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### **Solid waste management: environmental evaluation of land-applied pulp mill biosolids: monitoring the fate of sludge constituents in forest ecosystems and assessing the impact using ecologically-relevant organisms: executive summary**

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Steven Liss, and Trevor Stuthridge**

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# **Sustainable Forest Management Network**

## **Solid Waste Management**

### **Environmental Evaluation of Land-Applied Pulp Mill Biosolids: Monitoring the Fate of Sludge Constituents in Forest Ecosystems and Assessing the Impact using Ecologically-Relevant Organisms**

#### **Executive Summary**

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## **RESEARCH QUESTIONS AND OBJECTIVES**

In recent years, a movement towards minimizing pulp mill discharges into surface receiving waters has resulted in an increase in biosolids or sludge produced from the pulping process. Current technologies include the landfilling or incineration of these solid wastes, but increasingly high costs have led to an evaluation of viable and cost-effective alternatives. The Canadian pulp and paper industry generates 1.7 million dry tones of mill biosolids every year (OFIA 1996; Reid 1997) and the land-application of this material to forest soil is considered a highly-effective alternative to land-filling (Henry & Cole 1997; Bates 1999). Organic waste components are potentially degraded by indigenous microbial populations and nutrient constituents are utilized by the region's vegetation (Kays *et al.* 1997). Many studies have been conducted attesting to the benefits of land-disposal of sludges (Henry *et al.* 1993; McDonald *et al.* 1994; Beyer *et al.* 1997; Camberato *et al.* 1997; Macyk 1999) suggesting increased tree growth, increased crop nutrition, and improved soil physical conditions. However, while the high nutrient and carbon content of the biosolids might offer significant benefits to soil productivity and tree growth, biosolids application systems which exceed the retention and assimilation capacity of the soil and associated vegetation will have impacts on receiving waters and vegetative productivity. Research conducted by Stanosz and Trobaugh (1996) suggest that forest productivity can be threatened where sludge is applied as a result of nutritionally-intensified effects of moisture stress. Also, while soil has a very limited function as a transport medium, it can be an important source for contamination of groundwater should sludge constituents prove to have polluting properties (Lokke & van Gestel 1998).

Most research on land application of municipal or pulp and paper biosolids has focussed on nutrient cycling and the ability of vegetative cover or plantation forests to utilize the added nutrients. However, the long-term environmental impacts of these disposal practices are unknown and there are potential problems which must be addressed if sustainable forest management practices are to continue with the land-application of biosolids. There is a paucity of information on the fate and impact of the biosolid constituents themselves, both to terrestrial organisms native to forest biomes and to aquatic organisms when biosolids runoff impacts surrounding receiving waters. Surface receiving waters and groundwater quality may also be affected by high nutrient level leachate from land-applied material (Ferrari *et al.* 1999). Certainly, the capacity of a forest ecosystem to process biosolids without causing adverse environmental impact cannot be predicted until a holistic environmental evaluation of the ecosystem receiving the biosolids has been conducted. Thus, this study investigated the potential impact that land application of biosolids may have on selected terrestrial and aquatic organisms.

The original study had hoped to address three main concerns. These included 1) determining the acute and chronic impacts of applied biosolids on soil ecosystems using ecologically-relevant bioindicators and whole organism responses, 2) examining soil and sludge microbial community structure prior to, and upon application of the biosolids with regards to changes in species composition and biodegradative capabilities of the populations, and 3) evaluating the physicochemical behaviour of the biosolids constituents, including their mobility to surface receiving waters and leachability into ground water.

As the research progressed, it became clear that a holistic biological assessment needed to include a more extensive suite of organisms and test protocols than originally proposed. While the latter two Objectives were important, the first Objective had highest priority and became the focus for much of the study. It was broken down into two components. These included the multi-organism assessment of the land-applied biosolids (Objective 1A) and a series of experiments examining the induction of mixed function oxygenase (MFO) activity in the livers of juvenile rainbow trout (Objective 1B). While a microbial ecological examination was conducted to address Objective 2, the results remain preliminary, and Objective 3 was never achieved. This was noted in various progress reports and remains an important issue for future study. Lastly, it is tempting to report on all of the findings from this study, but space precludes this. Further details thus can be found in Bostan *et al.* (2003), McCarthy *et al.* (2003a, 2003b), and Spearin (2003).

## **PROGRESS and KEY FINDINGS**

### **OBJECTIVE 1A (McCarthy *et al.*):**

When the study was first proposed, the organisms suggested as ecotoxicological assessors of impact included the earthworm *Lumbricus terrestris* and the plant *Lactuca sativa* for the terrestrial component. Details of the latter bioassays are included in Alvarez (2000) and will not be discussed in this report. Impact from constituents desorbing from the land-applied biosolids and contributing to runoff into surface receiving waters was initially assessed using the zooplankter *Daphnia magna*. As the study progressed, more bioassay organisms were added. Due to the unusual nature of this study that included an extensive variety of food-web biota, greater space than usual has been allocated below as their relevance to this study is detailed. The experimental methodologies are not included here and can be found elsewhere (Bostan *et al.* 2003; McCarthy *et al.* 2003a, 2003b, and Spearin 2003).

### **Biosolids Collection**

The biosolids for this study came from a thermomechanical newsprint mill in western Canada where hardwood and softwood chips are pressurized into high-quality pulp. Total retention time of effluent is 6 days, and the secondary clarifier is an aeration lagoon. While the biosolids constitute a mixture of primary (70%) and secondary material (30%), the bulk originates from the primary clarifier. Under normal conditions, the biosolids are land-applied within 12 hours of being removed from the treatment system. Biosolids arrived in the laboratory within 48 hours of being collected and were stored up to 2 weeks at 4°C before being incorporated into bioassays.

### **Bioassay Organisms**

*Lumbricus terrestris* or earthworms are globally-distributed annelids, spending most of their time beneath the soil surface but emerging to feed on organic detritus. It has a life-cycle of approximately six years and becomes sexually mature within its first year (Edwards & Bohlen, 1996). Earthworms are thought to be good indicators of soil quality and while an environmentally-relevant bioassay organism, 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 (Berry & Jordan, 2001).

*Brassica rapa* - the genus *Brassica* is comprised of various species of plants with economic significance, such as broccoli and cabbage (Musgrave, 2000). A plant life-cycle test has been developed using rapid-cycling *Brassica rapa*, a species morphotype that completes its life cycle in 35 to 45 days (Kloepper-Sams *et al.* 1996; Musgrave 2000). The short life cycle of rapid-cycling *Brassica* plants provides an ideal opportunity for detecting effects at specific developmental stages, including impacts to the subsequent generation.

*Phaseolus vulgaris* or the common bean is the most widely-cultivated type of bean in temperate regions of the world (Fitter 2002). A 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. Two other vegetables used in the field in this study were *Cucurbita moschata* (squash) and *Raphanus sativus* (radish) respectively. A pretest on these seeds showed good germination capacity (over 90% in forest soil under artificial conditions). It is also worthwhile mentioning that the use of higher plants in environmental risk assessment has only recently gained the attention of the scientific community (Gong *et al.* 2001).

*Gambusia affinis* or mosquitofish have been used extensively in monitoring bodies of water for toxicity impact and are considered to be as sensitive as invertebrates to many contaminants (Reynoldson & Day 1993). *Gambusia affinis* belong to the Poeciliidae family, which are now native to North America, having been introduced two centuries ago from Europe (Krumholz 1948). They are of practical value in the current study due to their small size (3-4cm), ease of culturing, and relative insensitivity to changes in the physicochemical parameters of water.

*Daphnia magna* are small freshwater crustaceans that are typically found among weeds of ponds and lakes and are widely distributed throughout the northern hemisphere (USEPA 2002). *D. magna* are filter feeders and are frequently employed for ecotoxicological testing of water quality using various standard protocols (Environment Canada 1990; USEPA 2002). The ease of culturing and short life-cycle of *D. magna* make them an ideal bioassay organism.

*Hyalella azteca* are small freshwater amphipods that inhabit permanent lakes, ponds, and streams in regions of North and South America and are detritivores that burrow into the sediment and selectively feed on bacteria, algae, and organic debris (USEPA 2000). Reproduction occurs only during favourable conditions and due to their behaviour and feeding habits, *H. azteca* are commonly-used test organisms for contaminated sediment. Many organizations have developed standard test methods using them (USEPA 2000).

*Elodea densa* or the common waterweed has occupied the littoral zones of North American freshwaters for the past century (Carter & Sytsma 2001). It is a fast-growing dioecious vascular plant with a system of roots and shoots with leaves that provide food and habitat for invertebrates and small fish. Since the roots bury into the benthic zone and the leaves are submerged, *E. densa* is able to take up nutrients through these systems, which allows for ecotoxicological assessment of both the water column and sediment (Eugelink 1998). Ecotoxicological endpoints include overall length of plant, length of roots and shoots, and chlorophyll measurements.

*Lemna minor* is an emergent aquatic macrophyte that is among the world's smallest flowering plants and is free-floating, thereby obtaining nutrients from the surrounding water (Linton & Goulder 1998). Under ideal, nutrient-rich conditions, a single plant will reproduce approximately every three days. *L. minor* serves as food for various waterfowl and fish species and in terms of phytotoxicity testing, it has become a commonly-used aquatic plant (USEPA 1996). 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. Frond number is the most common endpoint and proves to be a good indicator of growth (Linton & Goulder 1998).

*Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) is a freshwater, unicellular green algae, obtaining its nutrients from the water in which it lives. Several test methods have been standardized for this organism both in North America and internationally (Lewis 1995). A study conducted by Bailey & Young (1997) compared the efficacy of various toxicity tests to evaluate the effects of pulp and paper mill effluents on fish and fish habitat. The 72-hour *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.

### **Soil Run-off Collection (Laboratory)**

Ramps were used to simulate biosolids run-off into adjacent receiving waters. The ramps were 90cm x 25cm x 15cm and were inclined to simulate a 15% slope. The Ontario Ministry of Environment (OMOE) 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 was chosen to simulate a worst-case scenario in terms of run-off risk. To emulate land-application, a predetermined amount of biosolids was hand-spread atop the soil in one ramp based on a typical 20 tonne/hectare/year dry weight application rate (USEPA 1997). The resulting run-off was collected in 10L aquaria placed at the bottom of the ramp and used in bioassays. Another ramp was set up as above but contained reference soil only.

### **Laboratory Mixed-Tank Bioassays**

In order to evaluate multi-species synergism, selected aquatic organisms used both in laboratory experiments and in the field campaign were assembled in mix tanks and included *Gambusia affinis*, *Daphnia magna*, *Hyalella azteca*, *Lemna minor* plants, and *Pseudokirchneriella subcapitatum*. Due to its voracity, the fish was caged in a 1mm x 1mm mesh basket, which prevented them from preying on the invertebrate population in the tank. The size of the mesh allowed some of invertebrates to enter the cage, thus completing the food chain inside the aquarium. After a three-week acclimation period for the mix populations, runoff was added to the tanks. In two replicate tanks, 10% volume

ratio of runoff from biosolids-amended soil was added. In the other two replicate tanks, 10% runoff from reference soil was added.

### **Laboratory Monoculture Bioassays**

The *Phaseolus vulgaris* and *Brassica rapa* bioassays were conducted in plastic planting containers with either non-amended (reference) soil or pulp mill biosolids-amended soil mixed in a 1-to-1 ratio with reference soil. The endpoint observations included the number of germinated plants, flower development, seed pod development, root length, and overall survival at the end of 8 weeks of the F<sub>0</sub> plants. The seed pods were collected, sorted, and allowed to desiccate for two weeks. They were then planted and an F<sub>1</sub> germination assessment was made.

The *Elodea densa* bioassay was conducted by establishing plants in 18L holding-capacity plastic food-grade containers. Reference soil was added to each vessel, with sand/gravel spread across the soil in an attempt to simulate a river bed adjacent to a biosolids-amended field site. Subsequently, dechlorinated water was added to each container and substrate was allowed to settle for two days, after which point, the plants were added. The lengths of the plants, shoots, and roots were measured prior to adding the *E. densa* and then the experiment was terminated 7 weeks later and endpoint observations included lengths and number of plants, shoots, and roots, and chlorophyll *a* and *b* determinations were made.

### **Field Bioassays**

In July of 2002, field experiments were conducted to study the effects of land-applying biosolids on forest ecosystems originating from the western Alberta pulp and paper mill that had been sending biosolids to our laboratory for testing. *Lumbricus terrestris*, *Brassica rapa*, *Phaseolus vulgaris*, *Cucurbita moschata*, and *Raphanus sativus* were used for the terrestrial bioassays while *Daphnia magna*, *Hyalella azteca*, and *Lemna minor* were employed in aquatic bioassays. The terrestrial bioassays were carried out at the field site and the aquatic tests were conducted at the pulp mill using biosolids runoff collected from the field site. The plant bioassays were run at both the field site (planted directly in the ground) and in a laboratory at the pulp-mill (in plant trays and under artificial, fluorescent grow-lights).

The site chosen for field experiments 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 40 meters away. Despite an extensive drought, the study area received 45-60mm precipitation less than a week before field experiments were launched (Alberta Ministry of Agriculture, Food and Rural Development, 2002). The topography of the forest block 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.

## **Observations**

### **1. Laboratory Mixed-Tank Bioassays**

Figure 1 indicates the results obtained from the mixed-tank bioassays. No statistically significant differences were noted between the pulp mill biosolids-amended runoff and the reference soil, both after 7 days and after 7 months. *Daphnia* survival was limited throughout the bioassays but it was concluded that they managed to squeeze into the *Gambusia* cage and were subsequently eaten. *Pseudokirchneriella subcapitatum* were part of the mixed assemblage but a measurable population of cells disappeared within 48 hours. It was speculated that the *Daphnia* and *Hyalella* consumed most of them during that time period. A subsequent monoculture experiment exposing the phytoplankton to pulp mill biosolids runoff and reference soil runoff indicated no statistically significant differences between the two treatments.

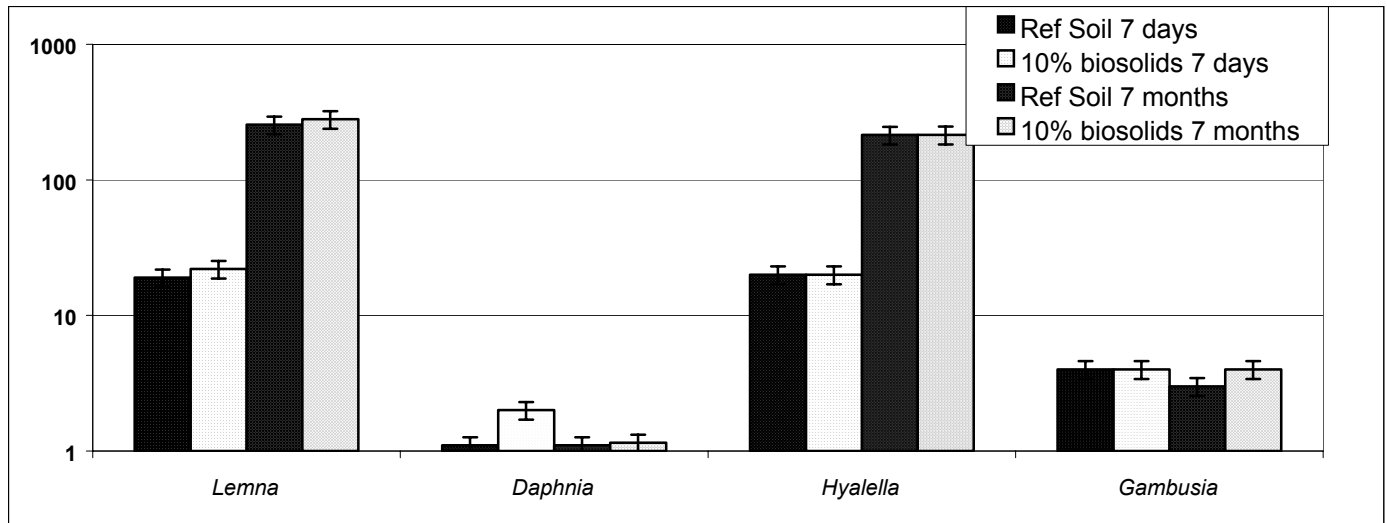


Figure 1: Mixed population of aquatic organisms exposed to laboratory-collected runoff (pulp mill biosolids and reference soil) after 7 days and 7 months.

## 2. Laboratory Monoculture Bioassays

The results from six different growth parameters for *Phaseolus vulgaris* grown in pulp mill biosolids-amended soil compared to reference soil are shown in Figure 2. Statistical analyses indicated no significant differences between the treatments with respect to all endpoints except for root-length index. This would be considered a significant impact if survival of the overall plant had been affected, but this is not apparent. Also, the increased germination rate of the second-generation seeds compared to reference treatment is noteworthy. Similar results were noted for *Brassica rapa*.

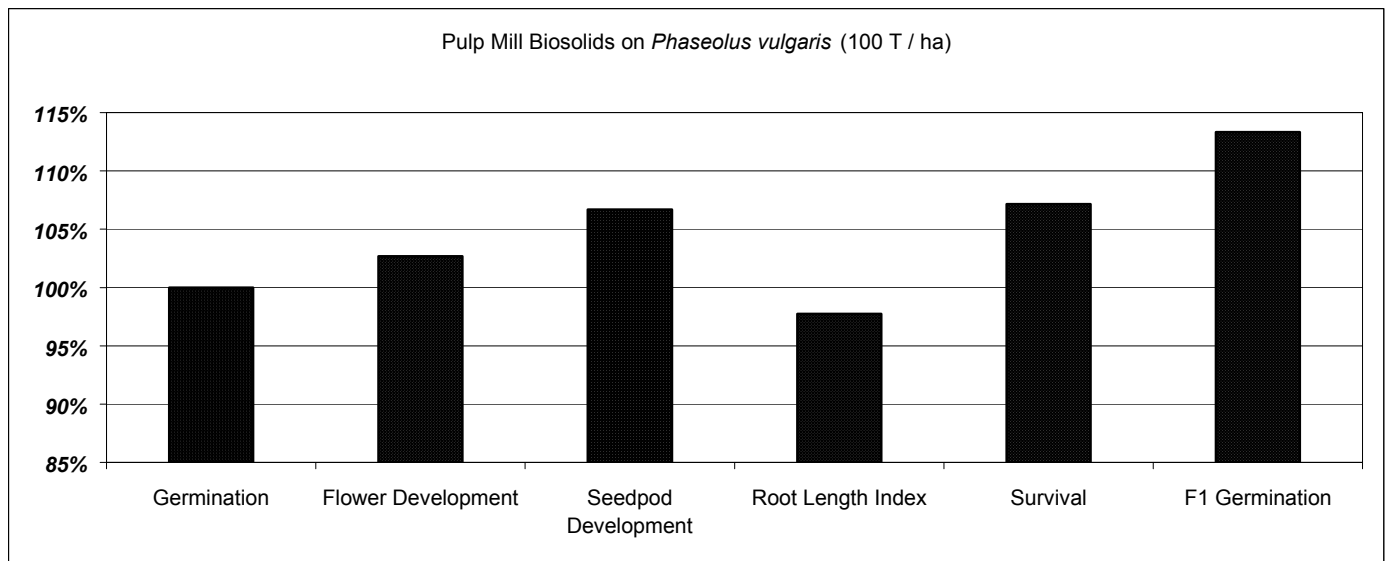
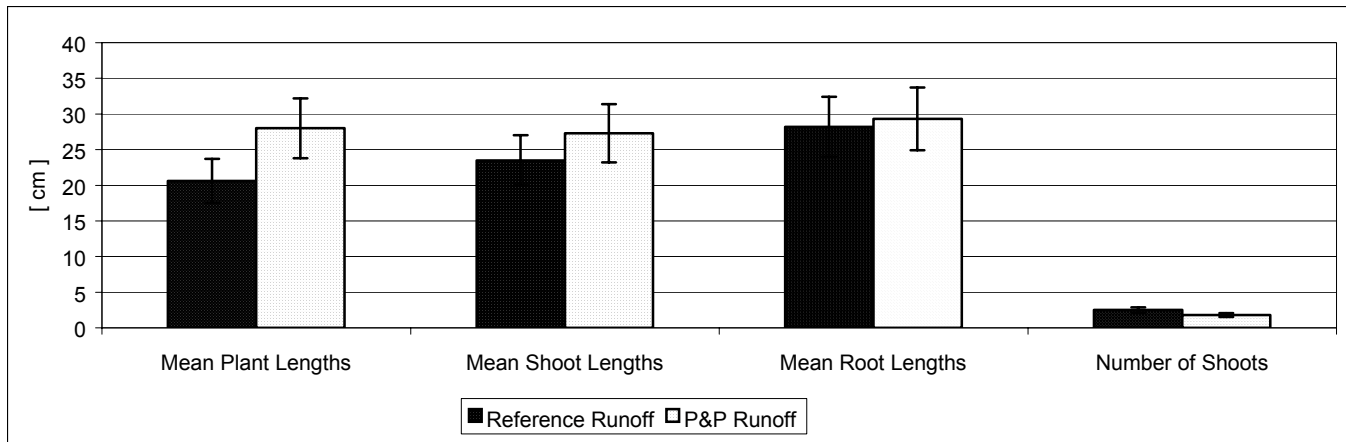


Figure 2: A comparison of six growth parameters for *Phaseolus vulgaris* grown in pulp mill biosolids-amended soil compared to reference soil (100%).

The results of bioassays conducted using *Elodea densa* are presented in Figure 3. The mean plant length is slightly greater in pulp mill biosolids-amended soil runoff. The rest of the parameters were, from a statistical point of view, unaffected by the addition of pulp mill biosolids to the containers with *E. densa*. The significant difference seen in mean plant lengths may be due to the potentially greater nutrient levels in pulp mill biosolids, but this is speculative and further experiments must be conducted, accompanied by chemical analysis.

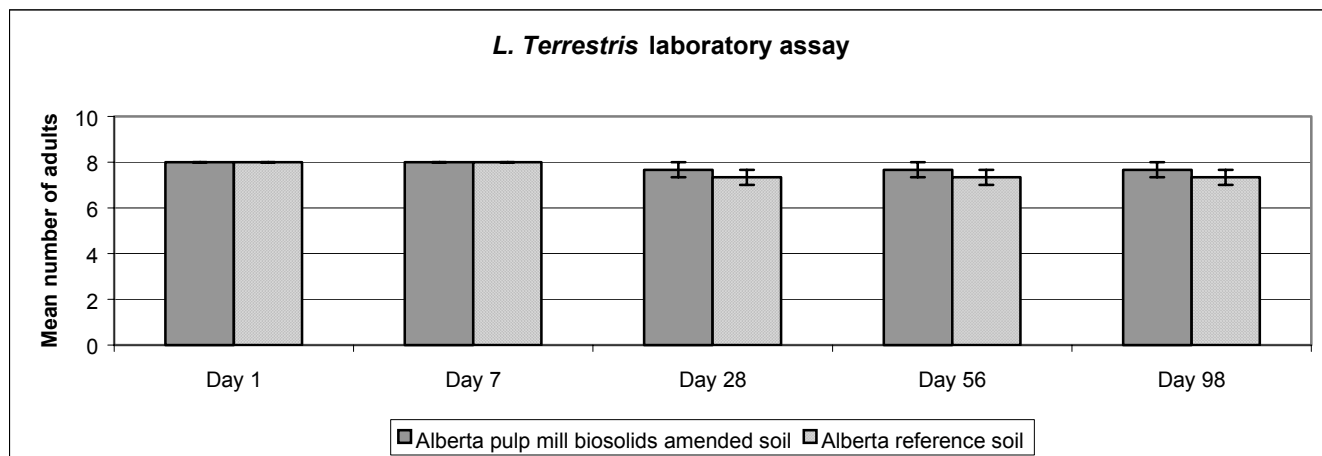


**Figure 3: Morphological measurements of the submerged macrophyte *Elodea densa* exposed to pulp mill biosolids-amended soil and reference soil.**

When measuring the concentrations of chlorophyll *a* and *b* in *E. densa* from the two treatments, they were similar. The results indicate that addition of pulp mill biosolids does not lead to significant differences in chlorophyll content.

### 3. Field Bioassays

Figure 4 indicates results from the field setup for the earthworm experiment which incorporated pulp mill biosolids-amended soil from an Alberta forest and the adjacent reference site. After 98 days, there were no significant statistical differences in survival of the organisms between the two treatments. Also, upon inspection, earthworms from all containers appeared healthy (ie. plump, moist, coated in mucus), and responded well to physical stimuli.

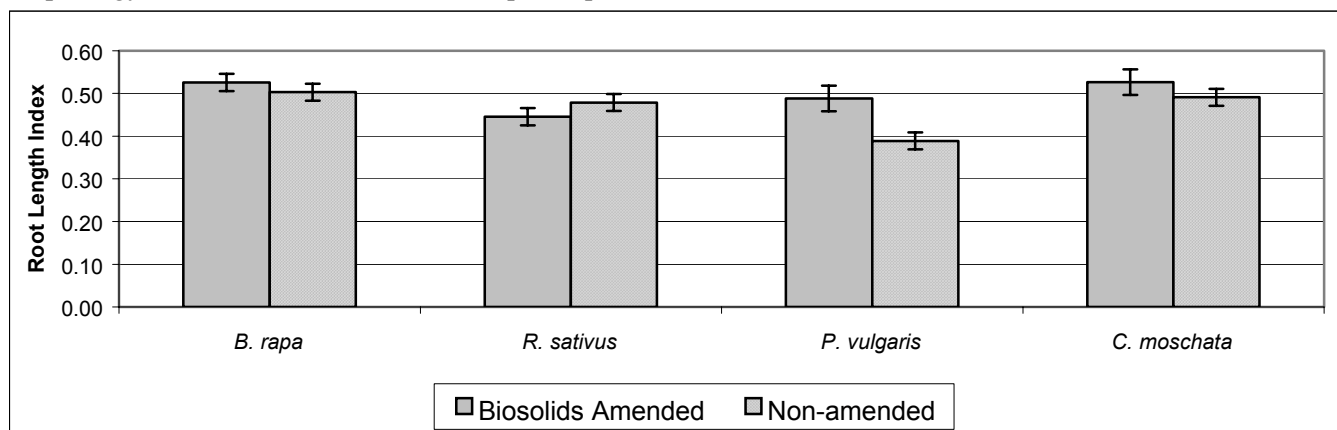


**Figure 4: Mean survival of earthworms after a 98-day exposure to pulp mill biosolids-amended soil from an Alberta forest and the reference soil adjacent to the treatment plot.**

*Brassica rapa*, *Raphanus sativus*, *Phaseolus vulgaris*, and *Cucurbita moschata* were grown in field plots containing either unamended forest soil (reference treatment) or pulp mill biosolids-amended soil adjacent to the former site. In the field plots, as well as comparative bioassays conducted in the mill, percent germination of *B. rapa* in biosolids-amended soil was significantly lower than in the reference soil. For all other plant species, there were no significant statistical differences between treatments. It is speculated that the tiny size of the *B. rapa* seed lead to physical limitations in pushing through the compacted biosolids-applied soil. The germination of the other plant species leads us to conclude that biosolids-amended soil is not detrimental to the germination of a variety of vegetable seeds.



Figure 5 shows the mean root lengths indices for the four plant species. The only plant to show statistically significant differences was the *P. vulgaris*, where the mean root length index was greater in the biosolids-amended field plot than in the reference site. While we are uncertain of the reason for this difference, it is clear that major differences in root morphology are not obvious with the other plant species.



**Figure 5: Mean root length indices (+/-SE) for plants grown at the biosolids-amended and reference field sites in Alberta.**

Lastly, the field runoff experiments were uneventful. *Daphnia magna*, *Hyalella azteca*, and *Lemna minor* indicated no significant statistical differences in survival and reproduction between treatments.

**OBJECTIVE 1B (Hodson et al.):**

Research was conducted to determine whether biosolids contain or release mixed function oxygenase (MFO)-inducing compounds upon land-application. Water-extracted leachate samples were tested on juvenile (eyed-egg to swim-up stages) rainbow trout in 96-hour assays. Results indicated acute toxicity to the fish over a four-day period when the biosolids were added to the water in concentrations as little as 1g/L. However, toxicity was reduced when suspensions of biosolids were replaced daily. The 96-h LC<sub>50</sub> (lethal concentration to 50% of the population) for daily biosolids renewal was 2.12g/L, while the LC<sub>50</sub> for four-day non-renewal testing was 0.875 g/L. This suggests that toxicity was generated by component(s) leached slowly from the biosolids. Ammonia was immediately suspected, but measured concentrations of the un-ionized form were 1/10<sup>th</sup> lethal concentrations. Thus, while ammonia may have contributed some toxic impact, this compound alone could not account for the fish mortality.

Further experiments using biosolids containing primary clarifier and secondary treatment waste showed an age-dependent toxicity response when bioassayed with rainbow trout. Fresh biosolids (age less than 1 week) produced an LC<sub>50</sub> of 0.56g wet weight biosolids/L while aged biosolids (44 weeks) had an LC<sub>50</sub> of greater than 15.6g ww/L. Mortality did not correlate with amount of suspended biosolids in the bioassay, suggesting that toxicity was not due solely to physical interactions. There were no significant increases in MFO induction or changes in the liver somatic index at biosolid concentrations up to and including those where mortality occurred. The mechanism of toxicity has yet to be elucidated. However, primary symptoms such as lethargy, loss of equilibrium, and full recovery following removal to clean water, indicate narcosis.

Fresh biosolids were dried and extracted with methanol or water. Trout exposed to water extracts equivalent to up to 5g ww/L displayed no changes in MFO induction or toxicity. Trout exposed to methanol extracts equivalent to 1.6 to 5g ww/L showed concentration-dependant increases in both MFO induction and mortality, suggesting hydrophobicity of the responsible compounds. Further fractionation using liquid-liquid extraction (LLE) with cyclohexane was performed, and the subsequent fractions were tested at a 1.32g ww/L biosolids equivalent. The toxicity of the methanol fraction following LLE was greatly exacerbated, and complete mortality occurred within 15 minutes of

initial exposure. No toxicity or MFO induction was observed from the compounds extracted into cyclohexane. The raw (non-LLE extracted) methanol extract caused 3.5x greater MFO induction than occurred in the water control. The repeat of this experiment with extract solids yielded the same results, with the exception of the complete absence of mortality. Therefore, there appears to be interactive effects between the compounds extracted into the cyclohexane and methanol fractions with regard to MFO induction and toxicity. The toxicity from these biosolids may be from high resin acid concentrations (83mg/g dw) although further testing must be done to prove this hypothesis. It is suggested that impact was due to chemical, as opposed to physical, interactions and that the toxic constituents are at least moderately water-soluble.

## **OBJECTIVE 2 (Fulthorpe *et al.*):**

### **i) Microbial community phylogenic analysis**

Techniques were explored throughout the study to determine the best way to examine distinct microbial consortia in the biosolids and forest soils. One such methodology had rRNA being extracted from biosolids and reference soil, reverse-transcribed into ribosomal DNA (rDNA) and amplified by polymerase chain reaction. rDNA was then run on polyacrilimide gels with the resulting profiles potentially revealing distinct microbial communities. Another technique used DGGE (denaturing gradient gel electrophoresis) which is a method by which fragments of DNA of the same length but different sequence can be resolved electrophoretically (Muyzer *et al.* 1993). This technique allowed the separation of a heterogeneous mixture of PCR amplified genes on a polyacrilimide gel. Individual bands may be excised, reamplified and sequenced, or challenged with a range of oligonucleotide probes to give an indication of the composition and diversity of the microbial population (Head *et al.* 1998).

Four kinds of treatments were set up using two types of soil: (i) commercial potting-soil and (ii) forest soil. The four treatments were: (i) 10% biosolids on reference soil (ii) 50% biosolids on reference soil (iii) 100% biosolids and (iv) reference soil (control soil without any application of biosolids). The soils were incubated at room temperature (approximately 22°C) for 12 weeks and samples were taken at 0, 3, 6 and 12 weeks of incubation. The extraction of community DNA from potting soil samples posed a few challenges, mainly due to the extremely high amount of coloured, presumably humic, substances in this soil. Total community DNA was extracted from potting soil samples using various methods (chemical extraction (Zhou *et al.* 1996), and bead-beating (Miller *et al.* 1999) and was further purified by passing through two sets of S-300 HR MicroSpin columns (Amersham-Pharmacia). However, there was an inability to remove all of the brown colour of humic compounds and DNA could not be amplified by polymerase chain reaction (PCR). Thus, Mo Bio Ultra Clean Soil DNA kit (Mo Bio Laboratories Inc., California) was tested. This reduced the DNA extraction time from one day to one hour and also gave clean, amplifiable DNA. This method was chosen for both forest soil and potting soil samples. After six weeks incubation and DGGE analysis, the control soils (both potting soil and forest soil) were found to be only 60-70% similar to those soils receiving 10% or 50% Biosolids. After twelve weeks incubation, all samples seem to converge. Soils from the same source are 90% or more similar to each other, regardless of the amendment, and the potting soil and forest soils have also become more similar to each other overall.

### **ii) Viability of microorganisms in biosolids**

A preliminary assessment of the viability of microorganisms in the biosolids compared to reference soils has been conducted. Firstly, the Contact Slide method of Cholodny and Rossi (Rossi *et al.* 1936) was employed, which is a simple technique for qualitatively assessing the spatial relationships between microorganisms. With nutrient amendments such as glucose and nitrogen, rapid proliferation of growth can be seen within a short period of time. Glass microscope slides were buried in vessels containing various substrates and nutrient amendments. After incubation, the slides were carefully removed, stained, and examined microscopically to observe microorganisms growing and interacting in their natural environments. Gram-negative and Gram-positive rods and cocci were very apparent in both the biosolid material and the Reference soil. Also, fungal hyphae with attendant fruiting bodies and actinomycetes were present in both substrates. A preliminary quantitative assessment indicated greater growth in the biosolids than in the Reference soil.

Secondly, an experimental setup attempted to segregate culturable microorganisms in order to facilitate greater ease of enumerating the biota. Biosolids and Reference soil were individually homogenized with phosphate buffer and aliquots of the resulting liquid were added to Tryptic Soy Agar (elucidate bacteria), Glycerol Yeast Agar (actinomycetes), and Sabourand Agar (fungi). Preliminary results verified the findings of the Contact Slide experiment.

Lastly, a microbial assessment technique was used to examine the overall metabolic activity of the microorganisms in the various substrates. Replicate test vessels were set up that contained biosolids and Reference soil alone and also the two substrates mixed together in a 1:1 ratio. The theory was to observe whether metabolic activity changed in the last test (containing both biosolids and Reference soil mixed together) when compared to the two substrates alone. The Dehydrogenase Activity bioassay (Trevors 1984) examines the amount of dehydrogenase enzymes present in all living organisms. These enzymes serve to transfer electron pairs to an acceptor (ie. NAD+) during the catabolism of macromolecules. Determination of this activity can be carried out by incubating substrate with 2,3,5-triphenyltetrazolium chloride (TTC), a competitive inhibitor of NAD+. Transfer of electrons to TTC results in reduction to triphenyl formazan (TPF), an insoluble red dye which can be measured spectrophotometrically. While respiring (living) cells will eventually be poisoned, the amount of TPF production can be correlated with the metabolic activity of soil microorganisms. The results from the experiment indicated that biosolid metabolic activity was approximately 10-fold higher compared to activity observed in Reference soil alone.

### **RESEARCH CONTRIBUTIONS AND OPPORTUNITIES**

It cannot be stressed how important the results of this study, particularly from Objective 1A are on many fronts. First and foremost, it is unusual in most environmental assessments to use the variety of bioassay organisms found in this research. A complete procedures guideline for such complex experiments as the mixed tank consortia constitute one of the major objectives of our ongoing research. The encouraging preliminary results obtained in this first phase of the study indicate that further protocols must be developed. Secondly, when bioassays are traditionally conducted, the endpoints are to assess either acute or chronic impact. We conducted whole lifecycle tests to assess reproductive potential as well. Thirdly, where land-application studies have been conducted, generally, only the health of the trees has been examined. We not only looked at effects on other vegetation and soil organisms, but also considered impact to the adjacent receiving water.

Based on the results from Objective 1A, 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 and incineration. Our research did not find any obvious impact from either pulp mill land-application on selected terrestrial species or effects on aquatic organisms from run-off into receiving waters. However, results from Objective 1B did find toxicity when biosolids were assayed with juvenile rainbow trout and therefore, parts of this study should be repeated to elucidate the origin of the impact. While this study conducted preliminary experiments to examine the impact of microbial consortia from the pulp mill biosolids on indigenous forest soil microorganisms (Objective 2), it is difficult to draw major conclusions at this point and more work must be conducted in this area. The importance of assessing whether land-applied additions of biosolid microbial populations causes deleterious impact to forest microbiota cannot be stressed enough. While obvious effects may not be immediately apparent, destroying indigenous microorganisms with competitive invader species can only lead to serious forest problems in the future. Lastly, the impact to groundwater from leaching of the biosolids was not looked at and these areas deserve further investigation. Again, however, it is important to reiterate that this study is the first of its kind to utilize a suite of biota to assess terrestrial and receiving-water impact and as the necessity increases to find safe disposal methods for biosolids, this study will serve as a baseline for future research.

### **Industry and Academic Partners**

Land-application of biosolids is clearly of broad interest to the Canadian pulp and paper industry and the key players involved in this study were Alberta Newsprint Company in Whitecourt, Alberta, Tembec Pulp and Paper in

Temiscaming, Quebec, and from an international standpoint, (formerly) Fletcher Challenge Paper from New Zealand. Mill personnel were enthusiastically co-operative and helped to ensure the successful completion of the study with in-kind contributions. We visited Alberta Newsprint twice during the project and presented our preliminary results for their comments. Also, this study would not have been possible without the funding from the Sustainable Forest Management Network and financial assistance from Ryerson University. In-kind support was also provided from the University of Toronto and Queen's University.

### **Knowledge transfer**

Throughout the study, industry personnel were kept apprised of the results. Research observations were also disseminated at the following institutions, workshops, and conferences:

**Ceric, N.** – *Effects of land-applied pulp and paper solids on selected terrestrial and aquatic biota*. 4<sup>th</sup>-year thesis defence at Ryerson University. April 2001. Supervisor: L.H. McCarthy.

**Hawkins, S.A.**, R. Wehrell, L.H. McCarthy, M. van den Heuvel, P.V. Hodson. 2001. Acute toxicity and CYP1A induction capacity of pulp mill biosolids to rainbow trout. 28<sup>th</sup> Aquatic Toxicity Workshop. Sept 30-Oct. 3. Winnipeg, Manitoba.

**Bostan, V.**, E. Bandelj, L.H. McCarthy, K. Yambao, M. Partyka. 2001. Environmental evaluation of land-applied pulp mill biosolids: Monitoring fate of sludge constituents in forest ecosystems and assessing impact using ecologically-relevant organisms. Sustainable Forest Management Network Student Workshop. Oct. 18-20. Edmonton, Alberta.

**Hawkins, S.A.**, C. Hedley, L. McCarthy, M. van den Heuvel, and P.V. Hodson. 2002. Acute toxicity and CYP1A induction capacity of pulp mill biosolids to rainbow trout. 12<sup>th</sup> Annual Meeting of the Society of Environmental Toxicology and Chemistry Europe. May 12-16. Vienna, Austria.

**Hawkins, S.A.**, C. Hedley, H. Orr, M. van den Heuvel, and P.V. Hodson. 2002. Do pulp mill biosolids pose a toxicity hazard to fish?. Poster, 29<sup>th</sup> Annual Aquatic Toxicity Workshop. Oct. 20-23. Whistler, British Columbia.

**Hawkins, S.A.**, C. Hedley, H. Orr, M. van den Heuvel, and P.V. Hodson. 2003. Hazard identification of pulp mill solid waste to aquatic species. 12<sup>th</sup> Annual Meeting of the Society of Environmental Toxicology and Chemistry Europe. Apr. 27-May 1. Hamburg, Germany. Also, 7<sup>th</sup> International Conference on Fate and Effects of Pulp and Paper mill Effluents. June 1-4. Seattle, Washington.

**Spearin, A.** 2003. Environmental evaluation of land-applied pulp mill and municipal biosolid. Great Lakes Research Student Symposium. March 13-15. State University, New York (SUNY) at Syracuse, New York. Won Best Presentation in Biology Category. Also, Master's Thesis, Ryerson University, Toronto, Ontario. August 2003. Supervisor: L.H. McCarthy.

**Videla, P.** 2003. Assessing toxicological impact of pulp mill and sewage treatment plant biosolids runoff to the macrophytes *Lemna minor* and *Elodea densa*. Great Lakes Research Student Symposium. March 13-15. State University, New York (SUNY) at Syracuse, New York. Also, 4<sup>th</sup>-year thesis defence. Ryerson University. April 2003. Supervisor: L.H. McCarthy.

**McCarthy, L.H.**, I.V. Bostan, S.N. Liss, A. Spearin, E. Bandelj, and K. Yambao. Evaluation of land-applied pulp mill biosolids: Monitoring the fate of sludge constituents in forest ecosystems and assessing impact using ecologically-relevant organisms. 5<sup>th</sup> International Conference on Fate and Effects of Pulp and Paper Mill Effluents. Jun 1-4, 2003. Seattle, Washington. Manuscript submitted for peer-reviewed publication in SETAC Press.

**Bostan, I.V.**, L.H. McCarthy, 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. Submitted. Waste Management.

**McCarthy, L.H.**, I.V. Bostan, and S.N. Liss. Rationale for selecting specific aquatic and terrestrial organisms in environmentally-relevant suite of bioassays to assess land-application of complex organic substrate. Submitted. Environ. Tox. Chem.

**Hawkins, S.A.**, C.Hedley, H. Orr, M. van den Heuvel, and P.V. Hodson. 2003. Pulp mill biosolid composition and toxicity hazard to rainbow trout. Platform paper, 23<sup>rd</sup> Annual Meeting for the Society of Environmental Toxicology and Chemistry. Nov. 16-20. Salt Lake City, Utah.

Currently, L.H. McCarthy and P.V. Hodson will co-chair a session on the land-application of biosolids at the 47<sup>th</sup> Annual Conference of the International Association of Great Lakes Research (May 2004). Industry, government, and academia will be invited to present a platform on this subject.

### Training Highly Qualified Personnel

From the funding of this study, many highly qualified personnel have been trained. **Nives Ceric** completed her fourth-year thesis in April 2001. **Patricia Videla** completed her fourth-year thesis in April 2003. Post-Doctoral Fellow **Dr. Vadim Bostan** was hired in September 2000 and completed his tenure in August 2003. He has been hired as faculty at Ryerson University. **Ashley Spearin** completed her Master's thesis in August 2003 and was hired by the Ontario Ministry of the Environment. Ryerson University Research Assistants included **Morgan Partyka**, **Emil Bandelj** and **Kathrina Yambao** (summer/fall 2000) and **Joseph Bautista** (summer/fall 2001/2002). **Stephanie Hawkins** is expected to complete her Master's thesis (under the supervision of Dr. Peter Hodson of Queen's University) in January 2004 and a short-term contract was developed for **Shobha Sharma** (under the supervision of Dr. Roberta Fulthorpe of the University of Toronto).

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