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AN ECOTOXICOLOGICAL AND MICROBIOLOGICAL STUDY OF THE YAMASKA RIVER

by

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ABSTRACT

Using Yamaska River waters and sediments, a battery of tests assessing microbial populations and genotoxic and toxic chemicals were used to evaluate spacial and temporal variations. The data resulting from these tests were also used to rank the sampling sites, based on the degree of contained hazards.

The general conclusions of this study are that temporal and spacial factors play a major role in the data collected and in the interpretation of these data. Also from the information collected in this study, it is recommended that a minimum of two or three closely spaced samples, 10-15 m apart should be collected from all river sampling sites and the data pooled to produce a more reliable, homogeneous data base upon which management decisions could be based.

RÉSUMÉ

Des échantillons de sédiments et d'eau provenant de la rivière Yamaska ont été soumis à une batterie de tests visant à évaluer les variations temporelles et spatiales des populations microbiennes et des substances chimiques toxiques et génotoxiques. Les données obtenues ont servi à classer les sites d'échantillonnage, en fonction du degré de risque de contamination.

La présente étude permet de conclure que les facteurs temporels et spatiaux jouent un rôle important sur les données recueillies et leur interprétation. Les auteurs recommandent de plus de prélever au moins deux ou trois échantillons rapprochés (10-15 m de distance) à tous les sites d'échantillonnage lotiques et de rassembler toutes les données, de manière à fournir une base homogène et plus fiable, en vue d'orienter les décisions gestionnelles.

MANAGEMENT PERSPECTIVE

One of the many problems researchers and managers face is to try and interpret data collected from various river studies. There is an underlying awareness of the fragility of most data bases due to a variety of problems outside of those concerning sample processing.

In this study we have attempted, by using the battery of tests approach (14 tests) and the examination of different types of samples such as water, Milli Q, water extracted sediments, and organic solvent extracted sediments, to explore the implication of spacial and temporal sampling programmes.

The results obtained were not unexpected, there are spacial and temporal influences, sometimes so great that it would appear that you are examining samples from different parts of the country. However, data arising from organically extracted sediments were found to be much less influenced by temporal sampling than by spatial variation. Based on the data collected in this study, it is recommended that a minimum of two or three closely spaced samples, not greater than 15 m apart should be collected from all river sampling sites and the data pooled to produce a more reliable, homogeneous data base.

In this study, the first field application of the Mutatox test (genotoxicity) was carried out and it was found to be a very responsive test in all three types of samples. This test shows great promise as laboratory studies indicate it is sensitive to many of the chemicals which trigger the Ames test.

PERSPECTIVE - GESTION

L'un des nombreux problèmes auxquels les chercheurs et les gestionnaires doivent faire face est de tenter d'interpréter les données provenant de diverses études sur les cours d'eau. On s'accorde à reconnaître la fragilité de la plupart des bases de données, causée par divers problèmes qui n'ont aucun rapport avec le traitement des échantillons.

Dans le cadre de cette étude, il s'est agi, à l'aide d'une batterie de 14 tests effectués sur plusieurs types d'échantillons (eau, eau purifiée par Milli Q, sédiments extraits à l'eau et sédiments extraits au solvant organique), d'examiner les conséquences des programmes d'échantillonnage spatial et temporel.

Les résultats obtenus n'étaient pas inattendus; en effet, des influences spatiales et temporelles se font sentir, parfois de manière si importante qu'on a l'impression d'examiner des échantillons qui proviennent d'endroits très éloignés. Les échantillons de sédiments extraits par solvant organique ont cependant montré une sensibilité beaucoup moins grande au facteur temporel qu'au facteur spatial. À la lumière des résultats obtenus, les auteurs recommandent de recueillir au moins deux ou trois échantillons rapprochés (pas plus de 15 m de distance) à tous les sites d'échantillonnage lotiques et de rassembler les données de manière à produire une base homogène et plus fiable.

Au cours de la présente étude, la première application sur le terrain du test Mutatox (génotoxicité) a été réalisée; nous avons obtenu de bons résultats pour tous les types d'échantillons. Ce test est donc très prometteur puisque les études en laboratoire montrent aussi qu'il est sensible à nombre des substances chimiques pour lesquelles le test d'Ames est positif.

YAMASKA RIVER

The Yamaska River has had a long history of water quality problems due to industrial and dairy industry concentrations in its basin. The Yamaska, a tributary of the St. Lawrence is approximately 63 km north-east of Montreal and flows south-east into Quebec's Eastern townships. The basin has been described (Tate, 1972) as Montreal's "recreation-shed" and "milk-shed" and is a major centre of a textile industry, and is also home to a variety of light manufacturing industries. In the early 1970's, the textile industry was the largest employer in the area, and today the industry is still a major employer. Many plants are considered to be technologically older (Tate, 1972) and continue to be major contributors to water pollution in the Yamaska River. Tate (1972) records that the dairy industry is important in the area for supplying local communities and Montreal; however, the industry also contributes a significant amount of bacterial and organic pollution. The large corn growing areas in the flat lands have a major impact in that at least 25% of the pesticides used in Quebec agriculture are used in the Yamaska basin, thus creating another environmental stress. The basin's rivers and creeks are also impacted by a variety of canneries, meat packing plants and light manufacturing industry discharges.

The siting of the various industries and their effluent's impact on the small rivers in the area have produced some interesting statistics. In the major municipalities the pollution loading from industry

is greater than that from the municipal population. The population equivalents for industry range from 1.2% to 767% (St. Damase, Tate, 1972). The location of relatively large textile mills in some of the smaller communities is another factor giving rise to the increase in the population equivalent ratio. Tate (1972) reports that in the town of Acton Vale, one textile mill generated a BOD which was 2.29 times greater than that produced by the municipal population. The conditions found in the Yamaska River basin (pesticides, dyes, industrial effluents, farmland runoff, domestic effluents) appeared to provide an excellent challenge for the battery of microbiological, biochemical and toxicant screening tests which we have been developing for environmental hazard assessment and priority setting (Dutka et al., 1988).

The goal of this research was to develop under diverse conditions a "battery of tests" which could be applied nationally and perhaps internationally to designate water bodies or sediments that are degraded or are being degraded. This battery of tests approach could also be used to assess the extent of the impact of specific discharges. Because of the variety of pollution sources and impacts in the Yamaska River it was decided to investigate the nature and extent of temporal and spacial distribution variability of contaminants as registered by the battery of tests approach in the waters and sediments of the rivers and streams of the Yamaska basin. Since many of the contaminants were organic in nature, it was also decided to evaluate the sensitivity of various sediment extraction procedures as they related to the various toxicant screening tests being used. A report of our findings is detailed in the following text.

STUDY AREA

The original intent of the sampling program was to select sites which were representative of the Yamaska River basin. Unfortunately either due to the inaccessibility of some parts of the rivers and creeks and the unavailability of sediments, the sampling program was skewed by the availability of accessible sediments (Figure 1, Table 1).

SAMPLING SITES

Site 15

This site on the North Yamaska River was upstream of Site 5, Granby and Lac Boivin and samples were collected on the eastern side of the bridge near Ranch Massawippi. The river at this site was fast flowing and varied in depth from 0.3-1 m, and had a rocky and gravel bottom. Sample A was collected approximately 60 m from the bridge and 2-3 m from the S.E. bank. Sample B was collected approximately 45 m from the bridge, and Sample C, approximately 30 m from the bridge. Both samples were collected 3-4 m from the S.E. bank.

Site 5

This sampling site was situated on the north Yamaska River with samples being collected on both sides of the bridge which is on the first side road south of Highway 10 which intersects with Highway 139. At this point the river was slow moving and approximately one meter deep. Sample A was collected approximately 15 m from the bridge and 1 m from the N.E. bank. Sample B was collected 1 m from the S.E. bank and 20 m from the bridge. The river was 15 m wide at this point. Sample C was collected 2 m from the S.W. bank and 20 m from the bridge.

Site 31

At the point where the Barbue River crosses Highway 112, three samples were collected. The river here was very narrow and windy, 3-5 m wide, with sandy banks and bottom with depths varying from 0.2 to 1.2 m. Sampling site C was in the centre of the river approximately 1 m north of the bridge. Sample B and Sample A were also in the centre of the river with B being 10 m downstream of C and A 15 m downstream of B.

Site 30

Samples were collected on the west side of the Yamaska River just upstream of where the Barbue River enters on the east side. The site

was near the junction of Martel Avenue and Highway 233 N, where the banks of the river are fairly high and steep (5-7 m). The river at this point was 40-50 m wide and three samples A, B and C were collected 3-4 m from the west bank in waters varying from 1-1.3 m deep with A being the upstream sample and B 20 m downstream of A, and C 20 m downstream of B.

Site 10

Samples were collected on the east side of the Riviere Noire on both sides of the bridge at the St. Pie exit on Highway 235 N. Sample A was collected upstream of the bridge, approximately 5 m from the bank. Sample B was 20 m downstream of A and 5 m from shore and C was 30 m downstream of B and 7 m from the shore. All sample sites were in approximately 1.2 m of water.

Site 11

These samples were collected from the west bank of the Yamaska River where Highway 233 crosses Decharge des Quinze Sud. The river at this point is approximately 100 m wide, slow moving and deep. Sample C was collected 4 m from the shore in 1.1 m deep water, 10 m downstream of Quinze Sud entrance. Samples B and A were also collected 4-5 m from shore in 0.9-1.1 m deep water, B being 20 m upstream of C and A being 30 m upstream of B.

Site 12

This site was situated near Highway 235 north of St. Hyacinthe on the east side of the Yamaska River between Ruis Rainville and Delome River entrances into the Yamaska. At this site the river is approximately 150 m wide, shallow and has a gravel and rocky bottom with no fine sediment. The river bank was very high (20 m) and steep, and had a stone retaining wall. Sample A was collected 20 m from the shore with B being collected 10 m west of A and C being 10 m west of B. The water varied from 0.2-0.5 m in depth.

Site 13

This single sampling site was situated off the southern tip Saint-Jean Island in the Yamaska River. The single sample was collected in 2.5 m deep water.

Site 14

This single sampling site was located at the entrance of Chenal du Dore into the western side of the Yamaska River near the southern tip of Rouche Is. At the sampling site the river was 1.5 m deep and had a fast current.

SAMPLE COLLECTION

Sediments were collected using a shovel or a Ekman dredge. Frequently, it was necessary to shovel or ekman many times before sufficient surface sediment (1 to 2 cm layer) was collected. At each sampling site the sediments were well mixed, placed into appropriate containers and refrigerated.

Surface water samples (500 mL) were collected at each site (3 per site) for fecal coliform and coliphage tests which were all completed within eight hours of collection. Also at each sampling site one litre of water was collected and preserved at 4°C for toxicant screening tests. Water samples for toxicant screening tests (with the exception of Daphnia and Ceriodaphnia tests) were concentrated 10 times (10X) by flash evaporating at 45°C.

SEDIMENT EXTRACTION AND PROCESSING

Prior to performing any toxicant screening tests, one of the sediment aliquots from each site was homogenized and split into two portions.

One portion of the sediment was sieved for size distribution, following the procedure of Duncan and LaHaie (1979). Basically the sample was sieved at 1/2 or 1/4 PH1 scale intervals (Krumbein and Pettijohn, 1938). The size distribution was determined with SIZDIST, a programme used in conjunction with an IBM PC computer (Sandilands and Duncan, 1980).

The second portion of the sediment (500 g) was extracted with Milli Q water (4 cartridge system, 1 Super C carbon cartridge, 2 Ion-ExTM cartridges, 1 Organex-Q cartridge and a Mill-StakTM filter with a glass distilled water feed), by mixing sediment and Milli-Q water in a 1:1 ratio (e.g., 100 g wet weight sediment:100 mL water), shaking vigorously for three minutes, then centrifuging at 10,000 rpm in a refrigerated centrifuge for 20 minutes. The supernatant was used in toxicity screening tests.

The above water extracted sediment was divided into two equal portions (by weight). One hundred grams of one portion 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 had been prerinsed twice with DCM, and shaken for approximately 24 hr on a Burrel wrist action shaker at position #2. After settling over night, the samples were filtered 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 to 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 testing, containing 250 DCM plus 1.0 mL DMSO evaporated to 1.0 mL DMSO. A method blank was also prepared as a control containing 250 mL DCM plus 1.0 mL DMSO, shaken, filtered, and evaporated as per total sample procedure.

The other portion of the water extracted sediment was processed in 50 g wet wt. aliquots or portion of 50 g in the following manner. Into a stainless steel beaker (250 mL), place 50 g sediment and 100 mL of 1:1 hexane:acetone (v/v) and sonicate for 3 min. After settling for 2 min., transfer the supernatant into a prewashed 5 cm celite column connected to a 1000 mL round bottom flask. Using the same sediment, repeat the previous sonication and extraction procedure. Repeat the above double extraction procedures on each 50 g portion of sediment, collecting the washings in the 1 L round bottom flask, until all the sediment has been processed. Place the 1 L flask in a rotary evaporator until the combined fractions are reduced to 200 mL. Transfer this predominantly hexane residual to a 500 mL separatory funnel to which 100 mL Milli Q water (4 cartridge) is added and shaken for two min. If an emulsion occurs, add 25 mL of a saturated sodium sulphate solution and shake again. Drain the aqueous layer into a 500 mL separatory funnel and extract once with 100 mL dichloromethane and twice with 50 mL methyl chloride which are retained. Recombine the retained methyl chloride washings with the 200 mL hexane and pass through 5 cm of sodium sulphate in an Allihn funnel and collect in a 500 mL round bottom flask. Add 2 mL of DMSO and concentrate the contents of the flask on a roto evaporator to 2 mL DMSO and transfer into a centrifuge tube. Wash the flask with three 1 mL aliquots of DMSO, and add to the centrifuge tube. Adjust the volume of extract, by evaporating with N_2, to 1 mL DMSO for each 100 g wet wt. of sediment extracted.

MICROORGANISM TESTS

Fecal coliform five tube MPN test using A-1 broth and Clostridium perfringens five tube MPN test using DRCM medium with confirmation in litmus milk were used to test each sediment (Dutka et al., 1986). Fecal coliform MF and coliphage tests were performed as described by Dutka (1988a).

TOXICITY SCREENING TESTS

With the exception of the Daphnia magna and Cereodaphnia reticulata tests, water samples for all other tests were concentrated 10X by flash evaporation at 42-45°C using a Buchi Rotovapor EL.

The Microtox test was performed using the luminescent bacterium Photobacterium phosphorium and the procedure detailed in Microtox Operation Manual (1982) with a 15 min. contact time (Dutka and Kwan, 1984). Spirillum volutans, a large bacterium with a rotating fasicle of flagella at each end, was used to test the water and sediment extracts, following a modification of the procedure developed in 1974 by Boudre and Krieg (Dutka and Kwan, 1984). A 24 hr direct agar diffusion toxicity test (spot plate), employing Bacillus cereus as the bacterial lawn and following the procedure of Liu and Kwasniewska (1981) was used to evaluate toxicant presence.

ATP-TOX System, a toxicity screening test based on the inhibition of bacterial growth and luciferase activity, was applied to water and

sediment extracts (Xu and Dutka, 1987). An algal-ATP toxicant screening test based on the inhibition of ATP production by the green alga Selenestrum capricornatum (Blaise et al., 1984) was applied to the samples also. The results are reported as a percentage of Relative Light Units (RLU) output by the tested sample, compared to the nonstressed control which is 100%.

A 48 hr Daphnia magna test, using ten organisms per sample and sample dilution was performed on water and sediment extracts to assess acute toxicant activity (APHA, 1985). The seven day Ceriodaphnia reticulata 3-brood life cycle chronic toxicity test using four cladocerans per sample or dilution was used to test water and sediment extracts (Rao, 1988).

Toxi-Chromotest rapid bacterial colorimetric assay based on the ability of toxicants to inhibit the de novo synthesis of an inducible enzyme-beta galactosidase- in an E. coli mutant was used to test water and sediment extracts (Orgenics, 1985).

A new unproven test, the Mutatox test based on the use of a dark mutant strain of Photobacterium phosphorium M169 to screen for genotoxic agents was evaluated in this study. This test will pack up chemicals which are (a) DNA damaging agents, (b) DNA intercalating agents, (c) direct mutagens which either cause base substitution or are frame shift agents, and (d) DNA synthesis inhibitors. These chemicals will restore the light emitting stage of the strain and can be measured in a modified Beckman Microtox Model 2055 analyzer. The test procedures are similar to those followed in the Microtox test

with incubation of M169 cells, cell media and sample being carried out at 20-24°C for 24 hr. Light level is read after 20 hrs contact and compared to negative and positive controls.

Two nonmicrobial toxicant screening tests were evaluated during this study, one was the seed germination and root elongation test using Prizehead leaf lettuce seeds and the other was the 14-day earthworm test using the redworm (Eisenia spp) (Dutka, 1988).

Ranking Scheme

A slightly revised format from that previously used (Dutka, 1988) to award points for specific data values in order to rank the sampled waters and sediments from those of most concern to least, is presented in Table 2. The revision concerns a modification of the point awarding scheme for Clostridium perfringens densities. In Table 2A, three tests new to our battery of tests approach are presented with their tentative point awards based on the degree of positiveness. The point allocations are biased, especially towards tests indicating genotoxic/mutagenic effects and contaminants which produce chronic toxicity effects. Notwithstanding the previous statement, the point allocations do reflect the authors' evolving experiences with data accumulated from the Canada-wide application of the battery of tests approach (Dutka, 1988).

RESULTS

Water Samples

Due to the number of tests applied, and the number of samples within sites and the seasonal sample collection format, the results obtained are discussed in general terms.

Tables 3A, B and C summarize the points awarded the various microbiological and genotoxic/toxic results obtained from the water samples tested. These results are in most instances the mean of duplicate tests.

Several tests proved to be either insensitive to the concentration of contaminants in the sample or they were not effected by the specific concentration of chemicals found in these 1X and 10X water samples. Specifically the Spirillum volutans, spot plate and Ceriodaphnia reticulata tests were all negative.

The Microtox test was negative with the August and November samples, and indicated the presence of triggering toxicants in only 3 of the June samples (3/21). A similar pattern of results were observed with the Toxi-Chromotest and Algal-ATP tests. The Toxi-Chromotest test was negative in all the June and November samples while the Algal-ATP test was negative in all the June and August samples, but surprisingly, indicated the presence of a low grade toxicity in every sample at each site during November, when the river was in flood.

The Mutatox test was accepted as positive if the number of revertants from the dark phase to the light phase was at least 3 times the negative control. In the positive water samples there were 3 to 11X the control rate of revertants and in sample 12C June, the number of revertants in replicate tests were 6X and 77X. This sole result, 77X the control rate, may have been a laboratory error or a sample varient.

The implication of these seasonal type responses observed with several of the screening tests are that toxicant presence may be related to seasonal events or practices. The two toxicant screening tests which appeared to be the most sensitive or responsive to the contaminant mix found in the Yamaska River basin were the ATP-TOX system and Daphnia magna test. The ATP-TOX System was positive in every sample with ATP inhibition varying from 5% to 60%. In one sample, 5B Nov, one of the replicated tests indicated 100% inhibition while the other indicated 45% inhibition.

The Daphnia magna results showed low grade toxicity in all the samples with November samples having the lowest toxicant concentrations. The greatest toxicant values as measured by the Daphnia test was found at site 11A in June where an EC_{50} value was obtained with natural water diluted to 70%.

Microbial pollution, with the exception of Site 15, was very high with definite seasonal effects. For instance, Site 12 in June had fecal coliform counts ranging between 23-190/100 mL and coliphage plague counts (PFU) ranging from 45 to 85/100 mL. Then in August, the

fecal coliform range was 310-470 and the coliphage 85-190 per 100 mL. In November, the fecal coliform count range was 130,000-240,000 and the coliphage range was 1400-2800 PFU/100 mL. Similar patterns were observed at all the other sites and usually the November samples had the greatest microbial populations and June the least.

Some of the highest coliphage counts encountered in our Canadian studies were seen at Site 31 during August and November with PFU/100 mL ranging from 2500-5200. The implications of these exceedingly high values are that (a) a sewage source is nearby and (b) there is a good probability that human enteric viruses will also be found (Grabow, 1968; Havelaar, 1986; Petrovicova et al., 1988). Several researchers have also indicated that the finding of coliphage in water should trigger studies for the presence of human enteric viruses (Petrovicova et al., 1988; Simkova and Cervenka, 1981).

These water sampling sites can be ranked using Table 2 and 2A and all the seasonal data from those with the greatest potential hazards to the least in at least two different ways. One approach is to rank the sites based on the averaged accumulated points from all tests. Following this procedure, Site 31 would contain the greatest potential hazards followed by Site 14, 12, 30, 10, 11, 13, 5, and finally, Site 15. Another method of ranking the sites is based on the total accumulated points for only the toxicant/genotoxicant screening tests. In this scheme Site 14 has been designated as having the greatest potential hazard load, followed by Sites 13, 31, 12, 10, 30, 15, 11, and Site 5.

An interesting pattern of contrary observations were seen during this study. It was noted that the 3 samples collected at the same site could show great variability in data, e.g., Site 11 August, fecal coliform counts 950-4450/100 mL range with an even greater variability between replicates 700-5500/100 mL and also show great stability in data, e.g., ATP-TOX System values of 31%, 33% and 35%. The data from almost any site at any season when carefully examined would show these contrary observations between one or more tests.

However, in general, the findings are supportive of the view that in flowing water there are at times great spacial variability in microbial populations and contaminant concentrations and seasonal variability is as great or greater than spacial variability. An excellent example of seasonal variability is the finding that all samples collected in November produced a positive Algal-Tox test while being completely negative in June and August.

Milli-Q Water-Extracted Sediments

Heavy rains produced flood conditions in the Yamaska River basin in November and as a result sediments were not able to be collected from sites 30, 10 and 11. Site 12 had no sediment, only rocks and gravel.

One of the first features that becomes apparent when examining the Milli-Q water extracted sediment data in Tables 4A, B and C, is the great variability and contrariness of the data. The same

sampling station, e.g., 5C, can vary from that with the highest concerns (greatest point score) to one with the lowest while other sampling sites e.g., 30, are very consistent in their responses to the various tests.

Fecal coliforms and Clostridium perfringens showed the greatest variations amongst all the tests of the battery of tests. At some sites there was a 100+ fold difference between stations and replicates, e.g., Site 5 in June had a fecal coliform range of 13-520/100 mL at stations 5A, 5B and 5C, while the range for Clostridium perfringens was 4600-28000/10 g. In November, Site 5 had a fecal coliform count ranging between 4-620/100 mL and a Clostridium perfringens range of 2200-92000/10 g. Similar types of variability were also seen in some of the toxicant screening tests, e.g., 5A Microtox test 1.5 points, 5B Microtox test 0 points and 5C Microtox test 7 points (a very toxic sample according to this test).

Only 2 sites, 15 and 5 were found to contain chemicals which produced a positive response in the chronic toxicity test, Ceriodaphnia reticulata, 5C in June, 15A, B and C in August and 15A in November.

The Mutatox Test for genotoxicity was found to be positive only in samples collected in August at Sampling Sites 15A, 10C and 11A, B and C. Based on these battery of tests results, stations 10C and 11A, B and C are believed to contain very low concentrations of other types of toxicants possibly not found at the other stations,

suggesting that a very specific type of contaminant is contaminating these samples. If so, the distribution pattern of this contaminant is so variable that two other samples 10A and B collected within 15 m of the positive 10C were negative.

Another anomaly in the distribution pattern of positive toxic responses is the absence of any positive Algal-ATP tests in August, then in June, 4 out of 18 samples were positive and in November 8 out of 8 samples tested were positive. The only toxicant screening tests which did not appear to have a seasonal effect were the Daphnia magna and ATP-TOX system toxicant screening tests.

Acknowledging that there is at times great spacial and seasonal variability, the sampling sites were ranked on their averaged accumulated points with the goal of ascertaining which sites contain the greatest amount of hazards/concerns (bacteriological, toxicants, genotoxicants or chronic toxicity) and which sites contain the least. Site 5 was considered to be the site with the greatest potential hazards, followed in order by 15, 11, 10, 31, 30, 13 and 14. However, if the microbiological data is excluded from the ranking process then the sites with greatest concern to least are 15, 11, 5, 10, 31, 13, 30, and 14.

Organically Extracted Sediments

Two methods of extracting sediments using organic solvent procedures were to be evaluated in this study. Our routine procedure

using DCM and 100% DMSO (with a 1% DMSO sample being tested for toxicant activity) was applied to all sediments with no problems. However, when we tried the hexane:acetone procedure, we found that the solvent controls, i.e., all the procedures and chemicals used in extracting the sediment without the presence of sediment, were positive in the majority of the toxicant screening tests used. Therefore, the data from the hexane: acetone extraction procedure were not considered here.

Examination of Table 5A, B and C, reveals several interesting features. The Daphnia magna test was positive in every sample tested, with most of the positives having a point score rating of 7 (Table 2), and samples collected during June showed the greatest variability with point ranking varying from 4 to 8. The Spirillum volutans and spot plate tests were consistently negative with the exception of one sample (water-extracted sediment 5C June, Spirillum volutans positive). These two tests were negative in all the samples tested.

Both the Microtox and ATP-TOX system tests gave positive results in all samples, and also produced a curious reversion of point ranking. Usually in any series of samples (Tables 3 and 4, Dutka et al., 1988), the ATP-TOX System produces a higher point ranking than the Microtox test, thus indicating a greater response to the toxicants. In these samples with the exception of Sample 30B June, all the samples produced a higher Microtox point ranking than observed for ATP-TOX System test results. It is suspected these results indicate that the Microtox system is responding to a chemical or group

of chemicals different from those triggering the ATP-TOX System, or are additive to the ATP-TOX System triggering chemicals. Sample 15B, August, produced one of the highest Microtox point values we have observed in our cross Canada studies (Dutka et al., 1987, 1988). This sample as part of the 3 samples collected at site 15 also illustrated the great variability of results obtained from samples collected 15-20 metres apart e.g., 15A - 6 points, 15B - 10 points (maximum) and 15C - 3 points.

Based on the pattern of results obtained, the Toxi-Chromotest appears to respond at a lower level to the contaminants in the samples and at best may provide supportive evidence for the other screening tests. This test did not seem to recognize the presence of toxicants different from those already indicating their presence by the other tests. Similar data patterns were observed with the water and water extracted sediment samples.

Due to the time consuming nature of the Ceriodaphnia reticulata chronic toxicity test, this test was only applied to specific June samples 15A, 5A, 31A, 30A, B, C, 10A, and 11A, and all these samples were positive. Two of the samples 30A and 11A, gave positive responses in 0.01% of the 1% DMSO sample. These preliminary applications of this test for chronic effects suggest that the whole upper river basin is contaminated with chemicals capable of chronic damage in target organisms.

The Mutatox test which screens for the presence of chemicals with genotoxic activity displayed excellent examples of spacial and

seasonal activity. All eight sites tested produced a positive response in at least one of the three seasons and in at least one of the three samples collected at each site.

In trying to establish a hierarchy of "worst" site (most points) to least contaminated site (least points), three approaches were tried. In the first approach we evaluated each sample and disregarded the fact that the Ceriodaphnia test was only performed on a few samples, and the following ranking of the top seven samples was developed. The ranking is from greatest potential hazard to least:

1. Sample 30A, June
2. Sample 11A, June
3. Sample 5C, August
4. Sample 13, August
5. Sample 11A, August
6. Sample 11B, August
7. Sample 10A, August

However, if the Ceriodaphnia test was removed from the system, then the top seven sites with the greatest potential hazards were:

1. 5C, August
2. 13, August
3. 11B, August
4. 11A, August
5. 10A, August
6. 15A, November
7. 5B, August

Since each sample is part of a site's total data, we averaged all the sample data from each site, thus blending seasonal effects and we obtain the following very interesting and supportive site ranking:

1. #13, site with greatest potential contained hazards
2. #14
3. #11
4. #5
5. #10
6. #30
7. #31
8. #15, site with least contained chemical hazards

By examination of Figure 1, the implications of these ranking results can be readily observed. Site 13 near the mouth of the Yamaska River is the repository of all upstream contaminants especially those with low or slow biodegradability, e.g., those soluble in organic solvents. Site 14 which should be similarly impacted as site 13, is also impacted by the Chenal du Dore and the faster water in this area probably dilutes and moves the finer sediments into the St. Lawrence River.

Site 11 is downstream of all the other sites, and is a potential repository of slow or nonbiodegradable contaminants from the upstream sites. Site 5 is downstream of Granby and Site 15 and these findings suggest that this area is a major source of organic contaminants which

produce toxic, genotoxic and chronic toxicity responses in test species. Site 10 reflects the contaminants from the St. Pie area and the whole Riviere Noire watershed. The ranking of this site may be indicative that the organic contaminants from this area of the province are similar to those arising in the North branch of the Yamaska River.

Site 30 is downstream of Site 31, 5 and 15, and is indicative of the downstream dilution effects of contaminants passing through Site 5 and Site 15 by the Centre Branch Yamaska, South East Branch Yamaska, South West Branch Yamaska and partially by the Barbue River in which Site 31 is found.

The site with the least contained organic hazards by this scheme is Site 15, which is in the middle of a woods, presenting a typical flowing, bubbling stream scene. However, the very sparse sediments from this site were found to yield chemicals with toxicant, genotoxicant and chronic toxicity effects. Similar findings were also found in the water and water extracted sediments. The responses to the battery of tests at this site were surprising as we had expected this would be our negative control site. The overall implications of these data are that the whole Yamaska River basin has been heavily impacted by chemicals of varying activities, e.g., genotoxic, toxic and those producing chronic effects.

Special Tests

Two additional exploratory toxicant screening tests were evaluated on Yamaska River samples.

One test was the 14 day earthworm test which was performed only on sediments from June stations 5A, 5B and 5C, and on 10X water sample concentrations on the same samples.

The results obtained indicated these samples were not lethal to the earthworms after 14 days of contact (Table 6).

The other exploratory test evaluated was seed germination and root length growth. Prizehead leaf lettuce seeds were tested with Milli Q water sediment extracts of the June samples. From the data obtained in this study (Table 7), it would appear that four samples 5C, 15B, 30C and 31C had definite seed germination inhibition effects. Also, it can be seen that samples 5C, 11B, 15A, and 11C produced inhibition of root length growth. An interesting observation of these data is that ability to sprout and ability to produce normal sized roots are not related, e.g., Samples 15A and 15B. It should be noted in Table 8 that the three sites with the greatest potential hazards from chemical contaminants based on Milli Q water extracted sediments were sites 15, 11 and 5.

GENERAL CONCLUSIONS AND OBSERVATIONS

1. The ranking of the various sites are summarized and presented in Table 8, in a format which shows the impact of

bacteriological pollution (both fecal and organic loading) on the site's rank. The sites are listed in decreasing potential hazard content (chemical and microbiological).

2. In the surface water site ranking, site 11 based on its situation in the site distribution pattern (Figure 1) clearly suggests a partial restoration of water quality at this point, possibly by sedimentation as shown by columns 3, 4 and 5 of Table 8.
3. The Milli-Q water extracted sediment data suggest that the upstream sites 15 and 5 are settling/deposition areas for water soluble toxicants and bacteria of fecal origin. The data also indicate that the two furthest downstream sites' (13 and 14) sediments were the least contaminated with fecal bacteria and water soluble contaminants.
4. The organically extracted sediments data from downstream sites 13 and 14, strongly suggest that these sites are one of the final repository areas for the Basin's non water soluble and slow biodegrading contaminants. These data also indicate that the upstream sampling sites are less polluted with non-water soluble and slow biodegrading contaminants than the downstream sites, e.g., 15, 30, 10.
5. Data obtained from water and water extracted sediments are indicative of temporal influences which may be moderated or exacerbated by hydrological events or local disposal practices.

6. Data obtained through the testing of organic extractions of sediments tended to show minimal seasonality. If seasonality was exhibited, it is believed this is in response to a result of major storm events.
7. Variations between sample data at the same site were no greater than replicate variations in the tests. This spacial variation, at times, could be so great that there appeared to be no relationship between samples collected 15-20 metres apart. Conversely at other sites and at different seasons, there was no apparent difference in results obtained from samples at the same site.
8. Based on the data collected during this study, it is recommended that a minimum of two or three closely spaced samples, 10-15 m apart should be collected from all river sampling sites and the data pooled to produce a more reliable, homogeneous data base for any studied river area.
9. Due to problems in the production of the SOS chromotest kit, the production of this kit for North American delivery has been temporarily suspended. As a result, comparisons between Mutatox and SOS chromotest were not performed.
10. The Mutatox test for genotoxicants, in the first-ever field evaluation of this procedure, was found to be a very responsive test in all three types of samples, water, Milli-Q water extracted sediments and organically extracted sediments.

11. The Mutatox test showed that chemicals with genotoxic activity are distributed throughout the studied area of the Yamaska River Basin, and that there appears to be accumulating deposition of these chemicals in sediments the further downstream one tests.

12. A comparison of Microtox and Toxi-Chromotest results indicated that the Microtox test is more sensitive or more chemicals trigger a response in the Microtox test. It was believed prior to this study that the Toxi-Chromotest would either complement and expand the toxicant sensing range or confirm Microtox test results, and it did neither.

13. The Spirillum volutans and spot plate tests were not responsive to the chemicals or chemical concentrations found in Yamaska River water and sediments.

14. The Ceriodaphnia reticulata test for chronic toxicity effects was not triggered by the concentration of contaminants found in the water column and only rarely by those removed from the sediments by Milli-Q water extraction. However, this test was very responsive and sensitive to chemicals found in the organic extract of the sediments. The results indicated that chemicals removable from sediments by using organic solvents have the capability of producing chronic toxic effects and these chemicals were distributed throughout the Yamaska River Basin.

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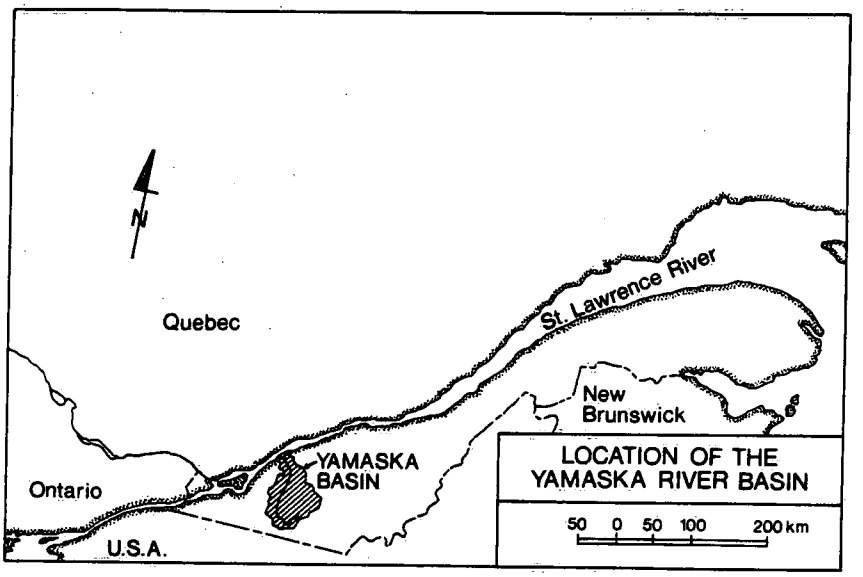
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LOCATION OF THE YAMASKA RIVER BASIN
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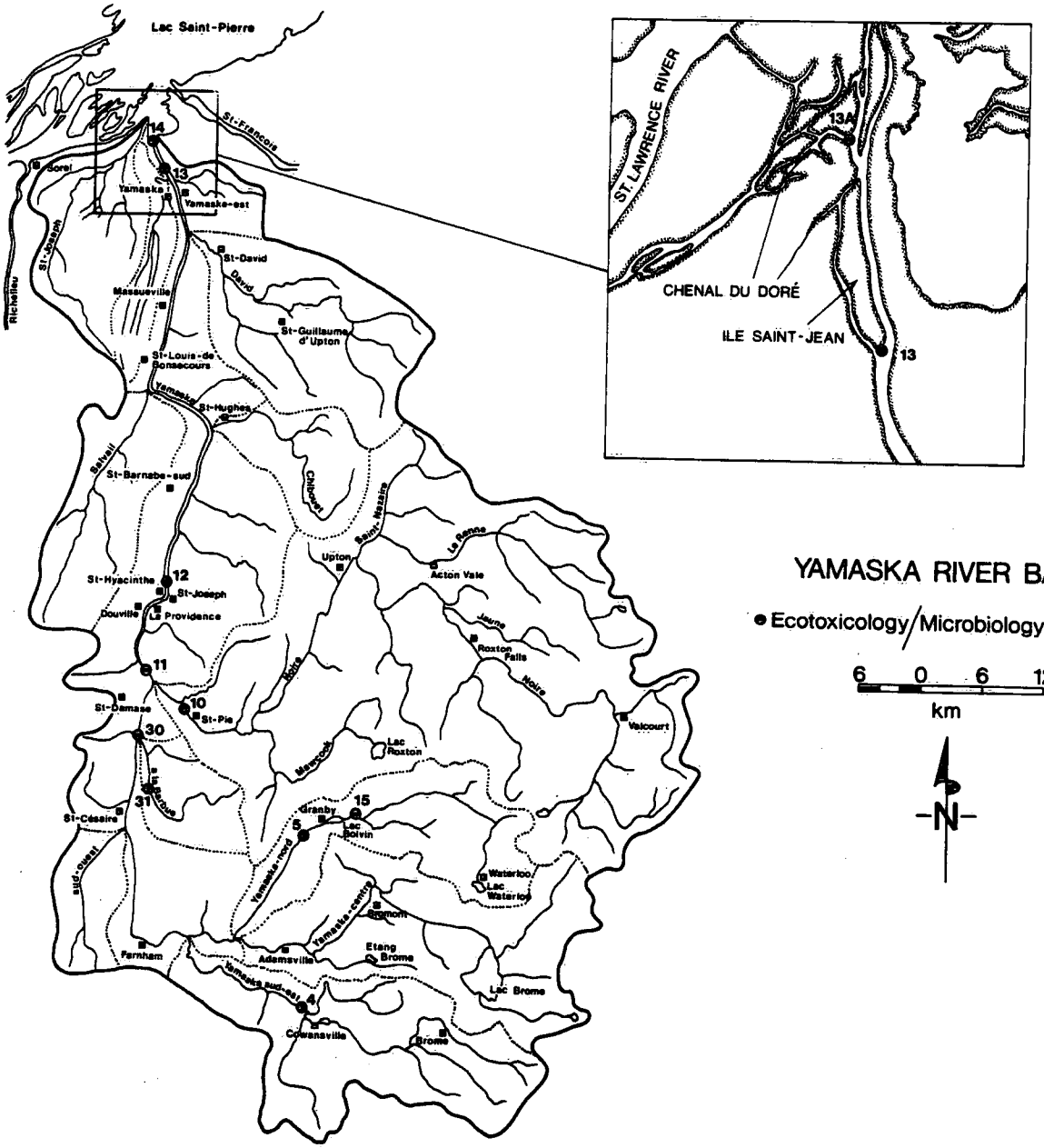
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St-David
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Noire
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St-Louis-de Bonsecours
Yamaska
St-Hughes
Salvail
St-Barnabé-sud
Chibouet
Saint-Nazaire
Upton
La Gance
Acton Vale
St-Hyacinthe
St-Joseph
Douville
La Providence
Jaune
Roxton Falls
Noire
Bois
St-Damase
St-Pie
Valcourt
Lac Roxton
Mascouche
St-Césaire
Granby
Lac Bolvin
Waterloo
Lac Waterloo
Sud-Ouest
Yamaska-nord
Yamaska-centre
Bromont
Farnham
Ademsville
Etang Brome
Yamaska sud-est
Lac Brome
Cowansville
Brome
ST. LAWRENCE RIVER
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Table 1. Site Location, Sediment Description and Classification

Site	Latitude and Longitude		Description and Shepherd Classification
5	45°20"35'	72°48"07'	Sand 20.34%, silt 64.72% clay 14.94%, SANDY SILT
10	45°29"51'	72°54"29'	gravel 2.04%, sand 88.38% silt + clay 9.58%, SAND
11	45°34"43'	72°58"59'	gravel 2.53%, sand 93.68% silt + clay 3.79%, SAND
12	45°11"33'	72°55"31'	NO SEDIMENT FOUND
13	46°03"36'	72°55"30'	sand 84.4%, silt 11.54% clay 4.01%, SAND
14	46°05"29'	72°56"58'	sediment no classified
15	45°25"00'	72°37"12'	gravel 6.62%, sand 82.88% silt + clay 10.5%, SAND
30	45°28"45'	72°58"48'	sand 4.78%, silt 21.15% clay 74.07%, SILTY CLAY
31	45°24"35'	72°56"08'	sand 98.71, silt + clay 1.39% SAND

Table 2. Point Awarding Scheme for Sampling 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 - 50	1	<1.0	1.0 - 1.92	100 - 50	1	<2.0	1
101 - 500	25 - 100	51 - 250	2	1 - 3.0	1.30 - 1.50	49 - 20	3	2.1 - 4.0	2
501 - 2500	101 - 250	251 - 1000	3	3.1 - 5.0	1.51 - 2.0	19 - 1.0	5	4.1 - 6.0	3
2501 - 16000	251 - 1000	1001 - 5000	4	5.1 - 7.0	2.1 - 3.0	0.9 - 0.1	7	6.1 - 8.0	4
16001 - 160000	1001 - 5000	5001 - 20000	7	7.1+	3.1+	0.09	10	8.1+	5
160000+	5001+	20001+	10						

ATP-TOX System % Inhibition per mL 10X Water Sample 1:1 Milli-Q Water Sediment Extract 1% DMSO Sediment Extract	Microtox EC_{50} % per mL 10X Water Sample 1:1 Milli-Q Water Sediment Extract 1% DMSO Sediment Extract	Points	*Daphnia magna* EC % per mL 1:1 Milli-Q Water Sediment Extract 1% DMSO Sediment Extract	Points	*Daphnia magna* EC % per mL Water Sample	Points	*Spirillum volutans* 10X Water Sample 1:1 Milli-Q Water Sediment Extract 1% DMSO Sediment Extract	Points
1 - 30	40.0+	1	EC_{20} at 100%	1	EC_{20} at 100%	1	negative	0
31 - 60	40.0 - 25.0	3	EC_{40} at 100%	2	EC_{40} at 100%	2	positive	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	Nature water sample	
100	0.9	10	at 50%	6	at 50%	8	negative	0
			at 25%	8	at 25%	10	positive	10
			at 10%	10				

Table 2A. Supplementary Point Awarding Scheme for Sampling Ranking Based on Suspected Contained Hazards.

Ceriodaphnia reticulata % of sample or sediment-extract able to produce reproduction inhibition	Points	Toxi-Chromotest % inhibition color production 10X water, 1:1 Milli Q water, sediment extract 1% DMSO Sediment Extract	Points
100%	2		
50%	3	0.1 - 10%	1
10%	5	11 - 25%	3
1%	10	26 - 50%	5
0.1%	15	51 - 75%	7
0.01%	20	76 - 100%	10

Mutatox Genotoxicity measured by production of light by revertants, 3X control 10X water sample, 1:1 Milli Q Water Sediment extract. 1% DMSO Sediment Extract	Points	Mutatox Revertants 3X Control 10X Water Sample 1:1 Milli Q Water Sediment Extract 1% DMSO Sediment Extract	Points
- (less than 3X control revertants)	0	3 - 6	5
		7 - 14	7
+ (more than 3X control revertants)	10	15 - 25	10
		26 - 49	15
		50 - 5000	20

Table 3A. Summary of Yamaska River Water Data Based on Points Allocated for Specific Values (Table 2). June Data.

Site and Sample	Fecal* Coliforms	Coliphage*	Daphnia magna*	Spirillum volutans*	Spot Plate[1]*	Toxi-Chromotest*	Microtox*	ATP-TOX* System	Algal* ATP	Ceriodaphnia reticulata	Mutatox* >3X revertants	Mutatox[2]* X times control level	Total Points Station	Site
15A	1	0	1	0	0	0	0.5	1	0	0	10	5X	13	
15B	1	0	0.5	0	0	0	0	1	0	0	10	4.5X	12.5	
15C	1	0	0.5	0	0	0	0.5	1	0	0	5	3X	8	33.5
5A	3	4	1.5	0	0	0	0	3	0	0	0	0	11.5	
5B	3	3	0.5	0	0	0	0	3	0	0	0	0	9.5	
5C	3	3	1.5	0	0	0	0	3	0	0	0	0	11.5	32.5
31A	3	3	0.5	0	0	0	0	5	0	0	10	4.5X	21.5	
31B	3	4	1	0	0	0	0	5	0	0	10	5.5X	23	
31C	3	4	0.5	0	0	0	0	3	0	0	10	4.5X	20.5	65
30A	3	0	0	0	0	0	0	3	0	0	0	0	6	
30B	3	1	0.5	0	0	0	0	3	0	0	5	3X	12.5	
30C	3	0	1	0	0	0	0	3	0	0	10	4X	17	35.5
10A	2	0	0.5	0	0	0	0	3	0	0	10	5X	15.5	
10B	2	0	1	0	0	0	0	3	0	0	10	5.5X	16	
10C	2	0	0.5	0	0	0	0	3	0	0	10	6X	15.5	47
11A	2	3	3	0	0	0	2.5	5	0	0	10	6X	25.5	
11B	2	3	1	0	0	0	0	3	0	0	5	3.5X	14	
11C	2	4	0.5	0	0	0	0	3	0	0	0	0	9.5	49
12A	2	2	1.5	0	0	0	0	3	0	0	5	3X	13.5	
12B	2	2	0.5	0	0	0	0	3	0	0	10	4X	17.5	
12C	1	2	0.5	0	0	0	0	3	0	0	10	42X	16.5	47

[1]point value not established
[2]not included in point value or ranking
*mean of 2 values

Table 3B. Summary of Yamaska River Water Data Based on Points Allocated for Specific Values (Table 2). August Data.

Site and Sample	Fecal* Coliforms	Coliphage*	Daphnia magna*	Spirillum volutans*	Spot Plate[1]*	Toxi-Chromotest*	Microtox*	ATP-TOX* System	Algal* ATP	Ceriodaphnia reticulata	Mutatox* >3X revertants	Mutatox[2]* X times control level	Total Points Station	Site
15A	2	0	1.5	0	0	0.5	0	1	0	0	5	3X	10	
15B	1	0	1.5	0	0	0.5	0	1	0	0	5	3.5X	9	
15C	1	0	2	0	0	0.5	0	3	0	0	10	4.5X	16.5	35.5
5A	3	4	0.5	0	0	0	0	3	0	0	0		10.5	
5B	3	4	0.5	0	0	0.5	0	3	0	0	5	3X	16	
5C	3	4	0	0	0	0.5	0	1	0	0	10	7X	18.5	45
31A	10	7	0.5	0	0	0	0	3	0	0	5	3.5X	25.5	
31B	10	7	0	0	0	0	0	1	0	0	10	10X	28	
31C	7	7	1.5	0	0	0	0	1	0	0	10	6.5X	26.5	80
30A	4	1	0.5	0	0	0.5	0	3	0	0	10	5.5X	19	
30B	4	1	0.5	0	0	0	0	3	0	0	10	6.5X	18.5	
30C	4	1	0.5	0	0	0	0	3	0	0	5	3X	13.5	51
10A	2	4	1	0	0	1	0	3	0	0	10	11X	21	
10B	3	7	1	0	0	0	0	3	0	0	0	0	14	
10C	3	7	1.5	0	0	1	0	3	0	0	10	9X	24.5	59.5
11A	3	2	0.5	0	0	0	0	1	0	0	10	6.5X	16.5	
11B	4	2	1	0	0	0	0	1	0	0	5	3X	13	
11C	3	1	1	0	0	0	0	1	0	0	0	0	6	35.5
12A	2	2	1	0	0	0	0	3	0	0	10	4.5X	18	
12B	2	3	0.5	0	0	0.5	0	3	0	0	10	8	19	
12C	2	3	1	0	0	0.5	0	3	0	0	10	7.5X	19.5	56.5
13	1	0	0	0	0	0.5	0	3	0	0	10	5X	14.5	
14	2	1	5	0	0	0	0	3	0	0	10	5X	21	

[1]point value not established
[2]not included in point value or ranking
*mean of 2 values

Table 3C. Summary of Yamaska River Water Data Based on Points Allocated for Specific Values (Table 2). November Data.

Site and Sample	Fecal* Coliforms	Coliphage*	Daphnia magna*	Spirillum volutans*	Spot Plate[1]*	Toxi-Chromotest*	Microtox*	ATP-TOX* System	Algal* ATP	Ceriodaphnia reticulata	Mutatox* >3X revertants	Mutatox[2]* X times control level	Total Points Station	Total Points Site
15A	1	1	2	0	0	0	0	1	1	0	0		6	
15B	1	1	1.5	0	0	0	0	1	1	0	0		5.5	
15C	1	1	0	0	0	0	0	2	1	0	0		5	16.5
5A	3	2	0	0	0	0	0	3	0.5	0	0		8.5	
5B	3	2	0	0	0	0	0	6.5	1	0	0		12.5	
5C	3	3	0	0	0	0	0	3	1	0	0		10	31
31A	7	7	0	0	0	0	0	5	1	0	0		20	
31B	7	7	1	0	0	0	0	5	1	0	0		21	
31C	7	10	0	0	0	0	0	5	1	0	0		23	64
30A	7	7	0	0	0	0	0	3	1	0	0		18	
30B	7	7	1	0	0	0	0	3	1	0	0		19	
30C	7	7	0	0	0	0	0	3	1	0	0		18	55
10A	3	3	0	0	0	0	0	3	1	0	0		10	
10B	4	2	0	0	0	0	0	3	1	0	0		10	
10C	4	3	0	0	0	0	0	4	2	0	0		13	33
11A	7	4	0	0	0	0	0	3	1	0	0		15	
11B	7	4	0	0	0	0	0	2	1	0	0		14	
11C	7	4	0	0	0	0	0	3	2	0	0		16	45
12A	7	7	0	0	0	0	0	3	1	0	0		18	
12B	10	7	0	0	0	0	0	3	1	0	0		21	
12C	10	7	0	0	0	0	0	3	1	0	0		21	60

[1]point value not established
[2]not included in point value or ranking
*mean of 2 values

Table 4A. Summary of Data from Milli Q Water Extracted Yamaska River Basin Sediments, Based on Points Allocated for Specific Values (Table 2). June Data.

Site and Sample	Fecal* Coliforms	Clostridium*	Daphnia magna*	Spirillum volutans*	Spot Plate[1]*	Toxi-Chromotest*	Microtox*	ATP-TOX* System	Algal* ATP	Ceriodaphnia reticulata	Mutatox* >3X revertants	Mutatox[2]* X times control level	Total Points Station	Site
15A	1	3	1	0	0	0	0	0	3	0	0		8	
15B	1	4	1.5	0	0	0	0	0.5	4	0	0		11	
15C	1	4	2	0	0	0	0	0.5	1.5	0	0		9	28
5A	1	7	1.5	0	0	1.5	1.5	2	0	0	0		14.5	
5B	2	10	1.5	0	0	2	0	1	0	0	0		16.6	
5C	3	7	0	5	0	0.5	7	4	0	5	0		31.5	62.5
31A	1	3	0.5	0	0	0	0	1	0	0	0		5.5	
31B	1	4	0.5	0	0	0	0	1	1.5	0	0		8	
31C	2	3	0.5	0	0	0	0	0.5	0	0	0		6	19.5
30A	2	3	0.5	0	0	0	0	1	0	0	0		6.5	
30B	3	3	0	0	0	0	0	0.5	0	0	0		6.5	
30C	2	3	0.5	0	0	0	0	1	0	0	0		6.5	19.5
10A	1	4	4	0	0	1	0	0.5	0	0	0		9.5	
10B	1	7	1	0	0	0	0	0	0	0	0		9	
10C	1	4	4	0	0	1	0	0.5	0	0	0		9.5	28
11A	3	3	0	0	0	0	0	0.5	0	0	0		6.5	
11B	2	3	0	0	0	0	0	1	0	0	0		5	
11C	3	4	0.5	0	0	0	0	1	0	0	0		7.5	19

[1]point value not established
[2]not included in point value or ranking
*mean of 2 values

Table 4B. Summary of Data from Milli Q Water Extracted Yamaska River Basin Sediments, Based on Points Allocated for Specific Values (Table 2). August Data.

Site and Sample	Fecal* Coliforms	Clostridium*	Daphnia magna*	Spirillum volutans*	Spot Plate[1]*	Toxi-Chromotest*	Microtox*	ATP-TOX* System	Algal* ATP	Ceriodaphnia reticulata	Mutatox* >3X revertants	Mutatox[2]* X times control level	Total Points Station	Site
15A	1	3	1	0	0	0	0	0.5	0	10	10	7X	25.5	
15B	1	4	1.5	0	0	0	0	1	0	5	0		12.5	
15C	1	4	0	0	0	0	0	0.5	0	2	0		7.5	45.5
5A	1	10	0	0	0	0	0	0	0	0	0		11	
5B	1	10	0	0	0	0	0	0.5	0	0	0		11.5	
5C	1	10	0	5	0	0	0	0	0	0	0		11	33.5
31A	1	4	0	0	0	0	0	0.5	0	0	0		5.5	
31B	2	7	0	0	0	0	0	0.5	0	0	0		9.5	
31C	1	4	4	0	0	0	0	0	0	0	0		9	19.5
30A	1	4	0	0	0	0	0	0.5	0	0	0		5.5	
30B	1	4	0	0	0	0	0	0	0	0	0		5	
30C	1	4	0	0	0	0	0	0	0	0	0		5	15.5
10A	1	4	0	0	0	0	0	0	0	0	0		5	
10B	1	4	2	0	0	0	0	1	0	0	0		8	
10C	3	4	0	0	0	0	0	0.5	0	0	10	7X	17.5	32.5
11A	1	3	0	0	0	0	0	0	0	0	10	7X	14	
11B	1	3	2	0	0	0	0	0.5	0	0	10	6X	16.5	
11C	1	4	0	0	0	0	0	0	0	0	10	7X	15	45.5
12A														
12B														
12C														
13			0	0	0	0	0	1	0	0	0		1	1
14			0	0	0	0	0	0	0	0	0		0	0

[1]point value not established
[2]not included in point value or ranking
*mean of 2 values

Table 4C. Summary of Data from Milli Q Water Extracted Yamaska River Basin Sediments, Based on Points Allocated for Specific Values (Table 2). November Data.

Site and Sample	Fecal* Coliforms	Clostridium*	Daphnia magna*	Spirillum volutans*	Spot Plate[1]*	Toxi-Chromotest*	Microtox*	ATP-TOX* System	Algal* ATP	Ceriodaphnia reticulata	Mutatox* >3X revertants	Mutatox[2]* X times control level	Total Points Station	Site
15A	3	4	1	0	0	0	0	0	3	2	0		13	
15B	3	3	2	0	0	0	0	0.5	2	0	0		10.5	
15C	4	4	0	0	0	0	0	0.5	1	0	0		9.5	33
5A	1	10	0	0	0	0	0	0	2	0	0		13	
5B	3	10	5	0	0	0	0	0.5	3	0	0		21.5	
5C	1	10	0	0	0	0	0	1.5	2	0	0		14.5	49
31A	1	4	0	0	0	0	0	0	2	0	0		7	
31B														
31C	2	4	0	0	0	0	0	1	2	0	0		12	19

[1]point value not established
[2]not included in point value or ranking
*mean of 2 values

Table 5A. Summary of Data from DOM-DMSO Extracted Yamaska River Basin Sediments, Based on Points Allocated for Specific Values (Table 2). June Data.

Site and Sample	Daphnia magna*	Spirillum volutans*	Spot Plate[1]*	Toxi-Chromotest*	Microtox*	ATP-TOX* System	Algal* ATP	Ceriodaphnia reticulata	Mutatox* >3X revertants	Mutatox[2]* X times control level	Total Points Station	Site
15A	7	0	0	0	6	2	3	3	0		21	
15B	7	0	0	0	6	3	1	-[3]	0		17	
15C	7	0	0	0	5	4	1	-	0		17	55
5A	4	0	0	1.5	7	2	1.5	5	0		21	
5B	7	0	0	1.5	6	3	1.5	-	0		19	
5C	8	0	0	3	7	5	0	-	0		23	63
31A	6.5	0	0	0	6	3	3	10	0		28.5	
31B	6	0	0	0	6	4	0.5	-	0		16.5	
31C	6.5	0	0	0.5	6	3	1.5	-	0		17.5	62.5
30A	5.5	0	0	0	5	3	0.5	20	10	3X	44	
30B	5	0	0	0	4	5	1.5	10	0		25.5	
30C	5.5	0	0	0	5	3	2	10	0		25.5	95
10A	6.5	0	0	1.5	5	4	2.5	3	0		22.5	
10B	6	0	0	1.5	5	5	1.5	-	0		19	
10C	6	0	0	3	5	5	0.5	-	0		19.5	61
11A	7	0	0	0	6	2	0.5	20	0		35.5	
11B	5	0	0	0	4	4	0.5	-	0		13.5	
11C	7	0	0	0.5	5	4	0.5	-	0		17	66

[1]point value not established
[2]not included in point value or ranking
*mean of 2 values
[3]not tested

Table 5B. Summary of Data from DLM-DMSO Extracted Yamaska River Basin Sediments, Based on Points Allocated for Specific Values (Table 2). August Data.

Site and Sample	Daphnia magna*	Spirillum volutans*	Spot Plate[1]*	Toxi-Chromotest*	Microtox*	ATP-TOX* System	Algal* ATP	Ceriodaphnia reticulata	Mutatox* >3X revertants	Mutatox[2]* X times control level	Total Points Station	Site
15A	7	0	0	0.5	6	1	1.5		0		16	
15B	7	0	0	2	10	1	0.5		0		20.5	
15C	7	0	0	1	3	3	2.5		0		15.5	52
5A	7	0	0	1	5	3	1.5		10	8X	27.5	
5B	7	0	0	1	6	2	4		10	12X	30	
5C	8	0	0	1.5	6	3	5		10	12X	33.5	91
31A	7	0	0	0	5	2	2.5		10		26.5	
31B	7	0	0	0	5	2	2.5				16.5	
31C	7	0	0	0	5	2	-		10	12X	24	67
30A	7	0	0	0	5	2	2.5		0		16.5	
30B	7	0	0	0	5	3	2.5		0		17.5	
30C	7	0	0	0	5	3	2.5		0		17.5	51.5
10A	8	0	0	0.5	5	2	5		10	13X	30.5	
10B	7	0	0	0.5	6	2	3		10	12X	28.5	
10C	7	0	0	0	4	2	6		0		19	78
11A	8	0	0	0	6	2	5		10	3X	31	
11B	8	0	0	0	6	3	4		10	10X	31	
11C	7	0	0	0	6	3	2.5		10	15X	28.5	90.5
13	7	0	0	1	6	3	5		10	17X	32	32
14	7	0	0	0	4	3	2.5		10	16X	26.5	26.5

[1]point value not established
[2]not included in point value or ranking
*mean of 2 values

Table 5C. Summary of Data from DLM-DMSO Extracted Yamaska River Basin Sediments, Based on Points Allocated for Specific Values (Table 2). November Data.

Site and Sample	Daphnia magna*	Spirillum volutans*	Spot Plate[1]*	Toxi-Chromotest*	Microtox*	ATP-TOX* System	Algal* ATP	Ceriodaphnia reticulata	Mutatox* >3X revertants	Mutatox[2]* X times control level	Total Points Station Site	
15A	7	0	0	0.5	4	3	6		10	3X	30.5	
15B	7	0	0	0.5	4	2	7				20.5	
15C	7	0	0	0.5	5	2	5				19.5	69.5
5A	7	0	0	1	5	4	1.5		0		18.5	
5B	7	0	0	1	5	1	2.5		0		16.5	
5C	6	0	0	0.5	5	2	5		0		18.5	53.5
31A	7	0	0	0	4	1	0		0		12	
31B												
31C	7	0	0	0	6	2	2.5		10	3X	27.5	39.5

[1]point value not established
[2]not included in point value or ranking
*mean of 2 values

Table 6. Result of Earthworm (Eisonia spp) Test.

Sample	Site	Incubation Days 7 day	14 day
Sediment	5A	100% survival	100% survival
Sediment	5B	100% survival	80% survival
Sediment	5C	100% survival	100% survival

Table 7. Seed Germination and Root Length Assay.

Sample	Percentage Sprouted (as % of control)	Percentage root length growth inhibition compared to control
5A	100	0 - stimulation
5B	105	0 - stimulation
5C	0	100
10A	94	0 - stimulation
10B	105	6
10C	94	25
11A	111	25
11B	105	44
11C	88	31
15A	94	40
15B	50	0
15C	89	0
30A	100	20
30B	94	27
30C	78	13
31A	83	0 - stimulation
31B	89	0 - stimulation
31C	72	0

Table 8 Summary of Site Rankings Based on Sample Treatment.

Rank	Water		Sediment-Mill Q Water Extract		Sediment-DCM-DMSO Extract
	Total Tests	Toxicant Screening Tests	Total Tests	Toxicant Screening Tests	Total Tests
1	31[1]	14	5	15	13
2	14	13	15	11	14
3	12	31	11	5	11
4	30	12	10	10	5
5	10	10	31	31	10
6	11	30	30	13	30
7	13	15	13	30	31
8	5	11	14	14	15
9	15	5			

[1]See Table 1, Figure 1