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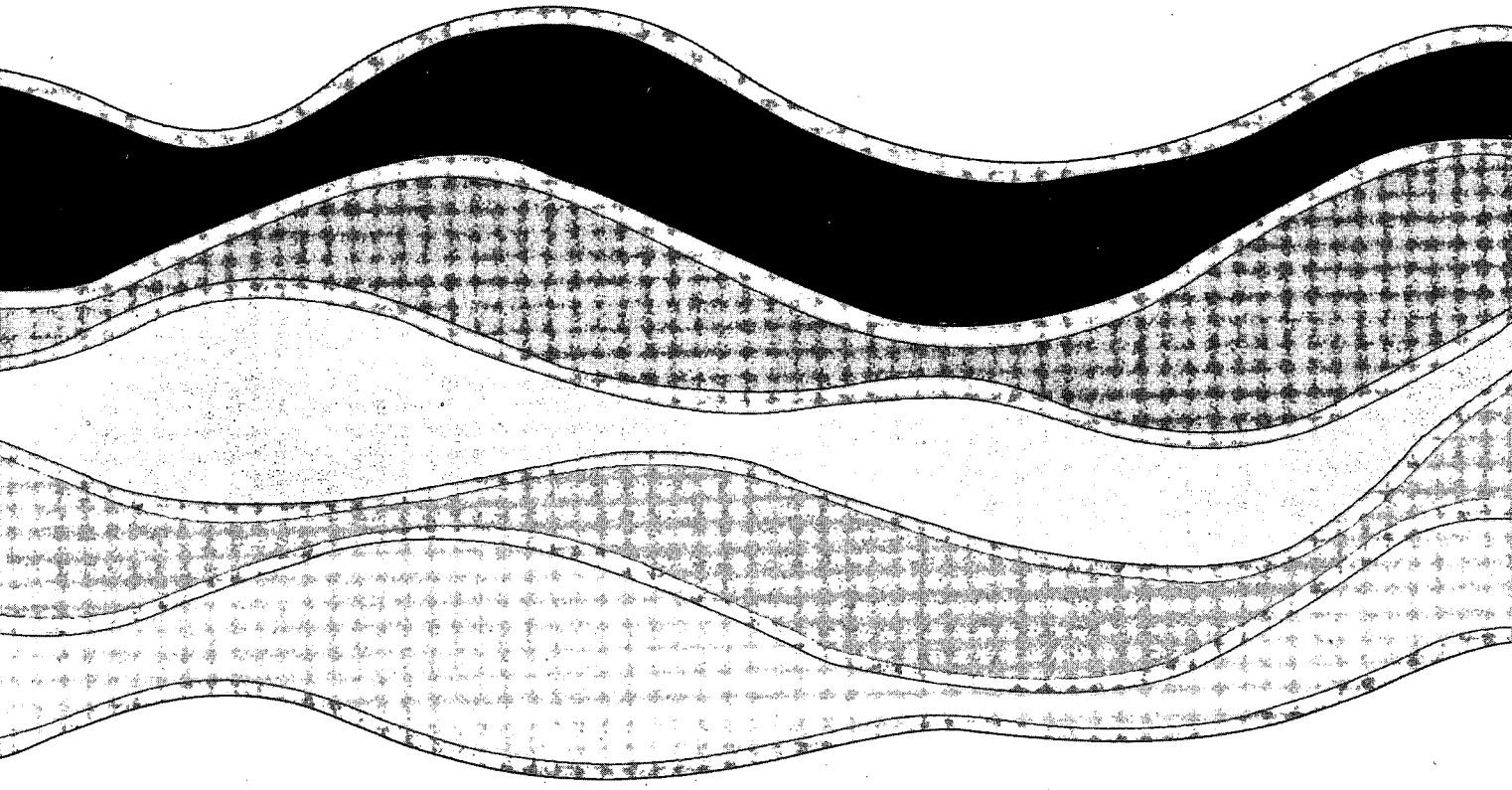

NITH RIVER BIOASSAY STUDY

B.J. Dutka, A. Jurkovic, R. McInnis,
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NWRI Contribution No. 91-73

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MANAGEMENT PERSPECTIVE

In a mass balance approach to the management of the Great Lakes, tributary loadings to the lakes need to be evaluated. One of the monitoring methodologies proposed for assessing tributary loading was the use of bioassay procedures to evaluate the bioavailability of toxicants/genotoxicants in tributaries. Since these contaminants would eventually impact on one of the Great Lakes, it is believed that knowing the degree of bioavailable toxicant loading at key upstream points and the percentage of this loading reaching the lakes, that this would provide modelers with a better insight into mass balance predictions.

For this study a minimally polluted river (Nith River), draining primarily an agricultural watershed was selected as the tributary representative of south western Ontario. The battery of tests approach combined with various simple extraction/concentration procedures was applied to the waters and suspended particulates to evaluate the sensitivity of these combinations to indicate the presence of bioavailable toxicants/genotoxicant. The results of this study indicated that only a portion of the bioassays were responsive in these agriculturally polluted waters. The most sensitive bioassays were the ATP-TOX System, SOS-Chromotest with and without S9 and the Nematode test.

SOMMAIRE À L'INTENTION DE LA DIRECTION

Si l'on choisit de gérer les Grands lacs en fonction des bilans massiques, il faut d'abord évaluer les charges dues aux tributaires. L'une des méthodes de surveillance proposée pour évaluer la charge dues aux tributaires consiste à utiliser des épreuves biologiques permettant d'évaluer la biodisponibilité des produits toxiques/génotoxiques dans les tributaires. Comme ces polluants finiront par avoir un impact sur l'un ou l'autre des Grands lacs, on pense qu'en connaissant la charge de produits toxiques biodisponibles en des points clés situés en amont ainsi que le pourcentage de cette charge qui atteint les lacs, les modélisateurs disposeraient d'une meilleure base pour prévoir les bilans massiques.

Pour les besoins de cette étude, on a choisi un cours d'eau présentant un degré minimal de pollution (rivière Nith), drainant principalement un bassin versant agricole, comme représentant des tributaires du sud-ouest de l'Ontario. On a appliqué la série d'essais combinés aux divers procédés d'extraction/concentration de manière à évaluer dans quelle mesure ces combinaisons étaient suffisamment sensibles pour déceler la présence des produits toxiques/génotoxiques biodisponibles. Les résultats de cette étude ont indiqué qu'une partie seulement des essais biologiques étaient sensibles dans ces eaux polluées par l'agriculture. Les essais biologiques les plus sensibles étaient les suivants : Système ATP-TOX, SOS-Chromotest avec et sans S9 et l'essai avec nématodes.

ABSTRACT

Studies were carried out to evaluate the battery of tests approach and the use of simple solvent extraction procedures on waters and suspended particulates within a relatively unpolluted agricultural area. Many of the bioassays used, which were very responsive in other more polluted areas, were non responsive with the samples collected from the Nith River. The bioassay tests which were responsive were the ATP-TOX System, SOS-Chromotest with and without S9 activation and the Nematode test (Panagrellus redivivus). Solvent extraction procedures using 100% DMSO, 100% methanol and 10% DMSO with 10% methanol were also shown to be equally effective in extracting toxicants/genotoxicants from suspended particulates. They also produced greater responses in the bioassays compared to pore water and Milli-Q water extracts.

RÉSUMÉ

On a cherché à évaluer l'approche consistant à utiliser la série d'essais et l'utilisation de simples procédés d'extraction par solvant avec des eaux et des particules en suspension provenant d'une région agricole relativement non polluée. Un grand nombre des essais biologiques utilisés, qui étaient très sensibles dans d'autres régions plus polluées, ne l'étaient pas dans le cas des échantillons prélevés dans la rivière Nith. Les essais biologiques sensibles étaient les suivants : Système ATP-TOX, SOS-Chromotest avec et sans activation de S9 et essai avec nématodes (Panagrellus redivivus). Les procédés d'extraction par solvant avec DMSO à 100 %, méthanol à 100 % et DMSO à 10 % avec méthanol à 10 % se sont révélés également efficaces en ce qui concerne l'extraction des produits toxiques/génotoxiques à partir de particules en suspension. On a également obtenu des réponses plus fortes avec les essais biologiques que dans le cas des extraits d'eau interstitielle et d'eau Milli-Q.

INTRODUCTION

In a mass balance approach to the management of the Great Lakes, tributary loadings to the lakes need to be evaluated. Such evaluations have been produced for selected streams in the past, however, the site-specific data obtained did not allow for a systematic development of a general methodology for tributary monitoring and load computations. Consequently it was proposed to refine the existing methodologies for evaluations of tributary loadings through a fundamental study of three interrelated problems, loading computations, ecosystem health indicators and monitoring procedures. All of these three tasks are listed in the Great lakes Water Quality Annex (GLWQA) as a federal responsibility.

One of the monitoring methodologies proposed for assessing tributary loading was the use of bioassay procedures to evaluate the bioavailability of toxicants/genotoxicants in tributaries. Since these contaminants would eventually impact on one of the Great Lakes, it is believed that knowing the degree of bioavailable toxicant loading at key upstream points and the percentage of this loading reaching the lakes, that this would provide modelers with a better insight into mass balance predictions.

One of the simpler procedures to estimate bioavailability of toxicants/genotoxicants is to establish the response that water, sediment and sediment extracts produce in various bioassays. A variety of short term bioassays hase been developed to assess the ecological impact of domestic and industrial effluents, land wash and airborne contaminants on waters and sediments (Bitton and Dutka 1986, Dutka and Bitton 1986. Liu and Dutka 1984). However, the application

of these short term bioassays to environmental samples soon revealed that there was no single test which was responsive to all contaminants or mixtures of contaminants (Bitton and Dutka 1986). This realization led to the concept of using a battery of tests to ascertain the bioavailability and impact of environmental contaminants.

Of the two main goals of this study, the major one was to evaluate a battery of toxicant/genotoxicant screening tests and propose a core group of tests which is responsive to the contaminants in this watershed. Realizing from many years' experience that the methods used to concentrate or extract contaminants from water and suspended particulates greatly influence the bioassay test results, the second goal of this study was to evaluate some simple quick inexpensive extraction/concentration procedures.

The Nith River which drains a typical agricultural area in south western Ontario (Province of Ontario) was selected as the study site (Fig. 1). A report of our findings is present below.

Study Site Area

The Nith River (Fig. 1) originates as small intermittent streams and ditches that drain the clay lands of Morington township east of the Milverton moraine. The Nith drains over 1146 square km and removes the equivalent of 31.24 cm of rainfall and with its tributaries drains the lands of eighteen townships.

Within the Nith River Basin there are several small towns and villages; Ayr, Drumbo, Millbank, Milverton, New Hamburg, Paris, Plattsville, Princeton and Wolverton, with a total population of approximately 29,000 people of which 25% are urban and 75% rural.

The main sources of pollution impacting the Nith River are agricultural practices and land runoff and the sewage from four sewage treatment plants; Ayr, Plattsville, New Hamburg and Petersburg.

Sampling Site

For this study the Nith River was sampled approximately 1 km downstream of the Ayr sewage treatment plant at a point where the oil pipe line crosses the river, longitude 80°30'25" and latitude 43°16'35".

Sample Collection

Three 20 litre samples of surface river water were collected for bioassay testing on August 1, 15 and Sept. 25/90. On the same dates, suspended particulates were also collected by means of an Alfa-Laval centrifuge. The water for the centrifuge was obtained by placing a submersible pump 0.3 m below the surface in the center flow point of the river. On Aug 1, 188 gm wet weight of suspended particulate slurry was collected, on Aug. 25 123 gm wet weight of suspended particulate slurry was collected and on Sept 25, 126 gm wet weight of suspended particulate slurry was collected after four hours of pumping at the rate of 6 litres per minute.

All waters and suspended particulates samples were maintained at 4°C until extraction/concentration procedures were initiated.

Concentration and Extraction Procedures

Water samples were concentrated 10 times (10x) and 25 times 25(x) by flash evaporation at 45°C using a Buchi Rotovapor EL.

Suspended particulates were extracted by the following procedures. In the first procedure the suspended particulates were allowed to settle out of the slurry. This was done by placing the slurry in a large graduated cylinder at 4°C and allowing settling to occur for 7 to 10 days. After settling, the surface water was carefully removed and the settled suspended particulate mass was slightly compressed to remove the trapped water. In the next step a specific weight of this suspended particulate mass (e.g. 2 gm wet weight) was centrifuged at 10,000 rpm at 4°C for 20 minutes and the supernatant (pore water) was carefully decanted for toxicity/genotoxicity testing. In the second procedure, this dewatered suspended particulate mass was weighed and Milli-Q water (Dutka and Kwan 1988) was added to the concentrated suspended particulate mass at the ratio of 1 gm sediment to 1 mL Milli-Q water, and then, after through mixing with a clean stainless steel spatala it was vigorously hand shaken for one minute. The slurry was then centrifuged at 10,000 rpm at 4°C for 20 minutes and the supernatant was used for toxicity/genotoxicity testing.

After the suspended particulate sediment was dewatered (above) portions of this sediment were subjected to various extraction procedures, which are described. Sediment was mixed with

(a) 100% dimethylsulfoxide (DMSO) in 1:1 ratio (solvent to sediment), hand shaken for 3 minutes, centrifuged at 10,000 rpm at 4°C for 10 minutes and the supernatant was collected and diluted to 1% DMSO with Milli-Q water for bioassay testing;

(b) 100% methanol in 1:1 ratio, hand shaken and centrifuged as above and the supernatant was collected and diluted to 1% methanol;

(c) solution of 10% DMSO and 10% methanol in 1:1 ratio, was mixed with the suspended particulate sediment in 1:1 ratio, hand shaken and centrifuged as above and the supernatant was collected and diluted to 1% solvent concentration, and

(d) 1% DMSO in 1:1 ratio (solvent:sediment), hand shaken and centrifuged as above and the supernatant was used directly in bioassay tests.

To evaluate our impression that flash evaporated water samples, while producing more concentrated samples for bioassay testing do not make the particle bound water insoluble chemicals, (which may have genotoxic or mutagenic effects) any more available for bioassay screening procedures, the following solvent-based extracting procedures were applied to 10x and 25x flash evaporated water samples. One or two mL volumes of 10x flash evaporated water sample were individually treated with equivalent amounts of 10% DMSO, 100% DMSO, 10% methanol or 100% methanol. Similarly 25x flash evaporated samples were treated with 10% DMSO, 100% DMSO, 10% methanol or 100% methanol. After mixing each sample was vigorously hand shaken for 2-3 minutes then slowly diluted with Milli-Q water additions until the mixture reached a 1% DMSO or 1% methanol concentration. These 1% concentration were subjected to SOS-Chromotest, Mutatox and Nematode survival and maturation tests.

Toxicity Screening Tests

Water samples were tested with the following bioassays previously described by Dutka et al. (1990),; ATP-TOX System, Microtox, Toxi-Chromotest, Mutatox with and without S9, SOS Chromotest with and without S9, ECHA dip stick, Daphnia magna, Ceriodaphnia dubia, Nematode (Panagrellus redivivius), Spirillum volutans and seed germination and root elongation. The Daphnia and Ceriodaphnia tests were performed on unconcentrated water samples while all the other tests were applied to 10x and 25x concentrated water samples.

Due to the small amounts of suspended particulates available after centrifugation; the battery of tests was reduced in the number of tests used. Some or all of the following bioassays were used to estimate contaminant bioavailability; ATP-TOX System, Mutatox with and without S9, SOS-Chromotest with and without S9, ECHA dip stick and Nematode.

RESULTS AND DISCUSSION

Water Samples 10x and 25x

Results of 10x and 25x concentrated water samples (flash evaporated) subjected to bioassay screening tests are shown in Table 1. From this table it can be seen that the following bioassays were non responsive to these samples; Daphnia magna, Ceriodaphnia dubia, Microtox, Toxi-Chromotest, Mutatox and ECHA dip stick.

ATP-TOX System results were unexpected as the replicated data of all three sets of sample indicated that with increasing concentrations

there was a decrease in toxicity. Also, the ATP-TOX data suggest that there was a variability in toxicant level during this eight week study.

The SOS-Chromotest, the genotoxicant assay, showed a negative response with the concentrated water samples when S9 was used, however, without S9 addition, the SOS-Chromotest results suggest that there was genotoxicants were present in all three samples, Aug. 1, 25 and Sept. 25. The sample with the greatest toxicant load was the Aug. 1 10x sample with an induction Factor of 2, and the second highest genotoxicant response was found in the Aug. 25 10x sample. Thus in two of the three samples, the 10x concentrated samples appear to contain the greatest toxicant loads although the 10x and 25x concentrates of Aug. 25 and Sept. 25 produced similar genotoxic responses. From the ATP-TOX System and SOS-Chromotest data it would appear that additional sample concentration, 10x to 25x, does not necessarily produce a greater toxic/genotoxic response in these bioassays. Two potential explanations for this observation may be the loss of some more firmly bound volatiles or the greater concentration of chemicals antagonistic to the toxic chemicals.

In the nematode, bioassay (Panagrellus redivivus)(Samoiloff 1990), contrary to ATP-TOX System and SOS-Chromotest results, there was a significant difference between 10x and 25x samples with the 25x being much more toxic than the 10x concentrated water samples. Similarly, maturation inhibition, which is believed to be a genotoxic effect (Samoiloff 1990) was inhibited by both 10x and 25x concentrates with the 25x concentrates having the greater effect. In the Sept. 25 sample the 25x concentrate completely inhibited the maturation of the few surviving nematodes.

Seed germination did not appear to be influenced by sample concentration. However, root length was affected by the 10x and 25x concentrated Nith River samples with the 25x samples appearing to have a slightly greater root length inhibiting effect in two of the three samples.

The Spirillum volutans 120 minute test for toxicants was positive in two 25x concentrates, Aug. 1 and Aug. 25, all other samples proved to be non toxic within the time frame of this test.

Using the point scoring scheme (Dutka 1988), even though we do not have sufficient data to accommodate the seed test in this scheme, it can be seen (Table 1), that all 10x samples were very similar and the 25x samples were also similar with the 25x samples having double the point score of the 10x samples. The greater point score by the 25x samples was mainly due to the strong responses seen in the nematode test and the Spirillum volutans tests.

Thus in this agriculturally oriented watershed, it would appear that the SOS-Chromotest without S9, the nematode and percent seed root length inhibition tests are the most responsive tests and could effectively screen the waters in this area for toxicants/ genotoxicants. Increasing sample concentration to 25x did effect the degree of response in some tests e.g. provided the only positive responses in the Spirillum volutans test, however, the benefit of evaluating two concentrations of water would be difficult to justify. By using the appropriate tests the less time consuming 10x sample concentrates probably give sufficient information to make judgements i.e. which sampling site requires more detailed studies.

Solvent Extracts of 10x and 25x Water Samples

In Table 2 the results of the various solvent extracting combinations are shown. The data and point scores indicate that there was litle or no difference between 10% and 100% concentrations of DMSO and Methanol, and if there was any preference or bias it would be toward the use of 100% concentrations of either solvent.

The Aug. 1 samples were the most responsive to the concentration - solvent procedures, which suggests that these samples may have contained low concentrations of particle-bound organic contaminants which were brought into solution by these procedures. By comparison, direct testing of the 10x and 25x flash evaporated concentrates provided a greater response than the concentrate-solvent procedures with the exception of the SOS-Chromotest with S9 addition. In Table 2 it can be seen in the Aug. 1 and Aug. 25 samples that there was a slight increase in some Induction Factors mainly in the 100% solvent extractions. Perhaps this is an indication that there are chemicals present in these samples which require metabolic enzyme activity (S9) to activate their effect.

From the data distribution pattern shown in Table 2, we suspect that the necessity of diluting the solvent (DMSO or Methanol) to a 1% level before bioassay testing negates the possibility of the solvents solubilizing strongly reacting organic compounds which are still reactive at the dilution required for testing.

In Table 3, the results of the various extracting procedures used on the suspended particulates are shown. Due to the small volume of concentrated suspended particulates it was decided to use only the ATP-TOX System and Microtox tests on the pore water and Milli-Q extracts.

Pore water ATP-TOX System results were similar and indicate a very low level toxic effect while the Microtox test was negative for toxicants in all three samples. Similar toxicant patterns were observed with the Aug. 1 and Aug. 25 Milli-Q water extracts, however, Sept. 25 Milli-Q water extracts indicated the strong presence of toxicants which induced responses in both the ATP-TOX System and Microtox test. Unfortunately a laboratory accident destroyed the remainder of the Sept. 25 suspended particulates and thus no data are available from the solvent treatment studies.

Extraction of the suspended particulates with 1% DMSO produced a negative set of data, very similar to 1% DMSO control results shown in Table 2. For the 100% solvents and 10% solvent mixture there appeared to be differences in solvent efficiency and the Aug. 25 samples appeared to have a greater toxicant/genotoxicant load than the Aug. 1 samples. All ATP-TOX System data from the solvent treated samples were 1.5 to almost 2 times greater than those observed in the pore water and Milli-Q water extracts. The Aug. 25 Microtox tested samples were all strongly positive while the Aug. 1 samples were negative which suggests that there was a new contaminant or greater concentration of previously not detected chemical during the Aug. 25 sampling period. The Aug. 25 SOS-Chromotest test data also supports this belief. Contrary to SOS-Chromotest results shown in Tables 1 and 2, Table 3 data indicate that the presence of S9 produces higher Induction Factors which suggests the suspended particulates are carrying different genotoxicants than are found in the water.

Realizing that only small volumes of suspended particulates are usually available for extracting and bioassay testing, we recommend the following bioassay tests in order of priority for use in these

agriculturally impacted waters: ATP-TOX System, SOS-Chromotest with and without S9, Nematode test and Microtox test. It would appear that 100% DMSO or Methanol or the 10% Methanol-DMSO mixture are all equally effective in extracting water insoluble toxicants/genotoxicants.

Overviewing Tables 1, 2 and 3, it can be seen that the ATP-TOX System, SOS-Chromotest with and without S9 and the Nematode test could be used as the core group of bioassays within the Nith River watershed. Auxillary supporting tests would be the Microtox and seed germination and root alongation tests.

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Figure 1. Nith River Basin and Sampling Site

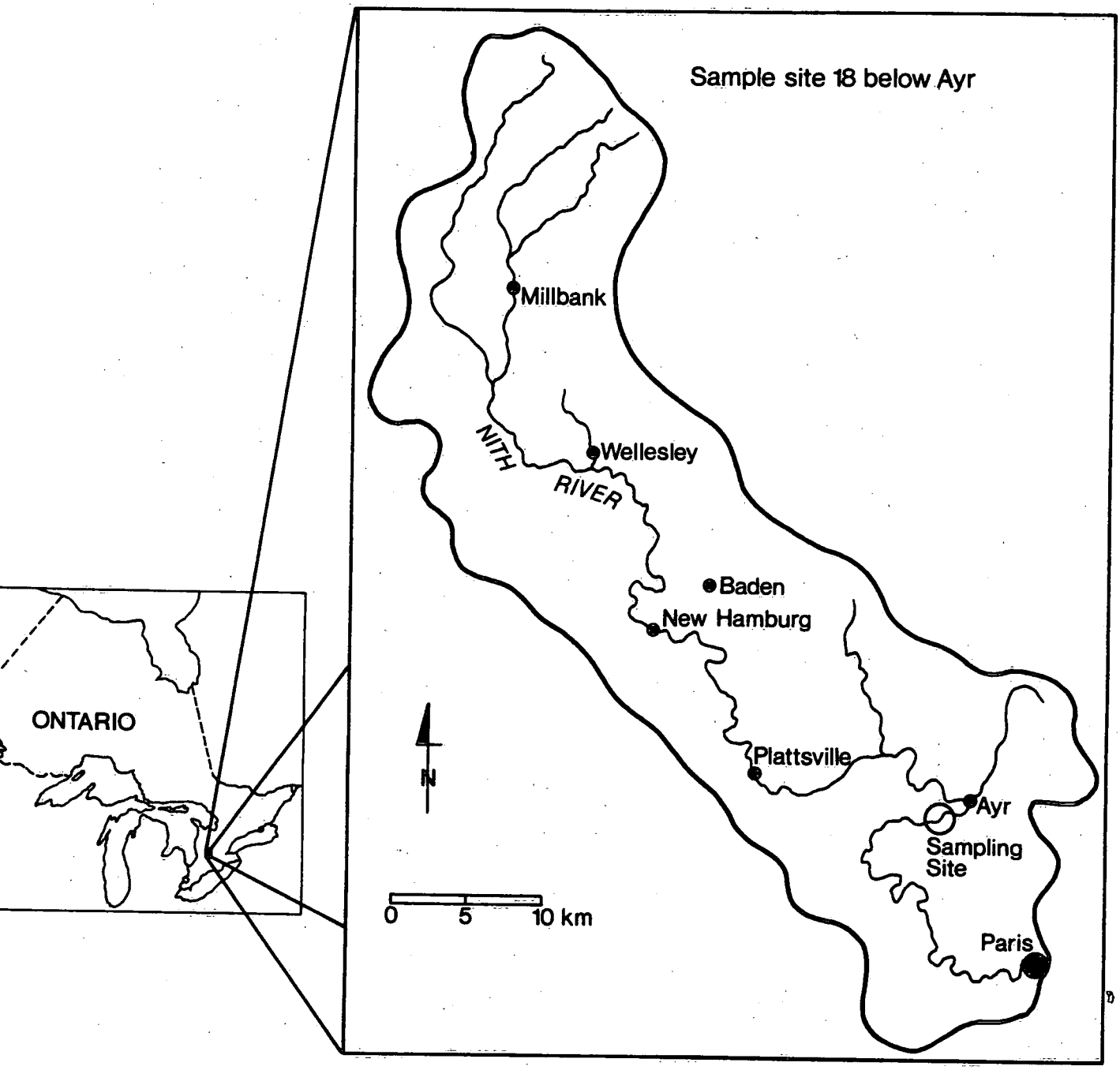
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Table 1. Bioassay Responses to Nith River Water Samples.

Date	Concentration	ATP-TOX % Inhibition	SOS-Chromotest Induction Factor -S9	SOS-Chromotest Induction Factor +S9	Nematode % Survival	Nematode % Maturation	Seed Test Percent Seed Germination	Seed Test Percent Root length Inhibition	Spirillum volutans 120 min test	Points[1]
Aug. 1	10x	36.2	2.0	0.8	85	76.9	100	75.5	negative	10
	25x	24.3	1.2	1.2	28	27.3	94.7	65.4	positive	24
Aug. 25	10x	20.8	1.6	1.0	93	59.1	100	69.8	negative	11
	25x	19.5	1.5	0.9	29	40.0	105	69.5	positive	22
Sept. 25	10x	37.8	1.2	1.0	92	50.0	105	64.1	negative	9
	25x	10.0	1.3	1.0	17	0	94.7	54.2	negative	23

The following tests did not show a positive (toxic or genotoxic) response:
Daphnia magna, Ceriodaphnia dubia, Microtox, Toxi-Chromotest, Mutatox, ECHA Dip stick.
Points[1] - no point allocation established for seed test.

Table 2. Comparison of solvent extracts of 10x and 25x flash evaporated concentrates from Nith River water.

Date	Concentration Flash Evaporation	Solvent Concentration	Mutatox ± S9	SOS Chromotest Induction Factor -S9	SOS Chromotest Induction Factor +S9	Nematode % Survival	Nematode % Maturation	Nematode Points
Aug. 1	10X	-	neg	2.0	0.8	85	76.9	9
	10x	10% DMSO	neg	1.0	1.2	79.8	50.8	10
	10x	100% DMSO	neg	1.0	1.3	63.6	70.9	10
	10x	10% Methanol	neg	1.0	0.8	88.9	81.8	3
	10x	100% Methanol	neg	1.1	1.1	79.8	62.3	8
	25x	-	neg	1.2	1.2	28	27.3	19
	25x	10% DMSO	neg	1.0	1.2	83.8	49.3	8
	25x	100% DMSO	neg	1.0	1.3	68.7	52.8	12
	25x	10% Methanol	neg	0.9	1.0	88.9	27.5	9
	25x	100% Methanol	neg	1.1	1.3	71.7	47.2	12
Aug. 25	10x	-	neg	1.6	1.0	93	59.1	11
	10x	10% DMSO	neg	1.0	1.2	90.9	88.1	3
	10x	100% DMSO	neg	1.1	1.1	85.9	94.7	3
	10x	10% Methanol	neg	1.1	1.0	99.0	84.4	3
	10x	100% Methanol	neg	1.1	1.0	92.9	90.2	2
	25x		neg	1.5	0.9	29	40	18
	25x	10% DMSO	neg	1.0	1.1	81.8	92.0	3
	25x	100% DMSO	neg	1.1	1.1	76.8	91.9	5
	25x	10% Methanol	neg	1.0	1.2	87.9	95.4	3
	25x	100% Methanol	neg	1.1	1.1	84.8	94.9	3
Sept. 25	10x		neg	1.2	1.0	92	50	7
	10x	100% DMSO	neg	1.1	1.1	90.9	81.9	3
	10x	100% DMSO	neg	1.1	1.0	91.9	80.3	3
	10x	10% Methanol	neg	1.1	0.8	92.9	97.7	1
	10x	100% Methanol	neg	1.0	1.0	94.9	95.2	2
	25x		neg	1.3	1.0	17	0	24
	25x	10% DMSO	neg	1.0	1.1	86.9	76.4	4
	25x	100% DMSO	neg	1.1	0.9	87.9	88.1	3
	25x	10% Methanol	neg	1.0	0.9	90.9	77.5	4
	25x	100% Methanol	neg	1.1	1.0	85.9	76.6	4
	Control	1% DMSO	neg	.9	.8	99.0	97.9	0
	Control	1% Methanol	neg	.9	.8	99.0	95.9	0

Table 3. Bioassay Responses to Extracts from Nith River Suspended Particulates

Date	Sample	ATP-TOX % Inhibition	Microtox EC_{50} %mL	Nematode % Survival	Nematode % Maturation	SOS-Chromotest Induction Factor -S9	SOS-Chromotest Induction Factor +S9	Points
Aug. 1	Pore water	23.1	neg	-	-	-	-	1
Aug. 25		19.1	neg					1
Sept. 25		24.4	neg					1
Aug. 1	Milli-Q water	22.4	neg					1
Aug. 25		20.3	neg					1
Sept. 25		72.3	46.9					6
Aug. 1	100% DMSO	31.5	neg	82.8	45.7	.8	1.2	10
Aug. 25		33.2	21.8	80.8	82.6	.9	1.6	15
Aug. 1	100% Methanol	36.3	neg	78.8	56.5	.9	1.2	12
Aug. 25		31.7	12.4	77.8	73.9	1.0	2.0	19
Aug. 1	10% DMSO+10%	40.8	neg	78.8	68.2	.9	1.1	10
Aug. 25	Methanol	43.8	17.6	81.8	60.9	1.2	1.3	15
Aug. 1	1% DMSO	1.7	neg	99.0	97.9	.9	.8	0
Aug. 25		neg	neg	99.0	95.9	.8	.9	0

The following tests did not show a positive (toxic or genotoxic) response.
Mutatox with and without S-9, ECHA Dip Stick.

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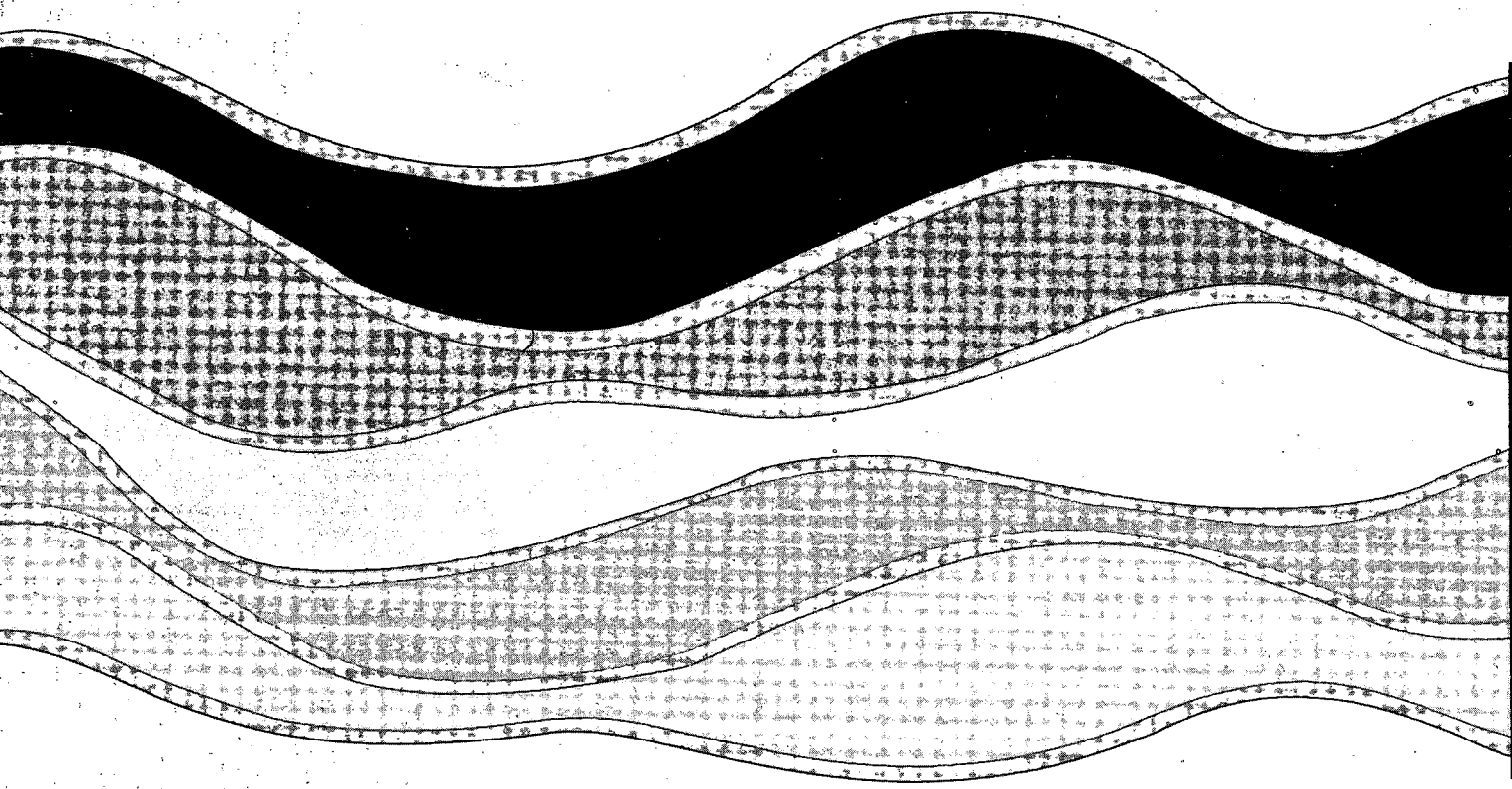

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