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# Environmental Evaluation of Land-Applied Pulp Mill and Municipal Biosolids

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by

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***Abstract***

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In terms of disposal options, a form of waste that has received much attention in recent years is sludge, the by-product of wastewater treatment from both industrial and municipal sources. Negative issues associated with traditional sludge disposal practices (e.g. landfilling or incineration) have resulted in an increased interest to find disposal alternatives such as applying the sludge, or biosolids, to land as a soil amendment for purposes such as agriculture, horticulture, and silviculture. The objective of this study was to assess the environmental impact of pulp mill and municipal biosolids land-application using a suite of ecologically-relevant biota. Based on the results of this study, it can be concluded that the practice of pulp mill and municipal biosolids land-application may indeed be a viable and environmentally-sound alternative to other traditional disposal methods. This study did not detect any obvious impact on biota from pulp mill and municipal biosolids land-application and run-off into receiving-water when compared to reference bioassays.

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# 1.0: INTRODUCTION

## *1.1 Overview of Biosolids Disposal*

Disposal of wastes and residuals is an obstacle faced by all types of industries and is governed primarily by economic and environmental considerations. Since the early 1970s, a decade when widespread awareness of water pollution problems was initiated, one type of waste in particular that has gained worldwide attention in terms of disposal options is sludge (Cole *et al.*, 1986). Sludge is the liquid, semi-solid, or solid by-product of wastewater treatment and originates from both industrial and municipal sources (USEPA, 2002a). As awareness of water quality issues grew, innovative solutions for the utilization of such waste sludges were sought as alternatives to landfilling and the once common practice of disposal in watercourses (Bright and Healey, 2003). Contributing to this need for alternative disposal methods was the implementation of more stringent wastewater treatment requirements for municipalities as well as industries. These new requirements, made law by such acts as the Canada Water Act of 1970 and the Federal Water Pollution Control Act of 1972 in the United States, also contributed to sludge disposal issues due to the consequent increase in the quantity of waste residuals (Cole *et al.*, 1986). An alternative disposal method that began receiving serious attention was land-application, or the use of sludge as a soil amendment. In addition, the realisation that sludge may potentially be viewed as a valuable resource for various applications strengthened the commitment by industries and municipalities to strive to manage wastes in a more cost-effective and environmentally-sound manner (Rechcigl and MacKinnon, 1997).

At present, the three main disposal options for sludge from all sources are landfilling, incineration, and land-application. Landfilling is the predominant means for managing sludges throughout most of Canada and the United States; however, landfills are becoming more difficult to site and more costly to construct and operate because of increasingly stringent regulations, diminishing land availability, and public opposition (Thacker, 1986). This subsequently results in an increased disposal cost for sludge and

thus a heightened interest in disposal alternatives such as applying the sludge to land (Feldkirchner *et al.*, 2003). For clarification, sludge that is destined for land-application is now commonly referred to as "biosolids" (USEPA, 2002a), a less offensive, more environmentally-favourable term than "sludge". Various avenues of biosolids land-application are being explored and, currently, biosolids are utilized as a soil amendment for such purposes as agriculture, horticulture, silviculture, and disturbed land reclamation (Forste, 1997). From an economic standpoint, land-application of biosolids has been shown to be less expensive than incineration or landfilling, the two traditional disposal methods (MacConnell *et al.*, 1986). In terms of quantity, it is estimated that over half of the sewage sludge produced annually in the United States is now used in a beneficial manner, primarily as a soil amendment on agricultural land (USEPA, 2002a).

In general, land-application projects of all types deal with three main issues. Environmental safety must be considered, the project must be cost-effective and economically feasible, and the issue of gaining public acceptance must be addressed. Public acceptance is the most difficult of these three issues to achieve based on perceptions that the biosolids are toxic (Burd, 1986).

Land-application of biosolids is a controversial issue. A better understanding of the economic benefits and costs of land-applying biosolids, the possible beneficial effects on vegetation growth responses, and potential adverse effects on water quality and health of organisms inhabiting the ecosystems in which land-application occurs is necessary before widespread application of biosolids can commence safely and in a manner that is publicly acceptable (Binder *et al.*, 2002; Feldkirchner *et al.*, 2003).

## *1.2 Land-Application of Biosolids*

### 1.2.1 - Physiochemical Processes

Beneficial use of biosolids as a soil amendment is based on their potential to positively alter such soil properties as plant nutrient availability, water holding capacity, tilth (physical condition of soil related to tillage, seedbed, and rooting media), and cation exchange capacity (related to enhanced soil organic matter status) (Camberato *et al.*, 1997). Improved soil structure, as well as the inherent water retaining ability of the biosolids themselves, can subsequently enhance the water holding capability of a soil (Piearce and Boone, 1998). Furthermore, biosolids serve as a source of nutrients to living organisms in the soil to which it is applied (Piearce and Boone, 1998).

Supporting the land-application disposal alternative is the fact that soils serve as a good chemical filter. They possess the properties necessary for ion exchange, adsorption or precipitation, and chemical alterations (MacConnell *et al.*, 1986). In addition to filtering the biosolids, soils may also serve as a physical barrier to prevent harmful by-products from contaminating adjacent land or water systems (MacConnell *et al.*, 1986).

While the benefits of using pulp mill biosolids as a soil amendment may be numerous, potential negative effects also abound. These include reduced short-term nitrogen availability, and thus a possible requirement for addition of fertilizer, as well as soil compaction during the spreading process (Piearce and Boone, 1998). A shift of the nutrient balance in the soil could ultimately affect the organisms inhabiting the soil (Lokke and Van Gestel, 1998). Sludge microbiota could potentially out-compete indigenous soil microbiota, resulting in widespread ecosystem alterations. Moreover, ground water and surface receiving-water quality may also be compromised as a result of run-off from areas of land on which biosolids are applied (Ferrari *et al.*, 1999).

### 1.2.2 - Public Perceptions

As previously mentioned, one of the most significant barriers to widespread biosolids land-application is public concern regarding the addition of potentially toxic substances and, in some cases perhaps, pathogens to forests, farmlands, and watersheds (Bastian, 1986). For this reason, it is imperative that the practice of land-application of biosolids be thoroughly studied in terms of impacts on human health effects, the environment, land productivity, and vegetation quality.

Commonly, concerns are also often expressed with respect to the odours, aesthetic problems, increased traffic and noise, and the potential impact on property value associated with biosolids land-application (Bastian, 1986). In addition, there is often psychological conflict associated with the idea of applying human wastes to land. In the case of sewage treatment plant biosolids; most North American people have an "out-of sight, out-of mind" attitude toward sewage treatment and tend to favour the more highly engineered disposal alternatives (Bastian, 1986).

Since it is often the case that biosolids land-application must take place in rural rather than urban areas, the reluctance of rural areas to accept waste that is not their own can be a significant problem. Land-application project proposals often result in the expression of "Not in my backyard" (NIMBY) and "What do I get out of it" attitudes that have to be addressed (Bastian, 1986).

## *1.3 Composition of Biosolids*

Biosolids contain various nutrients such as nitrogen, phosphorus, and potassium that are essential for plant growth, making them highly suitable as a soil amendment. Nutrient levels govern application rate since excessively high nutrient levels can have numerous detrimental effects on the environment, including ground and surface water contamination (USEPA, 1997). Although biosolids generally have many components

in common, the source from which they originate dictates their exact composition and, as a result, municipal biosolids are quite different in character from pulp mill biosolids.

The following section will discuss nitrogen, one of the main components of both industrial and municipal biosolids, and its relationship to land-application. Subsequent to that, the composition of pulp mill and municipal biosolids will be addressed individually.

### 1.3.1 - Nitrogen and its relationship to land-application rates

The rate at which biosolids must be land-applied depends on many factors, including site conditions and concentrations of metals, toxic organic compounds, pathogens, and nutrients, all of which can vary greatly among biosolids originating from different sources (Gilmour and Skinner, 1999). Whereas metals, toxic organic compounds, pathogens, or nutrients such as phosphorus do not limit application rate, the amount of biosolids that can be applied at one time is limited by, and therefore determined by the amount of plant-available-nitrogen (PAN) contained in the biosolids (Gilmour and Skinner, 1999).

The PAN in the biosolids must closely match crop or forest nitrogen needs. Biosolids contain both inorganic as well as organic forms of nitrogen that contribute to the total PAN (Gilmour and Skinner, 1999). The inorganic nitrogen pool consists of ammonium ($NH_4$) and nitrate ($NO_3$). Ammonium is plant-available but can volatilize as ammonia ($NH_3$) upon surface application (USEPA, 1997). Nitrate is a highly mobile and water-soluble form of nitrogen and therefore may be associated with groundwater contamination (USEPA, 1997). The organic pool of nitrogen, which is often much larger than the inorganic pool in biosolids, is unavailable for use by plants until it is decomposed by soil microorganisms or mineralized to inorganic $NH_4$ and $NO_3$, making it a slow-release form of nitrogen (USEPA, 1997). Thus, the amount of total PAN is calculated as the sum of the portion of initially-applied ammonium that does not volatilize plus the amount of organic nitrogen that is mineralized to inorganic forms

during a given time period (Gilmour and Skinner, 1999). The actual amount of nitrogen taken up by a plant will be slightly less than the PAN of the biosolids as there are various loss mechanisms and uptake efficiency rates that occur (Gilmour and Skinner, 1999). Land-application regulations are based primarily on nitrogen content of the biosolids. In Ontario, for example, the rate of PAN that is permitted to be supplied by municipal biosolids is limited to 135 kg of nitrogen per hectare every five years for agricultural operations (Payne, 2000).

Plant nitrogen deficiencies resulting from the application of high carbon-to-nitrogen (C:N) ratio biosolids can be due to nitrogen immobilization by microbes, a condition which occurs when the nitrogen content of the sludge is not adequate enough to meet the demands of the soil microbial community (Camberato *et al.*, 1997). As the decomposition of the sludge occurs, carbon is evolved as carbon dioxide ($CO_2$), and the C:N ratio is gradually diminished, enhancing nitrogen availability (Camberato *et al.*, 1997). Camberato *et al.* (1997) have identified numerous strategies to overcome this nitrogen limitation, including:

i. Applying sludge well before crops are planted to ensure that the C:N ratio of the sludge has been reduced to the point that immobilization will no longer occur,

ii. Adding supplemental nitrogen to fulfil the microbial demand for nitrogen that is necessary for sludge decomposition, or

iii. Planting legumes so that soil nitrogen is not a crop requirement.

The timing of crop planting relative to biosolids land-application is dictated by the duration of nitrogen immobilization that occurs when sludge is applied to soil (Camberato *et al.*, 1997). Unfortunately, the immobilization period can vary and is quite unpredictable. It is essential that site-specific conditions be taken into account when planning all aspects of land-application.

### 1.3.2 - Composition of Pulp Mill Biosolids

Generally speaking, pulp mill biosolids contain a number of essential plant elements, including nitrogen, phosphorus, potassium, calcium, and magnesium, all of which may benefit the nutrient-limited soils of agricultural land and forests (Feldkirchner *et al.*, 2003). The nutrient content of biosolids originating from different pulp mills varies according to the pulping method employed.

Organic matter makes up a significant portion of pulp mill biosolids, which is why they are considered to be a very good option as a soil amendment (Thacker, 1986; Bellamy *et al.*, 1995). Not surprisingly, this organic fraction of biosolids consists primarily of wood fibre. Studies have shown that the organic matter content of agricultural soils to which pulp mill sludges are applied is greatly enhanced (Thacker, 1986).

Nitrogen, phosphorus, potassium, calcium, magnesium, and sulphur are typically found in lower concentrations in pulp mill biosolids compared to municipal biosolids (Thacker, 1986); however, concentrations of these nutrients are usually still high enough that land-application of pulp mill biosolids is beneficial. Mill sludges tend to exhibit great variety in macronutrient composition, which again is a consequence of differences in the type and operation of the mill as well as in the sludge treatment provided. Secondary sludges are typically much higher in nitrogen and phosphorus than primary ones, because of the addition of these two nutrients to wastewater prior to biological (secondary) treatment (Thacker, 1986).

Pulp mill biosolids are substantially different from municipal biosolids in that they tend to have much lower concentrations of heavy metals and other trace elements. Arsenic, cadmium, chromium, cobalt, copper, mercury, molybdenum, nickel, lead, selenium, tin, and zinc levels are significantly lower in pulp mill sludges than in their municipal counterparts (Thacker, 1986; Feldkirchner *et al.*, 2003). Furthermore, heavy metal concentrations that may at one time have been present in pulp mill biosolids have been

significantly reduced in recent years due to improved processing techniques (Camberato *et al.*, 1997). A number of short-term studies have demonstrated that pulp mill biosolids can be applied to land without adverse levels of heavy metals being absorbed into plant tissue (Thacker, 1986; Feldkirchner *et al.*, 2003).

Organic compounds that have been detected at significant levels in pulp mill biosolids (concentrations higher than 10 mg/dry kg) include naphthalene, some phthalates, chloroform, polychlorinated biphenyls (PCBs), and wood extractives or derivatives such as abietic acid or retene (Thacker, 1986; Camberato *et al.*, 1997). At present, the concentration of harmful organic compounds, such as dioxins, in pulp mill sludge is often far below the threshold levels at which land-application is permitted according to USEPA guidelines (Gillespie and Abbot, 1998).

### 1.3.3 - Composition of Municipal Biosolids

The constituents of municipal biosolids, like pulp mill biosolids, can vary widely and depend on such factors as the type and amount of discharge to the sewage treatment plant as well as the various treatment processes employed by the plant (Bastian, 1986). For this reason, it is quite difficult to generalise about the biological, chemical, and physical properties of municipal biosolids. Immense diversity in the content of heavy metals, toxic organic compounds, and pathogens reported in municipal biosolids has been a major impediment to the widespread use of these materials for land-application purposes (Bastian, 1986).

Municipal wastewater treatment systems are vulnerable to input from a wide range of sources, in particular when they receive flows from storms sewers (Bright and Healey, 2003). Municipal biosolids typically contain a vast assortment of inorganic and organic contaminants that result from such inputs as disposal of hazardous or industrial wastes down drains or run-off of atmospheric deposition (Bright and Healey, 2003). In addition, the metals arsenic, cadmium, chromium, lead, zinc, copper, and nickel are

often present in appreciable quantities (MacConnell *et al.*, 1986; Hirsch, 1998; Bright and Healey, 2003). Unlike pulp mill biosolids, anthropogenic chemicals such as pesticides and polycyclic aromatic hydrocarbons (PAHs) that enter waste treatment systems through catchment basins are also present at significant level in most municipal biosolids (MacConnell *et al.*, 1986; Bright and Healey, 2003).

Municipal biosolids generally contain substantial amounts of nitrogen, phosphorus, and potassium, the primary plant nutrients, along with the exchangeable bases calcium, magnesium, and sodium (MacConnell *et al.*, 1986; Hirsch, 1998). There are fourteen mineral elements known as essential micro- or macronutrients, all of which are present in sufficient quantity in municipal biosolids and can potentially contribute significantly to a soil/crop system (Forste, 1997; Hirsch, 1998).

Much of the earlier literature on organic contaminants in municipal biosolids focuses on past-use chemicals such as polychlorinated biphenyls (PCBs), chlorophenols, and chlorinated pesticides (Bright and Healey, 2003). However, in recent decades there has been a transition away from the use of many of these contaminants; as a result, scientific interest has shifted to implications of current-use chemicals that are currently detected in significant amounts in municipal biosolids (Bright and Healey, 2003). Current-use chemicals include a number of known or suspected endocrine-disrupting substances (e.g. β-estradiol and alkyphenol ethoxylates) as well as a number of commonly used potential toxicants (e.g. linear alkyl benzenesulfonates) (Bright and Healey, 2003).

### *1.4 Land-Application of Biosolids for Agricultural Purposes*

From an agricultural standpoint, land-application of biosolids can effectively provide the farmer or landowner with numerous potential benefits, including lower fertilizer costs, improved soil characteristics, and increased crop productivity and yield (Bastian, 1986). Although biosolids are not considered a high-quality fertilizer, the substantial

amounts of nitrogen and phosphorus they contain can eventually, in some cases, partially or even fully diminish the need for commercial inorganic fertilizers (Bastian, 1986).

Despite the potential for enhanced growth of agricultural crops resulting from the effects of the primary constituents of biosolids (nitrogen and phosphorus will be discussed later), some components of biosolids may also be quite harmful in some cases. For instance, sewage treatment plant (municipal) biosolids often contain heavy metals (Hirsch, 1998). Several studies have shown that plants grown in soils amended with municipal biosolids exhibit high concentrations of elements such as zinc, cadmium, copper, nickel, and molybdenum in their tissues (Hirsch, 1998). These elements may pose a threat to the plants and, consequently, animals and humans. As a result, Canada, the United States, and other countries in which land-application occurs have set limits for the concentrations of various elements in biosolids destined for land-application (Hirsch, 1998).

Although the potential benefits of biosolids land-application for agriculuture have been well-documented, it has been identified that more research is necessary with respect to the economic costs and benefits to farmers, long-term nutrient availability, environmental impacts, as well as crop yeild responses (Binder *et al.*, 2002). Clearly, there is a considerable need for further research concerning land-application of biosolids for agricultural purposes.

### *1.5 Land-Application of Biosolids in the Forest*

Forest ecosystems are attractive alternatives for biosolids land-application for a variety of reasons. Firstly, there are fewer public health concerns related to plant uptake of contaminants from biosolids land-application projects that occur in forests since trees are not destined for human consumption, as are crops from other agricultural operations (Burd, 1986). Secondly, much research has indicated that certain tree species are quite

tolerant to contaminants that may pose problems for agricultural crops (Burd, 1986). Thirdly, since paper companies often own forests in proximity to the mill, the associated high costs of transporting biosolids destined for land-application are minimized (Feldkirchner *et al.*, 2003). This may also be true for municipal sewage treatment plants since it is common for municipalities to own forest lands that are located on the outskirts of their city (Burd, 1986). Finally, the perennial nature of a forest allows for a less stringent land-application schedule than for agricultural operations, which operate on a seasonal basis (Burd, 1986).

Bastian (1986) has identified a number of additional reasons for considering forested sites as potential recipients of biosolids. They are as follows:

1. Considering that forests occupy a significant portion of land in North America, there is a great deal of potential land-application sites. For instance, in the contiguous United States, forests occupy approximately 40% of the landscape.
2. Compared to agricultural sites, forests are not as susceptible to flooding since they typically have better drainage.
3. Forests are characteristically deficient in some of the major nutrients found in biosolids, most notably nitrogen and phosphorus. Forest productivity is primarily limited by lack of sufficient nutrition.
4. There are numerous characteristics of forest soils that make them well-suited to receive biosolids, including the fact that they tend to have high infiltration rates, thus minimizing the potential for surface run-off. In addition, the perennial root systems of forests enable nutrient uptake to occur on a year-round basis.

Gathering information on the way soil nutrients affect the health of a forest is vital to the sustainable management of forest ecosystems. During harvests, significant nutrient removal can occur, depending on such factors as the intensity of the harvest as well as the rates of nutrient inputs to the ecosystem (Feldkirchner *et al.*, 2003). Nutrient deficiencies in forest soil can result in decreased photosynthesis, decreased amount of foliage per tree, and a shift in biomass allocation from the stems to fine roots.

Biosolids land-application may serve as a reliable means of counteracting these detrimental nutrient deficiencies (Feldkirchner *et al.*, 2003).

A substantial amount of research has taken place with respect to the impacts of biosolids land-application on forested sites. It has been suggested that biosolids can be effectively used to increase forest productivity with limited associated environmental impacts, provided that proper management practices are adhered to (Bastian, 1986). Research has shown that biosolids can aid in reducing wood production cycles, increasing forest productivity (especially on soils that are only marginally productive to begin with), and revegetating and stabilizing clearcut areas or those destroyed by forest fires (Bastian, 1986).

Problems associated with using biosolids as a soil amendment in forests include the characteristic high infiltration rates of forest soils. Although considered beneficial in terms of minimizing surface run-off, high infiltration rates can cause excessive leaching of nitrates and movement of undesirable sludge constituents into groundwaters or surface waters (Bastian, 1986). Steps taken to alleviate these problems include limiting the application rates and restricting application to areas devoid of steep slopes.

In addition to many of the concerns associated with application to farmland mentioned previously, land-application of biosolids to forest areas raises its own concerns. These include the adverse impacts on wildlife, uptake of contaminants by edible berries and mushrooms, and public access restrictions to some areas that receive biosolids (Bastian, 1986).

Henry and Cole (1997) concluded that "*the future of biosolids applications to forests is bright*" and cited a number of reasons for this. Research has shown that a favourable growth response is indeed possible, the technology is well-developed, and the economics appear to be encouraging.

## *1.6 Previous Studies of Biosolids Land-Application*

Biosolids land-application research has typically been conducted using either pulp mill or municipal biosolids and therefore the two types will be addressed separately in the following sections.

### 1.6.1 - Pulp Mill Biosolids Land-Application Studies

Research has shown that land-application of pulp mill biosolids has created plant yields of agricultural crops that are greater than those from control areas receiving no biosolids amendment or commercial fertilizer, or equal to or greater than areas receiving fertilizer alone (Thacker, 1986). Research has also shown that pulp mill biosolids used as a soil amendment for agricultural, silvicultural and horticultural purposes effectively improve soil texture and structure while aiding drainage, aeration, and root penetration (Piearce and Boone, 1998).

Crop responses to land-application of pulp mill biosolids have been found to be extremely variable depending on nitrogen content, C:N ratio, and amount applied (Camberato *et al.*, 1997). Some studies have shown an increased crop yield from application of low carbon-to-nitrogen (C:N) ratio sludges, whereas other studies have demonstrated decreased crop productivity from high C:N ratio sludges (Camberato *et al.*, 1997). C:N ratios vary amongst pulp mill biosolids from different origins; however, the ratio tends to be much higher for pulp mill biosolids than for municipal biosolids (Camberato *et al.*, 1997).

Feldkirchner *et al.* (2003) conducted a study to assess the potential for pulp mill sludge to increase tree growth in sugar maple and aspen stands in a northern Michigan hardwood forest. They did not observe any beneficial effects of pulp mill sludge on tree growth in these forest types and found that changes in nutrient pools resulting from the amendments were generally quite small. It was concluded that a long-term study

was necessary to better quantify the nutritional constraints on the growth of hardwood forests (Feldkirchner *et al.*, 2003).

In a study by Macyk (1996), it was found that pulp mill biosolids land-application was beneficial to grass yield, in proportion to application rate (i.e. higher application rate resulted in higher yield). This study also demonstrates the ongoing beneficial effects of land-applied biosolids over the course of a number of years. Macyk (1996) found that this lengthy (three- to four-year) period of nutrient release is beneficial not only in terms of plant nutrition, but also with respect to groundwater quality protection. Furthermore, in another study by Macyk (1999), it was found that pulp mill biosolids are beneficial as soil amendments based on six years of field trial results. Significant increases in forage crop yields were seen in sludge-amended soil over five- to six-year periods (Macyk, 1999). In addition, it was found that the height and diameter of lodgepole pine and white spruce seedlings increased significantly in the biosolids-amended treatments compared to non-amended soils.

Zibilske *et al.* (2000) conducted a five-year field study to determine the long-term effects of five different biosolids application rates in addition to multiple application effects on the chemical and physical properties of soil. Their results show that significant increases in moisture-holding properties and soil aggregation occurred at higher application rates and when cumulative sludge additions reached 225 Mg/hectare. In another study, Jackson *et al.* (2000) used application rates of 0, 20, 40, and 60 Mg/hectare to determine which resulted in the greatest increase in stem diameter in a radiata pine (*Pinus radiata*) plantation. It was concluded that the highest application rate (60 Mg/hecatre) was most beneficial pine tree stem diameter growth. In an additional study, O'Brien *et al.* (2002) conducted a greenhouse experiment with corn (*Zea mays* L.) grown in various mixtures (0 to 561 Mg/hectare) of pulp mill biosolids and soil. They studied germination of the seeds as well as biomass of the seedlings and found that delaying of sowing (sowing 21 days post sludge/soil mixing) increased the number of seeds that germinated. They also discovered that plant biomass declined as the amounts of biosolids increased.

Research by Feagley *et al.* (1994) assessed the impact of pulp mill biosolids combined with fertilizer on the growth and nutrient content of bermudagrass (*Cyanodon dactylon* L.) grown on a mine soil. They concluded that there exists a rate of biosolids application at which only half of the recommended amount of fertilizer be used, thus reducing potential negative impacts associated with fertilizer use, including ground and surface water pollution.

Phillips *et al.* (1997) conducted a three-year study to assess the various factors that influence the applicability of pulp mill sludges as an agricultural soil amendment. The factors studied included crop type, soil type, sludge type, application method, and application rate. They concluded that the chief reason for farmers to make use of pulp mill biosolids as a soil amendment should be based on the ability of the biosolids to improve soil condition. This beneficial impact is the most readily achieved and it is suggested that subsequent increases in crop yield should in turn result from the successive improvements in soil condition when the biosolids are land-applied on an annual basis (Phillips *et al.*, 1997).

Studies examining the effects of pulp mill biosolids land-application on wildlife in forests are quite limited. In one of the few studies of this kind, Vera and Servello (1994) studied the impact of land-application on various songbirds (11 species) and small mammals (6 species) at typical application sites in spruce-fir (*Picea* spp.-*Abies balsamea*) forests in Maine. They found no evidence to suggest that land-application of pulp mill biosolids has any negative impact on breeding bird or small mammal communities (Vera and Servello, 1994).

### 1.6.2 - Municipal Biosolids Land-Application Studies

Municipal biosolids, when applied to land, alter many of the physical and chemical properties of the soil. The organic matter in the sludge improves the aggregation of soil and reduces surface run-off and erosion, while increasing nutrient loading and

infiltration rates (MacConnell *et al.*, 1986). Nutrients and heavy metals are concentrated in the foliage of trees and recycled when the leaves drop, thereby increasing the concentration of these substances in the organic ped. Nitrogen in the form of nitrates is the only nutrient leached in large quantities, and the concentration of nitrogen decreases with soil depth (MacConnell *et al.*, 1986).

When biosolids are land-applied in forests, it is quite possible that surface run-off and deep leaching (i.e. penetrating to water table) can impact water quality. Surface run-off and deep leaching transport both suspended and dissolved materials of biosolids, including microorganisms, organic compounds, heavy metals, and nitrates (Zasoski and Edmonds, 1986). The potential impact on water quality depends primarily on site characteristics and the composition of the biosolids being applied. Since site design criteria should, if effective, restrict surface run-off, leaching is typically the main concern (Zasoski and Edmonds, 1986). It has been shown that metals from municipal biosolids do not leach downward in significant quantities in the short term (Zasoski and Edmonds, 1986); however, long-term studies are needed.

Although it has been shown that direct effects on wildlife due to contact with land-applied biosolids are minimal, Haufler and West (1986) concluded that indirect impacts on wildlife can result from changes in productivity, composition, and structure of vegetation, changes in quality and quantity of forages, as well as toxicities from elements entering into the food chain.

Binder *et al.* (2002) studied the effects of five different municipal biosolids application rates on irrigated maize (*Zea mays* L.) and rainfed sorghum (*Sorghum bicolour* L.) and determined that maximal yields were achieved at a 62 Mg/hectare application rate on irrigated maize and a 36 Mg/hectare application rate on rainfed sorghum. In another study, Walter *et al.* (2000) used application rates of 40, 80, and 120 Mg/hectare to assess the effects of municipal biosolids land-application on chemical properties of soil, total plant cover, and total above-ground biomass one year following application. They found that the most favourable vegetation and soil results were achieved with the 40

and 80 Mg/hectare application rates. An additional study by Labrecque and Teodorescu (2001) assessed the impact of municipal biosolids land-application on plant response (productivity and growth) of two willow species (*Salix discolor* and *Salix viminalis*) on abandoned farmland sites in southern Quebec. For both species, optimal yield was obtained on sites receiving biosolids as a soil amendment compared to control sites. The authors also examined the nutrient status of the trees by foliar analysis and found that heavy metal accumulation from municipal biosolids land-application did not occur and does not pose a threat to the environment (Labrecque and Teodorescu, 2001).

A study conducted by Veerina *et al.* (2002) attempted to determine if run-off from croplands fertilized with municipal biosolids was toxic to aquatic biota, representing a potential threat to public health and the environment. They conducted seven-day bioassays with *Ceriodaphnia dubia* and found that run-off during rain events has the potential to detrimentally influence reproduction in daphnids and therefore the environment by way of the food chain (Veerina *et al.*, 2002).

### *1.7 Review of Current State of Knowledge*

It appears that the bulk of current literature in the areas of land-application of municipal and pulp mill biosolids focuses specifically on application rates with the ultimate goal of determining which rate is optimal for the practice of using biosolids as a soil amendment. As illustrated by the results of these various studies, the relationship of application rates to soil properties and plant growth seems to be largely a function of soil type and plant species.

In general, current knowledge tends to be based predominately on studies of single impact indicators. For the most part, biosolids land-application research examines effects on one specific organism rather than a suite of various indicator species. The literature reviewed does not reveal any studies in which assemblages of

environmentally-relevant species are employed. This is a very important concept in that a multiple-species approach potentially creates much greater ecological relevance.

The field and laboratory experiments cited above indicate that continued studies are paramount in order to better comprehend the environmental impacts of biosolids land-application. Current literature suggests that future studies need to focus on such issues as long-term availability of the nutrients supplied by biosolids, plant responses and impacts, and overall environmental impact when using biosolids as a soil amendment (Binder *et al.*, 2002). There is a general consensus in the existing literature that emphasis must be placed on long-term study so that the broad impacts of land-application can be understood. In addition, the need for *in situ* (field) experimentation at sites where biosolids are being land-applied is highlighted. It is crucial that field experiments be undertaken to verify results that, to date, have been largely based on laboratory study alone.

### *1.8 Bioassay Organisms*

This section will examine the various bioassay organisms employed in our research, including a brief description of the biology of the organism, its use in bioassays and any standard methods that have been developed for it, a brief explanation of the test procedure and common endpoints assessed, as well as its applicability to the environmental evaluation of biosolids land-appplication. The rationale behind using a battery of different species is that a single universal indicator organism does not exist; sensitivity to a particular contaminant will not be consistent among species. Rather, considering data collectively from a suite of ecologically-relevant species is a much more logical and intuitive approach from an environmental standpoint and serves as the basis of this study.

### *1.8.1 - Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*)

*Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) is a freshwater, unicellular green algae that is crescent-shaped and non-motile in character and obtains its nutrients from the water in which it lives. Inhabiting the freshwater regions of North America, these algae are commonly found in clumps of four to sixteen but may also be solitary. Algal species such as *P. subcapitata* are types of primary producers that play a vital role in aquatic ecosystems, where they serve as essential energy sources (Lewis, 1995).

*P. subcapitata* is a common microalgae employed for the purposes of toxicity testing. Several test methods have been standardized for this organism. These tests function to assess toxicity of various chemicals, effluents, contaminated sediments, and hazardous waste leachate and have been standarized by such agencies as Environment Canada, the European Economic Community, and the International Organization for Standardization (Lewis, 1995). To illustrate their widespread use, data from algal phytotoxicity testing are considered crucial in the development of water quality critera, municipal and industrial effluent assessment, and registration of commercial chemicals, for example (Lewis, 1995; Linton and Goulder, 1998; Pun *et al.*, 1995). In fact, almost all phytoxicity data of this nature is based on results from only a few freshwater green algae, primarily *P. subcapitata* (Lewis, 1995).

Although various standard methodologies differ slightly in nature, all basically consist of exposing a known concentration of algae to varying concentrations of contaminant or test media during its log phase of growth for 72 to 96 hours. Stimulatory or inhibitory effects are ascertained by microscopic counting of cells (Lewis, 1995). Conditions other than the toxicological effects of the contaminant that may impact the algal growth rate include the type and concentration of supplemental nutrient media used, the volume of test solution, pH, and temperature (Lewis, 1995). An advantage of algal bioassays is that they are rather simplistic, provide rapid results, and are more

economical to conduct when compared with fish and invertebrate bioassays (Pun *et al.*, 1995).

A study conducted by Bailey and Young (1997) under Canada's Environmental Effects Monitoring program employed and compared the efficacy of various toxicity tests to evaluate the effects of pulp and paper mill effluents on fish and fish habitat. The *S. capricornutum* algal growth inhibition test (Environment Canada 1992) was included and their results suggested that the algal toxicity test was quite reliable in terms of its ability to detect adverse effects (Bailey and Young, 1997).

### *1.8.2 - Lemna minor*

The aquatic macrophyte *Lemna minor* (commonly termed 'duckweed' because of their adaptation to aquatic habitats) is among the world's smallest flowering plants and is free-floating, thereby obtaining nutrients from the surrounding water (Linton and Goulder, 1998). *L. minor* has no true leaves but does possess a photosynthetic leaf-like body, termed a "frond", which is oval in shape, usually measures less than 6 mm across, and floats on the surface of the water (Newmaster *et al.*, 1997). Each plant has a short "rootlet" that hangs from its underside and extends downward into the water. Plants may be found on their own or in clumps and new plants arise from buds that form on either side of the parent plant; new plants may stay attached or eventually break free (Newmaster *et al.*, 1997). Under ideal, nutrient-rich conditions, a single plant will reproduce approximately every three days (Newmaster *et al.*, 1997).

*P. subcapitata*, *L. minor*, and other aquatic plants are important components of an ecosystem in that they play a key role in oxygen production, controlling water quality, nutrient cycling, stabilization of sediment, as well as providing habitat for various type of aquatic organisms (Lewis, 1995). Specifically, *L. minor* serves as food for various waterfowl and fish species.

In terms of phytotoxicity testing, *L. minor* is another commonly used aquatic plant (Linton and Goulder, 1998) and its testing protocol has become a USEPA standard. However, its use as a bioassay organism is not as widespread as *P. subcapitata* (Lewis, 1995). In the past, aquatic plants of any type have not been employed as bioassay organisms to assess toxicity as commonly as animal species. This mediocre status of aquatic plants as test species is contradictory to their tremendous ecological significance. In recent years, the use of plants to gauge environmental hazards is receiving more attention from, and is being regarded more highly by, the scientific community (Lewis, 1995).

The *L. minor* bioassay is quite popular as a result of its sensitivity as well as the simplicity of culturing the plant in the laboratory (Oanh and Bengtsson, 1995). Frond number is the most common endpoint in the *L. minor* bioasssay and proves to be a good indicator of growth (Linton and Goulder, 1998). Dry weight is another potential endpoint; however, frond number can be assessed much more rapidly (Linton and Goulder, 1998).

### *1.8.3 - Brassica rapa*

The genus *Brassica* is comprised of various species of plants having worldwide economic significance. Vegetable and crop varieties of *Brassica* include broccoli, Brussels sprouts, cabbage, cauliflower, collard, kale, mustard greens, rapeseed, rapini, and turnip (Musgrave, 2000). The species *Brassica rapa* contains a number of morphotypes including turnip, Chinese cabbage, pak choi, and rapid-cycling *Brassica* and is a member of the Cruciferae (Brassicaceae) family, referred to as the mustard family (Musgrave, 2000). The name Cruciferae, from crucifer, was chosen because of the cross-like shape of the flowers made up by four diagonally opposite petals (Canadian Food Inspection Agency, 1999). *B. rapa* is a dicotyledon and an obligate outcrosser, meaning that pollen must be transferred from plant to plant (typically through direct physical contact between neighbouring plants) in order for fertilization

to occur (Canadian Food Inspection Agency, 1999). Growth is influenced by air and soil temperatures, and is optimal at just over 20°C.

In nature, *B. rapa* is a primary colonizer of disturbed habitats, meaning that it is among the first plant species to inhabit disturbed land and competes against plants of similar type for space (Canadian Food Inspection Agency, 1999). It is reported that *B. rapa* is widely distributed throughout all provinces in Canada (Canadian Food Inspection Agency, 1999).

In recent years, a plant life-cycle test has been developed using rapid-cycling *Brassica*, a species morphotype that completes its life-cycle in 35 to 45 days, compared to six months to two years for most of the economically important *Brassica* species (Kloepper-Sams *et al.*, 1996; Musgrave, 2000). Due to their short life-cycle, small size, and ease of culturing in the laboratory, rapid-cycling *Brassica* seeds were employed for the purposes of this investigation and have experienced widespread use in plant and crop physiology research (Musgrave, 2000). Commonly measured endpoints in chronic toxicity tests with higher plant species include flower and seed production and vegetative growth (Kloepper-Sams *et al.*, 1996). In addition to making them well-suited to laboratory use, the short life-cycle of rapid-cycling *Brassica* plants provides an ideal opportunity for detecting effects at specific developmental stages, including impacts to subsequent generations (Kloepper-Sams *et al.*, 1996).

The use of higher plants in environmental risk assessment has only recently gained the attention of the scientific community, despite the essential role they play in the ecosystem. Currently, three higher plant phytotoxicity bioassays endpoints have been developed and implemented by regulatory agencies for the testing of new chemical products. These bioassays assess root elongation, seed germination, and early seedling growth (Gong *et al.*, 2001).

*1.8.4 - Phaseolus vulgaris*

The common bean, *Phaselous vulgaris*, is the most widely cultivated type of bean in temperate regions of the world (Fitter, 2002). The green seed pods produced by the bean plant are a popular vegetable and are marketed in many forms, including fresh, frozen, or canned. Beans tend to tolerate most environmental conditions in temperate and tropical zones but do poorly in very wet climates. They are desirable as crop species since they germinate and mature very rapidly; they can typically be harvested four to six weeks post-sowing (Fitter, 2002).

*P. vulgaris* is a dicotyledon and is a highly polymorphic species with many varieties. It generally grows to 20 to 60 cm in height, has ovate leaves 6 to 15 cm in length, has a small number of tiny flowers, and produces slender pods that may be 8 to 20 cm long and contain 4 to 12 seeds (Fitter, 2002).

Although it appears that higher plants in general have not yet gained the attention of ecotoxicologists in the scientific community, *P. vulgaris* has been used in a limited number of studies. One such study conducted by Gong *et al.* (2001) used *P. vulgaris* (bush bean variety) along with three other higher plant species to assess the applicability of germination and seedling growth bioassays for the ecotoxicological assessment of soils. Gong *et al.* (2001) stated that the relatively short life-cycle of *P. vulgaris* makes this plant well-suited to laboratory testing. This, along with its ability to germinate and grow rapidly under a wide range of conditions, is why *P. vulgaris* was chosen as a terrestrial plant bioassay organism for the purposes of this study.

*1.8.5 - Daphnia magna*

*Daphnia magna* are small freshwater crustaceans that are typically found in open water among weeds of ponds and lakes and are widely distributed throughout the northern hemisphere (White and Borror, 1998; USEPA, 2002b). *D. magna* generally reach a

maximum size of about five millimetres in length and are laterally flattened. Their movement is mainly vertical through the water column, propelling themselves with a relatively large pair of antennae. *D. magna* are filter-feeders that derive their nutrition from a variety of organisms such as bacteria, microalgae, protozoa, and yeast (White and Borror, 1998). Their life span is highly variable depending on environmental conditions and generally increases with decreasing temperatures. For example, they have a life span of approximately 40 days at 25°C, and 56 days at 20°C (USEPA, 2002b).

Throughout most of the year, *D. magna* populations consist almost entirely of females; males are only abundant during the spring or fall and are distinguishable from females by their smaller size, larger antennules, and first legs (used for clasping) (USEPA, 2002b). Males are only produced during unfavourable environmental conditions such as low temperatures, high population densities resulting in an elevated concentration of excrement, or decreased food availability (USEPA, 2002b). Adverse conditions may also result in the production of ephippia, which are essentially resting eggs that can hatch when conditions return to more favourable levels (USEPA, 2002b).

*D. magna* reproduce by cyclic parthenogenesis, a form of reproduction in which the organism develops without fertilization by the male gamete (USEPA, 2002b). The eggs develop and hatch within the brood chamber of the parent organism; this process takes about two days and a typical clutch consists of about 6 to 10 eggs. The resulting live-born offspring, as well as the ephippial offspring, are genetically identical to their mothers and will reach maturity, or be able to produce their first offspring, in approximately eight days (USEPA, 2002b).

*D. magna* are frequently employed for ecotoxicological testing of water quality. There are various standard methods that exist, including the USEPA's *Methods for Measuring the Acute Toxicity of Effluents and Receiving-waters to Freshwater and Marine Organisms* (2002b). The ease of culturing and short life-cycle of *D. magna* makes them an ideal bioassay organism.

### *1.8.6 - Hyalella azteca*

*Hyalella azteca* are small freshwater amphipods that inhabit permanent lakes, ponds, and streams in regions of North and South America (USEPA, 2000). They range in size from three to eight millimetres and they crawl, swim, and wriggle about quickly amongst vegetation and rocks using their numerous appendages. *H. azteca* are detritivores that burrow into the sediment and selectively feed on bacteria, algae, and organic debris (USEPA, 2000).

*H. azteca* reproduce sexually; males pair with females and feed together in this manner for one to seven days, after which time the female is ready to molt. She will then shed her exoskelton and reunite with the male so that copulation can occur. The fertilized eggs develop and hatch within the female and are released about five to ten days after fertilization (USEPA, 2000). Reproduction occurs only during favourable conditions, when temperatures range from 10°C to 18°C (USEPA, 2000).

Due to their behaviour and feeding habits, *H. azteca* are commonly used test organisms for evaluating adverse biological impacts associated with exposure to contaminated sediment (Steevens and Benson, 1998). Many organizations have developed standard test methods using *H. azteca*, including the United States Environmental Protection Agency.

### *1.8.7 - Lumbricus terrestris*

*Lumbricus terrestris*, (common names include earthworm, night crawler, and dew worm), are widely distributed throughout numerous regions of the world, including Ontario, where they are found mainly in wheat, corn and soybean fields, grasslands, meadows, pastures, and golf courses (Stephenson *et al.*, 1998). They are a highly mobile and tactile species that are capable of burrowing deep into the soil, up to depths of two meters or more (Ehlers, 1975). *L. terrestris* spend most of their time beneath the

surface but do come to the surface to selectively feed on organic matter such as leaves, small sticks, and straw (Stephenson *et al.*, 1998). Copulation also takes place at the soil surface.

The earthworm *L. terrestris* has a life-cycle of approximately six years and becomes sexually mature within approximately one year (Edwards and Bohlen, 1996). Sexual maturity is indicated by the presences of the *clitellum*, a saddle-shaped, swollen region found one third of the way back from the head. The clitellum contains gland cells that secrete mucus necessary to form the cocoons in which the worm embryos will be contained (Edwards and Bohlen, 1996). Earthworms are hermaphroditic but possess a mechanism to prevent self-fertilization and reproduce sexually to produce offspring. Fertilized eggs are released on or near the surface in tiny cocoons that are amber in colour, leather-like in texture, and very resistant to damage and desiccation (Edwards and Bohlen, 1996). Young earthworms develop in these cocoons and will eventually hatch; sexually mature earthworms will produce approximately two cocoons per year with one to two young each (Edwards and Bohlen, 1996).

Since earthworms are closely associated with the soil and are considered to be representative terrestrial organisms, they are considered good indicators of soil quality (Reinecke and Posthuma, 1998). *L. terrestris* contribute significantly to soil processes since they effectively incorporate organic matter, increase soil aggregation, and positively influence both water infiltration and soil aeration (Berry and Jordan, 2001).

Since many countries and regulatory agencies are becoming increasingly interested in evaluating the effects of contaminants on soil fauna, and since earthworms possess a number of characteristics (i.e. large size, behaviour) that make them an appropriate and key bioassay organism, earthworms have been implemented as standard test organisms for ecotoxicological testing by such organizations as the European Union, the Organization for Economic Co-operation and Development, and the International Standards Organization (Arnaud *et al.*, 2000; Kula, 1998). The species most commonly used in these standard tests are *Eisenia fetida* and *Eisenia andrei* since

mass-rearing of these species is relatively straightforward and since they have a relatively short reproductive cycle of about six weeks at 20°C (Kula, 1998; Walker *et al.*, 2001.).

Despite their inherent importance to the soil and their status as a representative soil species, there is little published information using *L. terrestris* as a test species due to the fact that mass-rearing techniques have not been fully established and because there is limited information available regarding the optimal environmental conditions (i.e. temperature and humidity) necessary for survival and growth (Berry and Jordan, 2001). A study conducted by Berry and Jordan (2001) found that the optimum temperature and soil moisture content for mass-rearing of *L. terrestris* was 20°C and 30%, respectively. It has also been noted that soil moisture appears to be a more important factor in earthworm survival than temperature (Berry and Jordan, 2001; Wever *et al.*, 2001).

At present, there is only a small amount of published literature available on the impact of pulp mill sludge land-application on soil fauna. In a study conducted by Piearce and Boone (1998), the effects of paper sludge on the abundance of the earthworms *Aporrectodea caliginosa* and *Octolasion cyaneum* in the field was assessed, along with the behavioural response of *L. terrestris* to paper sludge in the laboratory. They concluded that the soil community was not adversely affected by paper sludge land-application, and also suggested that fertility of some soils may even be enhanced in the long term (Piearce and Boone, 1998).

### *1.9 Thesis Objectives*

Given the need for field studies and the importance of determining the widespread environmental effects of biosolids land-application, the objectives of this thesis are as follows:

- To assess the environmental impact of pulp mill biosolids land-application at a site in central Alberta by conducting field studies using ecologically-relevant species and based on results previously obtained by laboratory experiments conducted by Bostan *et al.* (in press).
- To conduct a preliminary laboratory assessment of municipal biosolids land-application employing a similar battery of species as used for the field studies.

The *in situ* (field) and laboratory studies will focus specifically on two aspects of biosolids land-application. Firstly, the direct impacts of land-application of biosolids on terrestrial organisms will be assessed. Secondly, the implications of rain events (and resulting run-off) on receiving-water biota will be evaluated.

It is predicted that field experimentation at the pulp mill biosolids land-application site will verify the laboratory results of Bostan *et al.* (in press) who found no significant negative impact on organisms at or below industry-suggested concentrations for pulp mill biosolids land-application. It is also predicted that there will be no significant impact on organisms at environmentally-relevant concentrations for municipal biosolids land-applications.

### *1.10 Outline of Thesis*

Section 2.0 provides a description of the field study site for evaluating pulp mill biosolids land-application as well as the methodology used for conducting all experiments, in both the field and the laboratory. Section 3.0 presents the results obtained from all experiments. Section 4.0 consists of an analysis and discussion of the results obtained in this study and presents some recommendations for further research in the area of biosolids land-application.

## 2.0: METHODOLOGY

This study was divided into three subsections: pre-tests, field work bioassays, and laboratory bioassays. A summary of the organisms used for each subsection is presented in Table 1.

**Table 1.** Summary of pre-test, field, and laboratory bioassays.

| | | | Pre-Test | Field Bioassays | Laboratory Bioassays | |
|---|---|---|---|---|---|---|
| **Bioassay Organism** | **Kingdom** | **Phylum** | | | **Pulp Mill Biosolids** | **Municipal Biosolids** |
| *Lactuca sativa* | Plantae | Tracheobionta | • | | | |
| *Phaseolus vulgaris* | Plantae | Tracheobionta | • | • | • | • |
| *Raphanus sativus* | Plantae | Tracheobionta | • | • | | |
| *Cucurbita moschata* | Plantae | Tracheobionta | • | • | | |
| *Brassica rapa* | Plantae | Tracheobionta | | • | • | • |
| *Lemna minor* | Plantae | Tracheobionta | | • | • | • |
| *Pseudokirchneriella subcapitata* | Plantae | Chlorophyta | | | • | • |
| *Lumbricus terrestris* | Animalia | Annelida | • | • | • | • |
| *Daphnia magna* | Animalia | Arthropoda | | • | • | • |
| *Hyalella azteca* | Animalia | Arthropoda | | • | • | • |

(Note: pre-test and field bioassays conducted with pulp mill biosolids only)

### *2.1 Pre-Tests*

#### 2.1.1 - *Lumbricus Terrestris* Pre-Test

Six months prior to field work conducted in Alberta during July 2002, a *Lumbricus terrestris* pre-test was launched in the laboratory to assess earthworm survival in the soil that would be used for field bioassays. The two soils used were forest reference soil and pulp mill biosolids-amended forest soil from Alberta. The soil samples were collected and sent to the lab from a pulp mill biosolids land-application site on a forest block near Whitecourt, Alberta (see 2.2.1 for a detailed description of the study site). Reference soil was obtained from an adjacent non-amended site.

*L. terrestris* were obtained from Ward's Natural Science Ltd. two weeks prior to launching the pre-test. Upon receipt, the earthworms were placed into food grade, high-density polyethylene, cylindrical containers (10 L capacity) filled with reference soil (ASB Greenworld Topsoil). Water was added to the container to moisten the soil to approximately 30% (dry weight) via a hand-held spray bottle. Water-soaked sphagnum moss was then scattered on the surface of the soil in each container as a food source for the earthworms. The culture vessel was placed in a walk-in refrigerator, where temperature were maintained at 9°C. The earthworms were kept in the culture vessel for two weeks prior to launching the bioassay in order to acclimate.

Following the 14-day acclimation period, two 10 L containers (as described above) were filled three-quarters full with either Alberta reference soil or Alberta pulp mill biosolids-amended soil. Immediately following preparation of the test containers, eight healthy *L. terrestris* from the culture population were added to the soil surface of each of the containers. The containers were covered with perforated plastic lids (of the same composition as the containers) to prevent the earthworms from escaping and were placed in the refrigerator.

After one week, earthworms were checked for acute toxicity (Kula, 1998). This was accomplished by carefully emptying the contents of the containers one at a time and hand sifting through the soil to locate and record the number of the live organisms. The soil was then returned to the test containers and the surviving *L. terrestris* were once again placed on the soil surface. The containers were covered and returned to their original location.

Subsequent checks for chronic toxicity were performed bi-weekly. At each examination, the soil was also visually inspected for the presence of cocoons or young *L. terrestris* in order to assess reproductive capacity. The number of surviving earthworms, as well as the number of cocoons or young present, was recorded. Following this, the soil and all organisms were returned to the corresponding test container and location. Bi-weekly assessments of this nature continued for a total of

three months, at which time a final count of surviving organisms and cocoons was made and the pre-test terminated.

2.1.2 - Plant Pre-Test: Determination of Suitable Plant Species

Two months prior to field work that was conducted in Alberta during July 2002, a plant pre-test was launched in the laboratory to determine which common garden vegetable seed varieties would germinate successfully in the soil that would be used for field bioassays. The two soil types used were Alberta forest reference soil and pulp mill biosolids-amended forest soil. The soil samples were collected and sent to the lab from a pulp mill biosolids land-application site on a forest block near Whitecourt, Alberta (see 2.2.1 for a detailed description of the study site). Reference soil was obtained from an adjacent non-amended site.

Seeds of two varieties of lettuce (*Lactuca sativa* 'Early Great Lakes' and *L. sativa* 'Grand Rapids'), one variety of bush bean (*Phaseolus vulgaris* 'Improved Golden Wax'), one variety of radish (*Raphanus sativus* ' Crimson Giant'), and one variety of squash (*Cucurbita moschata* 'Winter squash') were obtained from a local garden center. Ten seeds of each variety were placed in a separate cell of a seed growing grid-type tray (tray size 65 cm x 35 cm; 50 cells/tray). Two trays were prepared in this manner, one containing Alberta reference soil and the other containing Alberta pulp mill biosolids-amended soil. Immediately following sowing, the trays were watered and placed under fluorescent lights in the laboratory. Germination was assessed and the trays watered every second day for 10 days, at which time a final count was made of seeds that had germinated. Upon termination, qualitative observations were also made and the plant varieties that grew the fastest in the Alberta soils were recorded in order to determine which garden variety vegetable seeds would be best-suited to Alberta field tests.

## *2.2 Field Experimentation – Pulp Mill Biosolids*

Over the course of a two-week period in July of 2002, field experiments were conducted in Whitecourt, Alberta to study the effects of land-application of biosolids from an Alberta paper mill. Various environmentally-relevant organisms were used in a battery of ecotoxicological field tests, including *Daphnia magna*, *Hyalella azteca*, *Lemna minor*, *Lumbricus terrestris*, *Brassica rapa*, *Phaseolus vulgaris*, *Cucurbita moschata*, and *Raphanus sativus*. Aquatic bioassays employing *D. magna*, *H. azteca*, and *L. minor* were conducted in a laboratory at the pulp mill using biosolids run-off collected from the field site (see 2.2.1). All terrestrial bioassays were carried out at the field site. The plant bioassays were duplicated and run at both the field site (planted directly in the ground) and in a laboratory at the pulp mill (in plant trays under artificial, fluorescent lights).

### 2.2.1 – Description of Study Site

The field site on which this study was conducted is located west of the town of Whitecourt (Figure 1), approximately 2.5 hours northwest of Edmonton, Alberta and is owned and managed by a newsprint manufacturing company. More specifically, this particular site was a five-year-old plantation of lodgepole pine (*Pinus contorta*) on which biosolids from the pulp mill were applied in 2001 as a soil amendment. The application rate used was 30 tons/hectare (wet weight) and the mode of application employed was aerospraying. Since biosolids were land-applied to only a fraction of this particular forest block, we were able to conduct our bioassays using the amended site and an adjacent reference site located only 20 meters away. Terrestrial bioassays were conducted on-site and aquatic bioassays were conducted in a lab at the pulp mill using run-off collected from the reference and biosolids-amended field sites.

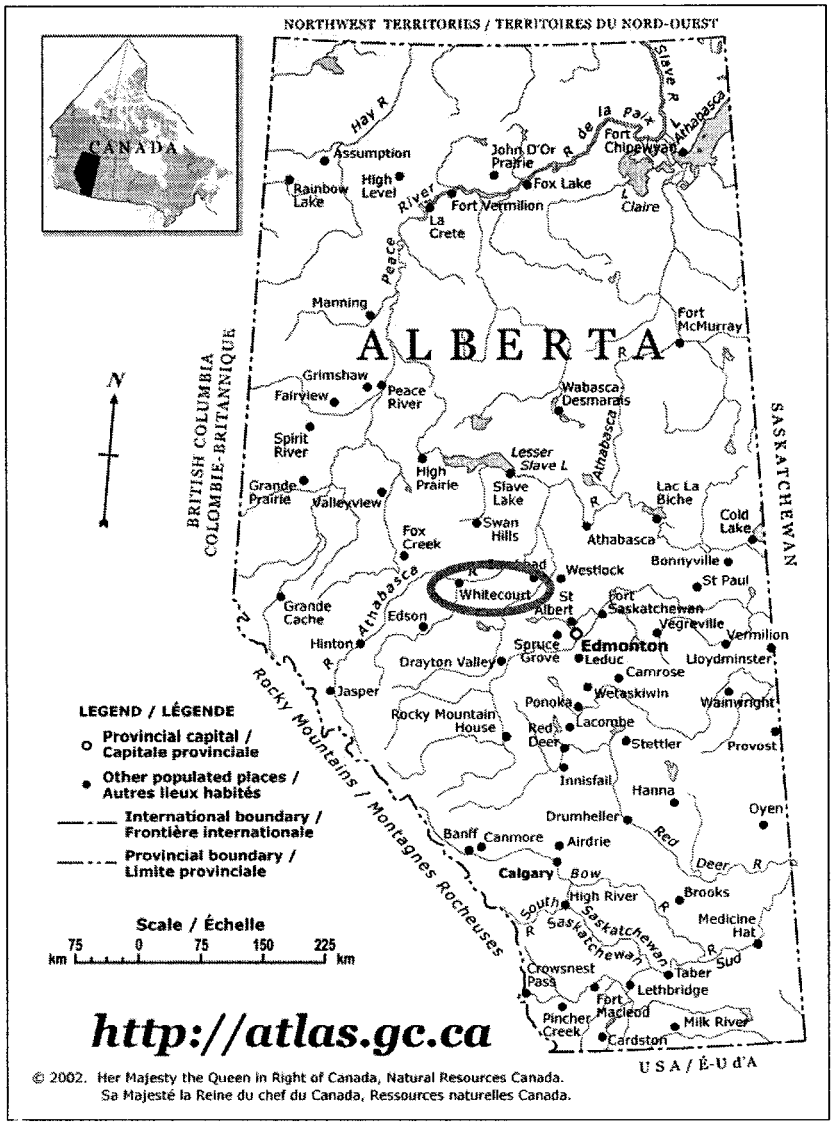


**Figure 1. Context map of Alberta showing Whitecourt**
(Taken from Natural Resources Canada website, 2002)

Although the summer months had endured drought conditions, the area did receive a significant amount of precipitation in the days prior to our arrival. According to Alberta Ministry of Agriculture, Food and Rural Development (2002), Whitecourt and the surrounding area received between 45 and 60 mm precipitation in total for the month of July 2002. Most of that was received at the beginning of the second week of July when a wide band of precipitation brought rain to most of the central region of

Alberta (Alberta Ministry of Agriculture, Food and Rural Affairs, 2002). This occurred less than a week before field experiments were launched.

The topography of the forest block where field-testing was carried out included an intermittent shallow creek to the south which naturally collected run-off originating from the biosolids-amended portion of the block. Due to the amount of precipitation prior to our arrival, it was possible to collect run-off from this creek for use in aquatic bioassays.

### 2.2.2 - Food Preparation for *Daphnia magna* and *Hyalella azteca* Bioassays

In culturing *Daphnia magna* and *Hyalella azteca* and for using these species as bioassay indicator organisms, it is essential that they receive the appropriate quantity and quality of food. There exists a fine balance between too little food and too much food for these aquatic organisms. Food is required to provide sufficient nutrition and to maintain reproductive function, however, excess food can physically harm the organisms, reduce the toxicity of the test media, and/or greatly decrease the dissolved oxygen concentration (USEPA, 2002b). Suitable nutrition is provided by feeding with a mixture prepared from yeast, alfalfa leaves, and flake fish-food according to USEPA (2002b) guidelines. This food is commonly referred to as YCT, an acronym that stands for "Yeast, CEROPHYLL®, and Trout Chow". Althought substitutions for these three components were made (described below), all are USEPA-approved (2002b). For our purposes, the *D. magna* and *H. azteca* food was referred to as YCT food.

The food was prepared by first weighing out 1.25 g of flake fish-food (TetraMin® brand) and grinding it into a fine powder using a mortar and pestle. The powder was then added to a beaker containing 250 mL of water. The solution was mixed well and aerated at ambient laboratory temperature in a fume hood for one week. After one week, the solution was allowed to settle and the supernatant was collected by filtering through a fine mesh screen (sediment was discarded). Then 1.25 g of dry yeast was

combined with 250 mL of water in a separate 500 mL beaker. The mixture was then stirred vigorously until the yeast was well dispersed. The yeast suspension was immediately combined (in equal volumes) with the fish-food supernatant. Following this, 1.25 g of dried, powdered alfalfa leaves were combined with 250 mL of water in another 500 mL beaker and stirred vigorously. After allowing the mixture to settle, the supernatant was decanted and combined with the yeast and fish-food preparation (all three solutions were combined in equal volumes).

Aliquots of the YCT food mixture were placed in 200 mL screw-cap containers and frozen until needed. Thawed food was kept refrigerated between feedings and was used for a maximum of one week.

### 2.2.3 - *Daphnia magna* Run-off Field Bioassay

Test chambers for the 8-day *Daphnia magna* field bioassay were prepared by adding 40 mL of either pulp mill biosolids run-off water (see 2.2.1 for run-off collection detatils), Athabasca River Water (reference), or bottled spring water (blank) to cleaned, labelled 50 mL glass beakers. Twelve replicates were prepared for each of the three treatment types. Once the test chambers were filled accordingly, they were allowed to settle for 24 hours before *D. magna* were added.

The *D. magna* used for the bioassay were obtained from a 20 L culture tank containing aerated dechlorinated tap water and maintained in the laboratory according to Environment Canada and USEPA guidelines (Environment Canada, 1990; USEPA, 2002b). On the day of departure from Toronto (10 days prior to launching the field bioassays), approximately 200 *D. magna* were removed from the culture vessel and transferred to a 2 L container of dechlorinated tap water and fed YCT food according to USEPA (2002b) guidelines. This new culture container (also containing *H. azteca* and *L. minor* – see 2.2.4) was placed in a Styrofoam box and transported to the field site in

Alberta. For the duration of the travel period, *D. magna* were fed YCT daily and maintained at approximately 20°C.

Twenty-four hours after the preparation of the test solutions, a single *D. magna* was removed from the 2 L culture vessel and added to each test chamber. Immediately following addition of *D. magna* to the test chambers, and on a daily basis for the duration of the bioassay, each test chamber received 0.5 mL of YCT food. Test chambers were covered with a sheet of plexiglass to minimize evaporation. Every 24 hours, the surviving *D. magna* in each beaker and any new offspring were counted and recorded.

At the end of the 8-day test period, the bioassay was terminated and the total number of surviving organisms, offspring, as well as the total number of original *D. magna* that died over the 8-day period, were recorded.

### 2.2.4 - *Hyalella azteca* and *Lemna minor* Run-off Field Bioassays

Test chambers for the 8-day *Hyalella azteca* and *Lemna minor* field bioassay were prepared by adding 150 mL of either pulp mill biosolids run-off water (see 2.2.1 for run-off collection details), Athabasca River water (reference), or bottled spring water (blank) to cleaned, labelled 200 mL glass beakers. Five replicates were prepared for each of the three treatment types. Once the test chambers were filled accordingly, a 5 $cm^2$ single layer of gauze was added to the beaker to act as substrate for the amphipods. The contents of the test chambers were then allowed to settle for 24 hours prior to the addition of *H. azteca* and *L. minor*.

The *H. azteca* and *L. minor* used for the bioassay were obtained from a 20 L culture tank containing aerated dechlorinated tap water and maintained in the laboratory according to USEPA guidelines (USEPA, 1996; USEPA, 2000). On the day of departure from Toronto (10 days prior to launching the field bioassays), approximately

200 *H. azteca* and *L. minor* were removed from the culture vessel and transferred to the 2 L container of dechlorinated tap water containing the *D. magna* (see 2.2.3) and fed YCT food according to USEPA (2002b) guidelines. This new culture container was placed in a Styrofoam box and transported to the field site in Alberta. For the duration of the travel period, *H. azteca* were fed YCT daily and maintained at approximately 20°C.

Twenty-four hours after the preparation of the test solutions, 3 small *H. azteca* and 5 *L. minor* plants (2 fronds per plant) were removed from the 2 L culture vessel and added to each test chamber. Immediately following addition of organisms to the test chambers, each test chamber received 1 mL of YCT food. Test chambers were covered with a sheet of plexiglass to minimize evaporation.

Twenty-four hours after adding the organisms, the number of surviving *H. azteca* in each test chamber was recorded to assess acute toxicity. At the end of the 8-day test period, the bioassay was terminated and the total number of *H. azteca* per test chamber was recorded to assess chronic toxicity. In addition, the number *L. minor* plants in each test chamber as well as the number of fronds on each plant was counted and recorded.

### 2.2.5 – Terrestrial Plant Land-Application Field Bioassays

*Plants Grown in Field Plots*

Two 4 $m^2$ field plots were selected for the field plant bioassays. The first was located on soil amended with pulp mill biosolids and the second on an adjacent non-amended (reference) soil site (see 2.2.1 for a description of the study site). The plots were located approximately 20 m from one another. Each plot was cleared of existing vegetation, which included various grass, berry, and weed species. The exposed soil surface was then raked and loosened to make it possible to plant seeds in. A border of

the plot was formed using logs gathered from the field site. Each plot was then sub-divided into four equal-sized rows demarcated using string.

Each of the four rows was planted with 15 seeds. Row 1 was planted with *Brassica rapa*, Row 2 with *Phaseolus vulgaris* 'Improved Golden Wax', Row 3 with *Cucurbita moschata* 'Winter Squash', and Row 4 with *Raphanus sativus* ' Crimson Giant'. All seeds were pre-tested in the lab in order to select the varieties that were capable of germination in Alberta soils (amended and non-amended). The *B. rapa* seeds were obtained from Carolina Biological Supply Company and all other seeds (which are common vegetable varieties) were purchased at a garden centre. Seeds were planted at evenly-spaced intervals in a straight row, approximately 3 cm under the soil surface. Once covered with soil, both plots were watered using a plant-watering canister with water collected from the nearby Athabasca River. The amended and non-amended field plots were prepared, planted, and watered in an identical manner. Since a large quantity of water was needed for this and other field tests, it was decided that the Athabasca River, at a site upstream of the pulp mill, provided the best available and most suitable source of water for both plant watering and as reference water. In addition, the use of Athabasca River water lends credence to the objective of incorporating environmental relevance into the bioassays.

The field plots were examined and watered with river water every second day for a total of 12 days. Germination was assessed and recorded on the days the plants were watered. On Day 12, a final count of seeds that had germinated and/or grown successfully was made. All plants were then carefully removed in their entirety from the soil and root and shoot lengths were measured and recorded.

*Plants Grown in the Laboratory*

In addition to conducting plant bioassays in field plots, the bioassays were also conducted simultaneously under artificial light conditions in a laboratory at the pulp mill. This was done to provide a comparison between field and lab conditions and also

as a precautionary measure in the event of the field plots being compromised (i.e. the seeds being eaten by wildlife).

Plastic seed growing grid-type trays (tray size 65 cm x 35 cm; 50 cells/tray) were obtained from a local gardening centre and filled with either pulp mill biosolids-amended soil or non-amended (reference) soil. The soil was obtained from the field site, at a location adjacent to each field plot to ensure uniform composition. Each cell in the grid-type tray received one seed of either *B. rapa*, *P. vulgaris* 'Improved Golden Wax', *C. moschata* 'Winter Squash', or *R. sativus* ' Crimson Giant'. In total, 32 *B. rapa* seeds and 18 of each of the three garden vegetable variety seeds were planted in both amended and non-amended soil in the trays.

Once planted, the trays were watered using a plant-watering canister with water collected from the Athabasca River. The trays were then placed directly under fluorescent lights set on timers to provide a 16-hour light: 8-hour dark photoperiod.

Every second day for a total of 10 days, the trays were examined for seed germination and watered with Athabasca River water. On Day 10, a final assessment of germination was made and all plants were carefully removed in their entirety from the soil in the trays. Root and shoot length of each plant was measured and recorded.

### 2.2.6 - *Lumbricus terrestris* Land-Application Field Bioassay

*Lumbricus terrestris* to be used for the earthworm bioassay were purchased from a bait shop in Whitecourt, AB on the same day that the bioassay was launched. Three food-grade, high-density polyethylene, cylindrical containers (10 L capacity) were filled approximately three-quarters full with either pulp mill biosolids-amended soil or non-amended (reference) soil collected from the field site (see 2.2.1 for a description of the field site). Three replicates were prepared for each of the two treatments.

Immediately following preparation of the test containers, eight sexually mature (indicated by the presence of the clitellum) *L. terrestris* from the bait shop culture were added to each of the six containers. The earthworms were placed directly on the surface of either the amended or non-amended soil. Leaf litter was placed on the surface of the soil, the soil was moistened slightly with river water, and the containers were covered with perforated plastic lids (of the same composition as the containers) to prevent the earthworms from escaping. The containers were placed in shallow trenches from which the soil samples were obtained and covered with pine branches in an attempt to keep the temperature of the soil in the containers as low as possible.

After 48 hours, the containers were checked to verify that the earthworms had burrowed in the soil. After 12 days, the bioassay was terminated and *L. terrestris* were counted to assess acute toxicity (Kula, 1998). This was accomplished by carefully emptying the contents of the containers one at a time onto a large tarpaulin and hand sifting through the soil to locate and record the number of live organisms. The soil temperature in the buckets at the time of bioassay termination was also recorded.

### *2.3 Laboratory Experimentation – Pulp Mill and Municipal Biosolids*

#### 2.3.1 – Description of Study Site

Municipal biosolids used for the laboratory portion of this study were obtained from the Ashbridges Bay Treatment Plant, which is one of four wastewater treatment facilities located in Toronto, Ontario (Figure 2). It is also the largest of the four facilities, treating approximately 818,000 $m^3$ of wastewater per day (City of Toronto, 2001). In 2001, more than 24,000 dry tonnes of anaerobically-digested, dewatered biosolids from the Ashbridges Bay Treatment Plant were land-applied for agricultural purposes (City of Toronto, 2001).

Pulp mill biosolids used for the laboratory studies were the same Alberta pulp mill biosolids that were used in the field study portion of this study (see section 2.2.1).


**Figure 2. Context map of Ontario showing Toronto**
(Taken from Natural Resources Canada website, 2002).

## 2.3.2 - Soil Run-off Collection in the Laboratory

A 90 cm x 25 cm x 15 cm wooden tray was built out of plywood and 4x4 boards to hold topsoil (ASB Greenworld brand) which was hand-packed to a depth of approximately 5 cm. The tray was inclined to simulate a 15% slope by raising one end by 13.5 cm (15% of 90 cm, the length of the tray) to form a ramp. The end of the tray (ramp) was affixed at this height using a retort stand and clamps. Since the USEPA has

determined that the acceptable range for biosolids land-application in the forest ranges from a 10% to 20% slope, a 15% slope was chosen (USEPA, 1997). The Ontario Ministry of Environment (MOE) and Ministry of Agriculture, Food and Rural Affairs (OMAFRA) Guidelines for utilization of biosolids on agricultural land defines the maximum acceptable slope as 9% and states that 0% to 3% slopes are preferable (MOE/OMAFRA, 1996). Therefore, the 15% slope that was chosen simulated a worst-case scenario.

To simulate land-application, a predetermined amount of biosolids were hand-spread atop the soil in the ramp based on a typical 20 ton/hectare/year application rate (USEPA, 1997). Since this application rate is based on the dry weight of the biosolids, an average dry weight – to – wet weight ratio was calculated to determine the quantity of wet biosolids to be land-applied to the ramp (this is the condition they are in when they leave the mill or sewage treatment plant destined for land-application). To calculate this ratio, a scoop of either pulp mill or municipal biosolids was added to a 25 mL ceramic crucible, weighed and then placed in a desiccator cabinent at 60°C (Gallenkamp Oven, Model OV-330) for 24 hours to eliminate moisture. After 24 hours, the crucible and its contents were weighed again and a dry – to – wet weight ratio was determined for the biosolids (see Appendix A for calculations). Once the specific amount of biosolids was applied to the appropriate ramp (1620.52 g of pulp mill biosolids and 1772.67 g of municipal biosolids – see Appendix A), they were hand-packed to simulate the compaction that occurs when biosolids are land-applied via equipment such as aerosprayers.

The open end of the ramp at the base of the slope was covered with two layers of standard window screen mesh to prevent the soil and biosolids from shifting down the slope and a 10 L glass aquarium was placed at the base of the ramp in order to collect run-off. For run-off collection, a typical one-day rainfall of 20 mm (Environment Canada, 2002) was simulated (see Appendix B for calculations). Based on calculations, 4.5 L of dechlorinated tap water was sprinkled over the ramp from an approximate height of 1 m using a plant-watering canister. The resulting run-off was

collected in the aquarium and either promptly used in bioassays or stored in glass containers and refrigerated. Three 'rain events' of 20 mm each were simulated (with a week between each rain event) to collect approximately 3 L of run-off needed for bioassays.

Three ramps in total were used for run-off collection. The first was amended with pulp mill biosolids, the second with municipal biosolids, and the third with reference soil only. Thus, three corresponding run-off samples were collected with which to conduct aquatic bioassays.

### 2.3.3 - *Daphnia magna* Run-off Laboratory Bioassays

*Acute Toxicity Bioassay*

Test chambers for the *Daphnia magna* 48-hour acute bioassay were prepared by adding 100 mL of various concentrations of pulp mill biosolids, municipal biosolids, or reference soil run-off to labelled 150 mL glass beakers. Three replicates were prepared for each type of run-off at concentrations of 0, 25, 50, 75, and 100%. Various combinations of dechlorinated tap water and run-off collected from the land-application simulation ramps were used to prepare the range of concentrations. Once the test chambers received the correct amount of run-off and/or dechlorinated tap water, they were allowed to settle for 24 hours prior to the addition of *D. magna* neonates.

The *D. magna* used for bioassays were obtained from a 20 L culture tank containing aerated dechlorinated water and maintained according to Environment Canada and USEPA guidelines (Environment Canada, 1990; USEPA, 2002b). Ten *D. magna* neonates (less than 24 hours old) were added to each test chamber and allowed to grow under static conditions for 48 hours according to USEPA (2002b) guidelines. Immediately following addition of neonates to the test chambers, each beaker received

1 mL of YCT formula. Test chambers were covered with a plexiglass sheet to minimize evaporation.

After 48 hours, the number of surviving *D. magna* in each test chamber was recorded and the bioassay was terminated.

*Chronic Toxicity and Reproduction Bioassay*

Test chambers for the *D. magna* 21-day chronic toxicity and reproduction bioassay were prepared by adding 40 mL of various concentrations of pulp mill biosolids, municipal biosolids, or reference soil run-off to 50 mL glass beakers. Ten replicates were prepared for each of three types of run-off at concentrations of 0, 25, and 100%. Dechlorinated tap water and run-off collected from the land-application simulation ramps were used to prepare the test solutions. Once the test chambers received the correct amount of run-off and/or dechlorinated tap water, they were allowed to settle for 24 hours prior to the addition of *D. magna* neonates.

The *D. magna* used for bioassays were obtained from a 20 L culture tank containing aerated dechlorinated water and maintained according to Environment Canada and USEPA guidelines (Environment Canada, 1990; USEPA, 2002b). One *D. magna* neonate (less than 24 hours old) was added to each test chamber and allowed to grow under static conditions for 21 days according to USEPA (2002b) guidelines. Immediately following addition of neonates to the test chambers and on a daily basis for the duration of the bioassay, each beaker received 0.5 mL of USEPA-approved YCT formula. Test chambers were kept covered with a plexiglass sheet to minimize evaporation. Every second day, *D. magna* were counted and any new offspring were removed from the test chambers once they had been accounted for. At this time, water renewal with dechlorinated tap water was also carried out to compensate for evaporation and to maintain test solution volume at 40 mL.

After 21 days, the test was terminated and the total number of offspring, as well as the total number of original organisms that died over the 21-day period, were tabulated.

### 2.3.4 - *Hyalella azteca* Run-off Laboratory Bioassays

*Acute and Chronic Toxicity and Reproduction Bioassay*

Test chambers for the *Hyalella azteca* 48-hour acute and 21-day chronic toxicity and reproduction bioassay were prepared by adding 100 mL of various concentrations of pulp mill biosolids, municipal biosolids, or reference soil run-off to labelled 150 mL glass beakers. Five replicates were prepared for each of the three types of run-off at concentrations of 0, 10, 25, 50, 75, and 100%. Dechlorinated tap water and run-off collected from the land-application simulation ramps were used to prepare the test solutions. Once the test chambers received the correct amount of run-off and/or dechlorinated tap water, a 5 $cm^2$ single layer of gauze was added to the beaker to act as substrate for the amphipods. The contents of the test chambers were then allowed to settle for 24 hours prior to the addition of *H. azteca* neonates.

The *H. azteca* used for bioassays were obtained from a 20 L culture tank containing aerated dechlorinated water and maintained according to USEPA guidelines (USEPA, 2000). Three young *H. azteca* (0 to 1 week old) were added to each test chamber and allowed to grow under static conditions for 21 days. Immediately following addition of young *H. azteca* to the test chambers, each test chamber received 2 mL of YCT. For the remainder of the bioassay, each test chamber received 1 mL of YCT on a daily basis (USEPA, 2000). Test chambers were kept covered with watch glasses to minimize evaporation. Every second day, water renewal was carried out using dechlorinated tap water to compensate for evaporation and to maintain test solution volume at 100 mL.

Forty-eight hours after initiation of the bioassay, the surviving organisms in all test chambers were counted to assess potential acute toxicity. After 21 days, the test was terminated and all young and surviving *H. azteca* were counted to assess chronic toxicity and reproduction.

2.3.5 - *Pseudokirchneriella subcapitata* Run-off Laboratory Bioassay

Five separate stock nutrient solutions that make up the required algal culture medium were prepared according to USEPA protocol (USEPA, 2002c) (see Appendix C). The five solutions were autoclaved and stored in the refrigerator until needed. Fifty mL of the algal culture media (termed "AAP") was prepared from the five stock solutions, by a 1000-fold dilution of each stock solution in a 250 mL Erlenmeyer flask. The 50 mL flask containing the AAP media was then aseptically inoculated with *Pseudokirchneriella subcapitata* cells from a culture on solid medium (agar-agar 1.5% in AAP 1.5X concentrate standard). This flask represented Culture #0, an initial culture that was not used for algal tests (USEPA, 2002c). Culture #0 was then incubated for one week at 24°C under cool-white fluorescent light and agitated daily until it took on a green hue. Following incubation for one week, Culture #1 was launched by aseptically transferring 100 µL of Culture #0 to 50 mL of AAP media. Each week, a new culture was prepared in this manner to maintain a healthy culture of cells for bioassays until reaching Culture #7, at which time a new Culture #0 was launched. This is in light of the fact that testing is not recommended using cultures higher than Culture #7, due to increased contamination potential (USEPA, 2002c).

*P. subcapitata* 96-hour bioassays were conducted on micro-plates with 24 2-mL wells (Bostan, 2001). There were six replicates (i.e. 6 wells) for each of four concentrations of the three types of run-off: 0, 25, 50, and 100%. Dechlorinated tap water and run-off collected from the land-application simulation ramps was used to prepare the various concentrations of test solution. To each well, 2 mL of the appropriate test solution was added, followed by 2µL of each of the five stock nutrient solutions (in order to dilute the stock nutrient solutions 1000-fold) (Bostan, 2001). The test solutions were then

allowed to settle in the wells for 24 hours. After 24 hours, a count of the cells in the culture flask (culture #4 at the time of the test) was made to determine the volume of culture to add to each well. At the beginning of the bioassay, it is a requirement that the concentration of *P. subcapitata* be $10^5$ cells/mL in each well (Bostan, 2001). The cell count of culture #4 was made optically using a microscope and an Improved Neubauer hemacytometer and was determined to be 3.75 x $10^6$ cells/mL. Thus, in order for the concentration of cells in each well to equal $10^5$, every well was spiked with 53.33 µL of the culture (see Appendix D for calculations).

The samples were incubated for 96 hours at 24°C under cool-white fluorescent light (USEPA, 2000). After 96 hours, the algal cells in each microplate were resuspended (a clean pipette tip was used for each well) and counted optically (as above). Five counts were made from each well in order to minimize counting error and an average count of *P. subcapitata* cells/mL was tabulated.

### 2.3.6 - *Lemna minor* Run-off Laboratory Bioassay

*Lemna minor* 14-day bioassays were conducted on micro-plates with 24 2-mL wells. There were twelve replicates (i.e. 12 wells) for each of four concentrations of the three types of run-off: 0, 25, 50, and 100%. Dechlorinated tap water and run-off collected from the land-application simulation ramps was used to prepare the various concentrations of test solution. To each well, 2 mL of the appropriate test solution was added, followed by 10µL of each of the five stock nutrient solutions used in the *P. subcapitata* bioassay (see Appendix C) in order to make a 20X-AAP nutrient media (corresponds to a 200-fold dilution of each of the 5 concentrated nutrient media), according to USEPA protocol (USEPA, 1996). The test solutions were then allowed to settle in the wells for 24 hours.

The *L. minor* plants used for the bioassay were obtained from Ward's Natural Science Ltd. one week prior to the beginning of the test launch. They were placed in a 10 L

glass aquarium containing dechlorinated tap water and AAP media and allowed to acclimate at approximately 24°C under cool-white fluorescent light for one week. Twenty-four hours following the preparation of test solutions in the micro-plates, each well was inoculated with a single 2-frond *L. minor* plant using a sterile wire loop. The samples were incubated for 14 days at 24°C under cool-white fluorescent light. Visual observations and a frond count were performed for each well every 2 days until Day 14, when a final frond count was made and the test terminated.

### 2.3.7 - *Brassica rapa* Land-Application Laboratory Bioassay

One week after completing the soil run-off collection, the three ramps were used for planting of *Brassica rapa* seeds. The contents of each of the three ramps (pulp mill biosolids-amended soil, municipal biosolids-amended soil, or non-amended soil) were mixed using a hand-held spade. This was done to incorporate the biosolids into the soil, representing a worst-case scenario of biosolids dispersal in the soil following land-application. Once the soil was mixed and loosened, 30 *B. rapa* seeds were planted in an evenly-spaced manner in each of the three ramps. The seeds were planted approximately 3 cm below the surface, were loosely covered and placed on a laboratory bench under fluorescent lights. The lights were set on a timer to provide a 12-hour light: 12-hour dark photoperiod.

Immediately following planting, and every second day for the duration of the 8-week bioassay, the ramps containing the *B. rapa* seeds were watered with tap water using a plant-watering canister. Each week, a count of the number of seeds germinated was made. As the plants grew taller, they were staked in an upright position using wooden sticks to prevent stem breakage. As they emerged, the flowers were cross-pollinated with other flowers in the same treatment group. This was accomplished using a sterile swab; the pollen from one flower was picked up on the surface of the swab and physically transferred to another flower within the same treatment group. After eight weeks, the bioassay was terminated. Each plant was carefully removed from the ramps

and the shoot and root lengths, as well as the number of flowers and seedpods on each plant, was recorded.

The seedpods from all of the *B. rapa* plants were collected in beakers according to the three treatment groups and allowed to desiccate for a period of two weeks. Once dried, the seedpods were opened and the second generation ($F_1$) seeds were collected. Keeping the three treatment groups separate, the $F_1$ seeds were planted in plastic seed growing grid-type trays (50 cells per tray; tray size 65 cm x 35 cm) that were obtained from a local gardening centre and filled with topsoil (ASB Greenworld Brand). One seed was planted in each cell of the grid-type tray, covered loosely with soil, and watered with tap water. The trays were placed on a laboratory bench under fluorescent lights that were set on a timer to provide a 12-hour light: 12-hour dark photoperiod. Every day for 7 days, the trays were moistened with tap water and the number of seeds that germinated was recorded. At the end of the 7-day $F_1$ germination bioassay, a final count of the number of seeds germinated in each of the three treatment groups was made and the test was terminated.

### 2.3.8 - *Phaseolus vulgaris* Land-Application Laboratory Bioassay

The *Phaseolus vulgaris* bioassay was conducted by filling plastic planting pots (1.5 L capacity) three-quarters full with either pulp mill biosolids-amended soil, municipal biosolids-amended soil, or non-amended soil. The soil used for all three treatments was topsoil (ASB Greenworld brand) obtained from a local garden supply centre. For the biosolids-amended treatments, topsoil and biosolids (either pulp mill or municipal) were combined in a one – to – one ratio and the soil was mixed thoroughly so that the biosolids were incorporated in the soil. Five replicates were prepared for each of the three treatment groups.

Three *P. vulgaris* seeds ('Improved Golden Wax' variety) were planted in each of the test pots. The seeds were planted approximately 5 cm deep, covered with soil, and

placed on the laboratory bench under fluorescent lights set on a timer to provide a 12-hour light: 12-hour dark photoperiod.

Immediately following planting, and every second day for the duration of the 8-week bioassay, the pots containing the *P. vulgaris* seeds were watered with tap water using a plant-watering canister. Every week, a count of the number of seeds germinated was made. As the plants grew taller, they were staked in an upright position using wooden sticks to prevent stem breakage. After eight weeks, the bioassay was terminated. Each plant was carefully removed from the pots and the shoot and root lengths, as well as the number of seedpods on each plant, was recorded.

The seedpods from all of the *P. vulgaris* plants were collected in beakers according to the three treatment groups and allowed to desiccate for a period of two weeks. Once dried, the seedpods were opened and the second generation ($F_1$) seeds were collected. Keeping the three treatment groups separate, the $F_1$ seeds were planted in plastic seed-growing grid-type trays (50 cells per tray; tray size 65 cm x 35 cm) that were obtained from a local gardening centre and filled with topsoil (ASB Greenworld Brand). One seed was planted in each cell of the grid-type tray, covered loosely with soil, and watered with tap water. The trays were placed on a laboratory bench under fluorescent lights that were set on a timer to provide a 12-hour light: 12-hour dark photoperiod. Every day for 7 days, the trays were moistened with tap water and the number of seeds that germinated was recorded. At the end of the 7-day $F_1$ germination bioassay, a final count of the number of seeds germinated in each of the three treatment groups was made and the test was terminated.

2.3.9 - *Lumbricus terrestris* Land-Application Laboratory Bioassay

*Lumbricus terrestris* were obtained from Ward's Natural Science Ltd. two weeks prior to initiating the bioassay. Upon receipt, the earthworms were placed into food-grade, high-density polyethylene, cylindrical containers (10 L capacity) filled with reference soil (ASB Greenworld topsoil). Water was added to the container to moisten the soil to approximately 30% (dry weight) via a hand-held spray bottle. Water-soaked sphagnum moss was then scattered on the surface of the soil in each container as a food source for the earthworms. The culture vessel was placed on a north-facing window ledge of our laboratory (an area where temperature is maintained at approximately 20°C). The earthworms were kept in the culture vessel for two weeks prior to launching the bioassay in order to acclimate.

Following the 14-day acclimation period, nine 10 L containers (as described above) were filled with 6 kg (dry-weight) of either reference soil (ASB Greenworld topsoil), pulp mill biosolids-amended topsoil, or municipal biosolids-amended topsoil. Calculations were done to determine the quantity of biosolids the amended samples should receive. Based on a 20 ton/hectare/year application rate and taking into account the dry – to – wet weight ratios of the two types of biosolids, 509 g (wet-weight) of pulp mill biosolids or 556 g (wet-weight) of municipal biosolids were applied to the topsoil in the treatment containers (calculations done in the same manner as in Appendix A). A soil moisture content of 30% was then achieved by misting the surface of the soil (or biosolids) with dechlorinated tap water using a hand-held spray bottle. A moisture content of 30% was chosen as it has been identified as optimal for mass-rearing of *L. terrestris* (Berry and Jordan, 2001). Three replicates were prepared for each of the three treatments.

Immediately following preparation of the test containers, eight healthy *L. terrestris* from the culture population were added to each of the nine containers. The earthworms were placed directly on the surface of either the topsoil or the biosolids. Water-soaked

sphagnum moss was then scattered on the surface of the soil in each container as a food source for the earthworms. The containers were covered with perforated plastic lids (of the same composition as the containers) to prevent the earthworms from escaping and were placed in the same location as the culture vessel where they were maintained at 20°C (± 2°C) for the duration of the bioassay.

After 7 days, earthworms were checked for acute toxicity (Kula, 1998). This was accomplished by carefully emptying the contents of the containers one at a time and hand sifting through the soil to locate and record the number of the live organisms. The soil was then returned to the test containers and the surviving *L. terrestris* were again placed on the soil surface and covered with moss. The containers were covered and returned to their original location. This process of sifting through the soil inevitably resulted in the partial incorporation of the biosolids with the soil in the biosolids-amended treatments. It was felt that this was acceptable since it simulates conditions that may eventually occur naturally on land to which biosolids are applied.

Subsequent checks for chronic toxicity were performed bi-weekly. At each examination, the soil was also visually inspected for the presence of cocoons or young *L. terrestris* in order to assess reproductive capacity. The number of surviving earthworms, as well as the number of cocoons or young present, was recorded. Following this, the soil, along with all organisms, was returned to the corresponding test container and location. At every other bi-weekly check (i.e. every month), fresh water-soaked moss was added to the surface of the soil and the soil was again misted with a small amount of water. Bi-weekly assessments of this nature continued for a total of 20 weeks, at which time a final count of surviving organisms and cocoons was made and the bioassay terminated.

Data collected from all pre-test, field, and laboratory experiments were then analyzed for statistical significance using either two-sample t-tests or chi-square analysis, depending on the nature of the data. For all statistical analyses, the confidence interval was set at 95% ($\alpha = 0.05$).

## 3.0: RESULTS

### *3.1 Pre-Tests*

A *Lumbricus terrestris* pre-test was conducted to determine whether earthworms would survive in pulp mill biosolids-amended Alberta forest soil and Alberta forest reference soil. After 7 days, 100% survival was exhibited in both the biosolids-amended and non-amended (reference) soils. After 98 days, the mean number of organisms per test container was found to have decreased very slightly from 8 (the initial number of earthworms in each container) to 7.33 (± 0.33) in reference soil and 7.67 (± 0.33) in biosolids-amended soil; this corresponds to 91.6% and 95.9% survival, respectively. It was determined that these values were not significantly different (chi-square). It was also established that there was no significant difference between the number of *L. terrestris* per test container at Day 1 compared to Day 98, for both the biosolids-amended and non-amended soils (See Appendix E for data).

Plant pre-tests conducted to determine which common garden vegetable varieties would exhibit high germination rates in pulp mill biosolids-amended Alberta forest soil and Alberta forest reference soil yielded the results shown in Table 2. In order for results to be considered acceptable, at least 80% of all seeds in the reference soil had to have germinated. Eighty percent survival of control organisms is the standard lower limit for considering any type of toxicological test results acceptable (USEPA, 2002b). Since *Lactuca sativa* (Grand Rapids) seeds did not germinate to the required 80% success rate in reference soil, this species was eliminated as an appropriate field bioassay species. Also, since germination of *L. sativa* (Early Great Lakes) was only 80% in reference soil, and because of the limited success of the second *L. sativa* variety, it was decided that all varieties of *L. sativa* would be excluded from field plant bioassays (See Appendix F for data).

**Table 2.** Percent germination of common garden vegetable variety seeds in Alberta reference and pulp mill biosolids-amended soil.

| | PERCENT GERMINATION (10 DAYS) | |
|---|---|---|
| **PLANT SPECIES** | **Reference Soil** | **Biosolids-Amended Soil** |
| *Lactuca sativa* (Early Great Lakes) | 80.0 % | 90.0 % |
| *Lactuca sativa* (Grand Rapids) | 70.0 % | 80.0 % |
| *Phaseolus Vulgaris* (Improved Golden Wax) | 100.0 % | 100.0 % |
| *Raphanus sativus* (Crimson Giant) | 90.0 % | 90.0 % |
| *Cucurbita moschata* (Winter Squash) | 100.0 % | 90.0 % |

## *3.2 Field Experimentation*

As mentioned in section 2.2.1, the field site where this portion of the study was conducted was a five-year-old plantation of lodgepole pine (*Pinus contorta*) on which biosolids from the pulp mill were applied in 2001 as a soil amendment. The soil was very dry and firm at the time of this field study. It was also noted that ground cover in the area was dense and consisted of a number of species of grasses and berries growing amongst the pine trees.

### 3.2.1 – Run-off Bioassays

In the *Daphnia magna* run-off field bioassay, 100% survival of organisms was observed after 24 hours in all three of the test solutions (Athabasca River water (reference), spring water (blank), and pulp mill biosolids-amended soil run-off). Similarly, 100% survival was also observed after 8 days in all three treatments (see Appendix G for data).

Although reproductive success was not assessed due to logistic and time constraints (a 21-day period is required for conducting *D. magna* reproductive assessments), within

the 8-day bioassay period, there were neonates produced in each of the three treatment groups. It was noted that were no apparent differences in size and swimming ability among neonates from various treatments.

In the *Hyalella azteca* run-off field bioassay, 100% percent survival was observed after 24 hours in the three test solutions (Athabasca River water (reference), spring water (blank), and pulp mill biosolids-amended soil run-off). Results from the *H. azteca* 8-day field bioassay are presented in Figure 3. Statistical analysis confirmed that there was no significant difference between the mean number of organisms at Day 8 in the reference water and in the biosolids run-off (t-test). There was, however, a significant difference between the mean number of *H. azteca* per test container at Day 8 in the blank and in the biosolids run-off treatment (see Appendix H for data).

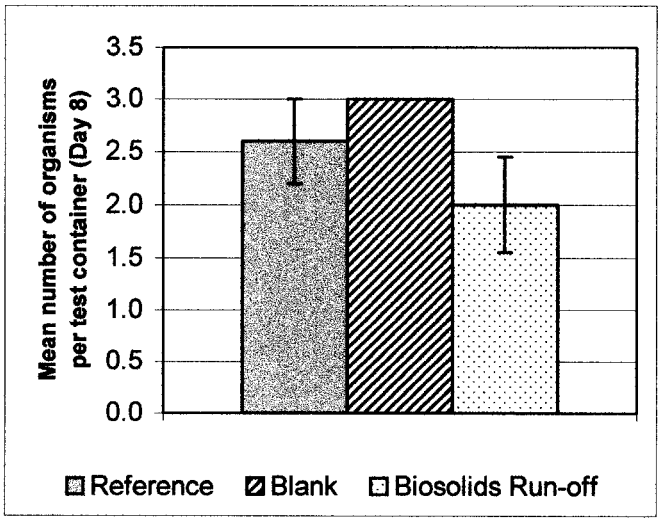


**Figure 3.** Mean number of *Hyalella azteca* per test container (± standard error; n=5) following eight days of exposure to Athabasca River water (reference), bottled spring water (blank), or pulp mill biosolids-amended soil run-off.

After 8 days, the mean number of *Lemna minor* fronds per test container in the run-off bioassay was found to have increased from 10 fronds to 23.2 (±0.86) fronds in reference water, 23.0 (± 1.00) fronds in the blank, and 25.8 (± 1.77) fronds in the biosolids-amended soil run-off (Figure 4). Statistical analysis of the data confirmed

that there was no significant difference between the three treatment groups at Day 8 (t-test). In addition, plants in all three treatment groups appeared healthy in that they were vivid green in colour and were continuing to divide and produce more fronds (see Appendix I for data).

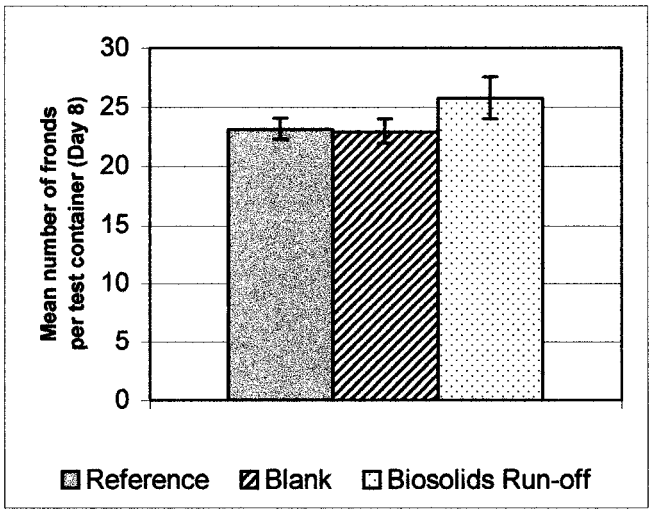


**Figure 4.** Mean number of *Lemna minor* per test container (± standard error; n=5) following eight days of exposure to Athabasca River water (reference), bottled spring water (blank), or pulp mill biosolids-amended soil run-off.

### 3.2.2 – Land-Application Bioasssays

The results of the terrestrial plant land-application field bioassays are displayed in Table 3 . In the field plots, as well as in the laboratory at the pulp mill, percent germination of *Brassica rapa* in biosolids-amended soil was significantly lower than in reference soil (chi-square). Percent germination of *Cucurbita moschata* in the reference soil field plot was significantly lower than in biosolids-amended soil in the field. For all other plant species (in both the field plots or in the laboratory at the mill) there were no significant differences between percent germination in reference soil and biosolids-amended soil (see Appendices J and K for data).

**Table 3.** Percent germination of plants at the field site and in the laboratory at the pulp mill planted in either reference soil or pulp mill biosolids-amended soil.

| | PERCENT GERMINATION (%) | |
|---|---|---|
| **FIELD PLOTS** | **Reference soil** | **Biosolids-amended soil** |
| *Brassica rapa* | 97% | 53% |
| *Raphanus sativus* | 100% | 100% |
| *Phaseolus vulgaris* | 87% | 87% |
| *Cucurbita moschata* | 53% | 87% |
| **LABORATORY** | **Reference soil** | **Biosolids-amended soil** |
| *Brassica rapa* | 88% | 63% |
| *Raphanus sativus* | 100% | 100% |
| *Phaseolus vulgaris* | 100% | 100% |
| *Cucurbita moschata* | 83% | 89% |

A comparison of root-length index (a ratio of root length to total length of plant) was made for all field plants, the results of which are shown in Table 4. The only significant difference was for *Phaseolus vulgaris* in the field plot. The root-length index for *P. vulgaris* in the biosolids-amended field plot was significantly higher than in the reference plot (t-test). There were no significant differences in mean root-length index between the biosolids-amended and reference treatments for any other plant species, whether grown in the field plot or at the laboratory at the pulp mill (see Appendices J and K for data).

**Table 4.** Mean root length indices (± standard error) for plants at the field site and in the laboratory at the pulp mill grown in either reference soil or pulp mill biosolids-amended soil.

| | MEAN ROOT-LENGTH INDEX (± S.E.) | |
|---|---|---|
| **FIELD PLOTS** | **Reference soil** | **Biosolids-Amended Soil** |
| *Brassica rapa* | 0.50 (± 0.02) | 0.53 (± 0.02) |
| *Raphanus sativus* | 0.48 (± 0.02) | 0.45 (± 0.02) |
| *Phaseolus vulgaris* | 0.39 (± 0.02) | 0.49 (± 0.03) |
| *Cucurbita moschata* | 0.49 (± 0.02) | 0.53 (± 0.03) |
| **LAB** | **Reference soil** | **Biosolids-Amended Soil** |
| *Brassica rapa* | 0.49 (± 0.01) | 0.47 (± 0.01) |
| *Raphanus sativus* | 0.30 (± 0.03) | 0.34 (± 0.03) |
| *Phaseolus vulgaris* | 0.31 (± 0.02) | 0.29 (± 0.02) |
| *Cucurbita moschata* | 0.48 (± 0.01) | 0.49 (± 0.01) |

After 12 days, the mean number of *Lumbricus terrestris* per test container was 7.00 (± 0.58) in reference soil and 8.00 (± 0.00) in pulp mill biosolids-amended soil; this corresponds to 87.5% and 100.0% survival, respectively. Statistical analysis confirmed that there was no significant difference between the two treatments (chi-square). In addition, organisms from both the biosolids-amended and non-amended treatments appeared healthy in that they were plump, moist (i.e. coated in mucus), and responded well to physical stimuli (i.e. wriggled immediately upon prodding) (see Appendix L for data).

### *3.3 Laboratory Experimentation*

#### 3.3.1 – Run-off Bioassays

Results of the *Daphnia magna* 48-hour run-off laboratory bioassay are displayed in Figure 5. Statistical analysis showed that there were no significant differences between treatments in the mean number of organisms per test container after 48 hours throughout the range of run-off concentrations (0, 25, 50, 75, 100%) for either reference soil, pulp mill biosolids-amended soil, or municipal biosolids-amended soil (chi-square). No significant differences were detected when equivalent concentrations of biosolids-amended and reference soil run-off were compared (see Appendix M for data).

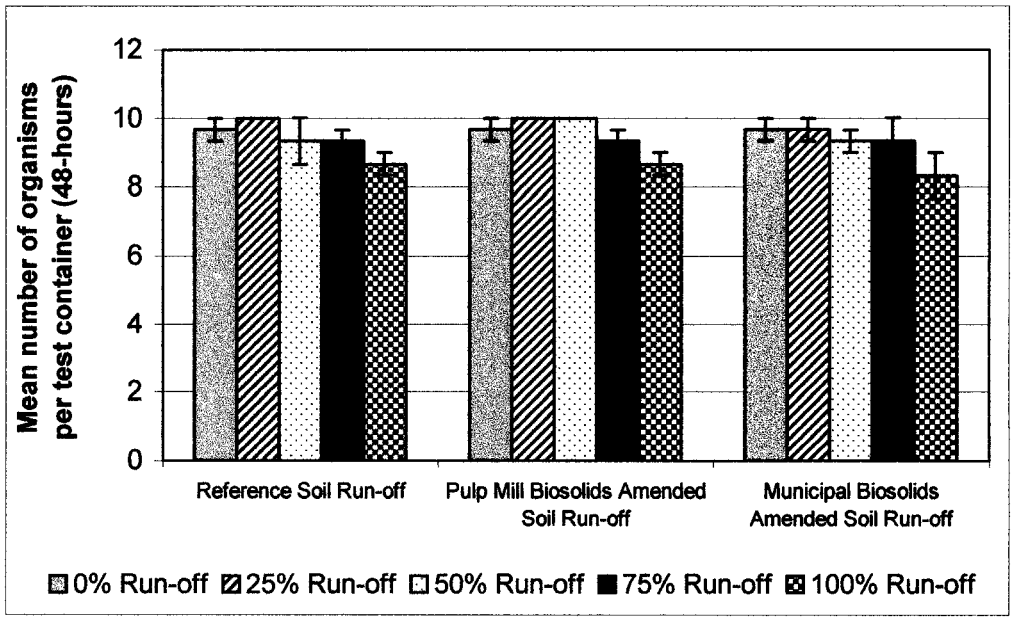


**Figure 5.** Mean number of *Daphnia magna* per test container (± standard error; n=3) following 48 hours of exposure to varying concentrations of reference soil run-off, pulp mill biosolids-amended soil run-off, or municipal biosolids-amended soil run-off.

After 21 days, the number of surviving *D. magna* out of ten (initial number of organisms) was 9 in the reference treatment, 8 in 25% pulp mill biosolids-amended soil run-off, 9 in 100% pulp mill biosolids-amended soil run-off, 8 in 25% municipal biosolids-amended soil run-off, 8 in 100% municipal biosolids-amended soil run-off, 10 in 25% reference soil run-off, and 10 in 100% reference soil run-off (see Appendix N for data). In all treatments, survival was greater than 80%, the standard lower limit for considering any type of toxicological test results acceptable (USEPA, 2002b).

The *D. magna* 21-day evaluation of reproductive fitness (Figure 6) showed that there was a significant difference between the mean number of neonates produced per living adult after 21 days when comparing the 0% and 25% reference soil run-off, 0% and 100% reference soil run-off, and 0% and 100% municipal biosolids-amended soil run-off (chi-square). A higher number of neonates were produced in these three treatments (100% municipal biosolids-amended run-off, and 25% and 100% reference soil run-off) compared to the blank. There was no statistical significance between concentrations (0, 25, 100%) for pulp mill biosolids-amended soil run-off or between 0% and 25% municipal biosolids-amended soil run-off. After 21 days, the mean number of neonates produced per living adult was significantly lower in both the 25% pulp mill and municipal biosolids-amended soil run-off than in 25% reference soil run-off. Similarly, significantly fewer neonates were produced in 100% pulp mill biosolids-amended soil run-off than in 100% reference soil run-off. In addition, visual observations indicated that there were no differences in size or swimming ability among neonates from the different treatments (see Appendix N for data).

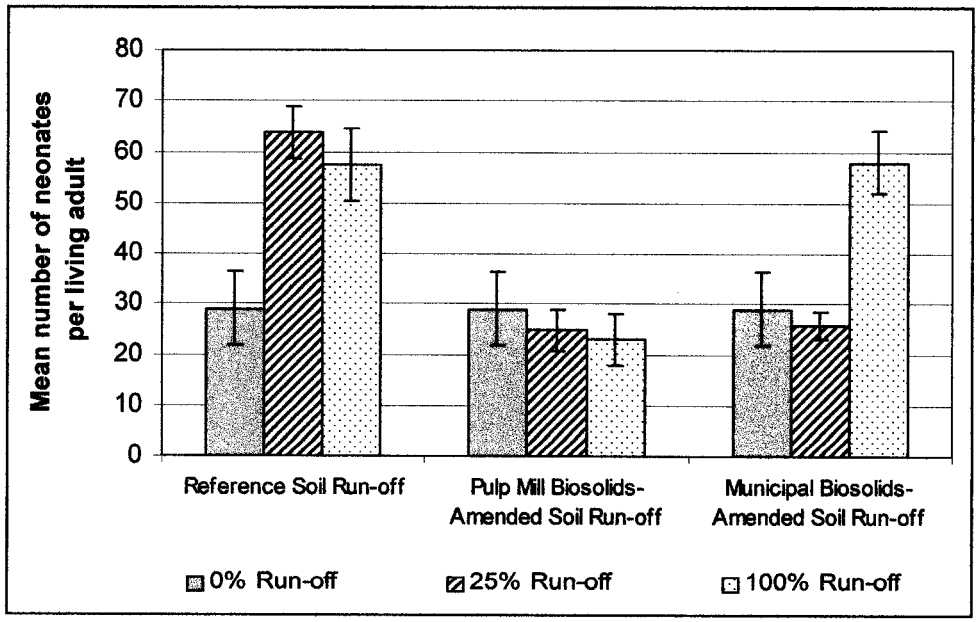


**Figure 6.** Mean number of *Daphnia magna* neonates per living adult (± standard error; n=10) following 21 days of exposure to varying concentrations of reference soil run-off, pulp mill biosolids-amended soil run-off, or municipal biosolids-amended soil run-off.

The results of the *Hyalella azteca* 48-hour run-off laboratory bioassay are displayed in Figure 7. Statistical analysis confirmed that there were no significant differences in the mean number of organisms per test container after 48 hours between concentrations (0, 10, 25, 50, 75, and 100%) for each of the three types of run-off (t-test), with the following exception. It was found that there were significantly fewer organisms in 100% pulp mill biosolids-amended soil run-off than in 0% run-off (blank). All concentrations of biosolids-amended soil run-off were not significantly different from corresponding concentrations of reference soil run-off (see Appendix O for data).

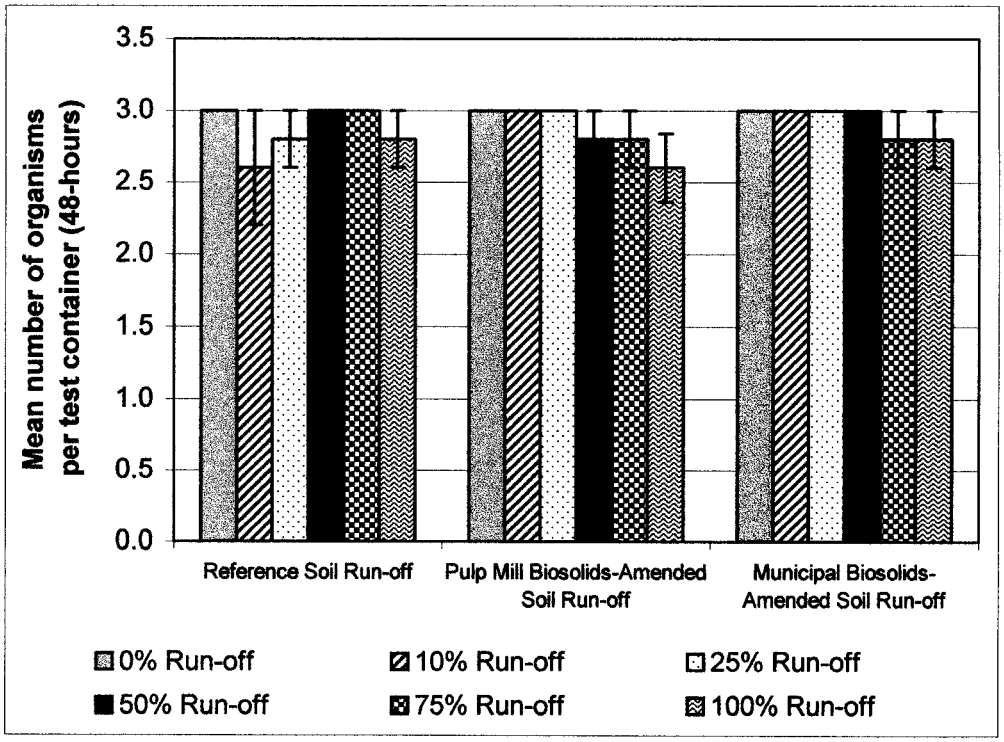


**Figure 7.** Mean number of *Hyalella azteca* per test container (± standard error; n=5) after 48 hours of exposure to varying concentrations of reference soil run-off, pulp mill biosolids-amended soil run-off, or municipal biosolids-amended soil run-off.

The results obtained from the *H. azteca* 21-day run-off laboratory bioassay are shown in Figure 8. After 21 days, there were no significant differences in the mean number of organisms per test container between concentrations (0, 10, 25, 50, 75, 100%) for each of the three types of run-off (t-test). The mean number of *H. azteca* was found to be significantly lower in 75% pulp mill biosolids-amended soil run-off than in 75% reference soil run-off. There were no significant differences for all other concentrations of pulp mill or municipal biosolids-amended soil run-off when compared to equivalent concentrations of reference soil run-off (see Appendix O for data).

Although a reproductive assessment for *H. azteca* was not conducted, it was noted that neonates were successfully produced in each concentration of all three types of run-off; observations indicated that there were no differences in size or swimming ability among treatments.

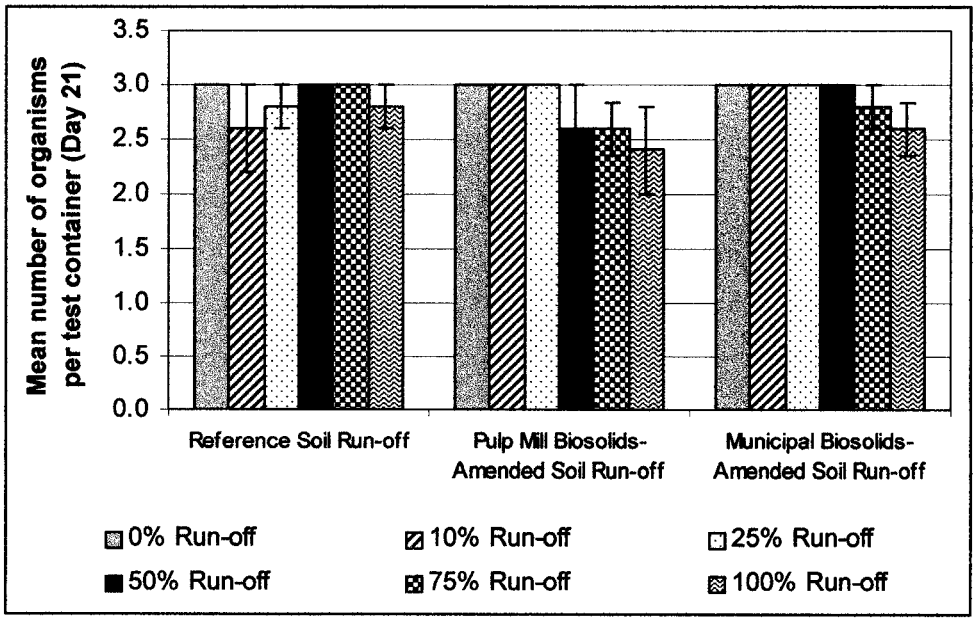


**Figure 8.** Mean number of *Hyalella azteca* per test container (± standard error; n=5) following 21 days of exposure to varying concentrations of reference soil run-off, pulp mill biosolids-amended soil run-off, or municipal biosolids-amended soil run-off.

Results from the *Pseudokirchneriella subcapitata* 96-hour run-off laboratory bioassay are displayed in Figure 9. For each of the three types of run-off, it was found that the 25, 50, and 100% concentrations all had significantly higher cell concentrations than the 0% treatment (chi-square). It was determined that there was no significant difference between the 25, 50,and 100% concentrations for each of the three types of run-off. The highest rate of growth was observed for the reference soil run-off treatments (25, 50, and 100%). In addition, higher growth rates were observed for the pulp mill biosolids-amended soil run-off treatments compared to the municipal biosolids-amended soil run-off treatments. When equivalent concentrations of biosolids-amended run-off and reference run-off were compared, a number of significant differences were detected. Algae growth was significantly lower in 25% municipal biosolids-amended soil run-off than in 25% reference soil run-off. Similarly, algae growth was significantly lower in 50% pulp mill and municipal biosolids-amended soil run-off than in 50% reference soil run-off. In addition, the number of cells per mL was significantly lower in 100% pulp mill and municipal biosolids-amended soil run-off than in 100% reference soil run-off (see Appendix P for data).

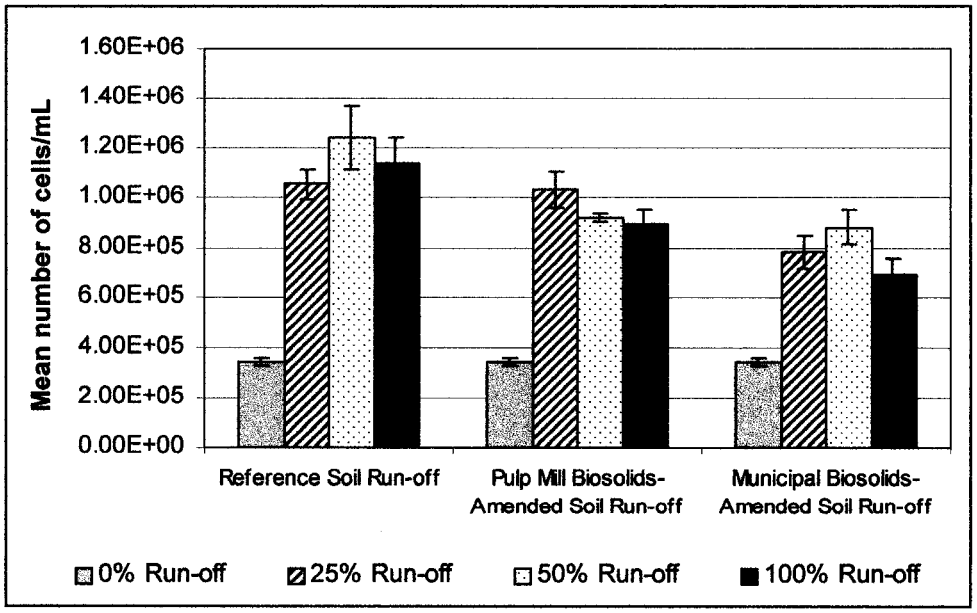


**Figure 9.** Mean number of *Pseudokirchneriella subcapitata* cells per mL (± standard error; n=6)of solution following 96 hours of exposure to varying concentrations of reference soil run-off, pulp mill biosolids-amended soil run-off, or municipal biosolids-amended soil run-off.

Visual observations indicated that colonization of *P. subcapitata* cells differed among concentrations; cells in treatments that had received run-off were clumped in larger groups than cells in 0% run-off (blank). No detectable difference in cell colouration was observed among treatments.

*Lemna minor* 14-day run-off laboratory bioassay results are shown in Figure 10. Statistical analysis revealed that there were significantly fewer fronds in the 25% and 50% reference soil run-off than in 0% run-off (t-test). There were significantly more fronds in 50% and 100% pulp mill biosolids-amended soil run-off than in 0% run-off. All other concentrations throughout the three treatment groups were not significantly different from the reference (0%) treatment. Significant differences were detected when equivalent concentrations of biosolids-amended soil run-off and reference soil run-off were compared. The mean number of fronds was significantly higher in 25% pulp mill and municipal biosolids-amended soil run-off than in 25% reference soil run-off. Similarly, the mean number of fronds was significantly higher in 50% pulp mill and municipal biosolids-amended soil run-off than in 50% reference soil run-off.

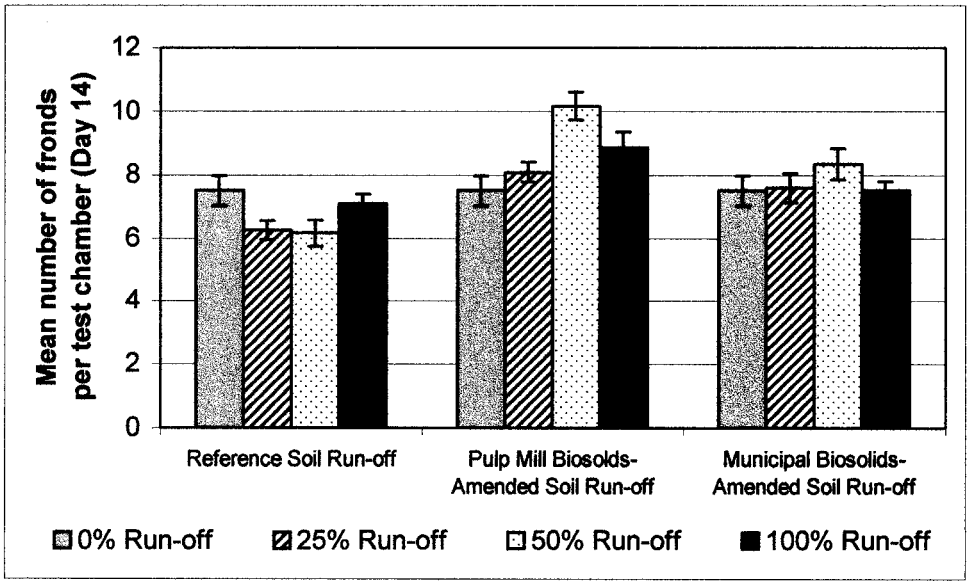


**Figure 60.** Mean number of *Lemna minor* fronds per test chamber (± standard errorl; n=12) following 14 days of exposure to varying concentrations of reference soil run-off, pulp mill biosolids-amended soil run-off, or municipal biosolids-amended soil run-off.

Upon visual observation, it was noted that there were no apparent differences between *L. minor* fronds; plants in all treatments were vivid green in colour and were continuing to divide and produce additional fronds (see Appendix Q for data).

### 3.3.2 – Land-Application Bioassays

The results of six different growth parameters for *Brassica rapa* grown in either pulp mill biosolids-amended soil, municipal biosolids-amended soil, or reference soil are shown in Table 5. Statistical analysis revealed that there were significantly more flowers produced per plant when grown in municipal biosolids-amended soil compared to pulp mill biosolids-amended soil or reference soil (t-test). In addition, there was a significant difference in root-length index between *B. rapa* grown in reference soil compared to those grown in biosolids-amended soil: the plants grown in reference soil had had a significantly higher mean root-length index than plants grown in soil amended with biosolids of either type (t-test). Statistical analyses also revealed that there were no significant differences between the three treatments in terms of percent germination (chi-square), mean number of seedpods developed per plant (t-test), percent survival over eight weeks (chi-square), or percent germination of $F_1$ generation *B. rapa* seeds (chi-square) (see Appendix R for data).

**Table 5. A comparison of six growth parameters for *Brassica rapa* grown in reference soil, pulp mill biosolids-amended soil, or municipal biosolids-amended soil.**

| | TYPE OF SOIL AMENDMENT | | |
|---|---|---|---|
| **GROWTH PARAMETER** | **Non-amended (Reference)** | **Pulp Mill Biosolids** | **Municipal Biosolids** |
| % Germination | 93% | 100% | 87% |
| Flower Development (mean/plant) | 4.68 (± 0.52) | 4.70 (± 0.60) | 6.68 (± 0.64) |
| Seedpod Development (mean/plant) | 2.68 (± 0.21) | 2.73 (± 0.17) | 3.25 (± 0.22) |
| Mean Root-length index | 0.31 (± 0.01) | 0.27 (± 0.02) | 0.21 (± 0.01) |
| % Survival (8 weeks) | 89% | 100% | 100% |
| F1 Generation % Germination | 85% | 90% | 80% |

(Note: means given with corresponding standard error)

The results of six different growth parameters for *Phaseolus vulgaris* grown in pulp mill biosolids-amended soil, municipal biosolids-amended soil, or reference soil are shown in Table 6. Statistical analysis revealed that *P. vulgaris* grown in reference soil had a significantly higher mean root-length index than plants grown in either type of biosolids-amended soil (t-test). Statistical analyses also showed that there were no significant differences between the three treatments with respect to percent germination (chi-Square), mean number of flowers developed per plant (t-test), mean number of seedpods developed per plant (t-test), percent survival over eight weeks (chi-square), or percent germination of $F_1$ generation *P. vulgaris* seeds (chi-square) (see Appendix S for data).

**Table 6. A comparison of six growth parameters for *Phaseolus vulgaris* grown in reference soil, pulp mill biosolids-amended soil, or municipal biosolids-amended soil.**

| | TYPE OF SOIL AMENDMENT | | |
|---|---|---|---|
| **GROWTH PARAMETER** | **Non-amended (Reference)** | **Pulp Mill Biosolids** | **Municipal Biosolids** |
| % Germination | 100% | 100% | 87% |
| Flower Development (mean/plant) | 1.43 (± 0.13) | 1.47 (± 0.13) | 1.30 (± 0.15) |
| Seedpod Development (mean/plant) | 1.00 (± 0.19) | 1.07 (± 0.15) | 0.90 (± 0.23) |
| Mean Root-length index | 0.25 (± 0.01) | 0.15 (± 0.01) | 0.15 (± 0.02) |
| % Survival (8 weeks) | 93% | 100% | 85% |
| F1 Generation % Germination | 85% | 80% | 75% |

(Note: means given with corresponding standard error)

After 20 weeks, the mean number of *Lumbricus terrestris* per test container was found to have decreased from 8 to 7.67 (± 0.33) in reference soil, 7.67 (± 0.33) in pulp mill biosolids-amended soil, and 6.67 (± 0.33) in municipal biosolids-amended soil; this corresponds to 95.9% survival in reference soil, 95.9% survival in pulp mill biosolids-amended soil, and 83.4% survival in municipal biosolids-amended soil. Statistical analysis revealed that there was no significant difference between treatments based on percent survival over the 20-week period (chi-square). In addition, organisms from all treatments appeared healthy in that they were plump, moist (i.e. coated in mucus), and responded well to physical stimuli (i.e. wriggled immediately upon prodding) (see Appendix T for data).

# 4.0: DISCUSSION

## *4.1 Field Experimentation*

Results from field experimentation indicate that there is relatively little difference in bioassay endpoints when comparing pulp mill biosolids-amended soil or run-off to reference soil or water with respect to the *Daphnia magna*, *Hyalella azteca*, *Lemna minor*, and *Lumbricus terrestris* bioassays. Figure 1 did indicate a slight decrease in the number of *H. azteca* in the biosolids run-off bioassay but the reasons for this are unknown. Significant differences among treatments were, however, observed in the plant bioassays. Germination of *Brassica rapa* (Table 3) was significantly reduced in biosolids-amended soil in the field plot as well as under artificial light conditions in the lab at the pulp mill. Since other plants showed high germination rates, a possible explanation for this is related to the physical characteristics of the soil. When biosolids are land-applied, it is possible for the soil to be compacted from the force of the application device (Piearce and Boone, 1998). For example, during aero-spraying (a method of land-application), biosolids are flung from the machinery at a high rate of speed thus causing the soil surface to become compacted. Soil compaction may have been responsible for the reduced germination of *B. rapa* seeds since these seeds are much smaller in size and more delicate than the other three terrestrial plant species used. In certain cases, the emerging shoots of the *B. rapa* plants may not have been able to physically push through and emerge from the soil.

Decreased germination was also observed for *Cucurbita moschata* in the non-amended field plots (Table 3). Since this was not the case for *C. moschata* grown in the same soil in the laboratory at the pulp mill, it is possible that birds or other wildlife may have eaten some of the *C. moschata* seeds in the biosolids-amended field plot. An increased number of replicates in the field (i.e. more than one field plot for each treatment) may have helped explain these results. In addition, employing screening or netting to control predators would be recommended for future study. This type of predator

control, however, was not possible during this particular study due to field site restrictions conveyed by pulp mill staff.

When examining mean root length index, statistical significance was found between biosolids-amended and non-amended soil for *Phaseolus vulgaris* grown in the field plots (Table 4). Root-length index was used as an endpoint since it is believed to be an important index of nutrient availability (Fitter, 2002). The plants in the amended plot had a significantly higher mean root-length index than the plants in the non-amended plot. It is difficult to speculate as to the cause of these results. A number of vegetation studies have shown that increased nutrient availability is directly correlated to proportionally decreased root growth (Fitter, 2002). If it were the case that the biosolids-amended soil was more nutrient-rich than the non-amended soil, the mean root-length index should have been higher in the non-amended soil. The roots of the plants in the non-amended soil should have, in theory, grown longer in the process of seeking out nutrients. This, however, was not the case. A possible explanation for *P. vulgaris* in the amended field plot having a higher root-length index than those plants in the non-amended plot may have to do with nutrient distribution in the soil. Land-application does not necessarily always result in an absolutely even distribution of biosolids on the site receiving the amendment. It is possible that the area of the amended field plot where the beans grew did not receive as high a quantity of biosolids as surrounding areas and thus the roots of the *P. vulgaris* plants grew longer in an effort to locate nutrients. This explanation is supported by the fact that the root-length index of *P. vulgaris* grown under artificial light was not significantly different between amended and non-amended soil, suggesting that the nutrient composition of the two soils may have been comparable.

Taking these explanations into consideration, it can be concluded that, based on these field observations, there is no obvious negative environmental impact at or below industry-suggested concentrations for pulp mill biosolids land-application. These field work results effectively verify the results obtained from prior pulp mill biosolids land-application laboratory studies by Bostan *et al.* (in press) employing the same bioassay

organisms. It is recognised that the field research conducted in this study was somewhat limited in its scope. The study assessed impact on representative terrestrial organisms that were in direct contact with the land-applied biosolids as well as the impact on selected aquatic biota in the run-off receiving-waters. A more broad-based field study incorporating additional assessments of land-application is necessary to further assess environmental impact (i.e. chemical, microbial, and ecological assessments).

### *4.2 Laboratory Experimentation*

The laboratory experiments that were completed subsequent to the field work were conducted in order to evaluate, from an environmental standpoint, the practice of municipal biosolids land-application using the same bioassay organisms as those used for the pulp mill biosolids field work. While planning the experiments using municipal biosolids, it was decided that bioassays using pulp mill biosolids would be conducted simultaneously in an attempt to further evaluate the practice of pulp mill biosolids land-application and to better understand the environmental impact of this disposal alternative.

Significant differences between treatments were detected in the *Daphnia magna* 21-day reproductive bioassay (Figure 6). *D. magna* in either the 25% or 100% reference soil run-off produced significantly more neonates than those organisms exposed to either concentration of pulp mill biosolids run-off. In addition, *D. magna* in 100% municipal biosolids-amended soil run-off produced significantly more neonates than organisms in the 25% municipal biosolids run-off, 25% pulp mill biosolids run-off, or 100% pulp mill biosolids run-off. These observed differences may be related to nutrient content differences between the types of run-off. However, based on the large number of neonates produced in the 100% municipal biosolids run-off treatment, it is difficult to speculate exactly how these nutrient – organism interactions might affect reproduction. For future research in this area, it would be prudent to test a wider range of run-off

concentrations and to utilize a higher number of replicates in order to verify the results obtained in this study. Also, extensive chemical analyses must be conducted on nutrient concentrations before correlative conclusions can be made. From an environmental perspective, it is difficult to conclusively state whether or not pulp mill biosolids-amended soil run-off has a detrimental effect on *D. magna* reproduction. When this bioassay is compared to 0% runoff within the treatment (Figure 6), there are no significant differences. This suggests that while enhanced reproduction did not occur (in contrast to results observed with 100% municipal biosolids-amended soil run-off), toxicity, based on inhibition of reproduction, was not evident either. Thus, these observations, alongside results from all additional aquatic bioassays conducted as part of this study, suggest that pulp mill and municipal biosolids run-off do not have a significant negative impact on receiving-water biota at or below industry-suggested concentrations (based on USEPA (1997) 20 ton/hectare/year application rate).

In the *Hyalella azteca* acute (48-hour) and chronic (21-day) toxicity bioassays (Figures 7 and 8), pulp mill or municipal biosolids-amended soil run-off did not have an impact on survival when compared to 0% run-off or to equivalent concentrations of reference soil run-off. Land-applied biosolids, at industry-suggested concentrations, do not appear to have an impact on survival of *H. azteca*.

In the *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) bioassay (Figure 9), it was found that run-off of all types (reference, pulp mill biosolids, and municipal biosolids) increased cell growth compared 0% run-off. Reference soil run-off treatments exhibited higher rates of growth than were observed in the biosolids-amended soil treatments of equivalent concentrations. In the *Lemna minor* bioassay (Figure 10), it was observed that biosolids-amended soil run-off did not have a significant impact on plant growth when compared to 0% run-off. Unlike *P. subcapitata* bioassay results, however, *L. minor* frond counts were higher in the biosolids-amended soil run-off (pulp mill and municipal) than in the reference soil run-off. These results could be a function of the nutrient requirements of *P. subcapitata* and *L. minor*, however, further speculation is unfruitful without nutrient analyses being

conducted. This experiment was also conducted to assess possible deleterious impact due to enhanced eutrophication from nutrient run-off. Figure 9 indicates that eutrophication from biosolids-amended soil run-off did not occur.

Results from *Brassica rapa* (Table 5) and *Phaseolus vulgaris* (Table 6) bioassays in which plants were grown in reference soil, pulp mill biosolids-amended soil, and municipal biosolids-amended soil suggest that the both types of biosolids-amended soils may function to enhance growth, in some circumstances, compared to reference soil. For both terrestrial plant bioassays, it was demonstrated that there was no negative impact of biosolids land-application for either pulp mill or municipal biosolids, based on industry-suggested quantities. These results are supported by numerous plant-response studies that have demonstrated the beneficial effects of pulp mill or municipal biosolids as a soil amendment. Such studies include those conducted by Phillips *et al.* (1997), Macyk (1999), Jackson *et al.* (2000), and Labrecque and Teodorescu (2001), the findings of which are presented in the Introduction.

Although the *Lumbricus terrestris* laboratory bioassay demonstrated 100% survival in pulp mill and municipal biosolids-amended soil, absolutely no evidence of reproduction was observed despite the fact that all earthworms were sexually mature for the duration of the bioassay (i.e. presence of clitellum). This was the case for both pulp mill and municipal biosolids-amended soils as well as for the reference soil, suggesting that some outside factor (other than soil composition) such as temperature or moisture may have been responsible for the lack of cocoons or young earthworms. These results validate the reason why *L. terrestris* is not widely used as a bioassay organism by the scientific community. The limited use of this species in bioassays is attributed to the lack of understanding of optimal conditions for mass-rearing (Berry and Jordan, 2001). Further research is needed to identify the most favourable conditions for growth and reproduction of *L. terrestris*.

### *4.3 Summary*

Based on the results of this study in its entirety, it can be concluded that the practice of biosolids land-application, when carefully regulated, may indeed be a viable and environmentally-sound alternative to other traditional disposal methods such as landfilling or incineration. This is true for both pulp mill and municipal biosolids as this study did not detect any impact from pulp mill and municipal biosolids land-application and run-off into receiving-water when compared to reference bioassays, based on the particular endpoints used. However, it is recognised that this study is limited in scope in that these conclusions are based solely on land-application and run-off bioassays. Various aspects of biosolids land-application were not addressed by this study. These include the impact of land-applied biosolids on indigenous soil microbial populations. It is well-known that forest biota exist in close, symbiotic, and often tentative relationships with microbial communities such as actinomycetes and may easily be disrupted with the addition of exotic microbial populations (such as those from biosolids that are land-applied). It may also take many generations to observe the impact of such an intrusion, but nonetheless, such practices as land-application of biosolids could be extremely detrimental to forest ecosystems in the long-term. It is thus suggested that a future study examine this issue in great detail. Also, the impact on groundwater due to leaching was not examined. As noted earlier in the Introduction, nitrate contamination and other chemical leaching could have a tremendously detrimental impact not only on human populations (via drinking water) but also on aquatic organisms in adjacent receiving-waters. Again, it is imperative that future research incorporates groundwater studies as well. Thus, it is difficult to resolutely conclude that land-application of biosolids does not impact the environment in any way.

It is difficult to compare the results obtained in this study to previous studies, as there is a lack of existing literature pertaining to ecotoxicological impact of biosolids land-application. A limited amount of research, including a study conducted by Brown *et al.* (1996), has examined the impact of heavy metal uptake on various fruits and vegetables

grown on biosolids-amended soils, however, studies of this nature have not yet assessed the ecotoxicological impacts of land-applied biosolids.

It is also very important to note that potential environmental impacts rely on a number of factors, including site characteristics, the composition of the biosolids being land-applied, and the application rates utilized. Furthermore, impacts may be species-specific and, as a result, each biosolids land-application program should be evaluated on a case-by-case, site-by-site basis.

There are a number of improvements that could be made to this study in order to gain even greater confidence that the practice of biosolids land-application does not have an adverse impact on the environment. Increasing the number of replicates in all bioassays is recommended to improve the statistical validity of the results. For this reason also, it is advised that that a wider range of land-application rates and concentrations of amended soil run-off be employed. With respect to the field studies, it would be beneficial to increase the duration of the bioassays. It is possible that impacts were not fully realised due to the short time frame available for field work. In addition, many of the bioassays could have been continued using a number of subsequent generations (i.e. *D. magna*, *H. azteca*, *B. rapa*, *P. vulgaris*) to determine the impacts of land-applied biosolids on offspring. Futhermore, it is recommended that chemical parameters be monitored closely in the aquatic bioassays in order to better quantify impacts. As previously mentioned, a broadened scope of study (i.e. assessing the impact of land-applied biosolids on indigenous soil microbial populations or the impact on groundwater due to leaching) is necessary to decisively conclude that the land-application of pulp mill or municipal biosolids is an environmentally-acceptable disposal alternative. However, this study is the first of its kind to utilize a suite of biota to assess terrestrial and receiving-water impact, not only of land-applied pulp mill biosolids, but also of municipal biosolids land-application. As the necessity increases to find safe disposal methods for biosolids, this study will serve as a baseline for future research.

## REFERENCES


Alberta Ministry of Agriculture, Food and Rural Affairs. 2002. Alberta Agricultural Weather Summary (July 2002). Government of Alberta, Edmonton, AB.

Arnaud, C., Saint-Denis, M., Narbonne, J.F., Soler, P., and D. Ribera. 2000. Influences of different standardised test methods on biochemical responses in the earthworm *Eisenia fetida andrei*. *Soil Biology and Biochemistry* **32**: 67-73.

Bailey, H.C. and L. Young. 1997. A comparison of the results of freshwater aquatic toxicity testing of pulp and paper mill effluents. *Water Science and Technology* **35**(2-3): 305-313.

Berry, E.C. and D. Jordan. 2001. Temperature and soil moisture content effects on the growth of *Lumbricus terrestris* (Oligochaeta: Lumbricidae) under laboratory conditions. *Soil Biology and Biochemistry* **33**: 133-136.

Bastian, R.K. 1986. Overview on Sludge Utilization. *In* The Forest Alternative for Treatment and Utilization of Municipal and Industrial Wastes. *Edited by* D.W. Cole, C.L. Henry, and W.L Nutter. University of Washington Press, Seattle, Washington, pp. 7-25.

Bellamy, K.L., C. Chong, and R.A. Cline. 1995. Paper sludge utilization in agriculture and container nursery culture. *Journal of Environmental Quality* **24**: 1074-1082.

Binder, D.L., Dobermann, A., Sander, D.H., and K.G. Cassman. 2002. Biosolids as nitrogen source for irrigated maize and rainfed sorghum. *Soil Science Society of America Journal* **66**: 531-543.

Bostan, I.-V. 2001. Bioavailability of nutrients associated with sediment and suspension of the Danube Delta; Assessment by Algal Bioassays. Ph.D. Thesis, University of Geneva, Switzerland.

Bostan, V., McCarthy, L.H., and S.N. Liss. Assessing the impact of land-applied pulp mill biosolids to a suite of terrestrial and aquatic bioassay organisms under laboratory conditions. In press.

Bright, D.A. and N. Healey. 2003. Contaminant risks from biosolids land-application: Contemporary organic contaminant levels in digested sewage sludge from five treatment plants in Greater Vancouver, British Columbia. *Environmental Pollution* **126**: 39-49.

Brown, S.L., Chaney, R.L., Lloyd, C.A., Angle, J.S., and J.A. Ryan. 1996. Relative uptake of cadmium by garden vegetables and fruits grown on long-term biosolids-amended soils. *Environmental Science & Technology* **30**: 3508-3511.

Burd, R.S. 1986. Forest Land-application of Sludge and Wastewater. *In* The Forest Alternative for Treatment and Utilization of Municipal and Industrial Wastes. *Edited by* D.W. Cole, C.L. Henry, and W.L Nutter. University of Washington Press, Seattle, Washington, pp.3-6.

Camberato, J.J., Vance, E.D., and A.V. Someshwar. 1997. Composition and Land-application of Paper Manufacturing Residuals. *In* Agricultural Uses of By-Products and Wastes. *Edited by* J.E. Rechcigl and H.C. MacKinnon. American Chemical Society, Washington, DC, pp. 185-202.

Canadian Food Inspection Agency. 1999. The biology of *Brassica rapa* L. Regulatory Directive No. 1999-02. Plant Health and Production Division, Canadian Food Inspection Agency, Nepean, Ontario.

City of Toronto. 2001. Annual Report: Ashbridges Bay Treatment Plant. City of Toronto, Works and Emergency Services Department, Water and Wastewater Services Division, Toronto, Ontario.

Cole, D.W., Henry, C.L. and W.L. Nutter. 1986. Preface. *In* The Forest Alternative for Treatment and Utilization of Municipal and Industrial Wastes. *Edited by* D.W. Cole, C.L. Henry, and W.L Nutter. University of Washington Press, Seattle, Washington, p. ix.

Edwards, C.A. and P.J. Bohlen. 1996. Biology and Ecology of Earthworms (3rd Edition). Chapman and Hall, London, UK.

Ehlers, W. 1975. Observations on earthworm channels and infiltration on tilled and untilled loess soil. *Soil Science* **119**: 242-249.

Environment Canada. 2002. Weekly average (1951-1980) of observed and normal daily precipitation: Toronto (station 6158733 YYZ 71624, Lester B. Pearson International Airport) and Edmonton (station 3012205 YEG 71123, Edmonton International Airport). Canadian Climate and Water Information, Meteorological Service of Canada.

Environment Canada. 1992. Biological test method: growth inhibition test using freshwater algae *Selenastrum capricornutum*. Environmental Protection Series, Report EPS/1/RM/25, November 1992. Environment Canada, Conservation and Protection, Ottawa.

Environment Canada. 1990. Biological test method: Acute lethality test using *Daphnia* spp. Environmental Protection Series, Report EPS/1/RM/11, July 1990. Environment Canada, Conservation and Protection, Ottawa.

Feagley, S.E., Valdez, M.S., and W.H. Hundall. 1994. Bleached, primary papermill sludge effect on bermudagrass grown on a mine soil. *Soil Science* **157**: 389-397.

Feldkirchner, D.C., Wang, C., Gower, S.T., Kruger, E.L., and J. Ferris. 2003. Effects of nutrient and paper mill biosolids amendments on the growth and nutrient status of hardwood forests. *Forest Ecology and Management* **177**: 95-116.

Ferrari, B., M.R. Claudemir, A. Veber, and J. Ferard. 1999. Ecotoxicological assessment of solid wastes. *Environmental Toxicology and Chemistry* 18: 1195-1202.

Fitter, A. 2002. Environmental Physiology of Plants (3rd Edition). Academic Press, San Diego, CA.

Forste, J.B. 1997. Biosolids Processing, Products, and Uses. *In* Agricultural Uses of By-Products and Wastes. *Edited by* J.E. Rechcigl and H.C. MacKinnon. American Chemical Society, Washington, D.C., pp. 50-62.

Gillespie, W.J. and J.D. Abbot. 1998. Progress in reducing the TCDD/TCDF content of effluents, pulps and wastewater treatment sludges from the manufacturing of bleached chemical pulp. *Chemosphere* **37**: 1973-1985.

Gilmour, J.T. and V. Skinner. 1999. Predicting plant available nitrogen in land-applied biosolids. *Journal of Environmental Quality* **28(4)**: 1122-1127.

Gong, P., Wilke, B.M., Strozzi, E. and S. Fleischmann. 2001. Evaluation and refinement of a continuous seed germination and early seedling growth test for the use in the ecotoxicological assessment of soils. *Chemosphere* **44**: 491-500.

Haufler, J.B. and S.D. West. 1986. Wildlife Responses to Forest Application of Sewage Sludge. *In* The Forest Alternative for Treatment and Utilization of Municipal and Industrial Wastes. *Edited by* D.W. Cole, C.L. Henry, and W.L Nutter. University of Washington Press, Seattle, Washington, pp. 110-116.

Henry, C.L. and D.W. Cole. 1997. Use of biosolids in the forest: technology, economics and regulations. *Biomass and Bioenergy* **13**: 269-277.

Hirsch, M.P. 1998. Availablity of sludge-borne silver to agricultural crops. *Environmental Toxicology and Chemistry* **17**: 610-616.

Jackson, M.J., Line, M.A., Wilson, S., and S.J. Hetherington. 2000. Application of composted pulp and paper mill sludge to a young pine plantation. *Journal of Environmental Quality* **29**: 407-414.

Kloepper-Sams, P., Torfs, F., Feijtel, T., and J. Gooch. 1996. Effects assessments for surfactants in sludge-amended soils: a literature review and perspectives for terrestrial risk assessment. *The Science of the Total Environment* **185**: 171-185.

Kula, C. 1998. Endpoints in laboratory testing with earthworms: experience with regard to regulatory decisions for plant protection products. *In* Advances in Earthworm Ecotoxicology. *Edited by* S. Sheppard, J. Bembridge, M. Holmstrup, and L. Posthuma. Society of Environmental Toxicology and Chemistry (SETAC) Press, Pensacola, FL. Pp. 3-14.

Labrecque, M. and T.I. Teodorescu. 2001. Influence of plantation site and wastewater sludge fertilization on the performance and foliar nutrient status of two willow species grown under SRIC in southern Quebec (Canada). *Forest Ecology and Management* 150: 223-239.

Lewis, M.A. 1995. Use of freshwater plants for phytotoxicity testing: a review. *Environmental Pollution* **87**: 319-336.

Linton, S. and R. Goulder. 1998. The duckweed *Lemna minor* compared with the alga *Selenastrum capricornutum* for bioassay of pond-water richness. *Aquatic Botany* **60**: 27-36.

Lokke, H. and C.A.M. Van Gestel. 1998. Soil Toxicity Tests in Risk Assessment of New and Existing Chemicals. *In* Handbook of Soil Invertebrate Toxicity Tests. John Wiley and Sons Ltd., Chichester, UK.

MacConnell, G.S., Wells, C.G., and L.J. Metz. 1986. Influence of Municipal Sludge on Forest Soil Mesofauna. *In* The Forest Alternative for Treatment and Utilization of Municipal and Industrial Wastes. *Edited by* D.W. Cole, C.L. Henry, and W.L Nutter. University of Washington Press, Seattle, Washington, pp. 177-187.

Macyk, T.M. 1999. Land application of mechanical pulp mill sludges in Alberta: research and operational activities. *Pulp & Paper Canada* **100**(6): 34-37.

Macyk, T.M. 1996. Research relative to land application of pulp mill wastes in Alberta. *Pulp & Paper Canada* **97**(3): 48-52.

Musgrave, M.E. 2000. Realizing the potential of rapid-cycling Brassica as a model system for use in plant biology research. *Journal of Plant Growth Regulation* **19**: 314-325.

Newmaster, S.G., Harris, A.G. and L.J. Kershaw. 1997. Wetland Plants of Ontario. Lone Pine Publishing, Edmonton, Alberta. P. 170.

O'Brien, T.A., Herbert, S.J., and A.V. Barker. 2002. Growth of corn in varying mixtures of paper mill sludge and soil. *Communications in Soil Science and Plant Analysis* **33**: 635-646.

Oanh, N.T.K. and B.E. Bengtsson. 1995. Toxicity to microtox, micro-algae and duckweed of effluents from the Bai Bang Paper Company (BAPACO), a Vietnamese bleached kraft pulp and paper mill. *Environmental Pollution* **90**(3): 391-399.

Ontario Ministry of Environment (MOE) and Ministry of Agriculture, Food and Rural Affairs (OMAFRA). 1996. Guidelines for the Utilization of Biosolids and Other Wastes on Agricultural Land. Publication No. 3425.

Payne, M. 2000. Factsheet: Land-application of Sewage Biosolids for Crop Production. Ontario Ministry of Agriculture and Food (OMAFRA) 00-023. Queen's Printer for Ontario, Ottawa.

Phillips, V.R., Kirkpatrick, N., Scotford, I.M., White, R.P., and R.G.O. Burton. 1997. The use of papermill sludges on agricultural land. *Bioresource Technology* **60**: 73-80.

Piearce, T.G. and G.C. Boone. 1998. Responses of invertebrates to paper sludge application to soil. *Applied Soil Ecology* **9**: 393-397.

Pun, K.C., Cheung, R.Y.H., and M.H. Wong. 1995. Characterization of sewage sludge and toxicity evaluation with microalgae. *Marine Pollution Bulletin* **31**(4-12):394-401.

Rechcigl, J.E. and H.C. MacKinnon. 1997. Preface. *In* Agricultural Uses of By-Products and Wastes. *Edited by* J.E. Rechcigl and H.C. MacKinnon. American Chemical Society, Washington, D.C., p.ix.

Reinecke, A.J. and L. Posthuma. 1998. Preface. *In* Advances in Earthworm Ecotoxicology. *Edited by* S. Sheppard, J. Bembridge, M. Holmstrup, and L. Posthuma. Society of Environmental Toxicology and Chemistry (SETAC) Press, Pensacola, FL. Pp.vi-vii.

Steevens, J.A. and W.H. Benson. 1998. *Hyalella azteca* 10-day sediment toxicity test: comparison of growth measurement endpoints. *Environmental Toxicology and Water Quality* **13**: 243-248.

Stephenson, G.L., Kaushik, A., Kaushik, N.K., Solomon, K.R., T. Steele, and R.P. Scroggins. 1998. Use of an avoidance-response test to assess the toxicity of contaminated soils to earthworms. *In* Advances in Earthworm Ecotoxicology. *Edited by* S. Sheppard, J. Bembridge, M. Holmstrup, and L. Posthuma. Society of Environmental Toxicology and Chemistry (SETAC) Press, Pensacola, FL. Pp. 67-81.

Thacker, W.E. 1986. Silvicultural Land-application of Wastewater and Sludge from the Pulp and Paper Industry. *In* The Forest Alternative for Treatment and Utilization of Municipal and Industrial Wastes. *Edited by* D.W. Cole, C.L. Henry, and W.L Nutter. University of Washington Press, Seattle, Washington, pp.41-54.

U.S. Environmental Protection Agency. 2002a. Land-application of Biosolids Status Report. EPA 2002-S-000004. Office of Inspector General, Washington, D.C.

U.S. Environmental Protection Agency. 2002b. Appendix A: Distribution, Life-cycle, Taxonomy, and Culture Methods. *In* Methods for Measuring the Acute Toxicity of Effluents and Receiving-waters to Freshwater and Marine Organisms (5th Edition). EPA 821-R-02-012. Office of Water, Washington, DC.

U.S. Environmental Protection Agency. 2002c. Test Method: Green Alga, *Selenastum capricornutum*, Growth Test Method. In Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving-waters to Freshwater Organisms (4th Edition). EPA 821-R-02-013. Office of Water, Washington, DC.

U.S. Environmental Protection Agency. 2000. Section 10: Collecting, Culturing, and Maintaining Test Organisms. In Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates (2nd Edition). EPA 600/R-99/064. Office of Water, Washington, DC.

U.S. Environmental Protection Agency. 1997. Land-application of Biosolids: Process Design Manual. Office of Research and Development, Cincinnati, Ohio.

U.S. Environmental Protection Agency. 1996. Ecological Effects Test Guidelines: Aquatic plant toxicity using *Lemna* spp., Tiers I and II (PPPTS 850.4400). EPA 712-C-96-156. Office of Prevention, Pesticides and Toxic Substances, Washington, DC.

Veerina. S.S., Parker, N.C., and C.B. Felder. 2002. Effects of sludge filtrate on the survival and reproduction of *Ceriodaphnia dubia*. *Ecotoxicology* **11**: 113-118.

Vera, C.J. and F.A. Servello. 1994. Effects of paper mill sludge in spruce-fir forests on wildlife in Maine. *Journal of Wildlife Management* **58**: 719-727.

Walker, C.H., Hopkin, S.P., Sibly, R.M., and D.B. Peakall (Eds.). 2001. Toxicity Testing. *In* Principles of Ecotoxicology (2nd Edition). Taylor and Francis Inc., New York. Pp.93-118.

Walter, I., Cuevas, G., Garcia, S., and F. Martinez. 2000. Biosolid effects on soil and native plant production in a degraded semiarid ecosystem in central Spain. *Waste Management & Research* **18**: 259-263.

Wever, L.A., Lysyk, T.J., and M.J. Clapperton. 2001. The influence of soil moisture and temperature on the survival, aestivation, growth and development of juvenile *Aporrectodea tuberculata* (Eisen) (Lumbicidae). *Pedobiologia* **45**: 121-133.

White, R.E. and Borror, D.J. 1998. Insects of America North of Mexico (A Peterson Field Guide Series). Houghton Mifflin, Boston, MA.

Zasoski, R.J. and R.L. Edmonds. 1986. Water Quality in Relation to Sludge and Wastewater Applications to Forest Land. *In* The Forest Alternative for Treatment and Utilization of Municipal and Industrial Wastes. *Edited by* D.W. Cole, C.L. Henry, and W.L Nutter. University of Washington Press, Seattle, Washington, pp. 100-109.

Zibilske, L.M., W.M. Clapham, and R.V. Rourke. 2000. Multiple applications of paper mill sludge in an agricultural system: soil effects. *Journal of Environmental Quality* **29**: 1975-1981.

## APPENDICES

### *Appendix A. Biosolids Dry to Wet Weight Ratio Calculations & Land-Application Quantity*

Soil surface area on run-off ramp = 90.0 cm x 25.0 cm
= 2250 $cm^2$

Application rate = 20 ton (dry weight)/hectare = 20000 kg (dry weight)/hectare

20000 kg (dry)/hectare X 1 hectare/1 x $10^8$ $cm^2$ = 20000 kg (dry weight)/ 1 x $10^8$ $cm^2$

Let **x** represent the amount of dry weight biosolids to be land-applied to the ramp:

20000 kg (dry)/1 x $10^8$ $cm^2$ = **x** kg (dry)/2250 $cm^2$
**x** = 0.450 kg (dry)

1. Pulp mill biosolids average dry weight to wet weight ratio:
**6.23 g wet: 1.73 g dry**

Let **y** represent the amount of wet pulp mill biosolids to be land-applied to the ramp:

6.23 g wet: 1.73 g dry = **y** g wet:450 g dry
**y** = 1620.52 g of wet pulp mill biosolids should be applied to the ramp

2. Municipal biosolids average dry weight to wet weight ratio:
**9.73 g wet: 2.47 g dry**

Let **y** represent the amount of wet pulp mill biosolids to be land-applied to the ramp:

9.73 g wet: 2.47 g dry = **y** g wet:450 g dry
**y** = 1772.67 g of wet municipal biosolids should be applied to the ramp

## *Appendix B. Simulated Rainfall Quantity Calculations*

For run-off collection a typical one-day rainfall of 20 mm (Environment Canada, 2002) was simulated.

Surface area of ramp = 2500 $cm^2$

Therefore, the volume of water needed = 2250$cm^2$ x 2 cm
= 4500 $cm^3$

= 4.5 L

*Appendix C. Synthetic Algal Nutrient Medium (USEPA, 2002).*

| Initial Solution | Substance | Conc. (mg/L) | Nutrient(s) | Conc. (mg/L) |
|---|---|---|---|---|
| I | $H_3BO_3$ | 185.520 | B | 32.43 |
| | $MnCl_2\ 4H_2O$ | 415.610 | Mn | 115.38 |
| | $ZnCl_2$ | 3.271 | Zn | 1.57 |
| | $CoCl_2\ 6H_2O$ | 1.428 | Co | 0.35 |
| | $CuCl_2\ 2H_2O$ | 0.012 | Cu | 0.004 |
| | $Na_2MoO_4\ 3H_2O$ | 7.260 | Mo | 2.88 |
| | $FeCl_3\ 6H_2O$ | 160.000 | Fe | 33.06 |
| | $Na_2EDTA\ 2H_2O$ | 300.000 | EDTA* | 234 |
| | $CaCl_2\ 2H_2O$ | 4410.000 | Ca | 1202.30 |
| | $MgCl_2\ 6H_2O$ | 12164.000 | Mg | 2904.00 |
| II | $NaNO_3$ | 25500.000 | N | 4203.00 |
| III | $MgSO_4\ 7H_2O$ | 14700.000 | S | 1912.63 |
| IV | $K_2HPO_4$ | 1044.000 | K | 469.00 |
| | | | P | 185.63 |
| V | $NaHCO_3$ | 15000.000 | Na | 11001.00 |
| | | | $HCO_3^-$ | 2144.44 |

* EDTA is not a nutrient; it is an organic compound.

*Appendix D.* **Pseudokirchneriella subcaptitata** *Algal Culture Calculations*

Initial count of algal culture #4 = $3.75 \times 10^6$ cells/mL

$C_1V_1=C_2V_2$,

Where $C_1 = 3.75 \times 10^6$ cells/mL,
$V_1$ = unknown (x),
$C_2 = 10^5$ cells/mL, and
$V_2$ = 2 mL

So, $3.75 \times 10^6$ cells/mL (X) = $10^5$ cells/mL (2mL)

Therefore, X = 0.05333 mL, or **53.33 µL**.
This corresponds to the amount of culture #4 that must be added to each well of the microplate.

## *Appendix E.* Lumbricus terrestris *Pre-test Bioassay Data*

| | Day 1 | Day 7 | Day 28 | Day 56 | Day 98 |
|---|---|---|---|---|---|
| | Count | Count | Count | Count | Count |
| **Alberta Reference Soil** | | | | | |
| Bucket # 1 | 8 | 8 | 8 | 8 | 8 |
| Bucket # 2 | 8 | 8 | 7 | 7 | 7 |
| Bucket # 3 | 8 | 8 | 7 | 7 | 7 |
| **Mean** | **8.00** | **8.00** | **7.33** | **7.33** | **7.33** |
| **Standard Error** | **0.00** | **0.00** | **0.33** | **0.33** | **0.33** |
| **Alberta Amended soil** | | | | | |
| Bucket # 1 | 8 | 8 | 8 | 8 | 8 |
| Bucket # 2 | 8 | 8 | 8 | 8 | 8 |
| Bucket # 3 | 8 | 8 | 7 | 7 | 7 |
| **Mean** | **8.00** | **8.00** | **7.67** | **7.67** | **7.67** |
| **Standard Error** | **0.00** | **0.00** | **0.33** | **0.33** | **0.33** |

## *Appendix F. Plant Pre-Test Data*

| | Number of Seeds Germinated (10 seeds of each planted) | |
|---|---|---|
| | **Reference Soil** | **Biosolids-Amended Soil** |
| **PLANT SPECIES** | **Day 10** | **Day 10** |
| *Lactuca sativa* (Early Great Lakes) | 8 | 9 |
| *Lactuca sativa* (Grand Rapids) | 7 | 8 |
| *Phaseolus Vulgaris* (Improved Golden Wax) | 10 | 10 |
| *Raphanus sativus* (Crimson Giant) | 9 | 9 |
| *Cucurbita moschata* (Winter Squash) | 10 | 9 |

## *Appendix G.* Daphnia magna *Run-off Field Bioassay Data*

| | Day 1 | Day 2 | Day 3 | Day 4 | | Day 5 | | Day 6 | | Day 7 | | Day 8 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | adults | adults | adults | adults | young | adults | young | adults | young | adults | young | adults | young |
| **Reference** | | | | | | | | | | | | | |
| A1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 2 | 1 | 2 | 1 | 9 |
| A2 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 3 | 1 | 3 | 1 | 3 |
| A3 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 1 |
| A4 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 8 |
| A5 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 |
| A6 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 0 |
| A7 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 |
| A8 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 2 | 1 | 3 |
| A9 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 4 | 1 | 5 |
| A10 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 2 |
| A11 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 |
| A12 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 |
| **total** | **12** | **11** | **11** | **12** | **0** | **12** | **0** | **12** | **5** | **12** | **12** | **12** | **31** |
| **mean** | **1.00** | **0.92** | **0.92** | **1.00** | **0.00** | **1.00** | **0.00** | **1.00** | **0.42** | **1.00** | **1.00** | **1.00** | **2.58** |
| **std error** | **0.00** | **0.08** | **0.08** | **0.00** | **0.00** | **0.00** | **0.00** | **0.00** | **0.29** | **0.00** | **0.41** | **0.00** | **0.92** |
| **Blank** | | | | | | | | | | | | | |
| B1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 7 |
| B2 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 9 |
| B3 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 |
| B4 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 |
| B5 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 |
| B6 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 10 |
| B7 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 |
| B8 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 2 | 1 | 3 |
| B9 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 |
| B10 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 |
| B11 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 |
| B12 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 |
| **total** | **12** | **12** | **10** | **12** | **1** | **12** | **1** | **12** | **1** | **12** | **3** | **12** | **29** |
| **mean** | **1.00** | **1.00** | **0.83** | **1.00** | **0.08** | **1.00** | **0.08** | **1.00** | **0.08** | **1.00** | **0.25** | **1.00** | **2.42** |
| **std error** | **0.00** | **0.00** | **0.11** | **0.00** | **0.08** | **0.00** | **0.08** | **0.00** | **0.08** | **0.00** | **0.18** | **0.00** | **1.13** |
| **Amended** | | | | | | | | | | | | | |
| C1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 |
| C2 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 |
| C3 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 |
| C4 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 10 | 1 | 10 |
| C5 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 |
| C6 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 |
| C7 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 |
| C8 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 10 |
| C9 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 13 |
| C10 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 3 | 1 | 3 | 1 | 3 |
| C11 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 |
| C12 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 |
| **total** | **12** | **12** | **12** | **12** | **0** | **12** | **2** | **12** | **5** | **12** | **15** | **12** | **37** |
| **mean** | **1.00** | **1.00** | **1.00** | **1.00** | **0.00** | **1.00** | **0.17** | **1.00** | **0.42** | **1.00** | **1.25** | **1.00** | **3.08** |
| **std error** | **0.00** | **0.00** | **0.00** | **0.00** | **0.00** | **0.00** | **0.11** | **0.00** | **0.26** | **0.00** | **0.84** | **0.00** | **1.42** |

## *Appendix H.* Hyalella azteca *Run-off Field Bioassay Data*

| | Initial | 24-hours | Day 8 |
|---|---|---|---|
| | adults | adults | adults |
| **Reference** | | | |
| A1 | 3 | 3 | 2 |
| A2 | 3 | 3 | 2 |
| A3 | 3 | 3 | 4 |
| A4 | 3 | 3 | 2 |
| A5 | 3 | 3 | 3 |
| **total** | **15** | **15** | **13** |
| **mean** | **3.00** | **3.00** | **2.60** |
| **standard error** | **0.00** | **0.00** | **0.40** |
| | | | |
| **Blank** | | | |
| B1 | 3 | 3 | 3 |
| B2 | 3 | 3 | 3 |
| B3 | 3 | 3 | 3 |
| B4 | 3 | 3 | 3 |
| B5 | 3 | 3 | 3 |
| **total** | **15** | **15** | **15** |
| **mean** | **3.00** | **3.00** | **3.00** |
| **standard error** | **0.00** | **0.00** | **0.00** |
| | | | |
| **Biosolids-Amended** | | | |
| C1 | 3 | 3 | 3 |
| C2 | 3 | 3 | 2 |
| C3 | 3 | 3 | 2 |
| C4 | 3 | 3 | 1 |
| C5 | 3 | 3 | 3 |
| **total** | **15** | **15** | **11** |
| **mean** | **3.00** | **3.00** | **2.20** |
| **standard error** | **0.00** | **0.00** | **0.37** |

## *Appendix I.* Lemna minor *Run-off Field Bioassay Data*

| | Initial | | Day 8 | |
|---|---|---|---|---|
| | **Plants** | **Fronds** | **Plants** | **Fronds** |
| **Reference** | | | | |
| A1 | 5 | 10 | 6 | 24 |
| A2 | 5 | 10 | 5 | 22 |
| A3 | 5 | 10 | 5 | 21 |
| A4 | 5 | 10 | 7 | 26 |
| A5 | 5 | 10 | 5 | 23 |
| **total** | **25** | **50** | **28** | **116** |
| **mean** | **5.00** | **10.00** | **5.60** | **23.20** |
| **standard error** | **0.00** | **0.00** | **0.40** | **0.86** |
| **spring water** | | | | |
| B1 | 5 | 10 | 5 | 26 |
| B2 | 5 | 10 | 5 | 23 |
| B3 | 5 | 10 | 5 | 24 |
| B4 | 5 | 10 | 5 | 20 |
| B5 | 5 | 10 | 7 | 22 |
| **total** | **25** | **50** | **27** | **115** |
| **mean** | **5.00** | **10.00** | **5.40** | **23.00** |
| **standard error** | **0.00** | **0.00** | **0.40** | **1.00** |
| **Biosolids** | | | | |
| C1 | 5 | 10 | 7 | 22 |
| C2 | 5 | 10 | 8 | 23 |
| C3 | 5 | 10 | 7 | 27 |
| C4 | 5 | 10 | 5 | 25 |
| C5 | 5 | 10 | 8 | 32 |
| **total** | **25** | **50** | **35** | **129** |
| **mean** | **5.00** | **10.00** | **7.00** | **25.80** |
| **standard error** | **0.00** | **0.00** | **0.55** | **1.77** |

## *Appendix J. Terrestrial Plant Land-Application Field Bioassay Data (Field Plots)*

| | DAY 12 | | | | |
|---|---|---|---|---|---|
| | **# germinated** | **total length (cm)** | **shoot length (cm)** | **root length (cm)** | **RLI** |
| **Reference** | | | | | |
| *Brassica rapa* | | | | | |
| 1 | 1 | 5.30 | 2.60 | 2.70 | 0.51 |
| 2 | 1 | 4.90 | 2.20 | 2.70 | 0.55 |
| 3 | 1 | 3.90 | 1.80 | 2.10 | 0.54 |
| 4 | 1 | 4.80 | 2.20 | 2.60 | 0.54 |
| 5 | 1 | 5.40 | 2.00 | 3.40 | 0.63 |
| 6 | 1 | 4.10 | 1.90 | 2.20 | 0.54 |
| 7 | 1 | 4.60 | 2.10 | 2.50 | 0.54 |
| 8 | 1 | 5.40 | 2.70 | 2.70 | 0.50 |
| 9 | 1 | 6.00 | 3.30 | 2.70 | 0.45 |
| 10 | 1 | 5.70 | 3.40 | 2.30 | 0.40 |
| 11 | 1 | 5.80 | 3.40 | 2.40 | 0.41 |
| 12 | 1 | 2.90 | 1.30 | 1.60 | 0.55 |
| 13 | 1 | 4.30 | 2.70 | 1.60 | 0.37 |
| 14 | 1 | 3.60 | 1.80 | 1.80 | 0.50 |
| 15 | 0 | | | | |
| **% germination** | **93.00** | | | | |
| **mean** | | **4.76** | **2.39** | **2.38** | **0.50** |
| **std error** | | **0.24** | **0.17** | **0.13** | **0.02** |
| *Raphanus sativus* | | | | | |
| 1 | 1 | 7.30 | 4.00 | 3.30 | 0.45 |
| 2 | 1 | 6.50 | 3.40 | 3.10 | 0.48 |
| 3 | 1 | 7.50 | 3.70 | 3.80 | 0.51 |
| 4 | 1 | 6.00 | 3.70 | 2.30 | 0.38 |
| 5 | 1 | 8.30 | 3.80 | 4.50 | 0.54 |
| 6 | 1 | 4.50 | 2.10 | 2.40 | 0.53 |
| 7 | 1 | 5.50 | 2.60 | 2.90 | 0.53 |
| 8 | 1 | 7.30 | 3.00 | 4.30 | 0.59 |
| 9 | 1 | 6.10 | 3.00 | 3.10 | 0.51 |
| 10 | 1 | 5.80 | 3.50 | 2.30 | 0.40 |
| 11 | 1 | 4.90 | 3.10 | 1.80 | 0.37 |
| 12 | 1 | 6.30 | 3.10 | 3.20 | 0.51 |
| 13 | 1 | 7.00 | 4.10 | 2.90 | 0.41 |
| 14 | 1 | 7.50 | 3.00 | 4.50 | 0.60 |
| 15 | 1 | 5.30 | 3.30 | 2.00 | 0.38 |
| **% germination** | **100.00** | | | | |
| **mean** | | **6.39** | **3.29** | **3.09** | **0.48** |
| **std error** | | **0.28** | **0.14** | **0.23** | **0.02** |
| *Phaseolus vulgaris* | | | | | |
| 1 | 1 | 10.00 | 5.90 | 4.10 | 0.41 |
| 2 | 1 | 10.20 | 6.40 | 3.80 | 0.37 |
| 3 | 1 | 14.50 | 10.60 | 3.90 | 0.27 |
| 4 | 1 | 18.20 | 9.50 | 8.70 | 0.48 |
| 5 | 1 | 15.30 | 9.00 | 6.30 | 0.41 |
| 6 | 1 | 7.20 | 4.00 | 3.20 | 0.44 |
| 7 | 1 | 16.30 | 10.10 | 6.20 | 0.38 |
| 8 | 1 | 9.50 | 6.80 | 2.70 | 0.28 |
| 9 | 1 | 8.10 | 4.60 | 3.50 | 0.43 |
| 10 | 1 | 8.20 | 5.30 | 2.90 | 0.35 |
| 11 | 1 | 7.50 | 4.20 | 3.30 | 0.44 |
| 12 | 1 | 9.50 | 4.80 | 4.70 | 0.49 |
| 13 | 1 | 8.50 | 6.10 | 2.40 | 0.28 |
| 14 | 0 | | | | |
| 15 | 0 | | | | |
| **% germination** | **87.00** | | | | |

| | | DAY 12 | | | |
|---|---|---|---|---|---|
| | # germinated | total length (cm) | shoot length (cm) | root length (cm) | RLI |
| **mean** | | **11.00** | **6.72** | **4.28** | **0.39** |
| **std error** | | **1.03** | **0.64** | **0.50** | **0.02** |
| *Cucurbita moschata* | | | | | |
| 1 | 1 | 8.00 | 4.20 | 3.80 | 0.48 |
| 2 | 1 | 9.10 | 4.50 | 4.60 | 0.51 |
| 3 | 1 | 5.50 | 3.10 | 2.40 | 0.44 |
| 4 | 1 | 8.60 | 2.80 | 5.80 | 0.67 |
| 5 | 1 | 9.50 | 5.40 | 4.10 | 0.43 |
| 6 | 1 | 8.30 | 4.20 | 4.10 | 0.49 |
| 7 | 1 | 5.30 | 2.00 | 3.30 | 0.62 |
| 8 | 1 | 9.20 | 4.60 | 4.60 | 0.50 |
| 9 | 0 | 6.20 | 3.20 | 3.00 | 0.48 |
| 10 | 0 | 7.70 | 3.70 | 4.00 | 0.52 |
| 11 | 0 | 8.20 | 4.60 | 3.60 | 0.44 |
| 12 | 0 | 9.40 | 5.90 | 3.50 | 0.37 |
| 13 | 0 | 8.60 | 4.90 | 3.70 | 0.43 |
| 14 | 0 | | | | |
| 15 | 0 | | | | |
| **% germination** | **87.00** | | | | |
| **mean** | | **7.97** | **4.08** | **3.88** | **0.49** |
| **std error** | | **0.40** | **0.30** | **0.23** | **0.02** |
| **Biosolids-Amended** | | | | | |
| *Brassica rapa* | | | | | |
| 1 | 1 | 4.30 | 1.90 | 2.40 | 0.56 |
| 2 | 1 | 3.90 | 2.10 | 1.80 | 0.46 |
| 3 | 1 | 6.00 | 2.90 | 3.10 | 0.52 |
| 4 | 1 | 5.20 | 2.60 | 2.60 | 0.50 |
| 5 | 1 | 4.60 | 1.90 | 2.70 | 0.59 |
| 6 | 1 | 5.50 | 2.70 | 2.80 | 0.51 |
| 7 | 1 | 4.10 | 1.70 | 2.40 | 0.59 |
| 8 | 1 | 4.30 | 2.20 | 2.10 | 0.49 |
| 9 | 0 | | | | |
| 10 | 0 | | | | |
| 11 | 0 | | | | |
| 12 | 0 | | | | |
| 13 | 0 | | | | |
| 14 | 0 | | | | |
| 15 | 0 | | | | |
| **% germination** | **53.00** | | | | |
| **mean** | | **4.74** | **2.25** | **2.49** | **0.53** |
| **std error** | | **0.26** | **0.15** | **0.14** | **0.02** |
| *Raphanus sativus* | | | | | |
| 1 | 1 | 5.30 | 3.80 | 1.50 | 0.28 |
| 2 | 1 | 11.40 | 5.50 | 5.90 | 0.52 |
| 3 | 1 | 10.80 | 6.00 | 4.80 | 0.44 |
| 4 | 1 | 7.00 | 3.70 | 3.30 | 0.47 |
| 5 | 1 | 7.10 | 4.50 | 2.60 | 0.37 |
| 6 | 1 | 8.50 | 5.20 | 3.30 | 0.39 |
| 7 | 1 | 11.00 | 5.90 | 5.10 | 0.46 |
| 8 | 1 | 8.30 | 4.70 | 3.60 | 0.43 |
| 9 | 1 | 6.20 | 3.00 | 3.20 | 0.52 |
| 10 | 1 | 6.50 | 4.20 | 2.30 | 0.35 |
| 11 | 1 | 7.30 | 4.10 | 3.20 | 0.44 |
| 12 | 1 | 12.20 | 4.30 | 7.90 | 0.65 |
| 13 | 1 | 6.50 | 4.00 | 2.50 | 0.38 |
| 14 | 1 | 5.50 | 2.60 | 2.90 | 0.53 |
| 15 | 1 | | | | |
| **% germination** | **100.00** | | | | |
| **mean** | | **8.11** | **4.39** | **3.72** | **0.45** |

| | | DAY 12 | | | |
|---|---|---|---|---|---|
| | # germinated | total length (cm) | shoot length (cm) | root length (cm) | RLI |
| **std error** | | **0.62** | **0.27** | **0.45** | **0.02** |
| *Phaseolus vulgaris* | | | | | |
| 1 | 1 | 9.50 | 3.80 | 5.70 | 0.60 |
| 2 | 1 | 13.90 | 5.70 | 8.20 | 0.59 |
| 3 | 1 | 10.20 | 6.10 | 4.10 | 0.40 |
| 4 | 1 | 9.40 | 4.30 | 5.10 | 0.54 |
| 5 | 1 | 13.10 | 7.50 | 5.60 | 0.43 |
| 6 | 1 | 10.60 | 4.80 | 5.80 | 0.55 |
| 7 | 1 | 15.40 | 10.10 | 5.30 | 0.34 |
| 8 | 1 | 11.00 | 5.10 | 5.90 | 0.54 |
| 9 | 1 | 11.10 | 6.10 | 5.00 | 0.45 |
| 10 | 1 | 11.20 | 4.10 | 7.10 | 0.63 |
| 11 | 1 | 8.90 | 6.10 | 2.80 | 0.31 |
| 12 | 1 | 6.90 | 4.10 | 2.80 | 0.41 |
| 13 | 1 | 15.30 | 6.90 | 8.40 | 0.55 |
| 14 | 0 | | | | |
| 15 | 0 | | | | |
| **% germination** | **87.00** | | | | |
| **mean** | | **11.27** | **5.75** | **5.52** | **0.49** |
| **std error** | | **0.70** | **0.48** | **0.48** | **0.03** |
| *Cucurbita moschata* | | | | | |
| 1 | 1 | 10.10 | 3.00 | 7.10 | 0.70 |
| 2 | 1 | 11.60 | 4.60 | 7.00 | 0.60 |
| 3 | 1 | 8.90 | 2.80 | 6.10 | 0.69 |
| 4 | 1 | 15.00 | 6.30 | 8.70 | 0.58 |
| 5 | 1 | 10.50 | 5.20 | 5.30 | 0.50 |
| 6 | 1 | 12.70 | 6.80 | 5.90 | 0.46 |
| 7 | 1 | 14.30 | 6.50 | 7.80 | 0.55 |
| 8 | 1 | 8.00 | 4.20 | 3.80 | 0.48 |
| 9 | 1 | 12.70 | 4.60 | 8.10 | 0.64 |
| 10 | 1 | 12.30 | 6.90 | 5.40 | 0.44 |
| 11 | 1 | 10.30 | 6.60 | 3.70 | 0.36 |
| 12 | 1 | 7.50 | 4.30 | 3.20 | 0.43 |
| 13 | 1 | 11.80 | 6.80 | 5.00 | 0.42 |
| 14 | 0 | | | | |
| 15 | 0 | | | | |
| **% germination** | **87.00** | | | | |
| **mean** | | **11.21** | **5.28** | **5.93** | **0.53** |
| **std error** | | **0.63** | **0.41** | **0.49** | **0.03** |

## *Appendix K. Terrestrial Plant Land-Application Field Bioassay Data (Laboratory)*

| | | DAY 12 | | | |
|---|---|---|---|---|---|
| | plants | total length (cm) | shoot length (cm) | root length (cm) | RLI |
| **Reference** | | | | | |
| *Brassica rapa* | | | | | |
| 1 | 1 | 3.90 | 1.80 | 2.10 | 0.54 |
| 2 | 1 | 4.80 | 2.30 | 2.50 | 0.52 |
| 3 | 1 | 5.10 | 2.60 | 2.50 | 0.49 |
| 4 | 1 | 5.60 | 3.10 | 2.50 | 0.45 |
| 5 | 1 | 5.20 | 2.90 | 2.30 | 0.44 |
| 6 | 1 | 4.90 | 2.60 | 2.30 | 0.47 |
| 7 | 1 | 6.10 | 3.30 | 2.80 | 0.46 |
| 8 | 1 | 3.70 | 1.90 | 1.80 | 0.49 |
| 9 | 1 | 5.60 | 3.10 | 2.50 | 0.45 |
| 10 | 1 | 5.80 | 3.00 | 2.80 | 0.48 |
| 11 | 1 | 4.90 | 2.90 | 2.00 | 0.41 |
| 12 | 1 | 6.00 | 2.90 | 3.10 | 0.52 |
| 13 | 1 | 5.90 | 2.60 | 3.30 | 0.56 |
| 14 | 1 | 4.60 | 2.50 | 2.10 | 0.46 |
| 15 | 1 | 4.10 | 2.10 | 2.00 | 0.49 |
| 16 | 1 | 4.20 | 1.90 | 2.30 | 0.55 |
| 17 | 1 | 5.30 | 2.60 | 2.70 | 0.51 |
| 18 | 1 | 5.80 | 3.10 | 2.70 | 0.47 |
| 19 | 1 | 4.10 | 2.00 | 2.10 | 0.51 |
| 20 | 1 | 4.70 | 2.10 | 2.60 | 0.55 |
| 21 | 1 | 4.90 | 2.80 | 2.10 | 0.43 |
| 22 | 1 | 5.60 | 2.60 | 3.00 | 0.54 |
| 23 | 1 | 5.50 | 3.00 | 2.50 | 0.45 |
| 24 | 1 | 4.80 | 2.50 | 2.30 | 0.48 |
| 25 | 1 | 5.10 | 2.80 | 2.30 | 0.45 |
| 26 | 1 | 4.40 | 2.00 | 2.40 | 0.55 |
| 27 | 1 | 6.10 | 3.30 | 2.80 | 0.46 |
| 28 | 1 | 5.80 | 2.90 | 2.90 | 0.50 |
| 29 | 0 | | | | |
| 30 | 0 | | | | |
| 31 | 0 | | | | |
| 32 | 0 | | | | |
| **% germination** | **88** | | | | |
| **mean** | | **5.09** | **2.61** | **2.48** | **0.49** |
| **std error** | | **0.13** | **0.08** | **0.07** | **0.01** |
| | | | | | |
| *Raphanus sativus* | | | | | |
| 1 | 1 | 11.10 | 7.40 | 3.70 | 0.33 |
| 2 | 1 | 9.50 | 7.60 | 1.90 | 0.20 |
| 3 | 1 | 15.50 | 6.70 | 8.80 | 0.57 |
| 4 | 1 | 9.60 | 7.10 | 2.50 | 0.26 |
| 5 | 1 | 11.30 | 8.20 | 3.10 | 0.27 |
| 6 | 1 | 4.90 | 3.20 | 1.70 | 0.35 |
| 7 | 1 | 7.70 | 6.10 | 1.60 | 0.21 |
| 8 | 1 | 8.30 | 6.80 | 1.50 | 0.18 |
| 9 | 1 | 10.30 | 7.60 | 2.70 | 0.26 |
| 10 | 1 | 7.80 | 6.20 | 1.60 | 0.21 |
| 11 | 1 | 10.30 | 6.40 | 3.90 | 0.38 |

| | | DAY 12 | | | |
|---|---|---|---|---|---|
| | plants | total length (cm) | shoot length (cm) | root length (cm) | RLI |
| 12 | 1 | 9.60 | 7.10 | 2.50 | 0.26 |
| 13 | 1 | 12.40 | 6.80 | 5.60 | 0.45 |
| 14 | 1 | 11.10 | 8.50 | 2.60 | 0.23 |
| 15 | 1 | | | | |
| 16 | 1 | | | | |
| 17 | 1 | | | | |
| 18 | 1 | | | | |
| **% germination** | **100** | | | | |
| **mean** | | **9.96** | **6.84** | **3.12** | **0.30** |
| **std error** | | **0.66** | **0.34** | **0.53** | **0.03** |
| | | | | | |
| *Phaseolus vulgaris* | | | | | |
| 1 | 1 | 30.90 | 24.80 | 6.10 | 0.20 |
| 2 | 1 | 44.70 | 32.70 | 12.00 | 0.27 |
| 3 | 1 | 36.20 | 25.50 | 10.70 | 0.30 |
| 4 | 1 | 50.50 | 35.40 | 15.10 | 0.30 |
| 5 | 1 | 23.90 | 15.50 | 8.40 | 0.35 |
| 6 | 1 | 11.50 | 6.30 | 5.20 | 0.45 |
| 7 | 1 | 36.50 | 33.00 | 3.50 | 0.10 |
| 8 | 1 | 47.10 | 33.70 | 13.40 | 0.28 |
| 9 | 1 | 28.60 | 20.90 | 7.70 | 0.27 |
| 10 | 1 | 27.30 | 20.80 | 6.50 | 0.24 |
| 11 | 1 | 26.20 | 17.50 | 8.70 | 0.33 |
| 12 | 1 | 44.60 | 24.60 | 20.00 | 0.45 |
| 13 | 1 | 31.80 | 22.20 | 9.60 | 0.30 |
| 14 | 1 | 41.10 | 28.40 | 12.70 | 0.31 |
| 15 | 1 | 43.20 | 27.10 | 16.10 | 0.37 |
| 16 | 1 | 31.10 | 22.50 | 8.60 | 0.28 |
| 17 | 1 | 30.90 | 19.80 | 11.10 | 0.36 |
| 18 | 1 | 29.50 | 19.50 | 10.00 | 0.34 |
| **% germination** | **100** | | | | |
| **mean** | | **34.20** | **23.90** | **10.30** | **0.31** |
| **std error** | | **2.30** | **1.72** | **0.97** | **0.02** |
| | | | | | |
| *Cucurbita moschata* | | | | | |
| 1 | 1 | 12.60 | 6.30 | 6.30 | 0.50 |
| 2 | 1 | 11.80 | 5.90 | 5.90 | 0.50 |
| 3 | 1 | 13.00 | 7.20 | 5.80 | 0.45 |
| 4 | 1 | 10.20 | 6.10 | 4.10 | 0.40 |
| 5 | 1 | 10.90 | 5.20 | 5.70 | 0.52 |
| 6 | 1 | 12.50 | 6.40 | 6.10 | 0.49 |
| 7 | 1 | 13.10 | 7.00 | 6.10 | 0.47 |
| 8 | 1 | 12.30 | 6.60 | 5.70 | 0.46 |
| 9 | 1 | 11.40 | 6.20 | 5.20 | 0.46 |
| 10 | 1 | 13.30 | 7.30 | 6.00 | 0.45 |
| 11 | 1 | 9.80 | 4.50 | 5.30 | 0.54 |
| 12 | 1 | 10.90 | 5.20 | 5.70 | 0.52 |
| 13 | 1 | 12.40 | 6.30 | 6.10 | 0.49 |
| 14 | 1 | 13.30 | 6.40 | 6.90 | 0.52 |
| 15 | 1 | 11.60 | 6.60 | 5.00 | 0.43 |
| 16 | 0 | | | | |
| 17 | 0 | | | | |
| 18 | 0 | | | | |
| **% germination** | **83** | | | | |

| | | DAY 12 | | | |
|---|---|---|---|---|---|
| | plants | total length (cm) | shoot length (cm) | root length (cm) | RLI |
| **mean** | | **11.94** | **6.21** | **5.73** | **0.48** |
| **std error** | | **0.29** | **0.20** | **0.17** | **0.01** |
| | | | | | |
| **Biosolids-Amended** | | | | | |
| *Brassica rapa* | | | | | |
| 1 | 1 | 5.20 | 2.60 | 2.60 | 0.50 |
| 2 | 1 | 6.10 | 3.30 | 2.80 | 0.46 |
| 3 | 1 | 4.90 | 2.90 | 2.00 | 0.41 |
| 4 | 1 | 3.20 | 1.90 | 1.30 | 0.41 |
| 5 | 1 | 5.80 | 3.50 | 2.30 | 0.40 |
| 6 | 1 | 6.50 | 3.70 | 2.80 | 0.43 |
| 7 | 1 | 4.40 | 2.30 | 2.10 | 0.48 |
| 8 | 1 | 5.60 | 3.30 | 2.30 | 0.41 |
| 9 | 1 | 5.10 | 3.10 | 2.00 | 0.39 |
| 10 | 1 | 5.30 | 3.00 | 2.30 | 0.43 |
| 11 | 1 | 6.00 | 2.90 | 3.10 | 0.52 |
| 12 | 1 | 4.80 | 2.20 | 2.60 | 0.54 |
| 13 | 1 | 4.70 | 2.00 | 2.70 | 0.57 |
| 14 | 1 | 5.30 | 2.60 | 2.70 | 0.51 |
| 15 | 1 | 5.50 | 2.50 | 3.00 | 0.55 |
| 16 | 1 | 6.10 | 3.70 | 2.40 | 0.39 |
| 17 | 1 | 4.00 | 1.80 | 2.20 | 0.55 |
| 18 | 1 | 3.90 | 2.00 | 1.90 | 0.49 |
| 19 | 1 | 5.80 | 2.90 | 2.90 | 0.50 |
| 20 | 1 | 4.70 | 2.50 | 2.20 | 0.47 |
| 21 | 0 | | | | |
| 22 | 0 | | | | |
| 23 | 0 | | | | |
| 24 | 0 | | | | |
| 25 | 0 | | | | |
| 26 | 0 | | | | |
| 27 | 0 | | | | |
| 28 | 0 | | | | |
| 29 | 0 | | | | |
| 30 | 0 | | | | |
| 31 | 0 | | | | |
| 32 | 0 | | | | |
| **% germination** | **63** | | | | |
| **mean** | | **5.15** | **2.74** | **2.41** | **0.47** |
| **std error** | | **0.19** | **0.13** | **0.10** | **0.01** |
| | | | | | |
| *Raphanus sativus* | | | | | |
| 1 | 1 | 9.10 | 6.40 | 2.70 | 0.30 |
| 2 | 1 | 12.10 | 9.40 | 2.70 | 0.22 |
| 3 | 1 | 13.10 | 9.30 | 3.80 | 0.29 |
| 4 | 1 | 11.10 | 6.90 | 4.20 | 0.38 |
| 5 | 1 | 8.70 | 5.40 | 3.30 | 0.38 |
| 6 | 1 | 7.90 | 6.20 | 1.70 | 0.22 |
| 7 | 1 | 10.00 | 7.80 | 2.20 | 0.22 |
| 8 | 1 | 15.30 | 6.40 | 8.90 | 0.58 |
| 9 | 1 | 9.60 | 6.20 | 3.40 | 0.35 |
| 10 | 1 | 16.20 | 8.60 | 7.60 | 0.47 |
| 11 | 1 | 11.10 | 7.20 | 3.90 | 0.35 |
| 12 | 1 | 11.20 | 8.70 | 2.50 | 0.22 |

| | | DAY 12 | | | |
|---|---|---|---|---|---|
| | plants | total length (cm) | shoot length (cm) | root length (cm) | RLI |
| 13 | 1 | 8.20 | 5.50 | 2.70 | 0.33 |
| 14 | 1 | 10.20 | 5.60 | 4.60 | 0.45 |
| 15 | 1 | 12.20 | 8.00 | 4.20 | 0.34 |
| 16 | 1 | | | | |
| 17 | 1 | | | | |
| 18 | 1 | | | | |
| **% germination** | **100** | | | | |
| **mean** | | **11.07** | **7.17** | **3.89** | **0.34** |
| **std error** | | **0.63** | **0.36** | **0.51** | **0.03** |
| *Phaseolus vulgaris* | | | | | |
| 1 | 1 | 49.50 | 28.50 | 21.00 | 0.42 |
| 2 | 1 | 43.20 | 31.80 | 11.40 | 0.26 |
| 3 | 1 | 38.60 | 31.40 | 7.20 | 0.19 |
| 4 | 1 | 42.40 | 29.20 | 13.20 | 0.31 |
| 5 | 1 | 31.60 | 27.50 | 4.10 | 0.13 |
| 6 | 1 | 49.20 | 32.30 | 16.90 | 0.34 |
| 7 | 1 | 43.60 | 31.10 | 12.50 | 0.29 |
| 8 | 1 | 37.20 | 29.10 | 8.10 | 0.22 |
| 9 | 1 | 22.50 | 16.30 | 6.20 | 0.28 |
| 10 | 1 | 14.10 | 9.20 | 4.90 | 0.35 |
| 11 | 1 | 40.60 | 30.60 | 10.00 | 0.25 |
| 12 | 1 | 36.10 | 28.20 | 7.90 | 0.22 |
| 13 | 1 | 44.70 | 31.50 | 13.20 | 0.30 |
| 14 | 1 | 38.90 | 20.60 | 18.30 | 0.47 |
| 15 | 1 | 49.60 | 33.50 | 16.10 | 0.32 |
| 16 | 1 | 44.20 | 28.70 | 15.50 | 0.35 |
| 17 | 1 | 43.30 | 29.70 | 13.60 | 0.31 |
| 18 | 1 | | | | |
| **% germination** | **100** | | | | |
| **mean** | | **39.37** | **27.60** | **11.77** | **0.29** |
| **std error** | | **2.28** | **1.55** | **1.19** | **0.02** |
| *Cucurbita moschata* | | | | | |
| 1 | 1 | 12.30 | 5.90 | 6.40 | 0.52 |
| 2 | 1 | 14.20 | 6.90 | 7.30 | 0.51 |
| 3 | 1 | 15.30 | 8.10 | 7.20 | 0.47 |
| 4 | 1 | 11.00 | 6.10 | 4.90 | 0.45 |
| 5 | 1 | 10.90 | 5.90 | 5.00 | 0.46 |
| 6 | 1 | 12.10 | 6.40 | 5.70 | 0.47 |
| 7 | 1 | 9.80 | 5.10 | 4.70 | 0.48 |
| 8 | 1 | 13.30 | 6.80 | 6.50 | 0.49 |
| 9 | 1 | 12.60 | 6.10 | 6.50 | 0.52 |
| 10 | 1 | 10.10 | 4.90 | 5.20 | 0.51 |
| 11 | 1 | 9.90 | 4.80 | 5.10 | 0.52 |
| 12 | 1 | 12.50 | 5.90 | 6.60 | 0.53 |
| 13 | 1 | 13.10 | 7.10 | 6.00 | 0.46 |
| 14 | 1 | 11.70 | 5.90 | 5.80 | 0.50 |
| 15 | 1 | 14.80 | 7.10 | 7.70 | 0.52 |
| 16 | 1 | 13.60 | 7.70 | 5.90 | 0.43 |
| 17 | 0 | | | | |
| 18 | 0 | | | | |
| **% germination** | **89** | | | | |

| | DAY 12 | | | |
|---|---|---|---|---|
| | plants | total length (cm) | shoot length (cm) | root length (cm) | RLI |
| mean | | 12.33 | 6.29 | 6.03 | 0.49 |
| std error | | 0.42 | 0.24 | 0.23 | 0.01 |

## *Appendix L.* Lumbricus terrestris *Land-Application Field Bioassay Data*

| | Initial | Day 12 |
|---|---|---|
| | **Adults** | **Adults** |
| **Reference** | | |
| A1 | 8 | 6 |
| A2 | 8 | 8 |
| A3 | 8 | 7 |
| **Mean** | **8.00** | **7.00** |
| **Standard Error** | **0.00** | **0.58** |
| | | |
| **Biosolids-Amended** | | |
| B1 | 8 | 8 |
| B2 | 8 | 8 |
| B3 | 8 | 8 |
| **Mean** | **8.00** | **8.00** |
| **Standard Error** | **0.00** | **0.00** |

## *Appendix M.* Daphnia magna *Run-off Laboratory Bioassay Data (Acute)*

| | Initial | 48-hours |
|---|---|---|
| Blank (Dechlorinated Water) | | |
| A1 | 10 | 10 |
| A2 | 10 | 10 |
| A3 | 10 | 9 |
| **average** | | **9.67** |
| **std error** | | **0.33** |
| **% survival** | | **96.67** |
| 25% STP Biosolids Run-off | | |
| B1 | 10 | 9 |
| B2 | 10 | 10 |
| B3 | 10 | 10 |
| **average** | | **9.67** |
| **std error** | | **0.33** |
| **% survival** | | **96.67** |
| 50% STP Biosolids Run-off | | |
| B4 | 10 | 10 |
| B5 | 10 | 9 |
| B6 | 10 | 9 |
| **average** | | **9.33** |
| **std error** | | **0.33** |
| **% survival** | | **93.33** |
| 75% STP Biosolids Run-off | | |
| B7 | 10 | 8 |
| B8 | 10 | 10 |
| B9 | 10 | 10 |
| **average** | | **9.33** |
| **std error** | | **0.67** |
| **% survival** | | **93.33** |
| 100% STP Biosolids Run-off | | |
| B10 | 10 | 9 |
| B11 | 10 | 7 |
| B12 | 10 | 9 |
| **average** | | **8.33** |
| **std error** | | **0.67** |
| **% survival** | | **83.33** |
| 25% Pulp Mill Biosolids Run-off | | |
| C1 | 10 | 10 |
| C2 | 10 | 10 |
| C3 | 10 | 10 |
| **average** | | **10.00** |
| **std error** | | **0.00** |
| **% survival** | | **100.00** |

| | Initial | 48-hours |
|---|---|---|
| 50% Pulp Mill Biosolids Run-off | | |
| C4 | 10 | 10 |
| C5 | 10 | 10 |
| C6 | 10 | 10 |
| **average** | | **10.00** |
| **std error** | | **0.00** |
| **% survival** | | **100.00** |
| 75% Pulp Mill Biosolids Run-off | | |
| C7 | 10 | 9 |
| C8 | 10 | 9 |
| C9 | 10 | 10 |
| **average** | | **9.33** |
| **std error** | | **0.33** |
| **% survival** | | **93.33** |
| 100% Pulp Mill Biosolids Run-off | | |
| C10 | 10 | 8 |
| C11 | 10 | 9 |
| C12 | 10 | 9 |
| **average** | | **8.67** |
| **std error** | | **0.33** |
| **% survival** | | **86.67** |
| 25% Reference Run-off | | |
| C1 | 10 | 10 |
| C2 | 10 | 10 |
| C3 | 10 | 10 |
| **average** | | **10.00** |
| **std error** | | **0.00** |
| **% survival** | | **100.00** |
| 50% Reference Run-off | | |
| C4 | 10 | 8 |
| C5 | 10 | 10 |
| C6 | 10 | 10 |
| **average** | | **9.33** |
| **std error** | | **0.67** |
| **% survival** | | **93.33** |
| 75% Reference Run-off | | |
| C7 | 10 | 9 |
| C8 | 10 | 9 |
| C9 | 10 | 10 |
| **average** | | **9.33** |
| **std error** | | **0.33** |
| **% survival** | | **93.33** |
| 100% Reference Run-off | | |
| C10 | 10 | 8 |
| C11 | 10 | 9 |
| C12 | 10 | 9 |
| **average** | | **8.67** |
| **std error** | | **0.33** |
| **% survival** | | **86.67** |

## *Appendix N.* Daphnia magna *Run-off Laboratory Bioassay Data (Chronic)*

| | Day 0 adults | Day 2 adults | Day 21 adults | TOTAL NEONATES (over 21 days) |
|---|---|---|---|---|
| **Reference** | | | | |
| A1 | 1 | 1 | 1 | 0 |
| A2 | 1 | 1 | 1 | 1 |
| A3 | 1 | 1 | 1 | 41 |
| A4 | 1 | 1 | 1 | 47 |
| A5 | 1 | 1 | 1 | 0 |
| A6 | 1 | 1 | 1 | 33 |
| A7 | 1 | 1 | 1 | 47 |
| A8 | 1 | 1 | 1 | 48 |
| A9 | 1 | 1 | 1 | 44 |
| A10 | 1 | 1 | 0 | |
| **total** | | | **9.00** | **261.00** |
| **mean** | | | **0.90** | **29.00** |
| **std dev** | | | | **21.97** |
| **std error** | | | **0.10** | **7.32** |
| | | | | |
| **25% Pulp Mill Biosolids Run-off** | | | | |
| B1 | 1 | 1 | 0 | |
| B2 | 1 | 1 | 1 | 32 |
| B3 | 1 | 1 | 1 | 29 |
| B4 | 1 | 1 | 1 | 29 |
| B5 | 1 | 1 | 1 | 17 |
| B6 | 1 | 1 | 0 | |
| B7 | 1 | 1 | 1 | 0 |
| B8 | 1 | 1 | 1 | 40 |
| B9 | 1 | 1 | 1 | 26 |
| B10 | 1 | 1 | 1 | 26 |
| **total** | | | **8.00** | **251.0** |
| **mean** | | | **0.80** | **24.88** |
| **std dev** | | | | **11.93** |
| **std error** | | | **0.13** | **4.22** |
| | | | | |
| **100% Pulp Mill Biosoldis Run-off** | | | | |
| B11 | 1 | 1 | 1 | 50 |
| B12 | 1 | 1 | 1 | 5 |
| B13 | 1 | 1 | 1 | 25 |
| B14 | 1 | 1 | 1 | 30 |
| B15 | 1 | 1 | 1 | 24 |
| B16 | 1 | 1 | 1 | 35 |
| B17 | 1 | 0 | 0 | |
| B18 | 1 | 1 | 1 | 0 |
| B19 | 1 | 1 | 1 | 27 |
| B20 | 1 | 1 | 1 | 10 |
| **total** | | | **9.00** | **206.0** |
| **mean** | | | **0.90** | **22.89** |
| **std dev** | | | | **15.67** |
| **std error** | | | **0.10** | **5.22** |
| | | | | |
| **25% Municipal Biosolids Run-off** | | | | |
| C1 | 1 | 1 | 0 | |

| | Day 0 adults | Day 2 adults | Day 21 adults | TOTAL NEONATES (over 21 days) |
|---|---|---|---|---|
| C2 | 1 | 1 | 0 | |
| C3 | 1 | 1 | 1 | 29 |
| C4 | 1 | 1 | 1 | 27 |
| C5 | 1 | 1 | 1 | 19 |
| C6 | 1 | 1 | 1 | 22 |
| C7 | 1 | 1 | 1 | 21 |
| C8 | 1 | 1 | 1 | 18 |
| C9 | 1 | 1 | 1 | 42 |
| C10 | 1 | 1 | 1 | 27 |
| **total** | | | **8.00** | **230.0** |
| **mean** | | | **0.80** | **25.63** |
| **std dev** | | | | **7.74** |
| **std error** | | | **0.13** | **2.74** |
| | | | | |
| **100% Municipal Biosolids Run-off** | | | | |
| C11 | 1 | 1 | 1 | 73 |
| C12 | 1 | 1 | 0 | |
| C13 | 1 | 1 | 1 | 81 |
| C14 | 1 | 1 | 0 | |
| C15 | 1 | 1 | 1 | 64 |
| C16 | 1 | 1 | 1 | 43 |
| C17 | 1 | 1 | 1 | 45 |
| C18 | 1 | 1 | 1 | 28 |
| C19 | 1 | 1 | 1 | 61 |
| C20 | 1 | 1 | 1 | 69 |
| **total** | | | **8.00** | **488.0** |
| **mean** | | | **0.80** | **58.00** |
| **std dev** | | | | **17.78** |
| **std error** | | | **0.13** | **6.29** |
| | | | | |
| **25% Reference Soil Run-off** | | | | |
| D1 | 1 | 1 | 1 | 92 |
| D2 | 1 | 1 | 1 | 61 |
| D3 | 1 | 1 | 1 | 59 |
| D4 | 1 | 1 | 1 | 88 |
| D5 | 1 | 1 | 1 | 54 |
| D6 | 1 | 1 | 1 | 58 |
| D7 | 1 | 1 | 1 | 50 |
| D8 | 1 | 1 | 1 | 42 |
| D9 | 1 | 1 | 1 | 75 |
| D10 | 1 | 1 | 1 | 58 |
| **total** | | | **10.00** | **637.0** |
| **mean** | | | **1.00** | **63.70** |
| **std dev** | | | | **16.21** |
| **std error** | | | **0.00** | **5.13** |
| | | | | |
| **100% Reference Soil Run-off** | | | | |
| D11 | 1 | 1 | 1 | 2 |
| D12 | 1 | 1 | 1 | 66 |
| D13 | 1 | 1 | 1 | 38 |
| D14 | 1 | 1 | 1 | 66 |
| D15 | 1 | 1 | 1 | 66 |
| D16 | 1 | 1 | 1 | 67 |

| | Day 0 adults | Day 2 adults | Day 21 adults | TOTAL NEONATES (over 21 days) |
|---|---|---|---|---|
| D17 | 1 | 1 | 1 | 84 |
| D18 | 1 | 1 | 1 | 60 |
| D19 | 1 | 1 | 1 | 67 |
| D20 | 1 | 1 | 1 | 60 |
| **total** | | | **10.00** | **576.0** |
| **avg # young/living adult** | | | **1.00** | **57.60** |
| **std dev** | | | | **22.54** |
| **std error** | | | **0.00** | **7.13** |

*Note: Mean number of neonates based on number of living adults in treatment.

## *Appendix O.* Hyalella azteca *Run-off Laboratory Bioassay Data*

| Treatment | 48 hours | Day 21 | young |
|---|---|---|---|
| Reference | | | |
| A1 | 3 | 3 | 0 |
| A2 | 3 | 3 | 2 |
| A3 | 3 | 3 | 2 |
| A4 | 3 | 3 | 1 |
| A5 | 3 | 3 | 3 |
| **average** | **3.00** | **3.00** | **1.60** |
| **std error** | **0.00** | **0.00** | **0.51** |
| 10% STP Biosolids Run-off | | | |
| B1 | 3 | 3 | 1 |
| B2 | 3 | 3 | 0 |
| B3 | 3 | 3 | 0 |
| B4 | 3 | 3 | 1 |
| B5 | 3 | 3 | 1 |
| **average** | **3.00** | **3.00** | **0.60** |
| **std error** | **0.00** | **0.00** | **0.24** |
| 25% STP Biosolids Run-off | | | |
| B4 | 3 | 3 | 0 |
| B5 | 3 | 3 | 0 |
| B6 | 3 | 3 | 3 |
| B7 | 3 | 3 | 1 |
| B8 | 3 | 3 | 2 |
| **average** | **3.00** | **3.00** | **1.20** |
| **std error** | **0.00** | **0.00** | **0.58** |
| 50% STP Biosolids Run-off | | | |
| B7 | 3 | 3 | 1 |
| B8 | 3 | 3 | 1 |
| B9 | 3 | 3 | 0 |
| B10 | 3 | 3 | 0 |
| B11 | 3 | 3 | 1 |
| **average** | **3.00** | **3.00** | **0.60** |
| **std error** | **0.00** | **0.00** | **0.24** |
| 75% STP Biosolids Run-off | | | |
| B10 | 3 | 3 | 0 |
| B11 | 3 | 3 | 0 |
| B12 | 2 | 2 | 0 |
| B13 | 3 | 3 | 0 |
| B14 | 3 | 3 | 1 |
| **average** | **2.80** | **2.80** | **0.20** |
| **std error** | **0.20** | **0.20** | **0.20** |

| Treatment | 48 hours | Day 21 | young |
|---|---|---|---|
| 100% STP Biosolids Run-off | | | |
| B13 | 2 | 2 | 0 |
| B14 | 3 | 2 | 0 |
| B15 | 3 | 3 | 1 |
| B16 | 3 | 3 | 0 |
| B17 | 3 | 3 | 0 |
| **average** | **2.80** | **2.60** | **0.20** |
| **std error** | **0.20** | **0.24** | **0.20** |
| | | | |
| 10% Pulp Mill Biosolids Run-off | | | |
| C1 | 3 | 3 | 1 |
| C2 | 3 | 3 | 0 |
| C3 | 3 | 3 | 3 |
| C4 | 3 | 3 | 2 |
| C5 | 3 | 3 | 2 |
| **average** | **3.00** | **3.00** | **1.60** |
| **std error** | **0.00** | **0.00** | **0.51** |
| | | | |
| 25% Pulp Mill Biosolids Run-off | | | |
| C4 | 3 | 3 | 0 |
| C5 | 3 | 3 | 0 |
| C6 | 3 | 3 | 1 |
| C7 | 3 | 3 | 1 |
| C8 | 3 | 3 | 0 |
| **average** | **3.00** | **3.00** | **0.40** |
| **std error** | **0.00** | **0.00** | **0.24** |
| | | | |
| 50% Pulp Mill Biosolids Run-off | | | |
| C7 | 3 | 3 | 1 |
| C8 | 3 | 3 | 2 |
| C9 | 2 | 1 | 0 |
| C10 | 3 | 3 | 0 |
| C11 | 3 | 3 | 1 |
| **average** | **2.80** | **2.60** | **0.80** |
| **std error** | **0.20** | **0.40** | **0.37** |
| | | | |
| 75% Pulp Mill Biosolids Run-off | | | |
| C10 | 2 | 2 | 0 |
| C11 | 3 | 3 | 0 |
| C12 | 3 | 2 | 0 |
| C13 | 3 | 3 | 0 |
| C14 | 3 | 3 | 1 |
| **average** | **2.80** | **2.60** | **0.20** |
| **std error** | **0.20** | **0.24** | **0.20** |
| | | | |
| 100% Pulp Mill Biosolids Run-off | | | |
| C13 | 2 | 2 | 0 |
| C14 | 3 | 3 | 2 |

| Treatment | 48 hours | Day 21 | young |
|---|---|---|---|
| C15 | 2 | 1 | 0 |
| C16 | 3 | 3 | 0 |
| C17 | 3 | 3 | 1 |
| **average** | **2.60** | **2.40** | **0.60** |
| **std error** | **0.24** | **0.40** | **0.40** |
| | | | |
| 10% Reference Biosolids Run-off | | | |
| D1 | 3 | 3 | 0 |
| D2 | 3 | 3 | 2 |
| D3 | 1 | 1 | 0 |
| D4 | 3 | 3 | 0 |
| D5 | 3 | 3 | 0 |
| **average** | **2.60** | **2.60** | **0.40** |
| **std error** | **0.40** | **0.40** | **0.40** |
| | | | |
| 25% Reference Biosolids Run-off | | | |
| D4 | 3 | 3 | 0 |
| D5 | 3 | 3 | 0 |
| D6 | 2 | 2 | 0 |
| D7 | 3 | 3 | 0 |
| D8 | 3 | 3 | 1 |
| **average** | **2.80** | **2.80** | **0.20** |
| **std error** | **0.20** | **0.20** | **0.20** |
| | | | |
| 50% Reference Biosolids Run-off | | | |
| D7 | 3 | 3 | 0 |
| D8 | 3 | 3 | 0 |
| D9 | 3 | 3 | 0 |
| D10 | 3 | 3 | 1 |
| D11 | 3 | 3 | 0 |
| **average** | **3.00** | **3.00** | **0.20** |
| **std error** | **0.00** | **0.00** | **0.20** |
| | | | |
| 75% Reference Biosolids Run-off | | | |
| D10 | 3 | 3 | 0 |
| D11 | 3 | 3 | 1 |
| D12 | 3 | 3 | 1 |
| D13 | 3 | 3 | 0 |
| D14 | 3 | 3 | 2 |
| **average** | **3.00** | **3.00** | **0.80** |
| **std error** | **0.00** | **0.00** | **0.37** |
| | | | |
| 100% Reference Biosolids Run-off | | | |
| D13 | 3 | 3 | 5 |
| D14 | 2 | 2 | 0 |
| D15 | 3 | 3 | 1 |
| D16 | 3 | 3 | 3 |
| D17 | 3 | 3 | 0 |

| Treatment | 48 hours | Day 21 | young |
|---|---|---|---|
| average | 2.80 | 2.80 | 1.80 |
| std error | 0.20 | 0.20 | 0.97 |

## *Appendix P.* Pseudokirchneriella subcapitata *Run-off Laboratory Bioassay Data*

| Well # | Initial [cell] 0 hours | Final [cell] 96 hours | cells/mL 96 hours | Standard Error | % growth (compared to blank) | % std error |
|---|---|---|---|---|---|---|
| **BLANK (d H2O)** | **1.00E+05** | **1.38** | **3.44E+05** | **7034.143** | | |
| A1 | | 1.50 | 3.75E+05 | | | |
| A2 | | 1.30 | 3.25E+05 | | | |
| A3 | | 1.35 | 3.38E+05 | | | |
| A4 | | 1.35 | 3.38E+05 | | | |
| A5 | | 1.35 | 3.38E+05 | | | |
| A6 | | 1.40 | 3.50E+05 | | | |
| **25% STP BIOSOLIDS** | **1.00E+05** | **3.13** | **7.81E+05** | **62562.47** | **227.27%** | **18.20%** |
| B1 | | 2.75 | 6.88E+05 | | | |
| B2 | | 3.15 | 7.88E+05 | | | |
| B3 | | 3.00 | 7.50E+05 | | | |
| B4 | | 2.25 | 5.63E+05 | | | |
| B5 | | 3.65 | 9.13E+05 | | | |
| B6 | | 3.95 | 9.88E+05 | | | |
| **50% STP BIOSOLIDS** | **1.00E+05** | **3.52** | **8.79E+05** | **68134.87** | **255.76%** | **19.82%** |
| C1 | | 4.50 | 1.13E+06 | | | |
| C2 | | 3.30 | 8.25E+05 | | | |
| C3 | | 2.90 | 7.25E+05 | | | |
| C4 | | 3.20 | 8.00E+05 | | | |
| C5 | | 4.20 | 1.05E+06 | | | |
| C6 | | 3.00 | 7.50E+05 | | | |
| **100% STP BIOSOLIDS** | **1.00E+05** | **2.77** | **6.92E+05** | **63792.2** | **201.21%** | **18.56%** |
| D1 | | 2.60 | 6.50E+05 | | | |
| D2 | | 2.50 | 6.25E+05 | | | |
| D3 | | 2.90 | 7.25E+05 | | | |
| D4 | | 1.90 | 4.75E+05 | | | |
| D5 | | 3.80 | 9.50E+05 | | | |
| D6 | | 2.90 | 7.25E+05 | | | |
| **25% PULP MILL BIOSOLIDS** | **1.00E+05** | **4.12** | **1.03E+06** | **74558.89** | **299.39%** | **21.69%** |
| E1 | | 4.40 | 1.10E+06 | | | |
| E2 | | 3.30 | 8.25E+05 | | | |
| E3 | | 3.80 | 9.50E+05 | | | |
| E4 | | 3.70 | 9.25E+05 | | | |
| E5 | | 4.10 | 1.03E+06 | | | |
| E6 | | 5.40 | 1.35E+06 | | | |
| **50% PULP MILL BIOSOLIDS** | **1.00E+05** | **3.67** | **9.17E+05** | **16666.67** | **266.67%** | **4.85%** |
| F1 | | 3.70 | 9.25E+05 | | | |
| F2 | | 3.50 | 8.75E+05 | | | |

| | Initial [cell] | Final [cell] | cells/mL | Standard | % growth | % std error |
|---|---|---|---|---|---|---|
| Well # | 0 hours | 96 hours | 96 hours | Error | (compared to blank) | |
| F3 | | 3.60 | 9.00E+05 | | | |
| F4 | | 3.80 | 9.50E+05 | | | |
| F5 | | 3.90 | 9.75E+05 | | | |
| F6 | | 3.50 | 8.75E+05 | | | |
| **100% PULP MILL BIOSOLIDS** | **1.00E+05** | **3.58** | **8.96E+05** | **48911.77** | **260.61%** | **14.23%** |
| G1 | | 3.40 | 8.50E+05 | | | |
| G2 | | 3.40 | 8.50E+05 | | | |
| G3 | | 3.90 | 9.75E+05 | | | |
| G4 | | 2.80 | 7.00E+05 | | | |
| G5 | | 4.10 | 1.03E+06 | | | |
| G6 | | 3.90 | 9.75E+05 | | | |
| **25% REFERENCE RUN-OFF** | **1.00E+05** | **4.20** | **1.05E+06** | **57735.03** | **305.45%** | **16.80%** |
| H1 | | 4.80 | 1.20E+06 | | | |
| H2 | | 3.60 | 9.00E+05 | | | |
| H3 | | 3.40 | 8.50E+05 | | | |
| H4 | | 4.60 | 1.15E+06 | | | |
| H5 | | 4.40 | 1.10E+06 | | | |
| H6 | | 4.40 | 1.10E+06 | | | |
| **50% REFERENCE RUN-OFF** | **1.00E+05** | **4.95** | **1.24E+06** | **126614.6** | **360.00%** | **36.83%** |
| I1 | | 4.40 | 1.10E+06 | | | |
| I2 | | 7.40 | 1.85E+06 | | | |
| I3 | | 4.80 | 1.20E+06 | | | |
| I4 | | 3.90 | 9.75E+05 | | | |
| I5 | | 4.50 | 1.13E+06 | | | |
| I6 | | 4.70 | 1.18E+06 | | | |
| **100% REFERENCE RUN-OFF** | **1.00E+05** | **4.55** | **1.14E+06** | **98053.98** | **330.91%** | **28.52%** |
| J1 | | 4.40 | 1.10E+06 | | | |
| J2 | | 5.80 | 1.45E+06 | | | |
| J3 | | 5.40 | 1.35E+06 | | | |
| J4 | | 4.10 | 1.03E+06 | | | |
| J5 | | 4.50 | 1.13E+06 | | | |
| J6 | | 3.10 | 7.75E+05 | | | |

## *Appendix Q.* Lemna minor *Run-off Laboratory Bioassay Data*

| Well # | Frond Count | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Day 0 | Day 2 | Day 4 | Day 6 | Day8 | Day 10 | Day 12 | Day 14 |
| **Blank** | | | | | | | | |
| A1 | 2 | 4 | 5 | 6 | 7 | 7 | 8 | 8 |
| A2 | 2 | 3 | 4 | 4 | 5 | 5 | 6 | 6 |
| A3 | 2 | 3 | 4 | 4 | 5 | 6 | 7 | 9 |
| A4 | 2 | 3 | 5 | 6 | 6 | 7 | 8 | 9 |
| A5 | 2 | 3 | 6 | 6 | 7 | 7 | 8 | 8 |
| A6 | 2 | 2 | 3 | 3 | 3 | 4 | 4 | 5 |
| A7 | 2 | 3 | 3 | 3 | 4 | 5 | 5 | 5 |
| A8 | 2 | 3 | 6 | 7 | 7 | 8 | 9 | 9 |
| A9 | 2 | 4 | 5 | 6 | 8 | 8 | 9 | 10 |
| A10 | 2 | 3 | 3 | 3 | 4 | 4 | 5 | 7 |
| A11 | 2 | 3 | 5 | 5 | 5 | 5 | 5 | 6 |
| A12 | 2 | 4 | 6 | 6 | 7 | 7 | 8 | 8 |
| **avg** | **2.0000** | **3.1667** | **4.5833** | **4.9167** | **5.6667** | **6.0833** | **6.8333** | **7.5000** |
| **std dev** | | | | | | | | **1.6787** |
| **std err** | | | | | | | | **0.4846** |
| **25% municipal biosolids run-off** | | | | | | | | |
| B1 | 2 | 3 | 5 | 5 | 5 | 6 | 6 | 8 |
| B2 | 2 | 3 | 5 | 5 | 6 | 7 | 7 | 9 |
| B3 | 2 | 3 | 6 | 6 | 6 | 7 | 7 | 8 |
| B4 | 2 | 4 | 5 | 5 | 5 | 5 | 5 | 6 |
| B5 | 2 | 2 | 5 | 5 | 5 | 6 | 6 | 8 |
| B6 | 2 | 4 | 6 | 7 | 7 | 8 | 9 | 11 |
| B7 | 2 | 3 | 4 | 4 | 5 | 6 | 6 | 7 |
| B8 | 2 | 3 | 4 | 4 | 5 | 5 | 6 | 6 |
| B9 | 2 | 3 | 4 | 4 | 4 | 4 | 4 | 5 |
| B10 | 2 | 4 | 5 | 5 | 5 | 6 | 6 | 7 |
| B11 | 2 | 3 | 5 | 5 | 5 | 6 | 7 | 7 |
| B12 | 2 | 3 | 5 | 7 | 7 | 8 | 8 | 9 |
| **avg** | **2.0000** | **3.1667** | **4.9167** | **5.1667** | **5.4167** | **6.1667** | **6.4167** | **7.5833** |
| **std dev** | | | | | | | | **1.6214** |
| **std err** | | | | | | | | **0.4680** |
| **50% municipal biosolids run-off** | | | | | | | | |
| C1 | 2 | 3 | 5 | 6 | 7 | 7 | 8 | 9 |
| C2 | 2 | 3 | 5 | 5 | 7 | 7 | 8 | 10 |
| C3 | 2 | 4 | 7 | 8 | 8 | 9 | 11 | 12 |
| C4 | 2 | 3 | 5 | 5 | 5 | 6 | 6 | 7 |
| C5 | 2 | 2 | 5 | 5 | 5 | 6 | 6 | 8 |
| C6 | 2 | 3 | 5 | 5 | 6 | 7 | 7 | 8 |
| C7 | 2 | 3 | 3 | 3 | 3 | 4 | 4 | 6 |
| C8 | 2 | 3 | 5 | 5 | 6 | 6 | 7 | 9 |
| C9 | 2 | 2 | 4 | 4 | 4 | 5 | 5 | 6 |
| C10 | 2 | 3 | 5 | 5 | 6 | 7 | 7 | 9 |
| C11 | 2 | 3 | 5 | 5 | 5 | 6 | 7 | 8 |
| C12 | 2 | 3 | 4 | 4 | 6 | 6 | 6 | 8 |
| **avg** | **2.0000** | **2.9167** | **4.8333** | **5.0000** | **5.6667** | **6.3333** | **6.8333** | **8.3333** |
| **std dev** | | | | | | | | **1.6697** |
| **std err** | | | | | | | | **0.4820** |
| **100% municipal biosolids run-off** | | | | | | | | |
| D1 | 2 | 3 | 5 | 5 | 6 | 6 | 8 | 10 |
| D2 | 2 | 2 | 4 | 4 | 4 | 5 | 6 | 7 |
| D3 | 2 | 3 | 4 | 4 | 5 | 6 | 6 | 7 |
| D4 | 2 | 3 | 5 | 5 | 5 | 6 | 7 | 8 |
| D5 | 2 | 3 | 5 | 5 | 5 | 6 | 8 | 8 |
| D6 | 2 | 2 | 4 | 4 | 4 | 6 | 6 | 7 |
| D7 | 2 | 2 | 4 | 4 | 5 | 7 | 7 | 7 |
| D8 | 2 | 3 | 5 | 5 | 6 | 6 | 7 | 8 |
| D9 | 2 | 4 | 6 | 6 | 7 | 7 | 7 | 8 |
| D10 | 2 | 3 | 6 | 6 | 6 | 6 | 7 | 7 |

| Well # | Frond Count | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Day 0 | Day 2 | Day 4 | Day 6 | Day8 | Day 10 | Day 12 | Day 14 |
| D11 | 2 | 3 | 3 | 3 | 4 | 5 | 6 | 6 |
| D12 | 2 | 3 | 5 | 5 | 5 | 6 | 7 | 7 |
| **avg** | **2.0000** | **2.8333** | **4.6667** | **4.6667** | **5.1667** | **6.0000** | **6.8333** | **7.5000** |
| **std dev** | | | | | | | | **1.0000** |
| **std err** | | | | | | | | **0.2887** |
| **25% pulp mill biosolids run-off** | | | | | | | | |
| E1 | 2 | 3 | 5 | 5 | 6 | 6 | 8 | 9 |
| E2 | 2 | 3 | 5 | 5 | 5 | 7 | 8 | 8 |
| E3 | 2 | 3 | 5 | 5 | 6 | 6 | 7 | 7 |
| E4 | 2 | 3 | 4 | 4 | 5 | 6 | 6 | 8 |
| E5 | 2 | 3 | 5 | 6 | 6 | 8 | 10 | 10 |
| E6 | 2 | 3 | 4 | 4 | 5 | 5 | 6 | 8 |
| E7 | 2 | 3 | 5 | 5 | 6 | 8 | 9 | 9 |
| E8 | 2 | 3 | 4 | 4 | 4 | 5 | 5 | 7 |
| E9 | 2 | 4 | 5 | 5 | 5 | 7 | 8 | 9 |
| E10 | 2 | 3 | 4 | 4 | 6 | 6 | 7 | 8 |
| E11 | 2 | 3 | 4 | 4 | 4 | 5 | 6 | 8 |
| E12 | 2 | 3 | 4 | 4 | 5 | 5 | 6 | 6 |
| **avg** | **2.0000** | **3.0833** | **4.5000** | **4.5833** | **5.2500** | **6.1667** | **7.1667** | **8.0833** |
| **std dev** | | | | | | | | **1.0836** |
| **std err** | | | | | | | | **0.3128** |
| **50% pulp mill biosolids run-off** | | | | | | | | |
| F1 | 2 | 3 | 6 | 6 | 7 | 7 | 9 | 10 |
| F2 | 2 | 3 | 7 | 7 | 8 | 10 | 12 | 12 |
| F3 | 2 | 3 | 5 | 5 | 6 | 8 | 9 | 9 |
| F4 | 2 | 3 | 5 | 5 | 6 | 7 | 7 | 8 |
| F5 | 2 | 4 | 6 | 6 | 8 | 8 | 10 | 13 |
| F6 | 2 | 3 | 5 | 5 | 6 | 8 | 8 | 10 |
| F7 | 2 | 2 | 6 | 6 | 7 | 8 | 10 | 11 |
| F8 | 2 | 3 | 4 | 4 | 5 | 6 | 7 | 8 |
| F9 | 2 | 3 | 5 | 5 | 6 | 6 | 7 | 9 |
| F10 | 2 | 3 | 5 | 5 | 7 | 8 | 8 | 10 |
| F11 | 2 | 3 | 6 | 6 | 8 | 8 | 10 | 11 |
| F12 | 2 | 3 | 5 | 5 | 6 | 8 | 10 | 11 |
| **avg** | **2.0000** | **3.0000** | **5.4167** | **5.4167** | **6.6667** | **7.6667** | **8.9167** | **10.1667** |
| **std dev** | | | | | | | | **1.5275** |
| **std err** | | | | | | | | **0.4410** |
| **100% pulp mill biosolids run-off** | | | | | | | | |
| G1 | 2 | 3 | 6 | 6 | 7 | 9 | 10 | 11 |
| G2 | 2 | 2 | 3 | 3 | 4 | 5 | 5 | 6 |
| G3 | 2 | 2 | 4 | 4 | 5 | 7 | 8 | 9 |
| G4 | 2 | 3 | 5 | 5 | 5 | 6 | 7 | 9 |
| G5 | 2 | 3 | 5 | 5 | 6 | 6 | 8 | 8 |
| G6 | 2 | 3 | 5 | 5 | 5 | 6 | 6 | 7 |
| G7 | 2 | 3 | 5 | 5 | 6 | 6 | 8 | 9 |
| G8 | 2 | 3 | 5 | 5 | 6 | 8 | 9 | 10 |
| G9 | 2 | 2 | 3 | 4 | 4 | 6 | 7 | 8 |
| G10 | 2 | 3 | 5 | 6 | 9 | 11 | 12 | 12 |
| G11 | 2 | 4 | 6 | 6 | 7 | 9 | 9 | 10 |
| G12 | 2 | 2 | 3 | 3 | 4 | 5 | 5 | 7 |
| **avg** | **2.0000** | **2.7500** | **4.5833** | **4.7500** | **5.6667** | **7.0000** | **7.8333** | **8.8333** |
| **std dev** | | | | | | | | **1.7495** |
| **std err** | | | | | | | | **0.5050** |
| **25% reference soil run-off** | | | | | | | | |
| H1 | 2 | 3 | 4 | 4 | 5 | 5 | 5 | 6 |
| H2 | 2 | 2 | 3 | 3 | 3 | 4 | 4 | 5 |
| H3 | 2 | 2 | 4 | 4 | 4 | 5 | 5 | 6 |
| H4 | 2 | 2 | 3 | 3 | 3 | 4 | 4 | 5 |
| H5 | 2 | 3 | 4 | 4 | 5 | 5 | 5 | 6 |
| H6 | 2 | 3 | 4 | 4 | 4 | 5 | 5 | 7 |
| H7 | 2 | 3 | 4 | 4 | 5 | 5 | 5 | 6 |
| H8 | 2 | 4 | 4 | 5 | 5 | 6 | 6 | 8 |
| H9 | 2 | 3 | 4 | 4 | 4 | 5 | 6 | 6 |

| Well # | Frond Count | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Day 0 | Day 2 | Day 4 | Day 6 | Day8 | Day 10 | Day 12 | Day 14 |
| H10 | 2 | 3 | 3 | 3 | 4 | 5 | 5 | 7 |
| H11 | 2 | 3 | 3 | 3 | 3 | 4 | 4 | 5 |
| H12 | 2 | 4 | 4 | 4 | 5 | 5 | 5 | 8 |
| **avg** | **2.0000** | **2.9167** | **3.6667** | **3.7500** | **4.1667** | **4.8333** | **4.9167** | **6.2500** |
| **std dev** | | | | | | | | **1.0553** |
| **std err** | | | | | | | | **0.3046** |
| **50% reference soil run-off** | | | | | | | | |
| I1 | 2 | 3 | 3 | 3 | 3 | 3 | 4 | 6 |
| I2 | 2 | 2 | 3 | 3 | 4 | 4 | 4 | 5 |
| I3 | 2 | 3 | 5 | 5 | 6 | 6 | 7 | 9 |
| I4 | 2 | 3 | 4 | 4 | 4 | 4 | 4 | 5 |
| I5 | 2 | 3 | 4 | 4 | 5 | 5 | 5 | 7 |
| I6 | 2 | 3 | 3 | 3 | 4 | 4 | 5 | 5 |
| I7 | 2 | 3 | 5 | 5 | 5 | 6 | 6 | 7 |
| I8 | 2 | 3 | 5 | 5 | 6 | 6 | 7 | 8 |
| I9 | 2 | 3 | 3 | 3 | 4 | 5 | 5 | 6 |
| I10 | 2 | 2 | 3 | 3 | 3 | 4 | 4 | 5 |
| I11 | 2 | 2 | 4 | 4 | 5 | 5 | 5 | 7 |
| I12 | 2 | 2 | 3 | 3 | 3 | 3 | 3 | 4 |
| **avg** | **2.0000** | **2.6667** | **3.7500** | **3.7500** | **4.3333** | **4.5833** | **4.9167** | **6.1667** |
| **std dev** | | | | | | | | **1.4668** |
| **std err** | | | | | | | | **0.4234** |
| **100% reference soil run-off** | | | | | | | | |
| J1 | 2 | 3 | 4 | 4 | 5 | 5 | 7 | 8 |
| J2 | 2 | 3 | 4 | 4 | 5 | 5 | 6 | 8 |
| J3 | 2 | 2 | 4 | 4 | 5 | 5 | 7 | 7 |
| J4 | 2 | 2 | 4 | 4 | 5 | 5 | 6 | 6 |
| J5 | 2 | 3 | 5 | 5 | 6 | 6 | 7 | 8 |
| J6 | 2 | 2 | 4 | 4 | 4 | 5 | 5 | 6 |
| J7 | 2 | 3 | 3 | 3 | 4 | 4 | 4 | 6 |
| J8 | 2 | 3 | 3 | 3 | 3 | 4 | 4 | 6 |
| J9 | 2 | 3 | 4 | 4 | 6 | 8 | 8 | 9 |
| J10 | 2 | 3 | 5 | 5 | 6 | 6 | 6 | 7 |
| J11 | 2 | 2 | 6 | 6 | 6 | 7 | 7 | 8 |
| J12 | 2 | 3 | 4 | 4 | 5 | 5 | 6 | 6 |
| **avg** | **2.0000** | **2.6667** | **4.1667** | **4.1667** | **5.0000** | **5.4167** | **6.0833** | **7.0833** |
| **std dev** | | | | | | | | **1.0836** |
| **std err** | | | | | | | | **0.3128** |

*Appendix R.* Brassica rapa *Land-Application Laboratory Bioassay Data*

| | Root Length (cm) | Shoot Length (cm) | Total Length (cm) | # of Flowers | # of Seed Pods | Root-length index |
|---|---|---|---|---|---|---|
| **Reference** | 3.6 | 9.1 | 12.7 | 2 | 3 | 0.2835 |
| | 6.3 | 9.2 | 15.5 | 6 | 4 | 0.4065 |
| | 5.3 | 11.1 | 16.4 | 3 | 2 | 0.3232 |
| | 4.2 | 10.1 | 14.3 | 2 | 1 | 0.2937 |
| | 5.9 | 7.6 | 13.5 | 2 | 2 | 0.4370 |
| | 3.1 | 6.6 | 9.7 | 5 | 1 | 0.3196 |
| | 8.2 | 12.1 | 20.3 | 6 | 3 | 0.4039 |
| | 3.6 | 9.5 | 13.1 | 4 | 2 | 0.2748 |
| | 5.3 | 15.5 | 20.8 | 2 | 3 | 0.2548 |
| | 4.1 | 11.3 | 15.4 | 2 | 4 | 0.2662 |
| | 4.6 | 8.7 | 13.3 | 4 | 2 | 0.3459 |
| | 5.8 | 9.5 | 15.3 | 8 | 4 | 0.3791 |
| | 5.3 | 11.9 | 17.2 | 9 | 2 | 0.3081 |
| | 3.7 | 9.5 | 13.2 | 4 | 3 | 0.2803 |
| | 4.7 | 13.6 | 18.3 | 9 | 3 | 0.2568 |
| | 2.6 | 6.6 | 9.2 | 8 | 2 | 0.2826 |
| | 1.9 | 6.5 | 8.4 | 4 | 4 | 0.2262 |
| | 5.2 | 10.5 | 15.7 | 7 | 3 | 0.3312 |
| | 4.9 | 10.6 | 15.5 | 7 | 3 | 0.3161 |
| | 2.6 | 9.3 | 11.9 | 7 | 5 | 0.2185 |
| | 5.1 | 7.5 | 12.6 | 8 | 2 | 0.4048 |
| | 4.6 | 7.6 | 12.2 | 1 | 1 | 0.3770 |
| | 3.9 | 7.9 | 11.8 | 2 | 2 | 0.3305 |
| | 5.1 | 11.3 | 16.4 | 2 | 4 | 0.3110 |
| | 2.3 | 9.0 | 11.3 | 3 | 2 | 0.2035 |
| **Total** | | | | **117** | **67** | |
| **Average** | | | | **4.6800** | **2.6800** | **0.3134** |
| **Standard Error** | | | | **0.5187** | **0.2139** | **0.0125** |
| | | | | | | |
| **Pulp Mill Biosolids** | 5.3 | 18.5 | 23.8 | 2 | 3 | 0.2227 |
| | 6.3 | 26.3 | 32.6 | 2 | 4 | 0.1933 |
| | 5.6 | 12.5 | 18.1 | 7 | 4 | 0.3094 |
| | 6.1 | 22.1 | 28.2 | 5 | 3 | 0.2163 |
| | 6.2 | 35.5 | 41.7 | 13 | 3 | 0.1487 |
| | 4.5 | 26.1 | 30.6 | 4 | 2 | 0.1471 |
| | 7.2 | 14.5 | 21.7 | 5 | 3 | 0.3318 |
| | 7.1 | 29.1 | 36.2 | 5 | 2 | 0.1961 |
| | 9.3 | 25.5 | 34.8 | 6 | 3 | 0.2672 |
| | 4.3 | 12.4 | 16.7 | 6 | 4 | 0.2575 |
| | 4.4 | 11.3 | 15.7 | 6 | 2 | 0.2803 |
| | 5.1 | 16.1 | 21.2 | 6 | 2 | 0.2406 |
| | 4.2 | 17.2 | 21.4 | 6 | 3 | 0.1963 |
| | 2.7 | 12.1 | 14.8 | 2 | 5 | 0.1824 |
| | 6.2 | 19.1 | 25.3 | 3 | 2 | 0.2451 |
| | 6.0 | 19.2 | 25.2 | 2 | 3 | 0.2381 |
| | 3.4 | 9.8 | 13.2 | 2 | 4 | 0.2576 |
| | 2.9 | 12.1 | 15.0 | 6 | 3 | 0.1933 |
| | 5.9 | 18.1 | 24.0 | 8 | 2 | 0.2458 |
| | 6.1 | 13.1 | 19.2 | 7 | 2 | 0.3177 |
| | 4.4 | 14.1 | 18.5 | 9 | 2 | 0.2378 |
| | 5.5 | 10.0 | 15.5 | 11 | 3 | 0.3548 |

| | Root Length (cm) | Shoot Length (cm) | Total Length (cm) | # of Flowers | # of Seed Pods | Root-length index |
|---|---|---|---|---|---|---|
| | 5.5 | 9.0 | 14.5 | 7 | 4 | 0.3793 |
| | 4.3 | 10.1 | 14.4 | 2 | 2 | 0.2986 |
| | 3.6 | 18.1 | 21.7 | 2 | 2 | 0.1659 |
| | 6.2 | 8.1 | 14.3 | 0 | 3 | 0.4336 |
| | 3.2 | 5.4 | 8.6 | 0 | 2 | 0.3721 |
| | 4.4 | 5.5 | 9.9 | 1 | 1 | 0.4444 |
| | 6.9 | 7.9 | 14.8 | 3 | 2 | 0.4662 |
| | 3.6 | 10.1 | 13.7 | 3 | 2 | 0.2628 |
| **Total** | | | | **141** | **82** | |
| **Average** | | | | **4.7000** | **2.7333** | **0.2701** |
| **std error** | | | | **0.5696** | **0.1656** | **0.0157** |

| **Municipal Biosolids** | Root | Shoot | Total | Flowers | Seed Pod # | Root-length index |
|---|---|---|---|---|---|---|
| | 16.3 | 24.2 | 40.5 | 7 | 4 | 0.4025 |
| | 9.3 | 45.3 | 54.6 | 11 | 4 | 0.1703 |
| | 17.1 | 33.3 | 50.4 | 6 | 3 | 0.3393 |
| | 18.5 | 45.1 | 63.6 | 7 | 2 | 0.2909 |
| | 9.9 | 43.4 | 53.3 | 11 | 5 | 0.1857 |
| | 13.2 | 49.1 | 62.3 | 12 | 3 | 0.2119 |
| | 9.2 | 47.1 | 56.3 | 14 | 4 | 0.1634 |
| | 22.3 | 65.4 | 87.7 | 6 | 2 | 0.2543 |
| | 8.4 | 28.5 | 36.9 | 4 | 2 | 0.2276 |
| | 6.4 | 35.1 | 41.5 | 4 | 3 | 0.1542 |
| | 8.9 | 49.1 | 58.0 | 5 | 2 | 0.1534 |
| | 5.1 | 49.8 | 54.9 | 2 | 2 | 0.0929 |
| | 9.4 | 48.6 | 58.0 | 14 | 6 | 0.1621 |
| | 7.3 | 44.3 | 51.6 | 4 | 4 | 0.1415 |
| | 7.1 | 44.1 | 51.2 | 6 | 3 | 0.1387 |
| | 12.1 | 36.1 | 48.2 | 7 | 4 | 0.2510 |
| | 11.1 | 33.5 | 44.6 | 6 | 2 | 0.2489 |
| | 6.1 | 29.1 | 35.2 | 5 | 3 | 0.1733 |
| | 8.2 | 34.1 | 42.3 | 5 | 3 | 0.1939 |
| | 13.4 | 30.9 | 44.3 | 9 | 5 | 0.3025 |
| | 9.3 | 36.1 | 45.4 | 3 | 4 | 0.2048 |
| | 7.9 | 37.0 | 44.9 | 3 | 3 | 0.1759 |
| | 11.2 | 28.7 | 39.9 | 4 | 2 | 0.2807 |
| | 7.1 | 19.1 | 26.2 | 5 | 3 | 0.2710 |
| | 6.8 | 35.1 | 41.9 | 7 | 4 | 0.1623 |
| | 6.2 | 44.0 | 50.2 | 12 | 5 | 0.1235 |
| | 8.3 | 36.1 | 44.4 | 5 | 2 | 0.1869 |
| | 5.2 | 25.1 | 30.3 | 3 | 2 | 0.1716 |
| **Total** | | | | **187** | **91** | |
| **Average** | | | | **6.6786** | **3.2500** | **0.2084** |
| **std error** | | | | **0.6446** | **0.2159** | **0.0133** |

## *Appendix S.* Phaseolus vulgaris *Land-Application Laboratory Bioassay Data*

| | Root Length (cm) | Shoot Length (cm) | Total Length (cm) | # of Flowers | # of Seed Pods | Root-length index |
|---|---|---|---|---|---|---|
| **Reference** | 17.9 | 66.8 | 74.1 | 2 | 1 | 0.2113 |
| | 20.2 | 46.5 | 57.8 | 1 | 0 | 0.3028 |
| | 19.8 | 59.3 | 74.4 | 2 | 0 | 0.2503 |
| | 18.2 | 46.1 | 51.0 | 1 | 0 | 0.2830 |
| | 13.4 | 37.4 | 42.0 | 1 | 2 | 0.2638 |
| | 22.1 | 63.1 | 74.3 | 1 | 1 | 0.2594 |
| | 21.6 | 62.4 | 72.9 | 2 | 1 | 0.2571 |
| | 12.5 | 39.9 | 45.7 | 1 | 1 | 0.2385 |
| | 14.5 | 42.3 | 50.1 | 2 | 1 | 0.2553 |
| | 16.3 | 48.3 | 56.7 | 1 | 1 | 0.2523 |
| | 17.8 | 55.4 | 65.6 | 1 | 2 | 0.2432 |
| | 18.8 | 51.0 | 63.1 | 2 | 1 | 0.2693 |
| | 11.3 | 55.1 | 61.9 | 1 | 1 | 0.1702 |
| | 23.5 | 102.5 | 120.7 | 1 | 0 | 0.1865 |
| | 20.1 | 63.5 | 78.3 | 1 | 2 | 0.2404 |
| **total** | | | | **20** | **14** | |
| **average** | | | | **1.4286** | **1.0000** | **0.2456** |
| **std error** | | | | **0.1260** | **0.1817** | **0.0089** |
| **Pulp Mill Biosolids** | 9.2 | 50.1 | 59.3 | 1 | 1 | 0.1551 |
| | 9.8 | 55.1 | 64.9 | 2 | 2 | 0.1510 |
| | 15.0 | 65.2 | 80.2 | 2 | 1 | 0.1870 |
| | 4.0 | 60.3 | 64.3 | 1 | 1 | 0.0622 |
| | 5.5 | 55.3 | 60.8 | 1 | 0 | 0.0905 |
| | 13.5 | 45.2 | 58.7 | 2 | 2 | 0.2300 |
| | 10.2 | 66.2 | 76.4 | 1 | 1 | 0.1335 |
| | 6.4 | 65.2 | 71.6 | 1 | 1 | 0.0894 |
| | 6.4 | 32.1 | 38.5 | 2 | 1 | 0.1662 |
| | 7.2 | 45.1 | 52.3 | 1 | 2 | 0.1377 |
| | 14.3 | 64.2 | 78.5 | 2 | 0 | 0.1822 |
| | 6.3 | 49.1 | 55.4 | 2 | 1 | 0.1137 |
| | 10.2 | 48.7 | 58.9 | 1 | 1 | 0.1732 |
| | 12.2 | 65.8 | 78.0 | 1 | 1 | 0.1564 |
| | 12.1 | 60.5 | 72.6 | 2 | 1 | 0.1667 |
| **total** | | | | **22** | **16** | |
| **average** | | | | **1.4667** | **1.0667** | **0.1468** |
| **std error** | | | | **0.1333** | **0.1533** | **0.0112** |
| **Municipal Biosolids** | 6.9 | 54.7 | 61.6 | 1 | 0 | 0.1120 |
| | 9.6 | 51.9 | 61.5 | 2 | 0 | 0.1561 |
| | 10.3 | 52.8 | 63.1 | 1 | 1 | 0.1632 |
| | 4.3 | 62.6 | 66.9 | 1 | 1 | 0.0643 |
| | 9.4 | 76.3 | 85.7 | 1 | 2 | 0.1097 |
| | 12.2 | 43.2 | 55.4 | 2 | 0 | 0.2202 |

| | Root Length (cm) | Shoot Length (cm) | Total Length (cm) | # of Flowers | # of Seed Pods | Root-length index |
|---|---|---|---|---|---|---|
| | 16.1 | 62.6 | 78.7 | 1 | 1 | 0.2046 |
| | 2.2 | 27.5 | 29.7 | 2 | 1 | 0.0740 |
| | 14.2 | 42.0 | 56.2 | 1 | 2 | 0.2527 |
| | 6.6 | 64.4 | 71.0 | 1 | 1 | 0.0930 |
| **total** | | | | **13** | **9** | |
| **average** | | | | **1.3000** | **0.9000** | **0.1460** |
| **std error** | | | | **0.1528** | **0.2333** | **0.0205** |

*Appendix T.* **Lumbricus terrestris** *Land-Application Laboratory Bioassay Data*

| | Day 1 | Day 7 | Day 28 | Day 56 | Day 84 | Day 112 | Day 140 |
|---|---|---|---|---|---|---|---|
| **Reference Soil** | | | | | | | |
| **Bucket # 1** | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| **Bucket # 2** | 8 | 8 | 8 | 8 | 7 | 7 | 7 |
| **Bucket # 3** | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| **Mean** | **8.00** | **8.00** | **8.00** | **8.00** | **7.67** | **7.67** | **7.67** |
| **Standard Error** | **0.00** | **0.00** | **0.00** | **0.00** | **0.33** | **0.33** | **0.33** |
| **Pulp Mill Biosolids Amended soil** | | | | | | | |
| **Bucket # 1** | 8 | 8 | 8 | 8 | 8 | 7 | 7 |
| **Bucket # 2** | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| **Bucket # 3** | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| **Mean** | **8.00** | **8.00** | **8.00** | **8.00** | **8.00** | **7.67** | **7.67** |
| **Standard Error** | **0.00** | **0.00** | **0.00** | **0.00** | **0.00** | **0.33** | **0.33** |
| **Municipal Biosolids Amended soil** | | | | | | | |
| **Bucket # 1** | 8 | 8 | 8 | 8 | 8 | 8 | 7 |
| **Bucket # 2** | 8 | 8 | 7 | 7 | 7 | 6 | 6 |
| **Bucket # 3** | 8 | 8 | 8 | 8 | 7 | 7 | 7 |
| **Mean** | **8.00** | **8.00** | **7.67** | **7.67** | **7.33** | **7.00** | **6.67** |
| **Standard Error** | **0.00** | **0.00** | **0.33** | **0.33** | **0.33** | **0.58** | **0.33** |