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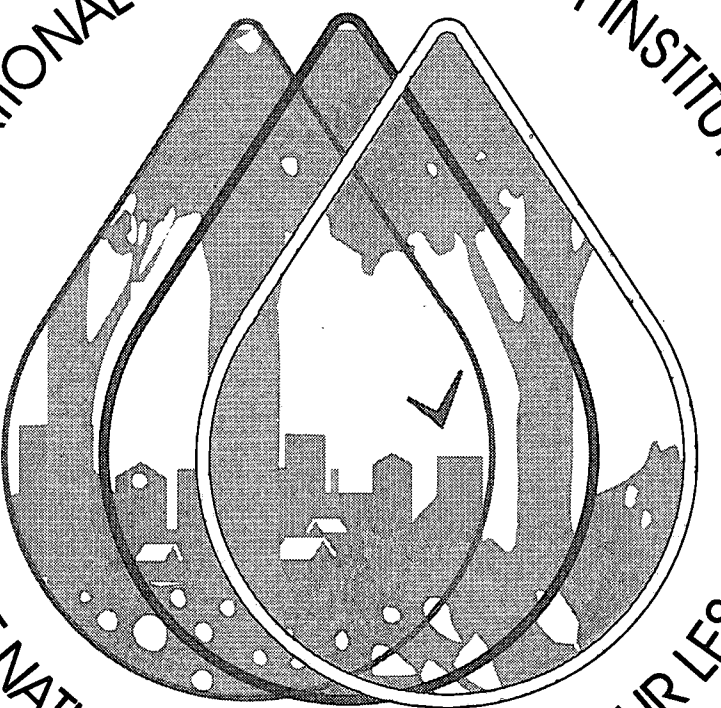

INSTITUT NATIONAL DE RECHERCHE SUR LES EAUX

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NWRI Contribution No. 96-157

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MANAGEMENT PERSPECTIVE

In previous studies the toxicant load of inlet and outlet sediments were evaluated in several Toronto area stormwater ponds. After these studies were completed Dutka and co-workers developed a direct sediment genotoxicity test. Because the history of the stormwater pond sediments was known it was believed they would provide ideal genotoxicological conditions to evaluate the direct sediment genotoxicity test versus the genotoxicity of an organic extract of the sediment.

Complete and unequivocal preservation of samples, whether domestic wastewater, industrial wastes, natural waters or sediments for microbiological or bioassay testing, is a practical impossibility. Realizing the problem, the effects of freezing and thawing on sediments as they relate to bioassay results were investigated by incorporating this preservation feature into a study designed to evaluate the relative sensitivities of direct sediment bioassays and bioassays performed on sediment extracts and the relative toxicant/genotoxicant load of inlet and outlet sediments at three Toronto area stormwater ponds.

The bioassay responses to stored sediments were very bioassay specific as two bioassays supposedly measuring similar effects often produced diametrically opposite results. There is a suggestion that the two week results are as different from the original samples as they are from the four week results. Also four week storage results are not predictably lower or higher than original sample results.

The superior sensitivity of the direct sediment SOS-Chromotest procedure over genotoxicity tests performed on sediment extracts is well illustrated in this comparison study and confirms earlier observations by Dutka and his co-workers.

SOMMAIRE À L'INTENTION DE LA DIRECTION

Lors d'études précédentes, Dutka et collaborateurs ont évalué la charge des substances toxiques dans les sédiments à l'entrée et à la sortie de plusieurs bassins d'eaux pluviales situés dans la région de Toronto. Une fois ces études terminées, l'équipe de chercheurs a mis au pont un test direct pour mesurer la génotoxicité des sédiments. Comme l'historique de ces derniers est bien connu, les chercheurs croyaient qu'ils pourraient évaluer le test direct de génotoxicité en le comparant au test pratiqué sur un extrait organique des sédiments.

Qu'il s'agisse d'eaux résiduaires domestiques, d'effluents industriels, d'eau douce ou de sédiments, il est pratiquement impossible de préserver des échantillons complètement et sans transformation en vue de procéder à des bio-essais ou à des tests microbiologiques. Conscients du problème, les chercheurs ont décidé d'étudier les effets sur les sédiments de la congélation et de la décongélation pour voir dans quelle mesure ils influencent les résultats des bio-essais. À cette fin, ils ont tenu compte de cette caractéristique de la préservation dans une étude destinée à évaluer les sensibilités respectives des bio-essais directs sur les sédiments et des bio-essais sur des extraits de sédiments, ainsi que la charge relative en substances toxiques/génotoxiques de sédiments prélevés à l'entrée et à la sortie de trois bassins d'eaux pluviales de la région de Toronto.

Avec des sédiments entreposés, les résultats produits par les bio-essais dépendaient étroitement de chaque type de bio-essai, deux tests qui devaient supposément mesurer des effets similaires donnant souvent des résultats diamétralement opposés. Il semble que les résultats obtenus au bout de deux semaines diffèrent de ceux obtenus sur des échantillons frais autant qu'ils diffèrent des résultats obtenus au bout de quatre semaines. De plus, ces derniers ne sont ni inférieurs ni supérieurs aux résultats obtenus sur des échantillons frais de manière prévisible.

ABSTRACT

Complete and unequivocal preservation of samples, whether domestic wastewater, industrial wastes, natural waters or sediments for microbiological or bioassay testing, is a practical impossibility. Realizing the problem, the effects of freezing and thawing on sediments as they relate to bioassay results were investigated by incorporating this preservation feature into a study designed to evaluate the relative sensitivities of direct sediment bioassays and bioassays performed on sediment extracts and the relative toxicant/genotoxicant load of inlet and outlet sediments at three Toronto area stormwater ponds.

The bioassay responses to stored sediments were very bioassay specific as two bioassays supposedly measuring similar effects often produced diametrically opposite results. The superior sensitivity of the direct sediment SOS-Chromotest procedure over genotoxicity tests performed on sediment extracts is well illustrated in this comparison study.

RÉSUMÉ

Qu'il s'agisse d'eaux résiduaires domestiques, d'effluents industriels, d'eau douce ou de sédiments, il est pratiquement impossible de préserver des échantillons complètement et sans transformation en vue de procéder à des bio-essais ou à des tests microbiologiques. Conscients du problème, les chercheurs ont décidé d'étudier les effets sur les sédiments de la congélation et de la décongélation pour voir dans quelle mesure ils influencent les résultats des bio-essais. À cette fin, ils ont tenu compte de cette caractéristique de la préservation dans une étude destinée à évaluer les sensibilités respectives des bio-essais directs sur les sédiments et des bio-essais sur des extraits de sédiments, ainsi que la charge relative en substances toxiques/génotoxiques de sédiments prélevés à l'entrée et à la sortie de trois bassins d'eaux pluviales de la région de Toronto.

Avec des sédiments entreposés, les résultats produits par les bio-essais dépendaient étroitement de chaque type de bio-essai, deux tests qui devaient supposément mesurer des effets similaires donnant souvent des résultats diamétralement opposés. Cette étude comparative fait ressortir la sensibilité supérieure de la méthode SOS-Chromotest appliquée directement aux sédiments par rapport à celle des tests de génotoxicité sur des extraits d'échantillons.

INTRODUCTION

Complete and unequivocal preservation of samples, whether domestic wastewater, industrial wastes, natural waters or sediments for microbiological or bioassay testing, is a practical impossibility (APHA, 1995). We know this intuitively, but choose to ignore it. This condition is best described by the Papuan term *mokita*, "truth we all know but agree not to talk about". Regardless of the sample, complete stability for every constituent can never be achieved. At best, preservation techniques only retard chemical and biological changes that inevitably continue after sample collection (APHA, 1995). Since we are dealing with biological testing systems, many of the preservation techniques which chemists use to stabilize samples e.g. pH change, use of organic or inorganic preservatives, etc., cannot be used because they are often toxic to the test organism.

Basically there are only a few options available for samples requiring bioassay testing: (1) test the samples immediately upon collection. For many samples and laboratories this is often an impossibility, especially if more than one bioassay is being done on each sample; (2) the sample may be preserved by holding it on melting ice or in the refrigerator at 1-4°C. Depending on the sample composition the sample may produce stable repeatable bioassay results for days or weeks; (3) the sample may be frozen. However the freezing and thawing processes may cause chemical changes in the sample e.g. disruption of cell membranes, activation of bacterial metabolism during thawing etc; (4) extracts or concentrates of samples may be stored in solvent where the solvent is part of the extracting /concentration process. The problems encountered with these samples are, we believe, related to the extracting procedures which can change the sample chemistry, selectively extract chemicals, and change concentrations due to volume manipulations and losses. Not all samples are amenable to this type of preservation nor can we be sure the bioassay results truly reflect the original sample.

In previous studies Dutka et al.(1994, 1994a) evaluated the toxicant load of inlet and outlet sediments in various Toronto area stormwater ponds. After these studies were completed Dutka

and co-workers (1995) had developed a direct sediment genotoxicity test. Because the history of the stormwater pond sediments was known it was believed they would provide ideal genotoxicological conditions to evaluate the direct sediment genotoxicity test versus the genotoxicity of an organic extract of the sediment.

Since there is a recognized problem with sediment preservation for bioassay testing, it was decided that these sediments and bioassay procedures could also be used to evaluate one of the more common sediment preservation techniques, freezing (as outlined above). For simplification simple freezing and thawing over two, two week cycles was incorporated into the above comparison study.

In this short evaluation study we will report on (a) the effects of freezing and thawing on sediments as they relate to bioassay results, (b) the relative sensitivities of direct sediment bioassays and bioassays performed on sediment extracts and (c) the relative toxicant/genotoxicant load of inlet and outlet sediments at three Toronto area stormwater ponds.

METHODS

Sampling sites

Benthic sediments were collected from the following three Metro Toronto stormwater ponds; Colonel Sam Smith outfall pond in Etobicoke, Heritage Estates (Phase V) pond in Richmond Hill and Grenadier Pond in High Park, Toronto. Figure 1 shows the various sampling sites.

Bioassays

The sediments were tested by the following direct tests (no extraction); Direct Sediment Toxicity Testing Procedure (DSTTP) (commercially available as Toxi-Chromopad, EBPI Brampton,

Ont.) (Kwan, 1993), SOS-Chromotest Pad Procedure (SCPP) (Dutka et al. 1995) and the Solid Phase Microtox test (SPT)(Tung et al. 1991).

The organic extracts from the sediments were subjected to the SOS-Chromotest and the Toxi-Chromotest (Dutka et al. 1994).

Sediment Extracts

A portion of sediment from each site was was weighed out and an equal wt/vol of a 10% DMSO (dimethyl sulphoxide) plus 10% methanol solution was added to the sediment. This was mixed thoroughly, shaken vigorously for 3 minutes, then centrifuged for 20 minutes at 10,000 rpm at 4° C. The supernatant was used with dilutions in the two bioassays.

Sediment Preservation

After each sediment was collected it was thoroughly homogenized and an aliquot taken for testing. The rest of the sample was frozen at -20° C. Two weeks later the sediment was placed at room temperature overnight to thaw. The thawed sediment was homogenized after which another aliquot was removed for bioassay testing and extraction. The sediment was refrozen, and two weeks later the final sample was assayed.

RESULTS AND DISCUSSION

All data from this study are summarized in Table 1.

Previous studies on Heritage and Colonel Sam Smith pond sediments (Dutka et al. 1994, 1994a) indicated that toxicity and genotoxicity showed unpredictable seasonal variation with no distinct

pattern indicating whether inlet or outlet sediments contained the greater load of toxicants.

In this study Colonel Sam Smith sediments were found to decrease in genotoxicants and toxicants (as measured by DSTTP) from inlet to outlet but toxicants as measured by the Solid Phase Microtox test increased in outlet sediments. At the Heritage Pond sites the reverse was observed, genotoxicity and DSTTP toxicity increased from inlet to outlet while SPT Microtox decreased from inlet to outlet. It can also be seen that genotoxicity of the solvent extract was maximum at one of the intermediate sites HE3.

In Grenadier pond (GP) samples, genotoxicity levels from extracts and sediment increased from inlet to outlet. Also the two intermediary points GP2 and GP3 have higher genotoxicity values than found at the inlet. Both toxicity tests DSTTP and SPT Microtox showed no difference between inlet and outlet sediments but the intermediate sediments GP2 and GP3 showed higher toxicant levels.

In general a variety of toxicant and genotoxicant responses were noted which seemed dependent on the bioassay used and whether the sediment or extract was tested. These results tend to confirm the earlier studies of Dutka et al. (1994 and 1994a) which indicated that there does not appear to be a consistent pattern in sediment toxicity /genotoxicity from inlet to outlet in Heritage and Colonel Sam Smith stormwater ponds.

All 33 sediment organic extracts were negative (non-toxic) in the Toxi-Chromotest while 18 of the 32 sediments tested by the DSTTP indicated the presence of toxicants. Two of the probable causes for this big difference in sensitivity between tests which use the same indicator organism may be the wrong extracting procedure (solvent or concentration) was used or the organic and heavy metal contaminants were not in a bioavailable state.

Eleven sediments were assayed by both the SPT Microtox and DSTTP methods. From Table 1 it can be seen that the SPT and DSTTP indicated the presence of toxicants in the same six sediments while the DSTTP was positive in three sediments where the STP Microtox was

negative. Both tests indicated no toxicity in two sediments, however one of these sediments, GP4 original, indicated the presence of low grade toxicity (2 CIP) in the cytotoxicity part of the SCPP test.

In comparing the three toxicity screening procedures the SCPP cytotoxicity indicated the presence of toxicants in three of the 11 comparable sediments while the STP was positive in six sediments and the DSTTP indicated the presence of toxicants in nine sediments. In one sample, S1 original, all three tests indicated the presence of toxicants.

When the preserved and original sediment sample bioassay data for DSTTP and cytotoxicity SCPP are compared (Table 1) it can be seen that both are positive in five samples, DSTTP is positive in 13 sediments and cytotoxicity SCPP is positive in only three sediments. Thus in this limited study the DSTTP was found to be more reactive than the STP Microtox procedure and the SCPP cytotoxicity test.

Genotoxicant presence in these sediments was evaluated directly as well as by testing the organic extract of these sediments. In Table 1 it can be seen that genotoxicity SCPP and extract Induction Factors (IF) were positive in six sediments (where 2 CIP units are considered to indicate a genotoxic effect and 1.25 IF and greater indicates genotoxic activity), and the genotoxicity SCPP indicated another 14 sediments which had genotoxic activity. The superior sensitivity of the direct sediment SOS-Chromotest procedure over genotoxicity tests performed on sediment extracts is well illustrated in this comparison study and confirms earlier observations by Dutka et al.(1995).

Storage effects were monitored with five bioassays, three direct sediment assays and two extract bioassay tests. One of the extract bioassays, Toxi-Chromotest was negative in all samples under all storage conditions and thus does not appear as part of Table 1. The other four bioassay results are shown in Table 1 and present a variety of results which will be discussed sediment by sediment.

SSI (Colonel Sam Smith Site 1 sediment) data indicate a decrease in genotoxicity over time and depending on the toxicity bioassay looked at there is both a decrease and increase with storage. SS2 shows no change in genotoxicity but a decrease in toxicity while SS3 produced a set of ambivalent results.

Grenadier Pond Site 1 (GP1) data suggest original and four week sediments are similar in toxicant load. GP2 results are dependent on the bioassay used and thus do not provide any clear trend. Interpretation of the freeze thaw and holding period on GP2 appears to be bioassay dependent. Cytotoxicity is maximum at four weeks while DSTTP toxicity is maximum in the original sample. SCPP genotoxicity results are more or less consistent over the whole period while extract genotoxicty values decrease greatly with storage. GP3 results are very similar to GP2 except toxicity increases with storage and GP4 results are similar to GP3 with no real change in toxicity with storage.

Heritage Estates Site 1 (HE1) four week data indicate that only two bioassays gave a response showing there was an increase in genotoxicity with storage but a decrease in toxicity. HE2 data mirrored HE1 data but at lower levels. Data from HE3 suggest that similar low levels of toxicity were found in the original and four week stored sediments while low level genotoxicity responses disappeared on storage. HE4 data was bioassay specific and indicated that both genotoxic and toxic activity decreased with storage.

These bioassay responses to stored sediments are very bioassay specific as two bioassays supposedly measuring similar effects often produce diametrically opposite results. There is a suggestion that the two week results are as different from the original samples as they are from the four week results. Also four week storage results are not predictably lower or higher than original sample results.

Another factor not considered in the effects of storage is the lack of homogeniety in sediment samples. One can mix the samples to try and create a homogenious sediment however there is no guarantee that this actually occurs in a natural sediment and during the mixing, aeration and

niche bacterial populations may create "hot spots" which are inadvertently selected for testing.

These storage studies while providing a "first look" at storage of sediments and the effects of these storage conditions on a battery of bioassays, raise more questions than answers. However the results do point out the dangers of trying to make conclusions on the results of a single bioassay. Hopefully this study will lead to a more thorough investigation of sediment preservation using the battery of bioassay tests approach.

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Table 1 Stormwater Pond Bioassay Data and Sediment Preservation (Freezing) Results

BIOASSAY

TESTING PERIOD AND SITE

	SS1			SS2			SS3		
	ORIGINAL	WEEK 2	WEEK 4	ORIGINAL	WEEK 2	WEEK 4	ORIGINAL	WEEK 2	WEEK 4
Cytotoxicity, CIP units	4	0	16	3	0	0	0	0	4
Genotoxicity, CIP units	3	6	0	0	0	0	0	0	2
Genotoxicity extract, IF units	1.38	1.02	1.04	1.02	1.01	1.05	1.04	1.04	1.21
DSTTP % sediment	1.6	12.5	25	6.3	0	25	12.5	0	0
Microtox, SP* %EC50	0.35			neg**			0.23		
Toxi-Chromo % extract =EC50	>50****	>50	>50	>50	>50	>50	>50	>50	>50

	GP1			GP2			GP3			GP4		
	ORIGINAL	WEEK 2	WEEK 4	ORIGINAL	WEEK 2	WEEK 4	ORIGINAL	WEEK 2	WEEK 4	ORIGINAL	WEEK 2	WEEK 4
Cytotoxicity, CIP units	0	0	0	0	0	12	0	0	4	2	0	0
Genotoxicity, CIP units	9	0	8	7	6	8	9	15	8	27	0	26
Genotoxicity extract, IF units	1.28	1.05	1.05	1.41	1.05	1.02	1.52	1.03	1.06	1.43	1.12	1.06
DSTTP % sediment	0	0	0	50	0	0	50	12.5	6.3	0	0	0
Microtox, SP* %EC50	neg			neg			0.12			neg		
Toxi-Chromo % extract =EC50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50

	HE1			HE2			HE3			HE4		
	ORIGINAL	WEEK 2	WEEK 4	ORIGINAL	WEEK 2	WEEK 4	ORIGINAL	WEEK 2	WEEK 4	ORIGINAL	WEEK 2	WEEK 4
Cytotoxicity, CIP units	0	0	0	0	0	0	0	NT***	2	0	0	0
Genotoxicity, CIP units	0	0	16	3	0	8	4	NT	0	18	12	7
Genotoxicity extract, IF units	1.02	1	1.02	1.02	1.02	1	1.25	1.04	1.06	1.06	1	1
DSTTP % sediment	25	25	0	50	50	0	50	NT	50	6.3	0	12.5
Microtox, SP* %EC50	0.07			0.04			neg			0.64		
Toxi-Chromo % extract =EC50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50

SP*= solid phase test
neg**= EC50 > 1%
NT***= not tested

DSTTP = Toxi-Chromopad
>50**** = negative

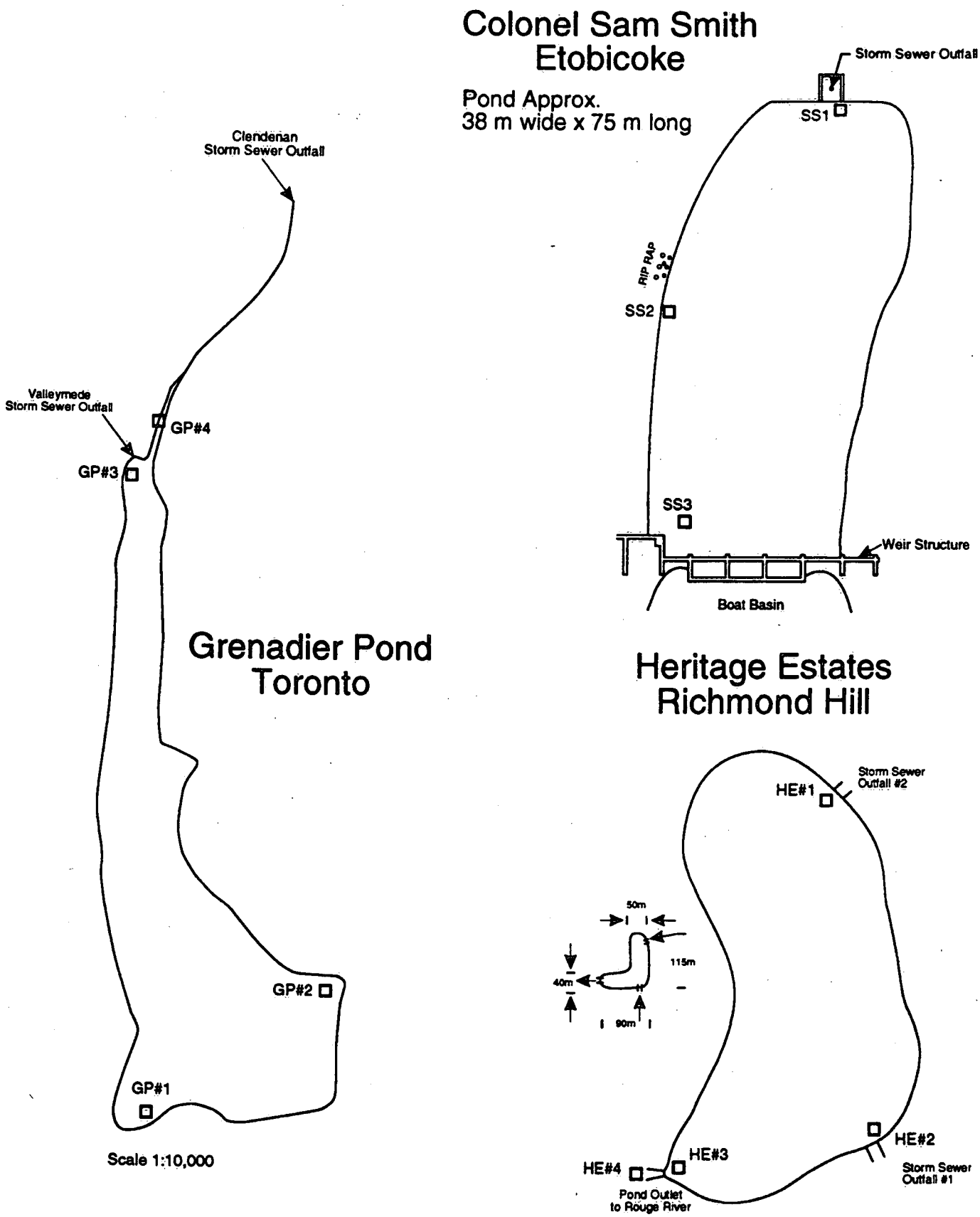

Figure 1. Sediment collection sites at stormwater runoff ponds.

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