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BATTERY OF SCREENING TESTS APPROACH APPLIED TO SEDIMENT EXTRACTS

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ABSTRACT

This paper reports the application of a battery of microbiological and toxicant screening tests to sediments collected from the Port Hope area. The sediments were extracted using Milli Q water and solvents. The solvent extracts indicated that the whole sampling area has been impacted by toxic chemicals. The <u>Daphnia</u> <u>magna</u> test was the most sensitive screening test in Milli Q water extracts. Microbiological studies indicated that the sampling area was and is continuing to be impacted by fecal pollution.

RÉSUMÉ

La présente communication décrit l'utilisation d'une batterie d'essais de dépistage d'agents microbiologiques et de toxiques pour des sédiments recueillis dans la région de Port Hope. Les sédiments on été soumis à l'extraction à l'aide d'eau Milli Q et de solvants. Les produits d'extraction au solvant ont révélé que toute la zone d'échantillonnage subissait les effets des produits chimiques toxiques. L'essai Daphnia magna constitue le moyen de dépistage le plus sensible pour les produits d'extraction à l'eau Milli Q. Les études microbiologiques ont montré que la zone d'échantillonnage est et continue à être contaminée par la pollution fécale.

MANAGEMENT PERSPECTIVE

A study of the Port Hope area sediments was carried out at the request of Environmental Protection, Ontario Region, Conservation and Protection, Department of Environment, to confirm earlier studies by the authors, that indicated the presence of toxicants in some unsuspected areas. A battery of microbiological and toxicant stress indicating tests were carried out on 19 sediments. These sediments were extracted by two procedures, Milli Q water and solvents (dichloromethane and DMSO).

Results of these studies showed that the Daphnia magna test was positive for toxicant activity (10 sediments) while in the solvent extracted sediments all of the samples tested were positive for toxicant stressing activities by the Spirillum volutans and algal growth inhibition tests and only one sample was negative by the Microtox test.

In summation, the application of the "battery of tests approach" to the sediments of Lake Ontario at Port Hope and Port Hope Harbour indicated that the whole area is impacted with toxicants and microbiological pollution of fecal origin with the area west of the sewage treatment plant being the most impacted. The make up of the "battery of toxicant screening tests" is very critical as noted by the results of the water extracted sediments. Daphnia magna is apparently sensitive to a range or concentration of chemicals to which the other tests are not. The Daphnia magna Milli Q water extract results were useful predictors of the high toxicant activity in the sediment solvent extracts as measured by the Microtox Test.

It is recommended that the waters and sediments of the Port Hope are be more thoroughly evaluated by the "battery of tests approach" to ascertain the true dimensions of the impacted area.

PERSPECTIVES DE GESTION

Une étude des sédiments de la région de Port Hope a été effectuée à la demande de la Protection de l'environnement, région de l'Ontario, Conservation et Protection, Environnement Canada, pour confirmer les résultats de recherches antérieures par les auteurs, qui avaient révélé la présence d'agents toxiques dans certaines régions non suspectées. Une batterie d'essais microbiologiques et de toxicité ont été effectués sur 19 échantillons de sédiments. Ces derniers ont été soumis à des extractions à l'aide d'eau Milli Q et de solvants (dichlorométhane et DMSO).

Les résultats de ces études ont montré que l'essai avec Daphnia magna était positif pour l'activité toxique (10 sédiments); les produits d'extraction des sédiments aux solvants ont tous donné des résultats positifs pour l'activité toxique, grâce aux essais avec Spirillum volutans et d'inhibition de la croissance algale; seulement un échantillon a donné un résultat négatif par essai Microtox.

En résumé, l'application de la technique de la "batterie d'essais" aux sédiments du lac Ontario de Port Hope et du port de Port Hope a révélé que toute la zone était contaminée par des toxiques et une pollution microbiologique d'origine fécale, la zone située à l'ouest de la station d'épuration des eaux usées étant la plus touchée. La composition de la "batterie d'essais pour le dépistage de toxiques" est très importante, comme le montrent les résultats des sédiments après extraction à l'eau. Il semble que Daphnia magna soit sensible dans un certain intervalle de concentration de produits chimiques, sensibilité que l'on ne retrouverait pas dans les autres essais. Les résultats de l'extraction à l'eau Milli Q, avec Daphnia magna, étaient très utiles pour prévoir l'activité toxique élevée dans les produits d'extraction aux solvants pour les mêmes sédiments, selon l'essai Microtox.

Il est proposé que les eaux et les sédiments de la région de Port Hope fassent l'objet de recherches et de mesures plus approfondies grâce à la "batterie d'essais" pour déterminer avec certitude l'étendue réelle de la zone contaminée.

APPLICATION OF THE BATTERY OF SCREENING TESTS APPROACH TO SEDIMENTS IN THE PORT HOPE AREA OF LAKE, ONTARIO, CANADA

INTRODUCTION

A series of studies were initiated in 1985 to identify degraded or degrading waterbodies by using a variety of microbiological, biochemical and bioassay tests (Dutka et al. 1986). The tests used in these environmental studies were also being evaluated as potential candidates for a "battery of test procedures" which could be used nationally or internationally to prioritize water bodies, sediments or selected areas within water bodies for remedial action or further investigation. It is also believed that this battery of tests could be used to indicate qualitative changes in conditions due to remedial action activities.

Port Hope, situated on Lake Ontario at the mouth of Ganaraska River was one of the areas sampled during the 1985 studies (Dutka et al. 1986) and several of the sediment sampling sites were found to show toxicant activity by some of the tests used. As Port Hope has been classified by the International Joint Commission (IJC) Great Lakes Water Quality Board, as a class B area of concern (Lake Ontario Surveillance Plan 1984) it was decided to more thoroughly evaluate the extent of the impacted area by pollutants entering and exiting Port Hope Harbour.

rotary evaporator to 1 mL. The sample was transferred to a test tube along 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 test containing 250 mL DCM plus 1.0 mL DMSO evaporated to 1.0 mL DMSO. A method blank was also prepared as control and contained 250 mL DCM plus 1.0 mL DMSO, shaken, filtered and evaporated as per total sample procedure. One sample, #11, was broken in the solvent extraction procedure and there was insufficient sediment to replace this sample.

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

Microorganism Tests

Fecal coliform MPN tests using A1 broth and Clostridium perfringens MPN tests using DRCM medium with confirmation in litmus milk were applied to each sediment (Dutka et al. 1987).

A uranium refining facility has been operating at Port Hope for a number of years. This plant takes water from Port Hope Harbour and discharges it as waste heat with some process wastes. As a result of the discharges of the Ganaraska River and harbour waters to Lake Ontario, the lake immediately adjacent to the harbour often contains unacceptable levels of gross and radiation and occasionally exceeds the limit for uranium (Lake Ontario Surveillance Plan 1984).

In addition, the Port Hope Harbour area has seen other industrial operations until very recently. Coal was stockpiled in the harbour area for shipment to inland markets, until the late 1960's, and until the seventies the turning basin received site drainage from agricultural fertilizer and feed grain industries as well as from a metal fabricating facility (McKee et al. 1985).

This report details the results of the application of toxicant, genotoxicant and microbiological screening tests to the sediments of Port Hope Harbour and surrounding area in April 1987.

METHODS

Sampling Sites

A total of 19 sediment collection sites were selected for this study (Fig. 1, Table 1) with sites 1 and 18 being selected as background level stations.

Sediments

All sediments were collected with an Ekman dredge. Usually several casts were required to obtain sufficient surface layer sediment (1-2 cm). At each site the surface layers were pooled, mixed and aliquots dispensed into containers for each group of testing procedures and the containers were then stored in melting ice, until processing (Dutka et al., 1986a).

Prior to performing toxicant screening tests the sediment samples from each site were homogenized and split into two portions.

One porition of the sediment was extracted with Milli Q water (4 cartridge system-1, Super C carbon cartridge, 2, Ion-Ex™ cartridges, 1, Organet-Q® cartridge and a Milli-Stak™ filter with a glass distilled water feed), by mixing sediment and Milli Q water in a 1:1 ratio, shaking vigorously for 3 minutes, then centrifuging at 500 rpm in a refrigerated centrifuge for 10 minutes. The supernatant was used in toxicity screening tests.

A 100 gram portion of the above water extracted sediment was freeze dried, then weighed on prefired aluminium foil (550°C overnight). The weighed freeze-dried sample was added along with 250 mL Dichloromethane (DCM) into a 1L Erlenmyer flask, which was prerinsed twice with DCM, and shaken for approximately 24 hr on a Burrell wrist action shaker at position #2. After settling overnight, the samples were filtered overnight through prewashed Na_2SO_4. To the filtrate 1.0 mL DMSO was added and the samples were evaporated in a

Toxicant Screening Tests

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

RESULTS

Latitudes and longitudes of the Port Hope sampling sites are presented in Table 1. Also shown in the Table is the composition of each sediment based on particle size distribution by sieve analyses. From Table 1 it can be seen that the majority of sediments were mainly composed of sand. Only sediments 8, 9, 9A and 15 had a significant portion of silt and clay. Sediments 8, 9 and 9A were all collected within the harbour area.

The format used to award points for specific data values, in order to rank the sediments from areas of most concern to least, is presented in Table 2. The point allocation scheme is biased, and not scientifically defensible, however it reflects the authors' experience with various levels of toxicant activity and health related bacterial densities in Canadian waters and sediments. The present point allocation scheme is a viable process which may change with increased data accumulations.

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

Table 3 is a complex table which presents all the microbiological and toxicological data obtained from the unaltered sediments. All toxicological data are based on 1:1 sediment:Milli Q water extracts. In this table it can be seen that the Microtox, Algal ATP and Spirillum volutans tests were all negative. Although there are indications of toxicant activity as shown by occasional slight increases above the normal control levels in the ATP-TOX System and SOS Chromotest, the level of toxicant activity is still considered to be in the doubtful range. Only the Daphnia magna, 48 hour acute toxicity test indicated the unqualified presence of toxicant activity in samples 3, 4, 5 and 9A. Samples 1, 2, 7, 8, 17 and 18 also indicated the presence of lower toxicant activity, EC20, EC30 and EC40 levels in the 100% concentrations of the Milli Q extract.

Fecal coliform densities, indicators of relatively recent fecal pollution, were greater than 1000/10 gm wet weight of sediment at stations 3, 4, 8, 9A and 15. Clostridium perfringens, microbial indicators of historical and ongoing pollution were found in all sediments, with sediments 4, 8, 9, 9A and 15 having densities greater than 100 spores/10 gm wet weight of sediment.

Table 4 presents the results of toxicant screening tests performed on organic extracts of the sediments. The tests to ascertain the presence of chemicals which could stress or inactivate the screening organisms were all performed on a 1% DMSO suspension of the extracted sediments originally suspended in 10 mL of 100% DMSO. In this table it can be seen that all the samples tested by the Spirillum volutants and algal growth inhibition tests were positive for toxicants. With the exception of sample no. 8 all the samples tested by the Microtox test indicated the presence of toxicants or stressing agents. The SOS Chromotest was negative in all samples while the ATP-TOX System tests indicated the presence of low grade toxicant stresses in all samples except nos. 5, 6 and 7. The toxicant levels shown by the ATP-TOX System are considered to be within the doubtful range.

DISCUSSION

In this study, all the sediment water extracts when tested for toxicant or stressing activity by the Microtox test, Algal ATP test, ATP-TOX System, Spirillum volutans test and the SOS Chromotest, were essentially negative. In an earlier study (Dutka et al. 1986) of the Port Hope area, two stations 9M and 10 which were similar to 9A and 12 (this study) were found to be negative for toxicant activity as measured by the Microtox test. Thus this study, also using water extracted sediments, supported the findings of the 1985 study.

However, when Daphnia magna were used to screen for toxicants using these water extracts, EC50 values of 70%, 50%, 40% and 30% were found. Also, using undiluted water extracts, sample nos. 1, 2, 6, 7, 17 and 18 indicated values of 20%, 30% and 40% (not listed in order). Thus without the Daphnia magna test, these water extracted sediments would have been reported as negative, and these data would have supported the first study (Dutka et al. 1986) indicating that toxicants were only found within the inner harbour sediments.

These findings are very supportive of our current practise of using a battery of tests to evaluate samples for the presence of toxicants or stressing activities. The problem lies in trying to establish a minimum battery of tests which would provide maximum information with the fewest number of tests and thus lowest cost.

In all previous studies by our laboratory, toxicant activity estimations had been carried out on water, effluents and water extracted sediments. This practice was followed due to problems with organic extraction techniques and the toxicity of the solvent carrier. In laboratory studies, DMSO at the 1% level was found not to be toxic to the various organisms used in our battery of screening tests, nor had a synergistic effect been noted between toxicant and 1%

DMSO. Therefore in this study organic extracts of the sediments were also tested. The format observed was to extract 100 gm of sediment 1:1 with Milli Q water, then use the same 100 grams of sediment for freeze drying prior to extraction with dichloromethane with final resuspension in 10 mL of 100% DMSO. In Table 4 the dramatic difference between water extracts and organic solvent extracted sediments can be seen. For instance, with the exception of sample no. 8, all the extracts were positive for toxicant activity via the Microtox test with sample nos. 3, 5, 6 and 13 being the most toxic. All the algal growth inhibition tests and S. volutans tests were also positive for toxicant activity. The SOS Chromotest was still negative for genotoxicity while the ATP-TOX System indicated increased activity but no definite positives for toxicants. Daphnia magna was not tested with the DMSO extracts as we have insufficient data on the effect of DMSO on Daphnia magna and the sample volume was too small.

From the solvent extracts, it can be seen that the whole sampling area is contaminated with chemicals having toxic activity and especially the area west of the sewage treatment plant outfall.

Examination of Tables 3 and 4, indicates that there are two major areas of concern, based on toxicant activity tests and fecal pollution indicators; the area of first concern would be that adjacent and west of the sewage treatment plant and the other is the mouth of the inner harbour (9A) through which the contaminated inner harbour sediments and water move into Lake Ontario.

The bacteriology data and especially Clostridium perfringens densities would lead one to suspect that the whole sampling area has been and is continuing to be impacted by sewage. Only a few sampling sites seem to be out of the main flow of microbial contaminants, e.g. nos. 16, 17, 18 and 6 and 11, and yet three of these stations, 6, 17 and 18, indicate the presence of a high level of toxicant activity in the solvent extracted sediments.

Sediments sample nos. 3, 4, 5 and 6 were almost totally composed of sand (Table 1) and yet ranked 3, 2, 3, and 9 based on water extracts and 1,1, 3 and 4 based on solvent extracts (Tables 3 and 4). Samples 8 and 9A which had the greatest concentration of silt and clay, ranked 4 and 1 based on water extracts and 7 and 3 based on solvent extracts. Accepting the premise that silt and clay are usually the major sites for bacterial and chemical adsorption, it may be surmised that sampling sites 3, 4, 5 and 6, if they had a different sediment composition, i.e. silt and clay, or if they had been sampled more finely, removing only the top 1-2 mm, would have contained a much greater toxicant load than samples 8 and 9A. The source of this toxicant load would appear to be the sewage treatment plant, or a combination depending on current flow, of Port Hope Harbour discharges and sewage treatment plant effluent.

In summation, the application of the battery of tests to the sediments of Lake Ontario at Port Hope and Port Hope Harbour indicated that the whole area is impacted with toxicants and microbial pollution of fecal origin with the area west of the sewage treatment plant being the most impacted. The make up of the "battery of toxicant screening tests" is very critical as noted by the results of the water extracted sediments (Table 3). Daphnia magna appears to be, in part, sensitive to a range of chemicals to which the other tests are not. The Daphnia magna water extract results were useful predicors of the high toxicant activity in the sediment solvent extracts as measured by the Microtox test. It is recommended that the waters and sediments of the Port Hope area be further evaluated by the "battery of tests approach" to ascertain the dimensions of the impacted area.

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Table 1. Port Hope Sampling Site Location and Sediment Description.

Sample Number	Latitude	Longitude	Sediment Size Distribution Based on Sieve Analysis			Comments
			% Gravel	% Sand	%Silt and Clay	
1	43°57'06"	78°15'54"	.00	99.35	.65	
2	43°57'01"	78°15'10"	.10	99.46	.44	
3	43°56'56"	78°16'24"	.02	99.86	.12	
4	43°56'52"	78°16'40"	.24	99.53	.23	
5	43°56'49"	78°16'54"	.03	99.77	.20	
6	43°56'39"	78°17'26"	.00	99.82	.18	
7	43°56'49"	78°17'33.5"	8.23	90.19	1.58	
8	43°56'31"	78°17'31"	.82	54.86	44.32	organic matter present
9	43°56'25"	78°17'27"	.00	86.65	13.35	
9A	43°56'39"	78°17'34.5"	5.21	57.11	37.68	organic matter present
10	43°56'19"	78°17'26.5"	.01	98.80	1.19	
11	43°56'25"	78°17'32.5"	.07	99.63	.30	
12	43°56'27"	78°17'36.5"	.02	99.46	.52	
13	43°56'21"	78°17'48"	.04	98.19	1.77	
14	43°56'29"	78°17'46.5"	.00	94.54	5.46	
15	43°56'29"	78°17'55"	.00	84.19	15.81	
16	43°56'30"	78°18'04"	.11	99.82	.07	
17	43°56'21"	78°18'12"	.00	99.88	.12	
18	43°55'57"	78°20'48"	.02	99.89	.09	

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

Fecal Coliform Sediment 10g/100 mL	*Clostridium perfringens* sediment 10 g /100 mL MPN	Points	Genotoxicity Induction Factor per 1:1 Milli Q Water Extract 1% DMSO	Algal-ATP % Relative light Units per 1:1 Milli Q Sediment Extract	Points
<100	<25	1	1.0-1.29	100-50	1
101-500	26-100	2	1.30-1.50	49-20	3
501-2500	101-500	3	1.51-2.0	19-1.0	5
2500-16000	501-2500	4	2.1-3.0	.9-.1	7
16000-160000	2501-10000	7	3.1+	.09-.01	10
160000+	10000+	10			

ATP-TOX System % Inhibition per mL sediment extract or 10x water	Microtox			*Daphnia magna*		*Spirillum volutans* and Algal Growth Inhibition Test			
	EC50/g wet wt Sediment	EC50 1%DMSO Extract	Points	EC/ 1:1 Milli Q Extract (100%)	Points	Sediment Extract 1:1 Milli Q Water	Points	Sediment Extract 1% DMSO	Points
1-30	.4+	40.0+	1	EC20 at 100%	1	neg	0	neg	0
31-60	.40-.31	40-25.0	3	EC40 at 100%	2	+	10	+	5
61-90	.30-.21	24-10.0	5	EC50 at 100%	4				
91-99	.20-.11	9-1.0	7	at 75%	5				
100	<.10	<1.0	10	at 50%	6				
				at 25%	8				
				at 10%	10				

Table 3. Results of Port Hope Area Sediment Analyses - Water Extracts.

Sample number	Fecal Coliform A1 Broth MPN 10g/100 mL	*Clostridium perfringens* 10g/100 mL MPN	Microtox EC50 /g.wet wt	Algal-ATP RLU[3] %	*Spirillum volutans* 120 min test	ATP-TOX System % Inhibition	SOS Chromotest[2] Induction Factor	*Daphnia magna* EC50 % 1:1 Extract	Points	Rank
1	330	13	neg	neg S[10]	neg	0	1.1	0[4]	6	6
2	170	8	neg	neg S	neg	0	1.2	0[5]	5	7
3	4900	21	neg	neg S	neg	7.8	1.1	50	11	3
4	11000	240	neg	neg S	neg	16	1.1	40	15	2
5	790	13	neg	neg S	neg	33	1.3	70	15	2
6	79	7	neg	neg S	neg	0	1.0	0	3	9
7	130	33	neg	neg S	neg	0	1.1	0[6]	6	6
8	4900	170	neg	neg S	neg	14	0.9	0[7]	9	4
9	700	920	neg	neg S	neg	23	1.2	0	9	4
9A[1]	3300	2200	neg	neg S	neg	37	1.0	30	18	1
10	11	170	neg	neg S	neg	0	1.0	0	4	8
11	49	17	neg	neg S	neg	0	1.1	0	3	9
12[2]	350	33	neg	neg S	neg	0	1.2	0	5	7
13	330	48	neg	neg S	neg	0	1.0	0	5	7
14	790	39	neg	neg S	neg	0	1.2	0	6	6
15	2200	280	neg	neg S	neg	0	1.1	0	7	5
16	49	5	neg	neg S	neg	0	1.1	0	3	9
17	17	11	neg	neg S	neg	16	1.2	0[8]	5	7
18	79	5	neg	neg S	neg	0	1.1	0[9]	4	8
[1]9M 1985	>1600	-	neg				.9			
[2]10 1985	>1600		neg				.9			

[3]RLU = relative light units

[4]EC40 at 100% [5]EC20 at 100% [6]EC30 at 100%

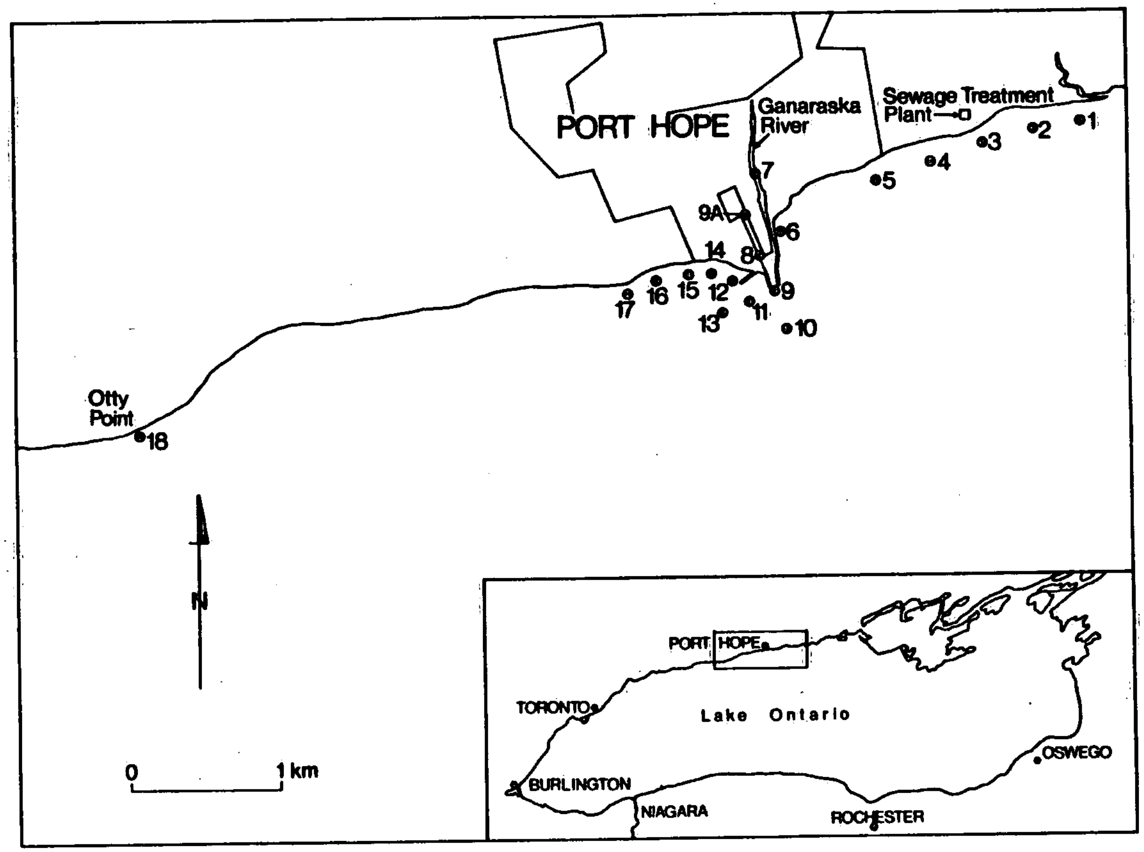
[7]EC30 at 100% [8]EC20 at 100% [9]EC20 at 100%

[10]No toxicity ATP production stimulated

Table 4. Results of Port Hope Area Sediment Analyses - Organic Extracts.

Sample number	Microtox EC50 per 1% DMSO Extract	Algal Growth Inhibition in 1% DMSO Extract	*Spirillum volutans* 120 min test in 1% DMSO Extract	SOS Chromotest Induction Factor	ATP-TOX System % Inhibition in 1% DMSO	Points[1]	Rank
1	16.29	positive	positive	.9	20	19	7
2	4.37	positive	positive	.9	28	21	5
3	0.72	positive	positive	.9	16	26	1
4	6.25	positive	positive	.9	32	26	1
5	0.50	positive	positive	.9	0	24	3
6	0.35	positive	positive	.9	0	22	4
7	1.04	positive	positive	.9	0	20	6
8	negative	positive	positive	.9	11	19	7
9	29.23	positive	positive	.9	8	21	5
9A	16.25	positive	positive	.9	22	24	3
10	15.85	positive	positive	.9	14	20	6
11	LOST	LOST	LOST	LOST	LOST		
12	21.51	positive	positive	.9	17	20	6
13	0.70	positive	positive	.9	7.5	25	2
14	26.78	positive	positive	.9	20	19	7
15	34.44	positive	positive	.9	27	20	6
16	26.08	positive	positive	.9	27	16	8
17	2.15	positive	positive	.9	21	20	6
18	3.46	positive	positive	.9	38	22	4

[1]Microbiological data included in point total.


PORT HOPE
Ganaraska River
Sewage Treatment Plant
1
2
3
4
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9A
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Otty Point
18
N
0
1 km
PORT HOPE
TORONTO
Lake Ontario
OSWEGO
BURLINGTON
NIAGARA
ROCHESTER

Fig. 1

Fig. 1 Port Hope Area Sediment Sampling Sites, April 1987.