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USE OF BIOASSAYS TO EVALUATE THAMES RIVER WATER AND SEDIMENT QUALITY

by

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ABSTRACT

In trying to establish the extent and degree of impact of point and non-point source contaminants on riverine systems, it is important to know the implications of the data obtained from various sampling points in a riverine system. Spatial variability between water and/or sediment samples collected in close approximation at the same sites was assessed by the battery of tests approach. In these samples there appeared to be no consistent relationship between sediment structure, microbial population and toxicant concentrations. Furthermore the ATP-TOX System and Mutatox tests were the most responsive tests in all types of samples. Since sediments with their bound contaminants may be an important factor in this data interpretation, different methods for releasing and concentrating the sediment bound contaminants were evaluated. The results and implications of these results are described.

RÉSUMÉ

Lorsqu'on cherche à établir l'étendue et l'importance de l'impact des sources ponctuelles et non ponctuelles de contaminants sur les systèmes riverains, il est important de connaître les implications des données obtenues à partir de divers points d'échantillonnage dans un système donné. La variabilité spatiale entre les échantillons d'eau et les échantillons de sédiments prélevés en approximation étroite dans les mêmes sites a été évaluée à l'aide d'une batterie de tests. Il ne semblait pas y avoir de rapport cohérent dans ces échantillons entre la structure des sédiments, la population microbienne et les concentrations de substances toxiques. De plus, le système ATP-TOX et les tests Mutatox étaient les plus sensibles dans tous les types d'échantillons. Étant donné que les sédiments et leurs contaminants liés peuvent constituer un facteur important dans cette interprétation des données, on a évalué différentes méthodes visant à libérer et à concentrer les contaminants liés aux sédiments. Les résultats et leurs implications sont décrits dans ce rapport.

MANAGEMENT PERSPECTIVE

The interpretation of bioassay data collected from riverine systems is never easy and rarely straight forward. As more and more riverine sediment data are collected it becomes increasingly obvious that the variability within samples collected in close proximity, is often as great as or greater than downstream variations. On the other hand, river water data, outside of major impacting events, are surprisingly stable and consistent for periods of at least 90 to 120 minutes.

Knowledge of the variability of data collected from riverine systems can help managers make judgements and decisions on the implications of various proposed scenerios.

In this report the battery of tests approach is used (1) to study the spatial variability of contaminant levels in water and sediment and (2) to evaluate the suitability of different sediment extraction/concentration procedures to produce suitable extracts for toxicant screening tests. Also, the lack of relationships between sediment structure, microbial populations and toxicant loads are noted and discussed.

PERSPECTIVE-GESTION

L'interprétation des données de bio-essais prélevés dans des systèmes riverains n'est jamais facile et rarement explicite. Il devient de plus en plus évident à mesure que les données sur les sédiments riverains s'accumulent que la variabilité entre les échantillons prélevés en proximité étroite est souvent aussi importante ou même plus que les variations en aval. D'un autre côté, les données sur l'eau des rivières, hors des principaux points d'impact, sont remarquablement stables et constants pendant des périodes d'au moins 90 à 120 minutes.

Le fait de connaître la variabilité des données rassemblées dans les systèmes riverains peut aider les gestionnaires à prendre des décisions relativement aux implications de divers scénarios proposés.

Dans ce rapport, la batterie de tests est utilisée (1) pour étudier la variabilité spatiale des teneurs en contaminants dans l'eau et les sédiments et (2) pour évaluer l'à-propos des divers procédés d'extraction et de concentration des sédiments pour produire des extraits appropriés aux tests de dépistage des substances toxiques. L'absence de rapport entre la structure des sédiments, les populations microbiennes et les charges en substances toxiques est également signalée et analysée dans ce rapport.

In 1988 a study (Dutka et al. 1989) was undertaken to evaluate the nature and extent of temporal and spatial distribution variability of point and non-point source contaminants as registered by the "battery of tests" approach (Bitton and Dutka 1986) in the waters and sediments of the rivers and streams of the Yamaska River Basin. Realizing that many of the contaminants were organic in nature, it was decided to incorporate into the study an evaluation of the sensitivity/selectivity of different sediment extraction procedures as monitored by the reaction of various bioassays to the extracts. The overall goal of the 1988 study was to establish a simple, inexpensive and reliable "core battery of bioassay tests" which could be applied universally to compare or monitor water bodies and sediments. This new concept of having a "core" group of tests which could be augmented with locally preferred or situationally warranted tests is important in that with these few bioassay tests a data bank could be established for national and international comparisons. Furthermore, when the data are supplemented with a point scoring and ranking scheme (Dutka 1988), inferences can be made on the state of degradation or rehabilitation of a water body. This integration of point scoring and ranking with the battery of bioassays approach would also provide managers with a judgemental vehicle for setting project priorities and intensive/extensive chemical analyses.

One of the main findings of the Yamaska River basin study (Dutka et al. 1989) was that sediment test results could vary to such an extent that some samples collected within 15 metres of each other were no more similar than samples collected 5 - 10 kilometers apart.

Two other major observations were that the type of extraction procedure used on sediments had a bearing on the test results and the Mutatox test for genotoxicants (Kwan et al. 1990) which had its first-ever field evaluation on Yamaska River samples, was found to be the most responsive test with all three sample types: water, Milli-Q water-extracted sediments and organic solvent extracted sediments.

In an attempt to generalize the major observations of the Yamaska River basin study, a smaller but more analytically intense study was carried out on the Thames River. The Thames is a major river of south western Ontario, Canada, and with its tributaries passes through prime agricultural land and the cities of Stratford, Woodstock, London and Chatham on its way to Lake St. Clair (Fig. 1).

Four different sediment extraction procedures were evaluated in this Thames River study, and in this report two of these procedures, Milli-Q water extract and HCl-KCl pH 2 buffer extract and their results will be discussed. Each of the sample extracts were assayed by the genotoxic bioassay Mutatox plus other components of the battery of tests approach. Spatial variation studies were also carried out at five of the six sampling sites. Results of this confirmation study are presented and discussed.

Methods

Sampling Sites

Two sampling sites were selected to assess the water and sediment quality before the river passes through, and is impacted by the city of London. Site #1 was placed in a man-made lake (Fanshawe) created

by building a dam on the North Thames River just outside the city limits. The second site chosen was at the south eastern city limits, where Waubuno Creek enters the Thames River. Site #3 was selected to monitor the combined flow impact of the Thames and North Thames Rivers within the city of London. The next two sites #4 and #5 were chosen to enable us to assess the changes, if any, in water and sediment quality after the combined river flow had passed through the city and meandered through 7 kilometers of woods and agricultural lands. Site #6, approximately 80 river kilometers from London was selected to hopefully allow us to assess the rate of river rehabilitation as it passes through more wooded and agricultural areas on its way to Chatham and Lake St. Clair.

Sample collection

Three sediment samples within a five metre linear distance e.g. A to C = 5 metres, were collected at each site with an Ekman dredge or flat bladed shovel. Frequently, it was necessary to sample many times before sufficient surface sediment (1 to 2 cm layer) was collected. Each of the sediment samples (A, B or C) were thoroughly mixed, placed in appropriate containers and refrigerated. Sub-surface water samples (500 ml) were collected at each site (3 per site) refrigerated and tested within 6 hours for microbiological content. Also at each sampling site one litre of subsurface water was collected and preserved at 4°C for toxicant screening tests.

Sediment Extraction and Processing

Figure 2 summarizes the extraction protocols followed with the sediment samples. Sediment size distribution (Table 1), and Milli-Q water extraction procedures are described in detail in Dutka and Kwan (1988). The HCl-KCl pH 2 buffer sediment extraction was a slightly modified version of that described by Atkinson et al. (1985). In the procedure followed, sediment was mixed with the HCl-KCl pH 2 buffer in a 1:1 ratio (wet wt: vol). The sediment slurry (250 gm wet wt. sediment + 250 ml buffer) was placed in a wrist action shaker for 24 hr. at room temperature (20-22°C). The flasks were stoppered with foam plugs to facilitate air and gas diffusion. After shaking, the mixture was decanted into 250 ml centrifuge tubes and centrifuged for 20 min at 10,000 rpm at 4°C. The supernatant was decanted, neutralized to pH 6.8 - 7.0, and stored at 4°C until tested.

Microbiological Tests

The five tube MPN fecal coliform test using A-1 broth (water and sediment), heterotroph spread plate test (water and sediment), four tube coliphage test (water) and the five tube MPN test for Clostridium perfringens, were performed on the water and sediment samples as detailed in Dutka (1989). A microscope technique for total and viable microbial counts in water was performed following the procedures detailed by Rao et al. (1984).

Toxicity Screening Tests

With the exception of the Daphnia magna and Ceriodaphnia dubia 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 phosphoreum and the procedure detailed in Microtox System Operating 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).

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 toxicity screening test based on the inhibition of ATP production by the green alga Selenastrum capricornutum (Kwan 1989) was applied to the samples also. The results are reported as a percentage of Relative Light Units (RLU) produced by the tested sample, compared to the non-stressed control which is accepted as 100% output.

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 dubia 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 a 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).

The Mutatox test based on the use of a dark mutant strain of Photobacterium phosphorium M169 to screen for genotoxic agents was field-tested in this study. This test will reveal the presence of 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. Genotoxic 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 20 hr. Light level is read after 20 hrs contact and compared to negative controls (Kwan et al. 1990).

Three non-routine toxicant screening tests were included in this study (a) a seed germination and root elongation test using prize head leaf lettuce seeds (Dutka 1989), (b) a 14 day earthworm (Eisenia) survival test (Dutka 1989) and the ECHA dip stick test (Dutka and Gorrie 1989) which included a one minute contact period with the sample.

Point Scoring and Ranking Scheme

The procedures detailed in Dutka (1988) with modifications described in Dutka et al. (1989) were used in this study to award points for specific data values and to rank the samples and sites.

RESULTS

Sediment descriptions and classifications shown in Table I illustrate the great variations one sees in riverbed sediments, even in those collected within a linear space of five metres. From the Table it can be seen that only the following sediments, structurally closely resembled each other: 2A and 2B; 3A, 3B and 3C; 4A and 4B; 5B and 5C; 2C and 5A; and 3A, 3B, 3C, 4A and 4B. These relationships will be reviewed as they impact on the microbiological and toxicological data obtained from these sediments.

Although, at each site water samples were collected at points within a 5 metre linear distance i.e. A = 0 metre, B = 3 metres from A, C = 5 metres from A, and were supposedly representative of the same specific water mass, they were in reality representative of completely different water masses collected at points less than five metres apart. This can easily be demonstrated. For instance, at site 2A where the river flow rate was 8 - 10 km per hour, it took 30-35 minutes to collect both sediment and water samples. Thus, approximately 40 minutes had elapsed from the initiation of sample collection, before collection of samples from site 2B were started. By this time a new water mass which had been 5-7 kilometres upstream when the sampling at 2A started, is now being sampled at 2B. Unless the whole river is homogeneous in regards to particulate matter, dissolved chemicals and bacteria, then each sampling point even though it is less than 2-3 metres from its neighbour, will/may contain a completely different chemical/microbiological/particulate distribution and ratio pattern. It is therefore important to recognize this reality when trying to establish relationships between samples collected at the same site, no matter how close they are to each other.

Microbiological data from surface water are presented in Table 2. It is apparent that fecal coliform concentrations, with the exception of Fanshawe Lake samples and 2B and 4B, exceed provincial recreational water quality guidelines of 100 fecal coliforms per 100 mL. . Site 3 which was situated immediately downstream of the confluence of the North Thames and Thames Rivers contained both the highest fecal coliform and coliphage densities. Only coliphage concentrations at sites 4 and 5 tended to meet the suggested coliphage guideline of 20 coliphage/100 ml (Dutka et al. 1987). Grabow et al. in 1984 reported "coliphage counts could give a useful estimate of numbers of other micro-organisms in sewage polluted waters" and in their studies "evidence is presented, that though counts of coliphages may not always correlate with those of enteric viruses, coliphages meet the basic requirements of an indicator for the virological safety of water". Earlier, Simkova and Cervenka (1981) were able to demonstrated a positive correlation between the levels of coliphages and the presence of enteroviruses in Czechoslovakian rivers. If the concentrations of coliphage in these Czechoslovakian studies (Simkova and Cervenka, 1981) are compared to the levels enumerated from these six Thames River sites, by extrapolation, it is possible that enteroviruses may also have been present in the sampled waters, and therefore these presences of coliphage should be further investigated in conjunction with an epidemiological and human enteric virus study.

In water microbiology enumeration procedures, especially at lower densities, where variations between replicates of 50-100% are commonly found, differences in counts, between most samples at each site, were well within normal environmental sample variations.

With the exception of Fanshawe lake samples, river fecal coliform levels tended to be fairly stable with densities in the 110-130 organism/100 mL range. Total bacterial densities, throughout the sampling area were stable at the 10^6/mL level while the microscopic viable count based on INT-formazan reduction (Rao et al. 1984) and the heterotrophic plate counts were similar with less than one log variation. The implications of these microbiological density observations, are suggestive that at least 10-15 kilometres of river water mass are fairly homogeneous microbiologically for at least a 90-120 minute period.

Results of the ecotoxicity tests with positive responses to the water samples collected from the Thames River are shown in Table 3. Four of the tests, Daphnia magna, Spirillum volutans, Algal ATP and Toxi-chromotest, indicated that these waters, at the concentrations tested, contained non detectable concentrations of toxicants as measured by these tests. The three samples collected at Site 3 were all negative when tested by the ATP-TOX System. This was a totally unexpected response, because this site was slightly downstream of the confluence of the N. Thames and Thames Rivers, where, further upstream, on these rivers (Sites 1 & 2) the highest ATP-TOX System values were found. From Table 3, it can also be seen that there was very little variation between sample responses to the ATP-TOX System at each of the sample sites.

The Microtox test was negative in 10X concentrated waters collected from sites 2, 5 and 6. Site 1, however produced the greatest toxicant response e.g. it look only ≈ 6% of the 1 mL 10X concentrated water sample to produce an EC_{50} effect.

The samples which produced the greatest genotoxic effect, as asssessed by the Mutatox test, were the three samples collected at Site 4. The addition of activated liver cells (S-9) to the Mutatox test did not effect the sensitivity of the test, since the results with and without (S-9) addition were vertually the same (Table 3). Mutatox test results showed greater variation in A, B and C samples of each site, than did the ATP-TOX system and microtox tests.

With the exception of water samples from Site 5 all sites had samples which gave a positive response in the test for chronic toxicity, Ceriodaphnia dubia. Site 1 samples produced the greatest response i.e. two samples (B and C) indicated the presence of chemicals which produced chronic toxicity even when the samples were diluted to the 1% level. Sample 4C also produced a similar response, even though 4A and 4B were negative. An interesting observation on Site 4 data, was that 4A and 4B produced positive responses in the Microtox test and a negative response in the Ceriodaphnia dubia test, while 4C showed a very strong chronic toxicity response in the Ceriodaphnia dubia test and a negative response in the Microtox test. Samples 4A B and C were also strongly positive in the Mutatox test.

The toxicant screening bioassays, with few minor exceptions tended to show the same variations from sample to sample within site as did the microbiological data. These toxicity screening test data seem to imply that, in this river under June flow regimes, at least 10-15 kilometres of river water mass are fairly homogeneous for at least a 90-120 minute period.

Based on Site mean point scores, Site 1 with or without the inclusion of the water bacteriological data, ranked #1 for potential contained hazards. Sites #2, #6 and #5 had much lower point scores (50% or slightly less) than Sites 1 and 4 which ranked #1 and #2 respectively. The ranking of each site was the same with or without the bacteriological data. The very strong responses of Site 1 waters in the chronic toxicity test (C. Dubia) and Site 4 waters in the genotoxicity test (Mutatox) were the main factors responsible for their #1 and #2 ranking as hazard containing sites.

The results, of microbiological and toxicity assessment tests performed directly on the sediments collected at the six Sites, are presented in Table 4. Heterotrophic bacterial counts at each Site were found to vary between samples from as little as 20% to more than 1000%. Differences in sediment structure may be an explanation for the differences in heterotrophic counts between samples 2A (70% silt) and 2B (72% silt) and 2C (7% silt and clay), however, it does not explain the greater than 800% heterotrophic count difference between 2A and 2B. Site 3 sediments, which combine the loadings of the North Thames and Thames River, and which were expected to contain higher or equivalent heterotrophic sediment bacterial populations to Sites 1 and 2, were instead, found to have the lowest site mean heterotrophic densities. The explanation for this we believe, is the slightly narrowed river at this point, with two combined river flows resulting in a slightly faster flow over a river bottom composed mainly of stones and gravel with pockets of sand. Each sample was obtained from bottom scrapings from an area of approximately 2 metres square, which were then mixed to produce the specific site sample. Sediment heterotrophic counts, which were all performed in duplicate, show no

consistent pattern between samples at each site or between sites. Similar observations were noted in our Yamaska River study (Dutka et al. 1989). From these combined data it must be concluded that sediment heterotrophic populations are insensitive indicator systems in fluvial systems.

Fecal coliform populations showed consistent increasing downstream populations with the greatest concentration (110,000 FC/100 gm wet sediment) being found at Site 6 the furthest downstream site. The lowest concentration was found at Site 1, Fanshawe lake. Fecal coliform density variations between samples at the same site illustrated the same order of variability as the heterotrophic populations and there appears to be no relationship between heterotroph densities and fecal coliform densities.

The fecal coliform distribution pattern suggest that all of the sampled area of the Thames River is impacted by fecal contamination, much of which appears to be associated with city of London inputs.

Clostridum perfringens spore densities tended to show an increasing downstream deposition trend. Site 1 data are also suggestive of a downstream accumulation due to the creation of Fanshawe Lake by damming the North Thames River. Fecal coliform populations which were the lowest at Site 1, and are much shorter lived than C. perfringens spores, support the belief that Fanshawe Lake sediments show long term buildup of C. perfringens spores from upstream population density sources i.e. St. Marys, Stratford and Mitchell.

C. perfringens spore densities, similar to heterotrophic bacterial and fecal coliform densities tended to show great variations (up to >1000%) between samples at the same site. There also appears to be no consistent relationship between the various microbiological

tests with the exception of the downstream build up of fecal coliforms and C. perfringens spores, one test indicating a renewable short term impact and the other an accumulating historical record. All the microbiological data indicate that there is a great variation in microbial populations between closely collected river sediment samples and surprisingly, the seemingly independence of the population densities to sediment structure.

Two toxicological bioassay tests were performed directly on the sediments, the ECHA Biocide Monitor (dipstick) and the earthworm test. The results shown in Table 4, indicate that with the exception of sites 5 and 6, samples at the other sites showed varying responses. There appears to be no relationship between sediment structure (Table 1) and toxic response, as assessed by the ECHA dipstick. If one assumes that toxicity is related to the finer sediments i.e. silt and clay, then samples 1A, B and C, and 2A and B and 6 have the greatest portion silt and clay, and yet only 2A produced a strong positive response in the dipstick test. Samples 5A, 5B and 5C were all positive with the dipstick test and the sand concentration varied from 94% to 40%. These observations seem to be contrary to the belief that the smaller the particle size, the greater the surface area and therefore the greater the potential contaminant load. A possible explanation is that toxicants which are inhibitory to the bacteria in the ECHA dip stick, are sporadically distributed with no relation to sediment structure.

The earthworm test which was performed on the B samples at each site indicated that only two sediments 4B and 5B, contained toxicants at concentrations harmful to the earthworm (Eisenia Spp). In the dipstick test, sample 4B was slightly positive for toxicant activity while 5B was positive for toxicants. Based on the results obtained from these two direct sediment toxicant screening tests it would appear that each of the samples testing positive for toxicants contained different chemicals or ratios of chemicals. The finding of positive (toxicity) responses in the dipstick and earthworm tests to samples 4B and 5B is suggestive of a downstream accumulation of specific contaminants or specific area inputs which have not been transported as far as Site 6 in sufficient concentrations to trigger these tests.

Table 5 is a summary of all the Milli-Q water extracted sediment data obtained from split sample analyses with replicates (Fig. 1). The results are presented as sample Site means. Data from the following tests are not shown as the results were all negative: Microtox, Toxi-chromotest, Algal-ATP and Spirillum volutans. The Mutatox test with S-9 addition produced only two positive sample results 3A and 4A, both of which were much lower than the test results without S-9 addition. Therefore, the Mutatox test with S-9 was also excluded from the table for clarity and ease of interpretation.

Sites 4, 5 and 3, based on the application of points (Dutka 1988) to specific toxicant response values and bacterial populations (Table 4) were deemed to contain the greatest potential hazards, chemical and microbiological. With or without the addition of the bacteriological points Site 4 is #1 and sites 3 and 5 reverse their ranking #2 and #3 to #3 and #2 depending on whether or not the bacteriological data are considered.

ATP-TOX System summarized mean data, (Table 5), illustrates the general trend in sample result variations we have observed with all samples collected at the same site. Few of the sample results from the same site vary less than 25%, most results vary by several hundred percent. If these variations are examined in the context of points allocated to specific toxicity values, then with the exception of sample 4A, (0 points), all the samples are in the 1 point value range (Dutka 1988), where 1-30% inhibition = 1 point.

It was surmised before the study was initiated that there might be a relationship between sediment structure and toxicant loading. We also realized that local inputs may possibly nullify any site to site and downstream trend comparisons. As can be seen from Table 1 and Table 5, there are no obvious consistent relationships between sediment structure and ATP-TOX System values.

Only six Milli-Q sediment extracts were found to produce reproduction inhibition in the Ceriodaphnia dubia test. Site 3 in which all three samples were positive, may be indicative of the combined toxicant load brought to this site by the two Thames branches (Fig. 1) and partially deposited at this site. Samples 4A and 4B produced the highest values i.e. produced the greatest reproduction inhibition. At this site we can see that the typical pattern of great variability between sample response still holds as the sample with the greatest amount of silt and clay 30% (4C) showed the least reaction (negative) while samples 4A and 4B (0.59% and 2.1% silt and clay) were positive. Similar to the results observed with the ATP-TOX System, there are no obvious consistent relationships between sediment structure and Ceriodaphnia dubia results, as well as between results and samples collected of the same site.

In the Daphnia magna acute toxicity test only sample 5B produced a weak positive (EC15) response. The observed result is a mean response, just above background level and probably indicates the presence of low level concentrations of toxicants to which Daphnia magna are sensitive.

In the Mutatox test, all the samples with the exception of sample 1C, produced a genotoxic response indicating that all these samples produced at least 3X the amount of light the control samples produced. Samples 1B and 4B produced the highest genotoxic responses and these two samples also showed similar ATP-TOX System and seed germination test responses. However, their responses in the Ceriodaphnia dubia test showed the two greatest extremes, the highest response (4B) and the lowest response (1B) as well as having completely different sediment structures, e.g. 4B with 98% sand and gravel and 1B having 99% silt and clay. Thus from these results (Table 5, Table 1) it can be seen that genotoxic agents are fairly evenly distributed throughout the whole sampling area with great variation from sample to sample within the collection site.

Seed germination tests, with the possible exception of samples 1A, 1B and 2A, basically showed that the chemical concentrations present in the Milli-Q extract did not produce an inhibitory effect on this test system. However, root growth inhibition was found to occur in samples 2A, 2B, 3B, 4A, 5B and 6. The seed germination test results were the least variable results observed between samples at the same site, possibly due to the insensitivity of the test. Root elongation test results however, were similar in pattern to the other toxicity screening test results i.e. no relationship between toxicant levels in samples at each site or sediment structure.

Sediments extracted by using HCl-KCl pH 2 buffer proved to be unsatisfactory for toxicant screening tests. When the HCl-KCl buffer blanks were neutralized with NaOH, the resulting solution proved to be very toxic in all bioassays. This toxicity was so great that when the neutralized HCl-KCl buffer blank was finally diluted to its maximum allowable concentration (MAC), (Kwan and Dutka 1990), and this same dilution applied to the extracted samples, all of the samples tested negative for toxicity in the seven tests being assessed; S. volutans, D. magna, Microtox, Mutatox, Toxi-chromotest, ATP-TOX System and Algal-ATP. This procedure, HCl-KCl pH 2 buffer, which is an excellent technique for freeing metals from sediments, under the regime followed with these samples, proved to be incompatible with the toxicant screening bioassays used.

Generally, the data obtained from these Thames River water and sediment samples tend to indicate the sites 4, 3 and 5 are the major river problem areas. Site 1 even though it ranks as #1 with the water data is in reality based on a set of lake type data or a partially immobilized mixing zone.

The analytical results obtained from these river sediments raises the question of what is the most appropriate sample and sample collection approach in rivers subject to many diverse point and non point sources. Due to the great variability in sediment structures within site and from site to site and the lack of clear relationships between sediment structure, microbial load and toxicant load in this study, we believe that before any river study for toxicant loadings is undertaken a thorough review of sample collection goals and the philosophy to be followed for establishing the type of samples to be collected as well as sample collection density and frequency.

In summation, the following observations have been made during this study:

(1) there appears to be no consistent relationship between sediment structure, microbial population and toxicant concentrations;

(2) there appears to be no relationship between toxicant loading as measured by bioassay responses and endemic microbial populations, perhaps implying an adapted population;

(3) sediment samples collected within very short distances of each other, with or without having similar composition structures appear to show distinctive toxicological and microbiological responses;

(4) contrary to previous studies (Dutka et al. 1989), the Daphnia magna test was relatively non responsive in these waters and Milli-Q water extracted sediments;

(5) the ATP-TOX System and Mutatox continue to be the most responsive tests in all types of samples;

(6) the ECHA dipstick continues to show that it is a simple responsive test for sediment contained toxicants. As soon as a greater data base has been accumulated, relationships with other bioassays will be evaluated; and

(7) river water microbiological and toxicological data seem to imply that at least 10-15 km of river water mass are fairly homogeneous for at least a 90-120 minute period.

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Table 1. Site Location. Sediment Description and Classification

Site	Latitude	Longitude	Sample	Description and Shepard Classification[1]
1.	43°02'32"	81°10'46"	A.	Sand .51%, silt 33.03%, clay 66.46% SILTY CLAY
			B.	Sand 1.05%, silt 46.25%, clay 52.70% SILTY CLAY
			C.	Sand 18.52%, silt 21.08%, clay 60.39% SILTY CLAY
2.	42°58'38"	81°07'48"	A.	Sand 1.37%, silt 70.82%, clay 27.81% CLAYEY SILT
			B.	Sand .10%, silt 72.89%, clay 27.01% CLAYEY SILT
			C.	Sand 93.02%, silt and clay 6.98% SAND
3.	42°58'10"	81°16'31"	A.	Sand 98.91%, silt and clay 1.09% SAND
			B.	Gravel .17%, Sand 97.91%, silt and clay 1.92% SAND
			C.	Gravel .62%, Sand 97.04%, silt and clay 2.3% SAND
4.	42°57'53"	81°23'12"	A.	Sand 99.41%, silt 0.33%, clay 0.26% SAND
			B.	Sand 97.90%, silt 1.14%, clay 0.96% SAND
			C.	Sand 69.50%, silt 14.40%, clay 16.10% CLAYEY SAND
5.	42°56'03"	81°25'07"	A.	Sand 94.07%, silt and clay 5.93% SAND
			B.	Sand 40.57%, silt 32.96%, clay 26.47% SAN SIL CLY
			C.	Sand 43.64%, silt 28.32%, clay 28.04% SAN SIL CLY
6.	42°38'23"	81°42'05"	A.	Sand 10.39%, silt 32.65%, clay 56.96% SILTY CLAY

1. Dalton 1989

Table 2. Thames River, Surface Water Microbiology June 1989

Site and Sample	Coliphage/ 100 mL	Fecal Coliform MPN/100 mL	Spread Plate Heterotrophs/mL	Acridine Orange Microscopic Total Count/mL	Microscopic Total Viable Count/mL
1 A	150	8	5.5×10^4	2.4×10^6	4.7×10^4
B	150	5	3.0×10^4	1.5×10^6	3.6×10^4
C	160	11	4.3×10^4	1.5×10^6	4.7×10^4
2 A	35	130	4.8×10^3	1.4×10^6	2.4×10^4
B	25	110	9.5×10^3	1.6×10^6	3.6×10^4
C	33	70	1.4×10^4	1.9×10^6	1.2×10^4
3 A	190	350	7.3×10^3	3.4×10^6	3.6×10^4
B	240	130	4.3×10^3	2.5×10^6	4.7×10^4
C	280	350	6.5×10^3	2.5×10^6	3.6×10^4
4 A	8	130	4.4×10^4	2.0×10^6	1.2×10^4
B	23	79	1.2×10^4	2.5×10^6	3.6×10^4
C	18	130	3.7×10^4	2.2×10^6	2.4×10^4
5 A	20	130	2.0×10^4	1.2×10^6	9.5×10^4
B	8	110	6.5×10^4	2.9×10^6	1.1×10^5
C	6	110	5.7×10^4	2.2×10^6	4.7×10^4
6	60	130	5.4×10^4	2.5×10^6	4.7×10^4
SITE MEANS					
1	153	8	4.3×10^4	1.8×10^6	4.3×10^4
2	31	103	9.4×10^3	1.6×10^6	2.4×10^4
3	236	276	6.0×10^3	2.8×10^6	3.9×10^4
4	16	113	3.1×10^4	2.2×10^6	2.4×10^4
5	11	116	4.7×10^4	2.1×10^6	8.4×10^4
6	60	130	5.4×10^4	2.5×10^6	4.7×10^4

Table 3. Thames River Surface Water Ecotoxicology Data with Point Scores and Ranking

Site and Sample	ATP-TOX % Inhibition	Microtox EC_{50} % mL	Mutatox, no (S-9) no. revertants > control	Mutatox with (S-9) no. revertants > control	*Ceriodaphnia dubia* % of sample producing reproduction inhibition	Without Bacteriology		With Bacteriology	
						Mean Site Point Score	Rank	Mean Site Point Score	Rank
1 A	35	6.3	1.3x[2]	1.3x	10 %				
B	41	6.4	6.4x	6.4x	1 %	21.3	1	25.3	1
C	43	6.3	6.1x	6.1x	1 %				
2 A	43	N.D	2.3x	2.3x	100 %				
B	45	N.D	2.9x	2.9x	100 %	5.3	6	9	6
C	44	N.D	1.5x	1.5x	50 %				
3 A	ND[1]	15.0	2.6x	2.0x	100 %				
B	ND	32.0	9.0x	9.0x	ND	10.3	3	15.3	3
C	ND	23.0	6.6x	6.6x	100 %				
4 A	22	41.5	23.8x	21.8x	ND				
B	17	35.0	43.8x	43.8x	ND	19	2	21	2
C	15	N.D.	27.8x	25.8x	1 %				
5 A	36	N.D.	9.0x	9.0x	ND				
B	38	N.D.	4.9x	4.9x	ND	9.3	4	12	4
C	34	N.D.	7.4x	7.4x	ND				
6	8.2	N.D.	2.5x	2.5x	10 %	6	5	10	5

ND[1] = No toxic effect detected

1.3x[2] - only tests showing 3x control number of revertants are considered having genotoxic effect.

Table 4. Microbiological and Toxicological Tests on Sediment Before Concentration and Extraction Procedures.

Site and Sample	Heterotrophs/mL wet wt sediment	Site Mean	Fecal Coliforms A-1 Broth /100 gm wet wt sediment	Site Mean	Clostridum perfringens /100 gm wet wt sediment	Site Mean	ECHA Dip Stick test	Earthworm Test % Survival 14 days
1 A	2.4×10^8		170		7.9×10^2		±[a]	
B	1.1×10^8		230		4.6×10^3		+[b]	100 %
C	1.3×10^8		230		3.5×10^3		-	
		1.6×10^8		210		2.9×10^3		
2 A	1.1×10^8		1300		7.9×10^2		+	
B	9.1×10^8		7900		2.2×10^3		-	90 %
C	1.1×10^7		3300		1.1×10^3		±	
		3.4×10^8		4170		1.4×10^3		
3 A	3.0×10^7		1300		9.5×10^3		+	
B	6.8×10^7		3300		7.0×10^2		±	100 %
C	1.1×10^8		13000		1.7×10^3		+	
		6.9×10^7		5870		3.9×10^3		
4 A	4.7×10^8		3300		3.3×10^3		±	
B	2.6×10^7		35000		4.9×10^3		+[c]	0 %
C	3.4×10^8		4900		4.6×10^3		-	
		2.7×10^8		14300		4.2×10^3		
5 A	1.2×10^8		35000		4.3×10^3		+	
B	2.2×10^8		24000		2.8×10^4		+	20 %
C	7.9×10^7		11000		1.3×10^4		+	
		1.4×10^8		23,300		1.5×10^4		
6	7.9×10^7	7.9×10^7	110,000	110,000	1.4×10^3	1.4×10^3	-	100 %

a = more white area then red
b all white - complete inhibion - toxic
c more red areas then white.

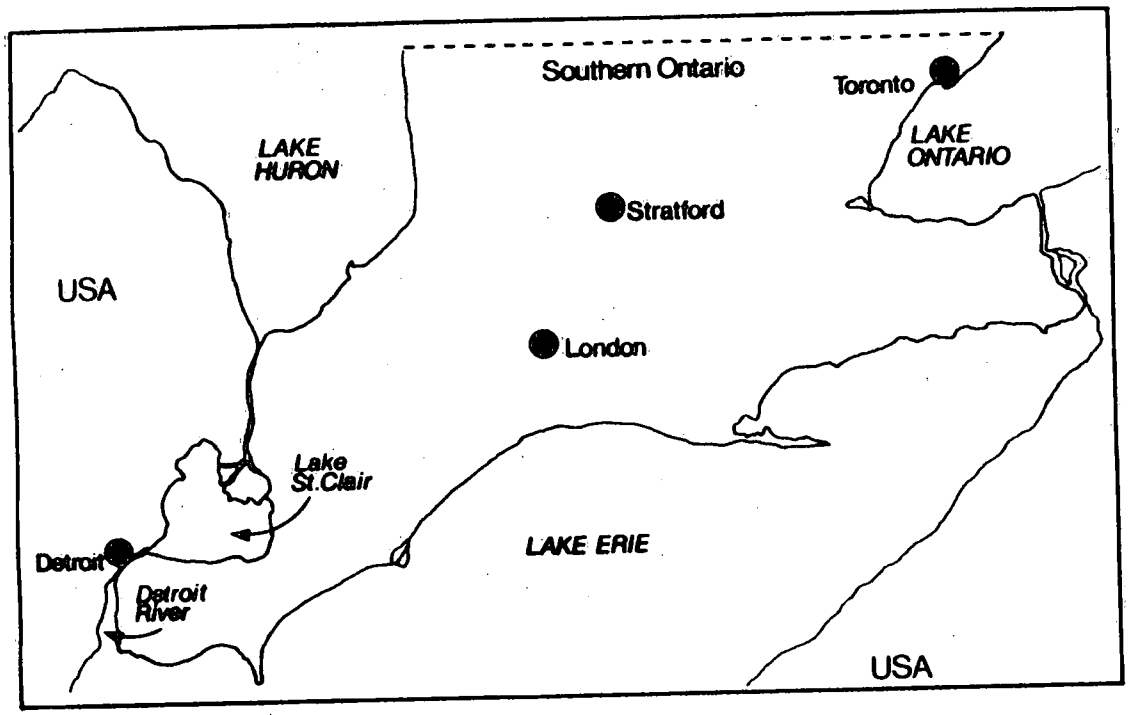
Table 5. Thames River, Summarized, mean split sample, Milli-Q Water sediment extract data with point scores and ranking.

Site and Sample	ATP-TOX % Inhibition	Mutatox no(S-9) no. revertants > control	Ceriodaphnia dubia % sample producing reproduction inhibition	Daphnia magna EC as % of sample	Seed Germination % Germinated	Seed Germination % root length inhibited	Without Bacteriology Mean Site Point Score	Without Bacteriology Rank	With Bacteriology Mean Site Point Score	With Bacteriology Rank
1 A	4.4	6.9x	100 %	ND	86.5	5.6				
1 B	12.6	21.2x	ND	ND	86.8	5.2	7	4	12.6	6
1 C	17.2	2.1x	ND	ND	100	0				
2 A	6.8	8.2x	ND	ND	88	33				
2 B	9.3	6.4x	ND	ND	94.3	19.2	7	4	14.0	5
2 C	14.5	3.7x	ND	ND	100	0				
3 A	2.1	10.1x	100 %	ND	97	5.5				
3 B	5.0	9.7x	100 %	ND	97.3	25.7	9.3	2	17.6	3
3 C	13.4	3.0x	100 %	ND	100	0				
4 A	N.D.[2]	10.1x	30 %	ND	94	21.2				
4 B	12.6	38.8x	.1 %	ND	97.3	2.2	16.3	1	25.3	1
4 C	15.5	3.9x	ND	ND	100	3				
5 A	12.0	8.2x	ND	ND	97	0				
5 B	11.7	8.4x	ND	EC15	90.3	35.7	7.3	3	19.3	2
5 C	20.7	3.2x	ND	ND	100	0				
6	21.2	6.5x	ND	ND	91.5	27.7	6	5	17.0	4

Data[1] from split samples and replicates presented as means.

N.D.[2] = not detected

Figure 1. Sediment and water sampling sites used to evaluate the battery of tests approach to prioritize areas of concern.



North Thames River

Stratford

Waubuno Creek

Fanshawe Lake

1

2

3

4

5

London

Thames River

6

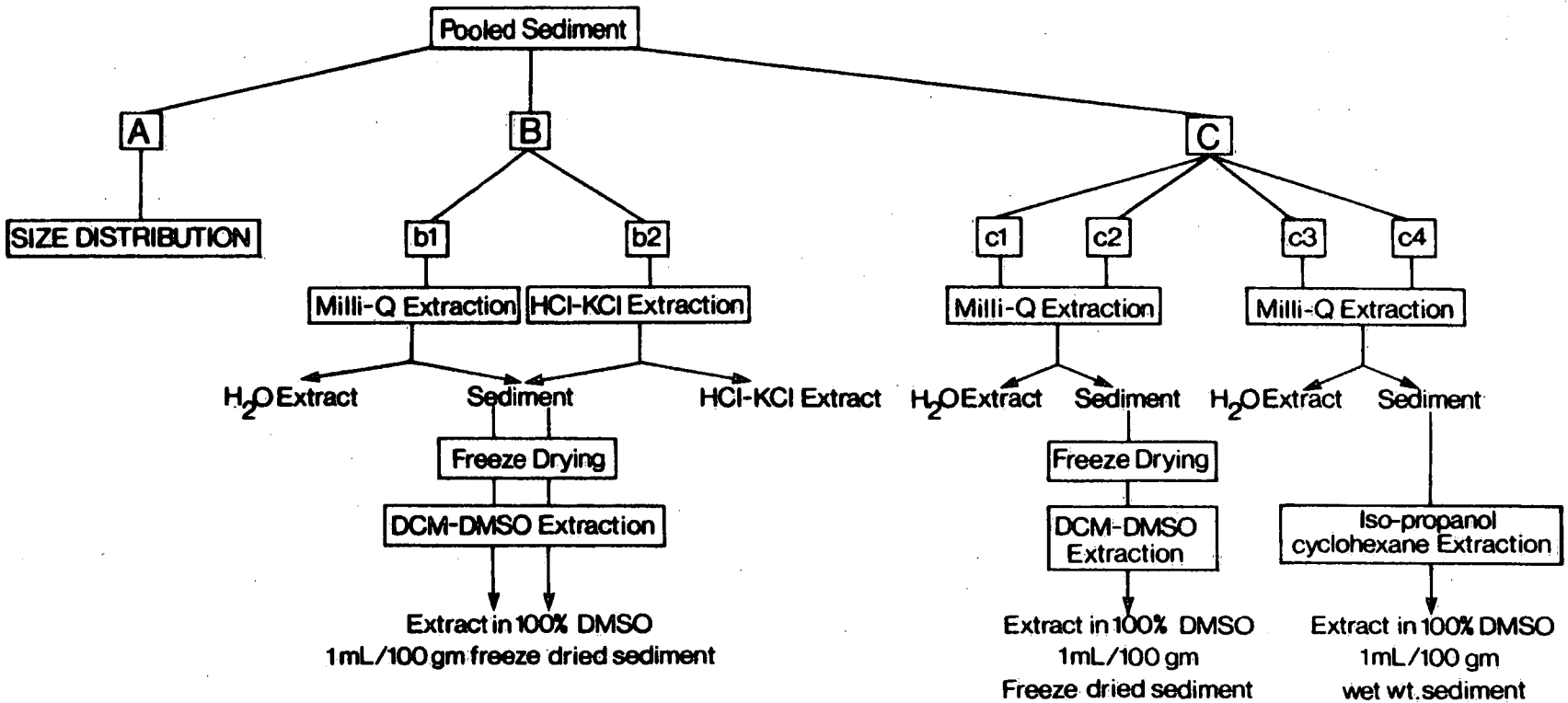
Lake St. Clair

Chatham

Lake Erie

N

Figure 2. Scheme used to extract sediments for toxicological examination by battery of tests approach.


Pooled Sediment
A
B
C
SIZE DISTRIBUTION
b1
b2
c1
c2
c3
c4
Milli-Q Extraction
HCl-KCl Extraction
Milli-Q Extraction
Milli-Q Extraction
H2O Extract
Sediment
HCl-KCl Extract
H2O Extract
Sediment
H2O Extract
Sediment
Freeze Drying
Freeze Drying
DCM-DMSO Extraction
DCM-DMSO Extraction
Iso-propanol cyclohexane Extraction
Extract in 100% DMSO
1mL/100 gm freeze dried sediment
Extract in 100% DMSO
1mL/100 gm
Freeze dried sediment
Extract in 100% DMSO
1mL/100 gm
wet wt. sediment