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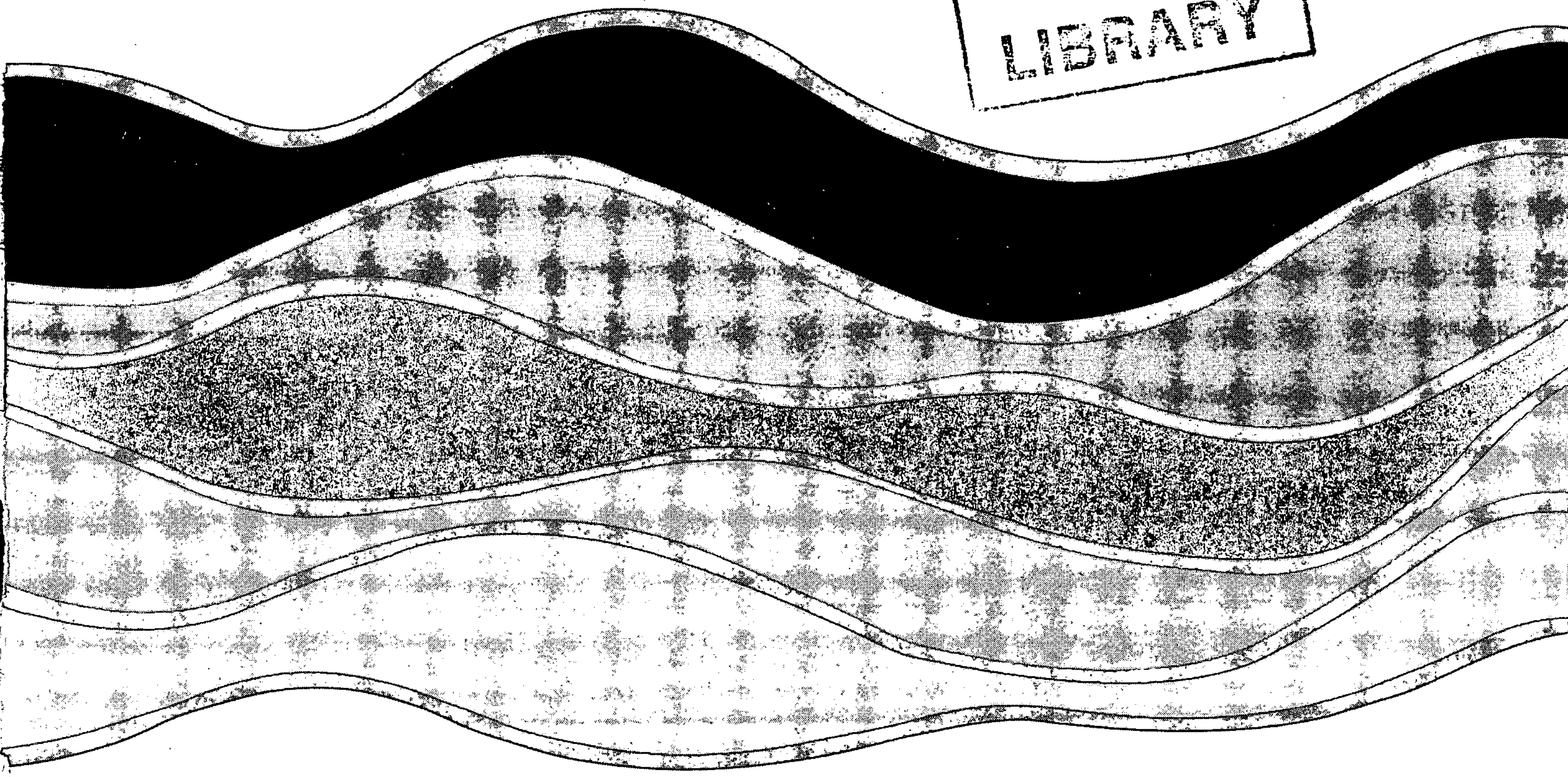

ACUTE TOXICITY OF COMBINED SEWER OVERFLOWS AND STORMWATER DISCHARGES

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MANAGEMENT PERSPECTIVE

The first attempts to describe environmental impacts of urban stormwater discharges and combined sewer overflows (CSOs) on receiving waters dealt with physical impacts, primarily flooding, arising from increased volumes and speed of runoff from urban areas. Subsequent research focussed on chemical and microbiological characterization of urban stormwater and CSOs, with successive emphasis on solids, biodegradable organics, nutrients, fecal bacteria, heavy metals, hydrocarbons, and trace organic contaminants. In this process, hundreds of chemicals including persistent, toxic and bioaccumulative substances, have been identified in urban stormwater. However, chemical protocols often fail to distinguish between toxic and non-toxic species of contaminants, their bioavailability or the synergistic toxic effects of chemical cocktails, and consequently, the environmental effects of stormwater and CSOs on the receiving waters as well as the effectiveness of stormwater quality enhancement by Best Management Practices should be assessed by toxicity testing.

To provide such an assessment, an exploratory study of stormwater and CSO toxicity was initiated in conjunction with several existing projects dealing with stormwater quality and its enhancement. The study sites included a highway bridge, freeway site, shopping plaza, several stormwater ponds, constructed wetland, biofilter, a small urban stream, and several CSO outfalls. These sites represent a whole spectrum of sources of stormwater and CSOs, and the associated pollution.

When selecting the appropriate toxicity tests, it was recognized that at the current level of knowledge, there is not a full agreement on which are the best toxicity tests to apply. Consequently, a battery of tests, which differ by their sensitivity in detecting various types and sources of toxicity, was used. The study concluded that toxicity was detected in up to 83% of all stormwater samples and 50% of all CSO samples collected at individual urban sites with various land use.

This report should be of interest to researchers and water managers dealing with abatement/management of urban non-point sources of pollution.

SOMMAIRE À L'INTENTION DE LA DIRECTION

Les premières tentatives de décrire les effets écologiques des eaux pluviales urbaines et des eaux des déversoirs d'orage sur les masses d'eau réceptrices portaient sur les effets physiques, en premier lieu les inondations, attribuables à l'accroissement du volume de l'eau déversée, et la vitesse d'écoulement des eaux de ruissellement provenant des secteurs urbains. La recherche subséquente s'est tournée vers la caractérisation chimique et microbiologique des eaux pluviales urbaines et des eaux des déversoirs d'orage, l'accent étant mis successivement sur les matières solides, les matières organiques biodégradables, les nutriments, les bactéries d'origine fécale, les métaux lourds, les hydrocarbures et les contaminants organiques à l'état de traces. De la sorte, des centaines de composés chimiques, notamment des substances persistantes, toxiques et bioaccumulables, ont été décelées dans les eaux pluviales urbaines. Toutefois, il arrive souvent que les méthodologies chimiques ne permettent pas de faire la distinction, sur le plan des contaminants, entre les substances toxiques et celles qui ne le sont pas, et ne déterminent pas la biodisponibilité ou les effets toxiques synergiques des mélanges chimiques. C'est pourquoi il faut évaluer, au moyen d'essais de toxicité, les effets sur les masses d'eau réceptrices des eaux pluviales et des eaux des déversoirs d'orage, aussi bien que déterminer l'efficacité des mesures d'amélioration de la qualité des eaux pluviales par l'application des meilleures pratiques de gestion.

Afin de préparer une évaluation de cette nature, nous avons effectué une étude exploratoire de la toxicité des eaux des déversoirs d'orage et des eaux pluviales parallèlement à plusieurs projets existants, portant sur la qualité des eaux pluviales et sur l'amélioration de leur qualité. Nous avons choisi des sites situés sur un pont routier, en bordure d'une autoroute, dans un centre commercial, dans plusieurs bassins d'eaux pluviales, dans un marécage artificiel, sur des lits bactériens, dans un petit cours d'eau situé en zone urbaine et à plusieurs exutoires de déversoirs d'orage. Ils correspondent à une vaste gamme de sources d'eaux pluviales et de déversoir d'orage, et sont représentatifs de la pollution associée à ces eaux.

Au moment de choisir des tests de toxicité appropriés, nous étions conscients du fait que, dans l'état actuel des choses, il n'y a pas unanimité relativement aux tests à appliquer. Par conséquent, nous avons eu recours à une batterie de tests qui varient par leur sensibilité aux types et aux sources de toxicité. L'étude indique que jusqu'à 83 % des échantillons d'eaux pluviales et que 50 % de ceux des eaux de déversoirs d'orage prélevés à des sites urbains déterminés dans des secteurs servant à différents usages, sont contaminés.

Ce rapport s'adresse aux chercheurs et aux gestionnaires de l'eau concernés par la gestion et la réduction des sources diffuses de pollution urbaine.

ABSTRACT

The impact of stormwater discharges and combined sewer overflow outfalls on receiving waters has been investigated in the past using chemical characterization. This has provided a great deal of information on the input of solids, nutrients, metals, hydrocarbons and trace organic compounds from these discharges. It does not, however, gauge their bioavailablity or the impact these chemical constituents are having on the biological organisms in the receiving waters. This research focused on the application of a battery of acute toxicity tests to a variety of stormwater and combined sewer overflow discharges, in order to better gauge the effects on the ecosystems in the receiving waters.

This battery of tests included *Daphnia magna*, Microtox™, Sub-mitochondrial particle bioassays (reverse and conventional electron transport methods) and SOS chromotest. Of these tests, *Daphnia magna* and Microtox™ exposed whole organisms (a freshwater cladoceran and bacteria respectively) to the effluent, demonstrating survival impacts. The sub-mitochondrial particle tests used cellular (beef heart) tissue to determine the impact of the effluent on cell biochemical processes. The SOS chromotest indicates the effects of the effluent on genetic repair processes (biochemical functions) and hence indicates the degree to which cellular genetic material may be affected.

Stormwater runoff and snowmelt samples were collected from five sites. These areas included highway runoff sites and a commercial parking lot. An urban stream which received runoff from a developing watershed was also included. Two stormwater ponds, a constructed wetland, a biofilter and two oil/grit separator units were assessed to determine the effectiveness of stormwater best management practices for removal of toxicity. Combined sewer overflow discharges were sampled in areas of varying land use and included industrial areas, commercial, high traffic flow and residential.

The results of the study indicated that stormwater discharges from highway runoff had the greatest potential for inducing a toxic response from the battery of tests, and therefore were most likely to have a negative impact on downstream ecosystems. Stormwater best management practices did appear to provide some reduction in toxicity, although further study is required to confirm these trends. Long term performance could not be assessed. Combined sewer overflow discharges were less likely to exert strong acute toxicity. The most toxic sites appeared to be in industrial areas, commercial sites, and those receiving hospital waste. The most sensitive test in these investigations appeared to be the sub-mitochondrial particle bioassay (reverse electron transport), which provided evidence of toxicity where other tests did not.

The variation of toxicity with time was investigated at the highway bridge runoff site. Samples taken successively over a period of two hours indicated a progressive reduction in the degree of sample toxicity. The sub-mitochondrial particle bioassay (reverse electron transport) still demonstrated toxicity in later phases of the runoff event when less sensitive tests showed that no toxicity was present in the sample.

Future investigations will focus on variation of toxicity in combined sewer overflow effluent during a discharge period, performance assessment of stormwater best management practices and evaluation of sources of effluent toxicity.

RÉSUMÉ

Antérieurement, on a étudié les répercussions écologiques des eaux pluviales urbaines et des eaux de déversoir d'orage sur les masses d'eau réceptrices par caractérisation chimique. Cela nous a permis d'en apprendre beaucoup sur les apports de cette origine en matières solides, en nutriments, en métaux, en hydrocarbures et en composés organiques à l'état de traces. Toutefois, cela ne nous a pas permis de mesurer la biodisponibilité de ces substances ou leurs effets sur les organismes qui vivent dans les eaux réceptrices. La recherche décrite ici traite de l'application d'une batterie de tests de toxicité aiguë à des eaux pluviales et de déversoir d'orage de diverses sources, afin de mieux évaluer leurs effets sur les écosystèmes des eaux réceptrices.

Il s'agit notamment du test avec *Daphnia magna*, du Microtox^{md}, des bioessais sur des particules submitochondriales (méthodes de transport normal et inverse des électrons) et du chromotest SOS. Le test avec *Daphnia magna* et le Microtox^{md} exposent des organismes entiers (un cladocère dulcicole et une bactérie, respectivement) aux effluents et en montrent les effets sur la survie. Les essais sur des particules submitochondriales utilisent des cellules (de coeur de boeuf) pour déterminer l'effet des effluents sur le fonctionnement biochimique des cellules. Le chromotest SOS montre les effets des effluents sur les mécanismes de réparation des dommages génétiques (fonctions biochimiques), ce qui revient à indiquer dans quelle mesure le matériel génétique des cellules peut être affecté.

Des échantillons d'eaux pluviales et d'eau de fonte de la neige ont été prélevés à cinq stations. Il s'agit notamment de sites situés à proximité d'une route et dans le stationnement d'un centre commercial. On a également prélevé des échantillons dans un cours d'eau qui s'écoule dans un bassin hydrographique dont le territoire est en développement. On a aussi évalué la situation dans deux bassins de retenue des eaux pluviales, dans un marécage artificiel, à la sortie d'un filtre biologique et à la sortie de deux extracteurs d'huile et de sable afin de déterminer l'efficacité des meilleures pratiques de gestion des eaux pluviales sur le plan de l'élimination de la toxicité. Nous avons aussi prélevé des échantillons d'eaux de déversoir d'orage dans des secteurs faisant l'objet de différents modes d'utilisation, notamment des secteurs industriels, commerciaux, résidentiels et à circulation intense.

L'étude montre que les eaux pluviales provenant de la route avaient le plus grand potentiel de toxicité mesuré par la batterie de tests; elles étaient donc les plus susceptibles d'exercer un effet nocif sur les écosystèmes situés en aval. Il semble bien que les meilleures pratiques de gestion des eaux pluviales aient contribué à réduire leur toxicité dans une certaine mesure, même si cette tendance doit être confirmée par d'autres travaux. Leur efficacité à long terme n'a pu être évaluée. Les eaux de déversoir d'orage risquaient moins d'exercer des effets marqués de toxicité aiguë. Les sites les plus toxiques tendaient à être situés dans les secteurs industriels et commerciaux ainsi qu'à être ceux recevant des déchets hospitaliers. Le bioessai sur des particules submitochondriales (méthodes de transport inverse des électrons) paraît avoir été le plus sensible; Il montrait la manifestation d'effets toxiques là où les autres ne le faisaient pas.

Les variations de la toxicité en fonction du temps ont été étudiées au site situé à proximité du pont routier. Les échantillons séquentiels, sur un intervalle de deux heures, ont mis en évidence une diminution progressive de la toxicité des échantillons. Le bioessai sur des particules submitochondriales (méthodes de transport inverse des électrons) indiquait encore l'existence d'effets toxiques vers la fin de la période d'écoulement de l'eau pluviale, alors que les autres tests n'indiquaient plus de toxicité.

De prochains travaux porteront sur la variation de la toxicité des effluents des déversoirs d'orage en période de fonctionnement, sur l'évaluation de l'efficacité des meilleures pratiques de gestion des eaux pluviales ainsi que sur l'évaluation des sources de toxicité des effluents.

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1.0 INTRODUCTION

1.1 Background

Urban wet-weather pollution, such as stormwater discharges and combined sewer overflows (CSOs), causes receiving water impacts, which depend on both the characteristics of urban sources producing such effluents (in terms of stormwater or CSO quantity and quality), and the characteristics of the receiving waters. The actual impacts need to be evaluated in terms of specific characteristics of each site, including physical habitat changes (changes in stream morphology), water quality deterioration (dissolved oxygen depletion, eutrophication, thermal enhancement, toxicity, fecal contamination), sediment contamination, impacts on biological communities and groundwater impacts (Ellis and Hvitved-Jacobsen, 1996).

In the Great Lakes Basin, the impacts of CSOs and stormwater discharges were assessed by Weatherbe and Sherbin (1994) and found to contribute strongly to impairments of beneficial water uses in a number of Areas of Concern (AOCs). In fact, the potential problems caused by stormwater and CSO pollution were rated as "medium" or "high" in 11 of the 17 Canadian AOCs, and in the case of Hamilton Harbour and Toronto Waterfront, as "very high". The delisting of many of these AOCs will depend on successful abatement of stormwater and CSO pollution. For example, the preliminary Stage 2 Report for the Toronto Waterfront AOC lists 35 recommendations for remediation of this area, 12 of which deal with stormwater and CSOs.

In developing remedial plans for stormwater and CSO pollution, Marsalek and Kok (1997) identified a number of challenges to be addressed, including (i) increasing regulatory demands on stormwater and CSO control, (ii) the cumulative nature of some of the stormwater and CSO impacts, (iii) the importance of stormwater and CSO pollution in relation to other sources, (iv) funding, and (v) limited experience with long-term operation and maintenance of stormwater and CSO control facilities in Canada. To overcome some of these challenges and to assist the Remedial Action Plan (RAP) teams in the development of remedial plans, Environment Canada's Great Lakes 2000 Cleanup Fund has been supporting demonstration and assessment studies of various processes for treatment of CSOs and stormwater, including the high-rate treatment of CSOs, stormwater settling in ponds or in flow balancing structures, and treatment by wetlands and biofilters.

Treatment of CSOs and stormwater has been traditionally evaluated by permissible concentrations of various chemicals or other materials, or their removals, or both. Such procedures do not address fully the issues of protection of the receiving waters against toxic impacts. This consideration is particularly important where the treatment processes involve additions of chemical aids (e.g. flocculants in the ongoing study of the high-rate treatment of CSOs), effluent UV irradiation, or open storage with habitat creation (e.g. in constructed wetlands).

Assessments of water quality or its improvement (through treatment) by chemical protocols does not distinguish between toxic and non-toxic species of some contaminants, and does not address the contaminant bioavailability, or synergistic toxic effects of chemical cocktails. To remedy this situation and strengthen the current and planned Cleanup Fund efforts in treatment of stormwater and CSOs, an exploratory study of toxicity of such effluents has been conducted.

When selecting the most appropriate tests for assessing the toxicity of CSOs and stormwater (i.e. intermittent sources of highly variable strength), the literature survey indicated that there was no agreement on such a selection and, consequently, it was desirable to use a variety of tests. This approach was adopted in this study by using such tests as *Daphnia magna*, Microtox™ and sub-mitochondrial particle bioassays which indicate potential acute cellular damage, SOS chromotest which suggests potential genetic damage, and fathead minnow and *Ceriodaphnia dubia* chronic tests, which demonstrate long term exposure effects.

1.2 Study Objectives

The study's objectives were to assess the toxicity of combined sewer overflows (sampled in Toronto and Hamilton), and stormwater runoff, both untreated or treated by the selected best management practices (sampled in Burlington, Kingston and Scarborough). Towards this end, suitable toxicity tests had to be selected and applied to CSO and stormwater samples collected mostly in conjunction with the existing projects. Finally, it was desirable to interpret the results obtained with reference to general sources of CSOs and stormwater and frequencies of toxic responses. Additional reports dealing with acute and chronic toxicity testing of CSOs, stormwater and the associated contaminated sediments are under preparation.

2.0 LITERATURE REVIEW

2.1 Aquatic Toxicity

Stormwater and combined sewer overflows may exert either acute or chronic toxicity to organisms in the receiving waters. Acute toxicity has generally been of greater concern, as the immediate effects of organism death are more severe. Recently, however the chronic buildup of toxic materials over time (bioaccumulation) that lead to reproductive effects, have also been noted. Concentration of toxic materials through food webs (biomagnification) has also been shown to occur for some of these chemicals (e.g. PCBs) and can lead to widespread ecosystem losses, including higher trophic level organisms.

The degree of toxicity of these effluents and sediments may not be easily assessed by just one test, but can be monitored effectively with a battery of screening tests (Dutka et al. 1995; Herricks et al. 1994). These tests are based on the rapid response of bacteria, tissues and enzymes to pollutant mixtures. Several tests are used, as some organisms are not as sensitive as others in their responses to some combinations of chemicals. The acute tests generally in use include Microtox™, sub-mitochondrial particle bioassay (conventional and reverse electron transport), SOS chromotest, *Daphnia magna* and rainbow trout. Most of these tests require exposure for less than 48 hours in order to induce a response. Herricks et al. (1994) suggest that the most suitable tests for stormwater and CSO discharge monitoring be able to respond to the rapid changes in pollutant concentrations and also have several measurable aspects for effect detection. They recommend using whole organism tests where biochemical, physiological and behavioural responses can be measured (such as *Gammarus pulex*), as well as using in place community or ecosystem response surveys.

When assessing the long term chronic toxicity, many organisms can be used, however these tests are generally much longer, encompassing a complete life cycle. These tests can take several months and are labour intensive to perform, therefore short term chronic toxicity tests, based on reproductive inhibition, survival and growth, have been developed with exposure times of approximately 7 days. These types of tests include *Ceriodaphnia dubia* and fathead minnow. Lewis et al. (1994) determined a series of short term methods for investigating chronic toxicity that used a suite of three tests: *Ceriodaphnia dubia*, *Selenastrum capricornutum* and fathead minnow

(Pimephales promelas). These tests were selected because the test endpoints (growth, mortality and reproduction) were more easily measured and less subjective than the measurements in other tests. Once these tests had been applied (in compliance with standardized USEPA guidelines), the most sensitive test was selected for use in monitoring specific changes in aquatic toxicity.

Many of the pollutants found in stormwater can have severe effects on the populations of fish and benthic invertebrates (e.g. *Asellus aquaticus*). Mulliss et al. (1993) investigated the effects of storm sewer and CSO discharges on in-stream caged *A. aquaticus* in London, UK. They observed weight change, mortality, total tissue concentrations of lead, cadmium, copper and zinc, weekly precipitation volume and dry days, and found that copper was a principal toxicant affecting organism survival, and that organism weight changes were affected by a complex interaction of in stream conditions.

Many other species that are higher up in the food chain can also be adversely affected. Suspended sediment can be deposited in various areas of the receiving stream, and cause reproductive effects, and reduction of spawning areas. There are also effects from the direct uptake from the sediment by the benthic biota. These act as a source of food for fish and other wildlife, which could result in possible bioaccumulation of various substances such as metals and trace organics. Many substances that are associated with these solids can be leached out easily by those that feed on them. Sediment accumulation and cleanout may be a very toxic project, requiring secure landfills for its disposal.

The toxicity of stormwater runoff on urban streams can be assessed using a wide range of toxicity tests. This helps ensure that bioassays do not over-predict or under-predict the degree of toxicity. It is highly important to use representative species from various tropic levels to properly assess the ecosystem effects. The toxicity of metals, salts, PAHs and pesticides/herbicides is varied, depending on the test organism used, and while a sensitive test may show toxicity in a sample, there is no certainty that the receiving system will be adversely affected. It is for this reason, that *in situ* bioassays and tests are the most informative, however their cost and lack of "control" makes them difficult to implement.

Blondin et al. (1987) suggests the use of sub-mitochondrial particle bioassays as a sensitive measure of aquatic toxicity as they are easy and inexpensive to perform, require small amounts of sample and rapid results can be achieved. Testing on "standard" solutions of toxicants such as pesticides and metals has shown that the sub-mitochondrial particle bioassay is a good predictor of aquatic toxicity in fish (rainbow trout 96 hour test), and may be suitable as a screening tool.

2.2 Sediments

Urban sediments contain adsorbed compounds, which may contribute to the toxicity of the stormwater. This sediment can usually be reduced or removed by settling under quiescent conditions, thereby reducing the toxicity of the stormwater. Large particles can settle quickly, but small, fine particles may remain in suspension for long periods of time. Many studies have shown that the sediment contains adsorbed organic constituents which include polynuclear aromatic hydrocarbons (PAH) and metals (Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Zn). Wei and Morrison (1991) investigated five urban rivers in Sweden by measuring ammonium acetate and EDTA extractable metals, as well as using bacterial enzyme activity bioassays. They determined that bacterial enzyme activity was reduced in stagnant streams impacted by urban storm drainage and combined sewer overflows. However, where the water was free flowing, lower metals and higher bacterial enzyme activities were recorded, showing that flushing of sediments downstream may help protect ecosystems subject to wet weather discharges.

For organic chemicals, sediment toxicity tests can be performed on samples using solvent extracts, in which the toxicity-generating chemical is preferentially dissolved in an organic solution (e.g. methylene chloride), which could then be used to test for the presence of toxicity. This method may be unrealistic in its representation of the true toxicity of the sample due to preferential extraction of organic materials. This type of test is better used to describe relative impacts between locations (Dutka and Kwan, 1988). It may be more beneficial to use a direct sediment toxicity test (without extraction) when assessing the potential toxicity of sediments (Dutka 1988).

Boxall and Maltby (1995) investigated the source of organic toxicity in sediment associated with urban runoff, using organic extraction and fractionation techniques coupled with chemical analysis. They found high levels of aliphatic hydrocarbons that may be acutely toxic to certain benthic invertebrates. Wet sediments were extracted using dichloromethane and diluted extracts were subject to Microtox™ bioassay and a *Gammarus pulex* 14-day test. A toxicity identification evaluation (TIE) was performed using these bioassays, and it was found that the fractions containing aliphatic hydrocarbons were the most toxic, followed by 2-5 ring PAH fractions and 4 and 5 ring PAH fractions.

Maltby et al. (1995) again used *Gammarus pulex* to identify the source of contamination in a stream receiving highway runoff. They determined that polynuclear aromatic hydrocarbons bound in the sediment were primarily responsible for the toxicity. It was found that the overlying water was not toxic to *G. pulex* when tested alone, and that upstream (non-impacted) sediments were non toxic. Metals, primarily copper and zinc from the sediment were also identified as contributing to overall toxicity.

Tests performed using *in situ* bioassays have shown that there is a strong increase in mortality of benthic invertebrates after a storm event. Seager and Abrahams (1990) investigated the water quality of a polluted river (Pendle water) in Lancashire, U.K., using in-situ bioassays and sampling of benthic invertebrates. Primary pollutant additions were from combined sewer overflows and sewage treatment plant discharges. Chronic effects were suggested by the reduced species number and diversity just downstream of the discharges, and an increase in oligochaetes (worms which feed on bacteria enriched sediments). Mortality of some invertebrates (*Asellus aquaticus* and *Baetis rhodani*), and increased gill ventilation frequencies in fish (*Salmo gairdneri*) after an event suggested that these discharges were having adverse effects on the river water quality. These effects were believed to be due in part from the increased turbidity, as well as the high biochemical oxygen demand (BOD) associated with the waste.

2.3 Water

While sediment may contribute significant pollutants to the system, there are also dissolved compounds in the wastewater stream which are easily bioavailable. These are not removed when sediment settles out in a stormwater pond or other detention facility, and as such, contribute to the downstream effects. As these compounds are dissolved in the water column, they become more bioavailable, and their presence can have a significant impact on the food web.

Metals may be present in high quantities, but their bioavailability generally depends on the pH and hardness of the water. Under certain circumstances, metals may leach from concentrated sources (e.g. sediments) and become dissolved. Many researchers point to strong correlations between sample toxicity and dissolved metal concentrations. Hall and Anderson (1988) investigated 12 stormwater runoff sites in the Brunette River drainage basin in British Columbia, which had a range of uses (industrial, commercial, urban and rural). *Daphnia pulex*, and soluble metals (iron, copper, lead and zinc) were used to classify the impacts of urban runoff. It was found that the most toxic runoff originated on commercial land, followed by industrial, urban and rural lands. Runoff collected within the first 20 minutes was generally highly toxic and its toxicity was related to suspended solids. Runoff that was toxic after the first 20 minutes was generally thought to be due to soluble material. It was also noted that the presence of iron reduced the toxicity of copper, lead and zinc. Bioavailability of these metals can change when in a mixture, and it is therefore very important in determining their toxic potential.

Organic chemicals may also be present in great enough quantities to induce a toxic response. Wei (1991) found that PAHs contributed to the stimulation of bacterial enzymes in streams receiving stormwater and combined sewer overflow discharges, which indicated high levels of toxicity in the river sediments. The research indicated that sediments could be disturbed during a storm event and release soluble PAHs and metals into the watercourse.

Jones et al. (1993) investigated hydrocarbon contamination in the Silk Stream catchment in NW London, U.K. using *Asellus aquaticus* bioassays. When tests were conducted *in situ* and in the laboratory with similar levels of measured PAHs and alkanes, it was found that those samples *in situ* exhibited greater toxicity than those in the lab. It was concluded that these PAHs may be acting synergistically with bioavailable metal species or other chemicals to induce this higher toxic response.

Herricks (1994) used field surveys to show that benthic and fish populations were under considerable stress downstream of CSO and stormwater discharges. Monitoring for seasonal changes using colonization and growth indices was suggested to be a highly sensitive measure of population stress.

Additional information may be gleaned from toxicity identification evaluations (TIE), which showed that the toxicity of stormwater was due mainly to soluble metals and this could be directly related to industrial rather than residential discharges. Cooke et al. (1995) determined that stormwater monitoring plans need to be highly structured in order to determine sources of pollutants and toxicity, including location of sampling stations, matrix (water/sediment/biota) and sampling methods used.

2.4 Best Management Practices

Best management practices have been described by Schueler et al. (1992) and encompass many different types of measures (ponds, wetlands, infiltration facilities and filters) which can help improve pollutant removal performance. The design guidelines for most of these systems and particularly stormwater ponds suggest to increase detention times to 24 hours or more to achieve significant improvements in water quality. These systems have been shown to provide some significant benefits over conventional detention ponds, however relatively little has been discovered about the toxicity reduction.

Some studies have shown that the suspended sediment (which often escapes conventional stormwater ponds) may contain more toxic material than settled sediment. Dutka et al. (1994a; 1994b) noted that seasonal factors (temperature, ice cover, light) did not appear to change the toxicity of the pond effluents from two stormwater ponds in industrial sites and two stormwater ponds in urban residential areas of Toronto, Canada. *Daphnia magna*, SOS Chromotest, Sub-mitochondrial particle bioassays and the Direct Sediment Toxicity Testing Procedure (DSTTP) were found to be the most suitable tests to determine the presence of toxicants in both bottom sediment and suspended sediment. This study indicates that although large sediment particles (along with associated pollutants) are removed in these ponds, most toxic substances are associated with the smaller suspended sediment which is not removed. It is therefore unlikely that stormwater ponds serve to reduce this toxicity.

It has been shown, however, that some stormwater BMPs do help remove suspended solids from the wastewater stream before release as well as reduce the velocity of the discharge. This lessens the scouring and redeposition of sediments downstream, protecting sensitive ecosystems from habitat damage. Wenholtz and Crunkilton (1995) used *Ceriodaphnia dubia* (48-hour acute test) and toxicity identification procedures to identify metals (zinc, iron and copper) as the primary toxicants in stormwater pond sediment pore water in Wisconsin. The data suggest that ammonia in the sediment may also contribute to toxicity.

Collins et al. (1992) found that four out of the five BMPs they tested in Virginia did not appear to reduce effluent toxicity significantly. *Ceriodaphnia dubia*, *Daphnia magna* and fathead minnow (*Pimephales promelas*) were used in determining effluent toxicity of freshwater discharges and sheepshead minnow (*Cyprinodon variegatus*) and mysid shrimp (*Mysidopsis bahia*) were used for marine discharges.

Katznelson et al. (1995) did find that constructed wetlands in California provided toxicity reduction of stormwater effluents. *Ceriodaphnia dubia* acute toxicity tests were used to determine the horizontal and vertical toxic gradients in the wetland, along with conductivity measurements. Toxicity reduction above that associated with dilution was observed, indicating that the marsh system did contribute to toxicity reduction.

2.5 Combined Sewer Overflows

Combined sewer overflow discharges can incorporate the pollutants from stormwater as well as those associated with municipal sewage, and as such, pollutant levels (and associated toxicity) may be quite high. These pollutants include increased levels of carbon, nitrogen and phosphorus, as well as metals, bacteria and viruses (Lijklema, 1993).

Many studies of combined sewer overflow toxicity have been done *in situ*, as it can be difficult to collect samples from sites with unpredictable discharges. Fabroulet et al. (1993) used two freshwater organisms; *Asellus aquaticus* (a deposit feeder) and *Dreissena polymorpha* (a filter feeder) *in situ* to determine the best indicator of metal pollution downstream of urban runoff and CSO discharges in the river Seine in Paris. It was found that the *Dreissena polymorpha* was able to avoid metal pollution (possibly due to reduced filtering during periods of high suspended solids), and that the *Asellus aquaticus* was more susceptible to metal toxicity and therefore more suited to CSO discharge monitoring.

Borchardt (1993) determined that low dissolved oxygen levels and high ammonium concentrations appeared to contribute to the toxic effects observed downstream of combined sewer overflow discharges in Germany. High shear stress associated with elevated velocities during storm events caused scouring of the river bed, which allowed fewer benthic species to colonize and resulted in overall degradation of the ecosystem. It was recognized that a reduction in the flow velocities and amounts of stormwater runoff would benefit the habitat and improve species abundance.

A notable comparison of enzyme activity between CSO and stormwater runoff in streams by Wei and Morrison (1991) showed that while combined sewer overflow discharges elevated the levels of enzyme, data from runoff sites showed reduced levels of enzyme.

Current management practices for combined sewer systems have been to build holding tanks for the wastewater, which allows the discharges to be held for a period of 24 hours before being discharged, or returned to the sewage treatment plant. This allows time for some solids settling, which could remove metals and biodegradable organics, thereby reducing effluent toxicity (Walker, 1993).

There has been a notable lack of investigations which use the "first flush" from a combined sewer overflow in Toxicity Identification Evaluations. Toxicity investigations where samples are taken after the first flush, or within the stream, may not be providing an accurate toxicity measurement for that discharge. Studies have shown that *in situ* tests demonstrate acute toxicity and therefore it would be expected that this toxicity could be measured in samples taken directly from the discharge. As this has not been demonstrated, it may be that the samples collected do not contain the toxicants present in the initial discharge and this may lead to inaccurate determination of the source of toxicity.

2.6 Concerns About Aquatic Toxicity Testing of Wet Weather Discharges

Many authors caution users about the interpretation of data and use of statistics. It has now been realized that many of the tests being used, cannot tell us as much as we originally anticipated and that we must not assume data to be "absolute", and to compare results with care (Dutka, 1988).

Morrison and Wei (1991) suggest that metals should be looked at with respect to bioavailability (soluble), and not just total concentrations.

Some authors focus on the applicability of tests to the periodic discharges from CSOs and stormwater. Milne et al. (1995) suggest that most chronic tests were developed based on "long term exposure" of the organism to a particular effluent. With stormwater discharges, the organisms are exposed to the effluent for only a short period before the toxic discharge is replaced with upstream waters. They suggest that these wet weather discharges warrant different standards (perhaps using only acute toxicity tests), for the evaluation of effluents. An alternative could be to use composite samples which represent the total effect of the storm and not just initial or final stages of the runoff event.

Pitt et al. (1995) and Jones et al. (1993) suggest that after toxicant types are identified, potential sources in the watershed should be assessed. These should then be minimized or eliminated as opposed to dealing with the pollutants with "end of pipe" solutions.

Paulson and Amy (1993) propose using a model to predict the bioavailability of metals in the receiving waters, based on concentration of total metals, metals bound in the solids, suspended solids levels, pH and dissolved organic carbon. In this way, the model can predict the degree to which metals will be leached from the sediment and become bioavailable.

Herricks et al. (1994) suggest that using a biochemical test (such as stimulation of hormone production in fish) produces a more useful result than one based on organism or cell mortality, and that these types of tests are more sensitive to synergistic effects of metals, solids and nutrients.

Lange and Lambert (1995) thought that using bioaccumulation would be a more "accurate" assessment of the long-term toxicity potential for a discharge, as it would determine the likelihood of persistent chemical toxicity.

Dutka et al. (1995) showed that a direct test of sediments without extractions would provide a more realistic assessment of toxicity and that there were less likely to be "masked" effects. In addition, they cautioned about the general interpretation of "biologically based tests". As organisms or cells respond very differently to subtle changes in environmental conditions, duplicate analyses may show quite different responses. Comparing results may be best done using a "scale" where response ranges are categorized as found in Dutka (1988).

Bascombe et al. (1990) were concerned that the criteria for toxicity for industrial discharges have been based on target chemicals (e.g. Cr) and that the overall toxicity of the effluents has not been adequately used to legislate discharges. It was also suggested that these toxicity guidelines should be applied to wet weather discharges to help alleviate the downstream pollution and improve treatment technology.

3.0 STUDY AREAS

Stormwater and combined sewer overflow sample sites were selected based on potential for creating water quality problems, range of input conditions, as well as ease of accessibility. Most samples were taken by hand (termed "grab" sample), during a storm event. Despite every effort to catch "first flush" conditions, some samples may have been taken after the outfalls had been discharging for a period of time. The following sections describe the field sites for stormwater outfalls and combined sewer overflows.

3.1 Stormwater Outfalls

3.1.1 Outfalls Without Treatment

Samples were collected at six different stormwater outfalls in Kingston, Scarborough and Burlington. Photographs of each outflow can be found in Appendix B. Water samples for ecotoxicity tests were collected by hand as well as using automated samplers, and consisted of 1.5 L of water in plastic (HDPE), acid washed bottles for acute toxicity tests and 4 L in amber glass bottles for PAH analysis.

In Kingston, two outfalls were selected which discharge into a stormwater pond located in Kingston Township. This pond is under co-operative investigation by Queen's University, Department of Civil Engineering, and the National Water Research Institute. The outfall from the pond feeds the Little Cataraqui Conservation area, which is a protected lakeshore marsh, and hence a sensitive ecosystem. A map which outlines the sampling locations can be seen in Figure 3.1.

The first outfall was the drain from a 12.6 ha parking lot at the Cataraqui Town Centre shopping plaza. This discharge pipe collected stormwater from catchbasins and roof runoff outlets located around the parking lot (Photo B1). A box weir was constructed around the outlet pipe for the purpose of monitoring flow and an automated sampler was programmed to collect runoff at the onset of flow over the weir (Van Buren 1994).

The second site was located at the other inflow to the stormwater pond, the West branch of the little Cataraqui Creek (Photo B2). This urban stream received road runoff and residential drainage from upstream developed and rural areas, including a small industrial park. The total drainage area was 4.5 km^2, with approximately 80 ha of connected impervious area. A weir was

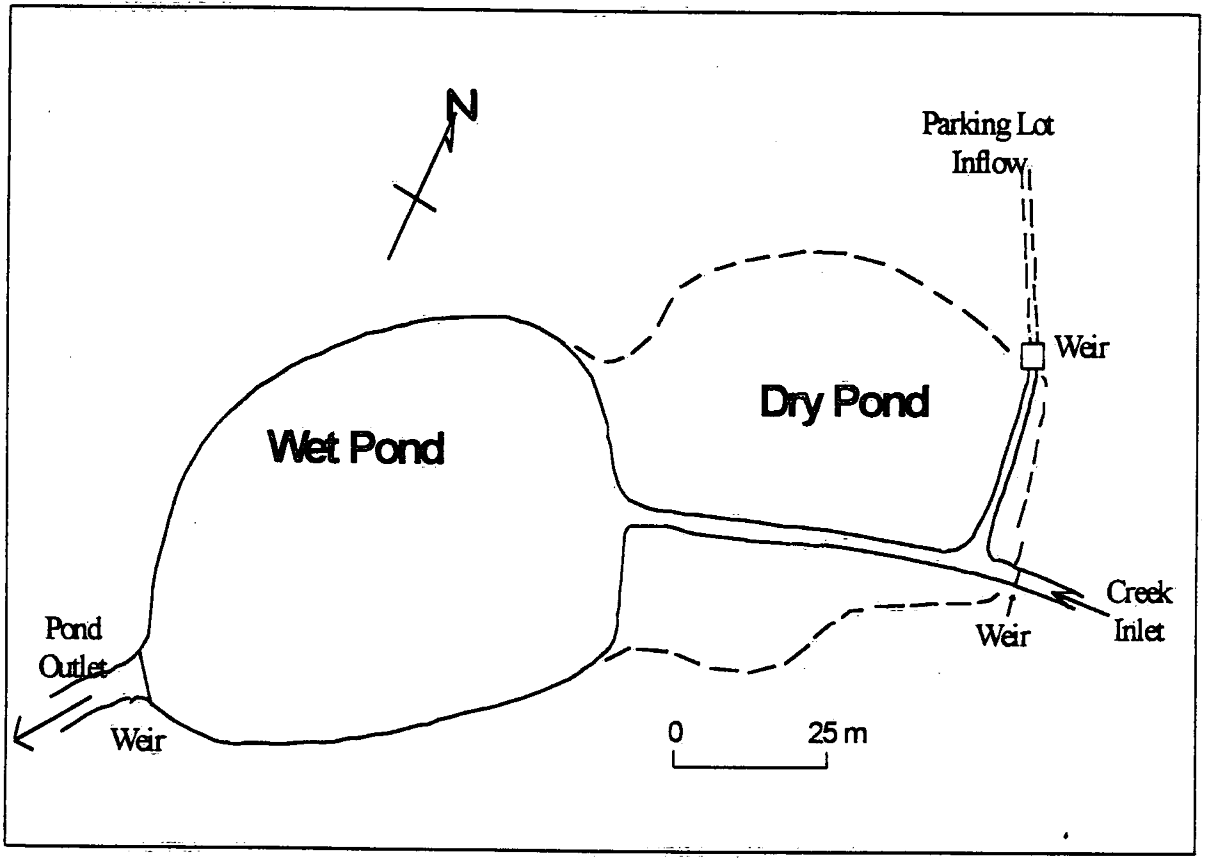

Figure 3.1: Cataraqui Stormwater Pond in Kingston Township, Ontario

placed in stream to measure flow, and an automated sampler installed to acquire water quality samples. These samplers were programmed to take 6 L of water in the first 6 minutes of flow, ensuring a "first flush" was captured.

In Scarborough, three sampling locations were chosen which represent highway runoff inputs to the Rouge River drainage basin (see Figure 3.2). The stormwater pond is located east of Port Union Road, immediately south of Highway 401. Samples from these locations were taken using "grab" techniques.

The first site is located at the inlet to the stormwater pond, and receives runoff from Highway 401 and surrounding roadways. Approximately 250 m north of the Scarborough pond is the Highway 2 bridge. Deck drains channel runoff into a 12" corrugated steel culvert, and samples were taken at this point (Photo B3). The third site was located at the Highway 401 bridge, immediately south of

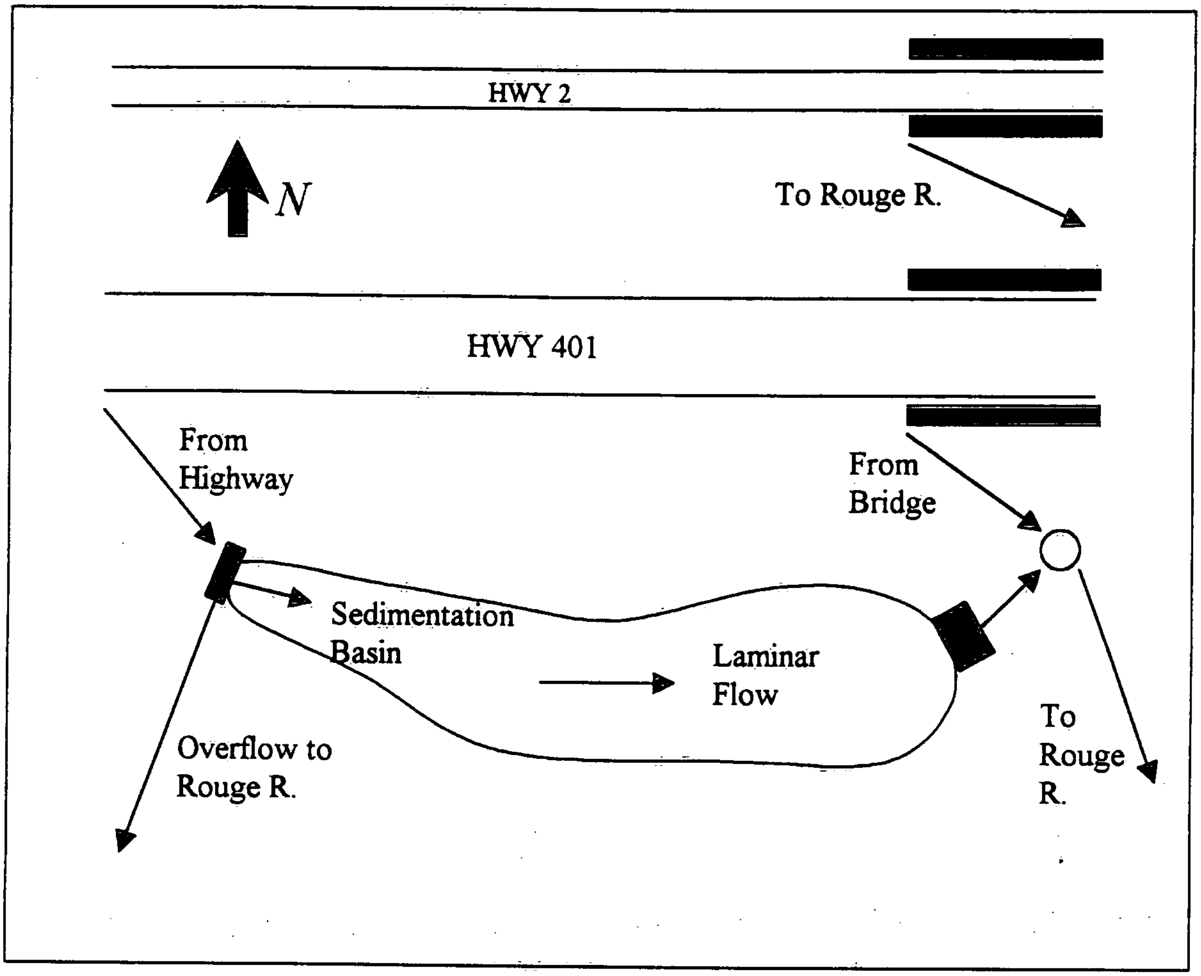

Figure 3.2: Scarborough Stormwater Pond – Rouge River, Scarborough, Ontario

Highway 2. Deck drains from the Highway 401 bridge convey the water to a storm sewer, which combines with the effluent from the Scarborough stormwater pond (Photo B4), and enters the Rouge River.

In Burlington, the James N. Allen Burlington Bay Skyway bridge was used as a sample collection site. Samples were taken from two 250 L barrels set up under the northbound lane deck drains (Photo B5). The site was located approximately 750 m south of the Highway 2 (Lakeshore Rd.) turnoff (Interchange 97). The bridge receives heavy traffic across the 4 northbound lanes. When required, samples were also collected by hand at the start of storm events.

3.1.2 Outfalls From Best Management Practices

Best management practices include an array of water quality improvement systems designed to improve the pollutant removal performance of stormwater management facilities. Examples of these methods investigated in this study are: stormwater ponds, constructed wetlands, a biofilter and an oil/grit separation device.

3.1.2.1 Stormwater Ponds

The performance of two stormwater ponds was analyzed during these investigations. Considerable emphasis has been placed on solids removal and pollutant removal performance of stormwater control measures, however there has been relatively little understood about the change in acute toxicity of the flow passing through these systems.

The Kingston pond was sampled at the outlet of the large detention basin, using automated and grab sampling techniques. This pond is an on-stream pond which receives continuous flow of water from upstream catchment sources. It was constructed in 1982 to control the runoff created by the 12.6 ha Cataraqui Town Centre parking lot. Since then, there has been considerable development in the 4.5 km^2 watershed upstream of the pond, resulting in much higher flows during rain events. The stormwater facility consists of a two stage pond: a wet pool (5000 m^2 by 1m deep) and a dry pond of similar size. The dry pond area floods when the pond exceeds its normal operation level by 0.2 m.

The Scarborough stormwater pond was constructed by the Ontario Ministry of Transportation to help address water quality and fisheries issues downstream. The system is an extended wet detention pond 300 m long and width varying between 25 to 40 m. It is equipped with a sediment forebay of 80 m long by 20 to 40 m wide, which is separated from the rest of the pond by a submerged weir. This forebay was designed to catch most of the large particulates. The permanent wet pool is 2.5 m deep with a maximum depth of 4.5 m. Any storms which are too large for the pond to handle (i.e. greater than 2 year frequency) are diverted.

3.1.2.2 Constructed Wetlands

Two constructed wetland cells had been established by Queen's University at the stormwater pond in Kingston Township. The field scale cells were 4.9 m long by 1 m wide by 0.6 m deep, and were constructed from 22 gauge galvanized sheet metal, reinforced with a welded aluminum frame. The frame was lined with polyethylene and filled with 10 mm ($^3/_8$") limestone gravel, which provided a medium of high hydraulic conductivity. The wetlands were planted with broad leaf cattail and arrowhead and later supplemented by reeds and spike rush (Photo B7). Colonization by other species was not discouraged. The wetlands were located at the outlet to the stormwater pond and received final effluent from this pond for polishing (Rochfort, 1996).

3.1.2.3 Biofilter

Queen's University also constructed a field scale submerged aerobic biological filtration (SABF) unit at the outlet of the stormwater pond. This unit was placed in a polyethylene plastic tank (diameter 1.048 m), with a total volume of 1 m^3. The filter medium was an expanded schist with a nominal diameter of 3 to 6 mm packed to a depth of 800 mm. Water was supplied to the filter from the outlet of the stormwater pond for polishing, and percolated downward over the medium to an underdrain system (Caldwell, 1994).

3.1.2.4 Oil Grit Separator

Two in-place oil/grit separator units were selected for this study. The unit in Burlington was located on Mainway near Walkers Line and received urban runoff from a developing industrial park. Traffic flow was limited in this area, and sampling inflow to the unit required co-operation from the City of Burlington. The outflow discharged into a small urban creek.

A unit in Waterdown received runoff from a Sunoco Gas station forecourt (Photo B8). Access to one of the inlets was through a manhole, however the other inlet drain was inaccessible. The main unit and outflow were easily accessible for sampling.

3.2 Combined Sewer Overflows

Combined sewer overflow sites were all located in Hamilton, and their exact location can be seen on the accompanying map (Figure 3.3). They were chosen to represent all types of land use. The industrial sites were located at Kenilworth Avenue and Parkdale Avenue. These systems discharged, indirectly, into Hamilton harbour.

Three residential/commercial sites discharged into Red Hill Creek, an ecologically sensitive area. Melvin Avenue outfall at the lower end, was considered to have the greatest commercial input. Queenston Road outfall was located higher up, and appeared to have less commercial activity in its sewershed, but greater traffic flow. Lawrence Avenue outfall was located at the top of the system, and was primarily serving residential land and a major traffic artery.

In the west end, two sites were selected. Sterling Avenue outfall received runoff from parking lots and major roads near MacMaster University, as well as the hospital and laboratory waste sewage. The outfall was located in a small creek. A purely residential site was found at the corner of Stroud and Royal Avenues.

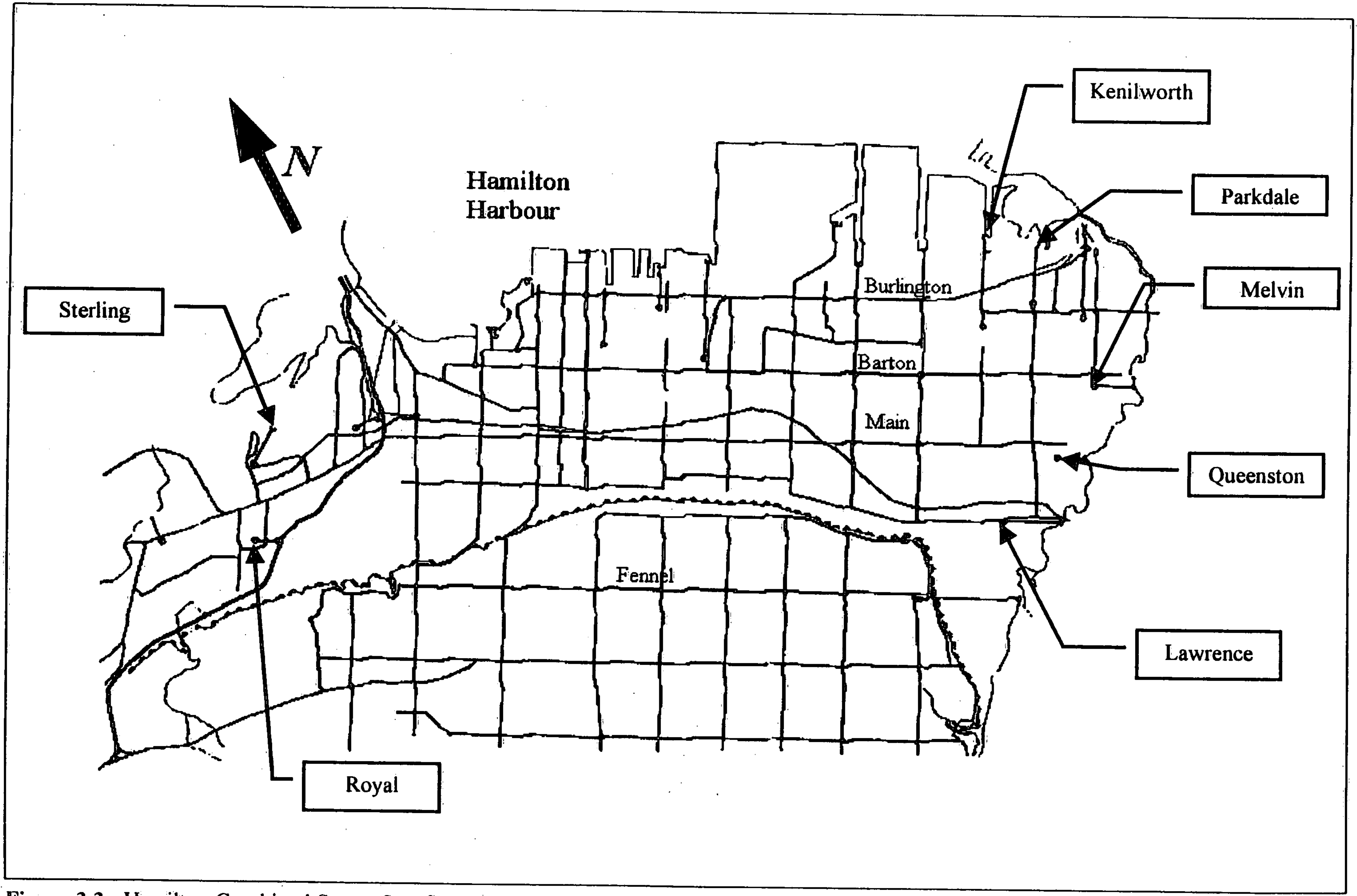

Figure 3.3: Hamilton Combined Sewer Overflow Site Location Map

4.0 METHODS

4.1 Bacteria

Bacterial counts of Fecal coliform and *Escherichia coli* were performed on samples which were less than 12 hours old, using A-1 broth with MUG. A five tube, three or four series, most probable number (MPN) test was used. In order to perform this test, the fresh water sample was used to inoculate the A-1 growth medium in 5 tubes. A series of 1:10 dilutions were made to lower the initial number of bacteria used in the inoculation. This range allows for more accurate estimates of total probable numbers. The samples were then capped and incubated for 24 hours at 44.5°C. A full description of these methods can be found in Dutka and Seidl (1993) and APHA (1989).

Fecal coliform bacteria are gram negative, non-spore forming bacilli which are cytochrome oxidase negative and ferment lactose. During the process of fermentation, gasses are released and trapped inside small inverted tubes. The number of positive tubes in each dilution was recorded and using a table, an estimate of fecal coliform numbers was achieved.

Escherichia coli are gram negative, oxidase negative bacteria which ferment lactose. They also produce the enzyme β-glucoronidase (which may also be found in *Salmonella* and *Shigella*), which degrades MUG (4-methylumbelliferyl-β-glucuronide) to produce 4-methylumbelliferone. This product fluoresces under long range UV light. The number of positive tubes in each dilution were recorded and then estimates were made as to the most probable number of *E. coli*.

4.2 Acute Toxicity Testing

The bioassays used on water samples for this study include: *Daphnia magna* 48 hour acute test, Microtox™ 15 minute test, Sub-mitochondrial particle bioassays (reverse and forward electron transport) and the SOS-Chromotest. With the exception of the *Daphnia magna* test, all other tests were performed on samples concentrated ten times (10X) by flash evaporation techniques. The concentration factor is commonly used in aquatic toxicity tests to eliminate the effect of the immediate dilution of the sample during preparation for testing. In many cases, the sample solution must be mixed with a medium that supports the life of the cell or organism. When a natural water sample is used, it is diluted when mixed with this medium, and therefore the toxic effects may also be reduced. In this way, the 10X concentration allows the original sample concentration of pollutant to be used in the test, rather than a diluted concentration. A detailed description of the toxicological techniques can be found in Dutka (1996) and Dutka (1989).

The reason behind using many tests was that these test organisms and tissues are sensitive to different concentrations and mixtures of pollutants. Some may show severe effects at very low levels, while others may not respond until much higher concentrations are experienced. As not all pollutants are bioavailable to all types of organisms, a battery of tests approach helps to reduce the chances that a sample will be registered non-toxic when in fact there may be some acute effect when using a different test. Table 4.1 shows a list of tests commonly used in aquatic toxicity testing, and the type of effects each one can measure.

Table 4.1: Toxicity Tests and Types of Toxicity Detected

Test	**Cytotoxicity**	**Genotoxicity**	**Acute**	**Chronic**
	Causes cellular damage	Causes genetic damage	Short Term	Long Term
Daphnia magna	✓		✓	
Microtox™	✓		✓	
Sub-mitochondrial particle bioassay	✓		✓	
SOS Chromotest		✓	✓	
Ames Fluctuation Test		✓	✓	
Rainbow Trout	✓		✓	
Fathead minnow	✓			✓
Ceriodaphnia	✓	✓		✓

4.2.1 *Daphnia magna*

The cladoceran *Daphnia magna* used in these tests is the largest of the *Daphnia*, often reaching 5 mm in size. The neonates (first-instar young) are approximately 0.9 mm long and can easily be observed by eye. Twelve to 24 hour old neonates are most commonly used in acute toxicity tests.

In the test, 10 neonates are used for each sample and sample dilution (usually 100, 75, 50, 25 and 10%) to be tested. The neonate organisms are observed after 1 hr, 4 hr, 24 hr and 48 hours incubation at 21±1 °C and the number of dead animals are recorded. A 48 hour LC_{50} or EC_{50} is derived from the pattern of deaths observed (Dutka, 1997). LC_X indicates the concentration at which X% of the organisms die (e.g. LC50 of 25% would indicate that when the test water is diluted to 25% of its original concentration, it would kill 50% of the test organisms). Here, "LC" stands for "lethal concentration". Similarly, the EC_X value shows the concentration at which X% of the organisms are inhibited (generally in growth or reproduction), where "EC" stands for "effective concentration".

4.2.2 Microtox™

Microbics Corporation has developed a photometric technique which uses a marine bioluminescent bacterium's (*Vibrio fischeri*) response to chemical exposure for assessing relative toxicity.

In the test the rehydrated bacteria are incubated (15°C) in the liquid sample and dilutions of the sample for 15-30 minutes. The samples are read in a Microtox 500M reader with computer print out and the toxicant concentration (% of sample) at which a fifty percent normalized light loss occurs for a certain exposure time is automatically calculated and reported as the EC_{50} (effective concentration for 50% light loss) of the toxicant (Dutka, 1997).

4.2.3 SMP (Reverse electron transport)

This procedure uses beef heart sub-mitochondrial particles (SMP) to screen for toxicants in liquid samples. The SMP are fragmented portions of the inner membrane of mitochondria and retain the ability to carry out the integrated enzymatic processes of electron transport and oxidative phosphorylation and are commonly called electron transport particles (ETP).

This bioassay is based on the ability of ETP to use energy supplied by adenosine tri-phosphate (ATP) to drive electrons supplied by succinate in a thermodynamically unfavourable direction through mitochondrial respiratory complex II to complex I where it reduces NAD to NADH. NAD is nicotinomide adenine dinucleotide, which acts as an electron acceptor in this biochemical reaction.

NADH is the reduced form of the NAD complex (containing one additional hydrogen atom).

To perform the test, thawed and reconstituted electron transport particles are added to a cuvette containing test reagent and the toxicant or environmental sample. ATP is added to drive the electron transport process and the reaction rate is monitored using a spectrophotometer. Toxicity is determined by comparing the rate of electron transport in the cuvettes containing the test samples to the rate observed in control cuvettes (Dutka, 1997).

4.2.4 SMP (Forward electron transport)

This procedure also uses beef heart sub-mitochondrial particles. The Forward (or Conventional) Electron Transport assay (FET or CET) is based on the forward movement of electrons from NADH through mitochondrial respiratory enzyme complexes I, III and IV. This is the direction of normal flow of electrons through these enzymes during cellular respiration. The conversion of NADH to NAD is monitored spectrophotometrically at 340 nm.

To perform the test, thawed and reconstituted electron transport particles are added to a cuvette containing test reagent and the toxicant or environmental sample. NADH is added as an electron donor and the rate of NADH oxidation is monitored using a spectrophotometer. The toxicity of the sample is determined by comparing the rate of NADH depletion in the sample cuvettes to the rate observed in control cuvettes (Dutka, 1997).

4.2.5 SOS Chromotest

This test for the presence of bioavailable genotoxicants, is based on a colorimetric assay of microbial enzymatic activities after incubating the genetically engineered tester strain (*E. coli* K12-PQ37) with a suspected liquid sample. The *E. coli* K12-PQ37 has been altered so that the ß-galactosidase gene (lacZ) is fused to the sulA gene. The sulA gene is part of the error-prone SOS repair system.

In the test an exponential growth phase culture of the *E.coli* is introduced into the wells of a microtitration plate containing samples and controls. After, a two hour incubation at 35°C for protein synthesis, ß-galactosidase activity (SOS response activity) is measured by changes in the optical density of the sample at 615 nm in a microtitration plate reader. This measures the level of ß-galactosidase via its effect on the indicator compound 5-bromo-4-chloro-3-indolyl-ß-D-galactoside. Thus the greater the amount of ß-galactosidase produced, the greater the SOS response pathway has been induced and thus the greater the genotoxicant concentration in the sample. This kit test can run with or without S-9 (Aroclor induced liver homogenate), and can be read visually or by a spectrophotometer (Dutka, 1997).

4.3 PAH Analysis

4.3.1 Filtration of Water Samples

A suitable portion (50-200 mL) of whole water sample was filtered through a 47 mm pre-washed, combusted (450°C) and pre-weighed Whatman GF/F glass fibre filter (pore size 0.7 µm) in order to determine the content (or concentration) of suspended solids in the whole sample. The remainder of the 4 L sample was pressure filtered through a 142 mm combusted (450°C) Gelman A/E filter (pore size 1 µm). The filtration apparatus was composed of stainless steel and PTFE components and was washed and rinsed with organic free water between samples. The filtered water was stored at 2°C until extracted and the filters were placed in test tubes and stored frozen.

4.3.2 Extraction of Water Samples

The samples were brought to room temperature and extracted in two-2 L portions with dichloromethane (DCM). Each portion was extracted with 75, 50 and 50 mL of DCM. The sample bottle was rinsed with this DCM to recover any hydrophobic compounds remaining in the container. The combined extracts were dried using combusted (450°C) sodium sulphate, concentrated on a rotary evaporator, transferred quantitatively to a 15 mL graduated tube, reduced to a few millilitres, and solvent exchanged into toluene (1.0 mL) containing 500 ng of dibenzofuran-d_8 as internal standard. This was then analyzed by gas chromatography-mass spectrophotometry in single ion mode, for polycyclic aromatic hydrocarbons (PAHs), alkyl-PAHs and dibenzothiophene. The results were quantified using Hewlett-Packard Chemstation™ software. Further details of this method can be found in Brownlee and Crosley (1996).

The instrument used was a Hewlett-Packard model 5890 gas chromatograph connected to a Hewlett-Packard model 5791 mass selective detector operating in electron impact mode at 70 eV.

The column was a 30 m x 0.25 mm capillary column coated with 0.25 μm of DB-5ms, helium carrier at ca. 35 cm/s (constant flow mode). The temperature program was 80-245°C at 3°C/min, then at 4°C/min to 280°C with a 10 minute hold. A 1 μL sample was injected by splitless injection.

4.3.3 Extraction of Particulate Material (Filters)

The filters were thawed, ground with combusted (450°C) sodium sulfate to adsorb water, and extracted with DCM in a Soxhlet apparatus for 18 hours (5-6 cycles per hour). The extracts were then evaporated and prepared for analysis in the same manner as the water extracts.

4.4 Toxicity Point Value Assessment of Data

The results from the battery of acute toxicity bioassays are presented in many different forms. Each test has specific initial indications of toxicity, definite indications and degrees of toxic response. As one of the goals of this research was to compare the sensitivity of the tests, it was necessary to be able to relate the results of one type of test to another. Dutka (1988) proposed the concept of a scaled response, which helps to identify the degree of toxicity of a sample, and makes it easy to relate one test response to another. This is a 10-point scale ranging from no toxicity (0) to strong acute toxicity (10). This Toxicity Point Value (TPV) could then also be used to determine the overall toxicity of a particular field site, which is useful in identifying consistently toxic sites.

The toxicity point value scale used in the interpretation of results from these battery of test results has been developed from Dutka (1988), and is now reduced to a four point scale (0, 1, 2 and 3). The original scheme used only data from SOS Chromotest, Microtox™ and *Daphnia magna*, where the sample had been concentrated 10 times. The new interpretation added the sub-mitochondrial particle bioassay (SMP) and allowed the final TPV to be subjectively altered by the results obtained using the 1X concentration. The conversion between test results and toxicity point values was based on guidelines outlined in Table 5.1 (Results Section).

5.0 RESULTS

This section details the results of the toxicity testing as well as some data from the organic chemical analysis that was performed. The raw data set (Appendix A) was converted into "Toxicity Point Values" to ease comparisons and aid in ranking sites. This toxicity point scale of 1 - 10 has been modified after Dutka et al. 1988, as discussed previously (Section 4.4). A summary of the limits for each toxicity range is listed in Table 5.1. Samples were considered to have no toxicity at level zero (0), suggested toxicity at level one (1), confirmed toxicity at level two (2) and strong toxicity at level three (3).

Table 5.2 shows a summary of the data gathered during the two years of field work. The data are presented as toxicity point values (scale of 0 to 3) for toxicity test results. Data concerning the precipitation, antecedent dry period and location of collection are also included in this table. Mean and standard deviation values refer to all tests combined (for all samples at that location), and therefore act as a guide for ranking the various sites.

5.1 Statistical Analysis of Toxicity Data

The data were analysed using basic statistical techniques, in order to interpret observed trends more accurately. Several different methods were used to compare the data. Samples which were generated by stormwater runoff, were generally analyzed together, as their sources were similar. Combined sewer overflows were treated as a group unless individual sites were being assessed. Determination of the frequency of positive detection, the most sensitive test (by way of response), ranking of sites and correlation of sample toxicity with various parameters, including rainfall, antecedent dry period, organic chemical analyses (for Phenanthrene, Fluoranthene and Pyrene), as well as suspended solids were performed on this data. The results from these analyses follow.

Table 5.1: Toxicity Point Values Corresponding to Raw Toxicological Data

Effect Level	**Toxicity Point Value**	***Daphnia magna*** **EC** *Percent Inhibition*	**Microtox EC50** (10X) *Percent Inhibition*	**Sub-mitochondrial Particle (RET and CET)** (10X) *Percent Inhibition*	**SOS Chromotest** (10X) *Genotoxicity Induction Factor*
No Toxicity Present	**0**	EC10 at 100%	> 100	0 - 9	< 1.00
Indication of Potential Toxicity	**1**	EC20 - EC40 at 100%	> 40	10 - 50	1.0 - 1.29
Confirmed Toxicity	**2**	EC50 at 100%	40.0 – 10.0	51 - 90	1.30 - 2.00
Severe Level of Toxicity	**3**	EC50 at 75% and below	9.0 and below	91 - 100	2.01 and above

EC - Effective concentration required to inhibit some percentage of the organism tested. (An EC20 at 100% indicates that 20% of the organisms were affected by the 100% solution)
10X - Test performed on sample which had been concentrated 10 times by flash evaporation.
RET - Reverse Electron Transfer
CET - Conventional (Forward) Electron Transfer

Table 5.2: Toxicity Point Values for Combined Sewer Overflows - Listed By site

Location	Date D-M-Y	Rainfall (mm)	Antecedent Dry (Days)	Precip Type	*Daphnia magna*	Microtox	SMP RET	SMP CET	SOS	TSS (mg/L)
Lawrence	28-Nov-95	0.4	0	R	0	0	1	0	3	121.5
Lawrence	28-Nov-95	0.4	0	R	0	0	1	0	2	-
Lawrence	19-Jan-96	18.6	0	R/S	0	2	1	0	0	207.6
Lawrence	19-Jan-96	18.6	0	R/S	1	0	1	0	1	430.6
Lawrence	24-Jan-96	11.6	0	R	0	0	1	0	0	90.1
Lawrence	22-Apr-96	7.7	1	R	0	0	1	0	1	-
					The mean TPV for all tests at this site (n=30) is 0.53 (Std. Dev.=0.78)					
Queenston	15-Dec-95	Trace	0	S	0	0	2	0	0	69.1
Queenston	17-Jan-96	1.8	0	S	0	0	2	0	0	243.3
Queenston	24-Jan-96	11.6	0	R	0	0	2	0	0	124.3
Queenston	23-May-96	15.4	0	R	0	0	2	0	1	-
Queenston	07-Sep-96	59.4	10	R	0	0	1	0	1	-
					The mean TPV for all tests at this site (n=25) is 0.44 (Std. Dev.=0.77)					
Melvin	15-Dec-95	Trace	0	S	0	0	1	1	1	11.8
Melvin	15-Dec-95	Trace	0	S	0	0	1	1	1	12.6
Melvin	24-Jan-96	11.6	0	R	0	0	2	0	0	134.6
Melvin	09-May-96	12.4	0	R	0	0	2	0	1	-
Melvin	09-Oct-96	11.4	7	R	0	0	2	0	0	
Melvin	18-Oct-96	35.8	8	R	0	0	1	0	0	
					The mean TPV for all tests at this site (n=30) is 0.47 (Std. Dev.=0.68)					
Parkdale	17-Jan-96	1.8	0	S	0	0	1	0	0	44.7
Parkdale	22-Apr-96	7.7	1	R	0	0	1	0	1	-
Parkdale	09-May-96	12.4	0	R	0	0	1	0*	1	-
Parkdale	11-May-96	11.1	0	R	0	0	1	0	1	-
Parkdale	26-Aug-96	3.8	4	R	0	0	3	2	1	-
Parkdale	07-Sep-96	59.4	10	R	1	0	1	0	1	-
					The mean TPV for all tests at this site (n=30) is 0.53 (Std. Dev.=0.73)					
Royal	17-Jan-96	1.8	0	S	0	0	1	1	1	36.1
Royal	17-Jan-96	1.8	0	S	0	0	1	0	1	35.5
Royal	24-Jan-96	11.6	0	R	0	0	1	0	1	230.3
Royal	24-Jan-96	11.6	0	R	0	0	2	0	1	211.8
Royal	22-Apr-96	7.7	1	R	0	0	1	0	1	-
Royal	07-Jun-96	10.2	0	R	1	0	1	0	1	-
					The mean TPV for all tests at this site (n=30) is 0.50 (Std. Dev.=0.57)					
Sterling	17-Apr-96	0	0	D	1	0	2	0	1	-
Sterling	22-Apr-96	7.7	1	R	0	0	2	2	1	-
Sterling	22-Apr-96	7.7	1	R	0	0	1	0	2	-
Sterling	07-Sep-96	59.4	10	R	0	2	1	0	1	-
					The mean TPV for all tests at this site (n=20) is 0.80 (Std. Dev.=0.83)					
Kenilworth	29-Apr-96	3.4	1	R	0	0	2	0	1	-
Kenilworth	30-Apr-96	10	0	R	0	0	2	0	1	-
Kenilworth	10-May-96	19.2	0	R	0	0	1	0	1	-
					The mean TPV for all tests at this site (n=15) is 0.53 (Std. Dev.=0.74)					

Precipitation Type R - Rain Event, S - Snowmelt, R/S - Rain and Snowmelt, D - Dry.
Data listed are Toxicity Point Values (scale of 0 to 3). Mean and Std. Dev. (shown in brackets) refer to results from all tests at that site.
Data complete as of March 31/97.

Table 5.2 Continued: Toxicity Point Values for Stormwater Sites - Listed By Site

Location	Date D-M-Y	Rainfall (mm)	Antecedent Dry (Days)	Precip Type	*Daphnia magna*	Microtox	SMP RET	SMP CET	SOS	TSS (mg/L)
Skyway Bridge	27-Nov-95	24.1	0	R	0	0	3	1	2	182.3
	28-Nov-95	0.4	0	R	0	0	2	0	2	183.1
	28-Nov-95	0.4	0	R	0	0	2	0	2	198.8
	01-Dec-95	0.4	0	R	3	0	3	2	2	145.7
	04-Dec-95	0.2	0	R	3	0	3	2	2	247.8
	04-Dec-96	0.2	0	R	0	0	3	0	1	204.7
	15-Dec-95	Trace	0	S	3	2	3	2	0	43.3
	17-Jan-96	1.8	0	S	3	0	3	2	0	86
	23-Jan-96	3.2	3	S	3	2	0	2	0	851.4
	24-Jan-96	11.6	0	R	2	0	3	2	0	106.75
	24-Jan-96	11.6	0	R	2	1	3	2	0	143.9
	24-Jan-96	11.6	0	R	2	2	3	2	0	-
	24-Jan-96	11.6	0	R	3	2	3	2	0	-
	24-Jan-96	11.6	0	R	3	1	2	0	0	-
	27-Jan-96	13.4	0	R	3	2	3	2	0	43.4
	19-Apr-96	6.2	2	R	0	0	3	3	1	-
	19-Apr-96	6.2	2	R	0	0	3	0	1	-
	22-Apr-96	7.7	1	R	0	0	2	0	1	-
	09-May-96	12.4	0	R	0	0	2	0	1	-
	09-May-96	12.4	0	R	0	0	3	0	1	-
	11-May-96	11	0	R	0	0	2	0	1	-
	21-May-96	16.6	0	R	0	0	1	0	1	-
	20-Jun-96	7.8	0	R	0	0	0	0	1	-
	19-Jul-96	9.4	0	R	0	0	2	0	1	-
	01-Dec-96	5.4	0	R	0	0	3	1	0	-
					The mean TPV for all tests at this site (n=125) is 1.18 (Std. Dev.=1.20)					
Skyway Bridge Time Series	22-Sep-96	10.2	5	R	0	1	3	2	1	89.3
	22-Sep-96	-	-	R	0	1	3	2	1	109.6
	22-Sep-96	-	-	R	0	0	3	1	1	106
	22-Sep-96	-	-	R	0	0	3	1	1	58.8
					The mean TPV for all tests at this site (n=20) is 1.20 (Std. Dev.=1.11)					

Precipitation Type R - Rain Event, S - Snowmelt, R/S - Rain and Snowmelt, D - Dry.

Data listed are Toxicity Point Values (scale of 0 to 3) Average and Standard Deviation (Std) refer to results from all tests at that site.

Data complete as of March 31/97.

Table 5.2 Continued: Toxicity Point Values for Stormwater Sites - Listed By Site

Location	Date D-M-Y	Rainfall (mm)	Antecedent Dry (Days)	Precip Type	*Daphnia magna*	Microtox	SMP RET	SMP CET	SOS	TSS (mg/L)
Urban Creek Kingston	10-Oct-95	18.6	6	R	0	0	1	0	2	83.2
	01-Nov-95	15.8	0	R	0	0	1	1	2	15.3
	07-Nov-95	6.9	3	R	0	0	2	0	0	40
	07-Nov-95	6.9	3	R	0	0	1	0	2	39.5
	26-Nov-95	1.4	0	R/S	0	0	2	1	2	54.8
	20-Feb-96	31.8	0	R/S	0	0	2	0	0	12.6
					The mean TPV for all tests at this site (n=30) is 0.63 (Std. Dev.=0.85)					
Parking Lot Kingston	10-Oct-95	18.6	6	R	0	0	3	2	2	105.9
	10-Oct-95	18.6	6	R	0	0	2	2	1	106.5
	01-Nov-95	15.8	0	R	0	0	2	0	0	44.7
	07-Nov-95	6.9	3	R	0	0	1	0	0	17.2
	07-Nov-95	6.9	3	R	0	0	2	1	1	60.8
	26-Nov-95	1.4	0	R/S	0	0	1	0	2	19
	20-Feb-96	31.8	0	R/S	0	0	2	0	0	73.8
	20-Feb-96	31.8	0	R/S	0	0	2	0	0	66.4
					The mean TPV for all tests at this site (n=40) is 0.65 (Std. Dev.=0.92)					
Pond Outflow Kingston	10-Oct-95	18.6	6	R	1	0	0	1	2	35
	10-Oct-95	18.6	6	R	1	0	0	1	2	35.6
	01-Nov-95	15.8	0	R	1	0	1	0	2	14.4
	07-Nov-95	6.9	3	R	0	0	1	0	0	17.2
	07-Nov-95	6.9	3	R	1	0	1	0	1	-
	26-Nov-95	1.4	0	R/S	0	0	1	0	2	19.4
	20-Feb-96	31.8	0	R/S	0	0	2	0	1	65.1
					The mean TPV for all tests at this site (n=35) is 0.63 (Std. Dev.=0.73)					

Precipitation Type R - Rain Event, S - Snowmelt, R/S - Rain and Snowmelt, D - Dry.
Data listed are Toxicity Point Values (scale of 0 to 3) Average and Standard Deviation (Std) refer to results from all tests at that site.
Data complete as of March 31/97.

Table 5.2 Continued: Toxicity Point Values for Stormwater Sites - Listed By Site

Location	Date D-M-Y	Rainfall (mm)	Antecedent Dry (Days)	Precip Type	*Daphnia magna*	Microtox	SMP RET	SMP CET	SOS	TSS (mg/L)
HWY 2 Bridge Scarborough	24-Oct-95	Trace	1	R	1	0	2	0	2	18.9
	27-Oct-95	10.4	0	R	1	0	2	0	1	18.9
	27-Oct-95	10.4	0	R	0	0	2	1	1	-
					The mean TPV for all tests at this site (n=15) is 0.87 (Std. Dev.=0.83)					
HWY 401 Bridge and Pond outfall	24-Oct-95	Trace	1	R	1	0	3	2	1	7.4
	27-Oct-95	10.4	0	R	1	3	2	1	2	64.9
	27-Oct-95	10.4	0	R	3	-	0	1	0	-
					The mean TPV for all tests at this site (n=14) is 1.43 (Std. Dev.=1.09)					
Pond Inflow Scarborough	24-Oct-95	Trace	1	R	1	0	2	0	0	38.15
	27-Oct-95	10.4	0	R	1	0	1	0	0	271.5
	27-Oct-95	10.4	0	R	1	0	2	0	0	-
					The mean TPV for all tests at this site (n=15) is 0.53 (Std. Dev.=0.74)					
Pond Outflow Scarborough	24-Oct-95	Trace	1	R	0	0	1	1	0	98.2
	27-Oct-95	10.4	0	R	0	0	2	1	0	29.35
	27-Oct-95	10.4	0	R	1	0	1	0	0	-
					The mean TPV for all tests at this site (n=15) is 0.47 (Std. Dev.=0.64)					
Oil/Grit Inlet	18-Jun-96	29	0	R	1	0	1	0	1	-
					The mean TPV for all tests at this site (n=5) is 0.60 (Std. Dev.=0.55)					
Oil/Grit Outlet	09-May-96	12.4	0	R	0	0	1	0	1	-
	18-Jun-96	29	0	R	1	0	1	1	0	-
	18-Jun-96	29	0	R	1	0	1	1	1	-
	18-Jun-96	29	0	R	0	0	1	0	1	-
					The mean TPV for all tests at this site (n=20) is 0.55 (Std. Dev.=0.51)					
Wetland Inlet	19-Oct-95	0	0	D	0	0	1	1	2	433.8
	07-Nov-95	6.9	3	R	1	0	1	0	2	1112.9
					The mean TPV for all tests at this site (n=10) is 0.80 (Std. Dev.=0.79)					
Wetland Outlet	19-Oct-95	0	0	D	0	0	2	1	2	2.7
	07-Nov-95	6.9	3	R	0	0	1	0	2	8.1
					The mean TPV for all tests at this site (n=10) is 0.80 (Std. Dev.=0.92)					
Biofilter Inlet	07-Nov-95	6.9	3	R	0	0	0	0	2	32.1
					The mean TPV for all tests at this site (n=5) is 0.40 (Std. Dev.=0.89)					
Biofilter Outlet	07-Nov-95	6.9	3	R	0	0	1	0	2	5.95
					The mean TPV for all tests at this site (n=5) is 0.60 (Std. Dev.=0.89)					

Precipitation Type R - Rain Event, S - Snowmelt, R/S - Rain and Snowmelt, D - Dry.
Data listed are Toxicity Point Values (scale of 0 to 3) Average and Standard Deviation (Std) refer to results from all tests at that site.
Data complete as of March 31/97.

5.1.1 Frequency of Toxicity Detection

Using TPV values, frequencies of toxicity detection were determined for each of the two sources studied, CSOs and stormwater, and for four classes of effects levels:

i. Non-toxic (TPV = 0)
ii. Potentially toxic (TPV = 1)
iii. Toxic (TPV = 2)
iv. Severely toxic (TPV = 3)

The broad category results (CSO, stormwater and highway bridge runoff) are summarized in Table 5.3. Table 5.4 presents individual sampling site summaries for combined sewer overflows, including land use, frequencies of toxicity detection and the mean toxicity point values for each site. Table 5.5 shows the same results for stormwater discharge sites.

Table 5.3: Frequency of Toxicity Detection in CSOs and Stormwater

	Combined Sewer Overflows		**Stormwater**		**Highway and Highway Bridge Runoff**	
	Number of Cases	Frequency (%)	Number of Cases	Frequency (%)	Number of Cases	Frequency (%)
Non-Toxic	106	58.9	219	49.3	62	42.8
Potentially Toxic	54	30	109	24.6	23	15.8
Toxic	18	10	84	18.9	32	22.1
Severely Toxic	2	1.1	32	7.2	28	19.3
Total Number of Samples	36		89		29	
Total Number of Tests	180	100.0	444	100.0	145	100.0

Table 5.4: Combined Sewer Overflow Sites

Site Name	**Land Use** *	Number of Samples	No Toxicity **TPV=0** (% of tests)	Suggested Toxicity **TPV=1** (% of tests)	Moderate Toxicity **TPV=2** (% of tests)	Extreme Toxicity **TPV=3** (% of tests)	**Mean TPV**	**Standard Deviation of TPV**
Lawrence	C, R	30	60	30	6.7	3.4	0.53	0.78
Queenston	C, R	25	72	12	16	0	0.44	0.77
Melvin	C, R	30	63.3	26.7	10	0	0.47	0.68
Parkdale	M	30	56.7	36.7	3.3	3.3	0.53	0.73
Royal	R	30	53.3	43.3	3.4	0	0.50	0.57
Sterling	C, R, I	20	45	30	25	0	0.80	0.83
Kenilworth	M	15	60	27	13	0	0.53	0.74

* B = BMP Outfall, C = Commercial, H = Highway, I = Institutional, M = Manufacturing and Industrial

Table 5.5: Stormwater Sites

Site Name	**Land Use** *	Number of Samples	No Toxicity **TPV=0** (% of tests)	Suggested Toxicity **TPV=1** (% of tests)	Moderate Toxicity **TPV=2** (% of tests)	Extreme Toxicity **TPV=3** (% of tests)	**Mean TPV**	**Standard Deviation of TPV**
Skyway Bridge	H	125	44.8	11.7	24.2	19.3	1.17	1.18
Urban Creek Kingston	C, R	35	57.1	20	22.9	0	0.63	0.85
Parking Lot Kingston	C	40	62.5	12.5	22.5	2.5	0.65	0.92
Pond Outflow Kingston	B	35	51.4	34.3	14.3	0	0.63	0.73
Highway 2 Scarborough	H	15	40	33.3	26.7	0	0.87	0.83
Highway 401 Scarborough	H	14	21.5	35.7	21.4	21.4	1.43	1.09
Stormwater Pond Inflow Scarborough	H, R	15	60	26.7	13.3	0	0.53	0.74
Stormwater Pond Outflow Scarborough	B	15	60	33.3	6.7	0	0.47	0.64
Oil/grit Separator Inlet	C	5	40	60	0	0	0.60	0.55
Oil/grit Separator Outlet	B	20	45	55	0	0	0.55	0.51
Wetlands Inlet	B	10	40	40	20	0	0.80	0.79
Wetlands Outlet	B	10	50	20	30	0	0.80	0.92
Biofilter Inlet	B	5	80	0	20	0	0.40	0.89
Biofilter Outlet	B	5	60	20	20	0	0.60	0.89

* B = BMP Outfall, C = Commercial, H = Highway, I = Industrial

It can be inferred from Table 5.3 that almost 60% of CSO toxicity tests and 50% of stormwater toxicity tests indicated no toxicity, and another 30% and 25%, respectively, were just potentially toxic. It is of interest to note that 10% of CSO tests had confirmed toxicity, as did 19% of all stormwater tests. Finally, while only 1% of CSO tests indicated severe toxicity, a higher proportion of stormwater tests (7%) indicated severe toxicity. It should be emphasized when interpreting these data that samples were typically collected after the first flush may have passed and this would affect toxicity results. So while the above data are probably fairly representative for most of the CSO and stormwater volumes discharged, they are not expected to cover short-duration, low volume first flush characterized by high pollutant concentrations and potential toxic impacts.

When comparing the CSO and stormwater toxicity, stormwater tests produced toxic responses more frequently, particularly at the severe toxicity level (seven times higher). Much of this difference was attributed to highway bridge runoff; after removing the highway data from the stormwater data set, the frequency of severe toxicity detection dropped to 1.3%, but the detection of toxicity in stormwater remains much higher than CSOs (Table 5.3). The highest detection of toxicity was noted for highway and highway bridge runoff (particularly the Skyway bridge and Highway 401 bridge - Table 5.5). In this case, 50% of samples were non-toxic, 16% potentially toxic, 20% toxic and 19% were severely toxic. Thus, road runoff appears to be a significant contributor to stormwater toxicity.

5.1.2 Frequency of Response by Test

The "battery of tests" approach was applied to this assessment of stormwater and CSO toxicity in order to better determine the most suitable tests for monitoring. Figure 5.1 shows the frequency of each level of toxic response (TPV of 0, 1, 2 or 3) of each test (*Daphnia magna*, Microtox™, Sub-mitochondrial particle reverse and forward electron transport and SOS chromotest), for CSO samples, and Figure 5.2 shows the results obtained for stormwater runoff samples. It can clearly be seen that for the most part, the Sub-mitochondrial Particle Reverse Electron Transport Test is the most "sensitive" test in both cases. The SOS Chromotest also gave a notable response in the CSO results, and could be used as an alternative. The *Daphnia magna* did pick up some strong evidence of toxicity from highway runoff, and may be worth considering as an alternative, only where facilities exist for culturing the organism. This test is labour intensive and therefore expensive to apply. Notably, the Microtox™ test did not provide a sufficient response to be considered useful in this case. As well, when analyzing CSO effluent, there was never a sample that did not register at least a TPV of 1 (suggestion of acute toxicity) with the sub-mitochondrial particle (reverse electron transport) test.

The mean toxicity point value for each test was determined using all samples within that group (CSOs or stormwater), and the result shows how sensitive the tests were for that "type" of sample. Figure 5.3 shows the mean response (TPV) for each test on CSO effluent and Figure 5.4 shows the mean response for each test on stormwater runoff samples. From this, it can be seen that on mean, no test showed strong toxicity (mean TPV >3) in CSO samples, but *Daphnia magna* and Sub-mitochondrial Particle (Reverse and Conventional Electron Transport) tests did show repeated toxicity in stormwater runoff samples.

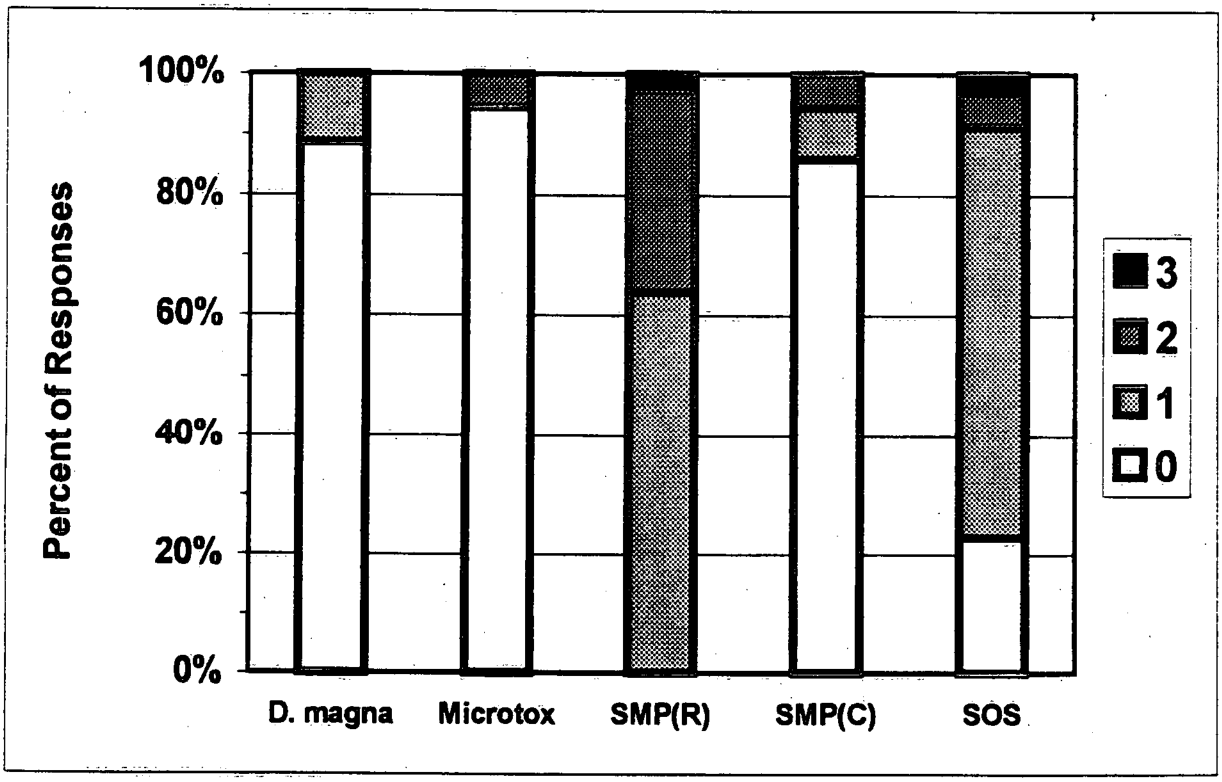

Figure 5.1: Frequency of Positive Detection of Toxicity (by Test) for Combined Sewer Overflows

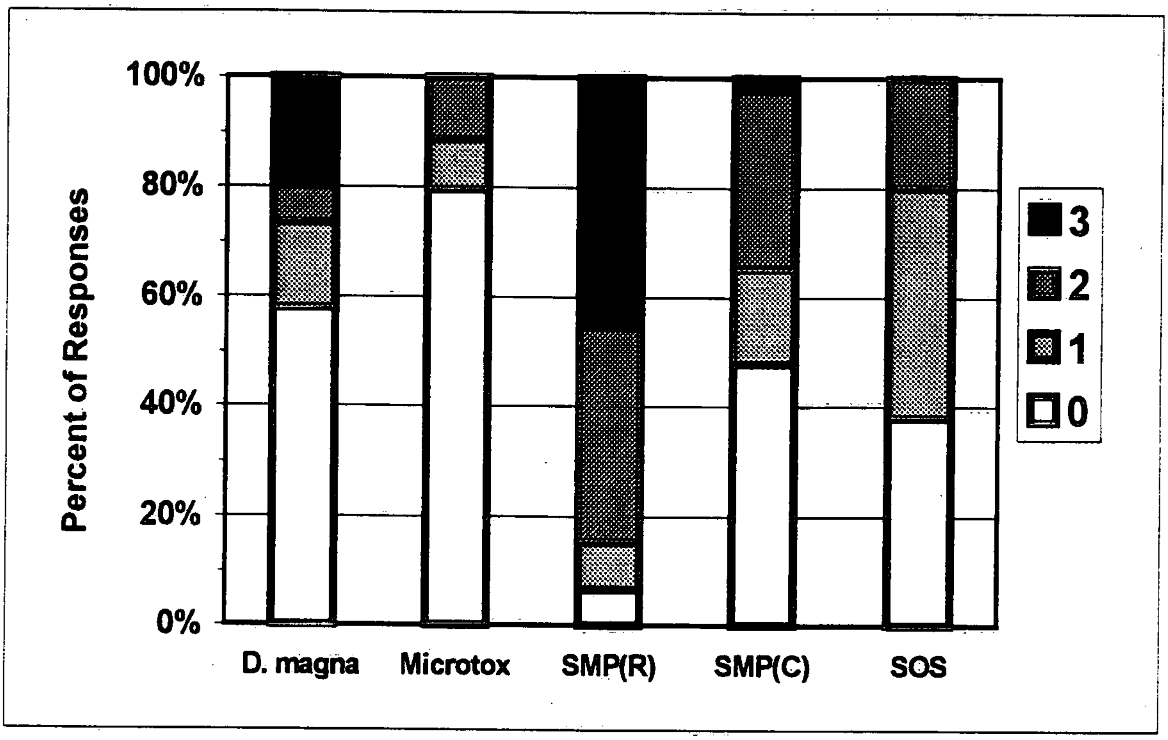

Figure 5.2: Frequency of Positive Detection of Toxicity (by Test) for Stormwater Runoff

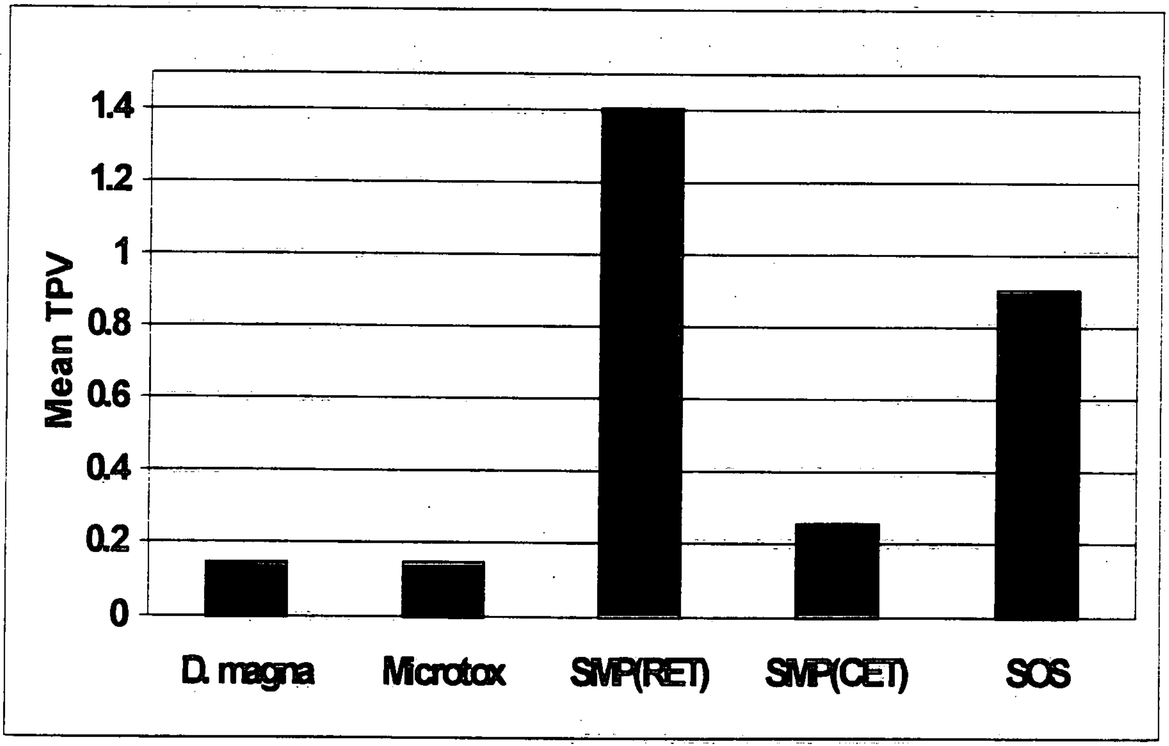

Figure 5.3: Mean Response of Toxicity Tests to Combined Sewer Overflow

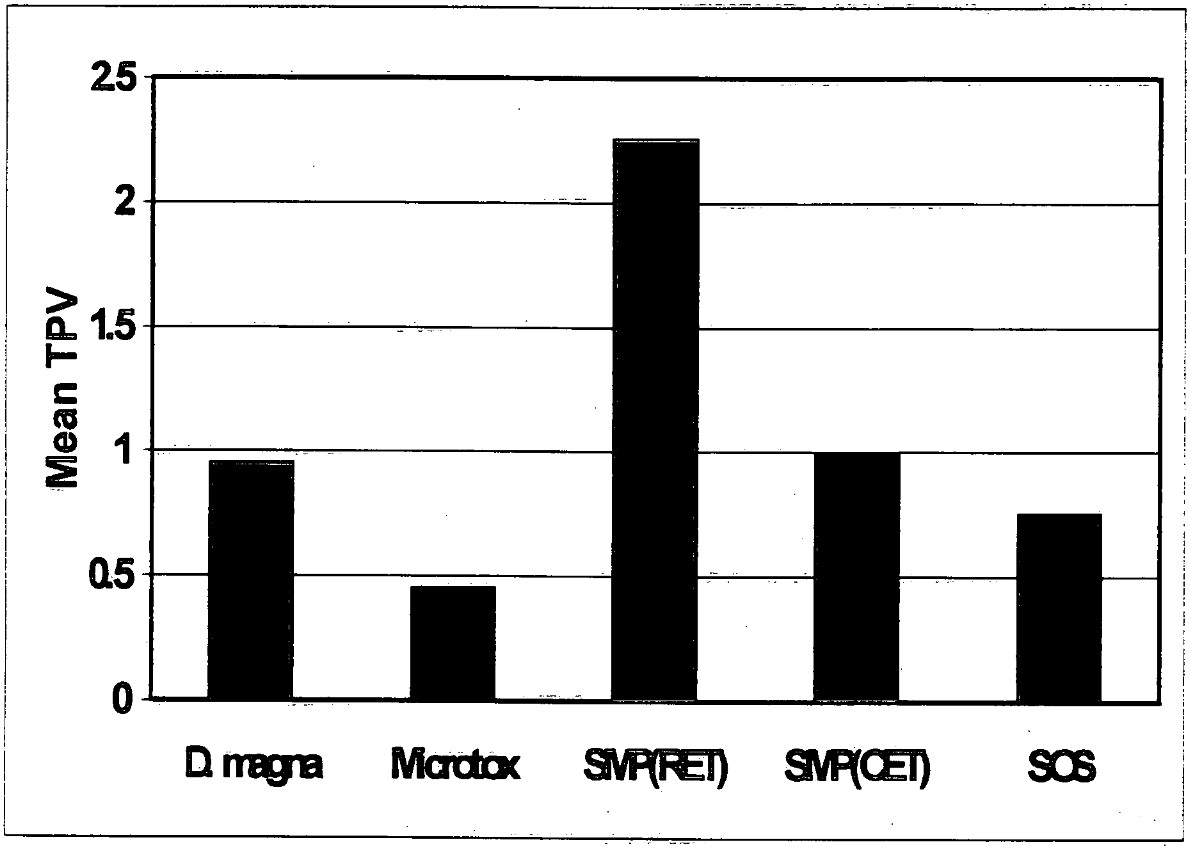

Figure 5.4: Mean Response of Toxicity Tests to Stormwater Runoff

5.1.3 Overall Sample Toxicity

The results from the battery of tests were aggregated to provide the overall value for each sample. These results were then used to rank the samples (by site or in general terms) as to their overall toxicity. Variables associated with these samples were then analysed using regression techniques, to determine if there were any factors which could be directly attributed to sample toxicity. The factors compared, were: total suspended solids (TSS), Phenanthrene, Fluoranthene and Pyrene concentration (liquid phase), antecedent dry period and rainfall amount. The analysis was divided up into sources of wastewater, including the following: CSOs, Skyway Bridge, Kingston creek, and Kingston storm flow (parking lot runoff). The results of these analyses (R^2 regression coefficients) are summarized in Table 5.6. The results show that based on the data collected, there was very minimal correlation among the factors examined. Suspended solids did not appear to have a great impact on the toxicity of the sample in most cases. However, samples from the Kingston storm outflow (runoff from the Cataraqui Town Centre parking lot), seemed to show a much higher correlation with both TSS and antecedent dry period. It is possible that this may be affected by the lower number of samples for this site. Stronger correlations were noted for the Skyway bridge samples with respect to the organic contaminants Fluoranthene and Pyrene. Very low correlations were observed for other sites and comparisons. In general there did not appear to be many strong correlations observed in the data. This could be a result of insufficient data or may be a true lack of correlation. Combined sewer overflows are more difficult to correlate, as in such cases sources vary with time of day, amount of rainfall or snowmelt and capacity of the sewer pipe network.

Table 5.6: Regression Correlation Coefficients (R^2) Between Selected Variables and Overall Toxicity Point Values, Based on Water Source.

Source	Combined Sewer Overflows	Skyway Bridge	Kingston Creek	Kingston Storm Flow
# Samples	15	12	7	7
Total Suspended Solids	0.006	0.035	0.097	0.517
Total Rainfall	0.000	0.007	0.004	0.019
Antecedent Dry Period	0.012	0.025	0.040	0.439
Phenanthrene	0.032	0.084	0.157	0.004
Fluoranthene	0.118	0.279	0.288	0.025
Pyrene	0.125	0.298	0.083	0.015

5.1.4 Individual Site Toxicity

Individual sites were assessed as to their overall acute toxicity potential using the mean toxicity point value for that site. This value was determined by taking the mean of all responses to all tests for all samples taken at that site. In addition to using all tests, the mean results of the Sub-mitochondrial particle (reverse electron transport) test were used in the same way to rank the sites. Mean toxicity point values and the relative standard deviations are listed for each site in Table 5.7. Ranking each site made it easier to assess the comparative toxicological impact of each site on the receiving waters. It can be seen from Table 5.7 that the Skyway Bridge in Burlington and the Highway 401 and Highway 2 bridges in Scarborough would produce the most toxic samples during wet weather conditions (using data from all tests combined or using just the results from the sub-mitochondrial particle bioassay). It appears that major roadways with high vehicular traffic generate the most toxic runoff.

For the most part, combined sewer overflows were not considered to be as toxic as highway runoff. However, samples obtained from Sterling CSO were considerably higher in toxicity than most of the other CSOs, when using all tests. This may be due to the fact that the University and the associated hospital contribute to this overflow. Samples from the industrial areas of Parkdale and Kenilworth were also higher in toxicity. When using only the SMP (RET) test to assess CSO toxicity, Queenston CSO was most toxic. All combined sewer overflows were sampled during larger storm events when there was enough accumulation of runoff to overload the drainage system and the outfalls were flowing. As such it was unlikely that a first flush was encountered during this sampling.

Table 5.7: Mean Toxicity Point Values for Each Sample Site.

Site	# Samples	All Tests		SMP(R)	
		TPV	S.D.	TPV	S.D.
Skyway Bridge Runoff	29	1.17	1.18	2.48	0.86
Highway 401 Bridge and Pond Outflow	3	1.33	1.07	2	0
Highway 2 Bridge Runoff	3	0.86	0.81	2.48	1.25
Scarborough Pond Inflow	3	0.53	0.72	1.67	0.47
Scarborough Pond Outflow	3	0.47	0.62	1.33	0.47
Kingston Storm Weir from Parking Lot	8	0.65	0.91	1.88	0.6
Kingston Creek Weir Inflow	8	0.66	0.83	1.43	0.5
Kingston Pond Outflow	8	0.63	0.72	0.86	0.64
Kingston Wetland Inflow	2	0.73	0.77	1	0
Kingston Wetland Outflow	1	0.77	0.79	1.1	0.33
Kingston Biofilter Inflow	1	0.40	0.89	0	0
Kingston Biofilter Outflow	6	0.60	0.89	1	0
Lawrence CSO	6	0.53	0.76	1	0
Queenston CSO	5	0.44	0.75	1.8	0.4
Melvin CSO	6	0.47	0.68	1.5	0.5
Parkdale CSO	6	0.53	0.72	1.3	0.75
Royal CSO	6	0.5	0.56	1.17	0.37
Sterling CSO	4	0.8	0.81	1.5	0.5
Kenilworth CSO	3	0.53	0.72	1.67	0.47
Waterdown Oil/grit Separator In	1	0.6	0.49	1	0
Waterdown Oil/grit Separator Out	4	0.55	0.5	1	0

TPV - Mean toxicity point value for that site
S.D. - Standard deviation of the mean toxicity point value for that site.

5.2 Time Series Analysis

The timing of sample collection during a runoff event is critical in determining the overall pollutant loading and toxicity impact on the receiving waters. For this reason, a study was conducted at the Skyway Bridge runoff site, to determine a time series for toxicity impacts. Figure 5.5 shows the % activity plot for the Microtox™ (MICRO), Sub-mitochondrial particle reverse electron transport (SMPR) and conventional electron transport (SMPC). Percent activity represents the degree to which the tissue or organism can function normally. If toxicity is present, normal functioning is affected, and the "activity" level decreases.

It can be clearly seen that there is an overall reduction in toxicity as the storm event continues. Microtox™ shows initial toxicity, but is reduced to a "non-toxic" state (100% of normal activity) after 30 minutes. The Sub-mitochondrial particle tests show a very strong reaction to the toxicity tests, with some reduction of toxicity over time. However, these tests still indicate significant toxicity in the sample after 2 hours of rainfall. This is a strong indication that by using these tests, sampling time may not be as critical as with the Microtox™ test, and offers further support for their use.

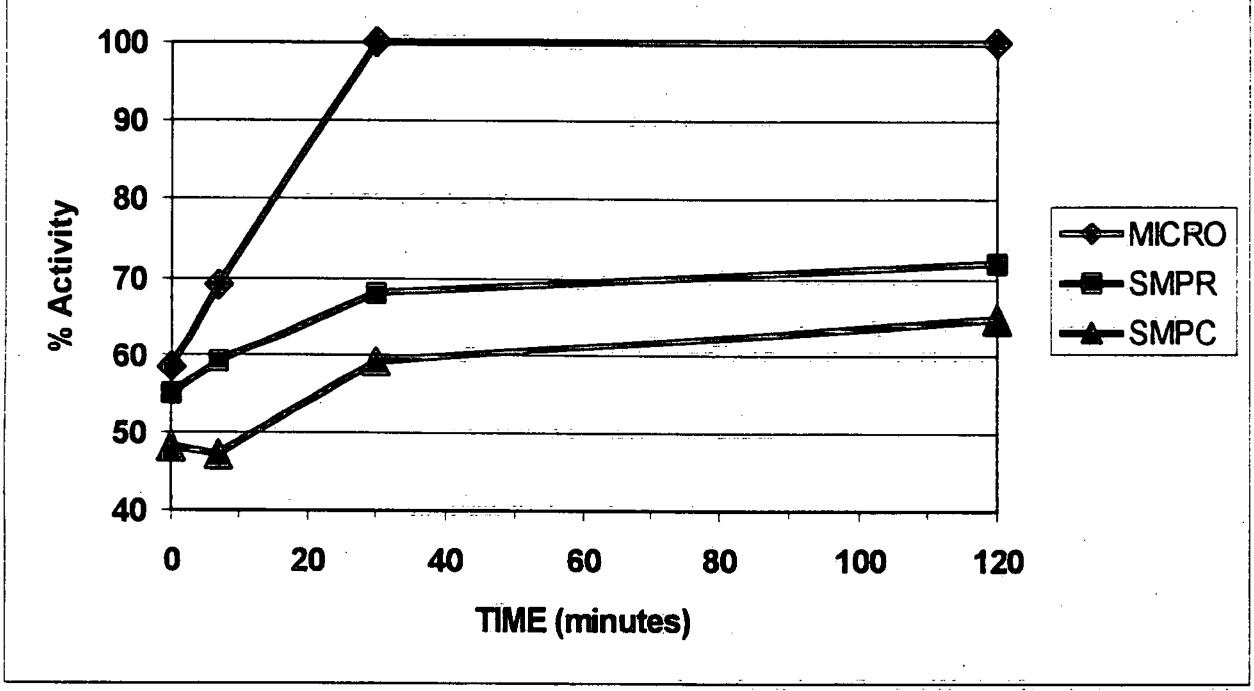

Figure 5.5: Time Series for Skyway Bridge Runoff Samples Showing Percent Activity for Microtox™, Sub-mitochondrial Particle Reverse and Conventional Electron Transport Bioassays

5.3 Repeatability of Toxicity Test Results

A series of five samples were submitted for analysis in order to determine the repeatability of the toxicity test results. A complete sample was subdivided into 5 sub-samples. It was assumed that the characteristics of all samples were identical. Comparative statistics was used to produce the mean, standard deviation and the co-efficient of variation for each set of sample results. The results of the repeated sample analysis are presented in Table 5.8. It can be seen that all tests produced variations in results, although the SOS Chromotest results appeared to be more reproducible than those from other tests. This demonstrates the inherent uncertainties in toxicity results and the need for a cautious interpretation of the results. The use of the Toxicity Point Value system of data provides a more robust interpretation of the data, and reduces the impact of the observed variance.

Table 5.8: Results from Multiple Sample Analysis

Sample	***D.magna***	**Microtox™**	**SMP (RET)**		**SMP (CET)**		**SOS Chromotest**
	EC50	*EC50-10X*	*1X*	*10X*	*1X*	*10X*	*Induction*
SW 10	80	100	47	100	0	55	0.68
SW 11	80	44.1	46	100	0	55	0.78
SW 12	80	39.7	44	100	0	55	0.79
SW 13	60	37.3	38	100	0	61	0.70
SW 14	50	40.9	10	51	0	0	0.79
Mean	**70**	**52.4**	**37**	**90**	**0**	**45**	**0.75**
S.D.	14.1	26.7	15.5	21.9	-	25.4	0.1
C.V.	20%	51%	42%	24%	-	56%	7%

SMP - Sub-mitochondrial Particle Bioassay (RET - Reverse electron transport, CET - Conventional electron transport)
EC - Effective concentration
S.D. - Standard Deviation
C.V. - Coefficient of Variance

5.4 Performance of Best Management Practices in Reducing Stormwater Toxicity

Several types of BMPs were examined during testing of stormwater effluents, however, there were no controls applied to the combined sewer overflows. The BMPs for stormwater management were: stormwater management ponds (Kingston and Scarborough), constructed wetlands (Kingston), biofiltration (Kingston) and Oil/grit Separator (Waterdown and Burlington).

5.4.1 Stormwater Management Ponds

The performance of stormwater ponds in toxicity reduction was assessed from 11 sets of samples collected at the Kingston Pond and the Scarborough Pond. The Kingston pond is an on-stream pond which receives two inflows - from the creek draining an upstream urbanizing catchment and passing through the pond, and from a storm sewer draining the adjacent shopping plaza. For reasons discussed elsewhere (Van Buren et al., 1997), this pond of an older design is considered under-sized for the current state of the catchment development. Furthermore, there are significant accumulations of marginally-to-significantly polluted sediments on the pond bottom (Marsalek et al., 1997) and such accumulations further reduce the pond's effectiveness in enhancement of stormwater quality. Consequently, the performance of this facility may be typical for older pond designs (with many of those in existence), but probably atypical for more recently built ponds. Thus the results presented below should not be interpreted as representative of all pond designs.

With this qualification, the data in Table 5.9 (eight sets of samples) indicate somewhat mixed results, with *Daphnia magna* indicating a minor increase in "suggested" toxicity as stormwater passes through the pond, and the three remaining tests, SMP(RET), SMP(CET) and SOS Chromotest indicating toxicity reduction in the pond, in most cases from "confirmed" or even "strong" level to just "suggested" toxicity levels. The only case of "confirmed" toxicity of the outflow was observed on Feb. 20, 1996. On that day, a very heavy rainfall (31.8 mm) caused toxic runoff passing through the ice-covered pond without much treatment. While this event demonstrates the potential toxic impacts of winter rainfall storms, its probability of occurrence is rather low.

In terms of comparing the toxicity of inflow and outflow, no confirmed toxicity was found in outflow in any of the samples collected. In terms of "suggested" toxicity, *Daphnia magna* showed some deterioration in quality of the pond outflow, SOS showed no change, and SMP (RET) and SMP (CET) showed some improvement in the effluent quality. Thus in the overall assessment based on all samples and toxicity tests, the pond outflow appeared to be less toxic than the inflow.

The data in Table 5.9 can also be used to compare the toxicity of the two inflows - the urban creek and the shopping plaza runoff. It was observed that some tests indicate a greater toxicity of the creek, and others of the plaza runoff. It would appear that these differences are not significant. The data set for the Scarborough pond (Table 5.10) was less extensive and produced similar results. Increased "confirmed" toxicity in outflow (compared to inflow) was noted in one out of 12 cases, and increased "suggested" toxicity was noted in two more cases. For other cases, there were either no changes in the inflow/outflow toxicity levels (five cases), or some toxicity reduction (4 cases).

Table 5.9: Kingston Stormwater Pond Toxicity Performance Data

Date	***Daphnia magna* EC 100**			**SMP (RET) 10X**			**SMP (CET) 10X**			**SOS Chromotest 10X**		
	In 1	**In 2**	**Out**	**In 1**	**In 2**	**Out**	**In 1**	**In 2**	**Out**	**In 1**	**In 2**	**Out**
14-Oct-95	0	0	1	3	1	0	2	1	1	1	1	1
14-Oct-95	0	0	1	2	1	0	2	0	1	0	1	1
19-Oct-95	-	0	1	-	1	1	-	1	0	-	1	1
1-Nov-95	0	1	0	2	1	1	0	0	0	0	0	0
7-Nov-95	0	0	1	1	2	1	0	0	0	0	0	0
7-Nov-95	0	0	-	2	1	-	1	0	-	0	2	-
26-Nov-95	0	0	0	1	2	1	0	1	0	1	0	0
20-Feb-96	0	0	0	2	2	2	0	0	0	0	0	1

Note: In 1 is West Branch of the Little Cataraqui Creek and **In 2** is Stormwater from the Cataraqui Town Centre. – Indicates data not available

Table 5.10: Scarborough Stormwater Pond Toxicity Performance Data

Date	*Daphnia magna* EC 100		SMP (RET) 10X		SMP (CET) 10X		SOS Chromotest 10X	
	In	Out	In	Out	In	Out	In	Out
24-Oct-95	1	0	2	1	0	1	0	0
27-Oct-95	1	0	1	2	0	1	0	0
27-Oct-95	1	1	2	1	0	0	0	0

Bacterial count reduction is also an important aspect of stormwater pond performance, as it may be necessary to protect downstream waters to meet recreational water quality standards. Both the Kingston and Scarborough ponds appeared to perform well with respect to bacterial reductions during the cold weather as shown in Tables 5.11 and 5.12. However, in warm weather, bacterial counts would be significantly higher and would exceed the Ontario Provincial Water Quality Objective of 100 *E. coli*/100 mL. The highest relative reduction was observed for samples taken on 14-Oct-95 when, even during periods of high flow (18.6mm rain) the pond was still able to reduce the bacteria to moderate levels. Both the inflow to and outflow from the Scarborough pond were characterized by relatively low bacterial counts, indicating fewer sources of fecal pollution.

Table 5.11: Kingston Stormwater Pond Bacteria Counts

Date	**Fecal Coliform** (counts/100mL)			*E. coli* (counts/100mL)		
	In 1	**In 2**	**Out**	**In 1**	**In 2**	**Out**
14-Oct-95	92000	11000	700	54000	11000	180
14-Oct-95	54000	790	4900	54000	790	2200
19-Oct-95	-	49	170	-	49	70
1-Nov-95	4900	330	130	4900	330	130
7-Nov-95	790	490	79	280	490	79
7-Nov-95	11000	1300	-	11000	1300	-
26-Nov-95	330	13	27	490	13	27
20-Feb-96	-	-	-	-	-	-

In 1 = Creek, **In 2** = Plaza runoff

Table 5.12: Scarborough Stormwater Pond Bacteria Counts

Date	**Fecal Coliform** (counts/100mL)		*E. coli* (counts/100mL)	
	In	**Out**	**In**	**Out**
24-Oct-95	7900	330	1400	70
27-Oct-95	3300	33	3300	17
27-Oct-95	3000	46	3000	46

5.4.2 Constructed Wetlands and Biofilter

The constructed wetlands and biofilter contain a granular medium of high hydraulic conductivity, and rely on biological growth (both plant and bacterial) in and filtration through this medium to achieve pollutant removal. These systems have been shown to be effective in removal of suspended solids and nutrients, however, it has not been conclusively determined if they reduce stormwater toxicity. The limited number of samples collected from the wetlands and biofilter do not permit a complete evaluation of performance with respect to toxicity reduction. Only one rainfall event was sampled for these systems in Kingston; all other samples were taken during dry weather baseflow from the stormwater pond. Some preliminary data suggest that toxicity reduction does occur (e.g. *Daphnia magna* in constructed wetlands), while others suggest a slight increase in toxicity (e.g. Sub-mitochondrial particle reverse electron transport bioassay in the biofilter). The low toxicity of influent waters reduced the chances of observing notable toxicity reductions. A more thorough investigation of these BMPs would be required before their performance in reducing toxicity could be confirmed.

5.4.3 Oil/grit Separator

Oil/grit separation devices offer a potential for removing sediment and oils from the storm runoff before they are conveyed into stormwater ponds and receiving waters. However, their ability to reduce effluent toxicity had not been thoroughly investigated. It was anticipated that the runoff from some of the sites selected would invoke a toxic response, and that the performance of the Oil/grit separator could then be assessed. Unfortunately all influent samples tested were relatively free from toxicity which made performance evaluation impossible (Table 5.2). The observed toxic responses at the "suggested" level will require further verifications by field measurements.

6.0 DISCUSSION

6.1 Rating of Stormwater and CSO Sites

Several different methods were used to characterize combined sewer overflow and stormwater toxicity at sampling sites. This allowed an assessment of these discharges under different environmental conditions, and despite the large variation in the results, some trends appeared to emerge.

Using the results from all five tests, a mean toxicity point value was obtained at each site and used to assess the site's toxicity potential. Using data from Tables 5.5 and 5.7, it can be seen that the most toxic sites were three stormwater outfalls; Skyway bridge (Burlington), Highway 2 bridge and Highway 401 bridge runoff (Scarborough). All of these sites are characterized by high volume highway traffic.

Combined sewer overflow sites in Hamilton were found to be less toxic than those with direct stormwater runoff; however, samples collected from Sterling, Kenilworth and Queenston CSO sites did demonstrate some low level toxicity, and consequently were ranked as the most toxic CSO sites. University and hospital waste as well as high volume traffic and many parking lots contributing to the overflow characterized the Sterling site. Kenilworth CSO was located in a high traffic and steel works area, which included many industrial sources of toxicants. Queenston Road and Lawrence Avenue CSOs were located in high traffic areas, with commercial developments surrounding them, which contribute to production of toxic runoff. In addition to the runoff component, combined sewer overflows contribute toxicity through sewage related toxicants, including suspended solids, biodegradable waste (consuming oxygen), as well as pathogens. The highly variable nature of stormwater and combined sewer overflows makes accurate characterization difficult, and therefore many combined sewer overflows could produce toxic effluent for short periods, although the potentially toxic "first flush" is likely to be conveyed to the sewage treatment facility.

From these results, the likelihood of finding toxicity in a sample collected from one of these sites (or in general) could also be determined. Stormwater samples results were aggregated, and it was found that there was an 83% chance that "confirmed" toxicity would be found for at least 1 test (TPV ≥ 2), and a 27% chance that the sample would be toxic for 2 tests. All combined sewer overflow data were also aggregated and it was found that a sample had a 50% probability of registering a toxic response in at least one test when analyzing these samples, and less than 6% probability of finding toxic response in two tests. At the most toxic site, the Sterling Avenue outfall, all samples were found to be toxic for at least one test, and 25% were toxic for at least two tests.

By pooling the data from all sites, a general probability can be assigned to "wet-weather discharges" (defined as any effluent produced during rainfall or snowmelt events) for detection of toxicity. It was found that there was a 70% chance of finding the sample toxic in at least one test. This value was strongly influenced by the data from stormwater runoff samples, but may offer a general guideline based on samples collected during these field investigations.

6.2 Sampling of Events

The time series of toxic response taken at the Skyway bridge showed how toxicity changed with time for several tests. This confirmed the general notion of the "first flush", characterized by high toxicant levels at the start of the stormwater runoff event, and their decline as the source of toxicants on the catchment surface is depleted. In most cases, the discharges are not sampled at the start of the runoff event. It is therefore important to know if the test result was an accurate representation of the storm event as a whole. It was noted (Figure 5.5) that the results from the Microtox™ test showed 100% activity (i.e. no inhibition and therefore no toxic effect) after 30 minutes. The sub-mitochondrial particle tests however, showed some reduction in toxicity over time, but still demonstrated toxic response after 2 hours. This makes these more sensitive tests more appropriate for monitoring (where grab sampling methods are commonly used) than Microtox™. It would have been beneficial to produce the same time series for a combined sewer overflow to determine how the effluent quality changes over time. However, overflow events were difficult to predict and their detailed analysis could not be initiated. It is anticipated that a similar first flush effect would be found for CSO discharges.

6.3 Toxicity Test Response

The battery of tests applied to these samples provided a range of different responses as to the toxicity of the samples. Some test results were negative while others displayed strong toxicity for the same sample. If only one test had been used (e.g. Microtox™), there could be many samples where a toxic effect went undetected. Each test is sensitive to different chemical constituents (or combinations of constituents), and at different concentrations, all of which may have toxic effects. It is therefore very difficult to use only one test to "screen" sites for potential monitoring and remedial action. The test results from these sites did show a much greater response to the sub-mitochondrial particle (Reverse electron transport) test (where cellular biochemical pathways are disrupted) than any other test in the group. However in some combined sewer overflow cases, the SOS chromotest (a "genotoxic" test where chromosomal or other genetic damage occurs) was more sensitive to the presence of toxicants.

The sub-mitochondrial particle tests are relatively straightforward, inexpensive to perform, and small sample aliquots are required (500 mL) for the test. This leads to the recommendation of this test as a potential screening tool in future surveys. The SOS chromotest was also sensitive and could be used as an additional or alternative test. It is also a straightforward test which can be performed quickly and inexpensively.

One test that did not show toxicity for many samples was the Microtox™ test. This test would not serve well (under these circumstances) as a single screening test. It may provide some key information on samples that did exhibit some toxicity, however, and should not be discounted as part of a battery of tests.

6.4 Performance of Best Management Practices

Wet detention ponds showed little change in overall sample toxicity between inlet and outlet. The stormwater ponds investigated (Kingston and Scarborough) were representative of typical water quantity control ponds installed in urban development to control downstream flooding and high flows. The main function performed by these ponds is sediment settling, and is likely to be largely responsible for some toxicity reductions, however their effectiveness may be improved by using retrofit techniques. This aspect requires further investigation.

There were insufficient data to comment on constructed wetlands and biofiltration, as these systems did not receive direct stormwater runoff, but pond effluent. It was noted, however, that constructed wetlands had been recently tested for toxicity reduction, and had proved successful (Katznelson et al. 1995). Future testing of wetlands for toxicity reduction is recommended.

6.5 Correlation of Toxic Response to Independent Variables

The data set was divided into runoff and combined sewer overflow sources for initial analysis. Additional investigations based on smaller subsets of the data provided a greater degree of correlation although the sample size (and hence confidence) was reduced. Overall, these results (Table 5.6) indicate that the toxicity data did not correlate well with suspended solids, rainfall amounts, PAH contamination or antecedent dry period. Rainfall amount could be discounted from further analysis in runoff events, as the correlations were so low. The Julian date was also examined as a potential factor affecting toxicity, however, no correlation was found.

It was expected that antecedent dry conditions would be strongly correlated due to pollutant buildup on the paved areas, but it is possible that there was not enough data for a proper correlation analysis. As the data collected did not include the first flush for many of these events, correlations between antecedent dry period and toxicity would not be generally detected. The exception to this was found in the "storm" flow from the Cataraqui Town Centre parking lot. There was a strong correlation between the sample toxicity and the antecedent dry period. Also, a strong correlation was found between total suspended solids and sample toxicity from the same site, which should be expected, as the accumulation of suspended solids increases with the antecedent dry period. A greater number of samples from this site would help further verify the demonstrated correlation between the toxicity and the antecedent dry weather period.

The samples from the Skyway bridge showed weak correlations for all measured variables, however, there were better correlations between the sample toxicity and the PAHs, Fluoranthene and Pyrene than for other sites. This may be a reflection of the high traffic volumes and oil and grease deposits. These samples were collected from 250 L plastic rain barrels and were therefore composite samples, which may have reduced the impact of the high "first flush" concentrations.

7.0 CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

On the basis of more than 600 toxicity measurements performed on 36 CSO and 125 stormwater samples, the following conclusions can be drawn about the toxicity of CSOs and stormwater:

1. Eighty-three percent of all stormwater samples and 50% of all CSO samples were found toxic in at least one test from the battery of five toxicity tests used in this study. These probabilities dropped to 27% and 6%, respectively, for detections of toxicity by at least two tests. Thus, grab or composite samples indicate higher toxicity of stormwater then CSOs in the areas studied.

2. Among the stormwater sites sampled, those with high contributions of highway runoff produced the highest frequencies of toxicity detection. Among the CSO sites, the Sterling CSO in Hamilton (receiving some wastewater from a university and hospital, and road surface runoff) produced the highest average toxicity responses.

3. Any single bioassay may not be able to detect all types of toxicity, so it is advisable to use a battery of tests capable of detecting various toxicants at various concentrations. In this study, five tests were used - *Daphnia magna*, Microtox™, Sub-mitochondrial Particle Bioassay (reverse and forward transport), and SOS Chromotest. In this group, the Sub-mitochondrial Particle Bioassay (reverse transport) proved to be the most sensitive; Microtox™ was the least sensitive test.

4. Time variation of stormwater toxicity was studied at only one site, a highway bridge, and indicated the existence of the first toxic flush occurring early during the runoff event. Less sensitive tests, applied to the samples collected during later phases of runoff, would not

have detected this first flush toxicity. The field methods used did not allow to apply a similar sampling procedure to CSO events.

5. The performance of several stormwater best management practices in reduction of stormwater toxicity was tested for a limited number of samples. Test results on inflow and outflow grab samples indicate that, in general, ponds, wetlands and a biofilter somewhat reduced the toxicity of stormwater. Under some conditions, the influent toxicity has not changed, and exceptionally, even marginal increases were observed. Such increases may be caused by displacement of contaminated content of a BMP structure by relatively clean inflow.

7.2 Recommendations

Future work on toxicity of stormwater and CSOs should focus on:

1. Toxicity variation in stormwater and CSOs during storm events, including the first flush assessment
2. Further monitoring of toxicity reduction by BMPs, focusing on periods of highly toxic inflow to the BMPs
3. Evaluations of sources of toxicity

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APPENDIX A:

Raw Data from Toxicity Tests

Table A1: Storm Drain - Cataraqui Town Centre, Kingston
Table A2: Urban Creek - Cataraqui Town Centre, Kingston
Table A3: Cataraqui Stormwater Pond Outfall, Kingston
Table A4: Stormwater Pond Inlet (Road Runoff) – Scarborough
Table A5: Outlet of Stormwater Pond – Scarborough
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Table A7: Outfall of Highway Runoff Overland Flow into Stormwater Pond – Scarborough
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Table A9: Constructed Wetland Inlet/Outlet – Cataraqui Stormwater Pond
Table A10: Biofilter Inlet and Outlet – Cataraqui Stormwater Pond
Table A11: Oil/Grit Separator Units – Mainway and Waterdown
Table A12: Combined Sewer Overflows – Hamilton

Table A1: Storm Drain - Cataraqui Town Centre, Kingston

Date	***D.Magna***	**Microtox 1X**		**Microtox 10X**		**SMP**		**SMP**		**SOS chromotest**		**A-1**	**A-1**
	48 hr	**EC50**	**EC10**	**EC50**	**EC10**	**RET**		**CET**		**1X**	**10X**	**F. coliform**	***E.coli***
	EC100					**1X**	**10X**	**1X**	**10X**				
10-Oct-95	0	>100%	---	>100%	---	71	7	34	27	(1:16)1.36	(1:128)1.20	92,000	54,000
10-Oct-95	10	>100%	---	>100%	---	84	34	31	46	1.29	0.96	54,000	54,000
01-Nov-95	10	---	---	>100%	---	79	34	---	117	1.01	0.93	4,900	4,900
07-Nov-95	0	---	---	>100%	---	88	70	---	91	0.98	0.78	790	280
07-Nov-95	0	---	---	>100%	---	77	30	60	58	(1:16)1.02	0.77	11,000	11,000
26-Nov-95	0	---	---	>100%	---	92	66	---	133	1.43	1.03	330	490
20-Feb-96	0	---	---	>100%	---	99	38	137	275	---	0.84	< 2	< 2
20-Feb-95	0	---	---	>100%	---	93	21	146	283	---	0.82	< 2	< 2

Table A2: Urban Creek - Cataraqui Stormwater Pond, Kingston

Date	***D.Magna***	**Microtox 1X**		**Microtox 10X**		**SMP**		**SMP**		**SOS chromotest**		**A-1**	**A-1**
	48 hr	**EC50**	**EC10**	**EC50**	**EC10**	**RET**		**CET**		**1X**	**10X**	**F.coliform**	***E.coli***
	EC100					**1X**	**10X**	**1X**	**10X**				
10-Oct-95	0	>100%	---	>100%	---	88	61	101	87	1.33	(1:32)1.24	11,000	11,000
10-Oct-95	10	>100%	---	>100%	---	93	64	122	120	1.34	(1:128)1.28	790	790
19-Oct-95	0	>100%	---	>100%	---	85	85	99	74	1.40	1.03	49	49
01-Nov-95	30	---	---	>100%	---	61	82	---	173	1.03	0.75	330	330
07-Nov-95	0	---	---	>100%	---	85	49	---	142	0.84	0.74	490	490
07-Nov-95	0	---	---	>100%	---	84	62	---	140	1.02	(1:16)1.31	1,300	1,300
26-Nov-95	0	---	---	>100%	---	90	34	129	68	1.47	0.95	13	13
20-Feb-96	0	---	---	>100%	---	93	21	146	283	---	0.87	< 2	< 2

Table A3: Cataraqui Stormwater Pond Outfall, Kingston

Date	***D.Magna***	**Microtox 1X**		**Microtox 10X**		**SMP**		**SMP**		**SOS chromotest**		**A-1**	**A-1**
	48 hr	**EC50**	**EC10**	**EC50**	**EC10**	**RET**		**CET**		**1X**	**10X**	**F.coliform**	***E.coli***
	EC100					**1X**	**10X**	**1X**	**10X**				
10-Oct-95	20	>100%	---	>100%	---	93	97	132	85	1.33	(1:32)1.23	700	180
10-Oct-95	20	>100%	---	>100%	---	88	93	54	81	1.36	(1:128)1.13	4,900	2,200
01-Nov-95	30	>100%	---	>100%	---	92	52	155	98	1.39	1.04	170	70
07-Nov-95	0	---	---	>100%	---	90	83	---	195	1.00	0.74	130	130
07-Nov-95	20	---	---	>100%	---	81	76	---	165	1.20	0.77	79	79
26-Nov-95	0	---	---	>100%	---	93	61	---	161	1.50	0.75	27	27
20-Feb-96	0	---	---	>100%	---	89	23	141	305	----	1.03	< 2	< 2

Table A4: Stormwater Pond Inlet (Road Runoff) - Scarborough

Date	*D.Magna*	Microtox 1X		Microtox 10X		SMP		SMP		SOS chromotest		A-1	A-1
	48 hr	EC50	EC10	EC50	EC10	RET		CET		1X	10X	F.coliform	*E.coli*
	EC100					1X	10X	1X	10X				
24-Oct-95	20	---	---	>100%	---	89	32	180	92	1.19	0.79	7,900	1,400
27-Oct-95	20	---	---	>100%	---	96	50	62	92	1.19	0.64	3,300	3,300
27-Oct-95	20	---	---	>100%	---	91	38	69	115	1.26	0.71	3,000	3,000

Table A5: Outlet of Stormwater Pond - Scarborough

Date	*D.Magna*	Microtox 1X		Microtox 10X		SMP		SMP		SOS chromotest		A-1	A-1
	48 hr	EC50	EC10	EC50	EC10	RET		CET		1X	10X	F.coliform	*E.coli*
	EC100					1X	10X	1X	10X				
24-Oct-95	0	---	---	>100%	---	94	42	138	89	1.3	0.78	330	70
27-Oct-95	10	---	---	>100%	---	105	28	170	90	1.06	0.84	33	17
27-Oct-95	20	---	---	>100%	---	105	45	160	180	1.06	0.83	46	46

Table A6: Outfall of Highway Runoff and Stormwater Pond - Scarborough

Date	*D.Magna*	Microtox 1X		Microtox 10X		SMP		SMP		SOS chromotest		A-1	A-1
	48 hr	EC50	EC10	EC50	EC10	RET		CET		1X	10X	F.coliform	*E.coli*
	EC100					1X	10X	1X	10X				
24-Oct-95	20	---	---	>100%	---	95	36	105	164	1.30	0.96	130	130
27-Oct-95	20	---	---	>100%	---	109	18	94	119	1.24	0.64	22	22
27-Oct-95	0	---	---	>100%	---	89	41	112	88	1.25	0.59	26	26

Table A7: Outfall of Highway Runoff Overland Flow into Stormwater Pond - Scarborough

Date	*D.Magna*	Microtox 1X		Microtox 10X		SMP		SMP		SOS chromotest		A-1	A-1
	48 hr	EC50	EC10	EC50	EC10	RET		CET		1X	10X	F.coliform	*E.coli*
	EC100					1X	10X	1X	10X				
24-Oct-95	20	---	---	>100%	---	73	0	94	23	1.28	0.72	7	4
27-Oct-95	20	pH7.6 12.9	8.2	---	---	112	35	86	83	1.31	0.71	< 2	< 2
27-Oct-95	EC50-70%	---	---	---	---	103	367	84	87	1.12	0.53	< 2	< 2

Table A8: Skyway Bridge Runoff - Burlington

Date	*D.Magna*	Microtox 1X		Microtox 10X		SMP		SMP		SOS chromotest		A-1	A-1
	48 hr	EC50	EC10	EC50	EC10	RET		CET		1X	10X	F.coliform	*E.coli*
	EC100					1X	10X	1X	10X				
27-Nov-95	0	---	---	>100%	---	62	.01	---	75	1.41	0.95	13,000	13,000
28-Nov-95	0	---	---	>100%	---	85	25	---	120	1.41	0.83	950	70
28-Nov-95	0	---	---	>100%	---	91	35	---	201	1.35	1.1	11,000	3,300
01-Dec-95	EC50-25%	---	---	>100%	---	13	0	84	11	(1:2)1.32	(1:16)1.03	24,000	4,900
04-Dec-95	EC50-55%	---	---	>100%	---	35	0	114	41	1.45	0.85	1,700	790
04-Dec-95	10	---	---	>100%	---	44	0	107	92	1.06	---	7,900	7,900
15-Dec-95	EC50-50%	>100%	---	15.22	9.72	0	.05	33	84	---	0.58	11	7
17-Jan-96	EC50-25%	>100%	---	>100%	---	37	4	179	32	---	0.77	< 2	< 2
23-Jan-96	EC50-5%	>100%	---	26.5	13.1	.01	118	83	16	---	0.58	< 2	< 2
24-Jan-96	EC50-80%	---	---	>100%	---	53	0	162	45	---	0.68	490	490
24-Jan-96	EC50-80%	>100%	---	44.1	15.3	54	0	159	45	---	0.78	700	700
24-Jan-96	EC50-80%	>100%	---	39.7	6.7	56	0	164	45	---	0.79	49,000	49,000
24-Jan-96	EC50-60%	>100%	---	37.3	5.3	62	0	292	39	---	0.7	7,000	7,000
24-Jan-96	EC50-50%	>100%	---	40.9	12.5	90	49	195	241	---	0.79	7,900	7,900
27-Jan-96	EC50-50%	>100%	---	23.9	12.1	28	0	188	31	---	0.84	4	5
19-Apr-96	0	---	---	>100%	---	40	0	107	46	---	1.05	24,000	24,000
19-Apr-96	10	>100%	---	41.3	13.7	42	0	108	0	---	1.23	24,000	13,000
22-Apr-96	0	---	---	>100%	---	70	10	---	149	---	1.06	1,100	490
09-May-96	0	---	---	>100%	---	69	23	---	121	---	1.27	3300	3300
09-May-96	0	---	---	>100%	---	80	9	---	104	---	1.27	4900	4900
11-May-96	10	---	---	>100%	---	95	44	---	151	---	1.13	n/a	n/a
21-May-96	10	---	---	>100%	---	112	76	---	142	---	1.17	1300	2300
20-Jun-96	10	---	---	>100%	---	102	59	---	144	---	1.09	7900	7900
19-Jul-96	0	---	---	>100%	---	88	39	---	110	---	1.03	54000	54000
01-Dec-96	n/a	---	---	>100%	---	70	0	101	56	---	1.05	n/a	n/a
Sep 22 /96 - Time Series													
5 min	0	---	---	58.3%	7.3%	55	0	95	48	---	1.11	>1600	>1600
12 min	0	---	---	69.0%	9.3%	59	0	88	47	---	1.01	>1600	>1600
30 min	0	---	---	>100%	9.4%	68	0	100	59	---	1.04	>1600	>1600
120 min	0	---	---	>100%	---	72	0	71	65	---	1.08	1600	>1600

Table A9: Constructed Wetland Inlet/Outlet - Cataraqui Stormwater Pond

Date	*D.Magna*	Microtox 1X		Microtox 10X		SMP		SMP		SOS chromotest		A-1	A-1
	48 hr EC100	EC50	EC10	EC50	EC10	RET		CET		1X	10X	F.coliform	*E.coli*
						1X	10X	1X	10X				
19-Oct-95	0	---	---	>100%	---	95	55	176	55	1.35	1.09	790	280
07-Nov-95	20	---	---	>100%	---	90	73	---	130	0.99	(1:128)1.31	230	230
19-Oct-95	10	---	---	>100%	---	90	48	121	57	1.41	1.17	17	< 2
07-Nov-95	0	---	---	>100%	---	87	67	---	189	0.95	(1:64)1.31	2	2

Inlet is first entry, outlet is second entry for each date

Table A10: Biofilter Inlet and Outlet - Cataraqui Stormwater Pond

Date Time (h)	*D.Magna*	Microtox 1X		Microtox 10X		SMP		SMP		SOS chromotest		A-1	A-1
	48 hr EC100	EC50	EC10	EC50	EC10	RET		CET		1X	10X	F.coliform	*E.coli*
						1X	10X	1X	10X				
07-Nov-95	10	---	---	>100%	---	83	141	---	141	1.38	0.73	170	170
07-Nov-95	10	---	---	>100%	---	85	68	---	145	1.31	0.74	49	49

Table A11: Oil/Grit Separator Units - Mainway and Waterdown

Date	*D.Magna*	Microtox 1X		Microtox 10X		SMP		SMP		SOS chromotest		A-1	A-1
	48 hr EC100	EC50	EC10	EC50	EC10	RET		CET		1X	10X	F.coliform	*E.coli*
						1X	10X	1X	10X				
09-May-96	0	---	---	>100%	---	87	63	---	92	---	1.09	13	13
18-Jun-96	20	---	---	>100%	---	95	77	---	102	---	1.08	330	79
18-Jun-96	20	---	---	>100%	---	95	71	---	89	---	0.94	790	140
18-Jun-96	20	---	---	>100%	---	97	56	---	93	---	1.01	1100	180
18-Jun-96	0	---	---	>100%	---	95	65	---	138	---	1.04	7900	7900
22-Jul-96	20	---	---	>100%	---	57	0	95	46	---	1.04	n/a	n/a

Table A12: Combined Sewer Overflows - Hamilton

Date	Location	*D.Magna*	Microtox 1X		Microtox 10X		SMP		SMP		SOS chromotest		A-1	A-1
		48 hr	EC50	EC10	EC50	EC10	RET		CET		1X	10X	F.coliform	*E.coli*
		EC100					1X	10X	1X	10X				
28-Nov-95	Lawrence	0	---	---	>100%	---	88	62	---	187	(1:2)1.34	1.69	350,000	79,000
28-Nov-95	Lawrence	0	---	---	>100%	---	88	79	---	311	1.34	1.39	79,000	79,000
19-Jan-96	Lawrence	10	100%	---	26.5	13.1	92	60	116	277	----	0.96	54,000	54,000
19-Jan-96	Lawrence	30	---	---	>100%	---	76	64	119	275	----	1.03	22,000	11,000
24-Jan-96	Lawrence	0	---	---	>100%	---	87	60	147	279	----	0.86	920,000	920,000
22-Apr-96	Lawrence	0	---	---	>100%	---	95	83	---	213	----	1.27	92,000	54,000
15-Dec-95	Queenston	0	---	---	>100%	---	75	11	133	>100	----	0.88	33,000	23,000
17-Jan-96	Queenston	0	---	---	>100%	---	84	30	161	225	----	0.98	17,000	17,000
24-Jan-96	Queenston	0	---	---	>100%	---	78	21	183	134	----	0.88	540,000	540,000
11-May-96	Queenston	0	---	---	>100%	---	95	52	---	188	----	1.19	n/a	n/a
23-May-96	Queenston	0	---	---	>100%	---	99	17	---	111	----	1.05	n/a	n/a
07-Sep-96	Queenston	0	---	---	>100%	---	104	83	---	109	----	1.02	n/a	n/a
15-Dec-95	Melvin	0	---	---	>100%	---	84	63	50	>100	----	1.04	1,700	1,700
17-Jan-96	Melvin	0	---	---	>100%	---	85	53	83	>100	----	1.04	4,900	1,400
24-Jan-96	Melvin	0	---	---	>100%	---	90	49	195	241	----	0.98	49,000	49,000
09-Oct-96	Melvin	0	---	---	>100%	---	84	34	---	101	----	0.99	< 2	< 2
18-Oct-96	Melvin	0	---	---	>100%		84	69	---	96	----		16000	16000
17-Jan-96	Parkdale	0	---	---	>100%	---	77	57	104	195	----	0.86	5	5
22-Apr-96	Parkdale	0	---	---	>100%	---	94	62	---	170	----	1.27	2	2
09-May-96	Parkdale	0	---	---	>100%	---	92	53	---	173	----	1.27	11000	11000
26-Aug-96	Parkdale	10	---	---	>100%	---	57	0	95	46	----	1.09	160000	160000
07-Sep-96	Parkdale	20	---	---	>100%	---	104	56	---	184	----	1.09	n/a	n/a
17-Jan-96	Royal	0	---	---	>100%	---	85	65	73	237	----	1.12	7,900	7,900
17-Jan-96	Royal	0	---	---	>100%	---	87	59	97	223	----	1.14	1,100	1,100
24-Jan-96	Royal	0	---	---	>100%	---	95	69	282	213	----	1.08	33,000	33,000
24-Jan-96	Royal	10	---	---	>100%	---	78	36	122	289	----	1.06	49,000	49,000
22-Apr-96	Royal	10	---	---	>100%	---	95	72	---	259	----	1.25	92,000	92,000
07-Jun-96	Royal	20	---	---	>100%	---	92	75	---	107	----	1.06	13000	3500
17-Apr-96	Sterling	40	---	---	>100%	---	89	35	---	249	----	1.17	240	240
22-Apr-96	Sterling	0	---	---	>100%	---	84	47	164	27	----	1.27	160,000	160,000
22-Apr-96	Sterling	0	---	---	>100%	---	73	50	---	387	----	1.33	160,000	160,000
07-Sep-96	Stirling	10	---	---	13.3	5.6	82	53	---	156	----	1.18	n/a	n/a
29-Apr-96	Kenilworth	0	---	---	>100%	---	100	44	---	267	----	1.27	2	< 2
30-Apr-96	Kenilworth	10	---	---	>100%	---	89	39	---	208	----	1.27	92000	92000
09-May-96	Kenilworth	0	---	---	>100%	---	83	26	---	115	----	1.1	2300	2300
10-May-96	Kenilworth	0	---	---	>100%	---	100	86	---	122	----	1.03	n/a	n/a

APPENDIX B:

Field Site Photographs

Figure B1: Cataraqui Town Centre Parking Lot Outfall (Kingston)

Figure B2: West Branch of Little Cataraqui Creek - Pond Inlet (Kingston)

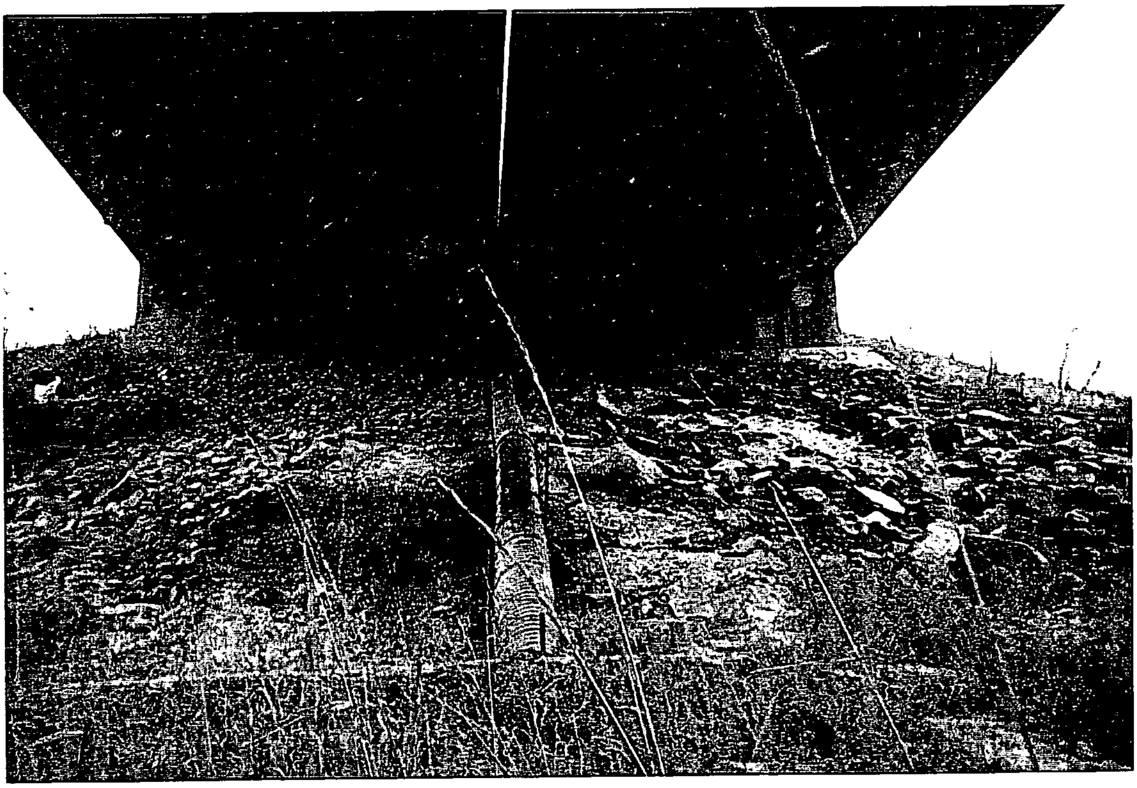


Figure B3: Highway 2 Bridge Drainage Outfall (Scarborough)

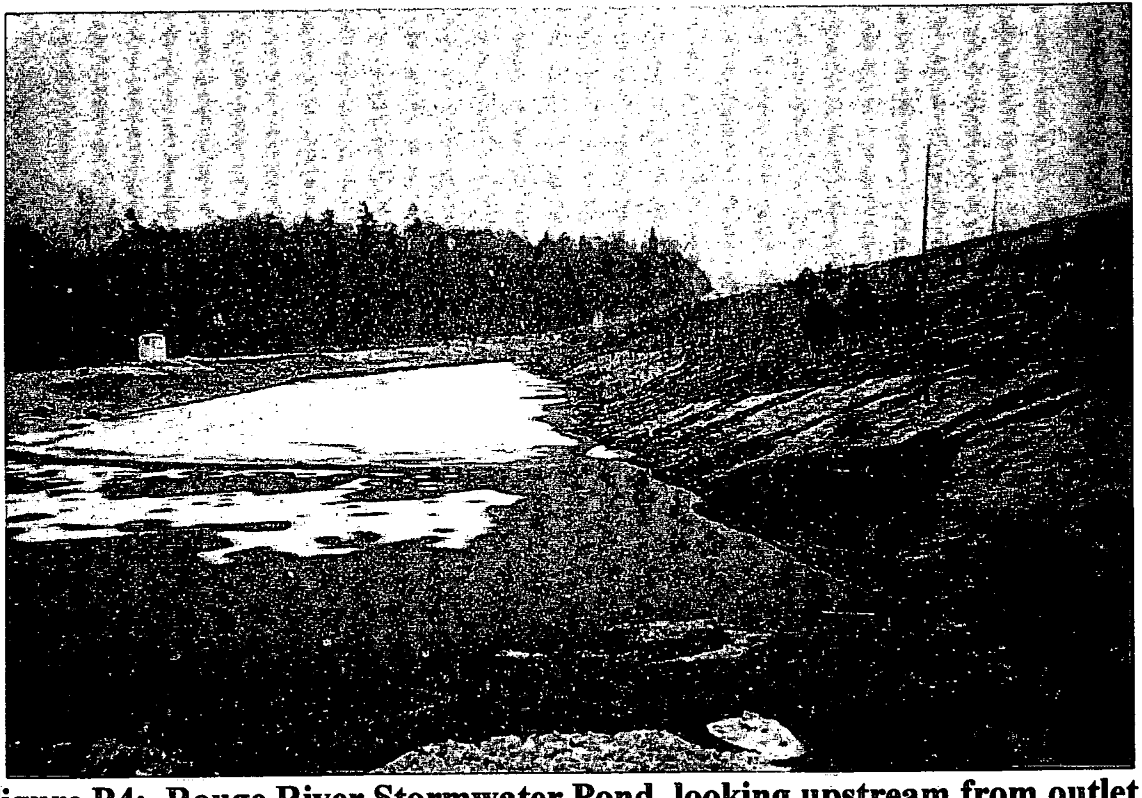


Figure B4: Rouge River Stormwater Pond, looking upstream from outlet (Scarborough)

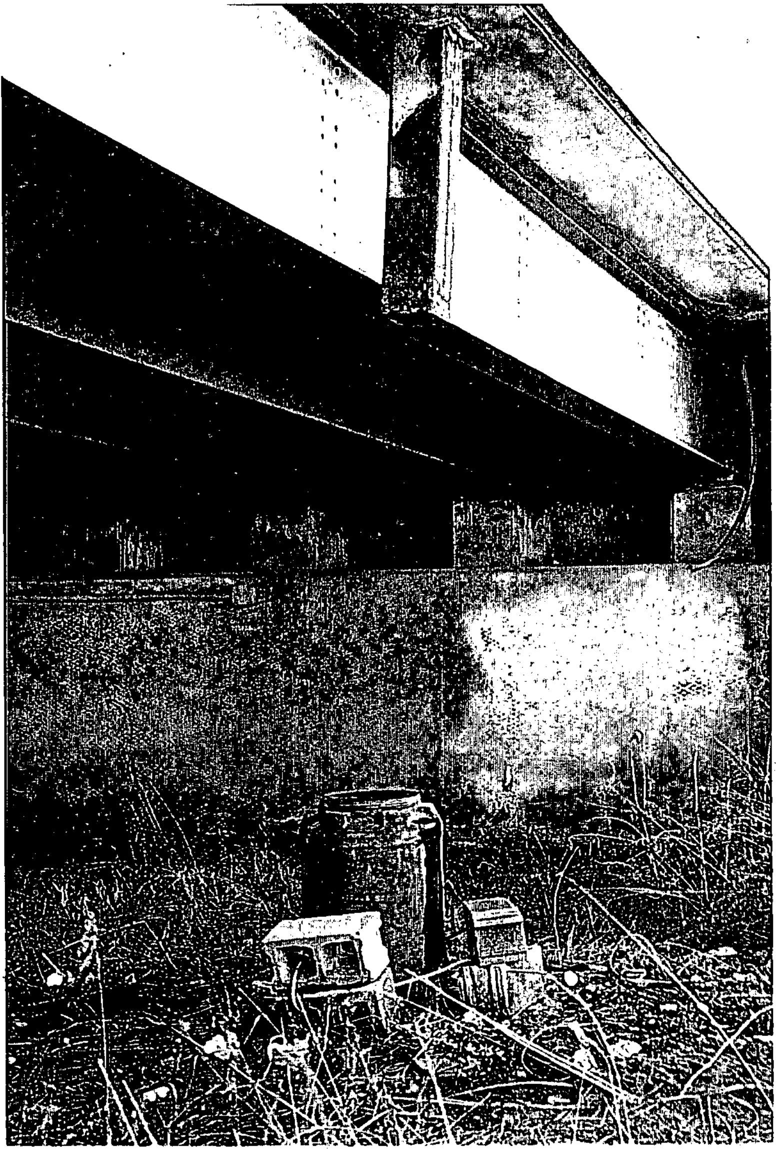


Figure B5: James N. Allen Burlington Bay Skyway Bridge Stormwater Runoff Site (Burlington)

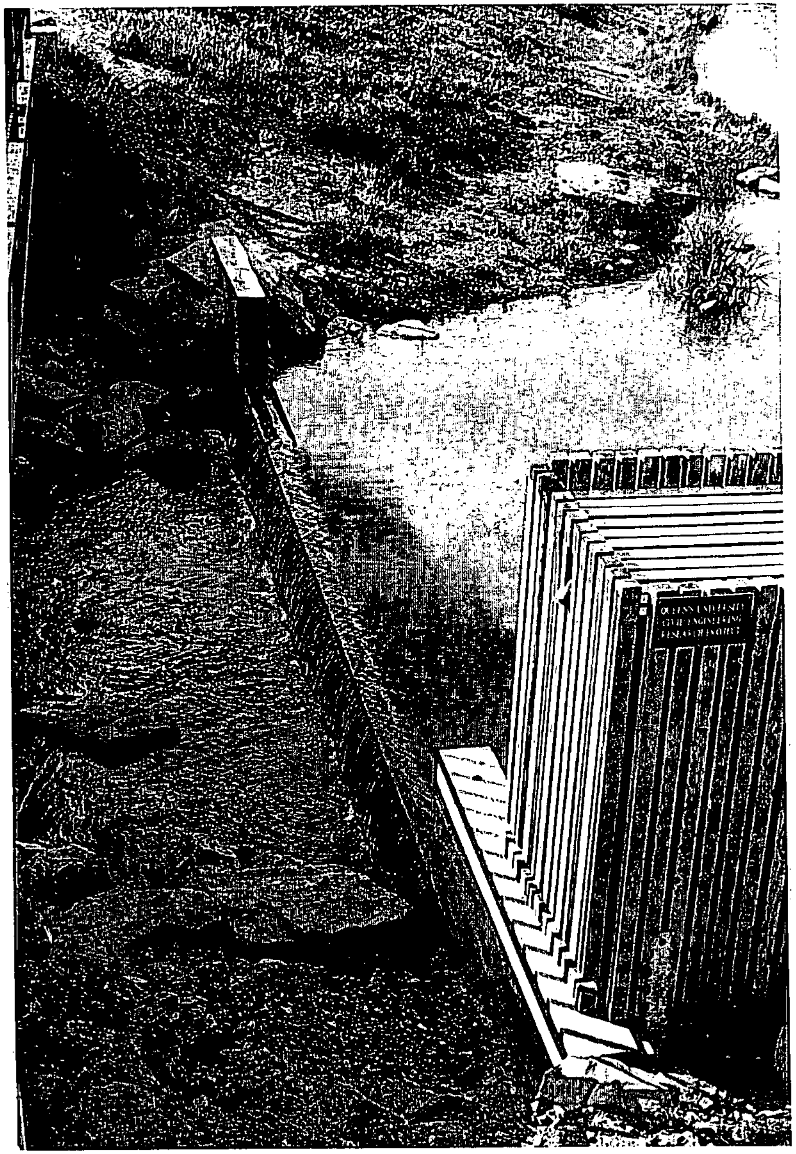


Figure B6: Cataraqui Stormwater Pond Outlet (Kingston)

Figure B7: Constructed Wetlands (Kingston)

Figure B8: Sunoco Station Forecourt (Waterdown)

Figure B9: Parkdale Avenue Combined Sewer Overflow (Hamilton)

Figure B10: Kenilworth Avenue Combined Sewer Overflow (Hamilton)

Figure B11: Melvin Avenue Combined Sewer Overflow (Hamilton)

Figure B12: Queenston Avenue Combined Sewer Overflow (Hamilton)

Figure B13: Lawrence Avenue Combined Sewer Overflow (Hamilton)

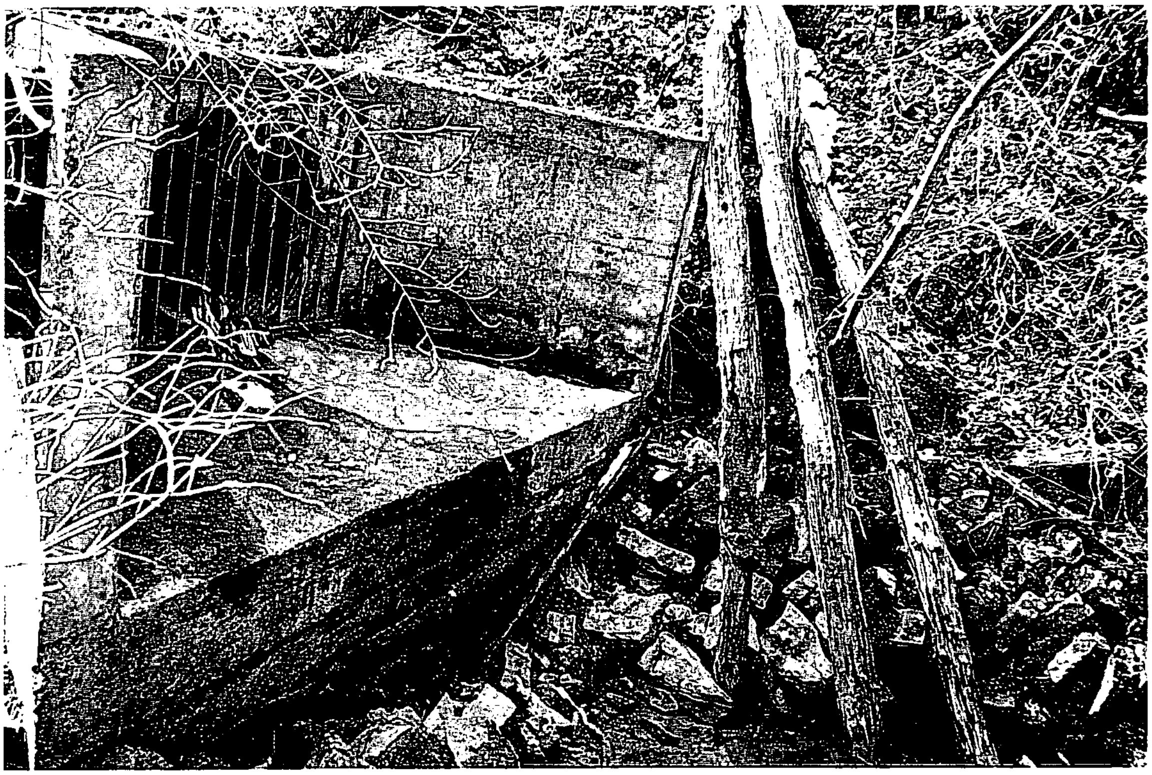
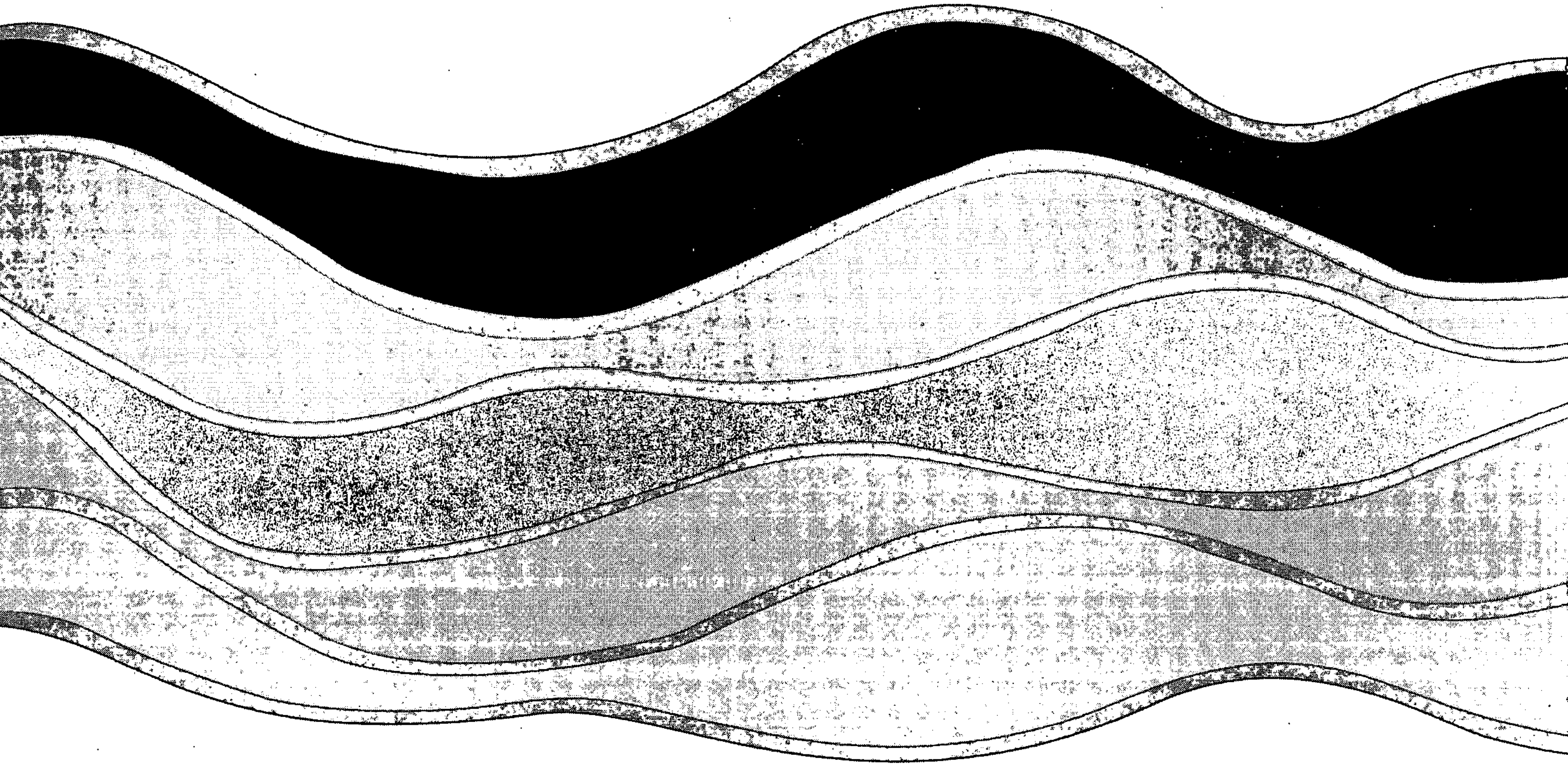


Figure B14: Stirling Avenue Combined Sewer Overflow (Hamilton)

Figure B14: Royal Avenue Combined Sewer Overflow (Hamilton)



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