The Toxicity of Harmony Landfill Leachate to Green Hydra (*Hydra viridissima*)

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ABSTRACT

Harmony Landfill is a former industrial waste disposal site located adjacent to Harmony Creek in Oshawa, Ontario, Canada. During active disposal, from 1957 until 1980, approximately 1 million tonnes of waste were land-filled at the site. Although past environmental monitoring had indicated localized contamination of ground and surface waters, the current level of impact remained unclear. In order to determine the potential of Harmony Landfill leachate to affect aquatic organisms in Harmony Creek, chemical analysis of field samples and laboratory toxicity testing were performed. Chemical analysis was completed on water samples from Harmony Creek and surface leachate samples collected seasonally at Harmony Landfill. Toxicity tests were conducted using the model freshwater invertebrate Green Hydra (*Hydra viridissima)*. Hydra were pulse-exposed for 24 hours to varying concentrations (0%, 3.2%, 10%, 32%, 100%) of monthly field-collected leachate samples diluted with laboratory water. Population growth, Hydra morphology and survival were recorded daily for 7 days. Results showed that creek waters generally had comparable analyte levels upstream and downstream of Harmony Landfill. Leachate samples contained iron, manganese and zinc at levels which may be toxic to aquatic invertebrates. Population growth was significantly inhibited compared to lab water (0%) controls at the 100% leachate concentration in December 2008 and July 2009. Hydra morphology (32% and 100%) and survival (100%) were also affected by the December 2008 leachate. Findings indicate that leaching is occurring at Harmony Landfill and that the leachate sampled and tested during this research program had the potential to negatively affect Green Hydra (*Hydra viridissima*).

Keywords: Green Hydra, *Hydra viridissima*, population growth, morphology, landfill leachate

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LIST OF ABBREVIATIONS

CCME = Canadian Council of Ministers of the Environment

CLOCA = Central Lake Ontario Conservation Authority

d = day

EC_{50} = median effective concentration for 50% of test organisms

$EC(I)_{50}$ = median effective concentration immobilizing 50% of test organisms

GPS = global positioning system

h = hour

LC_{50} = median lethal concentration for 50% of test organisms

m = metres

MAC = minimal affective concentration

mg/L = milligrams per litre

mL = milliliters

mm = millimetres

MOE = Ontario Ministry of the Environment

NOEC = no observed effect concentration

OEAB = Ontario Environmental Appeal Board

µg/L = micrograms per litre

µS/cm = microSiemens per centimetre

VOCs = volatile organic compounds

1.0 - INTRODUCTION

1.1 - Harmony Landfill

1.1.1 – Landfill Background

Harmony Landfill (Figure 1) is a former industrial waste disposal site located at the junction of Grandview Street North and Rossland Road East (GPS co-ordinates: 43°55'31.0" N, 78°50'07.0" W) in Oshawa, Ontario, Canada. It is bordered to the North and West by sub-urban development and to the South and East by Harmony Valley Park and Harmony Creek. The landfill was operated by owners Industrial Disposal (Oshawa) Limited from 1957 until its closure in 1980 (Crutcher & Mosher, 1991). The property is currently owned by Rossland Acres Incorporated (MOE, 2009). During active disposal, approximately 1 million tonnes of industrial waste were land-filled on a 9 hectare portion of the site (Crutcher & Mosher, 1991). The average depth of the waste mound, which was laid on top of a former sand and gravel pit, is estimated to be 15 metres (Crutcher & Mosher, 1991). The majority of the waste deposited at Harmony Landfill originated from General Motors' automotive manufacturing (Crutcher & Mateyk, 1994). Records show that the waste consisted of a mixture of industrial and operational materials including: metal sludges, paint sludges, industrial solvents, oils, paper, cardboard, wood and cafeteria wastes (Crutcher & Mateyk, 1994).

During the landfill's history a number of measures were taken in order to reduce the potential impact of leachate on the surrounding environment. By 1976, a leachate collection system, which involved piping emptying into a leachate lagoon located at the southwestern end of the site, had been installed (Sobanski, 1976 - 1982). Reports show that the system underwent upgrades in subsequent years, eventually culminating in a network of perforated pipes encircling the waste disposal area (Crutcher & Mosher, 1991; MOE, 2009). The newer collection system emptied directly into a sanitary sewer near the site of the former lagoon (Crutcher & Mosher, 1991). The current efficiency of leachate collection at Harmony Landfill is unclear. Putative components of the leachate collection system, observed during visits to the site, suggest that at least some components of the collection system remain intact (personal observation, 2010).

1.1.2 – Environmental Impact (1976 - 1983)

In 1976, concerns regarding the landfill's environmental impact resulted in a hearing held by the Ontario Environmental Appeal Board (OEAB, 1976). Required actions following that meeting included improvements to the leachate collection system as well as regular reporting of ground and surface water monitoring data to the Ontario Ministry of the Environment (OEAB, 1976). Reports indicate that Hydrology Consultants Limited conducted an environmental monitoring program at Harmony Landfill from July 1976 until July 1983 (Sobanski, 1976 - 1982; Sobanski, 1983 - 1984). A map from April 1981 (Appendix 1) shows that 13 ground water wells, situated around the waste perimeter, and 5 surface water sites, located in Harmony Creek, were sampled during that survey period (Sobanski, 1976 - 1982).

Hydrology Consultants' reports show levels of metals: copper, iron, lead and zinc exceeding present acceptable guidelines for the protection of aquatic life (CCME, 2007) in ground (lead) and ground and surface (copper, iron, zinc) waters immediately adjacent to Harmony Landfill (Sobanski, 1976 - 1982; Sobanski, 1983 - 1984; Appendix 2). Increased measurements of chloride, sulphate, hardness, alkalinity and conductivity, as well as decreased pH, also indicate that landfill leachate was reaching ground and surface waters during the 1976 to 1983 monitoring period (Sobanski, 1976 - 1982; Sobanski, 1983 - 1984; Appendix 3). A limited number of organic chemical compounds (phenols and aromatics) were also measured in ground and surface water samples. Detectable levels of total phenols exceeding guidelines for the protection of aquatic life (CCME, 2007) and measures of total aromatic compounds in ground and surface water samples also indicate organic chemical contamination (Sobanski, 1976 - 1982; Sobanski, 1983 - 1984; Appendix 4).

1.1.3 – Environmental Impact (1992 - 2008)

During the 1990s, several consultants' reports investigated the environmental impact of the former Harmony Landfill. Although the effects of leaching on adjacent lands were still a concern, housing developments were built to the North and West of Harmony Landfill (Crutcher & Mosher, 1991; Crutcher & Mosher, 1994). Ground and surface water monitoring completed by the Ontario Ministry of the Environment in 1992 identified elevated levels of iron and chloride in surface water collected at the landfill site (MOE, 2009). In that same year, the Ministry also noted iron-containing leachate discharges in Harmony Creek (MOE, 2009).

In 2008 public concern, over observable leachate at the southwestern end of the landfill, led to an investigation by the Ontario Ministry of the Environment (MOE, 2009). Surface water samples were taken by a local citizen during the summer of 2008, on which chemical analysis was performed by Maxxam Analytics (Mississauga, Ontario, Canada) (S. Ross, personal communication, October 24, 2008). Results of that analysis show aluminum, arsenic, cadmium, chromium, copper, iron, lead, nickel and zinc (Ross, 2008;

Appendix 2) at levels exceeding the current guidelines for the protection of aquatic life (CCME, 2007). Of the organic compounds tested in that analysis, only m/p-xylene was found at a detectable level, although it was below the recommended provincial water quality objective (Ross, 2008; MOE, 1994; Appendix 4). In November of 2008, the current owner of Harmony Landfill sampled onsite groundwater wells and reported levels of chloride, sodium, manganese and selenium exceeding the Canadian drinking water standards (MOE, 2009).

1.2 - Harmony Creek

1.2.1 – Water Quality

Harmony Creek is a fresh water stream whose tributaries are adjacent to the South, East, and West of Harmony Landfill. Its source is located North of the landfill in the Oak Ridges Moraine (CLOCA, 2009). As it travels southwards towards its mouth at Lake Ontario, Harmony Creek passes through a variety of landscapes capable of affecting its ecosystem health (Goodwin, 1979). These include: protected green spaces, agricultural land and sub-urban, urban and industrial developments (CLOCA, 2009). Harmony Creek forms part of the Harmony/Black/Farewell Creek watershed, which is monitored by the Central Lake Ontario Conservation Authority (CLOCA) (CLOCA, 2009). Water quality data from 2006 to 2007, collected from two sampling sites (CLOCA SWQ12 and SWQ3) considerably downstream of Harmony Landfill (Appendix 5) shows elevated levels of chloride, aluminum, cadmium and copper in Harmony Creek (CLOCA, 2008).

1.2.2 – Aquatic Biology

Aquatic invertebrate and fish species are present in Harmony Creek (CLOCA, 2002/2008). Invertebrates in the orders: amphipoda, diptera, ephemeroptera, coleoptera, megaloptera, odonata, trichoptera, hemiptera and decapoda, as well as oligochaeta and nematoda were detected using a kick net during the summer of 2002 at sampling sites (Appendix 5) upstream (CLOCA H403) and downstream (CLOCA H402) of Harmony Landfill (CLOCA 2002/2008). Fish found in Harmony Creek in 2008 included: rainbow trout (*Oncorhynchus mykiss*), creek chub (*Semolitus atromaculatus*), blacknose dace (*Rhinichthys atratulus*) and fathead minnow (*Pimephales promelas*) (CLOCA 2002/2008).

Figure 1: Map of Harmony Landfill study site. The broken black line represents the approximate location of the waste boundary and the position of the leachate collection system. Field sampling sites in Harmony Creek are marked in blue. Leachate sampling sites at Harmony Landfill are marked in yellow. Photo: Google Earth, June 2010.

2.0 – LITERATURE REVIEW

2.1 – Landfill Leachate

2.1.1 – General Characteristics

Land-filling continues to be a common method of disposal for industrial waste in Canada (Statistics Canada, 2009). Landfills may pose threats to human and ecosystem health through the leaching of gaseous and waterborne toxicants. Of primary concern for aquatic ecosystems is the movement of landfill leachate into ground and surface waters. Landfill leachate is created when rainwater filtering through the waste layers in a landfill picks up solid and dissolvable contaminants (Kjeldsen *et al.,* 2002). Although leachate composition is largely dependent upon the types of waste deposited and the decomposition processes occurring within a landfill, leachates share some general characteristics (Slack *et al.,* 2005).

Leachate samples, as well as ground and surface waters contaminated with leachate, generally have elevated measurements of hardness, alkalinity and conductivity (Christensen *et al.,* 2001). This is a reflection of the higher levels of ions present in leachate. Chloride, nitrate and sulphate are the major anions in landfill leachate while calcium, magnesium, potassium and sodium are the major cations (Öman & Junestedt, 2008). Due to its high mobility and limited tendency to complex, chloride is often used as a measure for the maximum migration distance of landfill leachate plumes (Christensen *et al.,* 2001). Depending on conditions, ammonia and other nitrogen compounds such nitrates and nitrites may also be present at high enough levels in landfill leachates to become toxic to aquatic organisms (Dave & Nilsson, 2005). Two other major components of landfill leachates which are generally of great concern to the health of aquatic organisms are metals and xenobiotic organic compounds (Kjeldsen *et al.*, 2002).

2.1.2 – Metals

Metals have been demonstrated to be universally present in landfill leachates. Commonly identified metals include: copper, cadmium, chromium, iron, manganese, nickel and zinc. (Plotkin & Ram, 1984; Assmuth & Penttilä, 1995; Rutherford *et al.*, 2000; Christensen *et al.*, 2001; Kjedlsen *et al.*, 2002; Baun & Christensen, 2004; Dave & Nilsson, 2005; Osaki *et al.*, 2006; Øygard *et al.*, 2007; Öman & Junestedt, 2008). Some sources of metals in landfills are: batteries, electronics and electrical appliances, scrap metal, paint sludges and post-industrial metal residues (Slack *et al.*, 2005; Östman *et al.*, 2008; Lambolez *et al.*, 1994).

Research has shown that metals, especially heavy metals (copper, cadmium, chromium, lead, nickel and zinc) are often found at low levels in landfill leachate (Kjeldsen *et al.*, 2002). Even after a period of 30 years, less than 0.02% of the heavy metals received at a landfill may have been removed through leaching (Kjeldsen *et al.*, 2002). Metals are believed to be immobilized chiefly by the processes of sorption to organic ligands and precipitation due to binding with inorganic ligands to form sulphides, carbonates, hydroxides, phosphates and chlorides (Kjeldsen *et al.*, 2002). Work with landfill leachates has demonstrated that, since landfill composition and conditions are so variable, a wide range of metal species exist in leachates (Baun & Christensen, 2004). Metal speciation is governed by leachate pH, redox status and the availability of binding organic material (Östman *et al.*, 2008). Lower pH, higher oxygen and a decreased amount of organic matter tend to result in an increase in mobile metal forms which are believed to be more bioavailable and thus more toxic to living organisms (Fraser *et al.,* 2000; Östman *et al.*, 2008).

2.1.3 – Xenobiotic Organic Compounds

Although many organic compounds may be lost through volatilization, diffusion, leaching and degradation early in a landfill's history, several xenobiotic organic compounds have been shown to persist in waste for decades (Kjeldsen *et al.,* 2002). Xenobiotic organic compounds commonly identified in landfill leachate include aromatic and halogenated hydrocarbons, phenols, pesticides, polychlorinated biphenyls and plasticizers (Christensen *et al.,* 2001; Kjeldsen *et al.*, 2002; Öman & Junestedt, 2008). In particular, chloroethanes, cholorobenzenes, ethylbenzenes, toluenes and xylenes, are slowly-reacting volatile organic compounds which are more likely to persist in landfills (Brack *et al.,* 1998; Slack *et al.,* 2005). If aerobic conditions are present, as may be the case in early and late stages of a landfill's history (Kjeldsen *et al.,* 2002), microbial degradation of xenobiotics may take place. For example, in soil and water, *Alcaligenes* sp. bacteria can biodegrade 1,3-dichlorobenzene and 1,4-dichlorobenzene and a *Pseudomonas* sp. bacteria isolated from sewage can degrade 1,2-diclorobenzene (Nishino *et al.,* 1993). Breakdown by these types of bacteria has been shown to lead to an increase in monochlorobenzene levels (Nishino *et al.,* 1993).

Organics in landfills originate from a wide variety of sources. Possible origins of xenobiotic organic compounds in industrial landfill leachates include: industrial solvents and substrates, paint solvents, paint adhesives, paint thinners, paint sludges, varnishes, degreasers, cleaning products and pesticides (Lambolez *et al.,* 1994; Slack *et al.*, 2005).

2.2 – Leachate Toxicity

2.2.1 – Toxicity to Aquatic Invertebrates

Although chemical analysis of leachates is an important tool in understanding their toxic potential, toxicity testing is often performed in tandem to obtain a more complete understanding of a landfill's environmental risk (Kjeldsen *et al.,* 2002). Toxicity testing allows for clarification of the bioavailability and possible synergistic, antagonistic and additive effects of toxicants in a complex mixture such as a landfill leachate (Kjeldsen *et al.,* 2002). Aquatic organisms may become naturally exposed to leachate as a result of surface leachate break-outs or through contamination of groundwater in connection with the water bodies in which they live (Dewhurst *et al.*, 2003; Slack *et al.*, 2007). As would be expected from the typical composition of leachate, discussed previously, field-collected leachate samples have been demonstrated to be toxic to aquatic invertebrates.

Contaminated surface water samples taken from ditches or creeks next to municipal and industrial waste co-disposal sites in Finland showed acute toxicity to water fleas (*Daphnia magna*), with an average 48 h $EC(I)_{50}$ of 50% leachate (50% dilution of the field-collected sample) (Assmuth & Penttilä, 1995). In this study toxicity testing was performed with samples from multiple sites, 75% of which showed toxicity to *Daphnia magna* (Assmuth & Penttilä, 1995). Leachate samples from 6 municipal solid waste landfills in the United States were also shown to be toxic to another cladoceran, *Ceriodaphnia dubia* with a 48 h $EC(I)_{50}$ of <10% leachate (range: 1.9% - 9.5% leachate) (Ward *et al.,* 2002). Groundwater samples from a well-documented leachate plume at a municipal and industrial co-disposal waste landfill in Denmark were acutely toxic to

Daphnia magna (Baun *et al.,* 1999). Interestingly, the samples decreased in toxicity as distance from the landfill increased with a 10 m 48 h $EC(I)_{50}$ of 13% leachate, a 15 m 48 h $EC(I)_{50}$ of 18% leachate and a 26 m 48 h $EC(I)_{50}$ of 28% leachate (Baun *et al.,* 1999).

Water–extractable fractions of solid industrial wastes accepted at controlled landfills in France, showed both acute and chronic toxicity to *Daphnia magna* (Lambolez *et al.,* 1994). A paint sludge extract had a 24 h $EC(I)_{50}$ of 40% leachate while two metal sludge extracts were acutely toxic with 24 h $EC(I)_{50}$ results of 0.6% and 2.5% leachate (Lambolez *et al.,* 1994). Chronic toxicity values for a 28 day reproduction experiment with *Daphnia magna* conducted with the same waste fractions continued to show that leachates from paint sludges (28 d EC_{50} of 5 - 20% leachate) may be less toxic to aquatic invertebrates than those from metal sludges (28 d EC_{50} of 0.2 - 2.5% leachate) (Lambolez *et al.*, 1994).

In tests with other freshwater invertebrates, landfill leachate collected from a closed industrial waste site in the United Kingdom was shown to be acutely toxic to freshwater crustaceans, amphipod *Grammarus pulex* and isopod *Asellus aquaticus*. The 96 h LC_{50} was 1% leachate for *Grammarus pulex* and 12.3% leachate for *Asellus aquaticus* (Bloor *et al.,* 2005). Leachates collected from a municipal solid waste landfill in Colombia also showed acute toxicity to a marine invertebrate, Brine Shrimp *Artemia franciscana*, with an average 48 h LC_{50} of 17.8% leachate (range: 3.2 - 39.0%) (Olivero-Verbel *et al.,* 2008). All of the above test results indicate that, even for significant dilutions of the field-strength (100%) leachate, exposure of aquatic invertebrates to landfill leachate may lead to acute and chronic toxic effects.

2.2.2 – Pulse-exposure in Toxicity Testing

Pulse-exposure refers to the exposure of test organisms to toxicants for a limited period of time, usually in terms of 1 to 24 hours. Pulse-exposures may be used experimentally in order to simulate the exposure of organisms to effluents (Diamond *et al.*, 2005). Studies have demonstrated that toxicant concentrations in landfill leachates are variable. They may vary daily or seasonally, with the concentration of possible toxicants increasing in dry weather and decreasing due to dilution during rain events (Ettler *et al.*, 2008). Pulse-exposures have been successfully used in other experiments to mimic episodic exposures of aquatic invertebrates and fish to metals (Zhao & Newman, 2006; Diamond *et al*., 2005), pesticides (Stoughton *et al*., 2008; Holdway *et al*., 2008) and urban runoff in stormwater ponds (Rosenkrantz *et al*., 2008).

2.3 – Hydra

2.3.1. – Hydra Background

Hydra are small invertebrates (~1-20 mm) which are difficult to detect but have repeatedly been described as ubiquitous inhabitants of freshwater environments (Holstein *et al.,* 1990; Slobodkin & Bossert, 2001). The literature shows that Hydra can be found in ponds (Schwartz *et al.,* 1983), rivers (Wang *et al.,* 2009) and lakes in both littoral (Walsh, 1995) and deep water (300 - 400 m) benthic zones (Nalepa *et al.,* 1987). They are generally attached to natural submerged substrates such as rocks (Nalepa *et al*., 1987) and plants (Elliott *et al.,* 1997). However, Hydra have also been shown to adhere to suitable manufactured substrates in the field (Bell & Wolfe, 1985) and under laboratory conditions (Lenhoff & Brown, 1970).

Hydra are members of the phylum Cnidaria, which includes many well-known marine species such as jellyfish, sea anemones and corals (Slobodkin & Bossert, 2001). Hydra belong to the class Hydrozoa, order Hydroida, family Hydridae and genus Hydra (Kovačević *et al.,* 2009). Unlike their cnidarian relatives, which have distinct floating (medusa) and sedentary (polyp) life stages, Hydra remain as sessile polyps during their entire lives (Slobodkin & Bossert, 2001). Floating, which has been observed to occur mainly during times of environmental stress, happens only periodically and is not accompanied by a change in life stage (Lomnicki & Slobodkin, 1966).

Like other cnidarians, Hydra are diploblastic organisms with 2 tissue layers: the outer ectoderm and inner endoderm, separated by a non-cellular mesoglea layer (Slobodkin & Bossert, 2001). The endoderm lines the gastrovascular cavity, a water-filled sac, which acts both as a hydrostatic skeleton and the site for food digestion and nutrient absorption (Slobodkin & Bossert, 2001). Hydra bodies (Figure 2) can be divided into two main functional sections: the hypostome which consists of the mouth and tentacles and the body column which contains the gastric and budding regions, the peduncle and the basal disk (Trottier *et al.,* 1997; Holdway, 2005).

Although capable of sexual reproduction, Hydra primarily reproduce asexually by budding (Loomis, 1954). Hydra are considered to be immortal animals (Stiven, 1962). This is because they have been shown to continually renew themselves by producing new cells in a growth zone around the hypostome (Loomis & Lenhoff, 1956). These new cells are used to replace lost or dead cells and to create buds instead of increasing the size of the adult animal (Stiven, 1962). As a result of this type of growth, it is not surprising that Hydra have a tremendous regenerative capacity. They have been shown to regenerate into a healthy adult polyp from either a seriously injured intact individual (Loomis & Lenhoff, 1956), a ball of disassociated Hydra cells (Johnson *et al.*, 1982) or a section of their gastric region (Quinn *et al.*, 2008a).

Feeding in Hydra occurs when live prey stimulate the release of nematocysts, located in the endodermal cells of the tentacles, which act to entangle and immobilize food organisms (Schwartz *et al.*, 1983). Following capture, reduced glutathione, which is released by injured prey items, activates the ingestion of prey through the mouth into the gastrovascular cavity (Loomis & Lenhoff, 1956). Once digestion is complete, undigested materials are expelled back out through the mouth (Slobodkin & Bossert, 2001).

Ecologically Hydra are assumed to play the role of invertebrate predators and prey in aquatic ecosystems (Slobodkin & Bossert, 2001). As predators, Hydra have been shown to ingest cladocerans (Schwartz & Hebert, 1989), copepods (Link & Keen, 1995), rotifers (Walsh, 1995) and larval fish (Elliott *et al.*, 1997), as well as their standard laboratory food, brine shrimp, *Artemia* sp. (Loomis & Lenhoff, 1956). They can be prey themselves for flatworms (Slobodkin & Bossert, 2001) and possibly small fish (personal observation, 2008). Although all Hydra feed exogenously, Green Hydra are unique in that they also have access to endogenous food resources (Slobodkin & Bossert, 2001).

2.3.2 – Green Hydra *(Hydra viridissima)*

Green Hydra, *Hydra viridissima*, are small (~1-5 mm) green-coloured Hydra which are widespread (Holstein *et al.*, 1990) and have been documented as being native to Ontario, Canada (D. Sutherland, personal communication, November 26, 2009). Their green colour is a result of their symbiotic relationship with the unicellular green algae, *Chlorella* sp. (Slobodkin & Bossert, 2001). The *Chlorella* inhabit vacuoles termed “symbiosomes” (Yellowlees *et al.,* 2008) located in the endodermal cells (10-40 algae/cell) of the Hydra (Muscatine & Lenhoff, 1965; Slobodkin & Bossert, 2001; Habetha *et al.,* 2003). The algae are believed to release photosynthetically-derived sugars, in the form of maltose (Yellowlees *et al.,* 2008) or glucose-6-phosphate (Habetha *et al.,* 2003) to their Hydra hosts. Although *Hydra viridissima* have been experimentally shown to be capable of normal growth without algal symbionts (Muscatine & Lenhoff, 1965; Habetha *et al.,* 2003; Karntanut & Pascoe, 2005), they are not thought to occur aposymbiotically in nature (Slobodkin & Bossert, 2001; Karntanut & Pascoe, 2005). The photosynthetic symbionts are believed to provide a competitive advantage by supplying their hosts with endogenous food sources during periods of starvation (Habetha *et al.*, 2003).

2.3.3 – Hydra Classification

Most basically, Hydra can be separated into 2 groups based on colour: Green Hydra and Brown or Pink Hydra. In the case of the species discussed in this work: *Hydra viridissima* is the only Green Hydra and *Hydra vulgaris/attenuata*, *Hydra littoralis, Hydra oligactis, Hydra pseudoligactis,* and *Hydra hexactinella* can all be considered to be Brown Hydra (Holstein *et al.*, 1990). Other distinguishing features used in Hydra classification are: the length of the body stalk, length of the tentacles relative to the body and the number of tentacles, although the most definitive feature, excluding the gene sequence, is the microscopic structure of the nematocysts (Holstein *et al.*, 1990). *Hydra viridissima, Hydra oligactis, Hydra pseudoligactis* and *Hydra littoralis* have all been identified as temperate Hydra species living in Canada (D. Sutherland, personal communication, November 26, 2009; Clifford, 2010) while *Hydra vulgaris,* which is often also known as *Hydra attenuata*, is native to North America and Europe (Campbell, 1989; Slobodkin & Bossert, 2001) and *Hydra hexactinella* is an Australian species (Murray-Darling Freshwater Research Centre, 2010).

2.3.4 – Laboratory Culture

Mass laboratory culture procedures were first developed and published for Hydra in the 1950s (Lenhoff & Brown, 1970). Given optimal conditions, Hydra populations can continue to reproduce asexually and grow logarithmically for an indefinite period of time (Loomis, 1954). Laboratory tests using *Hydra littoralis* and *Hydra attenuata* have demonstrated that Hydra may require a minimum of 6 mg/L of dissolved oxygen, a maximum water hardness of 750 mg $CaCO_3$/L, a pH range of 6 - 8, temperatures of 20 - 30°C and daily feeding of *Artemia* sp. in order to achieve logarithmic growth (Loomis, 1954; Fu *et al.*, 1991). Ions are also required for optimal growth of *Hydra viridissima* (Lenhoff & Brown, 1970). Those which are often added to Hydra culture medium include: chloride, calcium, magnesium, potassium, sodium and bicarbonate (Muscatine & Lenhoff, 1965).

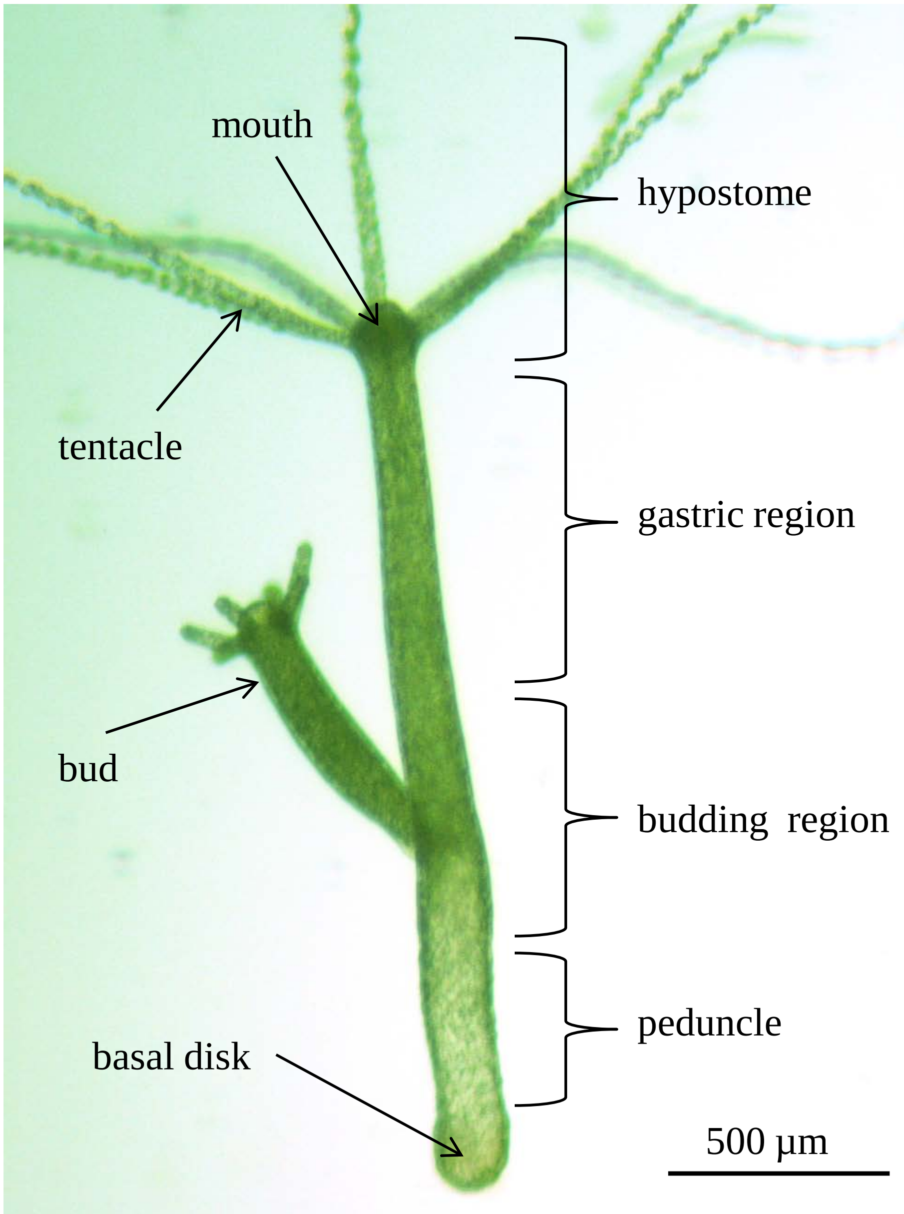


Figure 2: General body plan of a budding Hydra. The locations of major body parts (left side) and functional regions (right side) are indicated. Photograph depicts a Green Hydra (*Hydra viridissima*).

2.4 – Hydra in Toxicity Testing

2.4.1 – Toxicity Testing Background

Since Hydra were first described in the scientific literature in the early 1700s (Campbell, 1989), they have been used to advance knowledge in many areas of biological research (Slobodkin & Bossert, 2001). Standard culture methods (Lenhoff & Brown, 1970), consistent asexual reproduction leading to a population of genetically identical clones, exposure of cells directly to the environment due to a diploblastic structure (Loomis, 1954) and ubiquity in freshwater ecosystems (Slobodkin & Bossert, 2001), make Hydra a model test species for use in aquatic toxicology. Typical endpoints of toxicity testing in Hydra are: survival (mortality) and polyp structure (morphology of body and tentacles), budding (population growth) and polyp regeneration (teratogenicity) (Tarrant, 2007). Testing protocols have been developed for survival and morphology (Trottier *et al.,* 1997; Blaise & Kusui, 1997), population growth (Holdway, 2005) and teratogenicity (Johnson *et al.*, 1982; Quinn *et al.,* 2008a). The sensitivity of Hydra morphology as an indicator of sub-lethal toxicity and the rapidity with which population growth rate effects can be observed, make Hydra a uniquely useful toxicology test species. As such, Hydra have been used to test a variety of toxicants and have been demonstrated to be sensitive to metals, xenobiotic organic compounds and effluents.

2.4.2 – Hydra and Metals

In comparison studies testing the acute toxicity of heavy metals to a variety of Hydra species (*Hydra vulgaris, Hydra oligactis* and *Hydra viridissima*), copper was regularly found to be the most toxic with the order of toxicity from most to least being: copper > cadmium > zinc (Beach & Pascoe, 1998; Pollino & Holdway, 1999; Holdway *et* *al.*, 2001; Karntanut & Pascoe, 2000; Karntanut & Pascoe, 2002; Karntanut & Pascoe, 2005). *Hydra viridissima* was routinely found to be the most sensitive Hydra species with a 96 h LC_{50} range of 8.5 - 28 µg/L for copper, 3 - 210 µg/L for cadmium and 935 - 11,000 µg/L for zinc (Pollino & Holdway, 1999; Holdway *et al.*, 2001; Karntanut & Pascoe, 2002; Karntanut & Pascoe, 2005). Aluminum (72 h LC_{50} = 475,000 - 480,000 µg/L) and lead (>1000 µg/L lethal dose) have also been demonstrated to be lethally toxic to *Hydra viridissima* (Browne & Davis, 1977; Kovačević *et al.*, 2007). Morphological evidence recorded in several studies, including tentacle clubbing, tentacle shortening and full body contraction, support the toxicity of metals (aluminum, copper, cadmium, lead and zinc) to *Hydra viridissima* (Browne & Davis, 1977; Pollino & Holdway, 1999; Karntanut & Pascoe, 2002; Kovačević *et al.*, 2007).

Metals can have chronically toxic as well as hormetic effects on Hydra at lower concentrations, many of which are more environmentally relevant. Waterborne exposure of *Hydra viridissima* to metals led to reduced asexual budding, as measured by population growth, at levels of: 8 - 16 µg/L for copper (Pollino & Holdway, 1999; Karntanut & Pascoe, 2005), 0.8 µg/L for cadmium (Holdway *et al.*, 2001), 75 µg/L for zinc (Holdway *et al.*, 2001) and 50 µg/L for lead (Browne & Davis, 1977). Nickel tested on *Hydra littoralis* was also shown to inhibit population reproduction at 60 µg/L (Santiago - Fandiño, 1983). In their experiments with *Hydra viridissima*, Browne & Davis (1977) found that short exposures (5 - 60 minutes) to lead (10 - 1000 µg/L) had possible hormetic effects in that they increased asexual bud production.

Hydra may accumulate metals. After a 72 hour exposure to aluminum (25,000 - 475,000 µg/L), *Hydra viridissima* and *Hydra oligactis* were observed to have aluminum deposits in their gastrodermal, algal (*Hydra viridissima*) and ectodermal cells (*Hydra oligactis*) (Kovačević *et al.,* 2009). Deposits in the discharged nematocysts of *Hydra viridissima* were observed after a 24 hour exposure to 200 - 3900 µg/L of uranium in a single compound mixture as well as in an effluent (Hyne *et al.,* 1992a). These deposits were presumed to be responsible for the reduced post-exposure ability of the Hydra to capture live *Artemia* sp. (Brine Shrimp) (Hyne *et al.*, 1992a). In this study, inclusions of, aluminum, magnesium and zinc were also noted within the symbiotic algal cells of the Hydra (Hyne *et al.,* 1992a). In another study, copper, cadmium and zinc were demonstrated to accumulate in *Hydra vulgaris* through both waterborne and food-borne exposure routes (Karntanut & Pascoe, 2007).

2.4.3 – Hydra and Xenobiotic Organic Compounds

Hydra are generally not believed to be as sensitive to organic compounds as metals. Toxicity tests on *Hydra oligactis* using the polychlorinated biphenyls (PCBs) Aroclor 1016 and Aroclor 1254 resulted in a 72 h LC_{50} range of 5,000 - 20,000 µg/L, although sub-lethal inhibitory effects on reproduction and regeneration were seen at levels of 1,000 µg/L - 4,000 µg/L (Adams & Haileselassie, 1984). The 96 h LC_{50} of 4-chlorophenol from Mitchell & Holdway (2000), when used as a reference toxicant, was 34, 000 µg/L. However, 92 hour exposures of *Hydra attenuata* to organophosphates demonstrated that Hydra may be sensitive to some organics (Lum *et al.*, 2003). The 92 h minimal effective concentration range of that study was 0.003 – 100,000 µg/L with toxicity correlated with increasing compound hydrophobicity (Lum *et al.,* 2003). *Hydra attenuata* were also seen to be sensitive to 4-nonylphenol, with a 96 h LC_{50} of 97.5 µg/L and a "no observed effect concentration" (NOEC) for tentacle morphology of < 25 µg/L

(Pachura *et al.,* 2005). When a suite of chlorophenols were tested with *Hydra attenuata*, the 92 h minimal affective concentration (MAC) range was 40 – 500,000 µg/L with the more chlorine-substituted compounds generally being the most toxic (Mayura *et al.*, 1991). When tested with bisphenol A the 96 h LC_{50} for *Hydra vulgaris* was 6,900 µg/L, although sub-lethal effects occurred at a concentration of 42 µg/L (Pascoe *et al.,* 2002). Research has also indicated that *Hydra vulgaris/Hydra attenuata* are sensitive to pharmaceuticals typically found in wastewater effluents (Pascoe *et al.,* 2003; Quinn *et al.*, 2008a; Quinn *et al.*, 2008b; Quinn *et al.*, 2009).

2.4.4 – Hydra and Effluents

Hydra have also been used to test the environmental impact of industrial effluents. When exposed to retention pond water containing gold mine effluent, population reproduction in *Hydra viridissima* was reduced by 80 to 100% by a treatment of 0.1% pond water (pH 6.5), in which copper and zinc were the most likely toxic components (van Dam *et al*., 2008). A significant decrease in population growth, compared to controls for *Hydra viridissima*, was also noted when exposed to 100% retention pond water (pH 7.5 - 8.0) from a uranium mine (Hyne *et al.*, 1992a) although additional work showed the retention pond water to be toxic at 32% if the pH was reduced to 6.6 (Hyne *et al.,* 1992b). When *Hydra attenuata* were tested with a range of ten industrial effluents, four were found to be lethal and eight sub-lethal with a 96 h LC_{50} varying from 18.8 - 100% effluent (Blaise & Kusui, 1997). In a test of municipal sewage and industrial effluents on *Hydra attenuata* the 96 h LC_{50} varied from 17.5 - 98% effluent and the 96 h EC_{50} for tentacle clubbing from 4.9 - 98% effluent (Pardos *et al.,* 1999). In another study industrial wastewater samples tested on *Hydra attenuata* resulted in a MAC of 6 - 31%

effluent (Fu *et al.*, 1991). *Hydra hexactinella* has also been used to test the toxicity of urban runoff water collecting in stormwater basins (Rosenkrantz *et al.*, 2008). Researchers found that only one out of the three basin samples tested were toxic to Hydra with a 96 h LC_{50} of 61% stormwater (Rosenkrantz *et al.*, 2008). However, the basin water found to be toxic had the highest levels of copper, cadmium, lead, nickel and zinc (Rosenkrantz *et al.*, 2008). This supports the conclusion that, like the metal-laden mine effluents tested, effluents are most likely to be toxic to Hydra if they contain metals.

2.4.5 – Detoxification Processes in Hydra

Detoxification processes in Hydra are generally not well-documented. Since Hydra are small in size and their cells are in close contact with the aquatic environment, it is possible that diffusion is the main method for toxicant accumulation and detoxification (Walker *et al.*, 2006). An analogue of the metal binding protein metallothionein, which is responsible for transport and regulation of metals in other organisms, has not been discovered in Hydra (Andersen *et al.*, 1988). However, metals taken in by Hydra may be sequestered and expelled. This has been observed with uranium accumulated in discharged nematocyst cells which are routinely discarded as new cells replace them (Hyne *et al.*, 1992a). Molecular work has identified both phase I and phase II detoxification enzymes in *Hydra vulgaris/Hydra attenuata* (Quinn *et al*., 2004). Both mixed function oxidase (phase I) and glutathione S-transferase (phase II) activity were measured in *Hydra attenuata* exposed to the prescription drug, carbamazepine (Quinn *et al*., 2004). In addition, the anti-oxidant enzymes, glutathione peroxidase and superoxide dismutase, which are often upregulated in times of toxic stress, were characterized in Hydra and demonstrated to increase after toxicant exposure (Dash *et al*., 2006; Dash *et al*., 2007).

3.0 - RESEARCH OBJECTIVES

3.1 - Overall Objective

The overall objective of this research program was to assess the potential impact of Harmony Landfill leachate on the aquatic life of Harmony Creek.

3.1.1 – Specific Objectives

This was accomplished by:

(1) determining the current chemical composition of Harmony Creek surface waters upstream and downstream of Harmony Landfill,

(2) determining the current chemical composition of Harmony Landfill leachate, and

(3) determining the toxicity of multiple Harmony Landfill leachate samples to Green Hydra (*Hydra viridissima*) during the period from December 2008 to April 2010.

4.0 – EXPERIMENTAL METHODS

4.1 – Chemical Analysis

4.1.1 – Sample Collection

Field samples for chemical analysis were collected seasonally from December 2008 until January 2010 (Figure 3; Appendices 6 & 7). Samples were initially taken from Harmony Creek at both upstream and downstream sampling locations and at Harmony Landfill from Site 1. As a result of physical changes to the field site, all subsequent leachate collections were made uniquely at Harmony Landfill Site 2. Leachate and creek water was grab sampled in bottles provided by the York-Durham Regional Environmental Laboratory (Pickering, Ontario, Canada). Samples were always taken from running water using a clean glass beaker to fill bottles. Nitrile gloves were worn by researchers during sampling.

Creek water and leachate samples used for inorganics analysis were collected in 500 mL polyethylene plastic bottles. The bottle dedicated for metals analysis was acidified with 2 mL of concentrated nitric acid immediately upon collection in the field. No preservative was added to the bottle used to test general water characteristics (i.e. cations/anions). Samples for organics analysis were collected in 1000 mL amber glass bottles, with the exception of one 125 mL amber plastic Nalgene bottle, which was used to test for glyphosate and two 40 mL amber glass vials, precharged with sodium thiosulphate, which were used for analysis of volatile organic compounds (VOCs). Sulphuric acid (2 mL) was added to the 1000 mL amber glass bottle used to test for carburea.

All samples were transported to the University of Ontario Institute of Technology (UOIT, Oshawa, Ontario, Canada) on ice and stored at 4°C in the dark until they were brought on ice to the York-Durham Regional Environmental Laboratory for analysis. Samples were received at the testing lab within 1 day of sampling with the exception of the May 21, 2009 collection which was received 12 days after sampling.

4.1.2 – Sample Analysis

Based on results from the initial test date (December 11, 2008), in which inorganics (metals, cations/anions, general characteristics) and organics (VOCs, pesticides, chlorophenols, PCBs) were tested, only inorganics analysis was performed on the remaining samples. An exception was the May 21, 2009 collection in which a sample was also taken for VOCs analysis. Details of sample analysis performed at the York-Durham Regional Environmental Laboratory can be found in Appendix 8. Briefly, for the inorganic components, metals were detected using, inductively coupled plasma/mass spectrometry (ICP/MS), cations/anions using ion chromatography (IC) and pH, alkalinity and conductivity using a titralyzer. Organic components, organophosphorous and triazine pesticides were analyzed using gas chromatography/mass spectrometry (GC/MS), VOCs using purge and trap GC/MS (P&T GC/MS), chlorophenols, organochlorine pesticides, PCBs and phenoxy acid herbicides using GC/dual electron capture detection (GC/dual ECD) and carburea and glyphosate using liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS).

4.2 – Toxicity Testing

4.2.1 – Sample Collection

Glass and plastic bottles used for field sample collection for toxicity testing were soaked for a minimum of 24 hours in a 3% solution of Contrad70 (Decon Labs Inc., Pennsylvania, USA) and then washed on the intensive (glass) or plastics cycle in an industrial dishwasher (Miele Professional, Miele Ltd., Richmond Hill, Ontario, Canada) using LaboCleanF automatic dishwasher detergent (Dr. Weigert, Hamburg, Germany) before being air dried. Leachate and reference creek water samples used in toxicity testing were collected from Harmony Landfill sites 1 and 2 and the Harmony Creek upstream site during the period from December 2008 until April 2010 (Figure 3; Appendices 6 & 7). As mentioned previously, due to changes at the landfill site, all leachate collections after December 2008 were made at Site 2. Samples were always grab-sampled from running water using a clean glass beaker to fill collection bottles. Researchers wore nitrile laboratory gloves throughout the collection procedure. Samples were taken in either 1000 mL amber glass bottles (December 2008 – July 2009) or 1000 mL translucent high density polyethylene plastic bottles (August 2009 - April 2010).

Field samples were transported to the University of Ontario Institute of Technology on ice and stored at 4°C in the dark until they were used for toxicity testing in the Aquatic Toxicology Laboratory. Toxicity testing using *Hydra viridissima* occurred within 7 days of sample collection, with the exception of the May 21, 2009 leachate sample which was used up to 39 days after collection and the December 11, 2008 sample which was tested 16 months after collection on April 13, 2010.

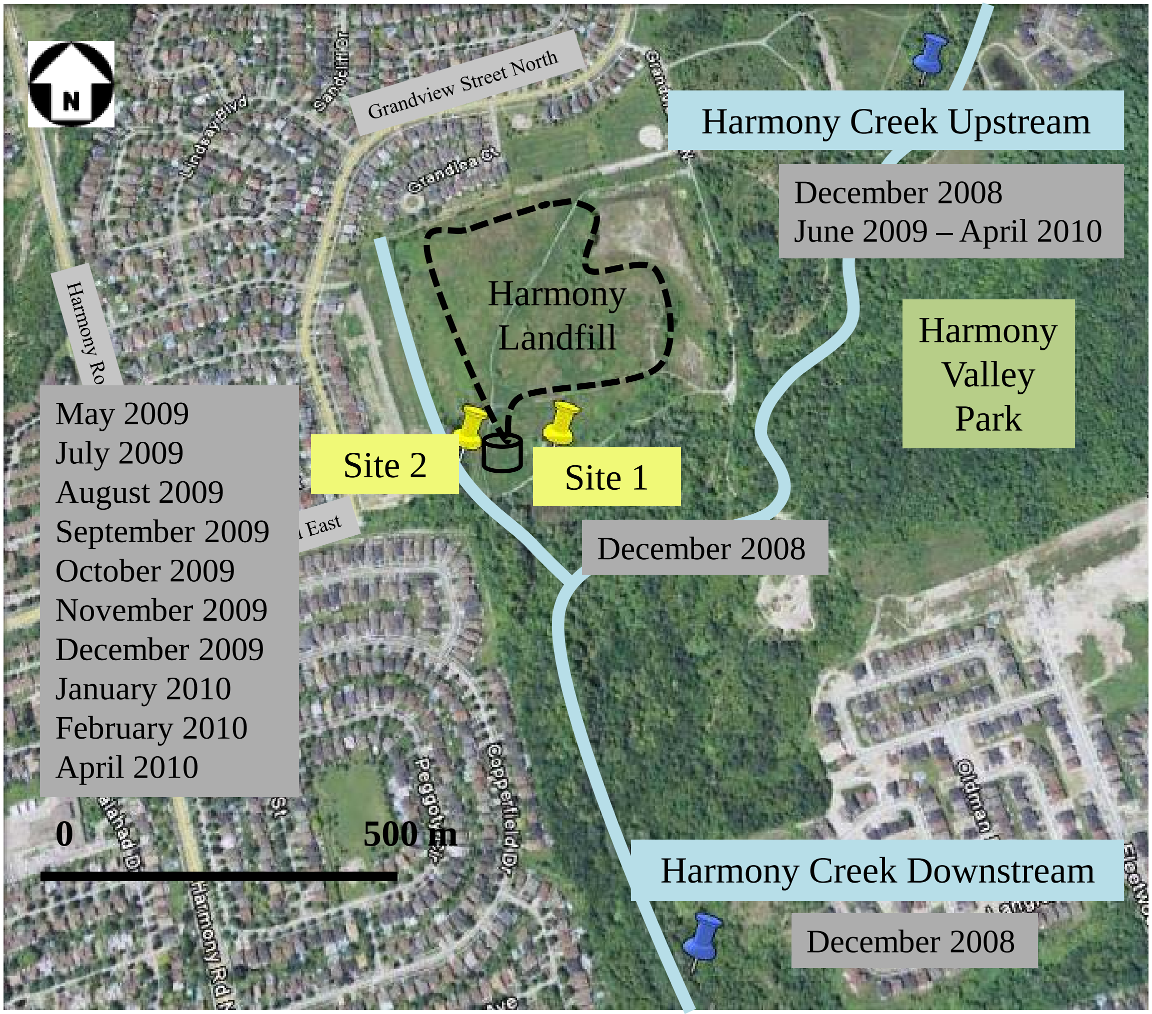


Figure 3: Map of Harmony Landfill study site with field-collection dates. The broken black line represents the approximate location of the waste boundary and the position of the leachate collection system. Field sampling sites in Harmony Creek are marked in blue. Leachate sampling sites at Harmony Landfill are marked in yellow.
Photo: Google Earth, June 2010.

4.2.2 – Hydra Culture

Hydra viridissima populations were initially obtained from Ward's Natural Science Incorporated (St. Catharines, Ontario, Canada). Prior to testing they were cultured in the UOIT Aquatic Toxicology laboratory, with direction from Lenhoff & Brown (1970). *Hydra viridissima* stock cultures were maintained in 10 L glass aquaria in a temperature control room at 25°C. The aquaria operated on a continous flow-through system with 25°C reverse-osmosis filtered laboratory water. The photoperiod was 8 hours light, 16 hours dark. During the experimental period, Hydra were fed daily with 0.5 – 2 mL newly hatched *Artemia franciscana* (Brine Shrimp) nauplii, depending on the density of the aquaria's population. Aquaria were cleaned using a turkey baster to remove excess food and wastes approximately 30 minutes to 2 hours post-feeding. Occasionally, water was completely removed from the tank and then refilled with fresh laboratory water. When aquaria became dirty, approximately every 3 months, the Hydra were gently removed and temporarily stored in plastic buckets. The glass aquaria were then completely cleaned manually with laboratory water and Liquinox™ soap (Alconox Inc., New York, USA) rinsed, refilled with laboratory water and then repopulated with healthy Hydra. Throughout the entire culture period, *Hydra viridissima* were observed to reproduce only asexually by budding.

4.2.3 – Brine Shrimp Culture

Brine Shrimp of the species *Artemia franciscana* were cultured as a food source for *Hydra viridissima*, used both for feeding stock cultures and experimental animals. *Artemia* were grown in aerated and heated (84°F, ~28°C) 15 L conical plastic Brine Shrimp hatchers (Aquatic Eco-Systems Inc., Florida, USA). The hatchers were set up every 24 hours by adding 2 teaspoons of dried *Artemia franciscana* egg cysts (Brine Shrimp Direct Inc., Utah, USA) to salt water created by dissolving 10 teaspoons of Instant Ocean® (Aquarium Systems Inc., Ohio, USA) into 2 L of 25°C laboratory water. After approximately 24 hours, aeration and heating of the culture was terminated and hatched *Artemia* were allowed to settle to the bottom of the cone. *Artemia* were harvested by filtering the culture water through a fine re-useable coffee filter and rinsing the hatched *Artemia* free of salt water with 25°C laboratory water. Prior to feeding, *Artemia* were stored temporarily in either plastic drinking cups or small glass beakers.

4.2.4 – Reference Toxicant

The reference toxicant used as a positive control in this experiment was tissue culture grade copper sulphate ($C_uSO_4{\cdot}5H_2O$) (Fisher Scientific, New Jersey, USA). Reference toxicants are used to check the sensitivity of stock cultures to toxicants over time (Environment Canada, 1990). Copper sulphate was used for this experiment because it is a reference toxicant recommended by Environment Canada and Hydra are sensitive to even small doses of copper (Environment Canada, 1990; Pollino & Holdway, 1999). A range-finding experiment (results not shown) demonstrated that a 24 hour pulse-exposure to 80 µg/L copper sulphate caused significantly reduced reproduction compared to controls, but not mortality in *Hydra viridissima*. Therefore a 80 µg/L concentration of copper sulphate was used as a positive control for Hydra toxicity tests performed in the period from January – April 2010, which included the December 2008 experiment. A 2064 µg/L stock solution of copper sulphate was prepared with Milli-Q water in a 500 mL clear glass laboratory bottle on October 7, 2009. The solution was stored at 4°C when not in use.

4.2.5 – Laboratory Glassware

All glassware used during experimentation was cleaned manually using laboratory water and Liquinox™ soap, rinsed with laboratory water and then washed on the intensive cycle in an industrial dishwasher, as described above, using LaboCleanF detergent before being air dried. Glass petri dishes used to expose and house *Hydra viridissima* during experiments were also soaked for a minimum of 24 hours in a 3% solution of Contrad70 between hand washing and dishwasher cleaning procedures.

4.2.6 – Test Exposure and Feeding Procedures

The Hydra reproductive toxicity test was performed with revisions to the protocol set out in Holdway (2005) (Figure 4). All experimental procedures were carried out in a temperature control room at 25°C with the exception of the May 2009 test which was run at 27°C. A 24 hour pulse-exposure period was chosen based on the results of preliminary experiments (results not shown). Test solutions were made up of logarithmic concentrations (0%, 3.2%, 10%, 32%, 100%) of field-collected leachate samples diluted with 25°C laboratory water. The negative control treatment (0%) was 25°C laboratory water. The positive control treatment was 80 µg/L copper sulphate. The Harmony Creek water used in toxicity testing was collected from the upstream sampling site. Just prior to testing, field samples and the copper sulphate stock solution were removed from storage at 4°C. A volume of 200 mL of each test solution (0%, 3.2%, 10%, 32%, 100% leachate, creek water, copper sulphate) was mixed with fresh 25°C laboratory water, where necessary, and heated in a 25°C water bath. Test solutions were not altered in any other way with the exception of the test involving the December 2008 leachate, which proved to be low in dissolved oxygen (~2 mg/L). Therefore, the laboratory water and the leachate were both aerated for approximately 30 minutes before test solutions were mixed and heated.

Working in triplicate from 0% - 100% leachate, creek water and then copper sulphate, each 5 cm diameter glass petri dish used for toxicity testing was rinsed with 5 mL of test solution before being re-filled with 15 mL of test solution. Then five Hydra, each with one tentacled bud, were randomly chosen and pipetted into each treatment dish. Once the 3 petri dishes for each treatment were set up, they were placed on a tray in their pre-determined randomly selected experimental locations. Feeding did not occur during the exposure period.

After 24 hours of exposure, working from 0% to 100% leachate, then creek water and copper sulphate when used, all of the test liquid was removed from each petri dish by decanting into a larger petri dish. Each test dish was immediately rinsed with 5 ml of 25°C laboratory water and then refilled with 15 mL of 25°C laboratory water. Any dislodged Hydra were returned to the test dish using a glass pipette. After the treatment solution had been refreshed with water, each Hydra dish was fed 1 mL of *Artemia franciscana* nauplii for a total of 30 minutes, during which time Hydra fed to satiation. Exceptions to this feeding regime were the May 2009 experiment in which Hydra were fed 0.5 mL of *Artemia* and the July 2009 experiment in which Hydra were fed 0.5 - 1 mL *Artemia.* After 30 minutes the feeding solution was removed, each dish rinsed with 5 mL of 25 °C laboratory water and then refilled with 15 mL of fresh laboratory water. Any remaining *Artemia* were removed and any missing Hydra were returned to the test petri dish. On subsequent experimental days the procedure of feeding and laboratory water renewal was repeated without the initial step of changing the 24 hour exposure test solution. Hydra were not fed on the final experimental day. All experiments lasted a minimum of 7 days and a maximum of 10 days.

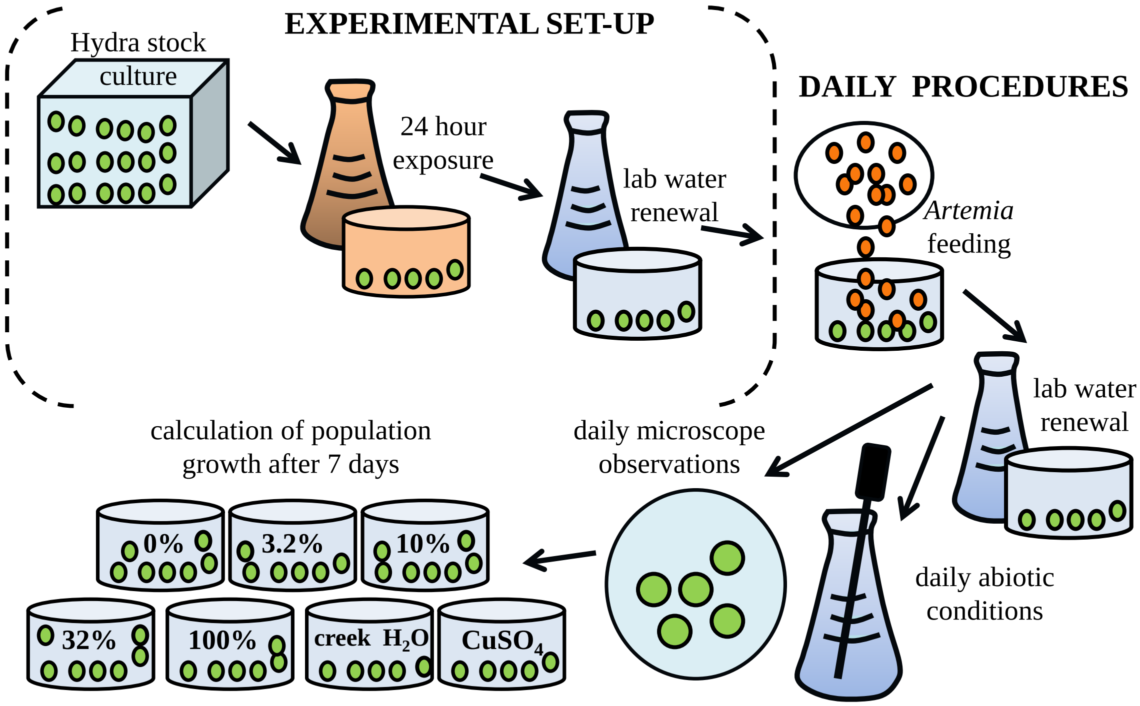


Figure 4: Diagram of toxicity testing method. The experimental set-up and daily procedures used during toxicity testing with Green Hydra (*Hydra viridissima*) are summarized visually.

4.2.7 – Abiotic Conditions

During experimental set-up, on Day 0, the temperature, pH, conductivity and dissolved oxygen of all test solutions was measured. After the 24 hour exposure period, on Day 1, the temperature, pH, conductivity and dissolved oxygen of the 25°C laboratory water used for renewals and the decanted test solutions were measured. Treatments were pooled for measurements on decanted test solutions. Multiple treatments were pooled to obtain the volume required for dissolved oxygen testing. Starting on Day 2, only treatment-pooled temperature and pH were measured on the decanted test solutions due to the presence of *Artemia* in the discarded solutions, which may have confounded readings of conductivity and dissolved oxygen. Measurements of renewal laboratory water continued on all parameters as described above. On the final day of experimentation (Day 7 - 10), the treatment-pooled temperature, pH, conductivity and multi-treatment pooled dissolved oxygen of decanted test solutions were measured.

Temperature readings were taken using a digital thermometer, pH and conductivity were measured using a portable combination pH and electrical conductance probe (Hanna® Instruments, Laval, Québec, Canada) and dissolved oxygen was measured using a titration-based testing kit (Dissolved Oxygen Test Kit, LaMotte, Maryland, USA). Although water hardness was not determined consistently throughout the study due to readings which were consistently below and beyond the detection limits of the instruments available, it was assayed in a limited amount of cases as mg $CaCO_3$/L using a titration-based testing kit (General Hardness and Carbonate Hardness Test Kit, Aquarium Pharmaceuticals™, Pennsylvania, USA).

4.2.8 – Hydra Morphology

Morphology of individual Hydra polyps, which is a measure of lethal and sub-lethal toxicity, was recorded for all experiments as based on the stages described in Johnson *et al.*, (1982). Progressive levels of toxicity were described as: normal (body stalk and tentacles extended), clubbed tentacles, shortened and clubbed tentacles, tulip and disintegrated (Figure 5). Approximately 2 hours post-exposure, on Day 0, and immediately after renewals on all subsequent days, Hydra morphology was observed using a stereomicroscope (Leica EZ4/EZ4D, Leica Microsystems, Wetzlar, Germany, magnification range 8-35x).

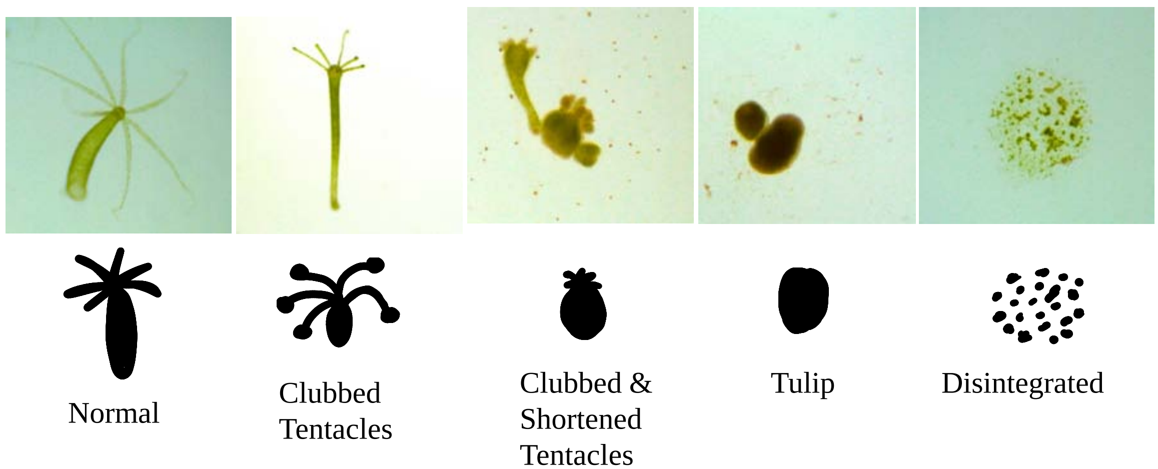


Figure 5: Progressive levels of toxicity based on Hydra morphology. Magnification of individual photographs (Leica EZ4/EZ4D) is approximately 10x.

4.2.9 – Hydra Population Growth

Population growth, which is a sensitive sub-lethal measure of toxicity, was also recorded for each experiment. Approximately 2 hours post-exposure, on Day 0, and immediately after renewals on all subsequent days, the number of Hydra in each petri dish was counted. For counting purposes, one Hydra was considered to be a single polyp including all of its attached buds. Recording of the number of Hydra in each treatment replicate allowed the mean population growth rate for all treatments to be determined upon the completion of each experiment. The population growth rate (K) was calculated as in Holdway (2005), using the formula:

$$K = \frac{\ln(ny) - \ln\ (nx)}{T}$$

where, *nx* represents the number of hydra at the beginning of the first day (t_x), *ny* represents the number of hydra after *y-x* days (t_y) ($n_0 = 5$), and *T* is the length of the test period in days (t_y-t_x).

4.3 – Data Analysis

4.3.1 – Statistics

When conditions for parametric analysis were met, main effects analysis of variance (ANOVA) and one-way ANOVA followed by the Tukey's Honest Significant Differences (HSD) post hoc test were used to determine significant differences between treatments. Normality was checked using the Shapiro-Wilk's test and homogeneity of variances was determined using the Levene's and Brown-Forsythe's tests. Nonparametric analysis was performed using the Kruskal-Wallis test (K-W). A p-value of 0.05 was used for all tests. Statistical analysis was done using the STATISTICA software program, version 8 (StatSoft Inc., Oklahoma, USA). Data spreadsheets were prepared using

Microsoft Excel 2007 (Microsoft Canada Co., Mississauga, Ontario, Canada) and graphing of results was completed using SigmaPlot version 11 (Systat Software Inc., Illinois, USA).

5.0 – RESULTS

5.1 – Chemical Analysis

5.1.1 – General Characteristics

A number of general characteristics were identified in upstream and downstream Harmony Creek water samples and Harmony Landfill leachate samples taken from Site 2 (Table 1). Similar readings were found for Harmony Creek samples collected both upstream and downstream of Harmony Landfill. Creek water parameters were generally within levels previously reported for Harmony Creek (CLOCA, 2008; Appendix 3).

Several parameters were found at higher levels in the leachate than in the creek water samples. These included: bromide, fluoride, nitrite, sulphate, calcium, magnesium, potassium, hardness, alkalinity and conductivity. Measures of pH in leachate samples were all lower than those found in upstream and downstream creek waters. When compared to the limited number of parameters available from the 1976 – 1983 period, the leachate samples collected in this study all had levels of chloride, sulphate, hardness, alkalinity and conductivity within but in the lower range of what was reported decades earlier (Sobanski, 1976-1982; Sobanski 1983-1984; Appendix 3). The pH levels recorded during the 1976 – 1983 period also encompassed the values seen in this study (Sobanski, 1976-1982; Sobanski 1983-1984). When Harmony Landfill leachate characteristics were compared to literature values, parameters were just below or within the low end of ranges discerned for other landfill leachates (Table 1). Ammonia was the only analyte found exceeding water quality guidelines (Table 1).

Table 1: General characteristics of creek water and leachate samples. Units are indicated for each parameter measured. Orange boxes indicate analytes exceeding water quality guidelines. Symbols: al = Canadian Water Quality Guidelines for the Protection of Aquatic Life (CCME, 2007), dw = Guidelines for Canadian Drinking Water Quality (Health Canada, 2008), SD = standard deviation, ND = not detectable, --- = data not available.

General Characteristics	Harmony Creek Upstream Dec 11/08	Harmony Creek Downstream Dec 11/08	Landfill Site 2 May 21/09	Landfill Site 2 July 30/09	Landfill Site 2 Oct 20/09	Landfill Site 2 Jan 22/10	Landfill Site 2 Mean (SD)	Landfill Literature (Refs: Appendix 9)	Guidelines
Bromide (mg/L)	ND	ND	ND	ND	0.54	0.10	0.32 (0.32)	0.13 – 10[14]	---
Chloride (mg/L)	142	138	30.2	65	94.9	55	61.2 (26.7)	8 – 6000[9,10]	250[dw]
Fluoride (mg/L)	ND	ND	0.09	0.05	0.04	0.07	0.06 (0.02)	0.05 – 12 [9,15]	1.5[dw]
Nitrate (mg/L)	4.7	3.8	0.21	ND	ND	ND	---	4.8 – 107 [9,14]	13[al]
Nitrite (mg/L)	ND	ND	0.03	ND	ND	ND	---	---	0.06[al]
Phosphate (mg/L)	ND	ND	ND	ND	ND	ND	---	0.03 – 17 [9,15]	---
Sulphate (mg/L)	36.4	35	24.5	35.7	56.1	53.3	42.4 (14.9)	1 – 7750 [7,13]	500[dw]
Calcium (mg/L)	128	126	143	164	178	152	159 (15.1)	6 – 7200 [7,10]	---
Magnesium (mg/L)	12.6	12.4	16.6	18.6	20.9	18.2	18.5 (1.7)	3 – 15,000 [7,10]	---
Potassium (mg/L)	2.73	2.72	3.14	1.69	1.74	1.25	1.95 (0.82)	1 – 3700 [7,10]	---
Sodium (mg/L)	82.4	81.2	29.3	45	62.1	36.1	43.1 (14.1)	3 – 7700 [7,9]	200[dw]
Ammonia (mg/L)	0.21	0.21	0.15	0.06	0.08	0.12	0.10 (0.04)	721[6]	0.02[al]
pH	8.15	8.2	7.58	7.25	7.29	7.22	7.33 (0.16)	4.5 – 9[10]	6.5 – 9[al]
Hardness (mg $CaCO_3$/L)	371	365	425	486	531	453	473 (45.5)	---	---
Alkalinity (mg $CaCO_3$/L)	281	283	421	435	458	427	435 (16.2)	120 – 705[9]	---
Conductivity (µS/cm)	1080	1060	880	1080	1210	998	1042 (138.8)	65 – 35,000[9,10]	---

5.1.2 – Metals

Metals were also detected in analysis of leachate and creek water samples (Table 2). Comparable metal concentrations were found at both upstream and downstream Harmony Creek sites, with the exception of nickel, which was 2.5 times higher and zinc which was 3 times higher at the downstream site. Metal values detected for Harmony Creek in this study were comparable to previously reported values (CLOCA 2008; Appendix 2).

Cadmium and chromium were the only metals assayed which were found at non-detectable levels, although 0.3 µg/L of chromium was detected in the December 2008 leachate sample. All other metals, excluding only aluminum, were found at higher concentrations in leachates than in creek waters. Iron and manganese were consistently found at higher levels than all other analytes with their maximum values being 61,600 µg/L and 1,020 µg/L respectively. When compared to available values from the 1976 – 1983 monitoring period, lead, zinc and most iron readings were within what had been previously reported (Sobanski, 1976-1982; Sobanski 1983-1984; Appendix 2). However, copper was below previous readings at levels of 0.7 – 3.3 µg/L versus 10 – 40 µg/L and the highest iron reading in this study of 61, 600 µg/L was greater than the highest reading of the earlier period which was reported at 27, 600 µg/L (Sobanski, 1976-1982; Sobanski 1983-1984). When compared to the July 2008 analysis, iron levels of 61, 000 µg/L were almost identical to this study's Site 1 value of 61, 600 µg/L (December 2008) (Ross, 2008; Appendix 2). Manganese concentrations of 880 µg/L in July 2008 were also very similar to the December 2008 Site 1 reading of 866 µg/L obtained in this study (Ross, 2008). However, generally the metals levels from the July 2008 analysis were much higher than those found for this study (Ross, 2008). When compared with literature values, metals in this study fell below or within the lower end of ranges reported for landfill leachates (Table 2). When considering only the December 2008 sample, all metals, excepting cadmium, chromium and copper, were found within ranges for landfill leachates. Iron and manganese exceed water quality guidelines for all leachate samples, while cobalt and selenium exceed guidelines in December 2008 and October 2009 and zinc only exceeded recommended levels in December 2008 (Table 2).

Table 2: Metals analysis of creek water and leachate samples. Orange boxes indicate analytes at levels exceeding water quality guidelines. Symbols: al = Canadian Water Quality Guidelines for the Protection of Aquatic Life (CCME, 2007), pwqo = Ontario Provincial Water Quality Objectives (MOE, 1994), dw = Guidelines for Canadian Drinking Water Quality (Health Canada, 2008), SD = standard deviation, ND = not detectable, --- = data not available.

Metals (µg/L)	Harmony Creek Upstream Dec 11/08	Harmony Creek Downstream Dec 11/08	Landfill Site 1 Dec 11/08	Landfill Site 2 May 21/09	Landfill Site 2 July 30/09	Landfill Site 2 Oct 20/09	Landfill Site 2 Jan 22/10	Landfill Site 2 Mean (SD)	Landfill Literature (Refs: Appendix 9)	Guidelines (µg/L)
Aluminum	86.3	93.7	20.1	21.8	2.6	21.6	12.3	14.5 (9.1)	8.5 – 3860[5,12]	100[al]
Antimony	0.5	0.5	3.8	1.6	0.3	0.4	0.8	0.7 (0.5)	0.2 - 6[15]	20[pwqo]
Arsenic	0.4	0.4	2.9	0.6	0.5	2.2	0.5	0.9 (0.8)	1 – 1000[7]	5[al]
Cadmium	ND	ND	ND	ND	ND	ND	ND	---	0.1 – 400[7]	0.017[al]
Chromium	ND	ND	0.3	ND	ND	ND	ND	---	1.4 – 2100[11,15]	1 - 8.9[al]
Cobalt	0.3	0.4	2.8	0.7	0.5	1.3	0.4	0.7 (0.4)	1.7 – 1500[7,15]	0.9[pwqo]
Copper	1.2	1.2	3.3	1.2	0.7	0.8	0.8	0.8 (0.2)	4 – 10,000[1,7]	4[al]
Iron	151	161	61,600	2,840	1,330	12,800	1,830	4,700 (5,436)	160 – 5500,000[7,15]	300[al]
Lead	0.2	0.2	3.3	0.5	0.1	0.2	0.2	0.2 (0.1)	2 – 5000[7,11]	7[al]
Manganese	57.4	45.4	866	179	314	1,020	107	405 (418)	30 – 1,400,000[7]	50[dw]
Molybdenum	0.5	0.5	0.6	0.5	0.4	1.0	0.5	0.6 (0.2)	---	73[al]
Nickel	0.4	1	30.6	4.8	0.8	1.9	0.8	2.1 (1.8)	9.8 – 13,000[7,15]	150[al]
Selenium	0.6	0.5	1.3	0.7	0.4	1.4	0.4	0.7 (0.4)	1 – 330[1]	1[al]
Zinc	1.7	5.3	124	5.2	0.6	1.7	1.8	2.3 (1.9)	10 – 1,000,000[2,7]	30[al]

5.1.3 – Xenobiotic Organic Compounds

Although xenobiotic organic compounds were found at detectable levels in the leachate sample collected from Site 1 on December 11, 2008, analysis failed to reveal xenobiotics in Harmony Creek water samples taken on the same date as well as a leachate sample taken at Harmony Landfill Site 2 on May 21, 2009 (Table 3).

When the December 2008 leachate sample was compared to records from the 1976 – 1983 monitoring period, levels of organics fell within the range for total aromatics (Sobanski, 1976-1982; Sobanski 1983-1984; Appendix 4). Levels of xenobiotic organic compounds in the December 2008 Harmony Landfill leachate were generally lower than or near minimum literature values (Table 3). Two exceptions were monochlorobenzene and m/p xylene. Monochlorobenzene was measured at 15 µg/L, which exceeds the suggested environmental guideline of 1.3 µg/L and m/p xylene was at a level of 6.3 µg/L which exceeds the guideline for m-xylene of 2 µg/L but is below the 30 µg/L recommended limit for p-xylene (Table 3).

Table 3: Xenobiotic organic compounds analysis of creek water and leachate samples. Orange boxes indicate analytes at levels exceeding water quality guidelines. Symbols: al = Canadian Water Quality Guidelines for the Protection of Aquatic Life (CCME, 2007), pwqo = Ontario Provincial Water Quality Objectives (MOE, 1994), SD = standard deviation, ND = not detectable.

Xenobiotic Organic Compounds (µg/L)	Harmony Creek Upstream Dec 11/08	Harmony Creek Downstream Dec 11/08	Landfill Site 1 Dec 11/08	Landfill Site 2 May 21/09	Landfill Literature (Refs: Appendix 9)	Guidelines (µg/L)
1,1 – dichloroethane	ND	ND	0.2	ND	0.6 – 46[7]	200[pwqo]
1,2 – dichloroethane	ND	ND	1.0	ND	212 – 20,000[4,7]	100[al]
Cis - 1,2 - dichloroethylene	ND	ND	0.2	ND	1.1 – 30,000[4,15]	200[pwqo]
monoclorobenzene	ND	ND	15	ND	0.1 – 20,000[4,7]	1.3[al]
1,2- dichlorobenzene	ND	ND	0.3	ND	0.1 – 4000[3,7]	0.7[al]
1,3-dichlorobenzene	ND	ND	0.2	ND	5.4 – 1000[4,7]	150[al]
1,4-dichlorobenzene	ND	ND	0.9	ND	0.1 – 4000[4,7]	26[al]
ethylbenzene	ND	ND	0.2	ND	1 – 20,000[4.6]	90[al]
m/p-xylene	ND	ND	6.3	ND	0.8 – 40,000[4,7]	2 (m), 30 (p) [pwqo]
o-xylene	ND	ND	0.6	ND	0.8 – 10,000[4,7]	40[pwqo]
toluene	ND	ND	0.6	ND	0.6 – 90,000[4,15]	2[al]

5.2 – Toxicity Testing

5.2.1 – Abiotic Conditions

Measurements of temperature, pH, conductivity and dissolved oxygen were recorded daily beginning on Day 0 and ending on the final day of each experiment (Day 7 - 10). A visual representation of Day 0 measurements obtained during the months of this study is provided in Figure 6. In general, the highest temperatures were recorded in May 2009 (when experiments were run at 27°C) and the lowest in January 2010. The 0%, 3.2%, 10% and positive control treatments had the highest pH values and lowest conductivites in contrast to the 32%, 100% and creek water treatments which had the lowest pH values and the highest conductivities. Dissolved oxygen readings were usually highest in creek water and lowest in the 100% leachate samples.

Temperatures taken on Day 0 (pre 24 hour pulse-exposure) had mean values from 24.9°C to 25.3°C (actual range: 23.8 - 27.1°C). Mean pH values were from 6.8 to 8.4 (actual range: 6.5 - 8.7). Mean conductivity readings varied considerably from 105 µS/cm to 975 µS/cm (actual range 72 - 1281 µS/cm). Mean dissolved oxygen ranged from 7.1 mg O_2/L to 8.5 mg O_2/L (actual range 4.8 - 10 mg O_2/L).

On Day 1, following the 24 hour pulse-exposure period, the mean pooled temperature readings for all treatments ranged from 24.9°C to 25.3°C (actual range: 24.1 – 26.9°C). Mean pH was 7.0 to 8.2 (actual range: 6.4 – 8.6). Mean conductivity values were 123 µS/cm to 638 µS/cm (actual range: 77 – 1142 µS/cm) and pooled dissolved oxygen means were 7.2 mg O_2/L to 7.4 mg O_2/L (actual range: 7.0 - 7.8 mg O_2/L. All Day 0 (pre-exposure) and Day 1 (post-exposure) data is summarized in Appendix 9.

From Day 1 onwards, all treatments were renewed daily with fresh 25°C laboratory water. Mean temperature values were 25.3°C to 27.1°C (actual range: 24.9 - 27.6°C). Mean pH values were 7.9 to 8.4 (actual range: 7.9 – 8.6). Mean conductivity was 67 µS/cm to 167 µS/cm (actual range: 34 - 196 µS/cm). Mean dissolved oxygen was 7.6 mg O_2/L to 8.2 mg O_2/L (actual range: 7.4 – 8.8 mg O_2/L).

From Day 2 onwards, the temperature and pH of water decanted from petri dishes post-feeding was taken daily. Measurements of conductivity and dissolved oxygen for decanted solutions were also made on the final experimental day (Day 7-10) after renewals. Mean temperatures in the decanted solutions ranged from 25.1°C to 27.0°C (actual range: 24.6 - 28.1°C). Mean pH was 7.9 to 8.3 (actual range: 7.7 - 8.6). Conductivity means were from 58 µS/cm to 226 µS/cm (actual range: 51 - 252 µS/cm). Mean dissolved oxygen values were 7.2 mg O_2/L to 7.8 mg O_2/L (actual range: 7.2 – 7.8 mg O_2/L). All Day 1+ (fresh renewal) and Day 2+ (24 hour old decanted) water data is summarized in Appendix 10.

Although water hardness was not measured consistently throughout the study, the approximate Day 0 range for all treatments was < 20 mg $CaCO_3$ /L to > 200 mg $CaCO_3$ /L. The 0% treatment was measured as <20 mg $CaCO_3$ /L, 3.2% as ~50 mg $CaCO_3$ /L, 10% as ~100 mg $CaCO_3$ /L, 32% as ~200 mg $CaCO_3$ /L and both 100% leachate and creek water as >200 mg $CaCO_3$ /L. External laboratory analyses confirmed that the hardness of 100% Harmony Landfill leachate and Harmony Creek samples was above 200 mg $CaCO_3$ /L with readings of 425 – 531 mg $CaCO_3$ /L for leachate and 365/371 mg $CaCO_3$ /L for creek water samples (Table 1).

Table 4: Day 0 (PRE) and Day 1 (POST) 24-hour exposure abiotic test conditions. Measurements for all 11 months reported (December 2008 – April 2010) are included. Mean values appear in bold font followed by the standard deviation (SD).

Mean (SD) (min-max)	**Temperature (C)**		**pH**		**Conductivity (µS/cm)**		**Dissolved Oxygen (mg O_2 /L)**	
	PRE (Day 0)	POST (Day 1)	PRE (Day 0)	POST (Day 1)	PRE (Day 0)	POST (Day 1)	PRE (Day 0)	POST (Day 1)
0%	**25.3** (0.94) (23.0 – 27.1)	**25.1** (0.56) (24.7 – 26.8)	**8.2** (0.14) (8.0-8.5)	**8.2** (0.12) (8.0-8.4)	**121** (29.1) (72-157)	**126** (28.1) (77-177)	**7.8** (0.25) (7.6-8.4)	**7.4** (0.26) (7.0-7.8)
3.2%	**25.3** (0.59) (23.8-25.9)	**25.2** (0.58) (24.7-26.9)	**8.1** (0.13) (8.0-8.4)	**8.2** (0.16) (7.9-8.5)	**147** (22.5) (114-180)	**143** (21.1) (107-178)	**7.8** (0.20) (7.6-8.2)	**7.4** (0.27) (7.0-7.8)
10%	**25.2** (0.61) (23.8-26.0)	**25.3** (0.51) (25.0-26.8)	**8.0** (0.19) (7.7-8.4)	**7.9** (0.15) (7.7-8.2)	**211** (28.2) (165-256)	**193** (19.5) (161-215)	**7.7** (0.16) (7.6-8.0)	**7.4** (0.16) (7.2-7.6)
32%	**25.2** (0.64) (23.8-26.0)	**25.3** (0.57) (24.8-26.9)	**7.2** (0.27) (6.9-7.7)	**7.7** (0.28) (7.3-8.1)	**408** (62.5) (312-497)	**343** (34.6) (290-389)	**7.7** (0.43) (6.6-8.2)	**7.4** (0.15) (7.2-7.6)
100%	**25.1** (0.51) (24.0-26.0)	**25.2** (0.65) (24.1-26.7)	**6.8** (0.23) (6.6-7.3)	**7.0** (0.34) (6.5-7.6)	**975** (188) (673-1266)	**553** (103) (419-684)	**7.1** (0.95) (4.8-8.2)	**7.4** (0.16) (7.2-7.6)
Creek Water	**25.1** (0.54) (24.4-26.3)	**25.3** (0.60) (24.7-26.9)	**6.9** (0.40) (6.5-7.6)	**7.3** (0.37) (6.4-7.7)	**900** (165) (742-1281)	**638** (197) (502-1142)	**8.5** (0.89) (7.4-10)	**7.4** (0.26) (7.0-7.6)
Copper Sulphate	**24.9** (0.83) (23.8-25.6)	**24.9** (0.30) (24.8-25.4)	**8.4** (0.25) (8.1-8.7)	**8.1** (0.31) (7.9-8.6)	**105** (32.9) (74-146)	**123** (26.1) (105-161)	**7.6** (0.32) (7.2-8.0)	**7.2** (0.30) (7.0-7.6)

Table 5: Daily Day 1+ (PRE) and Day 2+ (POST) abiotic test conditions. Measurements for all 11 months reported (December 2008 – April 2010) are included. Mean values appear in bold font followed by the standard deviation (SD). --- = data not available

Mean (SD) (min-max)	**Temperature (C)**		**pH**		**Conductivity (µS/cm)**		**Dissolved Oxygen (mg O_2 /L)**	
	PRE (Day 1+)	POST (Day 2+)	PRE (Day 1+)	POST (Day 2+)	PRE (Day 1+)	POST (Day 2+)	PRE (Day 1+)	POST (Day 2+)
December 2008	**25.7** (0.52) (24.9-26.4)	**25.2** (0.27) (24.6 – 25.8)	**8.3** (0.04) (8.3-8.4)	**8.2** (0.08) (7.9-8.4)	**146** (13.0) (127-169)	**137** (7.22) (126-147)	**7.8** (0.14) (7.6-8.0)	**7.4** (0.09) (7.4-7.6)
May 2009	**27.1** (0.38) (26.6-27.6)	**27.0** (0.41) (26.1-28.1)	**7.9** (0.07) (7.9-8.1)	**7.9** (0.08) (7.8-8.1)	**106** (27.0) (78-154)	**90** (3.97) (84-96)	**8.2** (0.48) (7.4-8.8)	---
July 2009	**25.7** (0.46) (24.5-26.3)	**25.2** (0.22) (24.8-25.9)	**8.3** (0.10) (8.2-8.5)	**8.0** (0.11) (7.7-8.3)	**67** (15.8) (34-91)	**58** (4.20) (51-64)	**8.1** (0.22) (7.8-8.4)	**7.2** (---)
August 2009	**25.6** (0.19) (25.4-26.0)	**25.4** (0.33) (24.7-26.1)	**8.2** (0.04) (8.1-8.3)	**8.0** (0.07) (7.8-8.1)	**134** (22.7) (105-160)	**113** (15.5) (101-146)	**7.6** (0.13) (7.4-7.8)	**7.6** (---)
September 2009	**25.8** (0.38) (25.5-26.5)	**25.6** (0.23) (25.2-26.1)	**8.2** (0.06) (8.2-8.4)	**8.1** (0.08) (7.9-8.3)	**112** (31.4) (61-162)	**68** (3.22) (64-74)	**7.8** (0.11) (7.6-8.0)	**7.8** (---)
October 2009	**25.9** (0.42) (25.4-26.6)	**25.5** (0.20) (25.1-25.9)	**8.2** (0.09) (8.1-8.4)	**8.0** (0.17) (7.5-8.2)	**135** (27.8) (95-180)	**183** (10.9) (173-203)	**8.1** (0.16) (7.8-8.4)	**7.6** (---)
November 2009	**25.7** (0.43) (25.0-26.4)	**25.4** (0.17) (25.0-25.7)	**8.2** (0.05) (8.2-8.3)	**8.1** (0.05) (8.1-8.3)	**139** (11.0) (125-165)	**147** (7.84) (137-160)	**7.9** (0.16) (7.6-8.2)	**7.4** (---)
December 2009	**25.4** (0.14) (25.1-25.6)	**25.2** (0.22) (24.7-25.5)	**8.2** (0.04) (8.2-8.3)	**8.1** (0.08) (8.0-8.3)	**142** (12.9) (127-168)	**132** (9.50) (115-145)	**7.9** (0.13) (7.8-8.2)	---
January 2010	**25.4** (0.23) (25.2-26.0)	**25.1** (0.24) (24.7-25.6)	**8.3** (0.05) (8.2-8.4)	**8.1** (0.05) (8.1-8.3)	**137** (7.80) (123-150)	**151**(5.30) (141-156)	**8.2** (0.13) (8.0-8.4)	**7.4** (0.0)
February 2010	**25.3** (0.52) (24.9-26.3)	**25.2** (0.36) (24.6-26.2)	**8.3** (0.05) (8.2-8.4)	**8.1** (0.12) (7.9-8.4)	**167** (15.4) (146-196)	**226** (14.5) (206-252)	**7.9** (0.17) (7.6-8.2)	**7.4** (0.30) (7.2-7.8)
April 2010	**25.5** (0.32) (25.0-25.9)	**25.3** (0.21) (24.7-25.8)	**8.4** (0.07) (8.4-8.6)	**8.3** (0.11) (8.1-8.6)	**128** (9.70) (112-138)	**101** (8.94) (87-118)	**7.7** (0.19) (7.4-8.0)	**7.3** (0.20) (7.2-7.6)

5.2.2 – Hydra Morphology

With the exception of the positive control treatment of copper sulphate, the December 2008 leachate from Site 1 at Harmony Landfill was the only sample which induced morphological signs of toxicity in *Hydra viridissima*. The December 2008 leachate sample, which was tested on *Hydra viridissima* in April 2010, caused morphological deviation from the norm at both the 32% and 100% leachate concentrations (Figure 6). At the first observation period, approximately 2 hours post-exposure the Hydra in the 32% and 100% treatments appeared normal. After the 24 hour exposure on Day 1, Hydra in the 32% treatment had shortened and clubbed tentacles and those in the 100% treatment had a variety of morphologies increasing in severity from shortened and clubbed tentacles to tulip stage and disintegrated (dead). In total two Hydra died in the 100% leachate treatment. By Day 2, Hydra in the 32% leachate treatment had returned to normal although those remaining in the 100% treatment were either at the tulip or shortened and clubbed tentacle stage. By Day 3, the Hydra in the 100% treatment were all at the shortened and clubbed tentacle stage and by Day 5 they had returned to their normal form. Feeding by 32% and 100% leachate-treated Hydra began on Day 1 (shortened and clubbed tentacle Hydra only) and budding was observed starting on Day 2 (32%) and Day 4 (100%).

Pulse-exposure of Hydra to 80 µg/L of copper sulphate for 24 hours initially caused shortened and clubbed tentacles as observed approximately 2 hours post-exposure, and then produced all tulip stage organisms by the end of the 24 hour exposure period (Figure 6). After laboratory water renewals on Day 1, the Hydra continued to exhibit tulip stage morphology and did not feed. However, by Day 2 (48 hours post-exposure) the Hydra had recovered to the shortened and clubbed tentacle stage and were observed feeding. By Day 5 (120 hours post-exposure) the copper exposed Hydra had recovered a normal appearance and were both feeding and budding. This occurred for positive control treatments run simultaneous to experiments conducted in January, February and April 2010 (including December 2008).

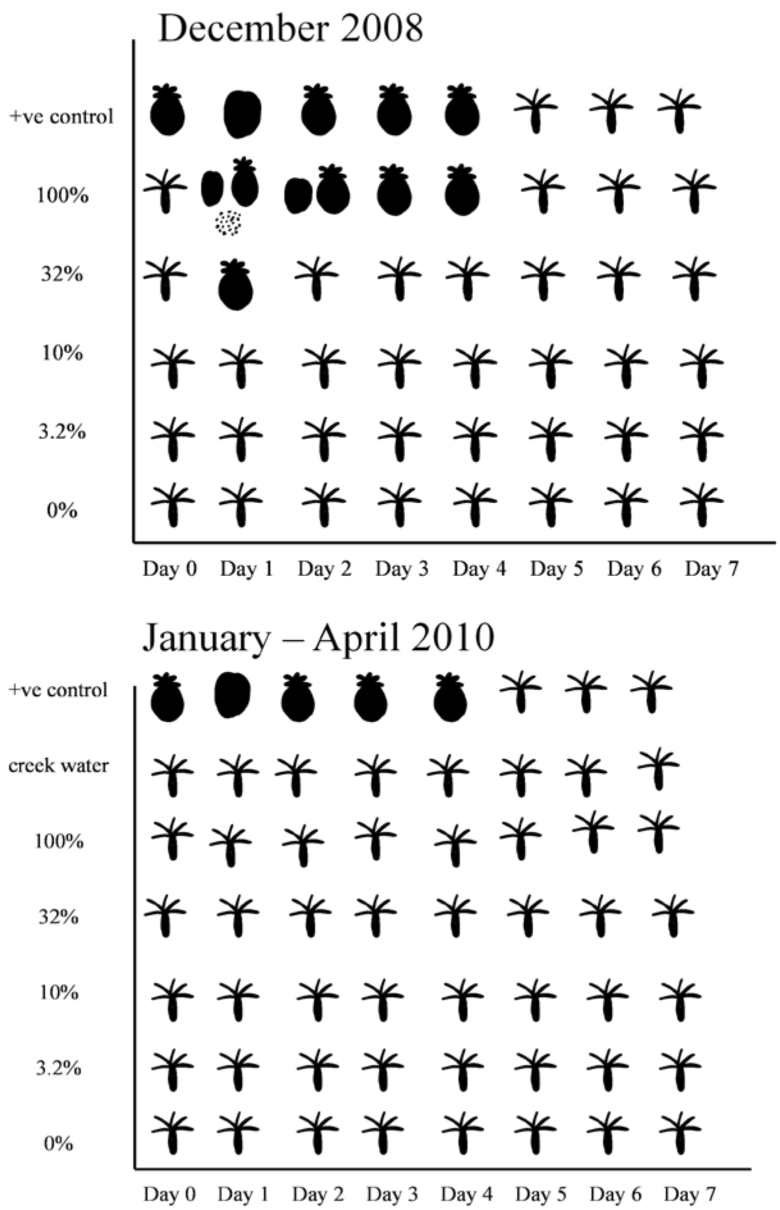


Figure 6: Leachate and positive control toxicity based on Hydra morphology.

The symbol represents a normal morphology, shortened and clubbed tentacles, tulip stage and complete disintegration, which indicates mortality.

5.2.3 – Hydra Population Growth

When the population growth rate (K) was calculated for each experiment on Day 7, there were no differences between replicates (ANOVA, Tukey's HSD, K-W, p=0.05). Therefore, mean K values were calculated and compared for each treatment. The majority of experiments showed no statistically significant differences between treatments (ANOVA, Tukey's HSD, p=0.05). These included samples taken and experiments performed for the months of May, August, September, October and December of 2009. Significant differences were seen between the negative control (laboratory water) and the positive control (copper sulphate) in the months of January and February 2010 and for the December 2008 test. In those cases the population growth for the positive control treatment was significantly decreased from the lab water controls (ANOVA, Tukey's HSD, p=0.05). Although population growth was also decreased for the April 2010 positive control, the difference was not statistically significant.

For the December 2008 leachate sample from Site 1, a statistically significant reduction in population growth was observed for the 100% leachate treatment as compared to the 0%, 3.2% and 10% leachate treatments (ANOVA, Tukey's HSD, p=0.05). For the July 2009 leachate sample from Site 2, a statistically significant reduction in population growth was observed for the 100% leachate treatment as compared to all other treatments, which included 0%, 3.2%, 10% and 32% leachate and upstream creek water (ANOVA, Tukey's HSD, p=0.05). For November 2009, which had a leachate sample also collected from Site 2, the 32% leachate treatment was statistically significantly decreased from 3.2% and 100% leachate and creek water treatments but not significantly different than the 0% or 10% leachate treatments (ANOVA, Tukey's HSD, p=0.05).

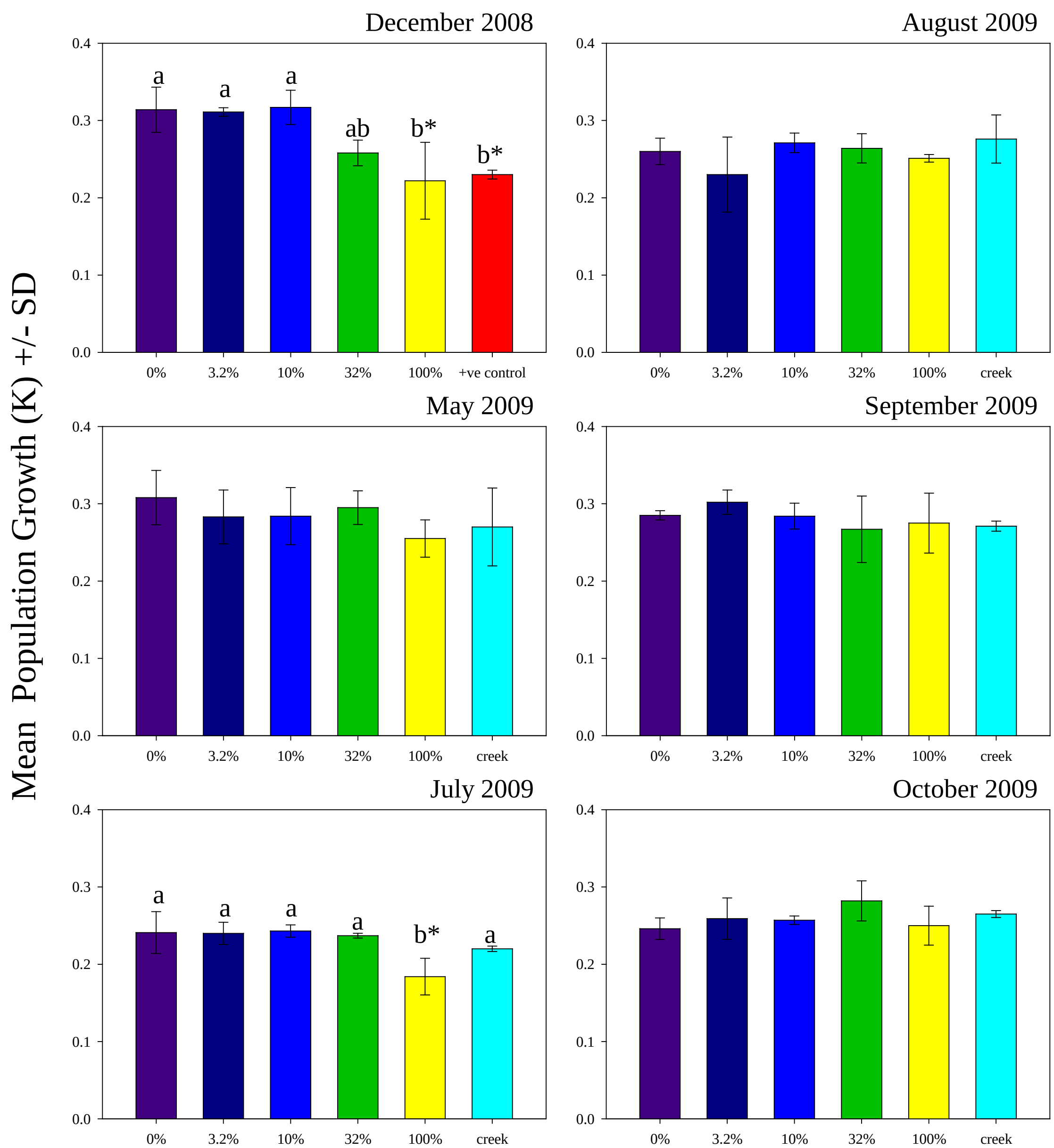


Figure 7: Mean population growth as calculated on Day 7. Letters are present when statistical significance was found and indicate treatment groupings (ANOVA, Tukey's HSD, p=0.05). Bars also marked with "*" indicate statistically significant difference from experimental controls (0% leachate). Error bars represent standard deviation.

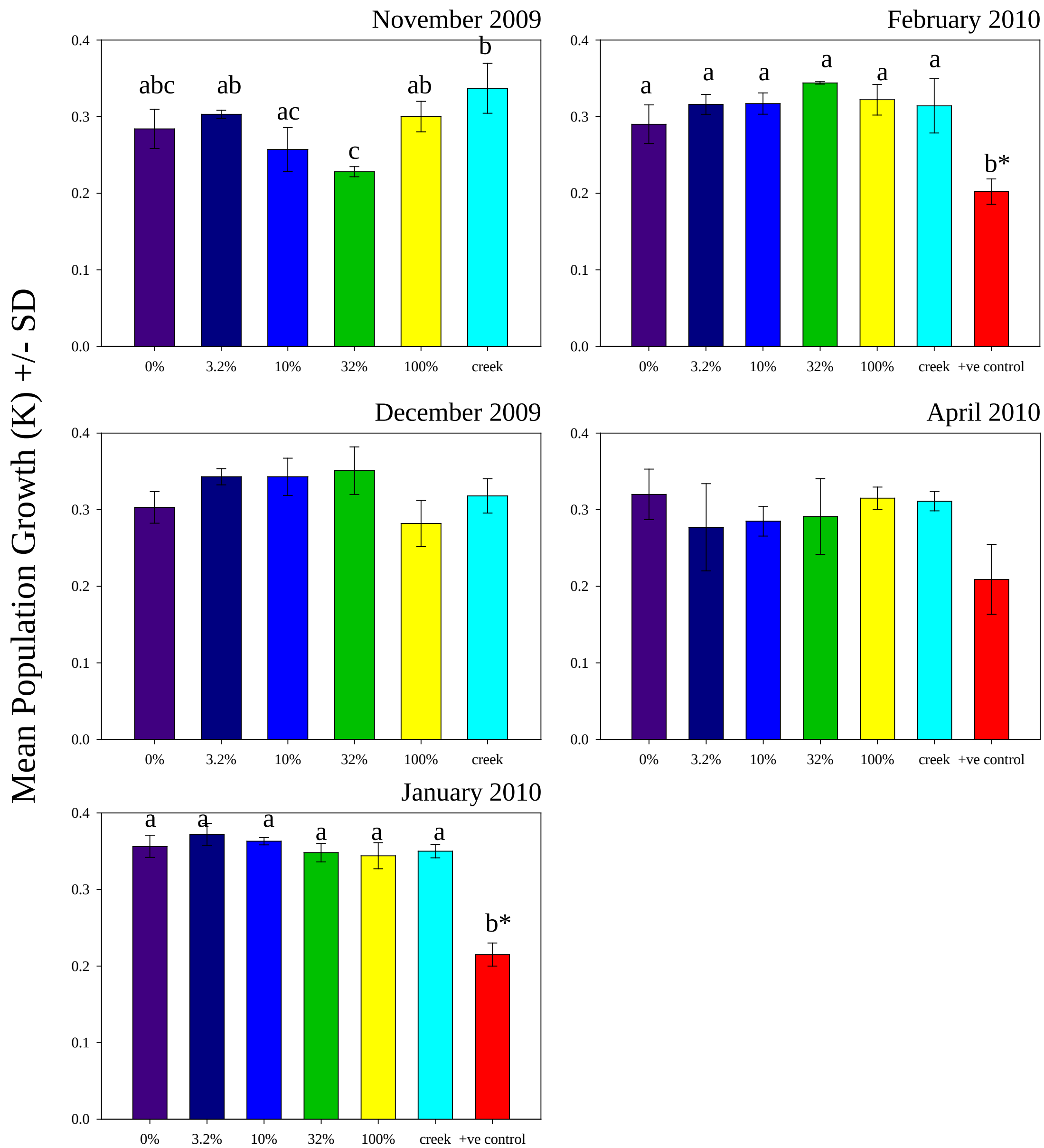


Figure 8: Mean population growth as calculated on Day 7. Letters are present when statistical significance was found and indicate treatment groupings (ANOVA, Tukey's HSD, p=0.05). Bars also marked with "*" indicate statistically significant difference from experimental controls (0% leachate). Error bars represent standard deviation.

6.0 – DISCUSSION

6.1 – Toxicity Testing

6.1.1 – Hydra Morphology

Although Hydra morphology remained normal throughout the majority of the toxicity tests, results of the December 2008 experiment show that Harmony Landfill leachate has the potential to cause lethal and sub-lethal morphological toxicity to *Hydra viridissima*. Since morphological effects of leachate exposure were observed in a dose-dependent fashion (32% less affected than 100%) for the December 2008 leachate and Hydra were affected morphologically by the positive control treatment, it can be concluded that the laboratory population of *Hydra viridissima* used in the present study was susceptible to toxicants.

A 24 hour pulse-exposure period provides adequate time to assess morphologically-indicated toxic effects. In this study, morphological toxicity was observed in the positive control treatment 2 hours post-exposure and in the December 2008 leachate treatments during observations at 24 hours post-exposure. Findings of other experiments indicate that, given adequate concentrations, tentacle clubbing in *Hydra viridissima* may be seen during the first hour of toxicant exposure (Pollino & Holdway, 1999). When they exposed *Hydra oligactis* and *Hydra viridissima* to copper and lead for 96 hours, Pyatt and Dodd (1984) found that the major morphological impact of those metals occurred within the first 24 hours (Pyatt & Dodd, 1984). Morphological signs of toxicity at 24 hours exposure to copper, cadmium and zinc were also noted for *Hydra vulgaris* (Karntanut & Pascoe, 2000). Exposure of *Hydra attenuata* to a xenobiotic organic chemical, 4-nonyphenol, as well as toxic industrial effluents also resulted in morphological toxicity during the first 24 hours (Pachura *et al*., 2005; Blaise & Kusui, 1997). Therefore, if the Harmony Landfill leachates tested during this study had contained concentrations of toxicants capable of affecting Hydra, changes in their morphology would most likely have been observed after the 24 hour pulse-exposure.

In this study *Hydra viridissima* were observed to recover fully, by Day 5 (120 hours post-exposure) from the tulip stage when placed in fresh laboratory water. The tulip stage is widely accepted in literature as a mortal endpoint (Trottier *et al*., 1997). This may be because most tests expose Hydra to toxicants for at least 96 hours (Blaise & Kusui, 1997). Exposure of *Hydra viridissima* to 80 µg/L of copper sulphate in this laboratory led to complete Hydra disintegration after 48 hours (Appendix 10). Given the capacity of Hydra for detoxification (Quinn *et al*., 2004) and regeneration (Johnson *et al*., 1982), it does not seem surprising that they may recover from a 24 hour pulse-exposure.

Since *Hydra viridissima* were able to recover from 24 hour toxicant exposures, this may indicate that other aquatic invertebrates could also withstand single short exposures to Harmony Landfill leachates. However, multiple exposures or pulses longer than 24 hours may have the potential to cause significant morphological impairment or mortality. In Hydra, the more subtle morphological changes accompanying toxicity, including clubbed and shortened tentacles, can have effects on survival and growth. In their 1998 work with *Hydra vulgaris*, Beach and Pascoe noted reductions in feeding behaviour after 48 h pulse-exposures to toxic concentrations of copper, cadmium and zinc. One consequence of reduced feeding may be decreased population growth.

6.1.2 – Hydra Population Growth

Calculations of population growth 7 days post-exposure also indicate that Harmony Landfill leachate has the potential to cause sub-lethal toxicity to *Hydra viridissima*. Exposure of Hydra to the positive control and 100% leachate samples from both December 2008 and July 2009, resulted in statistically significant decreases in population growth compared to lab water controls. Although the leachate from December 2008 also caused signs of morphological toxicity, as discussed previously, the July 2009 exposure did not. All other leachate samples failed to show significant reproductive toxicity, as based on population growth at Day 7.

Results from this study suggest that a 24 hour exposure can have significant effects on population growth as observed 7 days post-exposure. However, in the literature, changes in Day 7 population growth have generally been observed after longer-term exposures (Pollino & Holdway, 1999; Mitchell & Holdway, 2000; Rosenkrantz *et al*., 2008). When *Hydra viridissima* were exposed to three 90 minute pulses of cadmium, no significant effects on population growth were seen after 7 days in clean water (Holdway *et al*., 2001). However, a 7 h pulse-exposure of *Hydra hexactinella* to 100% stormwater led to a statistically significant increase in population growth as measured after 7 days in fresh Hydra medium (Rosenkrantz *et al*., 2008). In that same study 10% stormwater led to significantly reduced Day 6 population growth after a 6-day continuous exposure (Rosenkrantz *et al*., 2008). Reduced Day 7 population growth was also observed for *Hydra viridissima* exposed continuously to copper for 7 days (Pollino & Holdway, 1999) as well as *Hydra viridissima* exposed for 7 days to chemical dispersants and the water accommodated fraction of crude oil (Mitchell & Holdway, 2000). Although preliminary tests (results not shown) and a 7-day continuous exposure treatment with November 2009 leachate (Appendix 11) indicated that longer exposures of *Hydra viridissima* to 100% Harmony Landfill leachate would not have resulted in decreased population growth compared to pulse-exposed 100% leachate treatments, it is possible that more leachate samples would have shown significantly decreased population growth compared to controls given an extended exposure period.

The majority toxicants in Harmony Landfill leachate were detected at low concentrations. Therefore, this work may show that Hydra and other invertebrate populations would be unaffected by single leachate exposures, as investigated in this study. However, multiple exposures, which are likely to occur with landfill leachates, might have the potential to cause decreased population growth. Multiple low-level exposures have been demonstrated experimentally to have the potential to be more toxic than single exposures depending on their frequency, duration and the types and concentrations of toxicants involved (Diamond *et al*., 2005).

6.2 – Chemical Analysis

6.2.1 – General Characteristics

The presence of major leachate anions (chloride, nitrate, sulphate) and cations (calcium, magnesium, potassium, sodium) as well as increased measurements of alkalinity and conductivity and decreased pH in environmental samples collected at Harmony Landfill suggests that leaching from the waste mound is taking place (Dewhurst *et al.*, 2003). However, the low levels at which the major ions were found compared to published data for other landfill leachates shows that this leaching may be fairly insignificant (Christensen *et al*., 2001; Kjeldsen *et al*., 2002; Dewhurst *et al*., 2003; Baun

& Christensen, 2004; Øygard *et al*., 2007; Ettler *et al*., 2008; Öman & Junestedt, 2008). In particular chloride, which is usually elevated in landfill leachates (Christensen *et al.*, 2001), was detected at concentrations lower than those found in the adjacent Harmony Creek. Since levels of chloride, sulphate, hardness, alkalinity and conductivity were all higher in leachates sampled during the 1976-1983 period, when the landfill was still active or only recently closed, it is possible that, because it has been 30 years since the landfill's closure, major leaching has already occurred and the remaining ions have chiefly been immobilized (Kjeldsen *et al*., 2002).

Many of the components identified in Harmony Creek waters and Harmony Landfill leachates, such as chloride, calcium, magnesium, potassium and sodium, are required by Hydra for optimal growth (Muscatine & Lenhoff, 1965; Lenhoff & Brown, 1970). This may explain the observed, although not significant, increases in growth which occurred in some of the leachate and creek water treatments compared to the lab water controls.

Ammonia was the only general analyte found which exceeded water quality guidelines of 0.02 µg/L in both creek water and leachate samples (CCME, 2007). Creek water samples had the highest levels of ammonia with both upstream and downstream collections having readings of 0.21 µg/L while leachates ranged from 0.06 µg/L to 0.15 µg/L. Ammonia is a common component of landfill leachates (Kjeldsen *et al*., 2002). Research has shown that concentrations of ammonia may remain high in landfills even 30 years post closure (Kjeldsen *et al*., 2002). Leachate-derived ammonia has already been implicated in toxicity to aquatic invertebrates and fish (Dave & Nilsson, 2005). In an investigation of wastewater toxicity, ammonia levels were correlated with lethal and sub-lethal morphological toxicity to *Hydra attenuata* (Pardos *et al*., 1999). However, because Harmony Creek waters, which contained the highest concentrations of ammonia, did not elicit significant toxicity to *Hydra viridissima*, it is unlikely that ammonia was a major contributor to the Harmony landfill leachate toxicity seen in this study.

6.2.2 – Metals

Although most metals in landfills have limited mobility (Kjeldsen *et al*., 2002), some, such as copper, are capable of causing low concentration toxic effects in Hydra (Pollino & Holdway, 1999). In general, Harmony Landfill leachate samples contained higher levels of metals than those found in Harmony Creek. The elevated concentrations of metals in the December 2008 leachate sample, when compared to all other time points, may aid in explaining why it was the only leachate sample to have caused both morphological and reproductive toxicity in *Hydra viridissima*.

Cobalt, iron, manganese, selenium and zinc were detected in leachates above levels recommended by environmental guidelines (CCME, 2007; MOE 1994; Health Canada, 2008). Cobalt was found at a maximum level of 2.8 µg/L. This was above the guideline of 0.9 µg/L (MOE, 1994) but less than the 10 µg/L shown to impair reproduction in *Daphnia magna* (Biesinger & Christensen, 1972).

Iron was consistently found at high levels, exceeding the recommended 300 µg/L (CCME, 2007) by a range of 4 (1,330 µg/L) to 200 (61, 600 µg/L) times. In research which used ferrihydrate as an adsorbent, *Hydra vulgaris* was shown to be tolerant to that iron oxide (Taylor *et al*., 2009). However, reproductive effects on *Daphnia magna* have been observed at iron concentrations as low as 128 µg/L (Dave, 1984). Published 48 h LC_{50} values for *Daphnia magna* are in the range of 7,200 – 96,000 µg/L (Biesinger &

Christensen, 1972; Khangarot & Ray, 1989). When exposed to doses of iron sulphate (540 – 670 µg/L total iron) in flow-through river water, *Daphnia magna* showed increased mortality, decreased reproductive success and significantly higher iron accumulation compared to organisms at the reference site (70 µg/L total iron) (van Anholt, *et al*., 2002). Although detrimental effects to *Daphnia magna* have been observed within the levels of total iron recorded for Harmony Landfill leachates, it is difficult to extrapolate these findings to Hydra since they differ so greatly physically. In *Daphnia magna* accumulation of iron in the gut, which then interferes with digestion and nutrient uptake, is believed to contribute to its sub-lethal toxicity (van Anholt *et al*., 2002). A physical mechanism of iron toxicity has also been well-described in fish, at levels in excess of 350 µg/L total iron, where ferric hydroxides accumulating on gill surfaces cause sub-lethal and lethal effects (Lappivaara *et al*., 1999; van Anholt *et al*., 2002; Teien *et al*., 2008). Samples of Harmony Landfill leachate appeared to contain precipitated iron. If physical effects also contribute to iron toxicity in Hydra, this may explain some of the toxic effects seen with the December 2008 leachate which contained 61, 600 µg/L of total iron.

Manganese was well above the 50 µg/L drinking water guideline (Health Canada, 2008) but generally well below, except in December 2008 (866 µg/L) and October 2009 (1,020 µg/L) the 48 h LC_{50} of 972 µg/L for *Daphnia magna* (Mejía – Saavedra *et al*., 2005). However, the 48 h LC_{50} for the rotifer *Lecane quadridentata* was found to be 2,210 µg/L (Mejía – Saavedra *et al*., 2005) and another study found that reproductive effects in *Daphnia magna* were not seen until manganese levels of 4,100 µg/L (Biesinger & Christensen, 1972). *Hydra viridissima* has been demonstrated to exhibit normal population growth at manganese levels of 100 µg/L (Hyne *et al.*, 1992b), which corresponds to the levels seen in the May 2009 (179 µg/L) and January 2010 (107 µg/L) leachates but is below that of the July 2009 reading of 314 µg/L. Therefore, although toxic effects were not observed in October 2009 it is possible that manganese contributed to the effects seen with the December 2008 and July 2009 leachates.

Selenium was above the recommendation of 1 µg/L at two readings, one of 1.3 µg/L and the other of 1.4 µg/L. However, it was below the 348 µg/L shown to reduce the reproductive rate of *Daphnia magna* (Ingersoll *et al.*, 1990).

Zinc had one reading of 124 µg/L, in December 2008, which was both above the 30 µg/L guideline (CCME, 2007) and the 75 µg/L level shown to inhibit population growth in *Hydra viridissima* (Holdway *et al.*, 2001). Therefore, it is possible that it contributed to the toxicity of the December 2008 leachate.

Of the remaining detected metals tested on Hydra, aluminum (Kovačević *et al.*, 2007), arsenic (Taylor *et al.*, 2009), chromium (Arkhipchuk *et al.*, 2006), copper (Pollino & Holdway, 1999), lead (Browne & Davis, 1977), and nickel (Santiago-Fandiño, 1983) were all present at concentrations lower than those which have demonstrated sub-lethal toxicity to Hydra species. In addition, antimony was well below the 48 h EC_{50}(I) of 423,000 µg/L for *Daphnia magna* (Khangarot & Ray, 1989) and molybdenum was also much lower than the 75, 000 µg/L shown to inhibit *Daphnia magna* reproduction (Diamantino *et al.*, 2000).

Since records indicate that metals and metal sludges were deposited at Harmony Landfill, it is not surprising that they should be found in the leachates collected at that site (Crutcher & Mateyk, 1994). It is consistent with the literature on landfills to find iron and manganese at relatively high levels compared to other leachate metals (Christensen *et al*., 2001). Iron and manganese reduction zones in landfill leachate plumes result in greater concentrations of reduced forms of both metals (Fe^{2+} and Mn^{2+}) which may be more mobile and increasingly likely to be detected in leachate samples (Christensen *et al*., 2001). Nickel and zinc are two other more labile metals which were also found at higher concentrations in Harmony Landfill leachate (Christensen *et al*., 2001). Although difficult to attribute solely to leaching, nickel and zinc were the only two metals which were present at 2.5 times (nickel) and 3 times (zinc) greater concentrations at the downstream compared to the upstream Harmony Creek site.

6.2.3 – Xenobiotic Organic Compounds

The scarcity of xenobiotic organic compounds in Harmony Landfill leachates is probably related to the age of the landfill and the limited analysis (VOCs only) performed on the December 2008 leachate sample. Most organics were at low levels with the exception of monochlorobenzene at 15 µg/L which exceeded the recommended limit of 1.3 µg/L (CCME, 2007) and m/p xylene at 6.3 µg/L which exceeded the 2 µg/L (MOE, 1994) recommended limit for m-xylene.

The elevated levels of monochlorobenzenes, as well as the lesser amounts of dichlorobenzenes detected in the leachate samples, likely originated from the breakdown of more heavily chlorinated compounds, which were deposited at the landfill (Nishino *et al*., 1993). Reports indicate that organic compounds, including a variety of industrial solvents and paint sludges, were received at Harmony Landfill (Crutcher & Mateyk, 1994). Chlorobenzenes can originate from the disposal of industrial solvents and substrates, dichloroethanes from paint solvents, paint adhesives and degreasants, dichloroethylene from solvents, ethylbenzene from varnishes, adhesives and paints, xylene from paints and plastics and toluene from paint solvents (Slack *et al*., 2005).

As with most other toxicants in Harmony Landfill leachates, xenobiotic organic compounds were detected in the microgram per litre range. Therefore it is likely necessary to consider the entire leachate mixture as a whole in order to explain the observed toxicity. Interactive effects between pollutants, may contribute to the toxicity of mixtures which contain individual pollutants at non-toxic levels (Walker *et al*., 2006). In their 2009 study, Quinn *et al*., found that exposure of *Hydra attenuata* to a mixture of pharmaceuticals induced morphological toxic effects at levels below those demonstrated to affect Hydra individually. A metal and xenobiotic organic mixture of manganese and DDT, was also shown to be more toxic to *Daphnia magna* upon co-exposure (Mejía – Saavedra *et al*., 2005). The low levels of putative toxicants in Harmony Landfill leachate samples may explain their relative non-toxicity to *Hydra viridissima*. However, abiotic factors are also responsible for modifying toxicity in the aquatic environment.

6.3 – Abiotic Conditions

6.3.1 – Temperature

Temperature is not believed to have greatly influenced toxicity during this study. All experiments were operated within a temperature range of 23.8 - 27.6 °C. This falls in the general recommended range of 20 - 30°C for optimal growth in Hydra (Loomis, 1954) and published toxicity testing temperatures of 20 – 30°C for *Hydra viridissima* (Karntanut & Pascoe, 2005; Hyne *et al*., 1992).

6.3.2 – pH

The pH values of 6.4 - 8.7 recorded in this study are generally within the optimum pH range of 6 – 8 as suggested in Loomis (1954) and well within the 5.5 – 9.5 range of no morphological effects as described by Fu *et al.* (1991). Published pH values for toxicity tests with *Hydra viridissima* range from 5.5 – 7.8 (van Dam *et al*., 2010; Karntanut & Pascoe, 2005). Since pH values were lowest for the treatments with the highest concentrations of leachate (32% and 100%) it is possible that some toxicity in these treatments was due to pH. However, exposure of Hydra to lower pH creek water never resulted in a statistically significant reduction in population growth as compared to controls.

Although pH may not have been a factor on its own, it is well-known that pH may influence metal speciation and thus act to enhance or decrease toxicity. Lower pH values may increase the concentration of soluble and bioavailable free metal ions, which are more capable of causing internal toxic effects (Walker *et al*., 2006; Reithmuller *et al*., 2001). For example, uranium was determined to be more toxic to *Hydra viridissima*, as based on population growth, at a pH of 6.6 than a pH of 8.6 (Hyne *et al*., 1992b). The lower pH values measured in the 32% and 100% leachate treatments, minimum values 6.9 and 6.5 respectively, may explain some of the morphological and reproductive effects observed for those treatments.

6.3.3 – Conductivity

Conductivity, also known as specific conductance, is a measure of the ionic strength of a solution, which provides an estimate of the dissolved solids in a sample (AWWA, 1995). Conductivity values ranged most substantially throughout this study, particularly during the 24 hour pulse-exposure period (72 - 1281 µS/cm). This resulted from the generally low conductivity of the diluent laboratory water (34 – 252 µS/cm) when compared to environmental samples of both Harmony Creek water (742 - 1281 µS/cm) and Harmony Landfill leachate (673 – 1266 µS/cm). Conductivity values published for *Hydra viridissima* experiments range from 5 – 490 µS/cm (van Dam *et al*., 2010; Karntanut & Pascoe, 2005). The maximum value of this range falls considerably short of the maximum values in this study. However, Hydra exposed to Harmony Creek water, which had a similar conductivity to the 100% leachate, tended to demonstrate increased reproduction compared to lab water and leachate treatments. Therefore, conductivity alone is not considered to have contributed to Hydra toxicity. Although increased conductivity may indicate the presence of toxicants, higher conductivity values can also be associated with naturally high water hardness. Lower conductivity may have partially confounded population growth results for the 0%, 3.2%, 10% and 32% leachate treatments as they likely had lower levels of the ions and trace elements required for optimal growth, than the 100% leachate and creek water treatments.

Low laboratory water conductivity may aid in explaining the significant decrease in population growth of the 100% treatment in the July 2009 experiment. It is difficult to explain the toxicity of that leachate sample based on the July 2009 chemical analysis alone. Therefore, conductivity may have been a factor since all treatments were reared in lab water after the 24 hour pulse-exposure period. The July 2009 lab water conductivity readings were particularly low (range 34 – 91 µS/cm) as compared to the other months. Even though some Hydra may inhabit naturally low conductivity environments (van Dam *et al.,* 2010), perhaps it was an added stressor to a population acclimated to average conductivity values of over 100 µS/cm (Table 5).

6.3.4 – Water Hardness

Although not routinely recorded, measures of water hardness would also have varied quite substantially between treatments during the 24 hour pulse-exposure period from approximately 0 – 500 mg $CaCO_3$/L. Hardness values published for *Hydra viridissima* experiments range from 3 – 209 mg $CaCO_3$/L (vanDam *et al*., 2010; Karntanut & Pascoe, 2002). Fu *et al*., (2001) found that *Hydra attenuata* were unaffected by hardness values up to 750 mg $CaCO_3$/L.

Water hardness, which is a reflection of the concentrations of calcium and magnesium ions, can also act as a modifier of toxicity (Reithmuller at al., 2001). High water hardness tends to increase complexation of metals as well as provide competing cations (Ca^{2+} and Mg^{2+}) which can decrease the effects of toxic divalent metals (Reithmuller *et al*., 2001). The toxicity of uranium to *Hydra viridissima,* as measured by population growth, was significantly reduced when water hardness was increased from 6.6 mg $CaCO_3$/L to levels of both 165 and 330 mg $CaCO_3$/L (Reithmuller *et al*., 2001). The high water hardness of the 32% and 100% leachate treatments may help to explain their non-toxicity. In addition, water hardness is a possible explanation for the significant decrease in population growth of the 32% compared to the 100% leachate treatment observed in November 2009. Water hardness for the 0% and 3.2% treatments was ~0-60 mg $CaCO_3$/L so can be considered as "soft", the 10% treatment was within ~60–120 mg $CaCO_3$/L and therefore can be considered as "medium", 32% was ~120-180 mg $CaCO_3$/L and therefore was “hard” and the 100% leachate and creek water were both >180 mg $CaCO_3$/L and are therefore considered “very hard” (CCME, 2007).

6.3.5 – Dissolved Oxygen

With the exception of the February 2010 Day 0 measurement of 4.8 mg O_2/L (100% leachate) dissolved oxygen values (6.6 - 10 mg O_2/L) were above the 6.5 mg O_2/L level required for optimal Hydra growth as described by Loomis (1954). Literature values for dissolved oxygen in *Hydra viridissima* toxicity experiments range from 6.9 – 9.4 mg O_2/L (Karntanut & Pascoe, 2002; Holdway *et al*., 2001). Although 4.8 mg O_2/L is a low reading it falls above the minimal required level of 2 mg O_2/L as also suggested in Loomis (1954). In addition, *Hydra viridissima* contain photosynthetic algae, so are not dependent solely on diffusion of atmospheric oxygen into their environment. Furthermore, the February 2010 Day 1 pooled dissolved oxygen reading including the 100% leachate treatment had a dissolved oxygen level of above 7 mg O_2/L, which is the concentration recommended for optimal test conditions in Holdway (2005). That and the fact that the February 100% treatment did not appear to suffer morphologically or reproductively, leads to the conclusion that dissolved oxygen was not an important factor within this study.

7.0 – CONCLUSION

7.1 – Research Conclusions

The overall objective of this research program was to assess the potential impact of Harmony landfill leachate on the aquatic life of Harmony Creek. This was accomplished through several methods. Firstly, the chemical composition of Harmony Creek water samples, collected both upstream and downstream of Harmony Landfill, was determined in December 2008. Chemical analysis of general water characteristics, metals and xenobiotic organic compounds did not indicate that the downstream site was more impacted than the upstream location. Secondly, chemical analysis was performed on Harmony Landfill leachate samples collected in the period from December 2008 to January 2010. These analyses indicated that, although some components seemed to be leaching from Harmony Landfill into adjacent surface waters, the levels of potential toxicants in those leachates were low. Lastly, leachate samples collected from December 2008 until April 2010 were laboratory tested on *Hydra viridissima*. Although some morphological and reproductive toxicity was observed, the majority of leachate samples were demonstrated to be non-toxic to Hydra. Complementary experiments involving exposure of Brown Hydra (*Hydra littoralis*), embryonic and larval Flagfish (*Jordanella floridae*) and larval Rainbow Trout (*Oncorhynchus mykiss*) to Harmony Landfill leachates also showed them to be non-toxic within the conditions investigated (Appendix 12). Since the Harmony Landfill leachates as tested have been largely observed to be non-toxic to aquatic organisms, particularly *Hydra viridissima*, it can be concluded that at present and within the limitations of this research program, they do not pose a significant environmental risk to the aquatic life of Harmony Creek.

7.2 – Future Research

Future directions of research at Harmony Landfill may include analysis of groundwater samples. Due to its proximity to the landfill, Harmony Creek falls within the 1000 metres considered to be the average reach of landfill leachate plumes (Christensen *et al*., 2001). The creek is downgradient from the waste mound and, given the history of the landfill site as a former sand and gravel pit, the soil beneath Harmony Landfill may be fairly porous (Crutcher & Mosher, 1991). Surface leachate breakouts are likely to be mitigated by the presence of forested land and a wetland situated between the waste mound and the creek. However, hydraulic contiguity of groundwater beneath Harmony Landfill and Harmony Creek could introduce toxicants into surface waters via groundwater upwellings (Dewhurst *et al.*, 2003).

7.3 – Research Significance

Harmony Landfill was situated on a relatively isolated concession road during its operating years (Sobanski, 1976-1983). However, it is now located among residential neighbourhoods and next to a well-used public park. As has been demonstrated during the time period of this study, as residents become more aware of the landfill's history, public concern will most likely intensify. The City of Oshawa is currently investigating the Harmony Landfill property (S. Elston, personal communication, May 2010). As stated in the Harmony Valley Park Master Plan (2006) the landfill, if properly rehabilitated, is a target site for expansion of the city-owned park. The results of this research program, along with other reports, may be useful in determining the future land uses of Harmony Landfill.

8.0 – REFERENCES

Adams, J. A., & Haileselassie, H. M. (1984). The effects of polychlorinated biphenyls (Aroclors 1016 and 1254) on mortality, reproduction, and regeneration in *Hydra oligactis*. *Archives of Environmental Contamination and Toxicology, 13*, 493-499.

American Water Works Association (AWWA). (1995). *Water Quality, Principles and Practices of Water Supply Operations, Second Edition*. Denver, Colorado: American Water Works Association.

Andersen, R. A., Wiger, R., Daae, H. L., & Eriksen, K. D. H. (1988). Is the metal binding protein metallothionein present in the coelenterate *Hydra attenuata*? *Comparative Biochemistry and Physiology Part C, 91*(2), 553-557.

Arkhipchuk, V. V., Blaise, C., & Malinovskaya, M. V. (2006). Use of Hydra for chronic toxicity assessment of waters intended for human consumption. *Environmental Pollution, 142*, 200-211.

Assmuth, T., & Pentilla, S. (1995). Characteristics, determinants and interpretations of acute lethality in Daphnids exposed to complex waste leachates. *Aquatic Toxicology, 31*, 125-141.

Baun, A., Kloft, L., Berg, P. L., & Nyholm, N. (1999). Toxicity testing of organic chemicals in groundwater polluted with landfill leachate. *Environmental Toxicology and Chemistry, 18*(9), 2046-2053.

Baun, D. L., & Christensen, T. H. (2004). Speciation of heavy metals in landfill leachate: a review. *Waste Management and Research, 22,* 3-23.

Beach, M. J., & Pascoe, D. (1998). The role of *Hydra vulgaris* (Pallas) in assessing the toxicity of freshwater pollutants. *Water Research, 32*(1), 101-106.

Bell, G., & Wolfe, L. M. (1985). Sexual and asexual reproduction in a natural population of *Hydra pseudoligactis*. *Canadian Journal of Zoology, 63*, 851-856.

Biesinger, K.E., & Christensen, G.M. (1972). Effects of various metals on survival, growth, reproduction and metabolism in *Daphnia magna*. *Journal of the Fisheries Research Board of Canada 29*, 1691-1700.

Blaise, C., & Kusui, T. (1997). Acute toxicity assessment of industrial effluents with a microplate-based *Hydra attenuata* assay. *Environmental Toxicology and Water Quality, 12*, 53-60.

Bloor, M. C., Banks, C. J., & Krivtsov, V. (2005). Acute and sublethal toxicity tests to monitor the impact of leachate on an aquatic environment. *Environment International, 31*, 269-273.

Brack, W., Rottler, H., & Frank, H. (1998). Volatile fractions of landfill leachates and their effect on C*hlamydomonas reinhardtii : in vivo* chlorophyll a fluorescence. *Environmental Toxicology and Chemistry, 17*(10), 1982-1991.

Browne, C. L., & Davis, L. E. (1977). Cellular mechanisms of stimulation of bud production in *Hydra* by low levels of inorganic lead compounds. *Cell and Tissue Research*, *177*, 555-570.

Campbell, R. D. (1989). Taxonomy of the European Hydra (Cnidaria:Hydrozoa): a re-examination of its history with emphasis on the species *H. vulgaris* Pallas, *H. attenuata* Pallas and *H. circurncincta* Schulze. *Zoological Journal of the Linnean Society, 95*, 219-244.

Canadian Council of Ministers of the Environment (CCME) (2007). *Canadian water quality guidelines for the protection of aquatic life: summary table* (Update 7.1, December 2007). Winnipeg: Canadian Council of Ministers of the Environment.

Central Lake Ontario Conservation Authority (CLOCA) (2002/2008). *Benthic macroinvertebrate and fish sampling reports*. Received electronically: May 2009.

Central Lake Ontario Conservation Authority (CLOCA) (2008). *Water quality monitoring reports 2006-2007*. Received electronically: October 2008.

Central Lake Ontario Conservation Authority (CLOCA) (2009). *Black/Harmony/Farwell Creek watershed existing conditions - draft report*. Retrieved May 2009, from http://www.cloca.com

Christensen, T. H., Kjeldsen, P., Bjerg, P. L., Jensen, D. L., Christensen, J. B., Baun, A., Albrechtsen, H-J., & Heron, G. (2001). Biogeochemistry of landfill leachate plumes. *Applied Geochemistry*, *16*, 659-718.

Clifford, H. F. (2010). *Aquatic Invertebrates of Alberta.* Retrieved June 9, 2010 from http://sunsite.ualberta.ca/Projects/Aquatic_Invertebrates/?Page=9

Crutcher, A. J., & Mateyk, M. G. (1994). *Review of Hydrogeologic Studies, Industrial Disposal Landfill Site, Oshawa, Ontario* (No. 4149(4)). Conestoga-Rovers Associates.

Crutcher, A. J., & Mosher, F. (1991). *Engineered Systems Evaluation, Industrial Disposal Landfill Site, Oshawa, Ontario.* Conestoga-Rovers Associates.

Crutcher, A. J., & Mosher, F. (1994). *Engineered Systems Design Alternatives, Industrial Disposal Landfill Site* (No. 4149 (5)). Conestoga-Rovers and Associates.

Dash, B., Metz, R., Huebner, H.J., Porter, W., & Phillips, T.D. (2006). Molecular characterization of phospholipid hydroperoxide glutathione peroxidises from *Hydra vulgaris*. *Gene, 381*, 1-12.

Dash, B., Metz, R., Huebner, H.J., Porter, W., & Phillips, T.D. (2007). Molecular characterization of two superoxide dismutases from *Hydra vulgaris*. *Gene, 387*, 93-108.

Dave, G. (1984). Effects of waterborne iron on growth, reproduction, survival and haemoglobin in *Daphnia magna*. *Comparative Biochemistry and Physiology Part C, 78*(2), 433-438.

Dave, G., & Nilsson, E. (2005). Increased reproductive toxicity of landfill leachate after degradation was caused by nitrite. *Aquatic Toxicology, 73*, 11-30.

Dewhurst, R. E., Wells, N. C., Crane, M., Callaghan, A., Connon, R., & Mather, J. D. (2003). Multivariate relationships between groundwater chemistry and toxicity in an urban aquifer. *Environmental Toxicology and Chemistry, 22*(11), 2813-2821.

Diamantino, T. C., Guilhermino, L., Almeida, E., & Soares, A. M. V. M. (2000). Toxicity of sodium molybdate and sodium dichromate to *Daphnia magna* Straus evaluated in acute, chronic and acetylcholinesterase inhibition tests. *Ecotoxicology and Environmental Safety, 45*, 253-259.

Diamond, J., Bowersox, M., Latimer, H., Barbour, C., Bearr, J., & Butcher, J. (2005). Effects of pulsed contaminant exposures on early life stages of the fathead minnow. *Archives of Environmental Contamination and Toxicology, 49*, 511-519.

Elliott, J. K., Elliott, J. M., & Leggett, W. C. (1997). Predation by *Hydra* on larval fish: field and laboratory experiments with Bluegill (*Lepomis macrochirus*). *Limnology and Oceanogaphy, 42*(6), 1416-1423.

Environment Canada. (1990). *Environmental protection series: guidance document on control of toxicity test precision using reference toxicants* (Report EPS 1/RM/12). Ottawa: Minister of Supply and Services Canada.

Ettler, V., Mihaljevic, M., Matura, M., Skalova, M., Sebek, O., & Bezdicka, P. (2008). Temporal variation of trace elements in waters polluted by municipal solid waste landfill leachate. *Bulletin of Environmental Contamination and Toxicology, 80*, 274-279.

Fraser, J. K., Butler, C. A., Timperley, M. H., & Evans, C. W. (2000). Formation of copper complexes in landfill leachate and their toxicity to Zebrafish embryos. *Environmental Toxicology and Chemistry, 19*(5), 1397-1402.

Fu, L. J., Staples, E., & Stahl Jr., R. G. (1991). Application of the *Hydra attenuata* assay for identifying developmental hazards among natural waters and wastewaters. *Ecotoxicology and Environmental Safety, 22*, 309-319.

Goodwin, J. G. (1979). *Watershed Inventory, Watershed Plan Phase I*. Whitby, Ontario: Central Lake Ontario Conservation Authority.

Habetha, M., Anton-Erxleben, F., Neumann, K., & Bosch, T. C. G. (2003). The *Hydra viridis/Chlorella* symbiosis, growth and sexual differentiation in polyps without symbionts. *Zoology, 106*, 101-108.

Health Canada. (2008). *Guidelines for Canadian Drinking Water Quality Summary Table* (May 2008). Ottawa: Federal-Provincial-Territorial Committee on Drinking Water.

Holdway, D. A. (2005). *Hydra* population reproduction toxicity test method. In: C. Blaise & JF. Férard (Eds.), *Small-Scale Freshwater Toxicity Investigations, Volume 1* (pp. 395-411). Netherlands: Springer Publishers.

Holdway, D. A., Hefferman, J., & Smith, A. (2008). Multigeneration assessment of nonylphenol and endosulfan using a model Australian freshwater fish, *Melanotaenia fluviatilis*. *Environmental Toxicology, 23*, 253-262.

Holdway, D. A., Lok, K., & Seaman, M. (2001). The acute and chronic toxicity of cadmium and zinc to two Hydra species. *Environmental Toxicology, 16*, 557-565.

Holstein, T. W., Campbell, R. D., & Tardant, P. (1990). Identity crisis. *Nature, 346*, 21-22.

Hyne, R. V., Rippon, G. D., White, J., & Ellender, G. (1992a). Accumulation of uranium by freshwater Hydra into discharged nematocysts. *Aquatic Toxicology, 23*, 231-246.

Hyne, R. V., Rippon, G. D., & Ellender, G. (1992b). pH-dependent uranium toxicity to freshwater Hydra. *The Science of the Total Environment, 125*, 159-173.

Ingersoll, C. G., Dwyer, F. J., & May, T. W. (1990). Toxicity of inorganic and organic selenium to *Daphnia magna* (Cladocera) and *Chironomus riparius* (Diptera). *Environmental Toxicology and Chemistry, 9*(9), 1171-1181.

James, S. C. (1977). Metals in municipal landfill leachate and their health effects. *American Journal of Public Health, 67*(5), 429-432.

Johnson, E. M., Gorman, R. M., Gabel, B. E. G., & George, M. E. (1982). The *Hydra attenuata* system for detection of teratogenic hazards. *1982* (2), 263-276.

Karntanut, W., & Pascoe, D. (2000). A comparison of methods for measuring acute toxicity to *Hydra vulgaris*. *Chemosphere, 41*, 1543-1548.

Karntanut, W., & Pascoe, D. (2002). The toxicity of copper, cadmium and zinc to four different *Hydra* (Cnidaria: Hydrozoa). *Chemosphere, 47*, 1059-1064.

Karntanut, W., & Pascoe, D. (2005). Effects of removing symbiotic green algae to the response of *Hydra viridissima* (Pallas 1776) to metals. *Ecotoxicology and Environmental Safety, 60*, 301-305.

Karntanut, W., & Pascoe, D. (2007). A comparison of metal accumulation by the cnidarian *Hydra vulgaris* directly from water or through contaminated prey and effects upon reproduction and regeneration. *Songklanakarin Journal of Science and Technology, 29*(3), 869-880.

Khangarot, B. S., & Ray, P. K. (1989). Investigation of correlation between physiochemical properties of metals and their toxicity to the water flea *Daphnia magna* Straus. *Ecotoxicology and Environmental Safety, 18*, 109-120.

Kjeldsen, P., Barlaz, M. A., Rooker, A. P., Baun, A., Ledin, A., & Christensen, T. H. (2002). Present and long-term composition of MSW landfill leachate: a review. *Critical Reviews in Environmental Science and Technology, 32*(4), 297-336.

Kovačević, G., Želježić, D., Horvatin, K, & Kalafatić, M., (2007). Morphological features and comet assay of green and brown hydra treated with aluminum. *Symbiosis, 44*, 145-152.

Kovačević, G., Kalafatić, M., & Horvatin, K. (2009). Aluminum deposition in Hydras. *Folia Biologica (Krakow), 57*(3-4), 139-142.

Lambolez, L., Vasseur, P., Ferard, J. F., & Gisbert, T. (1994). The environmental risks of industrial waste disposal: an experimental approach including acute and chronic toxicity studies. *Ecotoxicology and Environmental Safety, 28*, 317-328.

Lappivaara, J., Kiviniemi, A., & Oikari, A. (1999). Bioaccumulation and subchronic physiological effects of waterborne iron overload on Whitefish exposed in humic and nonhumic water. *Archives of Environmental Contamination and Toxicology, 37*, 196-204.

Lenhoff, H. M., & Brown, R. D. (1970). Mass culture of H*ydra*: an improved method and its application to other aquatic invertebrates. *Laboratory Animals, 4*, 139-154.

Link, J., & Keen, R. (1995). Prey of deep-water *Hydra* in Lake Superior. *Journal of Great Lakes Research, 21*(3), 319-323.

Lomnicki, A., & Slobodkin, L. B. (1966). Floating in *Hydra littoralis*. *Ecology, 47*(6), 881-889.

Loomis, W. F. (1954). Environmental factors controlling growth in *Hydra*. *Journal of Experimental Zoology, 126*(2), 223-234.

Loomis, W. F., & Lenhoff, H. M. (1956). Growth and sexual differentiation of Hydra in mass culture. *Journal of Experimental Zoology, 132*(3), 555-568.

Lum, K. T., Huebner, H. J., Li, Y., Phillips, T. D., & Raushel, F. M. (2003). Organophosphate nerve agent toxicity in *Hydra attenuata*. *Chemical Research in Toxicology, 16*, 953-957.

Mayura, K., Smith, E. E., Clement, B. A., & Phillips, T. D. (1991). Evalution of the developmental toxicity of chlorinated phenols utilizing *Hydra attenuata* and postimplantation rat embryos in culture. *Toxicology and Applied Pharmacology, 108*, 253-266.

Mejía-Saavedra, J., Sánchez-Armass, S., Santos-Medrano, G. E., González-Amaro, R., Razo-Soto, I., Rico-Martínez, R., & Díaz-Barriga, F. (2005). Effect of coexposure to DDT and manganese on freshwater invertebrates: pore water from contaminated rivers and laboratory studies. *Environmental Toxicology and Chemistry, 24*(8), 2037-2044.

Mitchell, F. M., & Holdway, D. A. (2000). The acute and chronic toxicity of the dispersants Corexit 9527 and 9500, water accommodated fraction (WAF) of crude oil, and dispersant enhanced WAF (DEWAF) to *Hydra viridissima* (Green Hydra). *Water Research, 34*(1), 343-348.

Murray Darling Freshwater Research Centre (MDFRC). (2010). *Identification and Ecology of Australian Freshwater Invertebrates.* Retrieved June 9, 2010, from http://www.mdfrc.org.au/bugguide/index.htm .

Muscatine, L., & Lenhoff, H. M. (1965). Symbiosis of Hydra and algae. I. Effects of some environmental cations on growth of symbiotic and aposymbiotic Hydra. *The Biological Bulletin, 128,* 415-424.

Nalepa, T. F., Remsen, C. C., & Klump, J. V. (1987). Observations of *Hydra* from a submersible at two deepwater sites in Lake Superior. *Journal of Great Lakes Research*, *13*(1), 84-87.

Nishino, S. F., Spain, J. C., & Pettigrew, C. A. (1993). Biodegradation of chlorobenzene by indigenous bacteria. *Environmental Toxicology and Chemistry*, *13*(6), 871-877.

Olivero-Verbel, J., Padilla-Bottet, C., & De la Rosa, O. (2008). Relationships between physiochemical parameters and the toxicity of leachates from a municipal solid waste landfill. *Ecotoxicology and Environmental Safety, 70,* 294-299.

Öman, C. B., & Junestedt, C. (2008). Chemical characterization of landfill leachates - 400 parameters and compounds. *Waste Management*, *28*, 1876-1891.

Ontario Environmental Appeal Board (OEAB). (1976). *Decision Summary: Industrial Disposal (Oshawa) Co. v. Ontario (Ministry of Environment)* (No. 10). Retrieved June 6, 2010, from, http://www.ert.gov.on.ca/files/DEC/O7602d2.pdf

Ontario Ministry of the Environment (MOE). (1994). *Provincial Water Quality Objectives of the Ministry of Environment and Energy*. Toronto: Queen's Printer for Ontario.

Ontario Ministry of the Environment (MOE). (2009). *Decision Summary: Environmental Bill of Rights Application for investigation of Rossland Acres Inc., Oshawa* (EBRO File No: 08EBR009.I, April 2009). Retrieved May 2009, from http://www.waterkeeper.ca/documents/2009-04-Harmony_decision.pdf

Osaki, K., Kashiwada, S., Tatarazako, N., & Ono, Y. (2006). Toxicity testing of leachate from waste landfills using Medaka (*Oryzias latipes*) for monitoring environmental safety. *Environmental Monitoring and Assessment, 117*, 73-84.

Östman, M., Wahlberg, O., & Martensson, A. (2008). Leachability and metal-binding capacity in ageing landfill material. *Waste Management*, *28*, 142-150.

Øygard, J. K., Gjengedal, E., & Royset, O. (2007). Size charge fractionation of metals in municipal solid waste landfill. *Water Research, 41*, 47-54.

Pachura, S., Cambon, J. P., Blaise, C., & Vasseur, P. (2005). 4- nonylphenol-induced toxicity and apoptosis in *Hydra attenuata*. *Environmental Toxicology and Chemistry*, *24*(12), 3085-3091.

Pardos, M., Benninghoff, C., Gueguen, C., Thomas, R., Dobrowolski, J., & Dominik, J. (1999). Acute toxicity assessment of Polish (waste) water with a microplate-based *Hydra attenuata* assay: a comparison with the Microtox® test. *The Science of the Total Environment, 243-244*, 141-148.

Pascoe, D., Carroll, K., Karntanut, W., & Watts, M. M. (2002). Toxicity of 17 a-ethinylestradiol and bisphenol A to the freshwater cnidarian *Hydra vulgaris*. *Archives of Environmental Contamination and Toxicology, 43*, 56-63.

Pascoe, D., Karntanut, W., & Muller, C. T. (2003). Do pharmaceuticals affect freshwater invertebrates? A study with the cnidarian *Hydra vulgaris*. *Chemosphere, 51*, 521-528.

Plotkin, S., & Ram, N. M. (1984). Multiple bioassays to assess the toxicity of a sanitary landfill leachate. *Archives of Environmental Contamination and Toxicology, 13*, 197-206.

Pollino, C. A., & Holdway, D. A. (1999). Potential of two Hydra species as standard toxicity test animals. *Ecotoxicology and Environmental Safety, 43*, 309-316.

Pyatt, F. B., & Dodd, N. M. (1986). Some effects of metal ions on the freshwater organisms, *Hydra oligactis* and *Chlorohydra viridissima*. *Indian Journal of Experimental Biology, 24*, 169-173.

Quinn, B., Gagne, F., & Blaise, C. (2004). Oxidative metabolism activity in *Hydra attenuata* exposed to carbamazepine. *Fresenius Environmental Bulletin, 13*(8), 783-788.

Quinn, B., Gagne, F., & Blaise, C. (2008a). The effects of pharmaceuticals on the regeneration of the cnidarian, *Hydra attenuata*. *Science of the Total Environment, 402*, 62-69.

Quinn, B., Gagne, F., & Blaise, C. (2008b). An investigation into the acute and chronic toxicity of eleven pharmaceuticals (and their solvents) found in wastewater effluent on the cnidarian, *Hydra attenuata* . *Science of the Total Environment, 389*, 306-314.

Quinn, B., Gagne, F., & Blaise, C. (2009). Evaluation of the acute, chronic and teratogenic effects of a mixture of eleven pharmaceuticals on the cnidarian, *Hydra attenuata* . *Science of the Total Environment, 407*, 1072-1079.

Reithmuller, N., Markich, S. J., van Dam, R. A., & Parry, D. (2001). Effects of water hardness and alkalinity on the toxicity of uranium to a tropical freshwater Hydra (*Hydra viridissima*). *Biomarkers, 6*(1), 45-51.

Rosenkrantz, R. T., Pollino, C. A., Nugegoda, D., & Baun, A. (2008). Toxicity of water and sediment from stormwater retarding basins to *Hydra hexactinella*. *Environmental Pollution, 156*, 922-927.

Ross, S. (2008). *Harmony landfill water quality analysis summary*. Retrieved September 2009, from http://harmonylandfill.blogspot.com/

Rutherford, L. A., Matthews, S. L., Doe, K. G., & Julien, G. R. (2000). Aquatic toxicity and environmental impact of leachate discharges from a municipal landfill. *Water Quality Research Journal of Canada, 35*(1), 39-57.

Santiago-Fandiño, V. J. R. (1983). The effects of nickel and cadmium on the growth rate of *Hydra littoralis* and an assessment of the rate of uptake of ^{63}Ni and ^{14}C by the same organism. *Water Research, 17*(8), 917-923.

Schwartz, S. S., Hann, B. J., & Hebert, P. D. N. (1983). The feeding ecology of Hydra and possible implications in the structuring of pond zooplankton communties. *The Biological Bulletin, 164*, 136-142.

Schwartz, S. S., & Hebert, P. D. (1989). The effect of *Hydra* on the outcome of competition between *Daphnia* and *Simocephalus*. *The Biological Bulletin, 176*, 147-154.

Slack, R. J., Gronow, J. R., Hall, D. H., & Voulvoulis, N. (2007). Household hazardous waste disposal to landfill: using LandSim to model leachate migration. *Environmental Pollution, 146*, 501-509.

Slack, R. J., Gronow, J. R., & Voulvoulis, N. (2005). Household hazardous waste in municipal landfills: contaminants in leachate. *Science of the Total Environment, 337*, 119-137.

Slobodkin, L. B., & Bossert, P. E. (2001). Cnidaria. In: J. H. Thorp, & A. P. Covich (Eds.), *Ecology and Classification of North American Freshwater Invertebrates* (pp. 135-153). San Diego, California, United States of America: Academic Press.

Sobanski, A. A. (1976-1982). *Hydrogeologic Investigation of a Sanitary Landfill, Site 3, Lot 3, Concession 3, Township of East Whitby, for Industrial Disposal (Oshawa) Limited* (No. 8567). Mississauga: Hydrology Consultants Limited.

Sobanski, A. A. (1983-1984). *Industrial Disposal (Oshawa) Limited. Monitoring Program, Township of East Whitby* (No. 8567). Rexdale: Hydrology Consultants Limited.

Statistics Canada. (2009). *Human Activity and the Environment: Annual Statistics* (No. 16-201-X). Ottawa: Minister of Industry.

Stiven, A. E. (1962). The effect of temperature and feeding on the intrinsic rate of increase of three species of Hydra. *Ecology, 43*(2), 325-328.

Stoughton, S. J., Liber, K., Culp, J., & Cessna, A. (2008). Acute and chronic toxicity of Imidacloprid to the aquatic invertebrates *Chironomus tetans* and *Hyalella azteca* under constant- and pulse-exposure conditions. *Archives of Environmental Contamination and Toxicology, 54*, 662-673.

Svensson, B. M., Mathiasson, L., Martensson, L., & Bergstrom, S. (2005). *Artemia salina* as test organism for assessment of acute toxicity of leachate water from landfills. *Environmental Monitoring and Assessment, 102*, 309-321.

Tarrant, A. M. (2007). Hormonal signaling in cnidarians: do we understand the pathways well enough to know whether they are being disrupted? *Ecotoxicology, 16*, 5-13.

Taylor, J. F., Robinson, A., Johnson, N., Marroquin-Cardona, A., Brattin, B., Taylor, R., & Phillips, T.D. (2009). *In vitro* evaluation of ferrihydrite as an enterosorbent for arsenic from contaminated drinking water. *Environmental Science and Technology, 43*(14), 5501-5506.

Teien, H., Garmo, O. A., Atland, A., & Salbu, B. (2008). Transformation of iron species in mixing zones and accumulation on fish gills. *Environmental Science and Technology, 42*, 1780-1786.

Trottier, S., Blaise, C., Kusui, T., & Johnson, E. M. (1997). Acute toxicity assessment of aqueous samples using a microplate-based *Hydra attenuata* assay. *Environmental Toxicology and Water Quality, 12*, 265-271.

van Anholt, R. D., Spanings, F. A. T., Knol, A. H., van der Velden, J. A., & Wendelaar Bonga, S. E. (2002). Effects of iron sulphate dosage on the Water Flea (*Daphnia magna* Straus) and early development of Carp (*cyprinus carpio* L.). *Archives of Environmental Contamination and Toxicology, 42*, 182-192.

van Dam, R., Hogan, A., Harford, A., & Markich, S. (2008). Toxicity and metal speciation characterisation of waste water from an abandoned gold mine in tropical Northern Australia. *Chemosphere, 73*, 305-313.

van Dam, R., Hogan, A., McCullough, C.D., Houston, M.A., Humphrey, C.L. & Harford, A.J. (2010). Aquatic toxicity of magnesium sulphate, and the influence of calcium, in very low ionic concentration water. *Environmental Toxicology and Chemistry, 29* (2), 410-421.

Walker, C. H., Hopkin, S. P., Sibly, R. M., & Peakall, D. B. (2006). *Principles of Ecotoxicology* (Third Edition). Boca Raton, Florida, USA: Taylor Francis.

Walsh, E. J. (1995). Habitat-specific predation susceptibilities of a littoral rotifer to two invertebrate predators. *Hydrobiologia, 313/314*, 205-211.

Wang, A-T., Deng, L., Lai, J-Q., & Li, J. (2009). A new species of Green Hydra (Hydrozoa:Hydrida) from China. *Zoological Science, 26*, 664-668.

Ward, M. L., Bitton, G., Townsend, T., & Booth, M. (2002). Determining toxicity of leachates from Florida municipal solid waste landfills using a battery-of-tests approach. *Environmental Toxicology, 17*, 258-266.

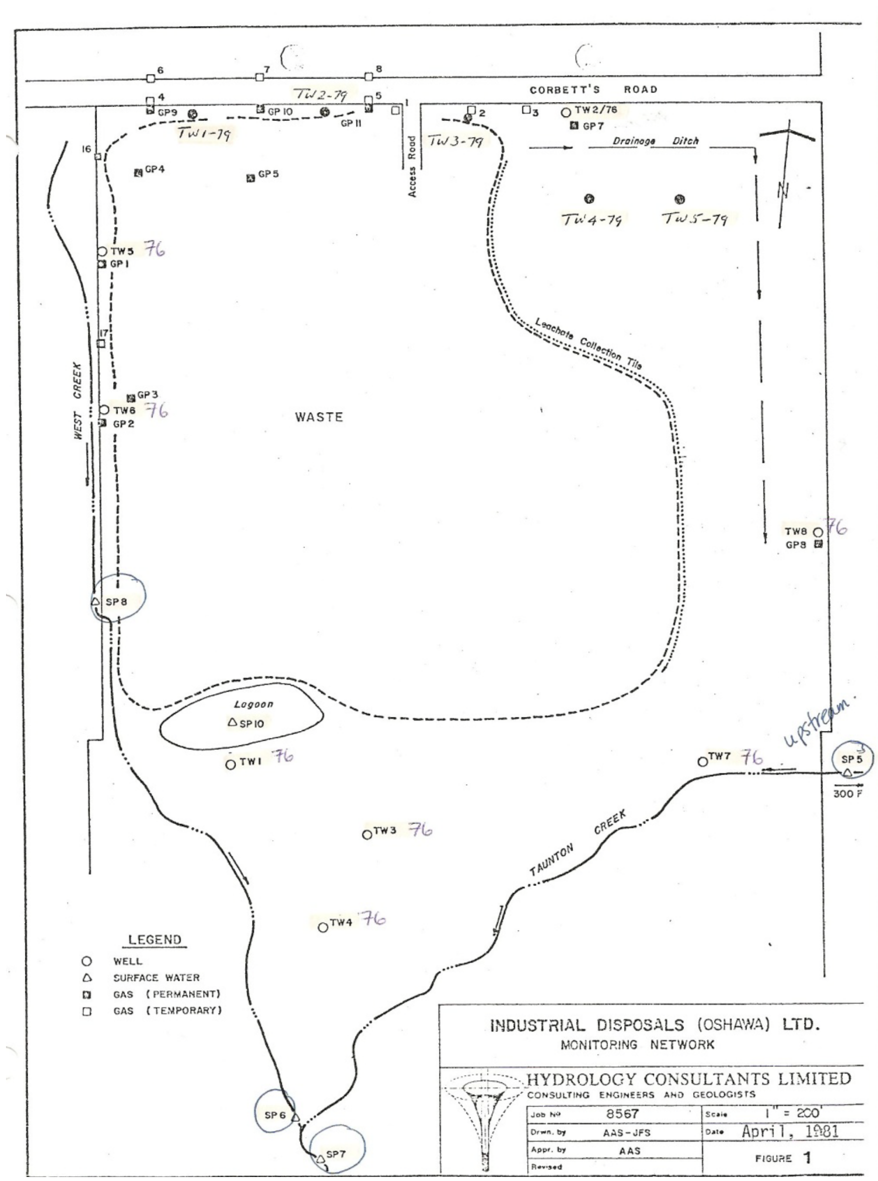
Yellowlees, D., Rees, T. A., & Leggat, W. (2008). Metabolic interactions between algal symbionts and invertebrate hosts. *Plant, Cell and Environment, 31*, 679-694.

Zhao, Y., & Newman, M. C. (2006). Effects of exposure duration and recovery time during pulsed exposures. *Environmental Toxicology and Chemistry, 25*(5), 1298-1304.

9.0 - APPENDICES

Appendix 1

A 1981 map of Harmony Landfill from Sobanski (1976 - 1982). The broken line represents the leachate collection system and demarcates the waste boundary. The leachate lagoon is labeled at its location on the southwestern corner of the site. Both groundwater sampling wells (TW) and surface water (SP) sampling sites used during the 1976 - 1983 environmental monitoring program are marked. Taunton Creek and West Creek refer to the tributaries of Harmony Creek which are adjacent to the landfill.



Appendix 2

Background metals monitoring data which gives an indication of metal levels in Harmony Creek and Harmony Landfill's environmental impact during the periods: 1976 - 1983 and summer 2008. If data was not available, cells are marked by NA = not available or ND = not detectable by analysis. Guideline values are marked by the following symbols: al = Canadian Water Quality Guidelines for the Protection of Aquatic Life (CCME, 2007), pwqo = Ontario Provincial Water Quality Objectives (MOE, 1994) and dw = Guidelines for Canadian Drinking Water Quality (Health Canada, 2008).

Metals (µg/L)	**Harmony Creek CLOCA 2006-2007 mean (min-max)**	**Harmony Landfill Sobanski 1976-1983 mean (min-max)**	**Harmony Landfill Maxxam Analytics July 27, 2008**	**Guidelines (µg/L)**
Aluminum	58.2 (19.2-128)	NA	17,000	100^{al}
Antimony	ND	NA	1.6	20^{pwqo}
Arsenic	ND	NA	9	5^{al}
Cadmium	0.06 (ND-0.1)	NA	1.5	0.017^{al}
Chromium	0.83 (0.7-0.9)	NA	39	$1 - 8.9^{al}$
Cobalt	0.26 (ND-0.5)	NA	11	0.9^{pwqo}
Copper	2.1 (1-4.1)	14 (10-40)	54	4^{al}
Iron	197 (172-238)	1,418 (ND-27,600)	61,000	300^{al}
Lead	ND	10 (ND-100)	130	7^{al}
Manganese	31.2 (21.8-39.8)	NA	880	50^{dw}
Molybdenum	0.36 (0.2-0.5)	NA	ND	73^{al}
Nickel	0.26 (ND-0.8)	NA	170	150^{al}
Selenium	ND	NA	ND	1^{al}
Zinc	3.4 (1.3-7.0)	3394 (ND-44,700)	320	30^{al}

Appendix 3

Background inorganics monitoring data which gives an indication of inorganics levels in Harmony Creek and Harmony Landfill's environmental impact during the periods: 1976 - 1983 and summer 2008. If data was not available, cells are marked by NA = not available or ND = not detectable by analysis. Guideline values are marked by the following symbols: al = Canadian Water Quality Guidelines for the Protection of Aquatic Life (CCME, 2007) and dw = Guidelines for Canadian Drinking Water Quality (Health Canada, 2008).

General Characteristics	**Harmony Creek CLOCA 2006-2007 mean (min-max)**	**Harmony Landfill Sobanski 1976-1983 mean (min-max)**	**Harmony Landfill Maxxam Analytics July 27, 2008**	**Guidelines**
Bromide (mg/L)	0.33 (ND-1)	NA	NA	NA
Chloride (mg/L)	244 (42-401)	313 (5-1500)	NA	250^{dw}
Fluoride (mg/L)	0.023 (ND-0.07)	NA	NA	1.5^{dw}
Nitrate (mg/L)	0.85 (ND–1.5)	NA	NA	13^{al}
Nitrite (mg/L)	ND	NA	NA	0.06^{al}
Phosphate (mg/L)	ND	NA	NA	NA
Sulphate (mg/L)	42.3 (17-56.7)	37.9 (ND-735)	NA	500^{dw}
Calcium (mg/L)	101 (54.8-133)	NA	350	NA
Magnesium (mg/L)	16.1 (8.4-21)	NA	18	NA
Potassium (mg/L)	3.5 (1.9-4.3)	NA	5.3	NA
Sodium (mg/L)	121 (26.9-193)	NA	4.5	200^{dw}
Ammonia (mg/L)	0.1 (ND-0.3)	NA	NA	0.02^{al}
pH	8.12 (7.95-8.25)	7.47 (2.2-9)	NA	$6.5 – 9^{al}$
Hardness (mg $CaCO_3$/L)	321 (172-420)	343 (45-1330)	NA	NA
Alkalinity (mg $CaCO_3$/L)	191 (139-226)	261 (ND-1300)	NA	NA
Conductivity (µS/cm)	1161 (445-1670)	1373 (ND-1600)	NA	NA

Appendix 4

Background organics monitoring data which gives an indication of organics levels in Harmony Creek and Harmony Landfill's environmental impact during the periods: 1976 - 1983 and summer 2008. If data was not available, cells are marked by NA = not available or ND = not detectable by analysis. Guideline values are marked by the following symbols: al = Canadian Water Quality Guidelines for the Protection of Aquatic Life (CCME, 2007) and pwqo = Ontario Provincial Water Quality Objectives (MOE, 1994).

Xenobiotic Organic Compounds (µg/L)	**Harmony Creek CLOCA 2006-2007 mean (min-max)**	**Harmony Landfill Sobanski 1976-1983 mean (min-max)**	**Harmony Landfill Maxxam Analytics July 27, 2008**	Guidelines (µg/L)
1,1 - dichloroethane	NA	NA	ND	200^{pwqo}
1,2 - dichloroethane	NA	NA	ND	100^{al}
Cis - 1,2 - dichloroethylene	NA	NA	ND	200^{pwqo}
monoclorobenzene	NA	NA	ND	1.3^{al}
1,2- dichlorobenzene	NA	NA	ND	0.7^{al}
1,3-dichlorobenzene	NA	NA	ND	150^{al}
1,4-dichlorobenzene	NA	NA	ND	26^{al}
ethylbenzene	NA	NA	ND	90^{al}
m/p-xylene	NA	NA	0.3	2 (m), 30 (p) ^{pwqo}
o-xylene	NA	NA	ND	40^{pwqo}
toluene	NA	NA	ND	2^{al}
Total phenols	NA	16.2 (ND-214)	NA	4^{al}
Total aromatics	NA	36.1 (ND-214)	NA	NA

Appendix 5

Background water quality data cited in this study originated from samples taken in Harmony Creek downstream of Harmony Landfill at CLOCA sites SWQ12 (GPS co-ordinates: 43°53'18.99" N, 78°49'29.68" W) and SWQ3 (GPS co-ordinates: 43°52'49.91" N, 78°49'17.10"). Aquatic biology data cited in this study originated from sampling in Harmony Creek at CLOCA site H403 upstream (GPS co-ordinates: 43°55'44.15" N, 78°49'41.99" W) and CLOCA site H402 downstream (GPS co-ordinates: 43°55'0.3" N, 78°50'0.98" W) of Harmony Landfill. Light blue markers represent CLOCA sampling sites, dark blue markers represent upstream and downstream sampling sites used in this study and the red marker represents Harmony Landfill. Photo retrieved from Google Earth, June 2010.

Appendix 6

Summary of the creek water and leachate collection dates and the chemical analysis and toxicity testing performed with each sample.

Creek Water Collection	Leachate Collection	Chemical Analysis	Toxicity Test
	December 2, 2008 SITE 1		Hydra Reproduction April 13 - 20, 2010
December 11, 2008 Up & downstream	December 11, 2008 SITE 1	Inorganics & Organics December 12, 2008	
June 26, 2009 Upstream	May 21, 2009 SITE 2	Inorganics & VOCs June 6, 2008	Hydra Reproduction June 29 – July 6, 2009
July 21, 2009 Upstream	July 21, 2009 SITE 2		Hydra Reproduction July 22 – August 1, 2009
	July 30, 2009 SITE 2	Inorganics July 31, 2008	
August 21, 2009 Upstream	August 21, 2009 SITE 2		Hydra Reproduction August 23 – September 1, 2009
September 16, 2009 Upstream	September 16, 2009 SITE 2		Hydra Reproduction September 17 – 26, 2009
October 20, 2009 Upstream	October 20, 2009 SITE 2	Inorganics October 21, 2008	Hydra Reproduction October 24 – November 2, 2009
November 27, 2009 Upstream	November 27, 2009 SITE 2		Hydra Reproduction November 30 – December 8, 2009
December 7, 2009 Upstream	December 7, 2009 SITE 2		Hydra Reproduction December 8 – 16, 2009
January 22, 2010 Upstream	January 22, 2010 SITE 2	Inorganics January 22, 2010	Hydra Reproduction January 22 – 29, 2010
February 20, 2010 Upstream	February 20, 2010 SITE 2		Hydra Reproduction February 27 – March 6, 2010
April 20, 2010 Upstream	April 20, 2010 SITE 2		Hydra Reproduction April 22 – 29, 2010

Appendix 7

GPS co-ordinates of sampling sites. The co-ordinates correspond to the sites pictured on the maps in Figures 1& 3. Co-ordinates were obtained by field-readings taken during sample collections with a hand-held GPS unit.

Sampling Site	**GPS co-ordinates**
Harmony Creek Upstream	43 55‘40.6’’ N, 78 49'45.0’’ W
Harmony Creek Downstream	43 55‘04.4’’ N, 78 49‘59.6’’ W
Harmony Landfill Site 1	43 55‘23.9’’ N, 78 50‘07.0’’ W
Harmony Landfill Site 2	43 55‘24.0’’ N, 78 50’12.4’’ W

Appendix 8

Details of chemical analysis performed on Harmony Creek water and Harmony Landfill Leachate samples at the York-Durham Regional Environmental Laboratory.

Components	Analysis	Method Details
metals	ICP/MS	Thermo Electron Corporation X Series
cations/anions	IC	Dionex ICS-2000 with AS40 automated sampler **Anions column** – IonPac AS18 (4x250mm) **Cations column** – IonPac CS14 (4x250mm)
pH, alkalinity, conductivity	titralyzer	
organophosphorous/ triazine pesticides	GC/MS	**GC** – HP6890N with EPC **MS** – LECO Pegasus III TOF **Column** - VB-5 TOF, 40mX0.18mmX0.18um
VOCs	P&T GC/MS	**GC** – HP5890 Series II Plus with EPC **MS** – HP 5972A **Purge & Trap unit** – Tekmar Dohrmann Acqua Tek 70 Liquid Autosampler+ 3100Concentrator with VOCARB 3000 trap **Column** – DB-624, 20mX0.18mmX1.0um
chlorophenols, organochlorine pesticides, PCBs, phenoxy acid herbicides	GC/dual ECD	**GC** – HP5890 Series II Plus with EPC **Detector** – 2 ECD **Dual Columns** - DB-5, 30m x 0.25mm x 0.25um and DB-17, 30m x 0.25mm x 0.25um
Glyphosate	LC/MS/MS	**LC** – Agilent 1200 Series **MS-MS** - MDS SCIEX API 3200 QTrap **Column** – Thermo Scientific Hypercarb column, 50x2.1mm, 5um
Carburea	LC/MS/MS	**LC** – Agilent 1200 Series **MS-MS** - MDS SCIEX API 3200 QTrap **Column** – Zorbax Eclipse XDB-C18

Appendix 9

Key to numbered references used in Tables 1, 2 and 3.

1. James, 1977

2. Plotkin & Ram, 1984

3. Scrab *et al*., 1992

4. Brack *et al.*, 1998

5. Rutherford *et al*., 2000

6. Christensen *et al.*, 2001

7. Kjeldsen *et al.*, 2002

8. Ward *et al.*, 2002

9. Dewhurst *et al.*, 2003

10. Baun & Christensen, 2004

11. Svensson *et al.*, 2005

12. Osaki *et al.*, 2006

13. Øygard *et al.*, 2007

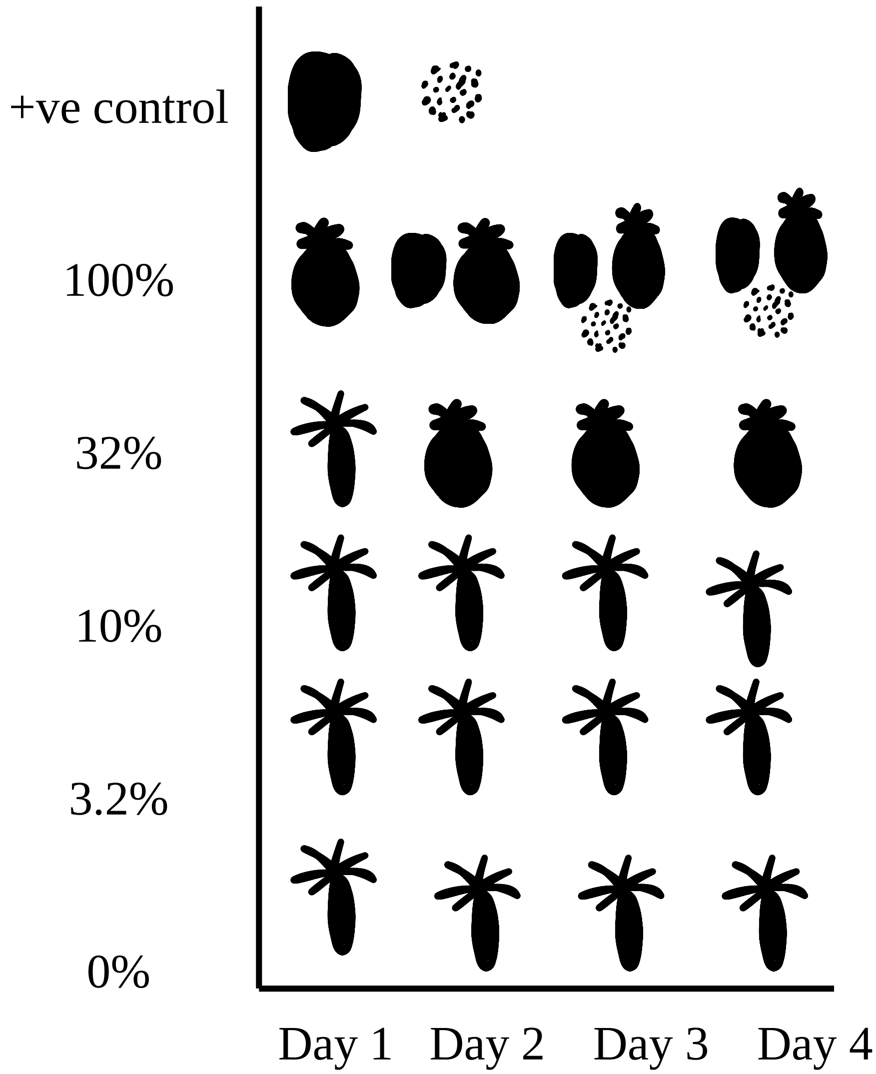
14. Ettler *et al.*, 2008

15. Öman & Junestedt, 2008

16. Olivero-Verbel *et al.*, 2008

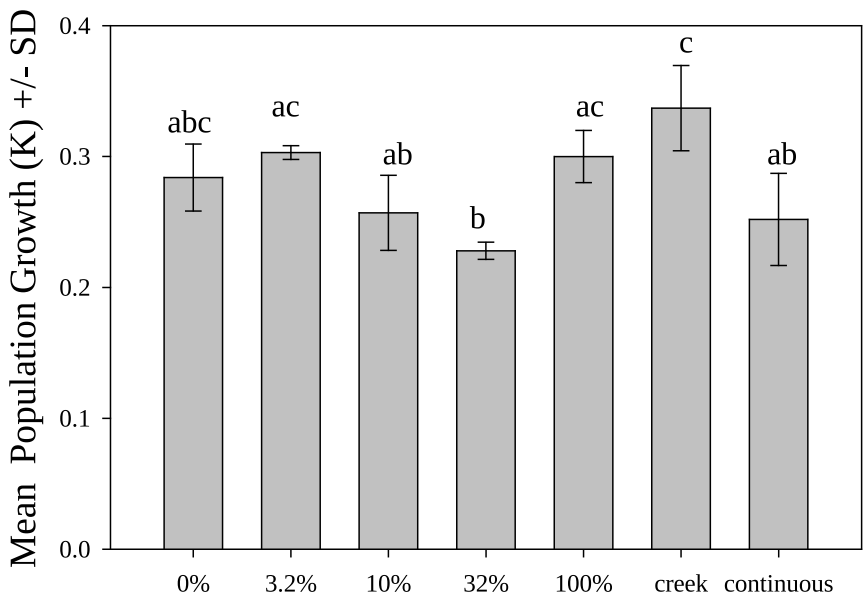
Appendix 10

Results of 96 h continuous exposure of *Hydra viridissima* to December 2008 Harmony Landfill leachate with copper sulphate as a positive control. The exposure was static-renewal and observations were taken every 24 hours. Ten non-budding Hydra were used for each treatment, in triplicate. Hydra were not fed during the experiment.
Test performed: April 26-30, 2010.



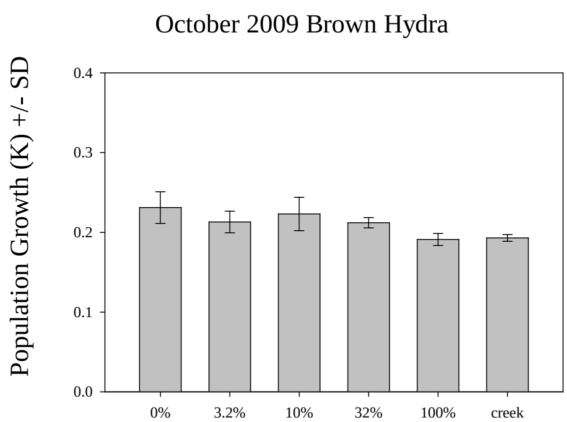
Appendix 11

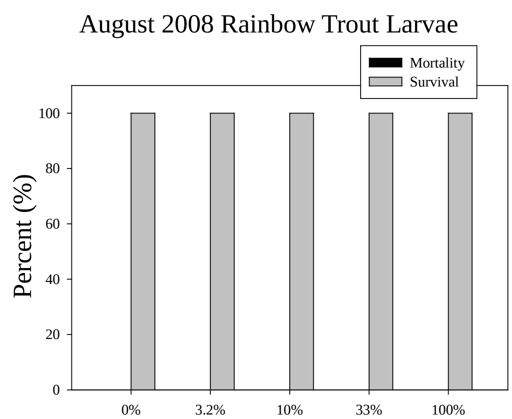
Additional results of the November 2009 leachate exposure to *Hydra viridissima* including the continuous exposure treatment. The continuous exposure was performed as a 7-day static renewal with 100% leachate. All other procedures were as per described in the experimental methods. The letters indicate statistically significant treatment groupings (ANOVA, Tukey's HSD, p=0.05).

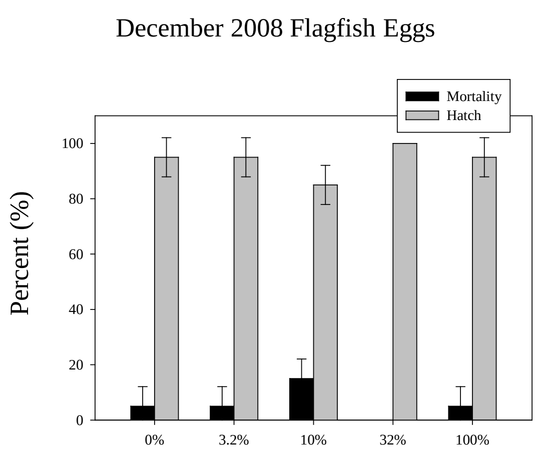


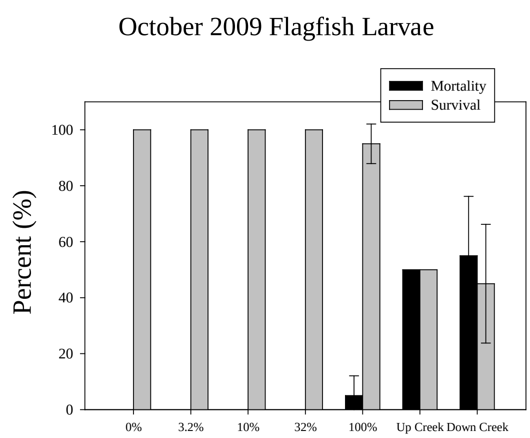
Appendix 12

Additional experiments. The Brown Hydra test was performed as per experimental protocol using October 2009 leachate and *Hydra littoralis* as a test species. The 7-day population growth is displayed. August 2008 leachate was used to expose 72 h post-hatch Rainbow Trout larvae. The test was static renewal with solutions changed every 24 hours. Trout were exposed for 96 h in beakers in a water bath at 11°C. There were 10 fish per treatment in duplicate. Shown are the results at 96h. The Flagfish Eggs were exposed 1 day post-fertilization until hatch at 25°C. The treatments were static renewal with renewals and observations made every 24 hours. There were 10 eggs per treatment in duplicate. The Flagfish larvae were exposed for 96 hours to October 2009 leachate at 25°C. The larvae were 1 day post-hatch and exposure was static renewal with observations and renewals every 24 hours. There were 10 fish per treatment in duplicate. Results at 96 hours are shown.









Appendix 13

Full chemical analysis results from samples collected on December 11, 2008.

York Region

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Regional Environmental Laboratory

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DURHAM REGION

INORGANICS ANALYSIS REPORT

Page 1 of 6

Work Order #: 2098380 **Submission #:** 137838

CLIENT INFORMATION

UOIT

Date Received: 2008-12-12
Date Printed: 2009-01-05
No. of Samples: 5

Sampled By: J. Guchardi

Report to:
John Guchardi
UOIT
2000 Simcoe Street North
Oshawa, ON
L1H 7K4

Comments:
Empty cell = Analysis not requested
< = Less than
^ = Result exceeds limit
MDL = Method detection limit
E = Sample exhausted
(R) = Resample

Sample I.D.:	428423
Waterworks No.:	N/A
Sample Location:	Aquatic Toxicology
Sub Location:	Landfill Upstream
Sample Type:	Raw
Date Sampled:	2008-12-11
Client/Field I.D.:	Site 4

Harmony Creek Upstream

	Analyte	MDL/Units	Limit	
Anions	Alkalinity as CaCO3	20.0 mg/L	500	281
	Bromide as Br	0.2 mg/L		<0.2
	Chloride as Cl	0.2 mg/L	250	142
	Fluoride as F	0.08 mg/L	1.5	<0.08
	Nitrate + Nitrite as N	0.06 mg/L	10.0	4.70
	Nitrate as N	0.06 mg/L	10.0	4.70
	Nitrite as N	0.05 mg/L	1.0	<0.05
	Phosphate as P	0.2 mg/L		<0.2
	Sulphate as SO4	0.3 mg/L	500	36.4
Calculations	Hardness as CaCO3	1.0 mg/L		371
	Ionic Balance	0.01 %		1.73
	Langelier Index	-2.0	neg. 2 to 2	1.9
	Total Anions	0.01 meq/L		10.7
	Total Cations	0.01 meq/L		11.1
	Calc. Conductivity	0.01 umho/cm		1160
	Calc. Dissolved Solids	20 mg/L		594
Cations	Calcium as Ca	0.3 mg/L		128
	Magnesium as Mg	0.06 mg/L		12.6
	Potassium as K	0.07 mg/L		2.73
	Sodium as Na	0.4 mg/L	20	82.4
	Ammonia +NH4 as N	0.05 mg/L		0.21
General 1	Colour	1 TCU	5	12
	pH (Units)	0.01 Units		8.15
	Conductivity	1.28 umho/cm		1080
	Turbidity	0.050 NTU	5	1.71
Mercury	Mercury as Hg	0.02 ug/L	1	<0.02
Metals Tests	Aluminum as Al	0.0002 mg/L	0.1	0.0863
	Antimony as Sb	0.0001 mg/L	0.006	0.0005
	Arsenic as As	0.0001 mg/L	0.025	0.0004
	Barium as Ba	0.0001 mg/L	1	0.0486
	Boron as B	0.005 mg/L	5	0.020
	Cadmium as Cd	0.0001 mg/L	0.005	<0.0001
	Chromium as Cr	0.0001 mg/L	0.05	<0.0001
	Cobalt as Co	0.0001 mg/L		0.0003
	Copper as Cu	0.0001 mg/L	1	0.0012
	Iron as Fe	0.0001 mg/L	0.3	0.151

YORK-DURHAM
Regional Environmental Laboratory

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INORGANICS ANALYSIS REPORT

Work Order #: 2098380 **Submission #:** 137838

428423
N/A
Aquatic Toxicology
Landfill Upstream
Raw
2008-12-11
Site 4

Metals Tests	Lead as Pb	0.0001 mg/L	0.010	0.0002
	Manganese as Mn	0.0001 mg/L	0.05	0.0574
	Molybdenum as Mo	0.0001 mg/L		0.0005
	Nickel as Ni	0.0001 mg/L		0.0004
	Selenium as Se	0.0001 mg/L	0.01	0.0006
	Uranium as U	0.0001 mg/L	0.02	0.0011
	Zinc as Zn	0.0001 mg/L	5	0.0017

Comments:

Empty cell = Analysis not requested
< = Less than
^ = Result exceeds limit
MDL = Method detection limit
E = Sample exhausted
(R) = Resample

Sample I.D.: 428424
Waterworks No.: N/A
Sample Location: Aquatic Toxicology
Sub Location: Landfill Downstream
Sample Type: Raw
Date Sampled: 2008-12-11
Client/Field I.D.: Site 5

Harmony Creek Downstream

	Analyte	MDL/Units	Limit	
Anions	Alkalinity as CaCO3	20.0 mg/L	500	283
	Bromide as Br	0.2 mg/L		<0.2
	Chloride as Cl	0.2 mg/L	250	138
	Fluoride as F	0.08 mg/L	1.5	<0.08
	Nitrate + Nitrite as N	0.06 mg/L	10.0	3.80
	Nitrate as N	0.06 mg/L	10.0	3.80
	Nitrite as N	0.05 mg/L	1.0	<0.05
	Phosphate as P	0.2 mg/L		<0.2
	Sulphate as SO4	0.3 mg/L	500	35.0
Calculations	Hardness as CaCO3	1.0 mg/L		365
	Ionic Balance	0.01 %		1.68
	Langelier Index	-2.0	neg. 2 to 2	1.9
	Total Anions	0.01 meq/L		10.5
	Total Cations	0.01 meq/L		10.9
	Calc. Conductivity	0.01 umho/cm		1130
	Calc. Dissolved Solids	20 mg/L		581
Cations	Calcium as Ca	0.3 mg/L		126
	Magnesium as Mg	0.06 mg/L		12.4
	Potassium as K	0.07 mg/L		2.72
	Sodium as Na	0.4 mg/L	20	81.2
	Ammonia +NH4 as N	0.05 mg/L		0.21
General 1	Colour	1 TCU	5	12
	pH (Units)	0.01 Units		8.20
	Conductivity	1.28 umho/cm		1060
	Turbidity	0.050 NTU	5	1.91
Mercury	Mercury as Hg	0.02 ug/L	1	<0.02
Metals Tests	Aluminum as Al	0.0002 mg/L	0.1	0.0937
	Antimony as Sb	0.0001 mg/L	0.006	0.0005
	Arsenic as As	0.0001 mg/L	0.025	0.0004
	Barium as Ba	0.0001 mg/L	1	0.0478
	Boron as B	0.005 mg/L	5	0.030
	Cadmium as Cd	0.0001 mg/L	0.005	<0.0001
	Chromium as Cr	0.0001 mg/L	0.05	<0.0001
	Cobalt as Co	0.0001 mg/L		0.0004

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INORGANICS ANALYSIS REPORT

Work Order #: 2098380 **Submission #:** 137838

				428424 N/A Aquatic Toxicology Landfill Downstream Raw 2008-12-11 Site 5
Metals Tests	Copper as Cu	0.0001 mg/L	1	0.0012
	Iron as Fe	0.0001 mg/L	0.3	0.161
	Lead as Pb	0.0001 mg/L	0.010	0.0002
	Manganese as Mn	0.0001 mg/L	0.05	0.0454
	Molybdenum as Mo	0.0001 mg/L		0.0005
	Nickel as Ni	0.0001 mg/L		0.0010
	Selenium as Se	0.0001 mg/L	0.01	0.0005
	Uranium as U	0.0001 mg/L	0.02	0.0011
	Zinc as Zn	0.0001 mg/L	5	0.0053

Comments:

Empty cell = Analysis not requested
< = Less than
^ = Result exceeds limit
MDL = Method detection limit
E = Sample exhausted
(R) = Resample

Sample I.D.: 428428
Waterworks No.: N/A
Sample Location: Aquatic Toxicology
Sub Location: Perculate
Sample Type: Raw
Date Sampled: 2008-12-11
Client/Field I.D.: Site 9

Harmony Landfill Site 1

	Analyte	MDL/Units	Limit	
Metals Tests	Aluminum as Al	0.0002 mg/L	0.1	0.0201
	Antimony as Sb	0.0001 mg/L	0.006	0.0038
	Arsenic as As	0.0001 mg/L	0.025	0.0029
	Barium as Ba	0.0001 mg/L	1	0.981
	Boron as B	0.005 mg/L	5	0.584
	Cadmium as Cd	0.0001 mg/L	0.005	<0.0001
	Chromium as Cr	0.0001 mg/L	0.05	0.0003
	Cobalt as Co	0.0001 mg/L		0.0028
	Copper as Cu	0.0001 mg/L	1	0.0033
	Iron as Fe	0.0001 mg/L	0.3	61.6
	Lead as Pb	0.0001 mg/L	0.010	0.0033
	Manganese as Mn	0.0001 mg/L	0.05	0.866
	Molybdenum as Mo	0.0001 mg/L		0.0006
	Nickel as Ni	0.0001 mg/L		0.0306
	Selenium as Se	0.0001 mg/L	0.01	0.0013
	Uranium as U	0.0001 mg/L	0.02	0.0001
	Zinc as Zn	0.0001 mg/L	5	0.124

Approved By:

B Gupta

Brij Gupta, Group Leader (Ext 4326)

J. Mirsch

J. Mirsch, Superintendent (Ext 4304)

ASSOCIATION OF THE CHEMICAL PROFESSION OF ONTARIO
CHARTERED
Jurgen Mirsch
CHEMIST

York Region

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DURHAM REGION

ORGANICS ANALYSIS REPORT

Work Order #: 2098380

Submission #: 137838

CLIENT INFORMATION

UOIT
Sampled By: J. Guchardi

Date Received: 2008-12-12
Date Printed: 2009-01-07
No. of Samples: 6

Report to:

John Guchardi
UOIT
2000 Simcoe Street North
Oshawa, ON
L1H 7K4

Comments:

Empty cell = Analysis not requested
< = Less than
^ = Result exceeds limit
MDL = Method detection limit
E = Sample exhausted
I = Possible interference interpret with caution

			Sample I.D.:	428423
			Waterworks No.:	N/A
			Sample Location:	Aquatic Toxicology
			Sub Location:	Landfill Upstream
			Sample Type:	Raw
			Date Sampled:	2008-12-11
			Client/Field I.D.:	Site 4
	Analyte	MDL/Units	Limit	Harmony Creek Upstream
CarbUrea	Aldicarb	6.0 ug/L	9	<6.0
	Bendiocarb	3.0 ug/L	40	<3.0
	Carbaryl	3.0 ug/L	90	<3.0
	Carbofuran	3.0 ug/L	90	<3.0
	Diuron	3.0 ug/L	150	<3.0
	Triallate	4.0 ug/L	230	<4.0
Chlorophenols (Chlorphen)	2,3,4,5-tetrachlorophenol	0.4 ug/L		<0.4
	2,3,4,6-tetrachlorophenol	0.5 ug/L	100	<0.5
	2,3,4-trichlorophenol	0.4 ug/L		<0.4
	2,3,5,6-tetrachlorophenol	0.4 ug/L		<0.4
	2,4,5-trichlorophenol	0.4 ug/L		<0.4
	2,4,6-trichlorophenol	0.5 ug/L	5	<0.5
	2,4-dichlorophenol	0.4 ug/L	900	<0.4
	Pentachlorophenol	0.4 ug/L	60	<0.4
Glyphosate and metabolites	Aminomethyl phosphonic acid (AMPA)	12.5 ug/L		<12.5
	Glyphosate	25 ug/L	280	<25
Organochlorine Pesticides (OCs)	(DDT) + Metabolites	0.008 ug/L	30	<0.008
	a-chlordane	0.006 ug/L		<0.006
	Aldrin	0.006 ug/L		<0.006
	Aldrin + Dieldrin	0.006 ug/L	0.7	<0.006
	alpha-BHC	0.006 ug/L		<0.006
	beta-BHC	0.005 ug/L		<0.005
	Chlordane (Total)	0.006 ug/L	7	<0.006
	Dieldrin	0.005 ug/L		<0.005
	Endosulphan I	0.005 ug/L		<0.005
	Endosulphan II	0.005 ug/L		<0.005
	Endosulphan sulphate	0.005 ug/L		<0.005
	Endrin	0.005 ug/L		<0.005
	g-chlordane	0.005 ug/L		<0.005
	Heptachlor	0.006 ug/L		<0.006
	Heptachlor + Heptachlor Epoxide	0.008 ug/L	3	<0.008
	Heptachlor epoxide	0.008 ug/L		<0.008
	Lindane	0.005 ug/L	4	<0.005
	Methoxychlor	0.009 ug/L	900	<0.009
	Mirex	0.006 ug/L		<0.006
	op-DDT	0.005 ug/L		<0.005

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Regional Environmental Laboratory
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ORGANICS ANALYSIS REPORT

Work Order #: 2098380 **Submission #:** 137838

				428423 N/A Aquatic Toxicology Landfill Upstream Raw 2008-12-11 Site 4
Organochlorine Pesticides (OCs)	Oxychlordane	0.005 ug/L		<0.005
	pp-DDD	0.005 ug/L		<0.005
	pp-DDE	0.006 ug/L		<0.006
	pp-DDT	0.008 ug/L		<0.008
	Toxaphene	0.5 ug/L		<0.5
	Trifluralin	0.006 ug/L	45	<0.006
Organophosphorus Pesticides (OPs)	Azinphos-methyl	0.3 ug/L	20	<0.3
	Benzo(a)pyrene	0.01 ug/L	0.01	<0.01
	Chlorpyrifos	0.2 ug/L	90	<0.2
	Chlorpyrifos-methyl (Reldan)	0.2 ug/L		<0.2
	Diazinon	0.2 ug/L	20	<0.2
	Dichlorvos	0.5 ug/L		<0.5
	Dimethoate	0.3 ug/L	20	<0.3
	Ethion	0.2 ug/L		<0.2
	Fenchlorphos (Ronnel)	0.2 ug/L		<0.2
	Malathion	0.2 ug/L	190	<0.2
	Methyl parathion	0.2 ug/L		<0.2
	Mevinphos (Phosdrin)	0.3 ug/L		<0.3
	Parathion	0.2 ug/L	50	<0.2
	Phorate	0.2 ug/L	2	<0.2
	Temephos	3 ug/L	280	<3
	Terbufos	0.2 ug/L	1	<0.2
PCBs	Polychlorinated Biphenyls (PCBs)	0.02 ug/L	3	<0.02
Phenoxy Acid Herbicides (Phenacid)	2,4,5-trichlorophenoxyacetic acid (2,4,5-T)	0.5 ug/L	280	<0.5
	2,4-dichlorophenoxyacetic acid (2,4-D)	0.8 ug/L	100	<0.8
	2,4-dichlorphenoxybutyric acid (2,4-DB)	0.4 ug/L		<0.4
	2,4-dichlorphenoxypropionic acid (2,4-DP)	0.5 ug/L		<0.5
	Bromoxynil	0.4 ug/L	5	<0.4
	Dicamba	0.4 ug/L	120	<0.4
	Diclofop-methyl	0.4 ug/L	9	<0.4
	Dinoseb	0.5 ug/L	10	<0.5
	Picloram	0.7 ug/L	190	<0.7
	Silvex (2,4,5-TP)	0.4 ug/L		<0.4
Triazine Pesticides (Triaz)	Alachlor	0.4 ug/L	5	<0.4
	Ametryn	0.4 ug/L		<0.4
	Atraton	0.2 ug/L		<0.2
	Atrazine	0.2 ug/L		<0.2
	Atrazine + N-dealkylated metabolites	0.2 ug/L	5	<0.2
	Cyanazine	0.3 ug/L	10	<0.3
	De-ethylated atrazine	0.2 ug/L		<0.2
	Desethyl simazine	0.2 ug/L		<0.2
	Metolachlor	0.2 ug/L	50	<0.2
	Metribuzin	0.3 ug/L	80	<0.3
	Prometon	0.2 ug/L		<0.2
	Prometryne	0.2 ug/L	1	<0.2
	Propazine	0.2 ug/L		<0.2
	Simazine	0.2 ug/L	10	<0.2
Volatile Organic Compounds (VOC)	1,1,1-trichloroethane	0.2 ug/L		<0.2
	1,1,2,2-tetrachloroethane	0.3 ug/L		<0.3
	1,1,2-trichloroethane	0.1 ug/L		<0.1

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Regional Environmental Laboratory

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ORGANICS ANALYSIS REPORT

Work Order #: 2098380 **Submission #:** 137838

				428423 N/A Aquatic Toxicology Landfill Upstream Raw 2008-12-11 Site 4
Volatile Organic Compounds (VOC)	1,1-dichloroethane	0.1 ug/L		<0.1
	1,1-dichloroethylene (vinylidene chloride)	0.3 ug/L	14	<0.3
	1,2-dibromoethane	0.2 ug/L		<0.2
	1,2-dichlorobenzene	0.1 ug/L	200	<0.1
	1,2-dichloroethane	0.1 ug/L	5	<0.1
	1,2-dichloropropane	0.1 ug/L		<0.1
	1,3-dichlorobenzene	0.1 ug/L		<0.1
	1,4-dichlorobenzene	0.1 ug/L	5	<0.1
	Benzene	0.1 ug/L	5	<0.1
	Bromodichloromethane	0.1 ug/L		<0.1
	Bromoform	0.2 ug/L		<0.2
	Bromomethane	0.5 ug/L		<0.5
	Carbon tetrachloride	0.2 ug/L	5	<0.2
	Chloroethane	0.2 ug/L		<0.2
	Chloroform	0.1 ug/L		<0.1
	Chloromethane	0.2 ug/L		<0.2
	cis-1,2-dichloroethylene	0.1 ug/L		<0.1
	cis-1,3-dichloropropylene	0.2 ug/L		<0.2
	Dibromochloromethane	0.1 ug/L		<0.1
	Dichloromethane	0.5 ug/L	50	<0.5
	Ethylbenzene	0.1 ug/L	2.4	<0.1
	m/p-xylene	0.2 ug/L		<0.2
	methyl-t-butyl ether	0.1 ug/L		<0.1
	Monochlorobenzene	0.1 ug/L	80	<0.1
	o-xylene	0.1 ug/L		<0.1
	Styrene	0.2 ug/L		<0.2
	Tetrachloroethylene (perchloroethylene)	0.3 ug/L	30	<0.3
	Toluene	0.2 ug/L	24	<0.2
	trans-1,2-dichloroethylene	0.1 ug/L		<0.1
	trans-1,3-dichloropropylene	0.1 ug/L		<0.1
	Trichloroethylene	0.1 ug/L	5	<0.1
	Trichlorofluoromethane	0.3 ug/L		<0.3
	Trihalomethanes (total)	0.2 ug/L	100	<0.2
	Vinyl Chloride	0.2 ug/L	2	<0.2
	Xylene; total	0.2 ug/L	300	<0.2

Comments:

Empty cell = Analysis not requested
< = Less than
^ = Result exceeds limit
MDL = Method detection limit
E = Sample exhausted
I = Possible interference interpret with caution

Harmony Creek Downstream

	Analyte	MDL/Units	Limit	Sample I.D.: 428424 Waterworks No.: N/A Sample Location: Aquatic Toxicology Sub Location: Landfill Downstream Sample Type: Raw Date Sampled: 2008-12-11 Client/Field I.D.: Site 5
CarbUrea	Aldicarb	6.0 ug/L	9	<6.0
	Bendiocarb	3.0 ug/L	40	<3.0
	Carbaryl	3.0 ug/L	90	<3.0
	Carbofuran	3.0 ug/L	90	<3.0
	Diuron	3.0 ug/L	150	<3.0
	Triallate	4.0 ug/L	230	<4.0

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ORGANICS ANALYSIS REPORT

Work Order #: 2098380 **Submission #:** 137838

				428424 N/A Aquatic Toxicology Landfill Downstream Raw 2008-12-11 Site 5
Chlorophenols (Chlorphen)	2,3,4,5-tetrachlorophenol	0.4 ug/L		<0.4
	2,3,4,6-tetrachlorophenol	0.5 ug/L	100	<0.5
	2,3,4-trichlorophenol	0.4 ug/L		<0.4
	2,3,5,6-tetrachlorophenol	0.4 ug/L		<0.4
	2,4,5-trichlorophenol	0.4 ug/L		<0.4
	2,4,6-trichlorophenol	0.5 ug/L	5	<0.5
	2,4-dichlorophenol	0.4 ug/L	900	<0.4
	Pentachlorophenol	0.4 ug/L	60	<0.4
Glyphosate and metabolites	Aminomethyl phosphonic acid (AMPA)	12.5 ug/L		<12.5
	Glyphosate	25 ug/L	280	<25
Organochlorine Pesticides (OCs)	(DDT) + Metabolites	0.008 ug/L	30	<0.008
	a-chlordane	0.006 ug/L		<0.006
	Aldrin	0.006 ug/L		<0.006
	Aldrin + Dieldrin	0.006 ug/L	0.7	<0.006
	alpha-BHC	0.006 ug/L		<0.006
	beta-BHC	0.005 ug/L		<0.005
	Chlordane (Total)	0.006 ug/L	7	<0.006
	Dieldrin	0.005 ug/L		<0.005
	Endosulphan I	0.005 ug/L		<0.005
	Endosulphan II	0.005 ug/L		<0.005
	Endosulphan sulphate	0.005 ug/L		<0.005
	Endrin	0.005 ug/L		<0.005
	g-chlordane	0.005 ug/L		<0.005
	Heptachlor	0.006 ug/L		<0.006
	Heptachlor + Heptachlor Epoxide	0.008 ug/L	3	<0.008
	Heptachlor epoxide	0.008 ug/L		<0.008
	Lindane	0.005 ug/L	4	<0.005
	Methoxychlor	0.009 ug/L	900	<0.009
	Mirex	0.006 ug/L		<0.006
	op-DDT	0.005 ug/L		<0.005
	Oxychlordane	0.005 ug/L		<0.005
	pp-DDD	0.005 ug/L		<0.005
	pp-DDE	0.006 ug/L		<0.006
	pp-DDT	0.008 ug/L		<0.008
	Toxaphene	0.5 ug/L		<0.5
	Trifluralin	0.006 ug/L	45	<0.006
Organophosphorus Pesticides (OPs)	Azinphos-methyl	0.3 ug/L	20	<0.3
	Benzo(a)pyrene	0.01 ug/L	0.01	<0.01
	Chlorpyrifos	0.2 ug/L	90	<0.2
	Chlorpyrifos-methyl (Reldan)	0.2 ug/L		<0.2
	Diazinon	0.2 ug/L	20	<0.2
	Dichlorvos	0.5 ug/L		<0.5
	Dimethoate	0.3 ug/L	20	<0.3
	Ethion	0.2 ug/L		<0.2
	Fenchlorphos (Ronnel)	0.2 ug/L		<0.2
	Malathion	0.2 ug/L	190	<0.2
	Methyl parathion	0.2 ug/L		<0.2
	Mevinphos (Phosdrin)	0.3 ug/L		<0.3
	Parathion	0.2 ug/L	50	<0.2
	Phorate	0.2 ug/L	2	<0.2

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ORGANICS ANALYSIS REPORT

Work Order #: 2098380 **Submission #:** 137838

				428424 N/A Aquatic Toxicology Landfill Downstream Raw 2008-12-11 Site 5
Organophosphorus Pesticides (OPs)	Temephos	3 ug/L	280	<3
	Terbufos	0.2 ug/L	1	<0.2
PCBs	Polychlorinated Biphenyls (PCBs)	0.02 ug/L	3	<0.02
Phenoxy Acid Herbicides (Phenacid)	2,4,5-trichlorophenoxyacetic acid (2,4,5-T)	0.5 ug/L	280	<0.5
	2,4-dichlorophenoxyacetic acid (2,4-D)	0.8 ug/L	100	<0.8
	2,4-dichlorphenoxybutyric acid (2,4-DB)	0.4 ug/L		<0.4
	2,4-dichlorphenoxypropionic acid (2,4-DP)	0.5 ug/L		<0.5
	Bromoxynil	0.4 ug/L	5	<0.4
	Dicamba	0.4 ug/L	120	<0.4
	Diclofop-methyl	0.4 ug/L	9	<0.4
	Dinoseb	0.5 ug/L	10	<0.5
	Picloram	0.7 ug/L	190	<0.7
	Silvex (2,4,5-TP)	0.4 ug/L		<0.4
Triazine Pesticides (Triaz)	Alachlor	0.4 ug/L	5	<0.4
	Ametryn	0.4 ug/L		<0.4
	Atraton	0.2 ug/L		<0.2
	Atrazine	0.2 ug/L		<0.2
	Atrazine + N-dealkylated metabolites	0.2 ug/L	5	<0.2
	Cyanazine	0.3 ug/L	10	<0.3
	De-ethylated atrazine	0.2 ug/L		<0.2
	Desethyl simazine	0.2 ug/L		<0.2
	Metolachlor	0.2 ug/L	50	<0.2
	Metribuzin	0.3 ug/L	80	<0.3
	Prometon	0.2 ug/L		<0.2
	Prometryne	0.2 ug/L	1	<0.2
	Propazine	0.2 ug/L		<0.2
	Simazine	0.2 ug/L	10	<0.2
Volatile Organic Compounds (VOC)	1,1,1-trichloroethane	0.2 ug/L		<0.2
	1,1,2,2-tetrachloroethane	0.3 ug/L		<0.3
	1,1,2-trichloroethane	0.1 ug/L		<0.1
	1,1-dichloroethane	0.1 ug/L		<0.1
	1,1-dichloroethylene (vinylidene chloride)	0.3 ug/L	14	<0.3
	1,2-dibromoethane	0.2 ug/L		<0.2
	1,2-dichlorobenzene	0.1 ug/L	200	<0.1
	1,2-dichloroethane	0.1 ug/L	5	<0.1
	1,2-dichloropropane	0.1 ug/L		<0.1
	1,3-dichlorobenzene	0.1 ug/L		<0.1
	1,4-dichlorobenzene	0.1 ug/L	5	<0.1
	Benzene	0.1 ug/L	5	<0.1
	Bromodichloromethane	0.1 ug/L		<0.1
	Bromoform	0.2 ug/L		<0.2
	Bromomethane	0.5 ug/L		<0.5
	Carbon tetrachloride	0.2 ug/L	5	<0.2
	Chloroethane	0.2 ug/L		<0.2
	Chloroform	0.1 ug/L		<0.1
	Chloromethane	0.2 ug/L		<0.2
	cis-1,2-dichloroethylene	0.1 ug/L		<0.1
	cis-1,3-dichloropropylene	0.2 ug/L		<0.2
	Dibromochloromethane	0.1 ug/L		<0.1
	Dichloromethane	0.5 ug/L	50	<0.5

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ORGANICS ANALYSIS REPORT

Work Order #: 2098380 **Submission #:** 137838

				428424 N/A Aquatic Toxicology Landfill Downstream Raw 2008-12-11 Site 5
Volatile Organic Compounds (VOC)	Ethylbenzene	0.1 ug/L	2.4	<0.1
	m/p-xylene	0.2 ug/L		<0.2
	methyl-t-butyl ether	0.1 ug/L		<0.1
	Monochlorobenzene	0.1 ug/L	80	<0.1
	o-xylene	0.1 ug/L		<0.1
	Styrene	0.2 ug/L		<0.2
	Tetrachloroethylene (perchloroethylene)	0.3 ug/L	30	<0.3
	Toluene	0.2 ug/L	24	<0.2
	trans-1,2-dichloroethylene	0.1 ug/L		<0.1
	trans-1,3-dichloropropylene	0.1 ug/L		<0.1
	Trichloroethylene	0.1 ug/L	5	<0.1
	Trichlorofluoromethane	0.3 ug/L		<0.3
	Trihalomethanes (total)	0.2 ug/L	100	<0.2
	Vinyl Chloride	0.2 ug/L	2	<0.2
	Xylene; total	0.2 ug/L	300	<0.2

Comments:

- Empty cell = Analysis not requested
- < = Less than
- ^ = Result exceeds limit
- MDL = Method detection limit
- E = Sample exhausted
- I = Possible interference interpret with caution

Harmony Landfill Site 1

	Analyte	MDL/Units	Limit	Sample I.D.: 428428 Waterworks No.: N/A Sample Location: Aquatic Toxicology Sub Location: Perculate Sample Type: Raw Date Sampled: 2008-12-11 Client/Field I.D.: Site 9
Volatile Organic Compounds (VOC)	1,1,1-trichloroethane	0.2 ug/L		<0.2
	1,1,2,2-tetrachloroethane	0.3 ug/L		<0.3
	1,1,2-trichloroethane	0.1 ug/L		<0.1
	1,1-dichloroethane	0.1 ug/L		0.2
	1,1-dichloroethylene (vinylidene chloride)	0.3 ug/L	14	<0.3
	1,2-dibromoethane	0.2 ug/L		<0.2
	1,2-dichlorobenzene	0.1 ug/L	200	0.3
	1,2-dichloroethane	0.1 ug/L	5	1.0
	1,2-dichloropropane	0.1 ug/L		<0.1
	1,3-dichlorobenzene	0.1 ug/L		0.2
	1,4-dichlorobenzene	0.1 ug/L	5	0.9
	Benzene	0.1 ug/L	5	15
	Bromodichloromethane	0.1 ug/L		<0.1
	Bromoform	0.2 ug/L		<0.2
	Bromomethane	0.5 ug/L		<0.5
	Carbon tetrachloride	0.2 ug/L	5	<0.2
	Chloroethane	0.2 ug/L		<0.2
	Chloroform	0.1 ug/L		<0.1
	Chloromethane	0.2 ug/L		<0.2
	cis-1,2-dichloroethylene	0.1 ug/L		0.2
	cis-1,3-dichloropropylene	0.2 ug/L		<0.2
	Dibromochloromethane	0.1 ug/L		<0.1
	Dichloromethane	0.5 ug/L	50	<0.5
	Ethylbenzene	0.1 ug/L	2.4	0.2
	m/p-xylene	0.2 ug/L		6.3
	methyl-t-butyl ether	0.1 ug/L		<0.1
	Monochlorobenzene	0.1 ug/L	80	15
	o-xylene	0.1 ug/L		0.6
	Styrene	0.2 ug/L		<0.2

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ORGANICS ANALYSIS REPORT

Work Order #: 2098380 **Submission #:** 137838

				428428 N/A Aquatic Toxicology Perculate Raw 2008-12-11 Site 9
Volatile Organic Compounds (VOC)	Tetrachloroethylene (perchloroethylene)	0.3 ug/L	30	<0.3
	Toluene	0.2 ug/L	24	0.6
	trans-1,2-dichloroethylene	0.1 ug/L		<0.1
	trans-1,3-dichloropropylene	0.1 ug/L		<0.1
	Trichloroethylene	0.1 ug/L	5	<0.1
	Trichlorofluoromethane	0.3 ug/L		<0.3
	Trihalomethanes (total)	0.2 ug/L	100	<0.2
	Vinyl Chloride	0.2 ug/L	2	<0.2
	Xylene; total	0.2 ug/L	300	6.9

Approved By:

Marco Giuliacci, Group Leader (Ext 4321)

J. Mirsch, Superintendent (Ext 4304)

Appendix 14

Full chemical analysis results from samples collected on May 21, 2009.

York Region

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DURHAM REGION

INORGANICS ANALYSIS REPORT

Page 1 of 2

Work Order #: 2105778 **Submission #:** 146380

CLIENT INFORMATION

UOIT

Date Received: 2009-06-02
Date Printed: 2009-06-09
No. of Samples: 1

Sampled By: C. Ginou

Report to:
John Guchardi
UOIT
2000 Simcoe Street North
Oshawa
L1H 7K4

Comments:
Empty cell = Analysis not requested
< = Less than
^ = Result exceeds limit
MDL = Method detection limit
E = Sample exhausted
(R) = Resample

Sample I.D.:	455140
Waterworks No.:	N/A
Sample Location:	Landfill Study
Sub Location:	Leachate Site 2
Sample Type:	Raw
Date Sampled:	2009-05-21
Client/Field I.D.:	1

Harmony Landfill Site 2

	Analyte	MDL/Units	Limit	Result
Anions	Alkalinity as CaCO3	20.0 mg/L		421
	Bromide as Br	0.05 mg/L		<0.05
	Chloride as Cl	0.1 mg/L		30.2
	Fluoride as F	0.01 mg/L		0.09
	Nitrate + Nitrite as N	0.06 mg/L		0.24
	Nitrate as N	0.01 mg/L		0.21
	Nitrite as N	0.01 mg/L		0.03
	Phosphate as P	0.01 mg/L		<0.01
	Sulphate as SO4	0.1 mg/L		24.5
Calculations	Hardness as CaCO3	1.0 mg/L		425
	Ionic Balance	0.01 %		0.30
	Langelier Index	-2.0		1.2
	Total Anions	0.01 meq/L		9.80
	Total Cations	0.01 meq/L		9.85
	Calc. Conductivity	0.01 umho/cm		942
	Calc. Dissolved Solids	20 mg/L		500
Cations	Calcium as Ca	0.3 mg/L		143
	Magnesium as Mg	0.06 mg/L		16.6
	Potassium as K	0.07 mg/L		3.14
	Sodium as Na	0.4 mg/L		29.3
	Ammonia +NH4 as N	0.05 mg/L		0.15
General 1	Colour	1 TCU		15
	pH (Units)	0.01 Units		7.58
	Conductivity	1.28 umho/cm		880
	Turbidity	0.050 NTU		0.986
Metals Tests	Aluminum as Al	0.0002 mg/L		0.0218
	Antimony as Sb	0.0001 mg/L		0.0016
	Arsenic as As	0.0001 mg/L		0.0006
	Cadmium as Cd	0.0001 mg/L		<0.0001
	Chromium as Cr	0.0001 mg/L		<0.0001
	Cobalt as Co	0.0001 mg/L		0.0007
	Copper as Cu	0.0001 mg/L		0.0012
	Iron as Fe	0.0001 mg/L		2.84
	Lead as Pb	0.0001 mg/L		0.0005
	Manganese as Mn	0.0001 mg/L		0.179
	Molybdenum as Mo	0.0001 mg/L		0.0005

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INORGANICS ANALYSIS REPORT

Work Order #: 2105778 **Submission #:** 146380

			455140 N/A Landfill Study Leachate Site 2 Raw 2009-05-21 1
Metals Tests	Nickel as Ni	0.0001 mg/L	0.0048
	Selenium as Se	0.0001 mg/L	0.0007
	Zinc as Zn	0.0001 mg/L	0.0052

Approved By:

Brij Gupta, Group Leader (Ext 4326)

J. Mirsch, Superintendent (Ext 4304)

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DURHAM REGION

ORGANICS ANALYSIS REPORT

Page 1 of 2

Work Order #: 2105778 **Submission #:** 146380

CLIENT INFORMATION

UOIT
Sampled By: C. Ginou

Date Received: 2009-06-02
Date Printed: 2009-06-08
No. of Samples: 1

Report to:

John Guchardi
UOIT
2000 Simcoe Street North
Oshawa
L1H 7K4

Comments:

Empty cell = Analysis not requested
< = Less than
^ = Result exceeds limit
MDL = Method detection limit
E = Sample exhausted
I = Possible interference interpret with caution

Sample I.D.:	455140		Harmony Landfill Site 2
Waterworks No.:	N/A		
Sample Location:	Landfill Study		
Sub Location:	Leachate Site 2		
Sample Type:	Raw		
Date Sampled:	2009-05-21		
Client/Field I.D.:	1		

	Analyte	MDL/Units	Limit	
Volatile Organic Compounds (VOC)	1,1,1-trichloroethane	0.2 ug/L		<0.2
	1,1,2,2-tetrachloroethane	0.3 ug/L		<0.3
	1,1,2-trichloroethane	0.1 ug/L		<0.1
	1,1-dichloroethane	0.1 ug/L		<0.1
	1,1-dichloroethylene (vinylidene chloride)	0.3 ug/L	14	<0.3
	1,2-dibromoethane	0.2 ug/L		<0.2
	1,2-dichlorobenzene	0.1 ug/L	200	<0.1
	1,2-dichloroethane	0.1 ug/L	5	<0.1
	1,2-dichloropropane	0.1 ug/L		<0.1
	1,3-dichlorobenzene	0.1 ug/L		<0.1
	1,4-dichlorobenzene	0.1 ug/L	5	<0.1
	Benzene	0.1 ug/L	5	<0.1
	Bromodichloromethane	0.1 ug/L		<0.1
	Bromoform	0.2 ug/L		<0.2
	Bromomethane	0.5 ug/L		<0.5
	Carbon tetrachloride	0.2 ug/L	5	<0.2
	Chloroethane	0.2 ug/L		<0.2
	Chloroform	0.1 ug/L		<0.1
	Chloromethane	0.2 ug/L		<0.2
	cis-1,2-dichloroethylene	0.1 ug/L		<0.1
	cis-1,3-dichloropropylene	0.2 ug/L		<0.2
	Dibromochloromethane	0.1 ug/L		<0.1
	Dichloromethane	0.5 ug/L	50	<0.5
	Ethylbenzene	0.1 ug/L	2.4	<0.1
	m/p-xylene	0.2 ug/L		<0.2
	methyl-t-butyl ether	0.1 ug/L		<0.1
	Monochlorobenzene	0.1 ug/L	80	<0.1
	o-xylene	0.1 ug/L		<0.1
	Styrene	0.2 ug/L		<0.2
	Tetrachloroethylene (perchloroethylene)	0.3 ug/L	30	<0.3
	Toluene	0.2 ug/L	24	<0.2
	trans-1,2-dichloroethylene	0.1 ug/L		<0.1
	trans-1,3-dichloropropylene	0.1 ug/L		<0.1
	Trichloroethylene	0.1 ug/L	5	<0.1
	Trichlorofluoromethane	0.3 ug/L		<0.3
	Trihalomethanes (total)	0.2 ug/L	100	<0.2

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ORGANICS ANALYSIS REPORT

Work Order #: 2105778

Submission #: 146380

				455140 N/A Landfill Study Leachate Site 2 Raw 2009-05-21 1
Volatile Organic Compounds (VOC)	Vinyl Chloride	0.2 ug/L	2	<0.2
	Xylene; total	0.2 ug/L	300	<0.2

Approved By:

Marco Giuliacci, Group Leader (Ext 4321)

J. Mirsch, Superintendent (Ext 4304)

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Jurgen Mirsch
CHEMIST

Appendix 15

Full chemical analysis results from samples collected on July 30, 2009.

York Region

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INORGANICS ANALYSIS REPORT

Page 1 of 2

Work Order #: 2109017 **Submission #:** 149522

CLIENT INFORMATION

UOIT

Date Received: 2009-07-31
Date Printed: 2009-08-17
No. of Samples: 1

Sampled By: C. Ginou
Report to:
John Guchardi
UOIT
2000 Simcoe Street North
Oshawa, ON
L1H 7K4

Comments:
Empty cell = Analysis not requested
< = Less than
^ = Result exceeds limit
MDL = Method detection limit
E = Sample exhausted
(R) = Resample

Sample I.D.: 466767
Waterworks No.: N/A
Sample Location: Landfill Study
Sub Location: Leachate Site 2 500ml No
Sample Type: Preservative @ 4 deg. C
Date Sampled: Raw
Client/Field I.D.: 2009-07-30

Harmony Landfill Site 2

	Analyte	MDL/Units	Limit	1
Anions	Alkalinity as CaCO3	20.0 mg/L	500	435
	Bromide as Br	0.05 mg/L		<0.05
	Chloride as Cl	0.1 mg/L	250	65.0
	Fluoride as F	0.01 mg/L	1.5	0.05
	Nitrate as N	0.01 mg/L	10.0	<0.01
	Phosphate as P	0.01 mg/L		<0.01
	Sulphate as SO4	0.1 mg/L	500	35.7
	Nitrate + Nitrite as N	0.06 mg/L	10.0	<0.06
	Nitrite as N	0.01 mg/L	1.0	<0.01
Calculations	Hardness as CaCO3	1.0 mg/L		486
	Ionic Balance	0.01 %		1.90
	Langelier Index	-2.0	neg. 2 to 2	1.0
	Total Anions	0.01 meq/L		11.3
	Total Cations	0.01 meq/L		11.7
	Calc. Conductivity	0.01 umho/cm		1130
	Calc. Dissolved Solids	20 mg/L		591
Cations	Calcium as Ca	0.3 mg/L		164
	Magnesium as Mg	0.06 mg/L		18.6
	Potassium as K	0.07 mg/L		1.69
	Sodium as Na	0.4 mg/L	20	45.0
	Ammonia +NH4 as N	0.05 mg/L		0.06
General 1	Colour	1 TCU	5	15
	pH (Units)	0.01 Units		7.25
	Conductivity	1.0 umho/cm		1080
	Turbidity	0.050 NTU	5	1.50
Metals Tests	Aluminum as Al	0.0002 mg/L	0.1	0.0026
	Antimony as Sb	0.0001 mg/L	0.006	0.0003
	Arsenic as As	0.0001 mg/L	0.025	0.0005
	Cadmium as Cd	0.0001 mg/L	0.005	<0.0001
	Chromium as Cr	0.0001 mg/L	0.05	<0.0001
	Cobalt as Co	0.0001 mg/L		0.0005

YORK-DURHAM
Regional Environmental Laboratory

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INORGANICS ANALYSIS REPORT

Work Order #: 2109017

Submission #: 149522

				466767 N/A Landfill Study Leachate Site 2 500ml No Preservative @ 4 deg. C Raw 2009-07-30 1
Metals Tests	Copper as Cu	0.0001 mg/L	1	0.0007
	Iron as Fe	0.002 mg/L	0.3	1.33
	Lead as Pb	0.0001 mg/L	0.010	0.0001
	Manganese as Mn	0.0001 mg/L	0.05	0.314
	Molybdenum as Mo	0.0001 mg/L		0.0004
	Nickel as Ni	0.0001 mg/L		0.0008
	Selenium as Se	0.0001 mg/L	0.01	0.0004
	Zinc as Zn	0.0001 mg/L	5	0.0006

Approved By:

B Gupta

Brij Gupta, Group Leader (Ext 4326)

J. Mirsch

J. Mirsch, Superintendent (Ext 4304)

ASSOCIATION OF THE CHEMICAL PROFESSION OF ONTARIO
CHARTERED
Jurgen Mirsch
CHEMIST

Appendix 16

Full chemical analysis results from samples collected on October 20, 2009.

York Region

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Regional Environmental Laboratory

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INORGANICS ANALYSIS REPORT

Page 1 of 2

Work Order #: 2113258 **Submission #:** 153696

CLIENT INFORMATION

UOIT

Date Received: 2009-10-21
Date Printed: 2009-11-19
No. of Samples: 1

Sampled By: C. Ginou

Report to:
John Guchardi
UOIT
2000 Simcoe Street North
Oshawa, ON
L1H 7K4

Comments:
Empty cell = Analysis not requested
< = Less than
^ = Result outside of guideline limits
MDL = Method detection limit
E = Sample exhausted
(R) = Resample

Sample I.D.:	482010
Waterworks No.:	N/A
Sample Location:	Landfill Study
Sub Location:	Leachate - Site 2 - 500ml
Sample Type:	Raw
Date Sampled:	2009-10-20
Client/Field I.D.:	1

Harmony Landfill Site 2

	Analyte	MDL/Units	Limit	Result
Anions	Alkalinity as CaCO3	20.0 mg/L		458
	Bromide as Br	0.05 mg/L		0.54
	Chloride as Cl	0.1 mg/L		94.9
	Fluoride as F	0.01 mg/L		0.04
	Phosphate as P	0.01 mg/L		<0.01
	Sulphate as SO4	0.1 mg/L		56.1
	Nitrate + Nitrite as N	0.06 mg/L		<0.06
	Nitrate as N	0.01 mg/L		<0.01
	Nitrite as N	0.01 mg/L		<0.01
Calculations	Hardness as CaCO3	1.0 mg/L		531
	Ionic Balance	0.01 %		1.38
	Langelier Index	-2.0		1.2
	Total Anions	0.01 meq/L		13.0
	Total Cations	0.01 meq/L		13.4
	Calc. Conductivity	0.01 umho/cm		1310
	Calc. Dissolved Solids	20 mg/L		689
Cations	Calcium as Ca	0.3 mg/L		178
	Magnesium as Mg	0.06 mg/L		20.9
	Potassium as K	0.07 mg/L		1.74
	Sodium as Na	0.4 mg/L		62.1
	Ammonia +NH4 as N	0.05 mg/L		0.08
General 1	Colour	1 TCU		5
	pH (Units)	0.01 Units		7.29
	Conductivity	1.0 umho/cm		1210
	Turbidity	0.050 NTU		20.5
Metals Tests	Aluminum as Al	0.0002 mg/L		0.0216
	Antimony as Sb	0.0001 mg/L		0.0004
	Arsenic as As	0.0001 mg/L		0.0022
	Cadmium as Cd	0.0001 mg/L		<0.0001
	Chromium as Cr	0.0001 mg/L		<0.0001
	Cobalt as Co	0.0001 mg/L		0.0013

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INORGANICS ANALYSIS REPORT

Work Order #: 2113258

Submission #: 153696

			482010 N/A Landfill Study Leachate - Site 2 - 500ml Raw 2009-10-20 1
Metals Tests	Copper as Cu	0.0001 mg/L	0.0008
	Iron as Fe	0.01 mg/L	12.8
	Lead as Pb	0.0001 mg/L	0.0002
	Manganese as Mn	0.005 mg/L	1.02
	Molybdenum as Mo	0.0001 mg/L	0.0010
	Nickel as Ni	0.0001 mg/L	0.0019
	Selenium as Se	0.0001 mg/L	0.0014
	Zinc as Zn	0.0001 mg/L	0.0017

Approved By:

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CHARTERED
Jurgen Mirsch
CHEMIST

J. Mirsch

Brij Gupta, Group Leader (Ext 4326)

J. Mirsch, Superintendent (Ext 4304)

Appendix 17

Full chemical analysis results from samples collected on January 22, 2010.

York Region

YORK-DURHAM
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DURHAM REGION

INORGANICS ANALYSIS REPORT

Work Order #: 2116920 **Submission #:** 159260

CLIENT INFORMATION

UOIT

Date Received: 2010-01-22
Date Printed: 2010-02-01
No. of Samples: 1

Sampled By: C. Ginou

Report to:
John Guchardi
UOIT
2000 Simcoe Street North
Oshawa, ON
L1H 7K4

Comments:
Empty cell = Analysis not requested
< = Less than
^ = Result outside of guideline limits
MDL = Method detection limit
E = Sample exhausted
(R) = Resample

Sample I.D.:	496245
Waterworks No.:	N/A
Sample Location:	Landfill Study
Sub Location:	Harmony Landfill at 4 Degrees
Sample Type:	Raw
Date Sampled:	2010-01-22
Client/Field I.D.:	1

Harmony Landfill Site 2

	Analyte	MDL/Units	Limit	
Anions	Alkalinity as CaCO3	20.0 mg/L	500	427
	Bromide as Br	0.05 mg/L		0.10
	Chloride as Cl	0.1 mg/L	250	55.0
	Fluoride as F	0.01 mg/L	1.5	0.07
	Phosphate as P	0.01 mg/L		<0.01
	Sulphate as SO4	0.1 mg/L	500	53.3
	Nitrate + Nitrite as N	0.06 mg/L	10.0	<0.06
	Nitrate as N	0.01 mg/L	10.0	<0.01
	Nitrite as N	0.01 mg/L	1.0	<0.01
Calculations	Hardness as CaCO3	1.0 mg/L		453
	Ionic Balance	0.01 %		2.43
	Langelier Index	-2.0	neg. 2 to 2	0.9
	Total Anions	0.01 meq/L		11.2
	Total Cations	0.01 meq/L		10.7
	Calc. Conductivity	0.01 umho/cm		1080
	Calc. Dissolved Solids	20 mg/L		572
Cations	Calcium as Ca	0.3 mg/L		152
	Magnesium as Mg	0.06 mg/L		18.2
	Potassium as K	0.07 mg/L		1.25
	Sodium as Na	0.4 mg/L	20	36.1
	Ammonia +NH4 as N	0.05 mg/L		0.12
General 1	Colour	1 TCU	5	18
	pH (Units)	0.01 Units		7.22
	Conductivity	1.0 umho/cm		998
	Turbidity	0.050 NTU	5	0.789
Metals Tests	Aluminum as Al	0.0002 mg/L	0.1	0.0123
	Antimony as Sb	0.0001 mg/L	0.006	0.0008
	Arsenic as As	0.0001 mg/L	0.025	0.0005
	Cadmium as Cd	0.0001 mg/L	0.005	<0.0001
	Chromium as Cr	0.0001 mg/L	0.05	<0.0001
	Cobalt as Co	0.0001 mg/L		0.0004

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INORGANICS ANALYSIS REPORT

Work Order #: 2116920

Submission #: 159260

496245
N/A
Landfill Study
Harmony Landfill at 4 Degrees
Raw
2010-01-22
1

Metals Tests	Copper as Cu	0.0001 mg/L	1	0.0008
	Iron as Fe	0.0001 mg/L	0.3	1.83
	Lead as Pb	0.0001 mg/L	0.010	0.0002
	Manganese as Mn	0.0001 mg/L	0.05	0.107
	Molybdenum as Mo	0.0001 mg/L		0.0005
	Nickel as Ni	0.0001 mg/L		0.0008
	Selenium as Se	0.0001 mg/L	0.01	0.0004
	Zinc as Zn	0.0001 mg/L	5	0.0018

Approved By:

B Gupta

Brij Gupta, Group Leader (Ext 4326)

Renu Joshi

Renu Joshi, Lab Superintendent (Ext 4325)