% E. coli.

The fecal sterol data provided very little useful information on the fecal pollution load on the waters in the Saint John River Basin. Only sampling site #2, Mill Stream at pond in St. Francois-de-Madawaska produced significant coprostanol and cholesterol

concentrations. These elevated levels were not reflected by the bacteriological data. Only the sediment fecal coliform and C. perfringens data at this site produced a suspicion that fecal pollution is a potential problem. Fecal sterol data continue to be an enigma. From the fecal sterol data obtained in this and earlier studies (Dutka et al., 1986; Dutka et al., 1986b) it would appear that these parameters are not amenable to random, one-time sample collection.

Water samples giving positive responses in all toxicant screening tests were only found at sites #3 and #34. Site #3, Lac Unique, was a suprise as there are no obvious toxicant inputs into the water column, while site #34 which drains a very heavily industrialized area was an expected positive.

The last three sites, #36, #37 and #38 (Saint John Harbour), were positive in all toxicant screening tests except the SOS Chromotest. These sites are also unique in that they produced the highest positive responses in the Microtox, Algal ATP, ATP-TOX System and Spirillum volutans tests. The S. volutans test was positive in both the neat and 10x concentrated samples. As these are marine water sites, the positive test response may have been influenced by the salinity of the freshwater-saltwater mixture found at these sites.

The SOS Chromotest and Microtox test were the least sensitive screening tests with these 10x concentrated water samples. The most sensitive test was the ATP-TOX System. However, if both the ATP-TOX System and Algal-ATP tests were reported as positive at EC_{50}

concentrations, then it would be found that the Microtox and S. volutans tests indicated the greatest number of positive toxic samples, 11 each, while the Algal-ATP and ATP-TOX Systems would suggest that 2 and 6 samples contained toxicants, respectively. The lack of positive responses with the SOS Chromotest appears to be related to batch to batch variations of the testing cells. In this study the positive control results were significantly lower than obtained with previous kit batch numbers.

Using the point scheme developed in Table 2, the ten water samples of the greatest potential concern are: 1, Little R. #34; 2, Saint John Harbour, outside #38,; 3, Saint John Harbour #36; 4, Saint John Harbour at Container Wharf #37; 5, Lac Unique #3; 6, Grand Bay (Saint John R.) near Boars Head #33; 7, Saint John R. at St. Bassle #9; 8, Madawaska R. below mill #7; 9, Mill Stream at St. Francois-de-Madawaska; #2; 10, Grand Bay, Saint John R., 1 km from St. John Marina #32.

Examination of the top ten ranking water sampling sites and top ten sediment sites revealed that there were four common sites which are listed below:

Sediment Sample Rank	Water Sample Rank	Sample Site
4	1	Little R. #34
3	6	Grand Bay, Saint John R. near Boars Head #33
2	8	Madawaska R. below mill #7
5	9	St. Francois-de-Madawaska: mill stream #2

Thus based on the rating scheme (Table 2), developed for ranking the various microbiological, biochemical and toxicant screening test responses to the samples, the four areas of highest concern would be sampling sites #34, #33, #7 and #2.

Use of the "battery of tests" approach in this study reemphasizes that individual bacteriological and toxicant screening tests do not provide a sufficient data base for realistic management decisions to be made on which of the many aquatic environments are of priority concern and require immediate or delayed remedial actions. Also from this study it would appear that the fecal sterol tests are not amenable to a "battery of tests" approach.

Further refinement of the present "battery of tests" will continue with emphasis on acute and chronic tests and techniques to assure the compatibility of reported test results. The eventual goal will be to select a maximum of three toxicant/mutagen screening tests and two microbiological tests as a core group. The ranking scheme will be reviewed after each study to ensure the points allocated to various response levels continues to reflect country wide conditions.

REFERENCES

Bitton, G. and Dutka, B.J. 1986. Introduction and review of microbial and biochemical toxicity screening procedures. in Toxicity Testing Using Microorganisms. Vol. I. eds. G. Bitton and B.J. Dutka. CRC Press, Boca Raton, Florida, pp 1-8.

Bitton, G. and Dutka, B.J. 1986a, Toxicity Testing Using Microorganisms. Vol. I. Bitton and Dutka eds. CRC Press Boca Raton. Florida U.S.A. 163 pp.

Blaise, C., Legault, R., Bermingham, N., von Collie, R. and Vasseur, P. 1984. Microtest mesurant l'inhibition de la croissance des alques (C150) par le dosage de l'ATP. Sciences et Techniques de l'eau 17.

Bonde, G.J. 1963. Bacterial Indicators of water pollution. A study of quantitative estimation. Tuknisk Forlag, Copenhagen.

Bringmann, G. and Kuhn, R. 1980. Comparison of the toxicity thresholds of water pollutants to bacteria, algae and protozoa in the cell multiplication inhibation test. Water Res. 14: 231-238.

Draggan, S. and Giddings, J.M. 1978. Testing toxic substances for protection of the environment. Sci. Total Environ. 9: 63-71.

Dutka, B.J. and Kwan, K. 1982. Application of four bacterial screening procedures to assess changes in the toxicity of chemicals in mixtures. Environ. Pollut. Series A 29: 125-134.

Dutka, B.J., Nyhold, N. and Petersen, J. 1983. Comparison of several microbiological toxicity screening tests. Water Res. 17: 1363-1368.

Dutka, B.J., Jones, K., Xu, H., Kwan, K.K. and McInnis, R. 1986(b). Phase II. Priority Site Selection for degraded areas based on microbial and toxicant screening tests. NWRI Contribution No. 86-174, NWRI, CCIW, Burlington, Ontario, Canada.

Dutka, B.J., Walsh, K., Kwan, K.K., El-Shaarawi, A., Liu, D.L. and Thompson, K. 1986. Priority site selection for degraded areas based on microbial and toxicant screening tests. Water Poll. Res. J. Canada. 21(2): 267-282.

Dutka, B.J. and Bitton, G. 1986. Toxicity Testing Using Microorganisms. Vol. 2. Dutka and Bitton eds. CRC Press Boca Raton, Florida, U.S.A. 202 pp.

Environment New Brunswick. 1977. Saint John River Coliform Survey. New Brunswick Dept. of the Environ. Technical Report Series No. T-7903. Environmental Services Branch, Environment N.B., Moncton, N.B., Canada.

Geldreich, E.E. 1972. Buffalo Lake recreational water quality: A study in bacteriological data interpretion. Wat. Res. 6: 913-924.

Kohn, G.K. 1980. Bioassay as a monitoring tool. Pest. Rev. 76: 99-104.

Luminescens Review 1983. Bulletin No. 204. Turner Designs Mountain View California, U.S.A.

Xu, H. and Dutka, B.J. 1987. ATP-TOX System - A rapid sensitive bacterial toxicity screening system based on the determination of ATP. Toxicity Assessment 2: 149-166.

Xu, H., Dutka, B.J. and Kwan, K.K. 1987. Genotoxicity studies on sediments using a modified SOS Chromotest. Toxicity Assessment 2: 79-88.

Table 1. Saint John River Basin, New Brunswick, Canada, Sampling Site Locations and Sediment Descriptions

Site No.	Location	Latitude	Longitude	Sediment Description
1	Glasier Lake, 200 m from outfall at centre	47°13'05"	68°58'50"	Brown clay and light grey silty clay
2	Mill Stream at Pond, St. Francois de Madawaska	47°14'40"	68°42'08"	Brown grey very light density surface layer of silty clay, little sand
3	Lac Unique, 150 m from outfall	47°20'24"	68°44'55"	Grey-brown silty clay, some sand
4	Lac Baker, 500 m N.W. of causeway	47°20'58"	68°40'26"	Dark grey to black silty clay, light density
5	SJR[1] below Babin Brook at Fifth Island, near shore	47°18'08"	68°29'39"	Brown grey silt with fine sand
6	Madawaska R. above mill	47°22'32"	68°20'58"	Grey to black silt with black streaks
7	Madawaska R. at Headpond below mill behind Lynne Motel	47°22'01"	68°19'16"	Grey brown silt with fibres, some sand, putred odour
8	SJR at mouth of Iroquois R. backwater	47°21'41"	68°16'49"	Sandy silt, grey with rust streaks
9	SJR at St. Basile	47°21'18"	68°14'00"	Sandy silty clay, dark grey with organic matter
10	SJR at Martin Siding, 10 km above Grand Falls	47°05'34"	67°50'45"	Grey brown soft silt.
11	SJR at Grand Falls Headpond	47°03'02"	67°44'30"	Grey brown silt
12	SJR at Grand Falls, below falls near Sewage treatment plant outfall	47°02'37"	67°44'23"	Soft grey brown silt

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Table 1. Saint John River Basin, New Brunswick, Canada, Sampling Site Locations and Sediment Descriptions Continued

Site No.	Location	Latitude	Longitude	Sediment Description
13	Aroostook R. headpond, at Canadian Border	46°47'42"	67°47'20"	Grey brown sandy clay with pebbles
14	SJR 200 m above Beechwood dam	46°32'35"	67°40'23"	Brown grey silty clay with shells
15	SJR 2 km below McCain's, Florenceville	46°26'29"	67°37'14"	Grey black sandy gravel with thin grey white overlay
16	Big Presque Isle stream at Tracy Mills, above old dam	46°26'17"	67°44'44"	Grey brown soft silt
17	SJR at Woodstock, inside enclosure formed at causeway to water treatment plant	45°26'15"	67°37'20"	Soft green-brown silt
18	SJR at Meductic, 1 km below Sabian	45°59'39"	67°27'58"	Brown grey soft silty clay
19	SJR at Pokiok, 0.5 km below Pokiok R.	45°58'02"	67°14'29"	Brown grey soft silty clay
20	SJR at Nackawic, 1 km above mill, 1/3 from left bank	45°59'02"	67°14'02"	Grey brown soft silty clay
21	SJR at Nackawic, 1 km below mill, 1/3	45°59'11"	67°12'42"	Grey brown soft silty clay
22	SJR at Longs Creek, headland in middle	45°52'23"	66°55'15"	Grey brown soft silty clay
23	SJR at Mactaquac Headpond, 500 m above dam, 100 m from right bank	45°56'56"	66°52'24"	Rusty brown soft silt
24	SJR at Fredericton, below Jewett Island	45°57'57"	66°40'46"	Brown soft silt

Table 1. Saint John River Basin, New Brunswick, Canada, Sampling Site Locations and Sediment Descriptions Continued

Site No.	Location	Latitude	Longitude	Sediment Description
25	Nashwaaksis Stream at mouth	45°58'12"	66°39'16"	Brown soft silty clay
26	Nashwaak R. at mouth upstream of hwy. bridge at left bank	45°57'11"	66°37'09"	Green-brown soft silt
27	SJR at Fredericton, 1 km below Princess Margaret Bridge, right bank	45°55'41"	66°37'03"	Green-brown soft silt
28	SJR, 0.5 km below confluence of Oromocto R., near right bank	45°51'23"	66°27'51"	Green brown soft silt under eel grass
29	Grand Lake near Douglas Harbour, 1 km from headland	45°54'02"	66°04'18"	Brown grey soft silt
30	SJR at Evandale Ferry, right bank	45°35'25"	66°01'55"	Brown grey soft silt
31	SJR at Westfield Ferry, 1 km below ferry, 100 m from shore	45°19'54"	66°13'00"	Grey brown with soft black silt overlay
32	Grand Bay, (SJR) 1 km from Saint John Marina	45°16'43"	66°09'00"	Grey brown soft silty clay
33	Grand Bay, (SJR) 1 km from Boars Head, left bank	45°17'10"	66°08'15"	Grey brown clay
34	Little River at Bayside Drive	45°16'26"	66°01'36"	Black soft odouriferous silt
35	Marsh Creek Pond at Red Bank	45°15'36"	66°01'02"	Brown sandy silt
36	Saint John Harbour, near wharf by Market Square	45°16'19"	66°04'09"	Brownish soft silt
37	Saint John Harbour, at slip next to container wharf	45°15'47"	66°03'54"	Grey black soft silt with brown streaks
38	Saint John Harbour, 1 km southwest of Courtenay Bay Breakwater	45°1[illegible]8"	66°02'00"	Grey brown silt

Table 2. Point Awarding Scheme for Sample ranking, Based on Suspected Contained Hazards

E. coli fecal coliform fecal strepto-cocci sediment MPN 10g/100 mL water /100 mL MF	Coliphage water/100 mL	*Clostridium perfringens* sediment 10 g /100 mL MPN	Points	Coprostanol ppb	Genotoxicity Induction factor per 0.5 mL 10x water or 1:1 Milli Q water extract	Algal-ATP % Relative light Units per 200 µL 10x water or 1:1 Milli Q sediment extract	Points
<100	5-24	<25	1	<1.0	1.0-1.29	100-50	1
101-500	25-100	26-100	2	1-3	1.30-1.50	49-20	3
501-2500	101-250	101-500	3	3.1-5	1.51-2.0	19-1.0	5
2500-16000	251-1000	501-2500	4	5.1-7	2.1-3.0	.9-.1	7
16000-160000	1000-5000	2501-10000	7	7+	3.1+	.09-.01	10
160000+	5001+	10000+	10				

ATP-TOX System % Inhibition per mL sediment extract or 10x water	Microtox EC_{50}/g wet wt sediment or/mL	Points	Cholesterol ppb	Points	*Spirillum volutans* Water Sample	Points	Sediment Extract and 10x water	Points
1-30	.4+	1	<2.0	1	neg	0	neg	0
31-60	.40-.31	3	2.1-4	2	+	10	+	5
61-90	.30-.21	5	4.1-6	3				
91-99	.20-.11	7	6.1-8	4				
100	<.10	10	8.+	5				

Table 3. Results of Saint John River Basin Sediment Analyses by Battery of Tests Approach

Sample number	Fecal coliform A1 broth 10g/100 mL MPN	Clostridium perfringens 10g/100 mL MPN	Microtox EC_{50} g.wet wt	Algal-ATP RLU[1] %	Spirillum volutans[2] 120 min test	SOS Chromotest[2] Induction Factor	ATP-TOX % Inhibition[2]	Points	Rank
1	<2	1,700	N.D.*	S**	+	.993	0	11	27
2	220	4,300	.17	45.0	+	.922	0	25	5
3	5	21	.40	28.1	+	.922	8	15	19
4	8	2,200	N.D.	29.4	+	1.08	14	14	20
5	3,500	540	.37	S	+	.893	5	22	6
6	1,100	92,000	.23	29.4	N.D.	.874	0	21	8
7	16,000	>160,000	.17	21.9	+	.922	14	33	2
8	16,000	330	N.D.	38.1	N.D.	.886	0	10	30
9	>16,000	4,300	N.D.	S	+	.893	0	19	12
10	5,400	1,100	N.D.	S	N.D.	.902	5	10	31
11	16,000	4,900	N.D.	S	N.D.	.893	0	10	28
12	>160,000	25,000	N.D.	S	N.D.	.979	14	10	9
13	2,200	35,000	N.D.	S	N.D.	.856	0	13	23
14	490	11,000	N.D.	S	N.D.	.863	0	17	17
15	92,000	22,000	.086	15.6	+	1.07	99	45	1
16	460	2,200	N.D.	S	N.D.	.856	22	7	35
17	170	35,000	N.D.	S	N.D.	.856	29	18	15
18	490	250	N.D.	S	+	.838	20	11	29
19	130	7,000	.27	100	N.D.	.783	20	16	18
20	790	28,000	N.D.	S	N.D.	.808	3	14	21

Table 3. Results of Saint John River Basin Sediment Analyses by Battery of Tests Approach continued

Sample number	Fecal coliform A1 broth 10g/100 mL MPN	Clostridium perfringens 10g/100 mL MPN	Microtox EC_{50} g.wet wt	Algal-ATP RLU[1] %	Spirillum volutans[2] 120 min test	SOS Chromotest[2] Induction Factor	ATP-TOX % Inhibition[2]	Points	Rank
21	330	11,000	.28	29.4	N.D.	.826	5	21	10
22	13	13,000	.35	30	N.D.	1.10	38	22	7
23	2	12,000	N.D.	S	N.D.	.816	38	18	14
24	490	790	N.D.	S	N.D.	.718	34	8	34
25	490	2,300	N.D.	S	N.D.	.816	0	6	36
26	230	170	N.D.	S	N.D.	.759	0	5	38
27	1,100	14,000	N.D.	S	N.D.	.869	0	13	22
28	1,700	4,900	N.D.	S	N.D.	1.09	0	11	25
29	0	700	N.D.	25.0	N.D.	1.40	5	11	26
30	49	11,000	N.D.	S	N.D.	.806	9	12	24
31	17	790	N.D.	S	N.D.	.816	10	6	37
32	7	4,900	N.D.	S	N.D.	.708	27	8	33
33	14	1,400	.29	54.4	N.D.	.718	30	33	3
34	>1,600,000	2,200	.052	S	N.D.	.716	57	27	4
35	330	72	N.D.	S	N.D.	.812	76	9	32
36	4,900	2,200	N.D.	12.5	N.D.	.534	76	18	16
37	11,000	700	N.D.	25.0	+	.479	71	21	11
38	2,200	280	N.D.	13.8	+	.550	54	19	13

N.D.* = Not detected
S** = Stimulated
RLU[1] = Relative light units

[2]Using 1:1 Milli Q water extract

Table 4. Results of Saint John River Basin Water Analyses by Battery of Tests Approach

Sample number	Fecal coliform MF-mFC /100 mL	Coliphage /100 mL	Fecal Streptococci MF-KF/100 mL	Fecal Sterols Coprostanol ppb	Fecal Sterols Cholesterol ppb	Microtox[2] EC50 /mL	Algal-ATP[2] RLU[1] %	*Spirillum*[2] volutans 120 min. test	SOS Chromotest[2] Induction Factor	ATP-TOX[2] % Inhibition	Points	Rank
1	1	0	5	<0.05	1.27	N.D.*	23.1	+	.947	86	16	14
2	61	0	83	3.46	3.81	N.D.	21.7	+	.778	65	22	9
3	0	0	0	<0.05	2.94	.26	15.4	+	1.17	100	28	5
4	2	0	0	<0.05	2.59	N.D.	18.2	N.D.	.854	43	16	14
5	9,400	0	52	<0.05	0.11	N.D.	22.4	+	.881	16	15	15
6	310	5	10	<0.05	0.74	N.D.	18.2	+	.976	42	18	12
7	50,000	0	212	0.15	0.49	N.D.	16.1	+	.881	29	22	8
8	680	0	13	<0.05	0.54	N.D.	26.6	N.D.	.851	65	13	17
9	3,300	95	101	0.24	0.87	N.D.	16.8	+	.822	42	23	7
10	800	45	12	<0.05	<0.05	N.D.	34.3	N.D.	.976	17	10	20
11	1,300	0	30	<0.05	<0.05	N.D.	30.1	N.D.	.881	49	10	20
12	1,100	0	22	<0.05	<0.05	N.D.	30.8	N.D.	.976	12	8	22
13	500	20	0	<0.05	0.06	N.D.	17.5	+	.794	36	17	13
14	340	0	26	<0.05	0.19	N.D.	25.2	N.D.	.794	33	10	20
15	630	15	308	<0.05	<0.05	N.D.	21.7	+	.881	33	17	13
16	100	0	2	<0.05	<0.05	N.D.	13.3	N.D.	.856	100	17	13
17	420	15	14	<0.05	<0.05	N.D.	18.8	N.D.	.881	26	9	21
18	80	5	2	<0.05	<0.05	N.D.	19.6	N.D.	.821	34	11	19
20	4	0	20	<0.05	<0.05	N.D.	22.4	+	.851	44	13	17
19	6	15	4	<0.05	<0.05	N.D.	60.9	N.D.	.881	68	9	21

Table 4. Results of Saint John River Basin Water Analyses by Battery of Tests Approach continued

Sample number	Fecal coliform MF-mFC /100 mL	Coliphage /100 mL	Fecal Streptococci MF-KF/100 mL	Fecal Coprostanol ppb	Sterols Cholesterol ppb	Microtox[2] EC50 /mL	Algal-ATP[2] RLU[1] %	Spirillum[2] volutans 120 min. test	SOS Chromotest[2] Induction Factor	ATP-TOX[2] % Inhibition	Points	Rank
21	830	0	30	<0.05	<0.05	N.D.	29.4	+	.767	41	15	15
22	6	0	6	<0.05	<0.05	N.D.	30.8	N.D.	.893	72	10	20
23	0	5	2	<0.05	<0.05	N.D.	68.6	+	.921	31	11	19
24	21	0	8	<0.05	<0.05	N.D.	22.4	N.D.	.950	44	13	17
25	280	0	24	<0.05	<0.05	N.D.	34.3	+	.845	60	14	16
26	62	0	2	<0.05	<0.05	N.D.	39.9	+	.969	23	11	19
27	276	0	17	<0.05	0.11	N.D.	50.4	+	.873	55	13	17
28	101	45	9	<0.05	0.96	N.D.	28.0	+	.901	85	19	11
29	0	0	0	<0.05	0.36	N.D.	27.3	+	.808	21	10	20
30	5	0	3	<0.05	<0.05	N.D.	30.1	+	1.19	43	14	16
31	17	0	9	<0.05	<0.05	N.D.	15.4	N.D.	.755	82	12	18
32	300	10	2	<0.05	<0.05	N.D.	14.7	+	.488	93	21	10
33	330	0	21	<0.05	0.12	.39	14.7	+	.466	100	27	6
34	>5,000,000	10	380	<0.05	0.19	.038	0.03	+	1.06	89	50	1
35	9	0	0	<0.05	1.31	N.D.	30.8	N.D.	1.05	61	11	19
36	570	0	87	<0.05	0.08	.21	0.01	+	.345	100	40	3
37	200	0	25	<0.05	<0.05	.24	0.01	+	.466	100	38	4
38	520	20	14	<0.05	0.17	.26.	0.01	+	.375	100	41	2

N.D.* = Not detected
RLU[1] = Relative light units
[2] = 10x water sample tested

Table 1. Saint John River Basin, New Brunswick, Canada, Sampling Site Locations and Sediment Descriptions Continued

Site No.	Location	Latitude	Longitude	Sediment Description
13	Aroostook R. headpond, at Canadian Border	46°47'42"	67°47'20"	Grey brown sandy clay with pebbles
14	SJR 200 m above Beechwood dam	46°32'35"	67°40'23"	Brown grey silty clay with shells
15	SJR 2 km below McCain's, Florenceville	46°26'29"	67°37'14"	Grey black sandy gravel with thin grey white overlay
16	Big Presque Isle stream at Tracy Mills, above old dam	46°26'17"	67°44'44"	Grey brown soft silt
17	SJR at Woodstock, inside enclosure formed at causeway to water treatment plant	45°26'15"	67°37'20"	Soft green-brown silt
18	SJR at Meductic, 1 km below Sabian	45°59'39"	67°27'58"	Brown grey soft silty clay
19	SJR at Pokiok, 0.5 km below Pokiok R.	45°58'02"	67°14'29"	Brown grey soft silty clay
20	SJR at Nackawic, 1 km above mill, 1/3 from left bank	45°59'02"	67°14'02"	Grey brown soft silty clay
21	SJR at Nackawic, 1 km below mill, 1/3	45°59'11"	67°12'42"	Grey brown soft silty clay
22	SJR at Longs Creek, headland in middle	45°52'23"	66°55'15"	Grey brown soft silty clay
23	SJR at Mactaquac Headpond, 500 m above dam, 100 m from right bank	45°56'56"	66°52'24"	Rusty brown soft silt
24	SJR at Fredericton, below Jewett Island	45°57'57"	66°40'46"	Brown soft silt

Table 1. Saint John River Basin, New Brunswick, Canada, Sampling Site Locations and Sediment Descriptions Continued

Site No.	Location	Latitude	Longitude	Sediment Description
25	Nashwaaksis Stream at mouth	45°58'12"	66°39'16"	Brown soft silty clay
26	Nashwaak R. at mouth upstream of hwy. bridge at left bank	45°57'11"	66°37'09"	Green-brown soft silt
27	SJR at Fredericton, 1 km below Princess Margaret Bridge, right bank	45°55'41"	66°37'03"	Green-brown soft silt
28	SJR, 0.5 km below confluence of Oromocto R., near right bank	45°51'23"	66°27'51"	Green brown soft silt under eel grass
29	Grand Lake near Douglas Harbour, 1 km from headland	45°54'02"	66°04'18"	Brown grey soft silt
30	SJR at Evandale Ferry, right bank	45°35'25"	66°01'55"	Brown grey soft silt
31	SJR at Westfield Ferry, 1 km below ferry, 100 m from shore	45°19'54"	66°13'00"	Grey brown with soft black silt overlay
32	Grand Bay, (SJR) 1 km from Saint John Marina	45°16'43"	66°09'00"	Grey brown soft silty clay
33	Grand Bay, (SJR) 1 km from Boars Head, left bank	45°17'10"	66°08'15"	Grey brown clay
34	Little River at Bayside Drive	45°16'26"	66°01'36"	Black soft odouriferous silt
35	Marsh Creek Pond at Red Bank	45°15'36"	66°01'02"	Brown sandy silt
36	Saint John Harbour, near wharf by Market Square	45°16'19"	66°04'09"	Brownish soft silt
37	Saint John Harbour, at slip next to container wharf	45°15'47"	66°03'54"	Grey black soft silt with brown streaks
38	Saint John Harbour, 1 km southwest of Courtenay Bay Breakwater	45°1[illegible]8"	66°02'00"	Grey brown silt

Table 2. Point Awarding Scheme for Sample ranking, Based on Suspected Contained Hazards

E. coli fecal coliform fecal strepto-cocci sediment MPN 10g/100 mL water /100 mL MF	Coliphage water/100 mL	*Clostridium perfringens* sediment 10 g /100 mL MPN	Points	Coprostanol ppb	Genotoxicity Induction factor per 0.5 mL 10x water or 1:1 Milli Q water extract	Algal-ATP % Relative light Units per 200 µL 10x water or 1:1 Milli Q sediment extract	Points
<100	5-24	<25	1	<1.0	1.0-1.29	100-50	1
101-500	25-100	26-100	2	1-3	1.30-1.50	49-20	3
501-2500	101-250	101-500	3	3.1-5	1.51-2.0	19-1.0	5
2500-16000	251-1000	501-2500	4	5.1-7	2.1-3.0	.9-.1	7
16000-160000	1000-5000	2501-10000	7	7+	3.1+	.09-.01	10
160000+	5001+	10000+	10				

ATP-TOX System % Inhibition per mL sediment extract or 10x water	Microtox EC_{50}/g wet wt sediment or/mL	Points	Cholesterol ppb	Points	*Spirillum volutans* Water Sample	Points	Sediment Extract and 10x water	Points
1-30	.4+	1	<2.0	1	neg	0	neg	0
31-60	.40-.31	3	2.1-4	2	+	10	+	5
61-90	.30-.21	5	4.1-6	3				
91-99	.20-.11	7	6.1-8	4				
100	<.10	10	8.+	5				

Table 3. Results of Saint John River Basin Sediment Analyses by Battery of Tests Approach

Sample number	Fecal coliform A1 broth 10g/100 mL MPN	Clostridium perfringens 10g/100 mL MPN	Microtox EC_{50} g.wet wt	Algal-ATP RLU[1] %	Spirillum volutans[2] 120 min test	SOS Chromotest[2] Induction Factor	ATP-TOX % Inhibition[2]	Points	Rank
1	<2	1,700	N.D.*	S**	+	.993	0	11	27
2	220	4,300	.17	45.0	+	.922	0	25	5
3	5	21	.40	28.1	+	.922	8	15	19
4	8	2,200	N.D.	29.4	+	1.08	14	14	20
5	3,500	540	.37	S	+	.893	5	22	6
6	1,100	92,000	.23	29.4	N.D.	.874	0	21	8
7	16,000	>160,000	.17	21.9	+	.922	14	33	2
8	16,000	330	N.D.	38.1	N.D.	.886	0	10	30
9	>16,000	4,300	N.D.	S	+	.893	0	19	12
10	5,400	1,100	N.D.	S	N.D.	.902	5	10	31
11	16,000	4,900	N.D.	S	N.D.	.893	0	10	28
12	>160,000	25,000	N.D.	S	N.D.	.979	14	10	9
13	2,200	35,000	N.D.	S	N.D.	.856	0	13	23
14	490	11,000	N.D.	S	N.D.	.863	0	17	17
15	92,000	22,000	.086	15.6	+	1.07	99	45	1
16	460	2,200	N.D.	S	N.D.	.856	22	7	35
17	170	35,000	N.D.	S	N.D.	.856	29	18	15
18	490	250	N.D.	S	+	.838	20	11	29
19	130	7,000	.27	100	N.D.	.783	20	16	18
20	790	28,000	N.D.	S	N.D.	.808	3	14	21

Table 3. Results of Saint John River Basin Sediment Analyses by Battery of Tests Approach continued

Sample number	Fecal coliform A1 broth 10g/100 mL MPN	Clostridium perfringens 10g/100 mL MPN	Microtox EC_{50} g.wet wt	Algal-ATP RLU[1] %	Spirillum volutans[2] 120 min test	SOS Chromotest[2] Induction Factor	ATP-TOX % Inhibition[2]	Points	Rank
21	330	11,000	.28	29.4	N.D.	.826	5	21	10
22	13	13,000	.35	30	N.D.	1.10	38	22	7
23	2	12,000	N.D.	S	N.D.	.816	38	18	14
24	490	790	N.D.	S	N.D.	.718	34	8	34
25	490	2,300	N.D.	S	N.D.	.816	0	6	36
26	230	170	N.D.	S	N.D.	.759	0	5	38
27	1,100	14,000	N.D.	S	N.D.	.869	0	13	22
28	1,700	4,900	N.D.	S	N.D.	1.09	0	11	25
29	0	700	N.D.	25.0	N.D.	1.40	5	11	26
30	49	11,000	N.D.	S	N.D.	.806	9	12	24
31	17	790	N.D.	S	N.D.	.816	10	6	37
32	7	4,900	N.D.	S	N.D.	.708	27	8	33
33	14	1,400	.29	54.4	N.D.	.718	30	33	3
34	>1,600,000	2,200	.052	S	N.D.	.716	57	27	4
35	330	72	N.D.	S	N.D.	.812	76	9	32
36	4,900	2,200	N.D.	12.5	N.D.	.534	76	18	16
37	11,000	700	N.D.	25.0	+	.479	71	21	11
38	2,200	280	N.D.	13.8	+	.550	54	19	13

N.D.* = Not detected
S** = Stimulated
RLU[1] = Relative light units

[2]Using 1:1 Milli Q water extract

Table 4. Results of Saint John River Basin Water Analyses by Battery of Tests Approach

Sample number	Fecal coliform MF-mFC /100 mL	Coliphage /100 mL	Fecal Streptococci MF-KF/100 mL	Fecal Sterols Coprostanol ppb	Fecal Sterols Cholesterol ppb	Microtox[2] EC50 /mL	Algal-ATP[2] RLU[1] %	*Spirillum*[2] volutans 120 min. test	SOS Chromotest[2] Induction Factor	ATP-TOX[2] % Inhibition	Points	Rank
1	1	0	5	<0.05	1.27	N.D.*	23.1	+	.947	86	16	14
2	61	0	83	3.46	3.81	N.D.	21.7	+	.778	65	22	9
3	0	0	0	<0.05	2.94	.26	15.4	+	1.17	100	28	5
4	2	0	0	<0.05	2.59	N.D.	18.2	N.D.	.854	43	16	14
5	9,400	0	52	<0.05	0.11	N.D.	22.4	+	.881	16	15	15
6	310	5	10	<0.05	0.74	N.D.	18.2	+	.976	42	18	12
7	50,000	0	212	0.15	0.49	N.D.	16.1	+	.881	29	22	8
8	680	0	13	<0.05	0.54	N.D.	26.6	N.D.	.851	65	13	17
9	3,300	95	101	0.24	0.87	N.D.	16.8	+	.822	42	23	7
10	800	45	12	<0.05	<0.05	N.D.	34.3	N.D.	.976	17	10	20
11	1,300	0	30	<0.05	<0.05	N.D.	30.1	N.D.	.881	49	10	20
12	1,100	0	22	<0.05	<0.05	N.D.	30.8	N.D.	.976	12	8	22
13	500	20	0	<0.05	0.06	N.D.	17.5	+	.794	36	17	13
14	340	0	26	<0.05	0.19	N.D.	25.2	N.D.	.794	33	10	20
15	630	15	308	<0.05	<0.05	N.D.	21.7	+	.881	33	17	13
16	100	0	2	<0.05	<0.05	N.D.	13.3	N.D.	.856	100	17	13
17	420	15	14	<0.05	<0.05	N.D.	18.8	N.D.	.881	26	9	21
18	80	5	2	<0.05	<0.05	N.D.	19.6	N.D.	.821	34	11	19
20	4	0	20	<0.05	<0.05	N.D.	22.4	+	.851	44	13	17
19	6	15	4	<0.05	<0.05	N.D.	60.9	N.D.	.881	68	9	21

Table 4. Results of Saint John River Basin Water Analyses by Battery of Tests Approach
continued

Sample number	Fecal coliform MF-mFC /100 mL	Coliphage /100 mL	Fecal Streptococci MF-KF/100 mL	Fecal Coprostanol ppb	Sterols Cholesterol ppb	Microtox[2] EC50 /mL	Algal-ATP[2] RLU[1] %	Spirillum[2] volutans 120 min. test	SOS Chromotest[2] Induction Factor	ATP-TOX[2] % Inhibition	Points	Rank
21	830	0	30	<0.05	<0.05	N.D.	29.4	+	.767	41	15	15
22	6	0	6	<0.05	<0.05	N.D.	30.8	N.D.	.893	72	10	20
23	0	5	2	<0.05	<0.05	N.D.	68.6	+	.921	31	11	19
24	21	0	8	<0.05	<0.05	N.D.	22.4	N.D.	.950	44	13	17
25	280	0	24	<0.05	<0.05	N.D.	34.3	+	.845	60	14	16
26	62	0	2	<0.05	<0.05	N.D.	39.9	+	.969	23	11	19
27	276	0	17	<0.05	0.11	N.D.	50.4	+	.873	55	13	17
28	101	45	9	<0.05	0.96	N.D.	28.0	+	.901	85	19	11
29	0	0	0	<0.05	0.36	N.D.	27.3	+	.808	21	10	20
30	5	0	3	<0.05	<0.05	N.D.	30.1	+	1.19	43	14	16
31	17	0	9	<0.05	<0.05	N.D.	15.4	N.D.	.755	82	12	18
32	300	10	2	<0.05	<0.05	N.D.	14.7	+	.488	93	21	10
33	330	0	21	<0.05	0.12	.39	14.7	+	.466	100	27	6
34	>5,000,000	10	380	<0.05	0.19	.038	0.03	+	1.06	89	50	1
35	9	0	0	<0.05	1.31	N.D.	30.8	N.D.	1.05	61	11	19
36	570	0	87	<0.05	0.08	.21	0.01	+	.345	100	40	3
37	200	0	25	<0.05	<0.05	.24	0.01	+	.466	100	38	4
38	520	20	14	<0.05	0.17	.26	0.01	+	.375	100	41	2

N.D.* = Not detected
RLU[1] = Relative light units
[2] = 10x water sample tested

Table 1. Saint John River Basin, New Brunswick, Canada, Sampling Site Locations and Sediment Descriptions

Site No.	Location	Latitude	Longitude	Sediment Description
1	Glasier Lake, 200 m from outfall at centre	47°13'05"	68°58'50"	Brown clay and light grey silty clay
2	Mill Stream at Pond, St. Francois de Madawaska	47°14'40"	68°42'08"	Brown grey very light density surface layer of silty clay, little sand
3	Lac Unique, 150 m from outfall	47°20'24"	68°44'55"	Grey-brown silty clay, some sand
4	Lac Baker, 500 m N.W. of causeway	47°20'58"	68°40'26"	Dark grey to black silty clay, light density
5	SJR[1] below Babin Brook at Fifth Island, near shore	47°18'08"	68°29'39"	Brown grey silt with fine sand
6	Madawaska R. above mill	47°22'32"	68°20'58"	Grey to black silt with black streaks
7	Madawaska R. at Headpond below mill behind Lynne Motel	47°22'01"	68°19'16"	Grey brown silt with fibres, some sand, putred odour
8	SJR at mouth of Iroquois R. backwater	47°21'41"	68°16'49"	Sandy silt, grey with rust streaks
9	SJR at St. Basile	47°21'18"	68°14'00"	Sandy silty clay, dark grey with organic matter
10	SJR at Martin Siding, 10 km above Grand Falls	47°05'34"	67°50'45"	Grey brown soft silt.
11	SJR at Grand Falls Headpond	47°03'02"	67°44'30"	Grey brown silt
12	SJR at Grand Falls, below falls near Sewage treatment plant outfall	47°02'37"	67°44'23"	Soft grey brown silt

Table 1. Saint John River Basin, New Brunswick, Canada, Sampling Site Locations and Sediment Descriptions Continued

Site No.	Location	Latitude	Longitude	Sediment Description
13	Aroostook R. headpond, at Canadian Border	46°47'42"	67°47'20"	Grey brown sandy clay with pebbles
14	SJR 200 m above Beechwood dam	46°32'35"	67°40'23"	Brown grey silty clay with shells
15	SJR 2 km below McCain's, Florenceville	46°26'29"	67°37'14"	Grey black sandy gravel with thin grey white overlay
16	Big Presque Isle stream at Tracy Mills, above old dam	46°26'17"	67°44'44"	Grey brown soft silt
17	SJR at Woodstock, inside enclosure formed at causeway to water treatment plant	45°26'15"	67°37'20"	Soft green-brown silt
18	SJR at Meductic, 1 km below Sabian	45°59'39"	67°27'58"	Brown grey soft silty clay
19	SJR at Pokiok, 0.5 km below Pokiok R.	45°58'02"	67°14'29"	Brown grey soft silty clay
20	SJR at Nackawic, 1 km above mill, 1/3 from left bank	45°59'02"	67°14'02"	Grey brown soft silty clay
21	SJR at Nackawic, 1 km below mill, 1/3	45°59'11"	67°12'42"	Grey brown soft silty clay
22	SJR at Longs Creek, headland in middle	45°52'23"	66°55'15"	Grey brown soft silty clay
23	SJR at Mactaquac Headpond, 500 m above dam, 100 m from right bank	45°56'56"	66°52'24"	Rusty brown soft silt
24	SJR at Fredericton, below Jewett Island	45°57'57"	66°40'46"	Brown soft silt

Table 1. Saint John River Basin, New Brunswick, Canada, Sampling Site Locations and Sediment Descriptions Continued

Site No.	Location	Latitude	Longitude	Sediment Description
25	Nashwaaksis Stream at mouth	45°58'12"	66°39'16"	Brown soft silty clay
26	Nashwaak R. at mouth upstream of hwy. bridge at left bank	45°57'11"	66°37'09"	Green-brown soft silt
27	SJR at Fredericton, 1 km below Princess Margaret Bridge, right bank	45°55'41"	66°37'03"	Green-brown soft silt
28	SJR, 0.5 km below confluence of Oromocto R., near right bank	45°51'23"	66°27'51"	Green brown soft silt under eel grass
29	Grand Lake near Douglas Harbour, 1 km from headland	45°54'02"	66°04'18"	Brown grey soft silt
30	SJR at Evandale Ferry, right bank	45°35'25"	66°01'55"	Brown grey soft silt
31	SJR at Westfield Ferry, 1 km below ferry, 100 m from shore	45°19'54"	66°13'00"	Grey brown with soft black silt overlay
32	Grand Bay, (SJR) 1 km from Saint John Marina	45°16'43"	66°09'00"	Grey brown soft silty clay
33	Grand Bay, (SJR) 1 km from Boars Head, left bank	45°17'10"	66°08'15"	Grey brown clay
34	Little River at Bayside Drive	45°16'26"	66°01'36"	Black soft odouriferous silt
35	Marsh Creek Pond at Red Bank	45°15'36"	66°01'02"	Brown sandy silt
36	Saint John Harbour, near wharf by Market Square	45°16'19"	66°04'09"	Brownish soft silt
37	Saint John Harbour, at slip next to container wharf	45°15'47"	66°03'54"	Grey black soft silt with brown streaks
38	Saint John Harbour, 1 km southwest of Courtenay Bay Breakwater	45°[illegible]08"	66°02'00"	Grey brown silt

Table 2. Point Awarding Scheme for Sample ranking, Based on Suspected Contained Hazards

E. coli fecal coliform fecal streptococci sediment MPN 10g/100 mL water /100 mL MF	Coliphage water/100 mL	*Clostridium perfringens* sediment 10 g /100 mL MPN	Points	Coprostanol ppb	Genotoxicity Induction factor per 0.5 mL 10x water or 1:1 Milli Q water extract	Algal-ATP % Relative light Units per 200 µL 10x water or 1:1 Milli Q sediment extract	Points
<100	5-24	<25	1	<1.0	1.0-1.29	100-50	1
101-500	25-100	26-100	2	1-3	1.30-1.50	49-20	3
501-2500	101-250	101-500	3	3.1-5	1.51-2.0	19-1.0	5
2500-16000	251-1000	501-2500	4	5.1-7	2.1-3.0	.9-.1	7
16000-160000	1000-5000	2501-10000	7	7+	3.1+	.09-.01	10
160000+	5001+	10000+	10				

ATP-TOX System % Inhibition per mL sediment extract or 10x water	Microtox EC_{50}/g wet wt sediment or/mL	Points	Cholesterol ppb	Points	*Spirillum volutans* Water Sample	Points	Sediment Extract and 10x water	Points
1-30	.4+	1	<2.0	1	neg	0	neg	0
31-60	.40-.31	3	2.1-4	2	+	10	+	5
61-90	.30-.21	5	4.1-6	3				
91-99	.20-.11	7	6.1-8	4				
100	<.10	10	8.+	5				

Table 3. Results of Saint John River Basin Sediment Analyses by Battery of Tests Approach

Sample number	Fecal coliform Al broth 10g/100 mL MPN	Clostridium perfringens 10g/100 mL MPN	Microtox EC_{50} g.wet wt	Algal-ATP RLU[1] %	Spirillum volutans[2] 120 min test	SOS Chromotest[2] Induction Factor	ATP-TOX % Inhibition[2]	Points	Rank
1	<2	1,700	N.D.*	S**	+	.993	0	11	27
2	220	4,300	.17	45.0	+	.922	0	25	5
3	5	21	.40	28.1	+	.922	8	15	19
4	8	2,200	N.D.	29.4	+	1.08	14	14	20
5	3,500	540	.37	S	+	.893	5	22	6
6	1,100	92,000	.23	29.4	N.D.	.874	0	21	8
7	16,000	>160,000	.17	21.9	+	.922	14	33	2
8	16,000	330	N.D.	38.1	N.D.	.886	0	10	30
9	>16,000	4,300	N.D.	S	+	.893	0	19	12
10	5,400	1,100	N.D.	S	N.D.	.902	5	10	31
11	16,000	4,900	N.D.	S	N.D.	.893	0	10	28
12	>160,000	25,000	N.D.	S	N.D.	.979	14	10	9
13	2,200	35,000	N.D.	S	N.D.	.856	0	13	23
14	490	11,000	N.D.	S	N.D.	.863	0	17	17
15	92,000	22,000	.086	15.6	+	1.07	99	45	1
16	460	2,200	N.D.	S	N.D.	.856	22	7	35
17	170	35,000	N.D.	S	N.D.	.856	29	18	15
18	490	250	N.D.	S	+	.838	20	11	29
19	130	7,000	.27	100	N.D.	.783	20	16	18
20	790	28,000	N.D.	S	N.D.	.808	3	14	21

Table 3. Results of Saint John River Basin Sediment Analyses by Battery of Tests Approach continued

Sample number	Fecal coliform A1 broth 10g/100 mL MPN	Clostridium perfringens 10g/100 mL MPN	Microtox EC_{50} g.wet wt	Algal-ATP RLU[1] %	Spirillum volutans[2] 120 min test	SOS Chromotest[2] Induction Factor	ATP-TOX % Inhibition[2]	Points	Rank
21	330	11,000	.28	29.4	N.D.	.826	5	21	10
22	13	13,000	.35	30	N.D.	1.10	38	22	7
23	2	12,000	N.D.	S	N.D.	.816	38	18	14
24	490	790	N.D.	S	N.D.	.718	34	8	34
25	490	2,300	N.D.	S	N.D.	.816	0	6	36
26	230	170	N.D.	S	N.D.	.759	0	5	38
27	1,100	14,000	N.D.	S	N.D.	.869	0	13	22
28	1,700	4,900	N.D.	S	N.D.	1.09	0	11	25
29	0	700	N.D.	25.0	N.D.	1.40	5	11	26
30	49	11,000	N.D.	S	N.D.	.806	9	12	24
31	17	790	N.D.	S	N.D.	.816	10	6	37
32	7	4,900	N.D.	S	N.D.	.708	27	8	33
33	14	1,400	.29	54.4	N.D.	.718	30	33	3
34	>1,600,000	2,200	.052	S	N.D.	.716	57	27	4
35	330	72	N.D.	S	N.D.	.812	76	9	32
36	4,900	2,200	N.D.	12.5	N.D.	.534	76	18	16
37	11,000	700	N.D.	25.0	+	.479	71	21	11
38	2,200	280	N.D.	13.8	+	.550	54	19	13

N.D.* = Not detected
S** = Stimulated
RLU[1] = Relative light units

[2]Using 1:1 Milli Q water extract

Table 4. Results of Saint John River Basin Water Analyses by Battery of Tests Approach

Sample number	Fecal coliform MF-mFC /100 mL	Coliphage /100 mL	Fecal Streptococci MF-KF/100 mL	Fecal Sterols Coprostanol ppb	Fecal Sterols Cholesterol ppb	Microtox[2] EC50 /mL	Algal-ATP[2] RLU[1] %	*Spirillum*[2] volutans 120 min. test	SOS Chromotest[2] Induction Factor	ATP-TOX[2] % Inhibition	Points	Rank
1	1	0	5	<0.05	1.27	N.D.*	23.1	+	.947	86	16	14
2	61	0	83	3.46	3.81	N.D.	21.7	+	.778	65	22	9
3	0	0	0	<0.05	2.94	.26	15.4	+	1.17	100	28	5
4	2	0	0	<0.05	2.59	N.D.	18.2	N.D.	.854	43	16	14
5	9,400	0	52	<0.05	0.11	N.D.	22.4	+	.881	16	15	15
6	310	5	10	<0.05	0.74	N.D.	18.2	+	.976	42	18	12
7	50,000	0	212	0.15	0.49	N.D.	16.1	+	.881	29	22	8
8	680	0	13	<0.05	0.54	N.D.	26.6	N.D.	.851	65	13	17
9	3,300	95	101	0.24	0.87	N.D.	16.8	+	.822	42	23	7
10	800	45	12	<0.05	<0.05	N.D.	34.3	N.D.	.976	17	10	20
11	1,300	0	30	<0.05	<0.05	N.D.	30.1	N.D.	.881	49	10	20
12	1,100	0	22	<0.05	<0.05	N.D.	30.8	N.D.	.976	12	8	22
13	500	20	0	<0.05	0.06	N.D.	17.5	+	.794	36	17	13
14	340	0	26	<0.05	0.19	N.D.	25.2	N.D.	.794	33	10	20
15	630	15	308	<0.05	<0.05	N.D.	21.7	+	.881	33	17	13
16	100	0	2	<0.05	<0.05	N.D.	13.3	N.D.	.856	100	17	13
17	420	15	14	<0.05	<0.05	N.D.	18.8	N.D.	.881	26	9	21
18	80	5	2	<0.05	<0.05	N.D.	19.6	N.D.	.821	34	11	19
20	4	0	20	<0.05	<0.05	N.D.	22.4	+	.851	44	13	17
19	6	15	4	<0.05	<0.05	N.D.	60.9	N.D.	.881	68	9	21

Table 4. Results of Saint John River Basin Water Analyses by Battery of Tests Approach continued

Sample number	Fecal coliform MF-mFC /100 mL	Coliphage /100 mL	Fecal Streptococci MF-KF/100 mL	Fecal Coprostanol ppb	Sterols Cholesterol ppb	Microtox[3] EC50 /mL	Algal-ATP[2] RLU[1] %	*Spirillum*[3] *volutans* 120 min. test	SOS Chromotest[3] Induction Factor	ATP-TOX[2] % Inhibition	Points	Rank
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22	6	0	6	<0.05	<0.05	N.D.	30.8	N.D.	.893	72	10	20
23	0	5	2	<0.05	<0.05	N.D.	68.6	+	.921	31	11	19
24	21	0	8	<0.05	<0.05	N.D.	22.4	N.D.	.950	44	13	17
25	280	0	24	<0.05	<0.05	N.D.	34.3	+	.845	60	14	16
26	62	0	2	<0.05	<0.05	N.D.	39.9	+	.969	23	11	19
27	276	0	17	<0.05	0.11	N.D.	50.4	+	.873	55	13	17
28	101	45	9	<0.05	0.96	N.D.	28.0	+	.901	85	19	11
29	0	0	0	<0.05	0.36	N.D.	27.3	+	.808	21	10	20
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33	330	0	21	<0.05	0.12	.39	14.7	+	.466	100	27	6
34	>5,000,000	10	380	<0.05	0.19	.038	0.03	+	1.06	89	50	1
35	9	0	0	<0.05	1.31	N.D.	30.8	N.D.	1.05	61	11	19
36	570	0	87	<0.05	0.08	.21	0.01	+	.345	100	40	3
37	200	0	25	<0.05	<0.05	.24	0.01	+	.466	100	38	4
38	520	20	14	<0.05	0.17	.26.	0.01	+	.375	100	41	2

N.D.* = Not detected
RLU[1] = Relative light units
[3] = 10x water sample tested

RIVERS RESEARCH BRANCH
MANUSCRIPT SELF-APPRAISAL

TITLE: Use of Microbial and Toxicant Screening Tests for Priority Site Selection of Degraded areas in water Bodies

AUTHORS: B. J. Dutka, K. Jones, K. K. Kwan, R. McInnis, H. Bailey

DATE:

SELF APPRAISAL (write one from each line)

Intellectual Significance: (Routine) 1 2 (3) 4 5 (Personal Best)

Application* (or potential for application)
(Nil) 1 2 3 (4) 5 (Outstanding Opportunity)

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* Circle '3' if you feel there is potential after further work and or development.

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RRB-87-05

USE OF MICROBIAL AND TOXICANT SCREENING TESTS FOR PRIORITY SITE SELECTION OF DEGRADED AREAS IN WATER BODIES

by

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ABSTRACT

In this study, a new approach has been taken to evaluate Saint John River water and sediment conditions. A battery of biochemical, microbiological and bioassay tests were used to identify degraded or degrading sediments and waters. Data were obtained from waters and sediments at 38 sites within the Saint John River Basin. The data suggested that the following four sites had the highest priority concern: Little River #34, Grand Bay, Saint John River near Boars Head #33, Madawaska River below mill #7 and St. Francois-de-Madawaska, mill stream #2. The data also indicate that microbial population, biochemical or bioassay tests performed independently do not provide realistic estimates of priority concern areas and that the "battery of tests" approach is necessary to provide management with information on which decisions can be made.

MANAGEMENT PERSPECTIVE

The goal of this study is to identify degraded or degrading water bodies so that managers will have a strong data base on which decisions can be made. This information is provided by using the "battery of tests" approach. Another goal of this study is to evaluate a variety of microbiological, biochemical and bioassay tests for their potential of becoming the core group of tests in the "battery of tests" approach. This core group of tests can and will be used nationally to prioritize water bodies and sediments or selected areas within water bodies for remedial action, further investigations or to monitor the effects of remedial actions.

The "battery of tests" approach should make it possible to establish "hot spots" areas of 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, 48-96 hours after collection, or later, 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 enteric 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.

INTRODUCTION

World wide there has been a dramatic increase in industrialization and population concentration over the past three decades. With these increased population and industry centres, both the developed and developing nations face increasing ecological and toxicological problems from the release of domestic wastes and contaminants into the environment. In response to these expanding stresses on the environment and in the belief that there is no single criterion to adequately judge the potential hazard (either to man or the environment) of an effluent or substance, (Draggan and Giddings, 1978), a multitude of biological and biochemical procedures have been and are being developed and used to assess these biological and toxicant impacts (Kohn, 1980; Bringmann and Kuhn, 1980).

Within the last two to three decades there has been an increasing awareness of the multitude of new chemicals being produced and eventually discharged to the environment. There has also been a slower but increasing realization that chemical analysis of all suspected effluents, emissions, waters and sediments is impractical and impossible. Therefore quick, inexpensive, simple screening tests must be developed to prioritize samples, water bodies or sediments for chemical analyses.

Many enzyme, bacterial and algal tests have been developed for the monitoring or screening of toxicant/genotoxciant effects in effluents, waters and sediments (Bitton and Dutka, 1986). The

majority of these tests are rapid, simple, relatively reproducible and inexpensive and require little space and time as compared to fish and Cladocern tests (Bitton and Dutka, 1986a; Dutka and Bitton, 1986). However, little information is available on comparative studies of short-term microbial assays for estimating the impact of toxicants on the aquatic environment. Such studies could give valuable information on reproducibility, sensitivity, cost and rapidity of the various tests.

Also, due to escalating costs and transportation problems, traditional and newer proposed microbiological tests for water and sediments must be re-evaluated. In this re-evaluation process, bias should be given to those tests which are amenable to short-term refrigeration/preservation (48-72 hr), are easy to perform and do not require excessively sophisticated equipment and specialized staff, and are cost effective.

In this paper, we continue to evaluate the suitability of a variety of microbiological, biochemical and toxicant screening tests to become part of a "battery of tests" approach. The final goal of these evaluation studies is to develop a "battery of tests" containing two or three toxicant/genotoxicant screening tests and two or three microbiological hazard screening tests which can be used internationally to designate and prioritize specific water bodies and sediments that are degraded or are being degraded for further investigation or remedial action.

In these Phase III studies, water and sediment from the Saint John River (New Brunswick, Canada) and rivers and lakes within the Saint John River Basin, as well as inshore marine waters influenced by the Saint John River, were used to evaluate the testing procedures.

Data from this Phase III study are presented and results discussed.

METHODS

Sampling Sites

A total of 38 sites were sampled in this study during late October 1986. Twenty-two of the samples were from the Saint John River, four of which were affected by salt water intrusion. Three of the samples were marine samples in Saint John Harbour and are influenced by the Saint John River. Nine samples were from tributaries of the Saint John River and four samples were from lakes within the drainage basin (Table 1, Fig. 1). Most of the Saint John River sites could be considered to be under various anthropogenic influences such as sewage, sewage treatment plant discharges, pulp and paper mill discharges, and food processing plant discharges.

Sample Collection

Sediments were collected with an Ekman dredge or shovel. Usually several drops or shovel loads were required to obtain sufficient

surface layer sediment (2-3 cm). At each site the surface layers were pooled, well mixed and aliquots dispensed for each testing procedure and refrigerated. Prior to performing toxicant screening tests, sediments were extracted with Milli Q water (4 cartridge system - A, Super C carbon cartridge, B, Ion-Ex^{tm}, C, Ion-Ex^{tm}, D, Organet-Q^{r} and E. Milli-Stak^{tm} filter; with a glass distilled water feed) by mixing sediment and Milli Q water in a 1:1 ratio, shaking vigorously by hand for 2 minutes, then centrifuging at 10,000 rpm in a refrigerated centrifuge for 20 minutes. The supernatant was used in toxicity screening tests.

Surface water samples were collected at each site, a 500 ml sample for fecal coliform, fecal streptococci and coliphage tests which were usually processed within 8 hours of collection, and another 500 ml sample which was preserved at 4°C for toxicant screening tests. Samples for toxicant screening tests were tested after being concentrated 10x by flash evaporation at 45°C.

Also at all sites a one litre surface water sample was collected and preserved with 1 ml H_2SO_4 for coprostanol and cholesterol analyses.

Microorganism Tests

Fecal coliform, fecal streptococci, E. coli and coliphage tests were performed as described by Dutka et al. (1986). Clostridium perfringens MPN enumeration techniques were performed as described by Bonde (1963) and Dutka et al. (1986b).

Lactose Fermenting Isolates

A total of 393 isolates were collected and identified from positive A-1 Broth tubes (MPN fecal coliforms in sediments) as well as from typical fecal coliform colonies on MF-mFC plates. Identification procedures included lactose fermentation, oxidase reaction, IMViC Tests, motility, H_2S production, inositol and sorbitol fermentation. Isolates were collected to ascertain the sensitivity of the two techniques for selecting and enumerating E. coli in these waters and sediments.

Biochemical and Toxicity Screening Tests

Coprostanol and cholesterol analyses were performed on water samples and the Microtox tests were performed on water and sediment extracts as detailed by Dutka et al. (1986). Genotoxicity tests on water and sediment 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 extracts (Xu and Dutka, 1987). Spirillum volutans, a large aquatic bacterium with a rotating fascicle of flagella at each pole was also used to test samples for toxicity following procedures described by Dutka and Kwan (1982).

The Algal-ATP toxicant screening test is based on the inhibition of ATP production in cultures of the green algae Selenastrum capricornutum (Blaise et al., 1984). The ATP content of the stressed

Selenastrum capricornutum was measured by the procedure described in the Turner Luminescence Review (1983). The results are reported as a percentage of Relative Light Output (RLU) of the non-stressed controls which is 100%.

Results - Discussion

In Table 1, a brief visual description of the 38 sediments is presented for comparison along with site description and the latitude and longitude of each sampling site. The format used to award points for specific data values in order to rank the waters and sediments from areas of most concern to least concern, is presented in Table 2. The point allocation scheme is biased, and not scientifically defensible, but it reflects the authors' experience with various concentration levels of toxicant activity and health related bacteria in Canadian waters and sediments. The present rating scheme is a viable entity which will change with increased inputs and when greater experience is gained.

Samples with the most points are deemed to contain the greatest potential hazard to man and organisms found in the aquatic ecosystem. High toxicant levels may have reduced microbial levels/activity in some sediment samples; however, cause and effect relationships were not investigated.

Table 3 presents the results of the sediment analyses. Fecal coliform densities were found to vary in the drainage basin from <2

(Glasier Lake #1 to >1,600,000 (Little River #34) per 10 gram wet weight sediment. The fecal coliform data suggest that there is a continuing large input of fecal material into the Saint John River from Babin Brook to Florenceville and also into the Saint John Harbour. Clostridium perfringens levels shown in Table 3 were the highest encountered in this series of studies (Dutka et al., 1986a) with densities varying from a low of 21 (Lac Unique #3) to a high of >160,000 (Madawaska River below paper mill #7). To illustrate the exceedingly high C. perfringens densities found in the Saint John River sediment, a study covering inshore Lake Erie and river and stream mouths entering this lake and the lower Detroit River and upper Niagara River, the maximum C. perfringens density found was 34/10 gram sediment. At some of the sampling sites, which are known recreational areas with mainly summer usage (Environ. New Brunswick, 1977), the data suggest, based on C. perfringens and fecal coliform densities that historical fecal pollution had occurred and has not continued to the same degree e.g. Glasier Lake #1, fecal coliforms <2, C. perfringens 1700; Lac Baker #4, fecal coliforms 8, C. perfringens 2,200; Longs Creek #22, fecal coliforms 13, C. perfringens 13,000; and Mactaquac head pond #23, fecal coliforms 2, C. perfringens 12,000.

A total of 140 positive A-1 broth tubes were subcultured onto MacConkey's agar for isolate identification and confirmation for the presence of fecal coliforms. A total of 36% of the tubes confirmed as having E. coli and 18% as having Klebsiella sp. These fecal coliform

estimates by A-1 broth, produced the lowest E. coli confirmation rate that we have ever encountered (usually 90%+, Dutka et al., 1986). This relatively low E. coli presence in the sediments may be an indication of the variety of the organic pollution reaching the Saint John River, e.g. piggery wastes, pulp and paper mill wastes, light industrial effluents, food processing wastes, and domestic sewage.

In Table 3, it can be seen that only six sites, #11, #13, #25, #26, #27 and #35 were completely negative for any toxicant and/or genotoxicant activity. Also sites #1 and #9 were only positive in the Spirillum volutans tests, an unexpected finding as the S. volutans test was usually found to be the least sensitive of the various toxicant screening tests studied (Dutka and Kwan, 1982; Dutka et al., 1983). Only three sites, #3, #7 and #15 were positive in all the toxicant screening tests, with #15 also showing a slight response in the genotoxicity test. A total of 11 sediment sites were positive for toxicants by the Microtox test, 13 sites by the algal-ATP test, 11 sites by the S. volutans test and 26 sites by the ATP-TOX System. These data suggest ATP-TOX System is the most sensitive screening test for indicating the possible presence of toxicants within the Saint John River basin. However, it must be pointed out that the points used to assess toxicant effects are different for each test, e.g. ATP-TOX System is recorded as a positive when there is only a 1% inhibition of ATP and growth while the Microtox test results are based on the concentration of toxicants that produce a 50% inhibition of light output (EC_{50}). In trying to relate ATP-TOX System positive sites to sites positive by the other tests, it can be seen that there

were nine sites positive by both the Microtox and ATP-TOX System, twelve sites by the algal-ATP and ATP-TOX System, and eight sites by the S. volutans and ATP-TOX System. These findings accentuate the need for the "battery of screening tests" approach to examine and prioritize environmental samples.

The SOS Chromotest kits used on these samples produced very low values and it is suspected from the positive controls that the test organisms were not operating at maximum efficiency. Nevertheless, site #29 sedimemt extract was found to have a substantive positive induction effect with four other sites, #4, #12, #15 and #19, showing weak inducing ability.

Based on the point scheme developed in Table 2 the ten sediments of the greatest potential concern are: 1, Saint John R. at Florenceville #15; 2, Madawaska R. below mill #7; 3, Grand Bay, Saint John R., 1 km from Boars Head #33; 4, Little R. #34; 5, Mill Stream pond, St. Francois-de-Madawaska #2; 6, Saint John R. below Babin Brook #5; 7, Saint John R. at Longs Creek #22; 8, Madawaska R. above mill site #6; 9, Saint John R., near Grand Falls S.T.P. outfall #12; and 10, Saint John R. at Nackwick below mill #21.

Table 4 displays the data obtained from the 38 water samples by the various techniques used. The microbiological data indicate tht the first definite signs of fecal pollution start at site #5, Saint John R. below Babin Brook at Fifth Island. This fecal pollution is shown in both the water and sediment sample. With the exception of sites #25 and #27, and down river from site #19 (Saint John R., .5 km below Pokiok R. mouth) to site #31 (Saint John R. at Westfield ferry)

the Saint John R. appears to be only slightly affected by microbiological pollution. However, within the Saint John River basin, waters at 24 of the 38 sampling sites produced elevated fecal coliform counts and fecal coliform; fecal streptococci ratios of 4:1 and greater. Based on Geldreich's hypothesis (1972) that 4:1 FC:FS ratios are indicative of human fecal pollution, data from these sites strongly suggest that the source of these elevated health related bacterial populations are human fecal material and sewage treatment plant discharges.

The highest water and sediment fecal coliform densities were found at site #34, Little River, with sediment fecal coliform concentrations of >1,600,000 and water column concentrations of >5,000,000 and only ten coliphage. Fecal coliform MF and MPN isolates collected from these samples were found to be 100% Enterobacter sp from the MF plate (11 isolates) and 80% Enterobacter sp and 20% Citrobacter sp from the MPN test (10 tubes).

Coliphage counts from the 38 water sample sites were inconsistent and showed no pattern or relation to fecal coliform concentrations in the water column or sediments. This may be a reflection of the lower E. coli concentrations in these samples as indicated by isolate identification from the A-1 broth MPN, 36% E. coli, and the membrane filter mFC agar, 77% E. coli.

The fecal sterol data provided very little useful information on the fecal pollution load on the waters in the Saint John River Basin. Only sampling site #2, Mill Stream at pond in St. Francois-de-Madawaska produced significant coprostanol and cholesterol

concentrations. These elevated levels were not reflected by the bacteriological data. Only the sediment fecal coliform and C. perfringens data at this site produced a suspicion that fecal pollution is a potential problem. Fecal sterol data continue to be an enigma. From the fecal sterol data obtained in this and earlier studies (Dutka et al., 1986; Dutka et al., 1986b) it would appear that these parameters are not amenable to random, one-time sample collection.

Water samples giving positive responses in all toxicant screening tests were only found at sites #3 and #34. Site #3, Lac Unique, was a suprise as there are no obvious toxicant inputs into the water column, while site #34 which drains a very heavily industrialized area was an expected positive.

The last three sites, #36, #37 and #38 (Saint John Harbour), were positive in all toxicant screening tests except the SOS Chromotest. These sites are also unique in that they produced the highest positive responses in the Microtox, Algal ATP, ATP-TOX System and Spirillum volutans tests. The S. volutans test was positive in both the neat and 10x concentrated samples. As these are marine water sites, the positive test response may have been influenced by the salinity of the freshwater-saltwater mixture found at these sites.

The SOS Chromotest and Microtox test were the least sensitive screening tests with these 10x concentrated water samples. The most sensitive test was the ATP-TOX System. However, if both the ATP-TOX System and Algal-ATP tests were reported as positive at EC_{50}

concentrations, then it would be found that the Microtox and S. volutans tests indicated the greatest number of positive toxic samples, 11 each, while the Algal-ATP and ATP-TOX Systems would suggest that 2 and 6 samples contained toxicants, respectively. The lack of positive responses with the SOS Chromotest appears to be related to batch to batch variations of the testing cells. In this study the positive control results were significantly lower than obtained with previous kit batch numbers.

Using the point scheme developed in Table 2, the ten water samples of the greatest potential concern are: 1, Little R. #34; 2, Saint John Harbour, outside #38,; 3, Saint John Harbour #36; 4, Saint John Harbour at Container Wharf #37; 5, Lac Unique #3; 6, Grand Bay (Saint John R.) near Boars Head #33; 7, Saint John R. at St. Bassle #9; 8, Madawaska R. below mill #7; 9, Mill Stream at St. Francois-de-Madawaska; #2; 10, Grand Bay, Saint John R., 1 km from St. John Marina #32.

Examination of the top ten ranking water sampling sites and top ten sediment sites revealed that there were four common sites which are listed below:

Sediment Sample Rank	Water Sample Rank	Sample Site
4	1	Little R. #34
3	6	Grand Bay, Saint John R. near Boars Head #33
2	8	Madawaska R. below mill #7
5	9	St. Francois-de-Madawaska: mill stream #2

Thus based on the rating scheme (Table 2), developed for ranking the various microbiological, biochemical and toxicant screening test responses to the samples, the four areas of highest concern would be sampling sites #34, #33, #7 and #2.

Use of the "battery of tests" approach in this study reemphasizes that individual bacteriological and toxicant screening tests do not provide a sufficient data base for realistic management decisions to be made on which of the many aquatic environments are of priority concern and require immediate or delayed remedial actions. Also from this study it would appear that the fecal sterol tests are not amenable to a "battery of tests" approach.

Further refinement of the present "battery of tests" will continue with emphasis on acute and chronic tests and techniques to assure the compatibility of reported test results. The eventual goal will be to select a maximum of three toxicant/mutagen screening tests and two microbiological tests as a core group. The ranking scheme will be reviewed after each study to ensure the points allocated to various response levels continues to reflect country wide conditions.

REFERENCES

Bitton, G. and Dutka, B.J. 1986. Introduction and review of microbial and biochemical toxicity screening procedures. in Toxicity Testing Using Microorganisms. Vol. I. eds. G. Bitton and B.J. Dutka. CRC Press, Boca Raton, Florida, pp 1-8.

Bitton, G. and Dutka, B.J. 1986a, Toxicity Testing Using Microorganisms. Vol. I. Bitton and Dutka eds. CRC Press Boca Raton. Florida U.S.A. 163 pp.

Blaise, C., Legault, R., Bermingham, N., von Collie, R. and Vasseur, P. 1984. Microtest mesurant l'inhibition de la croissance des alques (C150) par le dosage de l'ATP. Sciences et Techniques de l'eau 17.

Bonde, G.J. 1963. Bacterial Indicators of water pollution. A study of quantitative estimation. Tuknisk Forlag, Copenhagen.

Bringmann, G. and Kuhn, R. 1980. Comparison of the toxicity thresholds of water pollutants to bacteria, algae and protozoa in the cell multiplication inhibation test. Water Res. 14: 231-238.

Draggan, S. and Giddings, J.M. 1978. Testing toxic substances for protection of the environment. Sci. Total Environ. 9: 63-71.

Dutka, B.J. and Kwan, K. 1982. Application of four bacterial screening procedures to assess changes in the toxicity of chemicals in mixtures. Environ. Pollut. Series A 29: 125-134.

Dutka, B.J., Nyhold, N. and Petersen, J. 1983. Comparison of several microbiological toxicity screening tests. Water Res. 17: 1363-1368.

Dutka, B.J., Jones, K., Xu, H., Kwan, K.K. and McInnis, R. 1986(b). Phase II. Priority Site Selection for degraded areas based on microbial and toxicant screening tests. NWRI Contribution No. 86-174, NWRI, CCIW, Burlington, Ontario, Canada.

Dutka, B.J., Walsh, K., Kwan, K.K., El-Shaarawi, A., Liu, D.L. and Thompson, K. 1986. Priority site selection for degraded areas based on microbial and toxicant screening tests. Water Poll. Res. J. Canada. 21(2): 267-282.

Dutka, B.J. and Bitton, G. 1986. Toxicity Testing Using Microorganisms. Vol. 2. Dutka and Bitton eds. CRC Press Boca Raton, Florida, U.S.A. 202 pp.

Environment New Brunswick. 1977. Saint John River Coliform Survey. New Brunswick Dept. of the Environ. Technical Report Series No. T-7903. Environmental Services Branch, Environment N.B., Moncton, N.B., Canada.

Geldreich, E.E. 1972. Buffalo Lake recreational water quality: A study in bacteriological data interpretion. Wat. Res. 6: 913-924.

Kohn, G.K. 1980. Bioassay as a monitoring tool. Pest. Rev. 76: 99-104.

Luminescens Review 1983. Bulletin No. 204. Turner Designs Mountain View California, U.S.A.

Xu, H. and Dutka, B.J. 1987. ATP-TOX System - A rapid sensitive bacterial toxicity screening system based on the determination of ATP. Toxicity Assessment 2: 149-166.

Xu, H., Dutka, B.J. and Kwan, K.K. 1987. Genotoxicity studies on sediments using a modified SOS Chromotest. Toxicity Assessment 2: 79-88.