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ECOTOXICITY EVALUATION OF COMPOSTED SITE AND REFERENCE SOILS CONTAMINATED PREDOMINANTLY WITH PHCS AND ARSENIC

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1.0 Introduction

Komex International Ltd. (Worley Parsons) contracted Stantec Consulting Ltd. (Stantec) to conduct chronic ecotoxicity assessments of composted reference soils and composted site soils contaminated with petroleum hydrocarbons and arsenic. The contaminated site soils are subsurface soil (clay till) that originated from flare-pit plume material in a site located in northwest-central Alberta; therefore, it was predominantly contaminated with weathered petroleum hydrocarbons and arsenic. The reference soil was collected from the same vicinity and had physico-chemical characteristics similar to those of the contaminated site soil, but was free of contamination. Both the site and reference soils were amended with compost to promote degradation of the PHC residuals in soil. An ecotoxicity assessment of these soils was conducted between January and April, 2006.

Test species included one invertebrate earthworm species (*Eisenia andrei*) and five plant species, alfalfa (*Medicago sativa*), barley (*Hordeum vulgare* var. Chapais), northern wheatgrass (*Elymus lanceolatus*), red clover (*Trifolium pratense*) and red fescue (*Festuca rubra*). A field-collected reference soil and an experimental negative control soil were used as control treatments for this assessment. The field-collected reference soil had physical and chemical characteristics similar to those of the site soils under investigation, but was free of contamination. The reference soil delineated effects that might be attributable to the soil independent of the contamination. The experimental negative control soil was an artificial soil recommended for testing by OECD (1984), ASTM (1995), and EC (2005a,b). It serves primarily as a quality control for test organism health, test conditions and procedures, and technician proficiency.

Plant toxicity tests were conducted with three soil treatments; the artificial control soil, the reference and the contaminated site soil. The earthworm toxicity test design also included two additional soil treatments; the reference soil and the contaminated site soil amended with a soil conditioner. As subsurface clay till soils, the reference and site soils are fine-textured with high bulk density and little organic matter. These characteristics are known to provide suboptimal environments for reproduction of *Eisenia andrei*. *Eisenia andrei* normally inhabits soils with high organic matter content and moderate to low bulk density. Since soil physical and chemical characteristics can have a strong influence on earthworm reproduction, a soil conditioner (*Sphagnum* peat) was added to both the reference soil and the site soil to render soil conditions more amenable to earthworm reproduction. Testing peat-amended and unamended reference and site soils was conducted in an attempt to discriminate the effect on reproduction attributable to soil petroleum hydrocarbon contamination from that of soil characteristics. Because of the nature of the soil conditioner (*Sphagnum* peat is minimally used as a food source by *E. andrei* and is well-tolerated by this species) and the fact that the PHCs were well weathered and aged (i.e., tightly bound to soil particles), the soil conditioner was thought to minimally affect redistribution of the PHCs in the contaminated site soils and influence PHC toxicity to earthworm reproduction.

Petroleum hydrocarbon concentrations in the site soils were also measured at the beginning and the end of the earthworm ecotoxicity tests conducted by Stantec.

1.1 CONTAMINATED SITE SOIL AND REFERENCE SITE SOILS

Stantec Consulting, Ltd.'s terrestrial ecotoxicology laboratory (361 Southgate Drive, Guelph, ON, N1G 3M5) received the reference and contaminated site soils in December 6 2005. The soils were inspected and homogenized upon receipt. All soils (buckets) were kept in a temperature-controlled room for the duration of testing. The soil moisture content (Subsection A.2; Appendix A) and water holding capacity (Subsection A.4; Appendix A) of each soil were determined upon arrival to the lab.

1.2 NEGATIVE CONTROL SOIL

The negative control soil used for the toxicity assessment was an artificial soil (AS) formulated in the laboratory that is recommended by Environment Canada for toxicity testing (EC 2005a, 2005b). The AS is similar to that recommended by other standard protocols (OECD, 1984; ASTM, 1995). It is formulated from natural ingredients of silica sand, kaolinite clay, and *Sphagnum* peat and it is buffered to a neutral pH range (6.0 to 7.5) with calcium carbonate. This negative control soil served as an experimental QA/QC soil for test organisms, procedures, and conditions. The characteristics and formulation of artificial soil (AS) are reported in Tables A.1 and A.2. (Appendix A).

1.3 TEST ORGANISMS

Test species included one invertebrate earthworm species (*Eisenia andrei*) and five plant species, alfalfa (*Medicago sativa*), barley (*Hordeum vulgare* var. Chapais), northern wheatgrass (*Elymus lanceolatus*), red clover (*Trifolium pratense*) and red fescue (*Festuca rubra*). All species are recommended by the Environment Canada test methods (EC 2004 and 2005) and the plant species include monocotyledonous, dicotyledonous, leguminous, native, forage, reclamation and crop species.

2.0 Definitive Tests

Definitive plant tests were 14 or 21 days in duration depending on the species, and were conducted between January and February 2006. The chronic earthworm test was 63 days in duration and was conducted between February and April 2006. Measurement endpoints for the earthworm test included 35-d adult survival, number of progeny produced, and wet and dry mass of individual progeny, while the endpoints for the plant tests included seedling emergence, shoot and root lengths and shoot and root wet and dry masses. Tests were conducted with undiluted reference and site soil (plants and earthworms) and reference and site soils amended with *Sphagnum* peat (earthworms only).

The purpose of the longer-term tests (63-d earthworm; 14 to 21-d plant) was to examine the effects of prolonged exposure to PHC- and arsenic-contaminated soils on the survival and reproduction of earthworms and the emergence and growth of plants.

2.1 MATERIALS AND METHODS

All tests were conducted following the Environment Canada methods (EC 2004, 2005). The experimental design and test conditions for all tests are summarized in Table A.2 (Appendix A) and in the data summaries in Appendix B. The data summaries also contain the results of the toxicity tests and any modifications to the procedures and conditions recommended in the test methods.

2.2 PREPARATION OF TEST SOILS

The reference and contaminated soils were thoroughly homogenized upon receipt at Stantec. In addition, since both reference and contaminated soils were mixed with compost, some of the larger pieces of organic material were removed by hand in order to render the soil amenable for testing.

The methods and procedures used to complete the plant testing closely followed those recommended by Environment Canada (2004 and 2005). The experimental designs and test conditions are summarized in Table A.2 (Appendix A), as well as in the data summaries presented in Appendix B.

Each batch of test soil was comprised of a quantity of soil sufficiently large to accommodate the replicate requisite volumes. A pre-determined amount of the test soil was added on a dry weight basis into a mixing bowl, then mixed with a mechanical mixer and a stainless steel spoon. De-ionized water (DI) was also added to the batch of soil to hydrate the test soil to a moisture content, such that a crumbly aggregation was achieved. The batch of soil was considered to be homogenous when uniform in colour, texture, and wetness. The moisture content ranged from 49-100% of the water-holding capacity of the soil (Data Summaries; Appendix B). Once the soils and water were completely mixed, the soil was allocated to each test unit by weight.

A sub-sample of soil was collected from each treatment in order to measure soil moisture content (3 to 5 g), and soil pH and conductivity (25 g), according to the procedures described by EC (2004 and 2005). Sub-samples were also collected from each treatment for PHC F2-F4 analysis at the beginning and end of the earthworm reproduction test (Table 1). The earthworm test units were then sealed tightly and left to equilibrate for 24 hours at 20 ± 2ºC until the organisms were added the next day. For tests with plant species, the seeds were planted the same day as the test soils were prepared.

2.2.1 Peat Amendment of Soils for Earthworm Reproduction Test

The materials and methods used to prepare the peat-amended soils for the earthworm reproduction test were the same as those described above except that one batch each of reference or site soil was amended on a dry weight basis with predetermined amounts (4% peat on a dry weight basis) of *Sphagnum* peat prior to the addition of deionized water. Prior to the addition of peat, soil pH was measured for all test soils. After the pre-determined mass of peat was added to site soils and soils had been hydrated and thoroughly homogenized, soil pH was measured for each soil-peat treatment. Calcium carbonate was added to treatments so that all treatments were within 1 pH unit of the unamended reference and site soils (Table B.12.; Appendix B). The largest difference in pH was at the beginning of the test (0.86 units) and was between the contaminated ("impacted") site soil amended with 0% and 4% peat (6.51 and 7.37, respectively) which was not considered to be sufficiently large to influence test results.

2.3 PREPARATION OF ARTIFICIAL SOIL

The preparation of the artificial soil (AS) followed procedures identical to those described in subsection 2.2, except no sub-samples were collected for chemical analysis. The soil was hydrated to a moisture content of approximately 35%, or 60-70% of the water-holding capacity of the soil.

2.4 PREPARATION OF ORGANISMS

2.4.1 Plants

Plant seeds were stored in their original paper bags enclosed by a polyethylene zip-lock bag (Glad Zipper™) in a refrigerator at 6 ± 3°C. On Day 0, the seeds of the plant species to be used in a toxicity test were taken out of the refrigerator and acclimated to room temperature. A sub-sample of seeds was placed into a glass Petri dish and spread out over the bottom of the dish. The seeds were then "hand-sorted" or screened to ensure uniformity in size and "quality" of seed. Quality of seed simply refers to the selection of seeds without a blemished seed coat or irregularity of shape or colour, a relatively subjective procedure. The pre-sorted seeds were added to the test soils on Day 0 of a test. One seed was placed directly in the centre of the test unit and the remaining four seeds were distributed around the centre seed. Seeds were planted to a depth that was twice the depth of the diameter of the seed itself. Procedures and methodologies are described in detail in the test method (EC, 2005).

2.4.2 Earthworms

The earthworms used in these tests originated from the cultures at Stantec's laboratory. Organisms were added to the test units the day after the soils were prepared (EC, 2004). On the day the earthworms were to be added to the test units, the earthworms were removed from the cultures, washed with DI water, and placed onto a dry paper towel, or blotting paper, to remove excess water before adding worms to each test units. Only sexually mature adults with a clitellum and with no deformities (skinny, pale colour, constrictions, etc.) were used. The worms were added to the test units across treatments, randomly. Each mass of twenty randomly chosen worms was recorded and the average mass of the individuals was determined. If a worm appeared to be particularly small or large, it was also weighed to ensure that it fell within the required mass range of 250 to 600 mg. Two clitellated adults were placed into each of the test units using forceps.

2.5 TEST CONDITIONS

2.5.1 Plants

Once the seeds were planted in all of the test units, the test units were transported to the University of Guelph and placed randomly onto benches in an environmental growth chamber. The photoperiod was 16 h light: 8 h dark, and the corresponding temperatures were 24 $\pm$ 2°C and 15 $\pm$ 2°C, respectively. Water loss from the soil was minimized by keeping the 1-L container lids sealed until Day 7, or until the height of the shoots was about 4 cm, whichever came first. The containers did not interfere with the quality of light to which the seedlings were exposed. The seedlings were watered every 24 to 48 h. Historically, the phosphorus levels in the AS were adequate for plant growth; however, we have subsequently discovered that the commercial supplier for the sand used to formulate the AS changed their source. The origin of the sand is not the same as that in previous years. Chemical characterization of the AS showed the phosphorus levels in the AS were sub-optimal for plant growth of some species. To mitigate the lower phosphorus levels, the addition of a weak nutrient solution was used in the hydration of the AS test units (Subsection A.5; Appendix A). The dilution soils were hydrated with de-chlorinated municipal tap water. The locations of the test units on the benches in the environmental chamber were varied randomly each time water was added.

2.5.2 Earthworms

The toxicity test units containing earthworms were placed into an environmental chamber; the temperature was 20 $\pm$ 2 °C with a photoperiod of 16 h fluorescent illumination: 8 h dark (Data Summary; Appendix B). All test units were supplied with cooked oatmeal on days 0, 14, 28, and 42, and 56, according to the Environment Canada test method (EC, 2004). One tsp (5 mL) of cooked oatmeal was added to each test unit after the test units were visually inspected during feeding episodes, and the soils were misted gently with DI water to maintain the desired moisture content, as necessary.

2.6 MEASUREMENT ENDPOINTS

2.6.1 Physicochemical Measurements

The physico-chemical measurements recorded at the beginning and end of each test were soil pH and electrical conductivity. Percentage moisture of the soil was measured at the beginning and end of the earthworm test, but only at the beginning of each plant test, since the soils in the plant test units were watered throughout the test. Moisture content of the soil was expressed as a percent of the soil water-holding capacity as recommended by the EC methods (EC 2004 and2005). The methods for calculating water-holding capacity (Subsection A.4) and % moisture (Subsection A.2) are described in Appendix A. The pH and conductivity (Subsection A.3; Appendix A) were measured using a soil slurry method modified from the Soil Analysis Handbook (1992).

The test temperature in the environmental chambers and the room with controlled conditions was monitored with a maximum/minimum thermometer and temperature data loggers. Although the chambers had controlled humidity, watering of the soils in the test units was necessary.

2.6.2 Plant Measurement Endpoints

The measurement endpoints for the definitive tests included shoot and root lengths, shoot and root wet phytomasses, and shoot and root dry phytomasses. Seedling emergence was also measured. The criterion for emergence was a shoot height of 3 mm above the surface of the soil at the end of the test (14 or 21 days).

2.6.3 Earthworm Measurement Endpoints

The measurement endpoints were 35-day adult survival, number of progeny produced and individual progeny wet and dry masses at the end of the 63-day test. Progeny mass was determined with an Ohaus Analytical Plus electronic balance (0.1mg). Mortality of the earthworms was assessed as failure to respond (i.e., muscularly contract) upon gentle prodding with a glass rod. Dead worms rarely are observed because of the rapid rate of earthworm tissue decomposition.

2.7 TEST PROCESSING

2.7.1 Plants

At the end of the tests, the test units were transported from the University of Guelph to Stantec Consulting Ltd. and processed in the laboratory. Prior to processing the test units, photographs were taken to record the visual concentration-response relationship. The number of emerged seedlings at the end of the test was then recorded for each test unit. Composite sub-samples of soil within each treatment were also collected at this point for measurement of soil pH and conductivity. The plant roots were separated from the soil and, in some instances, from the roots of the other plants. The roots were washed in a pan with water and, held in the palm of

one hand, while water was sprayed onto the roots to dislodge soil particles. When the roots were clean (i.e., soil particles were dislodged), the plant was placed onto a sheet of moistened, labelled paper towel, one replicate (e.g., 5 plants) per towel, and the towel was covered with plastic to minimize water loss while the length measurements were recorded.

Shoot and root lengths were measured with a ruler and recorded in millimetres. The roots were separated from the shoots with a scalpel blade and the shoots and roots were each placed into pre-weighed, pre-labelled, aluminum pans (1-2.5 g) and the wet masses were determined using an Ohaus Analytical Plus electronic balance (0.1 mg). The seed and seed endosperm were not included in the measurements.

After the wet mass measurements were recorded, the aluminum pans with the plant material were placed into a drying oven and dried at 90°C for a minimum of 48 hours. The dry shoot and root mass measurements were then determined with the analytical balance.

2.7.2 Earthworms

In order to assess adult survival at Day 35 of the test, the contents of each test unit were emptied onto a tray and sorted with forceps, and the individual earthworms reported as either dead or alive. It is often not possible to observe dead earthworms because the bodies of the dead earthworms decompose rapidly. Therefore, the live earthworms were recorded and "missing" worms were considered dead. Surviving adults were removed from each test unit and the contents on the tray were then returned to the test units and the test units incubated for an additional four weeks.

To process the test units at the end of the experiment (e.g., Day 63), the contents of the test units were again distributed onto a tray. Sub-samples of soil were collected from each test unit and composited within treatments for determination of moisture content, soil pH, and electrical conductivity. Also, composite sub-samples of each treatment (RS, RS 4% peat, Impacted, Impacted 4% peat) were collected for F2-F4 PHC analysis. The soil was then carefully sorted with forceps to remove, count, and record the earthworms (neonates, juveniles, sub-adults = progeny) produced. The progeny were removed from the soil, rinsed with water, and then placed into a pre-weighed aluminum pan (~1g). All the progeny found in each test unit were weighed in the same weigh pan; therefore, for each test unit there existed only one wet and one dry weight measurement. The wet mass was measured with an Ohaus Analytical Plus electronic balance (0.1mg). The wet biomass was then placed into a drying oven at 90°C for 48 h after which the dry weights were measured. Mean individual progeny wet and dry metrics were calculated by dividing the wet or dry mass of one replicate by the corresponding number of progeny comprising that replicate.

2.8 STATISTICAL ANALYSES

Analyses of variance (ANOVA) procedures, followed by Fisher's protected LSD (Least Significant Difference), were applied to the data to determine if there were significant differences among site soil and reference soil treatments. Statistical tests for assumptions of normality and

heteroscedasticity were conducted in SYSTAT (SPSS, 1997) in order to satisfy the requirements of the ANOVA. Where data were not parametric, ANOVA procedures could not be applied. In these instances, a Kruskal-Wallis test, followed by a Mann-Whitney comparison test, were used. The assumptions of all statistical tests were tested and met, unless stated otherwise in the data summaries. All analyses were performed with SYSTAT 11 (SPSS, 2004).

3.0 Results and Discussion

3.1 ALFALFA

Seedling emergence was significantly reduced in the contaminated ("impacted") site soil compared to that in the reference and artificial control soil treatments (Table B.1., Figure B.1.; Appendix B). Shoot and root growth was significantly reduced for all growth endpoints in the site soil compared to growth of seedlings in the reference soil. With the exception of root length, shoot and root growth of seedlings grown in the reference soil was significantly less than that of seedlings grown in the artificial soil (Table B.1., Figure B.1.; Appendix B).

All performance criteria for test acceptability were met for the artificial soil treatment (EC 2005), indicating that the test procedures, conditions, seed quality and technical proficiency were acceptable. Growth of seedlings in the site-specific reference soil also met the Environment Canada test method validity criteria for a negative control soil, indicating that the soil characteristics were suitable for growth of this species; therefore the reference soil can be considered a suitable negative control soil for the contaminated site soil for this species.

The pH of the soil among treatments ranged from 6.85 to 7.12 at the beginning of the test and between 6.59 and 6.73 at the end of the test. Electrical conductivity (EC) increased with increasing contamination at the start of the test, but was well within a tolerable range for this species. Since the test units containing reference and site soils were hydrated with de-chlorinated municipal tap water, an overall increase in EC was observed at the end of the test for each treatment level (Table B.2; Appendix B). The initial moisture contents prior to watering were between 49% and 69% of the soil's water holding capacity.

3.2 BARLEY

Seedling emergence was not affected following growth of barley seedlings in the contaminated ("impacted") site soil compared to that in the reference and artificial control soil treatments (Table B.3., Figure B.2.; Appendix B). Seedling shoot growth was significantly reduced for the shoot wet mass metric only; for shoot length and shoot dry mass, there were no significant differences in shoot growth between seedlings grown in the reference and site soil treatments. Root growth was adversely affected in seedlings grown in the site soil compared to seedlings grown in the reference soil. Shoot and root growth of seedlings grown in both the reference and site soils was significantly less than that of seedlings grown in the artificial soil (Table B.3., Figure B.2.; Appendix B).

All performance criteria for test acceptability were met for the artificial soil treatment (EC 2005). Growth of seedlings in the site-specific reference soil met the Environment Canada test method validity criteria for a negative control soil for emergence and root length; the validity criterion for shoot length was not met. This indicates that the soil characteristics are somewhat less than optimal for growth of this species and; therefore, the reference soil cannot be used as a negative control soil for the contaminated site soil for this species without reservation.

The pH of the soil among treatments ranged from 6.85 to 7.12 at the beginning of the test and between 6.34 and 6.6.83 at the end of the test. Electrical conductivity (EC) increased with increasing contamination at the start of the test, but was well within a tolerable range for this species. Since the test units containing reference and site soils were hydrated with de-chlorinated municipal tap water, an overall increase in EC was observed at the end of the test for each treatment level (Table B.4.; Appendix B). The initial moisture contents prior to watering were between 49% and 69% of the soil's water holding capacity.

3.3 NORTHERN WHEATGRASS

Seedling emergence was significantly reduced in the contaminated ("impacted") site soil compared to that in the reference and artificial control soil treatments (Table B.5., Figure B.3.; Appendix B). Shoot and root growth was significantly reduced for all growth endpoints in the site soil compared to growth of seedlings in the reference soil. Shoot and root growth of seedlings grown in the reference soil was significantly less than that of seedlings grown in the artificial soil (Table B.5., Figure B.3.; Appendix B).

All performance criteria for test acceptability were met for the artificial soil treatment (EC 2005). Growth of seedlings in the site-specific reference soil met the Environment Canada test method validity criteria for a negative control soil for emergence and root length; the validity criterion for shoot length was not met. This indicates that the soil characteristics are somewhat less than optimal for growth of this species and; therefore, the reference soil cannot be used as a negative control soil for the contaminated site soil for this species without reservation.

The pH of the soil among treatments ranged from 6.61 to 7.11 at the beginning of the test and between 6.32 and 7.06 at the end of the test. Electrical conductivity (EC) increased with increasing contamination at the start of the test, but was well within a tolerable range for this species. Since the test units containing reference and site soils were hydrated with de-chlorinated municipal tap water, an overall increase in EC was observed at the end of the test for each treatment level (Table B.6; Appendix B). The initial moisture contents prior to watering were between 55% and 82% of the soil's water holding capacity.

3.4 RED CLOVER

Seedling emergence was not affected following exposure of red clover seedlings to the contaminated ("impacted") site soil compared to that in the reference and artificial control soil treatments (Table B.7., Figure B.4.; Appendix B).

Shoot and root growth was significantly reduced for all growth endpoints in the site soil compared to growth of seedlings in the reference soil. With the exception of root length, shoot and root growth of seedlings grown in the reference soil was significantly less than that of seedlings grown in the artificial soil (Table B.7., Figure B.4.; Appendix B).

All performance criteria for test acceptability were met for the artificial soil treatment (EC 2005). Growth of seedlings in the site-specific reference soil also met the Environment Canada test

method validity criteria for a negative control soil, indicating that the soil characteristics were suitable for growth of this species; therefore the reference soil can be considered a suitable negative control soil for the contaminated site soil for this species.

The pH of the soil among treatments ranged from 6.61 to 7.11 at the beginning of the test and between 6.50 and 6.87 at the end of the test. Electrical conductivity (EC) increased with increasing contamination at the start of the test, but was well within a tolerable range for this species. Since the test units containing reference and site soils were hydrated with de-chlorinated municipal tap water, an overall increase in EC was observed at the end of the test for each treatment level (Table B.8.; Appendix B). The initial moisture contents prior to watering were between 55% and 82% of the soil's water holding capacity.

3.5 RED FESCUE

There was no significant difference in seedling emergence between red fescue seedlings grown in the artificial soil and the reference control soil, and between seedlings grown in the artificial and the contaminated ("impacted") site soil. However, significantly fewer seedlings emerged in the site soil compared to the number that emerged in the reference control soil (Table B.9., Figure B.5.; Appendix B). In this instance, the emergence results in the reference control soil should be considered more relevant than the emergence results in the artificial soil. This is because all the performance criteria for test acceptability were met for the artificial soil treatment except emergence; the validity criterion for emergence for this species is 70% and emergence in this test was 60% (EC 2005). However, the validity criterion for emergence was met in the reference control soil.

Shoot and root growth was significantly reduced for all growth endpoints in the site soil compared to growth of seedlings in the reference soil with the exception of shoot length. Shoot and root growth of seedlings grown in the reference soil was significantly less than that of seedlings grown in the artificial soil (Table B.9., Figure B.5.; Appendix B).

All performance criteria for test acceptability were met for the artificial soil treatment except emergence; the validity criterion for emergence for this species is 70% and emergence in this test was 60% (EC 2005). However, all emerged seedlings appeared healthy and vigorous and all other validity criteria were met. Seedling emergence is in general poorly correlated to soil toxicity (or lack thereof) and therefore the effect on the seedling growth results of the toxicity test was considered negligible. Growth of seedlings in the site-specific reference soil met the Environment Canada test method validity criteria for a negative control soil for emergence and root length; the validity criterion for shoot length was not met. This indicates that while the soil characteristics did not influence red fescue emergence, they are somewhat less than optimal for the growth of this species. Therefore, the reference soil cannot be used as a negative control soil for the contaminated site soil for this species without reservation.

The pH of the soil among treatments ranged from 6.85 to 7.12 at the beginning of the test and between 6.59 and 6.82 at the end of the test. Electrical conductivity (EC) increased with increasing contamination at the start of the test, but was well within a tolerable range for this

species. Since the test units containing reference and site soils were hydrated with de-chlorinated municipal tap water, an overall increase in EC was observed at the end of the test for each treatment level (Table B.10.; Appendix B). The initial moisture contents prior to watering were between 49% and 79% of the soil's water holding capacity.

3.6 EISENIA ANDREI

There was no adverse effect of soil type on adult survival following 35 days of exposure. Adult survival was greater than or equal to 90% in all soil treatments (Table B.11., Figure B.6.; Appendix B). The number of progeny produced in the peat-amended and un-amended reference soils was not significantly different and ranged from 3.2 to 4.5 progeny/treatment. No progeny were produced in the contaminated ("impacted") site soil regardless of whether or not the soil was amended with *Sphagnum* peat. The difference between number of progeny produced in the reference and site soil treatments was significant. The number of progeny produced in the artificial soil treatment was significantly greater than that in all other soil treatments (mean number was 14.1 progeny) (Table B.11., Figure B.6.; Appendix B). There was no significant difference in the wet and dry mass of individual earthworm progeny between the earthworms produced in the peat-amended and un-amended reference soil (Table B.11., Figure B.6.; Appendix B). Wet and dry mass of progeny produced in the artificial soil treatment was significantly greater than progeny produced in the unamended reference soil, but not greater than those produced in the peat-amended reference soil treatment.

All performance criteria for test acceptability were met for the artificial soil treatment (EC 2004), indicating that the test procedures, conditions, seed quality and technical proficiency were acceptable. The validity criteria was met in the reference soil for adult survival, but not for the number of progeny produced. These results indicate that the reference soil is suboptimal for *E. andrei* reproduction. Amending the soil with 4% (on a dry weight basis) *Sphagnum* peat appeared to result in individual progeny that were slightly larger than those produced in the unamended reference soils; however, the difference was not significant. It is difficult to conclude that the lack of earthworm progeny in either the contaminated peat-amended or unamended site soil can be attributable solely to contamination by PHCs and arsenic; likely it is due to the combined effects of soil contamination and soil physical and chemical characteristics.

The pH of the soil among treatments ranged from 6.51 to 7.53 at the beginning of the test and between 6.77 and 7.63 at the end of the test. Electrical conductivity (EC) levels ranged from 283 to 501 µS/cm at the beginning of the test, and from 214 to 390 µS/cm at the end of the test (Table B.12.; Appendix B). All EC values were well within a tolerable range for this species. The initial moisture contents were between 41% and 100% of the soil's water holding capacity, and by the end of the test the moisture contents ranged from 63 to 123% of the soil's water holding capacity (Table B.12.; Appendix B). When the % mc was calculated on a wet weight basis (i.e., using % mc = ww-dw/ww) (Subsection A.2; Appendix A), the initial and final moisture contents ranged from 20 to 41% (Table B.12.; Appendix B).

3.7 CHEMISTRY

Sub-samples (~200 g) of test soil were collected at the beginning and end of the 63-day test with *Eisenia andrei* and submitted to AGAT Laboratories (Calgary, AB) for analyses of Fraction 2 to Fraction 4 using the CCME reference methods. The purpose for collecting these chemical data was to determine the background (e.g., 0% contamination) reference soil and initial measured exposure concentrations of the fractions in the contaminated ("impacted') site soil, and to determine the amount of each fraction that had dissipated over the duration of a test. PHC fractions were analyzed in both the peat-amended and unamended reference and site soils. The results of the F2 to F4 PHC analyses of the test soils are summarized in Table 1.

The concentrations of F2 to F4 was low and remained consistent in the unamended reference soil for the duration of the earthworm test; these values likely represent the naturally-occurring levels of non-petrogenic PHCs in the soil. The F2 to F4 concentrations in the peat-amended reference soil were similar to the non-amended reference soil at the beginning of the earthworm test; however, over time there was a decrease in the total amount of PHCs in the soil. Fraction 2 to Fraction 4 concentrations are high at the beginning of the test in both the peat-amended and unamended contaminated site soils; however, the concentrations of PHCs in the peat-amended soils was approximately 25% of those of the unamended soils. At the end of the earthworm test, the PHC concentrations in the unamended site soil decreased by Day 63 but stayed relatively constant in the peat-amended soils. At the end of the test, the PHC concentrations in the peat-amended site soils were approximately 75% of that of the unamended site soil. The PHC concentrations in soil expressed as a sum of the fractions were 185, 204, 37,000 and 9810 mg/kg for RS, RS 4% peat, Site, and Site 4% peat, respectively, at the beginning of the test and 167, 80, 16,900 and 13,140 mg/kg for RS, RS 4% peat, Site, and Site 4% peat, respectively, at the end of the test.

Table 1. PHC concentrations in peat-amended and unamended reference and contaminated site soils at the beginning (Day 0) and end of the earthworm reproduction test (Day 63).

Soil Treatment	Day 0			Day 63		
	Fraction 2 (mg/kg)	Fraction 3 (mg/kg)	Fraction 4 (mg/kg)	Fraction 2 (mg/kg)	Fraction 3 (mg/kg)	Fraction 4 (mg/kg)
RS	<10	143	42	<10	123	44
RS 4% peat	<10	153	51	<10	66	14
Site	5080	24600	7320	1540	10900	4460
Site 4% peat	1190	6580	2040	1130	8540	3470

4.0 Summary

The results of the definitive plant toxicity tests were in general similar among all five species; shoot and root growth in the contaminated ("impacted") site soil was adversely affected relative to growth in the reference soil, and growth in the artificial soil was greater than that in both the reference and contaminated site soils. In some cases, there was no effect of soil type upon seedling emergence (e.g., barley and red clover) and in other cases seedling emergence was reduced in the contaminated site soil (e.g., alfalfa, northern wheatgrass and red fescue).

The site-specific reference soil met two out of the three validity criteria for all plant test species (emergence and root length). However, the criterion for shoot length was not met for barley, northern wheatgrass and red fescue. There was no significant difference in barley and red fescue shoot length of seedlings grown in the reference and site soils; however, northern wheatgrass shoot length was significantly lower when grown in the site soil. However; in general, the reference soil acted as a reasonable negative control soil for the contaminated site soil.

There was no adverse effect on adult mortality following 35 days of exposure to the peat-amended and unamended reference and contaminated site soils. The number of progeny produced in the reference soils (peat-amended and un-amended) was significantly greater than that in the peat-amended and unamended site soils, but was less than that produced in the artificial soil. Wet and dry mass of progeny produced in the artificial soil treatment was significantly greater than that of progeny produced in the unamended reference soil, but not greater than those produced in the peat-amended reference soil treatment. Amending the reference and site soils did not significantly affect the results of the earthworm reproduction tests and did not appear to make the reference or site soils more amenable for earthworm reproduction. These results indicate that the reference soil is suboptimal for growth and reproduction of *E. andrei*. Amending the soil with 4% (on a dry weight basis) *Sphagnum* peat appeared to result in individual progeny that were slightly larger than those produced in the unamended reference soils; however the difference was not significant. The lack of earthworm progeny in either the contaminated peat-amended or unamended site soil is likely due to the combined effects of soil contamination and soil physical and chemical characteristics.

The concentration of F2 to F4 was low in the unamended reference soil for the duration of the earthworm test. The F2 to F4 concentrations in the peat-amended reference soil were similar to the non-amended reference soil at the beginning of the earthworm test; however, over time there was a decrease in the total amount of PHCs in the soil. F2 to F4 concentrations are high at the beginning of the test in both the peat-amended and unamended contaminated site soils; however, the concentrations of PHCs in the peat-amended soils was approximately 25% of those of the unamended soils. At the end of the earthworm test, the PHC concentrations in the unamended site soil decreased by Day 63 but stayed relatively constant in the peat-amended soils. At the end of the test, the PHC concentrations in the peat-amended site soils were approximately 75% of that of the unamended site soil.

So in conclusion, the results of the ecotoxicity assessment with five plant and one earthworm species indicate that exposure to the arsenic- and PHC-contaminated site soils represents a risk to plant growth, and to growth and reproduction of earthworms. However, the results also indicate that not all of the risk is directly attributable to the presence of arsenic or petroleum hydrocarbons in the site soil.

5.0 References

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Appendix A

Physico-chemical Characteristics of Test Soils and Test Design,Procedures and Conditions

Table A.1. Physico-chemical characteristics of the artificial soil.

Parameter	Artificial Soil (AS)	Analytical Method
Phosphorous (mg/kg)	23	Nitric/perchloric acid digestion
Potassium (mg/kg)	22	NH_4Ac extractable
Magnesium (mg/kg)	149	NH_4Ac extractable
Calcium (mg/kg)	1848	NH_4Ac extractable
Sodium (mg/kg)	67	NH_4Ac extractable
Sodium Absorption	0.57	
Total Carbon (%)	4.46	Leco furnace method
Total Nitrogen (%)	0.05	Kjeldahl method
C.E.C. (Cmol+/kg)	18.5	Barium chloride method
Soil Texture	Fine Sandy Loam	Gravimetric grain size distribution
Sand (%)	77.3	
Silt (%)	7.8	
Clay (%)	14.9	
Organic Matter (%)	9	Dichromate oxidation
Bulk Density (g/cm^3)	0.98	Clod method
pH (units)	6.09	Water method (1:2)
Conductivity (mS/cm)	0.3	Saturated paste method
Source	Formulated from constituents	

C.E.C = Cation exchange capacity

A.1. FORMULATION OF ARTIFICIAL SOIL

The artificial control soil (AS) was formulated in the laboratory by mixing the ingredients in their dry form, then gradually hydrating with deionized water, and mixing further until the soil was visibly uniform in colour and texture. The ingredients of AS were 70% silica sand (No. 200, Barco 71; Barnes Environmental International, Waterdown, ON), 20% kaolinite clay (Tuckers Pottery Supplies, Richmond Hill, ON), 10% *Sphagnum peat* (Horticulture Department, University of Guelph, Guelph, ON), and calcium carbonate (10-30 g per 1 kg peat). A 12 kg batch of AS was formulated on a dry weight basis by adding 7 kg of sand, 2 kg of kaolinite clay, 1 kg of sieved (2 mm) peat, approximately 30 g of $CaCO_3$ (sieved), and 2 L of deionized water. The amount of calcium carbonate required to achieve a soil pH in the range of 6-7.5, depended on the nature (i.e., acidity) of the *Sphagnum peat* and the silica sand. Each time a new bag of either of these ingredients was used, it was necessary to adjust the amount of $CaCO_3$ used in each batch of formulated soil.

A.2. DETERMINATION OF SOIL MOISTURE CONTENT

Prior to the day of test soil formulation (on Day -1), a 3 to 5 g sample of control soil wet weight (wet wt.) was placed into a pre-weighed aluminum weigh boat (1 or 2.5 g) and the wet mass recorded. Each weigh boat was then placed into a drying oven at 90°C for a minimum of 24 hours. The dry weight of each soil was then determined. Percent moisture content was calculated by expressing the dry mass as a percentage of the wet mass:

$$\text{Percent Moisture} = \frac{\text{wet mass (g wet wt.)} - \text{dry mass (g dry wt.)}}{\text{wet mass (g wet wt.)}} \times 100$$

The initial moisture content of the soils was needed in order to standardize the moisture content in the control soils.

A.3. MEASURING SOIL PH AND CONDUCTIVITY (WATER SLURRY) (MODIFIED FROM THE SOIL ANALYSIS HANDBOOK, 1992).

Approximately 25 g (wet wt.) of test soil and 50 mL of deionized water were placed into a glass beaker and stirred with a glass rod for two minutes. The beakers sat at room temperature in the laboratory for about one hour. Just prior to measuring pH and conductivity, the soil slurry was mixed again. Soil pH was measured with a pH and ATC probe submersed in the soil slurry that was gently agitated until the readings were constant. Conductivity was measured with a conductivity and ATC probe submersed in the freshly mixed slurry, and was recorded once the readings were constant. The slurry was not agitated while conductivity measurements were taken. The soil pH and conductivity were measured using an Accumet® Meter (Fisher Scientific Model 20) that had been calibrated before use with either two or three (pH 4, 7 and 10) external buffers and an external conductivity standard. The measurements were made first on the control treatments followed by the test soils, ensuring that the probes were washed between samples.

A.4. DETERMINATION OF WATER-HOLDING CAPACITY

The water-holding capacity of a soil was determined by placing ~130 g wet weight of soil sample into a large petri dish and drying the sample at 105°C to a constant weight. Subsequent to drying, the sample was removed from the oven and cooled in a desiccator for at least 20 minutes. 100 g of the dried soil sample were placed into a 250-mL glass beaker and 100 mL of de-ionized (or distilled) water were added to the sample and mixed thoroughly with a glass stir rod to ensure that all sample particles were wetted and that a slurry of soil and water existed. A circle of filter paper was folded into quarters and placed into a glass funnel; the folded filter paper was level with the top of the funnel. 7 mL of de-ionized (or distilled) water were slowly added, using a pipette, to the filter paper to wet the entire surface. The combined weight of the funnel and hydrated filter paper was measured. The weight of the dried soil, funnel and hydrated filter paper was recorded as the initial weight. The funnel was placed into a 500-mL Erlenmeyer flask and the slurry of soil and water was slowly poured onto the hydrated filter

paper held in the funnel. Any soil remaining on the beaker and stir rod was rinsed into the funnel with minimal amounts of deionized water to ensure that all of the solid material had been washed onto the filter. The funnel was covered tightly with aluminum foil and allowed to drain for 3 hours at room temperature. After the 3-hour drainage period, the funnel, hydrated filter paper, and soil were weighed and recorded as the final weight. The water-holding capacity, expressed as mL water/100 g soil, was equal to the difference between the final and initial weights of the funnel, filter, and sample.

A.5. NUTRIENT SOLUTION PREPARATION (FOR PLANTS GROWN IN ARTIFICIAL SOIL)

Artificial soil is low in the nutrients required by plants for growth. For testing purposes, plant test units containing artificial soil are formulated and irrigated with a dilute nutrient solution. The nutrient solution used is a 20-8-20 (N:P:K) formulation (Plant Products Company Ltd., 314 Orenda Road, Brampton, ON L6T 1G1) recommended by the Department of Plant Agriculture, University of Guelph. At the Stantec laboratory, a nutrient solution is made out of the powdered formulation to a concentration of 1 g/L. When preparing artificial soil for testing on Day 0 the soil is hydrated to a standard moisture content with nutrient solution at 1 g/L. For irrigating the plant tests (Day 7 – end of test), a half-strength nutrient solution is used (0.5 g/L) and the artificial soil treatments are misted with nutrient solution when necessary.

Table A.2. Summary of Test Design, Procedures and Conditions.

Test	Earthworm	Plant
Test type	Chronic Screening	Definitive Screening
Test duration (d)	63 (35-d adult survival)	14 or 21
Test unit (chamber)	Glass 270-mL mason jar	1-L polypropylene container
Amount of soil	270 g wet wt.	500 g wet wt.
Temperature (day/night)	20 $\pm$ 2°C	24/16 $\pm$ 3°C
Photoperiod (h)	16 L : 8 D	16 L : 8 D
Treatments (% contamination)	AS, RS, RS 4% peat, Impacted[1], Impacted 4% peat	AS, RS, Impacted[1]
Number of replicate test units per treatment	10	5
Number of organisms per test unit	2	5 -10
Lighting (Type & Intensity)	Fluorescent, 829 Lux	Full spectrum Durotest or Vita Lights; 341-398 μmoles/(m^2·s)
Physicochemical measurements	Conductivity, pH, % moisture	Conductivity, pH, % moisture, WHC
Biological endpoint measurements	Adult survival, no. progeny produced, progeny wet and dry mass	Emergence, shoot and root length, shoot and root wet and dry mass
Statistical endpoints	Significant difference among treatment means	Significant difference among treatment means
Description of methods	EC 2004	EC 2005

[1] "Impacted" soil refers to the contaminated site soil treatment

Appendix B

Test Conditions, Experimental Design, Data Summaries, and Results of Definitive Plant and Earthworm Toxicity Tests

Appendix B: Data Summary

Alfalfa Definitive Screening Test with Peat-amended Reference and PHC/As-Contaminated Site Soils

Sample Identification	
Client:	WorleyParsons Komex
Sample description:	Reference and contaminated site soils collected from Alberta
Date received:	December 6 2005
Date tested:	January 10 – January 31, 2006
Method of soil collection:	Grab samples
Soil storage:	20°C
Technicians:	Kelly Olaveson, Jill Crumb, Jessica Rahn
Analyst:	Kelly Olaveson, Natalie Feisthauer
QA/QC:	Gladys Stephenson
Test Organism:	Alfalfa *(Medicago sativa)*
Organism Source:	William Dam Seeds, Dundas, ON

Test Conditions and Procedures	
Test type:	Static, definitive
Test duration:	21 days
Number of treatments:	5, including 1 experimental control (AS)
Temperature:	22.8 ± 1.0°C (day) 15.2 ± 2.0°C (night)
Light intensity:	272 ± 38 µmol/(m^2·s)
Photoperiod:	16 h light; 8 h dark
Watering regime:	De-chlorinated municipal tap water, as required
Test unit description:	1-L clear polypropylene container
Soil mass/test unit:	500 g wet weight
No. organisms per test unit:	10
No. replicate test units/treatment:	5 replicates
Measured soil chemistry parameters:	Initial soil pH, electrical conductivity, and percent moisture content, final soil pH and electrical conductivity
Measured endpoint(s):	Day 21: Seedling emergence, shoot and root lengths, shoot and root wet and dry masses
Test Protocol:	Biological Test Method: Test for Measuring Emergence and Growth of Terrestrial Plants Exposed to Contaminants in Soil. Report EPS 1/RM/45, February 2005. Method Development and Applications Section, Environmental Technology Centre, Environment Canada, Ottawa, Ontario.
Test acceptability criteria met:	Yes

Results

Alfalfa Seedling Emergence and Growth

Table B.1. Effect of 21-day exposure of alfalfa to artificial soil and to peat-amended and unamended reference and contaminated site soils. Results are reported as treatment means, and the values in brackets indicate one standard deviation of the mean.

Soil Type	Percent Emergence (n = 10 seeds)	Shoot Length (mm)	Root Length (mm)	Individual Shoot Wet Mass (mg)	Individual Shoot Dry Mass (mg)	Individual Root Wet Mass (mg)	Individual Root Dry Mass (mg)
Artificial Soil	98 (4)	90.8 (3.1)	131.6 (4.9)	158.13 (20.22)	37.18 (4.89)	191.54 (39.41)	13.15 (1.51)
Reference Soil	90 (9)	58.2 (8.5)	189.3 (45.6)	73.68 (16.59)	15.69 (3.41)	77.02 (22.95)	6.37 (1.43)
Impacted Soil	78 (12)	34.2 (2.7)	114.3 (6.2)	33.84 (6.05)	8.05 (1.38)	23.86 (5.80)	2.73 (0.45)

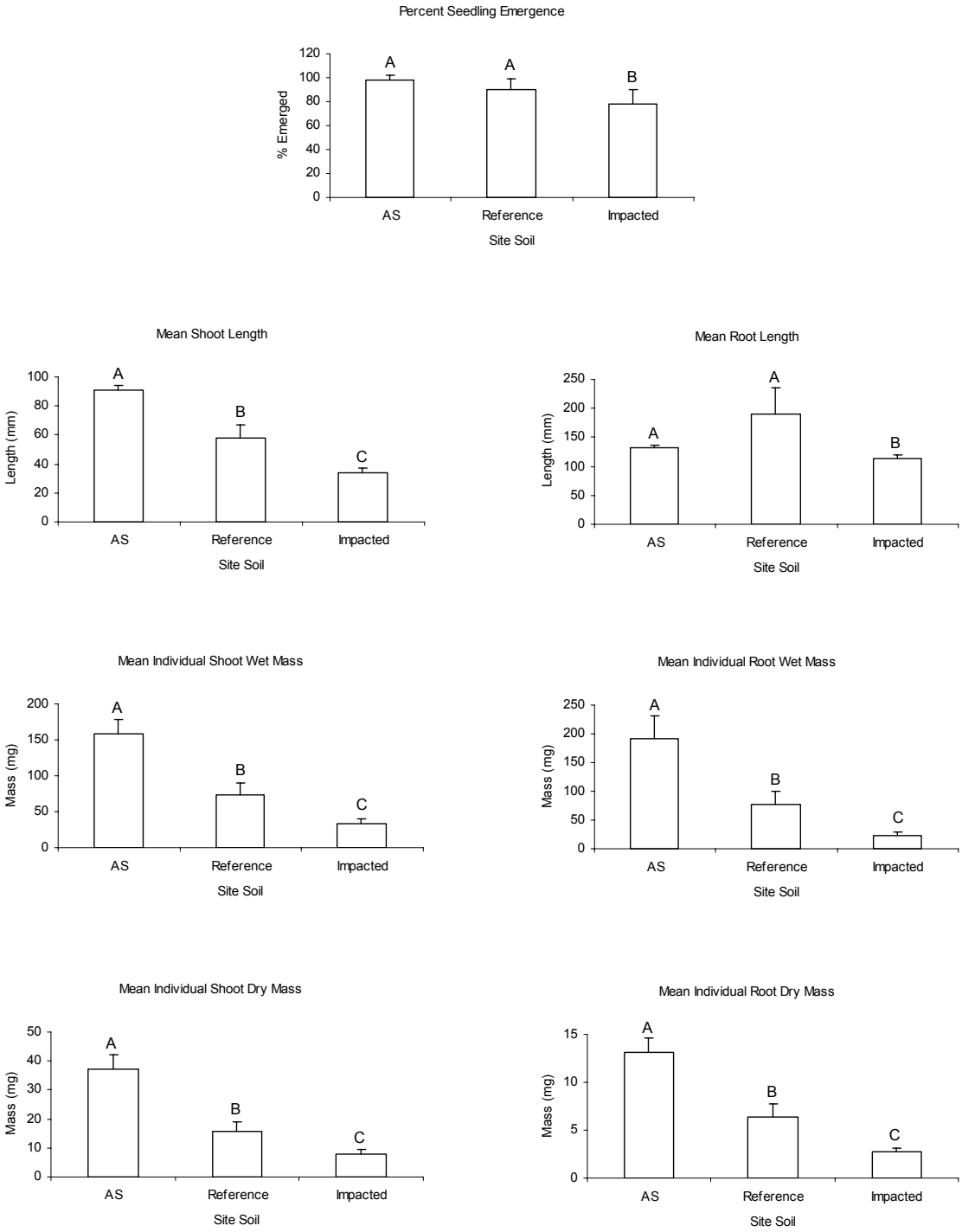

Figure B.1. Alfalfa seedling emergence and growth following 21 days of exposure to artificial soil and to peat-amended and unamended reference and contaminated site soils. Columns indicate treatment means. Bars above the columns represent one standard deviation of the mean. Letters that are different among treatment means indicate significant differences among the means at $P < 0.05$.

Soil Characteristics

Table B.2. Physical and chemical characteristics of test soils at test initiation (Day 0) and test end for alfalfa (Day 21).

Soil Type	Initial pH[1]	Final pH[1]	Initial Conductivity [1] (µS/cm)	Final Conductivity[1] (µS/cm)	Initial Soil Moisture (% WHC[2])	Initial Soil Moisture (% moisture content[3])
Artificial Soil	6.99	6.73	138	370	69	35
Reference Soil	7.12	6.85	268	2240	49	22
Impacted Soil	6.85	6.59	419	1400	79	23

[1] pH and conductivity were measured using a 2:1 water:soil slurry

[2] % WHC - percent of water-holding capacity of the soil

[3] % soil moisture content = (wet wt – dry wt / wet wt) x 100 This is an alternate measure of soil moisture of site soils that can be used when %WHC values do not make sense (e.g., > 100%). %WHC values > 100% are an artifact of the method used to derive %WHC, which calculates % moisture content as %mc = (wet wt – dry wt/dry wt) x 100.

Modifications of Test Protocol

1. Soil pH was measured using a soil-water slurry, which represents our normal practices and is a method modified from the Soil Analysis Handbook (1992), instead of using a $CaCl_2$ slurry, as recommended by Environment Canada (2005a). This had no impact on the results of the test. The method of using $CaCl_2$ was developed for soil scientists who were comparing the pH of different soils, and wished to minimize the variability of the pH measurements (McKeague, 1978). As a result, the $CaCl_2$ method will, by design, minimize the variability of the soil pH among soil samples, and will be less sensitive to differences in pH. In addition, soil pH measured in water is considered to be the pH closest to the pH of soil solution in the field (Hendershot *et al*., 1993).

Appendix B: Data Summary

Barley Definitive Screening Test with Peat-amended Reference and PHC/As-Contaminated Site Soils

Sample Identification	
Client:	WorleyParsons Komex
Sample description:	Reference and contaminated site soils collected from Alberta
Date received:	December 6 2005
Date tested:	January 10 – January 24, 2006
Method of soil collection:	Grab samples
Soil storage:	20°C
Technicians:	Kelly Olaveson, Jill Crumb, Jessica Rahn
Analyst:	Kelly Olaveson, Natalie Feisthauer
QA/QC:	Gladys Stephenson
Test Organism:	Barley (*Hordeum vulgare* var. Chapais)
Organism Source:	Rosebank Seed Farms, Staffa, ON

Test Conditions and Procedures	
Test type:	Static, definitive
Test duration:	14 days
Number of treatments:	5, including 1 experimental control (AS)
Temperature:	22.8 ± 1.0°C (day) 15.2 ± 2.0°C (night)
Light intensity:	272 ± 38 µmol/(m^2·s)
Photoperiod:	16 h light; 8 h dark
Watering regime:	De-chlorinated municipal tap water, as required
Test unit description:	1-L clear polypropylene container
Soil mass/test unit:	500 g wet weight
No. organisms per test unit:	10
No. replicate test units/treatment:	5 replicates
Measured soil chemistry parameters:	Initial soil pH, electrical conductivity, and percent moisture content, final soil pH and electrical conductivity
Measured endpoint(s):	Day 14: Seedling emergence, shoot and root lengths, shoot and root wet and dry masses
Test Protocol:	Biological Test Method: Test for Measuring Emergence and Growth of Terrestrial Plants Exposed to Contaminants in Soil. Report EPS 1/RM/45, February 2005. Method Development and Applications Section, Environmental Technology Centre, Environment Canada, Ottawa, Ontario.
Test acceptability criteria met:	Yes

Results

Barley Seedling Emergence and Growth

Table B.3. Effect of 14-day exposure of barley to artificial soil and to peat-amended and unamended reference and contaminated site soils. Results are reported as treatment means, and the values in brackets indicate one standard deviation of the mean.

Soil Type	Percent Emergence (n = 5 seeds)	Shoot Length (mm)	Root Length (mm)	Individual Shoot Wet Mass (mg)	Individual Shoot Dry Mass (mg)	Individual Root Wet Mass (mg)	Individual Root Dry Mass (mg)
Artificial Soil	100 (0)	241.6 (4.0)	354.4 (14.6)	968.91 (83.60)	144.94 (15.02)	1587.29 (137.05)	102.68 (6.71)
Reference Soil	93 (10)	138.0 (8.41)	223.0 (23.8)	196.32 (8.73)	32.64 (2.91)	242.83 (43.50)	27.31 (2.96)
Impacted Soil	100 (0)	127.4 (3.2)	155.1 (15.6)	176.75 (15.75)	30.65 (2.88)	168.84 (34.39)	20.95 (3.41)

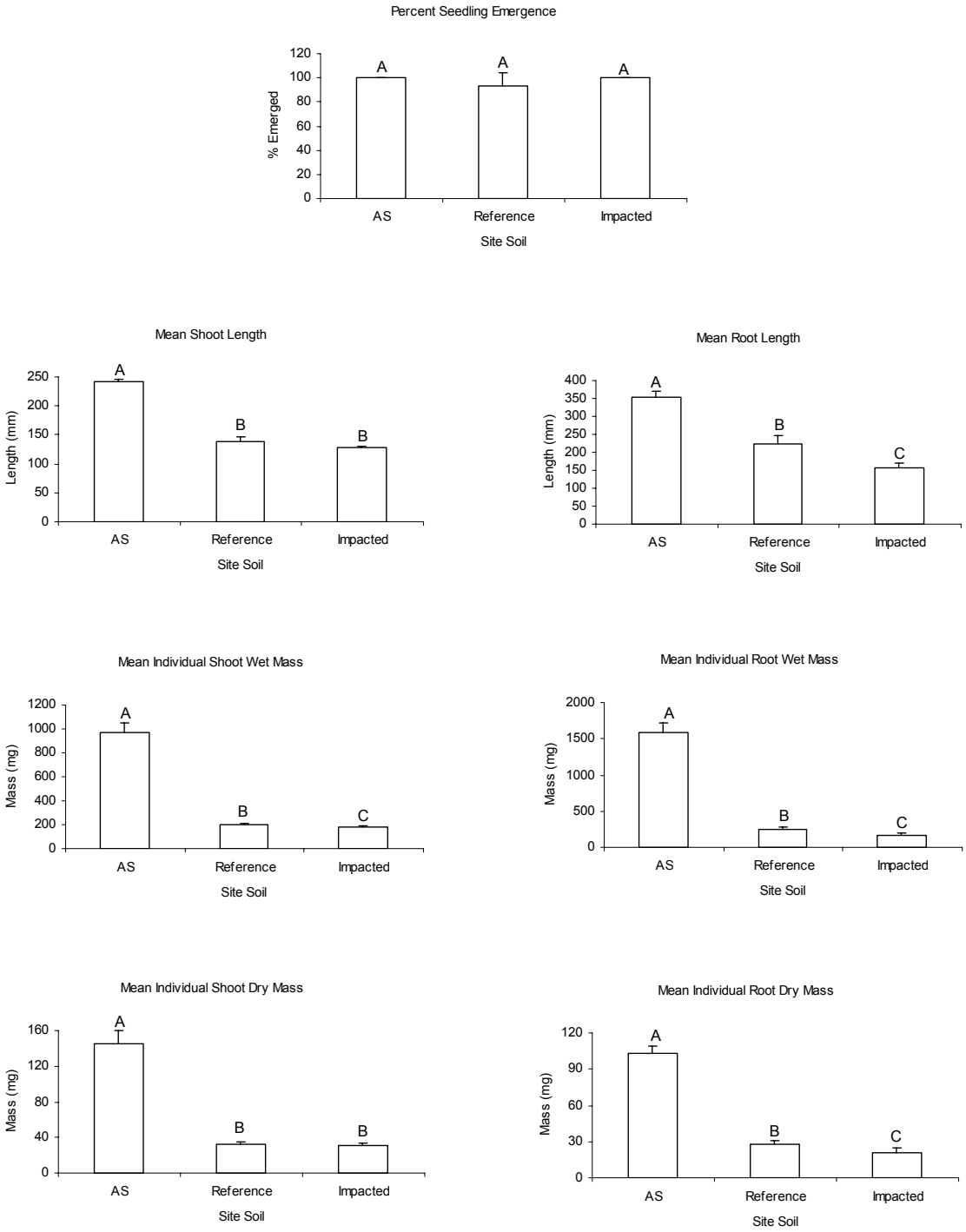

Figure B.2. Barley seedling emergence and growth following 14 days of exposure to artificial soil and to peat-amended and unamended reference and contaminated site soils. Columns indicate treatment means. Bars above the columns represent one standard deviation of the mean. Letters that are different among treatment means indicate significant differences among the means at $P < 0.05$.

Soil Characteristics

Table B.4. Physical and chemical characteristics of test soils at test initiation (Day 0) and test end for barley (Day 14).

Soil Type	Initial pH[1]	Final pH[1]	Initial Conductivity [1] (µS/cm)	Final Conductivity[1] (µS/cm)	Initial Soil Moisture (% WHC[2])	Initial Soil Moisture (% moisture content[3])
Artificial Soil	6.99	6.34	138	276	69	35
Reference Soil	7.12	6.83	268	2160	49	22
Impacted Soil	6.85	6.72	419	795	79	23

[1] pH and conductivity were measured using a 2:1 water:soil slurry

[2] % WHC - percent of water-holding capacity of the soil

[3] % soil moisture content = (wet wt – dry wt / wet wt) x 100 This is an alternate measure of soil moisture of site soils that can be used when %WHC values do not make sense (e.g., > 100%). %WHC values > 100% are an artifact of the method used to derive %WHC, which calculates % moisture content as %mc = (wet wt – dry wt/dry wt) x 100.

Modifications of Test Protocol

1. Soil pH was measured using a soil-water slurry, which represents our normal practices and is a method modified from the Soil Analysis Handbook (1992), instead of using a $CaCl_2$ slurry, as recommended by Environment Canada (2005a). This had no impact on the results of the test. The method of using $CaCl_2$ was developed for soil scientists who were comparing the pH of different soils, and wished to minimize the variability of the pH measurements (McKeague, 1978). As a result, the $CaCl_2$ method will, by design, minimize the variability of the soil pH among soil samples, and will be less sensitive to differences in pH. In addition, soil pH measured in water is considered to be the pH closest to the pH of soil solution in the field (Hendershot *et al*., 1993).

Appendix B: Data Summary

Northern Wheatgrass Definitive Screening Test with Peat-amended Reference and PHC/As-Contaminated Site Soils

Sample Identification	
Client:	WorleyParsons Komex
Sample description:	Reference and contaminated site soils collected from Alberta
Date received:	December 6 2005
Date tested:	January 17 – February 7, 2006
Method of soil collection:	Grab samples
Soil storage:	20°C
Technicians:	Kelly Olaveson, Jill Crumb, Jessica Rahn
Analyst:	Kelly Olaveson, Natalie Feisthauer
QA/QC:	Gladys Stephenson
Test Organism:	Northern wheatgrass (*Elymus lanceolatus*)
Organism Source:	Pickseed Canada Inc., Sherwood Park, AB

Test Conditions and Procedures	
Test type:	Static, definitive
Test duration:	21 days
Number of treatments:	5, including 1 experimental control (AS)
Temperature:	22.0 ± 1.8°C (day) 14.3 ± 1.2°C (night)
Light intensity:	241 ± 41 µmol/(m^2·s)
Photoperiod:	16 h light; 8 h dark
Watering regime:	De-chlorinated municipal tap water, as required
Test unit description:	1-L clear polypropylene container
Soil mass/test unit:	500 g wet weight
No. organisms per test unit:	10
No. replicate test units/treatment:	5 replicates
Measured soil chemistry parameters:	Initial soil pH, electrical conductivity, and percent moisture content, final soil pH and electrical conductivity
Measured endpoint(s):	Day 21: Seedling emergence, shoot and root lengths, shoot and root wet and dry masses
Test Protocol:	Biological Test Method: Test for Measuring Emergence and Growth of Terrestrial Plants Exposed to Contaminants in Soil. Report EPS 1/RM/45, February 2005. Method Development and Applications Section, Environmental Technology Centre, Environment Canada, Ottawa, Ontario.
Test acceptability criteria met:	Yes

Results

Northern Wheatgrass Seedling Emergence and Growth

Table B.5. Effect of 21-day exposure of northern wheatgrass to artificial soil and to peat-amended and unamended reference and contaminated site soils. Results are reported as treatment means, and the values in brackets indicate one standard deviation of the mean.

Soil Type	Percent Emergence (n = 5 seeds)	Shoot Length (mm)	Root Length (mm)	Individual Shoot Wet Mass (mg)	Individual Shoot Dry Mass (mg)	Individual Root Wet Mass (mg)	Individual Root Dry Mass (mg)
Artificial Soil	97 (8)	188.4 (9.7)	249.2 (9.5)	161.57 (14.59)	39.87 (4.70)	206.78 (51.17)	18.37 (3.07)
Reference Soil	87 (16)	87.3 (6.4)	187.7 (14.9)	16.77 (3.05)	6.18 (1.25)	14.87 (3.55)	3.84 (0.38)
Impacted Soil	60 (18)	64.8 (20.3)	61.8 (17.7)	9.68 (4.71)	3.21 (2.05)	3.86 (3.74)	1.02 (0.80)

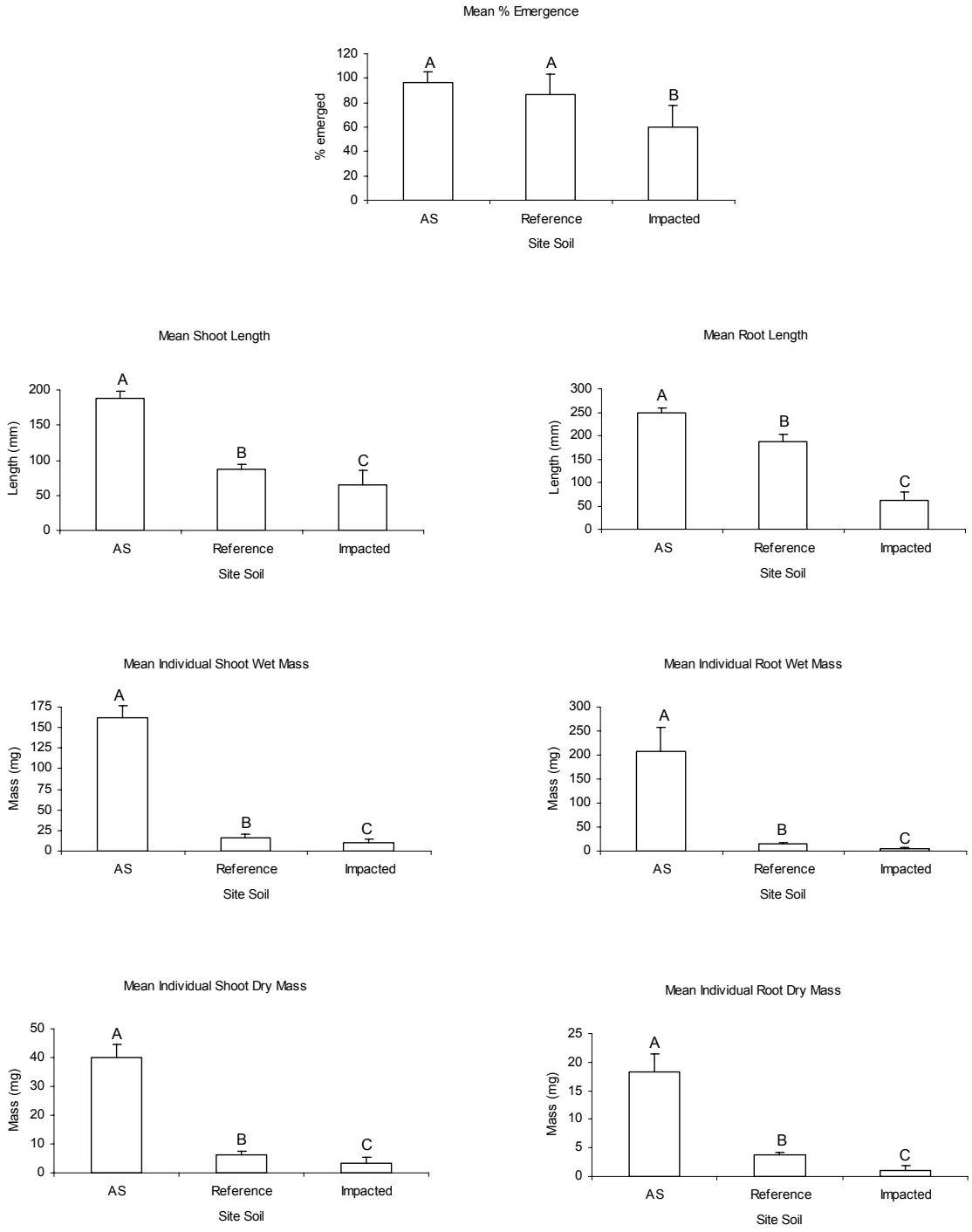

Figure B.3. Northern wheatgrass seedling emergence and growth following 21 days of exposure to artificial soil and to peat-amended and unamended reference and contaminated site soils. Columns indicate treatment means. Bars above the columns represent one standard deviation of the mean. Letters that are different among treatment means indicate significant differences among the means at $P < 0.05$.

Soil Characteristics

Table B.6. Physical and chemical characteristics of test soils at test initiation (Day 0) and test end for northern wheatgrass (Day 21).

Soil Type	Initial pH[1]	Final pH[1]	Initial Conductivity[1] (µS/cm)	Final Conductivity[1] (µS/cm)	Initial Soil Moisture (% WHC[2])	Initial Soil Moisture (% moisture content[3])
Artificial Soil	6.67	6.32	171	1120	63	33
Reference Soil	7.11	7.06	287	1800	55	24
Impacted Soil	6.61	6.58	338	1100	82	24

[1] pH and conductivity were measured using a 2:1 water:soil slurry

[2] % WHC - percent of water-holding capacity of the soil

[3] % soil moisture content = (wet wt – dry wt / wet wt) x 100 This is an alternate measure of soil moisture of site soils that can be used when %WHC values do not make sense (e.g., > 100%). %WHC values > 100% are an artifact of the method used to derive %WHC, which calculates % moisture content as %mc = (wet wt – dry wt/dry wt) x 100.

Modifications of Test Protocol

1. Soil pH was measured using a soil-water slurry, which represents our normal practices and is a method modified from the Soil Analysis Handbook (1992), instead of using a $CaCl_2$ slurry, as recommended by Environment Canada (2005a). This had no impact on the results of the test. The method of using $CaCl_2$ was developed for soil scientists who were comparing the pH of different soils, and wished to minimize the variability of the pH measurements (McKeague, 1978). As a result, the $CaCl_2$ method will, by design, minimize the variability of the soil pH among soil samples, and will be less sensitive to differences in pH. In addition, soil pH measured in water is considered to be the pH closest to the pH of soil solution in the field (Hendershot *et al*., 1993).

2. The Environment Canada test method requires the temperature for a plant test to be 15 $\pm$ 3^{o}C during the night and 24 $\pm$ 3^{o}C during the day. The minimum (day) temperature was slightly (0.8^{o}C) below the required range; the maximum (day and night) and minimum (night) temperatures were within the required range. The average minimum daily temperature was 14.3^{o}C and the average maximum daily temperature was 22.0^{o}C. The plants showed no signs of stress in the experimental negative control treatment at the experimental temperature range. Therefore, the effect on the results of the toxicity test was considered negligible.

Appendix B: Data Summary

Red Clover Definitive Screening Test with Peat-amended Reference and PHC/As-Contaminated Site Soils

Sample Identification	
Client:	WorleyParsons Komex
Sample description:	Reference and contaminated site soils collected from Alberta
Date received:	December 6 2005
Date tested:	January 17 – January 31, 2006
Method of soil collection:	Grab samples
Soil storage:	20°C
Technicians:	Kelly Olaveson, Jill Crumb, Jessica Rahn
Analyst:	Kelly Olaveson, Natalie Feisthauer
QA/QC:	Gladys Stephenson
Test Organism:	Red clover (*Trifolium pratense*)
Organism Source:	William Dam Seeds, Dundas, ON

Test Conditions and Procedures	
Test type:	Static, definitive
Test duration:	14 days
Number of treatments:	5, including 1 experimental control (AS)
Temperature:	22.0 ± 1.8°C (day) 14.3 ± 1.2°C (night)
Light intensity:	241 ± 41µmol/(m^2·s)
Photoperiod:	16 h light; 8 h dark
Watering regime:	De-chlorinated municipal tap water, as required
Test unit description:	1-L clear polypropylene container
Soil mass/test unit:	500 g wet weight
No. organisms per test unit:	10
No. replicate test units/treatment:	5 replicates
Measured soil chemistry parameters:	Initial soil pH, electrical conductivity, and percent moisture content, final soil pH and electrical conductivity
Measured endpoint(s):	Day 14: Seedling emergence, shoot and root lengths, shoot and root wet and dry masses
Test Protocol:	Biological Test Method: Test for Measuring Emergence and Growth of Terrestrial Plants Exposed to Contaminants in Soil. Report EPS 1/RM/45, February 2005. Method Development and Applications Section, Environmental Technology Centre, Environment Canada, Ottawa, Ontario.
Test acceptability criteria met:	Yes

Results

Red Clover Seedling Emergence and Growth

Table B.7. Effect of 14-day exposure of red clover to artificial soil and to peat-amended and unamended reference and contaminated site soils. Results are reported as treatment means, and the values in brackets indicate one standard deviation of the mean.

Soil Type	Percent Emergence (n = 5 seeds)	Shoot Length (mm)	Root Length (mm)	Individual Shoot Wet Mass (mg)	Individual Shoot Dry Mass (mg)	Individual Root Wet Mass (mg)	Individual Root Dry Mass (mg)
Artificial Soil	87 (16)	67.2 (5.7)	142.8 (19.8)	218.97 (19.68)	45.83 (5.47)	274.08 (71.59)	16.72 (4.89)
Reference Soil	80 (18)	38.1 (4.4)	161.1 (26.5)	57.23 (11.60)	11.63 (2.59)	46.22 (6.48)	4.44 (0.76)
Impacted Soil	77 (32)	29.8 (5.1)	104.1 (14.7)	30.68 (5.60)	6.74 (1.90)	25.36 (6.69)	3.03 (0.97)

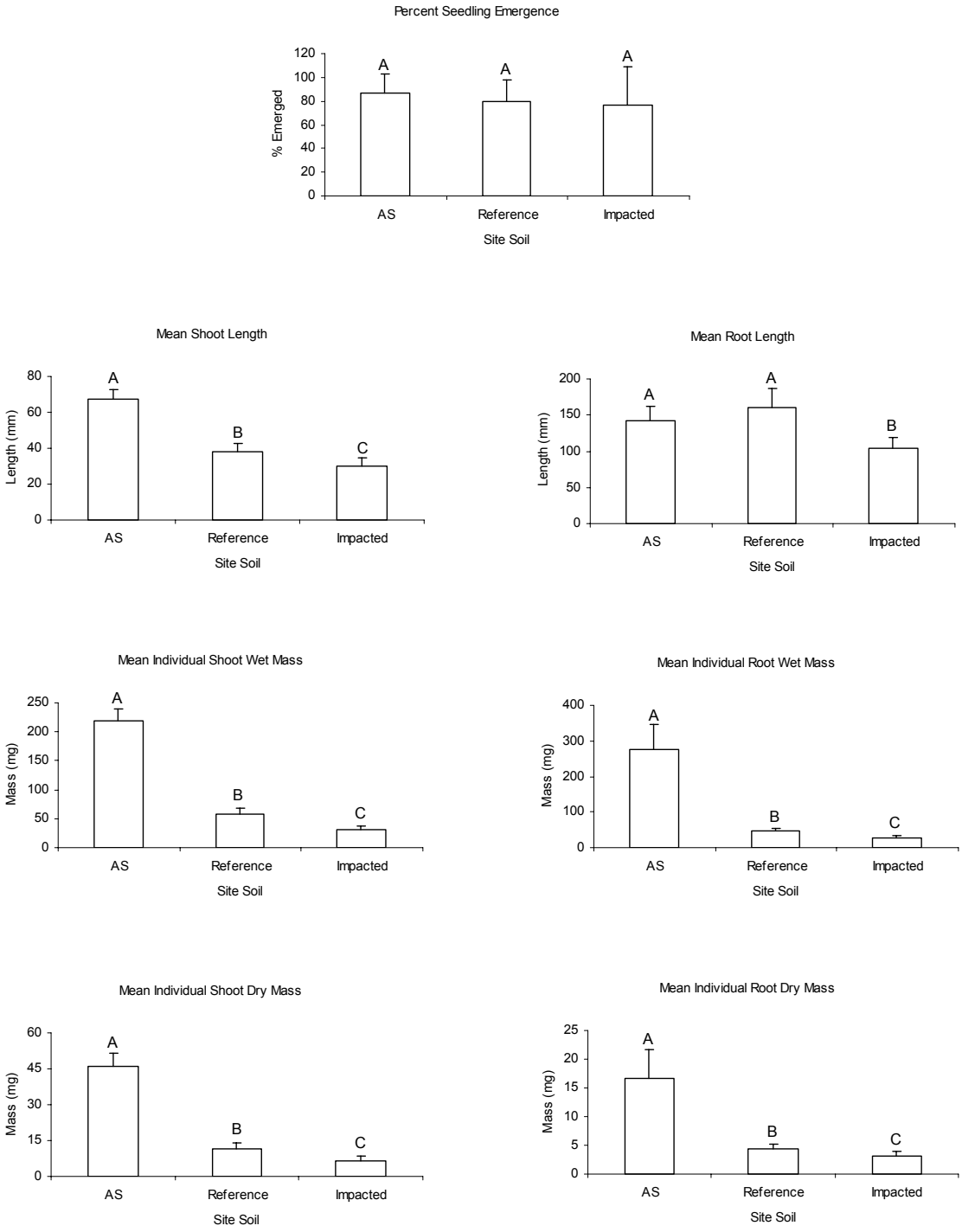

Figure B.4. Red clover seedling emergence and growth following 14 days of exposure to artificial soil and to peat-amended and unamended reference and contaminated site soils. Columns indicate treatment means. Bars above the columns represent one standard deviation of the mean. Letters that are different among treatment means indicate significant differences among the means at $P < 0.05$.

Soil Characteristics

Table B.8. Physical and chemical characteristics of test soils at test initiation (Day 0) and test end for northern wheatgrass (Day 21).

Soil Type	Initial pH[1]	Final pH[1]	Initial Conductivity[1] (µS/cm)	Final Conductivity[1] (µS/cm)	Initial Soil Moisture (% WHC[2])	Initial Soil Moisture (% moisture content[3])
Artificial Soil	6.67	6.67	171	235	63	33
Reference Soil	7.11	6.87	287	1440	55	24
Impacted Soil	6.61	6.50	338	1010	82	24

[1] pH and conductivity were measured using a 2:1 water:soil slurry

[2] % WHC - percent of water-holding capacity of the soil

[3] % soil moisture content = (wet wt – dry wt / wet wt) x 100 This is an alternate measure of soil moisture of site soils that can be used when %WHC values do not make sense (e.g., > 100%). %WHC values > 100% are an artifact of the method used to derive %WHC, which calculates % moisture content as %mc = (wet wt – dry wt/dry wt) x 100.

Modifications of Test Protocol

1. Soil pH was measured using a soil-water slurry, which represents our normal practices and is a method modified from the Soil Analysis Handbook (1992), instead of using a $CaCl_2$ slurry, as recommended by Environment Canada (2005a). This had no impact on the results of the test. The method of using $CaCl_2$ was developed for soil scientists who were comparing the pH of different soils, and wished to minimize the variability of the pH measurements (McKeague, 1978). As a result, the $CaCl_2$ method will, by design, minimize the variability of the soil pH among soil samples, and will be less sensitive to differences in pH. In addition, soil pH measured in water is considered to be the pH closest to the pH of soil solution in the field (Hendershot *et al*., 1993).

2. The Environment Canada test method requires the temperature for a plant test to be 15 $\pm$ 3^{o}C during the night and 24 $\pm$ 3^{o}C during the day. The minimum (day) temperature was slightly (0.8^{o}C) below the required range; the maximum (day and night) and minimum (night) temperatures were within the required range. The average minimum daily temperature was 14.3^{o}C and the average maximum daily temperature was 22.0^{o}C. The plants showed no signs of stress in the experimental negative control treatment at the experimental temperature range. Therefore, the effect on the results of the toxicity test was considered negligible.

Appendix B: Data Summary

Red Fescue Definitive Screening Test with Peat-amended Reference and PHC/As-Contaminated Site Soils

Sample Identification	
Client:	WorleyParsons Komex
Sample description:	Reference and contaminated site soils collected from Alberta
Date received:	December 6 2005
Date tested:	January 10 – January 31, 2006
Method of soil collection:	Grab samples
Soil storage:	20°C
Technicians:	Kelly Olaveson, Jill Crumb, Jessica Rahn
Analyst:	Kelly Olaveson, Natalie Feisthauer
QA/QC:	Gladys Stephenson
Test Organism:	Red fescue (*Festuca rubra*)
Organism Source:	William Dam Seeds, Dundas, ON

Test Conditions and Procedures	
Test type:	Static, definitive
Test duration:	21 days
Number of treatments:	5, including 1 experimental control (AS)
Temperature:	22.8 ± 1.0°C (day) 15.2 ± 2.0°C (night)
Light intensity:	272 ± 38 µmol/(m^2·s)
Photoperiod:	16 h light; 8 h dark
Watering regime:	De-chlorinated municipal tap water, as required
Test unit description:	1-L clear polypropylene container
Soil mass/test unit:	500 g wet weight
No. organisms per test unit:	10
No. replicate test units/treatment:	5 replicates
Measured soil chemistry parameters:	Initial soil pH, electrical conductivity, and percent moisture content, final soil pH and electrical conductivity
Measured endpoint(s):	Day 21: Seedling emergence, shoot and root lengths, shoot and root wet and dry masses
Test Protocol:	Biological Test Method: Test for Measuring Emergence and Growth of Terrestrial Plants Exposed to Contaminants in Soil. Report EPS 1/RM/45, February 2005. Method Development and Applications Section, Environmental Technology Centre, Environment Canada, Ottawa, Ontario.
Test acceptability criteria met:	Yes: % survival, % phytotoxicity, shoot and root length No: % emergence (must be > 70%). Emergence was 60%

Results

Red Fescue Seedling Emergence and Growth

Table B.9. Effect of 21-day exposure of red fescue to artificial soil and to peat-amended and unamended reference and contaminated site soils. Results are reported as treatment means, and the values in brackets indicate one standard deviation of the mean.

Soil Type	Percent Emergence (n = 5 seeds)	Shoot Length (mm)	Root Length (mm)	Individual Shoot Wet Mass (mg)	Individual Shoot Dry Mass (mg)	Individual Root Wet Mass (mg)	Individual Root Dry Mass (mg)
Artificial Soil	60 (22)	125.2 (22.3)	129.7 (19.7)	85.20 (21.56)	17.16 (4.52)	45.72 (18.60)	5.55 (1.77)
Reference Soil	80 (13)	63.0 (12.0)	90.5 (24.5)	6.43 (1.80)	1.81 (0.51)	3.62 (2.83)	1.10 (0.26)
Impacted Soil	40 (13)	48.2 (7.3)	61.0 (10.2)	3.70 (0.87)	0.96 (0.39)	1.11 (0.70)	0.21 (0.41)

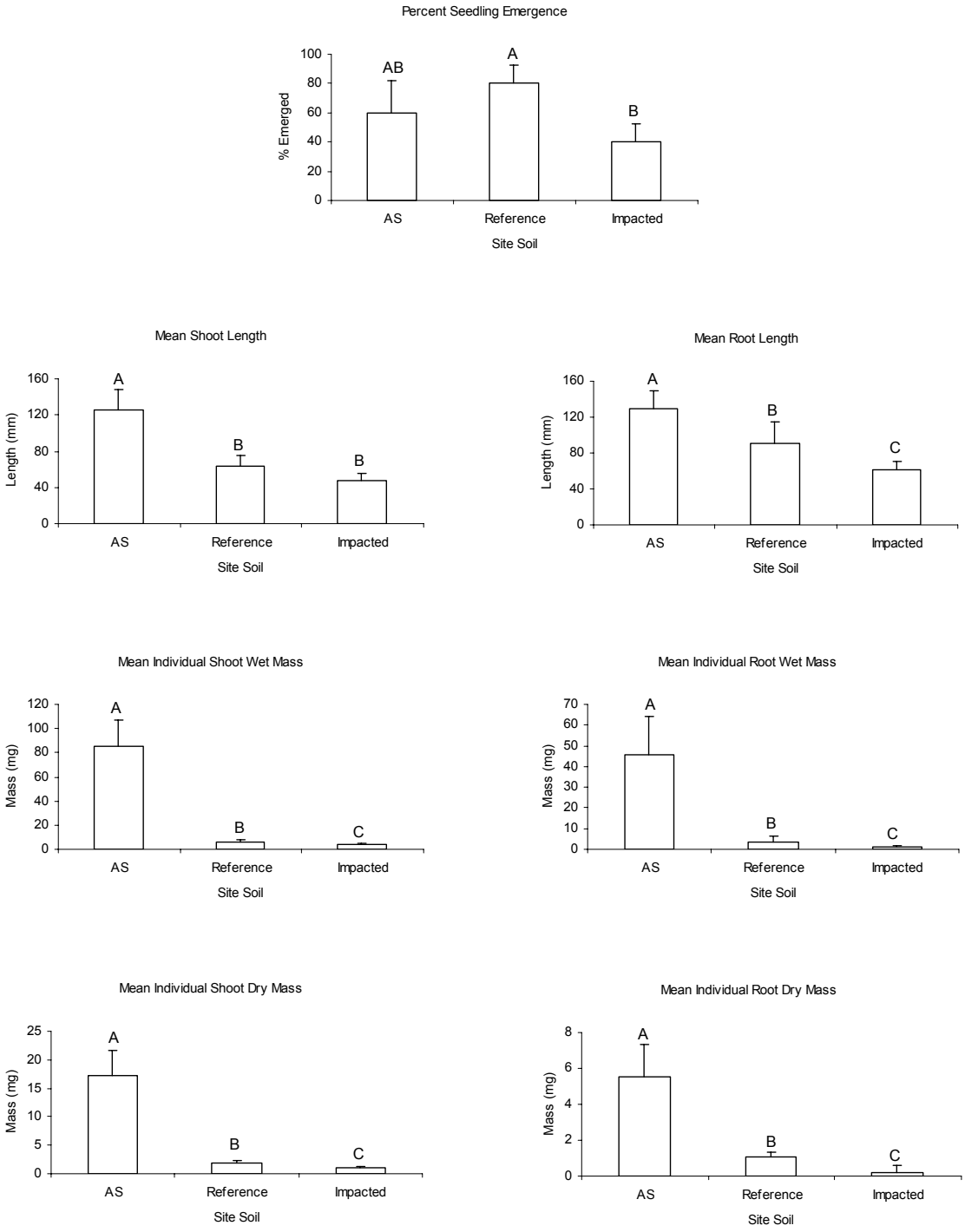

Figure B.5. Red fescue seedling emergence and growth following 21 days of exposure to artificial soil and to peat-amended and unamended reference and contaminated site soils. Columns indicate treatment means. Bars above the columns represent one standard deviation of the mean. Letters that are different among treatment means indicate significant differences among the means at $P < 0.05$.

Soil Characteristics

Table B.10. Physical and chemical characteristics of test soils at test initiation (Day 0) and test end for red fescue (Day 21).

Soil Type	Initial pH[1]	Final pH[1]	Initial Conductivity [1] (µS/cm)	Final Conductivity[1] (µS/cm)	Initial Soil Moisture (% WHC[2])	Initial Soil Moisture (% moisture content[3])
Artificial Soil	6.99	6.82	138	584	69	35
Reference Soil	7.12	6.59	268	1620	49	22
Impacted Soil	6.85	6.55	419	2190	79	23

[1] pH and conductivity were measured using a 2:1 water:soil slurry

[2] % WHC - percent of water-holding capacity of the soil

[3] % soil moisture content = (wet wt – dry wt / wet wt) x 100 This is an alternate measure of soil moisture of site soils that can be used when %WHC values do not make sense (e.g., > 100%). %WHC values > 100% are an artifact of the method used to derive %WHC, which calculates % moisture content as %mc = (wet wt – dry wt/dry wt) x 100.

Modifications of Test Protocol

1. The validity criterion for percent emergence in the negative control soil was not met in this test; emergence should be ≥ 70%; percent emergence in the negative control soil was 60%. However, all emerged seedlings appeared healthy and vigorous and all other validity criteria were met. Seedling emergence is in general poorly correlated to soil toxicity (or lack thereof) and therefore the effect on the results of the toxicity test was considered negligible.

2. Soil pH was measured using a soil-water slurry, which represents our normal practices and is a method modified from the Soil Analysis Handbook (1992), instead of using a $CaCl_2$ slurry, as recommended by Environment Canada (2005a). This had no impact on the results of the test. The method of using $CaCl_2$ was developed for soil scientists who were comparing the pH of different soils, and wished to minimize the variability of the pH measurements (McKeague, 1978). As a result, the $CaCl_2$ method will, by design, minimize the variability of the soil pH among soil samples, and will be less sensitive to differences in pH. In addition, soil pH measured in water is considered to be the pH closest to the pH of soil solution in the field (Hendershot et al., 1993).

Appendix B: Data Summary

Eisenia andrei Chronic Screening Test with Peat-amended Reference and PHC/As-Contaminated Site Soils

Sample Identification	
Client:	WorleyParsons Komex
Sample description:	Reference and contaminated site soils collected from Alberta
Date received:	December 6 2005
Date tested:	February 22 – April 26, 2006
Method of soil collection:	Grab samples
Soil storage:	20°C
Technicians:	Kelly Olaveson, Jill Crumb, Jessica Rahn, Natalie Feisthauer
Analyst:	Kelly Olaveson, Natalie Feisthauer
QA/QC:	Gladys Stephenson
Test Organism:	*Eisenia andrei*
Organism Source:	In culture

Test Conditions and Procedures	
Test type:	Static, chronic
Test duration:	63 days
Number of treatments:	5, including 1 experimental control (AS)
Temperature:	23.2 to 18.3 °C (24 hours)
Light intensity:	591 (± 174) lux
Photoperiod:	16 h light; 8 h dark
Watering regime:	de-ionized water, misted every 14 days, as required
Test unit description:	500-mL glass wide-mouthed mason jar
Soil volume/test unit:	~ 300 mL (3/4 of volume of test unit)
No. organisms per test unit:	2
No. replicate test units/treatment:	10
Measured soil chemistry parameters:	Initial and final soil pH, electrical conductivity, and percent moisture content
Measured endpoint(s):	Day 35 adult survival, number of progeny produced at Day 63, and wet and dry mass of individual juveniles at Day 63
Test Protocol:	Biological Test Method: Tests for Toxicity of Contaminated Soil to Earthworms (Eisenia andrei, Eisenia fetida, or Lumbricus terrestris). Report EPS 1/RM/43, June 2004. Method Development and Applications Section, Environmental Technology Centre, Environment Canada, Ottawa, Ontario.
Test acceptability criteria met:	Yes

Results

Eisenia andrei Reproduction and Chronic Survival

Table B.11. *Eisenia andrei* survival and reproduction following 63 days of exposure to artificial soil and to peat-amended and unamended reference and contaminated ("impacted") site soils. Results are reported as treatment means, and the values in brackets indicate one standard deviation of the mean.

Soil	Mean 35-d Adult Survival (n = 20)	Mean Number of Progeny	Mean Individual Wet Mass of Progeny (mg)	Mean Individual Dry Mass of Progeny (mg)
Artificial Soil	2.0 (0)	14.1 (10.7)	44.11 (26.41)	9.06 (5.83)
Reference Soil	1.9 (0.3)	4.5 (5.9)	14.28 (10.21)	2.94 (1.50)
Reference Soil - 4% peat	1.8 (0.4)	3.2 (4.7)	29.45 (14.68)	5.43 (2.14)
Impacted Soil	1.8 (0.4)	0	NA	NA
Impacted Soil – 4% peat	2.0 (0)	0	NA	NA

NA Data not available because there were no progeny produced in the treatment

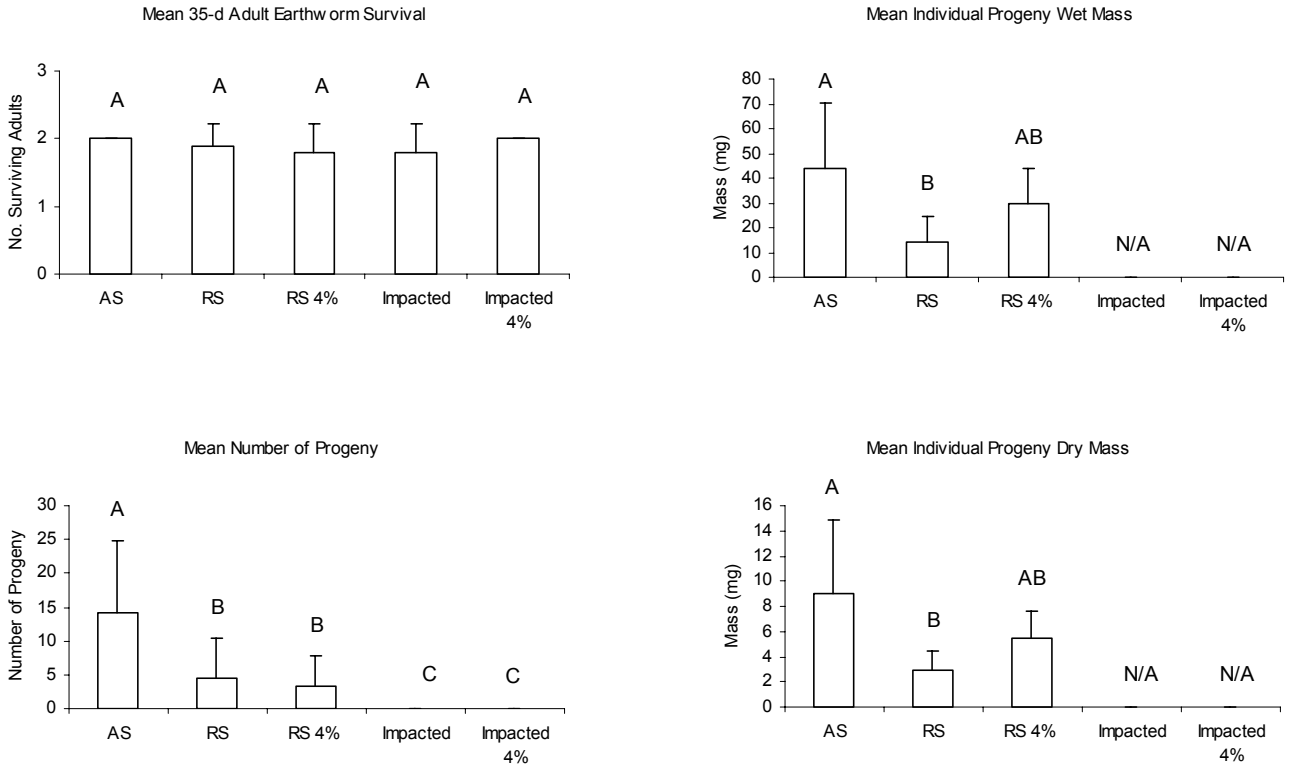

Figure B.6. *Eisenia andrei* survival and reproduction following 63 days of exposure to artificial soil and to peat-amended and unamended reference and contaminated ("impacted") site soils. Columns indicate treatment means. Bars above the columns represent one standard deviation of the mean. Letters that are different among treatment means indicate significant differences among the means at $P < 0.05$.

Soil Characteristics

Table B.12. Physical and chemical characteristics of test soils at test initiation (Day -1) and test end for *Eisenia andrei* (Day 63).

Soil Type	Initial pH[1]	Final pH[1]	Initial Conductivity[1] (µS/cm)	Final Conductivity[1] (µS/cm)	Initial Soil Moisture[2] (% WHC)	Final Soil Moisture[2] (% WHC)	Initial Soil Moisture[3] (% moisture content)	Final Soil Moisture[3] (% moisture content)
AS	7.18	6.93	283	214	85	92	40	41
RS	7.11	6.85	346	264	41	63	20	27
RS - 4% peat	7.53	7.59	372	325	NA	NA	31	32
IS	6.51	6.77	435	390	100	123	25	29
IS – 4% peat	7.37	7.63	501	390	NA	NA	25	33

AS Artificial Soil
RS Reference Soil
IS Impacted Soil
[1] pH and conductivity were measured using a 2:1 water:soil slurry
[2] % WHC - percent of water-holding capacity of the soil
[3] % soil moisture content = (wet wt – dry wt / wet wt) x 100 This is an alternate measure of soil moisture of site soils that can be used when %WHC values do not make sense (e.g., > 100%). %WHC values > 100% are an artifact of the method used to derive %WHC, which calculates % moisture content as %mc = (wet wt – dry wt/dry wt) x 100.
NA Data not available due to the limited volume of soil. Soil moisture was instead measured as % moisture content

Modifications of Test Protocol

1. Soil pH measurements were taken using a soil-water slurry, which represents our normal practices and is a method modified from the Soil Analysis Handbook (1992), instead of using a $CaCl_2$ slurry, as recommended by the method for pH. This had no impact on the results of the test. The method of using $CaCl_2$ was developed for soil scientists who were comparing the pH of different soils, and wished to minimize the variability of the different pHs (McKeague, 1978). As a result, the $CaCl_2$ method will, by design, minimize the variability of the soil pH among soil samples, and will be less sensitive to differences in pH. In addition, soil pH measured in water is considered to be the pH closest to the pH of soil solution in the field (Hendershot *et al*., 1993).

2. The Environment Canada test method requires the temperature for an earthworm test be 20 $\pm$ 2^{o}C over a 24-h period. The temperatures recorded for this earthworm test ranged from 18.3 to 23.2^{o}C. The maximum temperature was slightly (1.2^{o}C) above the required range; the minimum temperature was within the required range. The average minimum daily temperature was 18.6^{o}C and the average maximum daily temperature was 21.9^{o}C. This species is known to tolerate a wide range in temperature. In addition, the earthworms showed no signs of stress in the experimental negative control treatments at the experimental temperature range. Therefore, we conclude that the effect on the results of the toxicity test was negligible.