TOXICITY ASSESSMENT OF BTEX COMPOUNDS

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1. INTRODUCTION

The Canadian Council of Ministers of the Environment (CCME) Petroleum Hydrocarbon (PHC) Development Committee (DEVCOM) recently provided the scientific rationale for proposed Canada-wide Standards (CWS) for petroleum hydrocarbons (PHCs) in soil (CCME, 2000). These CWS were derived on a fraction-specific basis for different land uses and soil types, with consideration of the presence or absence of a groundwater exposure pathway and whether the location of the soil contamination was surface or subsurface. The four fractions for which standards were derived included the fraction of carbon constituents ranging from >C5-C10 (F1), nC10-C16 (F2), C17-C34 (F3), and >C34 (F4). In reality, F1 includes benzene, toluene, ethylbenzene, and xylene (BTEX); however, national and provincial standards for these compounds in soil already exist. It was decided that the BTEX compounds would be regulated based on the existing standards and, therefore, BTEX were not included in F1 (e.g., F1-BTEX).

The toxicity of fraction one was never determined directly; a surrogate mixture of additive-free motor gasoline (mogas) was evaluated along with naphthalene and hexane, all of which were highly volatile and believed to be representative of F1. The CWS for F1 were derived using the toxicity and effects data generated from tests with mogas, naphthalene, hexane, and the relevant literature values. BTEX comprised a significant portion of the mogas mixture (ESG, 2000a) and a significant part of the toxicity associated with mogas was likely attributable to the presence of these compounds.

There are established federal and provincial standards (e.g., guidelines) for each of the BTEX compounds. These standards were derived using an effects-based process and toxicity data from studies conducted in the early 1990s. Test procedures and methods for terrestrial toxicity testing were, at that time, in their infancy. Since then, test methods and procedures for assessing the toxicity of contaminants in soils to terrestrial organisms have been developed by Environment Canada (EC 1998a, 1998b, 1998c). The guidance provided in these test methods includes procedures and conditions specific to testing highly volatile organic substances, such as BTEX. In addition to methodological improvements, the statistical procedures used to describe the concentration-response relationships have also improved. Non-linear and linear regression models have been parameterized to include the determination of any ICp and the associated 95 % confidence intervals (Stephenson *et al.* 2000; EC 2001). These advances in the science of toxicity assessment have resulted in better tools with which to evaluate the fate and effects of contaminants in soils and, consequently, there is less uncertainty associated with the accuracy and precision of the estimates of toxicity.

Concurrent with the technical improvements describe above, the approach for deriving soil quality guidelines (SQGs) has also advanced toward risk-based derivation of standards from effects-based standards. The process used to derive the new CWS for PHCs was unique and involved a rank sensitivity analyses of the response data for the different fractions or fraction surrogates. Therefore, the existing BTEX standards for soils were derived using an effects-based process and data generated from tests with "inferior" methodologies. As a result, there is some concern (e.g., uncertainty) regarding the merit (i.e., the capacity to protect soil environments) of the existing BTEX soil standards.

The CCME, under the direction of Dr. Ted Nason (Alberta Environment) contracted ESG International to conduct a battery of acute toxicity tests with the earthworm (*Eisenia andrei*) and a species of plant known to be sensitive to hydrocarbons, northern wheatgrass (*Agropyron dasystachyum*). The test organisms were exposed to a series of exposure concentrations of each of the BTEX compounds in two soil types, a formulated sandy loam soil and a field-collected clay loam soil. The test methods used to assess the toxicity of BTEX were those currently recommended by Environment Canada. The results of the terrestrial toxicity tests with BTEX are summarized in this report.

2. MATERIALS AND METHODS

Acute screening toxicity tests were conducted with two terrestrial species, an earthworm species, *Eisenia andrei* and a plant species, northern wheatgrass (*Agropyron dasystachyum*). Tests with both species were conducted in two types of soil, a formulated sandy loam soil and a field-collected reference soil. The earthworms were obtained from in-house laboratory cultures at ESG International Inc. and the plant seed was purchased from Pickseed Canada Inc., Sherwood Park, AB. The BTEX compounds were purchased from Fisher Scientific and were certified (ethylbenzene) or certified ACS (benzene, toluene, and xylene) grade.

2.1 Control Soils

2.1.1 Sandy Loam Soil (AS)

The sandy loam soil used as a control soil in the acute tests with BTEX was an artificial control soil (AS) formulated in the laboratory by mixing thoroughly the ingredients in their dry form, then gradually hydrating with de-ionized water, and mixing again until the soil was visibly uniform in colour and texture. The ingredients of the AS were 70 % silica sand (No. 200, Barco 71; Barnes Environmental International, Waterdown, ON), 20 % kaolinite clay (Feldspar Corporation, Atlanta, GA), 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 kaolin, 1 kg of sieved (2-mm) peat, approximately 30 g of $CaCO_3$, and 2 L of de-ionized water. The amount of calcium carbonate required to achieve a soil pH in the range of 6-7, depended on the nature (i.e., acidity) of the sphagnum peat or 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 the formulation. Further reference to the formulation of artificial soil can be found in the ASTM Standard Guide E 1676-97 (ASTM, 1997). The physico-chemical characteristics of the artificial soil are listed in Table A.1, Appendix A.

2.1.2 Reference Soil (RS)

The field-collected control or reference soil (RS) originated from an area that had not been cultivated in the last 25 years and had not been, to the best of our knowledge, subjected to any direct application of pesticides during that time. The reference soil was a clay loam from Alberta, and was classified as a Delacour Orthic Black Chernozem. It was a fine loam soil with a relatively high organic matter content of 12.8 %. The reference soil was virtually free of any contaminants (Komex International, 1995). The physico-chemical characteristics of the reference soil are listed in Table A.1, Appendix A.

2.2 Determination of Soil Moisture Content

On the day prior to test soil formulation (e.g., Day-1 for plants and Day-2 for earthworms), a 3- to 5-g sample of each control soil wet weight (w.w.) was placed into a weighed aluminium pan (1 or 2.5 g) and the wet mass recorded. The pans were then placed into a drying oven at 90°C for a minimum of 24 h. The dry sample of each control soil was then determined. Percent moisture content was calculated by expressing the dry mass as a percentage of the wet mass:

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

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

2.3 Experimental Design

2.3.2 Earthworm Tests

Acute toxicity tests were conducted with *Eisenia andrei* to measure the effects of the individual BTEX compounds in both artificial and reference soils, on adult survival. Nominal exposure concentrations, test durations, and number of replicates per treatment were identical among all tests except benzene (Table 2.1). A second 14-d acute test was conducted, with benzene only, in both AS and RS using different nominal concentrations (Table 2.1).

The preparation of the test soils, the test units, and the test conditions in the second benzene tests were identical to those of the first tests with benzene. However, test methods deviated in how the test organisms were placed into the test units. Prior to the addition to a test unit, five clitellated *E. andrei* were removed from the cultures, washed with de-ionized water, and placed onto a tray lined with paper towel to remove excess water. The metal screw ring from a test unit was unscrewed, and the metal lid was held in place with a gloved hand. The washed earthworms were gently scooped up with forceps and transferred to the test unit with minimal opening of the lid (i.e., the lid was tipped open). After placing the worms into the test unit, the lid was quickly replaced. The metal lid was then immediately replaced with aluminium foil, and the test unit was resealed with the metal screw ring. The earthworms were added to each test unit independently across the treatment levels in order to minimize volatilization of the benzene. This is in contrast to the previous method whereby all the lids were removed from random replicates across a treatment level simultaneously. The earthworms were then added to each of the test units, and after all of the earthworms had been placed into all of the test units, aluminium foil was placed onto the test unit, and the test units were sealed one after the other with the metal screw rings.

A test unit for acute tests with earthworms consisted of 270 g w.w. (at 35% moisture content) of soil in a covered 500-mL glass, wide-mouth, mason jar. The cover for the test containers was aluminium foil held on with the metal screw ring. The aluminium foil was kept intact for the first 7 days of the experiment, after which, 5 pin-sized holes were poked into each foil lid to facilitate gas exchange until the termination of the test (Day 14).

Table 2.1. Exposure concentrations, duration, number of replicates and number of organisms per replicate used in each *Eisenia andrei* earthworm test.

Compound	Soil	Number of Replicates per Treatment	Number of Organisms	Duration (Days)	Nominal Exposure Concentrations (mg / kg soil d.w.)
Benzene Toluene Ethylbenzene Xylenes	AS	3	5	14	0, 100, 500, 1000, 2000, 3000
Benzene	AS	3	5	14	0, 1000, 2000, 3000, 6000, 8000, 12000
Benzene Toluene Ethylbenzene Xylenes	RS	3	5	14	0, 100, 500, 1000, 2000, 3000
Benzene	RS	3	5	14	0, 1000, 2000, 3000, 6000, 8000, 12000

AS Artificial Soil
RS Reference Soil

2.3.3 Plant Tests

Acute screening tests were conducted with northern wheatgrass to measure the effects of the individual BTEX compounds in both artificial and reference soils on seedling emergence and early seedling growth. Nominal exposure concentrations, test durations, and number of replicates per treatment were identical among tests (Table 2.2).

An individual test unit consisted of a 1-L glass parfait jar with hinged lids. The soil, 150 g w.w. (at 35% moisture content), was placed directly into the bottom of the jar, and the seeds planted within. This type of test unit was used to minimize loss of contaminant via volatilization from the soil during the initial few days of the test. The test units are presented in Figure 1. The lids remained closed until Day 7 of the toxicity test, after which, the test units were opened, and water was added to the test soils, as necessary (usually daily), until the end of the test (Day 14).

Table 2.2. Exposure concentrations, duration, number of replicates and number of seeds per replicate used in each northern wheatgrass plant test.

Compound	Soil	Number of Replicates per Treatment	Number of Seeds	Duration (Days)	Nominal Exposure Concentrations (mg / kg soil d.w.)
Benzene Toluene Ethylbenzene Xylenes	AS	6 (0 mg/kg) 4 (500-5000 mg/kg) 3 (6000-12000 mg/kg)	5	14	0, 500, 1000, 2000, 3000, 5000, 6000, 8000, 12000
Benzene Toluene Ethylbenzene Xylenes	RS	6 (0 mg/kg) 4 (500-5000 mg/kg) 3 (6000-12000 mg/kg)	5	14	0, 500, 1000, 2000, 3000, 5000, 6000, 8000, 12000

AS Artificial Soil
RS Reference Soil

2.4 Preparation of Test Soils

Preparation of test soils began either on Day -1 for earthworm tests or Day 0 for plant tests. Prior calculations were made, on a dry weight basis, to establish the quantity of a given BTEX compound required to achieve the desired nominal exposure concentration. Calculations also included the mass of soil (w.w.) to be used to make a batch of soil to which a known quantity of a given BTEX compound was added, as well as, the amount of deionized water necessary to standardize the percentage moisture content of the soil. Soils were hydrated to a level that was considered moist enough to provide a suitable environment for earthworms while still maintaining soil aggregate structure. The desirable moisture content for both test soils was 35 %.

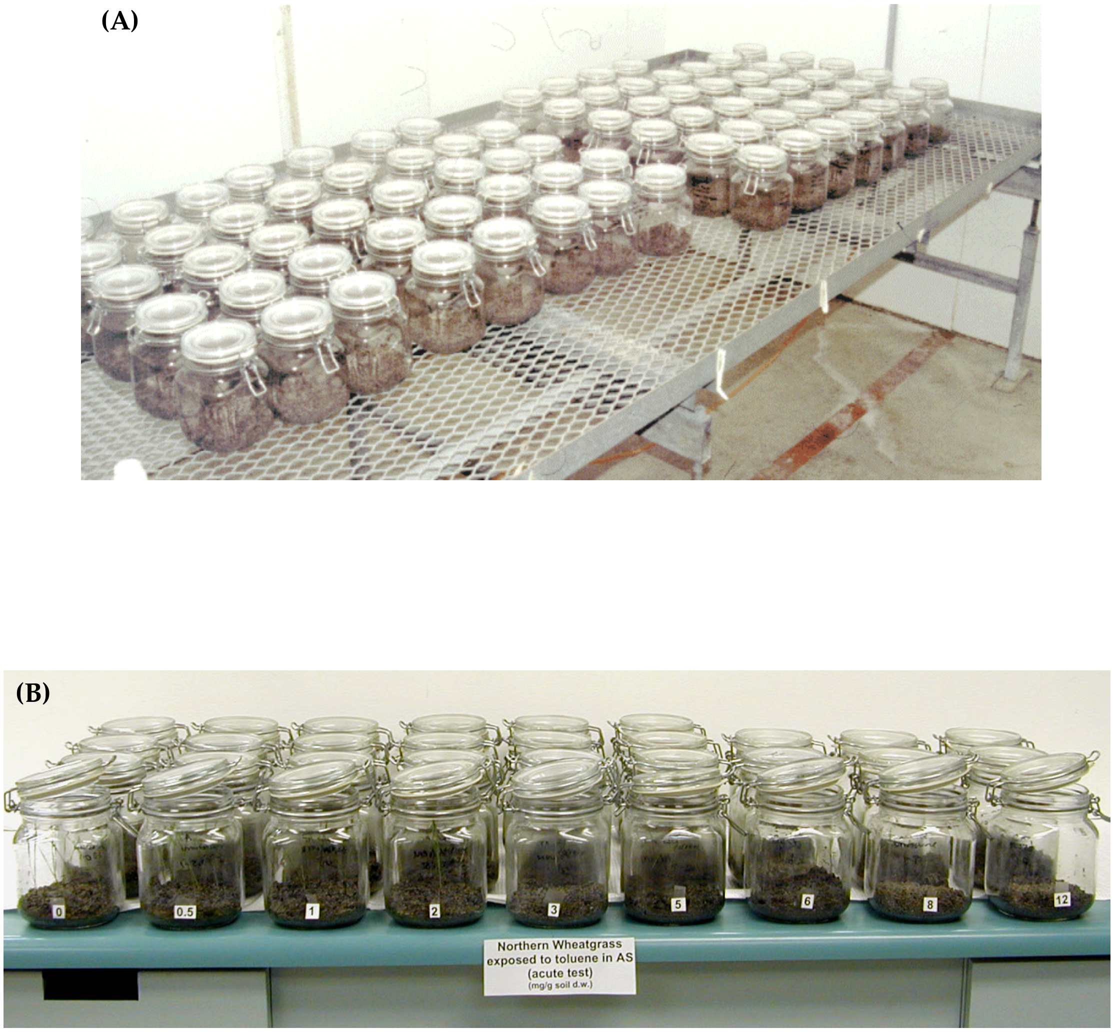

Figure 1. Test units consisting of closed 1-L parfait glass mason jars used in the plant toxicity tests. (A) The test units placed in an environmental growth chamber throughout the duration of an acute test. (B) The acute test units, prior to processing.

In order to minimize volatilization of the BTEX compounds from the spiked soil, soils were refrigerated at 6 ± 3 ºC for approximately 12 hours prior to each test set-up. A batch of soil sufficient to satisfy the replicate test-unit requisite for a treatment was weighed into a stainless steel mixing bowl. Depressions in the soil were made with a stainless steel scoopula, and the pre-calculated amount of a given BTEX compound was equally distributed (by volume) among these depressions with the use of a pipette. As soon as the test substance was added, the depressions were covered up with the surrounding soil. Water was added in increments, and the contents of the bowl were then mixed for approximately three minutes with a hand-held mixer and a stainless steel spoon when required, until homogeneity was achieved. The criterion for homogeneity of the mixture was visible uniformity of colour and texture. The soils were then divided equally by weight among the test units in the appropriate treatment. The sealed test units sat at room temperature for approximately 4 hours (plants) or 24 hours (earthworms) before the test organisms were added. Earthworm test units require a 24-hr equilibrium period prior to the addition of the organisms (ASTM, 1995).

2.5 Preparation of Organisms

2.5.1 Earthworms

On Day 0, the earthworms were removed from the cultures, washed with de-ionized water, and placed onto tray lined with paper towel, or blotting paper, to remove excess water. The worms were then added randomly to the test units across treatments. Five clitellate adult, or large juvenile, worms with no deformities (e.g. skinny, pale colour, constrictions, etc.) were added to each test unit.

2.5.2 Plants

The seeds of the plants that were used in the toxicity tests were kept in their original paper bags which were placed, as soon as they arrived at the laboratory, into a polyethylene zip-lock bag (Glad Zipper™) and stored in a refrigerator at 6 ± 3 °C. On Day 0, the seeds of the plant species to be used in the toxicity test were taken out of the refrigerator and acclimated to room temperature. A sub-sample of seed 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 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.

2.6 Test Conditions

2.6.1 Earthworm Tests

After the addition of the earthworms, the test units were placed into cardboard boxes that were then placed into a fumehood at room temperature (22 ± 2 ºC), with a photoperiod of constant fluorescent illumination. The test units were sealed for the first seven days of each test, after which, 5 pin-sized holes were poked into each foil lid to facilitate gas exchange until the termination of the test (Day 14).

2.6.2 Plant Tests

Once the seeds were planted, all the test units were transported to the University of Guelph and placed randomly into an environmental growth chamber. The photoperiod was 16 h light: 8 h dark, and the corresponding temperatures were 24 ± 2 °C and 16 ± 2 ºC, respectively. The light intensity was on average 400 μmoles/m^2/s, and the jars did not interfere with the quality of light to which the seeds were exposed. The 1-L glass parfait jars were kept sealed for the first seven days of each test and, since there was minimal loss of moisture, test units did not require watering during this period. After Day 7, the lids of each test unit were opened and water was added, as necessary, usually every 24 hours. Plants tested in artificial soil were watered using a dilute nutrient solution (Table A.2, Appendix A) and those tested in the reference soil were watered with de-chlorinated municipal tap water (Table A.2, Appendix A).

2.7 Measurement Endpoints

2.7.1 Physico-chemical Measurements

The physico-chemical measurements made at the beginning and end of each test were pH and conductivity. Percentage moisture content of the soil was also measured at the beginning and end of invertebrate tests in the same manner as described in Subsection 2.2. The pH and conductivity were measured using a soil slurry method modified from the Soil Analysis Handbook (1992). Approximately 25 g (w.w.) of test soil and 50 ml of de-ionized 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. The slurry in each beaker was agitated once more by stirring with a glass rod and then the pH and conductivity were measured using an IonCheck 65 (Radiometer Analytical, Villeurbanne, Cedex, France) meter 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 in sequence from the lowest concentration to the highest concentration within each type of soil.

The test temperature in the environmental chambers was monitored with a maximum/minimum thermometer as well as continually with a digital display.

2.7.2 Earthworm Tests

The measurement endpoint in acute toxicity tests with earthworms was adult survival at 7 and 14 days. Mortality of the earthworms was assessed as failure to respond (i.e. muscularly contract) upon gentle prodding with a glass rod. Dead earthworms were seldom observed because of the rapid rate of tissue decomposition.

2.7.3 Plant Tests

The measurement endpoints of the plant toxicity tests were seedling emergence, shoot and root length, shoot and root wet phytomass, and shoot and root dry phytomass. Seedling emergence was measured visually by counting the number of seedlings that had emerged from the soil in each test unit. The criterion for emergence was a shoot height of 3 mm above the surface of the soil. Shoot and root lengths were measured with a ruler and recorded in millimetres. The shoots and roots were separated, placed into pre-weighed, pre-labelled, aluminium pans (1 or 2.5 g) and the wet masses determined with an Ohaus Analytical Plus electronic balance (0.1 mg). The dry phytomass (root and shoot) was determined by drying the plants in an oven for 48 h at 90°C and measuring the dry mass with the balance.

2.8 Processing a Test

2.8.1 Earthworm Tests

The contents of each test unit were emptied onto a tray, where they were sorted with forceps, and the individual earthworms reported as either dead or alive. Dead earthworms were often not apparent because of their rapid decomposition, therefore any "missing" earthworms were considered dead. If the test continued with repeated measurements (e.g. 7- vs. 14-d exposure) to be made on the test unit, the contents on the tray were returned to the test units and the earthworms placed onto the soil surface. Otherwise, the test soils were discarded. Earthworm survival was determined on day 7 and on day 14 (the end of the test). Composite samples of soil from each treatment were collected for determination of pH, conductivity, and percent moisture content.

2.8.2 Plant Tests

At the end of the test, the test units were transported from the University of Guelph's environmental chambers to ESG International Inc., where they were processed in the laboratory.

The test units were placed onto the laboratory bench and blocked according to concentration. Photographs were taken to capture the visible concentration-response relationship and the relative seedling emergence. The number of emerged seedlings for each test unit was recorded. Composite samples of soil from each treatment were also collected for pH and conductivity measurements.

The test units were processed in sequence of increasing concentration, beginning with the controls. 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 then, 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., free of dislodgeable soil particles), 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, aluminium pans (1 or 2.5 g) and the wet masses were determined using a 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 aluminium pans with the plant material were placed into a drying oven and dried at 90°C for 48 hours. The dry shoot and root mass measurements were then determined with the analytical balance.

2.9 Statistical Analysis

2.9.1 Earthworm Tests

The results of the toxicity tests were manually entered onto data spreadsheets, graphed, and analyzed using the Probit, the Moving Average, and the Trimmed Spearman-Karber methods of analyses (Stephan, 1989) to determine the LC50. Analyses of variance procedures were applied to the data to determine differences among treatment means and the no or lowest observed adverse effect concentrations (SPSS, 1997).

2.9.2 Plants

Linear or nonlinear regression procedures were applied to the plant growth toxicity data after the data were entered into electronic spreadsheets and the emergence results graphed. The analyses consisted of using a linear and four non-linear regression models (i.e. logistic, gompertz, exponential, and logistic with hormesis) that had been re-parameterized to include the ICp and the associated 95% confidence limits. The ICp is the inhibiting concentration (IC) resulting in a specified percentage (p) effect. The residuals were examined for homogeneity of variance among treatments. If data showed heteroscedasticity among treatments, data were weighted with the inverse of the variance of each treatment (Myers, 1986; Stephenson *et al*., 2000). Emergence data were analyzed by analyses of variance procedures and a one-tailed Dunnett's test was used to compare each treatment mean to the mean of the control treatment. All analyses were performed with SYSTAT 7.0.1 (SPSS, 1997a). A more detailed description of the statistical procedures used to analyse plant test data can be found in either Environment Canada (1998) or Stephenson *et al*. (2000).

2.10 Chemical Analysis

The initial concentrations of the BTEX compounds in the artificial and reference soils were measured in order to establish the relationship between nominal and measured exposure concentrations in the test soils. Nominal concentrations measured were 0, 500, 3000 and 12000 mg/kg soil d.w.

Each type of soil was spiked with each BTEX compound separately in a manner identical to that described in Subsection 2.4. The BTEX compounds were added to separate, independent, replicate samples (n=2) of each type of soil, at each of the four concentrations (0, 500, 3000, and 12000 mg/kg soil d.w.). Immediately after each soil sample was spiked, two 60 mL glass jars were filled completely, to eliminate headspace. A total of 68 soil samples (2 soils x 4 compounds x 4 concentrations x 2 replicates) were then sent on ice by courier to Philips Analytical Services Corporation (Mississauga, Ontario) for analysis of total BTEX concentrations. The BTEX concentrations were determined via headspace analysis using gas chromatography with flame ionization detection.

Once results from the soil analyses were obtained, the data were corrected for background concentrations. This was accomplished by subtracting the amount of each BTEX compound in an experimental control soil from each of the measured concentrations in each corresponding test soil (i.e., all other treatments). The measured exposure concentrations of the individual BTEX compounds in a test soil were regressed, with the use of logarithmic scales, against the nominal exposure concentrations (SPSS, 1997b). The resultant relationships (i.e. the regression equations of the lines) could then be used to adjust the toxicity estimates (e.g., ICp and LCx) determined from the nominal exposure concentrations to more realistically reflect the concentrations to which the organisms were actually exposed. The adjusted toxicity estimates are referred to in this report as the estimated effect concentrations (EECs). It should be noted that this adjustment would result in EECs that are substantially lower than the nominal exposure concentrations and, in all probability, lower than the actual concentrations to which the organisms were exposed.

3. RESULTS

3.1 Acute Earthworm Tests

3.1.1 Sandy Loam Soil (AS)

Earthworm toxicity tests were performed in the artificial soil (AS), which acted as a surrogate for a sandy loam soil. The physical and chemical properties of the artificial soil are listed in Table A.1. (Appendix A). The mean number of *E. andrei* surviving in each treatment after 7 and 14 days of exposure to nominal concentrations of benzene, toluene, ethylbenzene, and xylenes in the sandy loam soil are depicted in Figures A.1 and A.2 (Appendix A). The estimates of toxicity based on the nominal exposure concentrations are listed in Table A.3 (Appendix A). The corresponding estimated effect concentrations (i.e., those adjusted for the measured values) are listed in Table A.4 (Appendix A).

Earthworm survival was not adversely affected by benzene in soil at the highest exposure concentration of 3,000 mg/kg soil d.w. in the first test, however LC50s were estimated in the second test conducted with exposure concentrations up to 12, 000 mg/kg soil d.w. (Figures A.1 and A.2; Appendix A). The 7- and 14-d LC50 estimates of benzene for *E. andrei* were identical at a nominal concentration of 1,247 mg/kg soil d.w. (Figure A.2; Appendix A). The estimated effect concentrations (EEC) were determined using the regression equation derived from all of the analytical data obtained through all tests with benzene in the artificial soil (Figure C.1; Appendix C). Therefore the corresponding EEC for the 7- and 14-day LC50s was 1.79 mg/kg soil d.w. The 7- and 14-d LC50s for *E. andrei* exposed to toluene in the sandy loam soil were identical at a nominal concentration of 707 mg/kg soil d.w., equivalent to an EEC of 5.13 mg/kg soil d.w. (Figure A.1, Tables A.3 and A.4; Appendix A). Estimated effect concentrations were determined using regression equations presented in Figure C.1 (Appendix C). Comparable results were observed for the ethylbenzene and xylenes whereby the 7- and 14-d LC50s did not differ. The 7- and 14-d LC50s for *E. andrei* exposed to ethylbenzene were both 261 mg/kg soil d.w., which corresponded to an EEC of 10.65 mg/kg soil d.w. (Figure A.1, Tables A.3 and A.4; Appendix A). The 7- and 14-d LC50s for *E. andrei* exposed to xylenes were both 224 mg/kg soil d.w., which corresponded to an EEC of 8.83 mg/kg soil (Figure A.1, Tables A.3 and A.4; Appendix A). In general, the toxicity of the four compounds were soil dependent, and the compounds in the sandy loam soils elicited effects at lower concentrations, when compared to the nominal and EECs generated from tests with clay loam soil.

The no observed adverse effect concentrations (NOAECs) for benzene, toluene, ethylbenzene, and xylenes did not differ between day 7 to day 14 (Tables A.3 and A.4; Appendix A). Comparable results were evident for the lowest observed adverse effect concentrations (LOAECs) (Tables A.3 and A.4; Appendix A).

The pH and electrical conductivity of the sandy loam soil at the beginning of the tests were 6.96 and 229 μS/cm, respectively (5.70 and 118 μS/cm for the second benzene test) and, at the end of the test, values ranged across treatments from 4.97 to 8.2 and from 127 to 234 μS/cm, respectively. The moisture content at the beginning of the tests with earthworms ranged across tests from 35.3 to 36.7 % and the moisture content in soils were comparable at the end of the test.

3.1.2 Clay Loam Soil (RS)

The physical and chemical properties of the clay loam soil are listed in Table A.1. (Appendix A). The 7-d LC50s for BTEX were the same as those determined on day 14, the end of the test. Therefore the effects of BTEX in clay loam soil on earthworms occurred within the first 7 days of the test. The 7- and 14-d LC50s for *E. andrei* exposed to benzene in the clay loam soil were both 2603 mg/kg soil d.w. in the first test, and both 2449 mg/kg soil d.w. in the second test, and both values are based on nominal concentrations only (Figures A.3 and A.4, Table A.3; Appendix A). The corresponding EEC values were calculated using the regression equation derived from the analytical data obtained in both tests with benzene in the reference clay loam (Figure C.2; Appendix C). The 7- and 14-day EECs for the first and second benzene tests were 43.84 and 41.87 mg/kg soil d.w., respectively (Table A.4; Appendix A). The 7- and 14-d LC50s for *E. andrei* exposed to toluene in the clay loam soil were both 1414 mg/kg soil d.w. (Table A.3; Appendix A), which corresponded to an EEC of 65.43 mg/kg soil d.w. (Figure A.3, Table A.4; Appendix A). The 7- and 14-d LC50s for *E. andrei* exposed to ethylbenzene were both 282 mg/kg soil d.w., which corresponded to an EEC of 29.40 mg/kg soil d.w. (Figure A.3; Tables A.3 and A.4; Appendix A). The 7- and 14-d LC50s for *E. andrei* exposed to xylenes in RS were both 413 mg/kg soil d.w., which corresponded to an EEC of 42.32 mg/kg soil (Figure A.3, Tables A.3 and A.4; Appendix A).

Like those toxicity estimates generated with the sandy loam soil, the no observed adverse effect concentrations (NOAEC) for benzene, toluene, ethylbenzene, and xylenes did not differ between day 7 and day 14 of the test (Tables A.3 and A.4; Appendix A). Comparable results were evident for the lowest observed adverse effect concentrations (Tables A.3 and A.4; Appendix A).

The pH and electrical conductivity of the clay loam soil at the beginning of the tests were 6.07 and 387 μS/cm, respectively, and at the end of the test values ranged across treatments from 5.84 to 6.55 and from 315 to 403 μS/cm, respectively. The moisture content at the beginning of the tests with earthworms ranged across tests from 35.34 to 37.42 % and, again, the moisture content of the soils did not change over the 14-d test.

3.2 Acute Plant Toxicity Tests

3.2.1 Sandy Loam Soil (AS)

Northern wheatgrass seedling emergence was insensitive to the range of effect concentrations used for benzene in the sandy loam soil (Figure B.1; Appendix B). However, seedling emergence was inhibited upon exposure to toluene at the highest nominal exposure concentration of 12,000 mg/kg soil d.w. (Figure B.1; Appendix B), equivalent to an EEC of 730.54 mg/kg soil d.w., when calculated using the regression equation for toluene measured in soils (Figure C.1; Appendix C). Seedling emergence was slightly more sensitive to ethylbenzene in soil, with significant inhibition of emergence beginning at a nominal concentration of 6,000 mg/kg soil d.w., which corresponded to an EEC of 1616.58 mg/kg soil d.w. Northern wheatgrass seedling emergence was also inhibited by xylenes in soil at a nominal concentration of 6,000 mg/kg soil d.w., which corresponded to an EEC of 1172.49 mg/kg soil d.w.

The results of the acute toxicity tests with northern wheatgrass exposed to nominal concentrations of benzene, toluene, ethylbenzene, and xylenes in the artificial sandy loam soil are summarized in Table B.1 (Appendix B); the corresponding EECs are summarized in Table B.2 (Appendix B). The concentration-response relationships are presented in Figures B.2 to B.5 (Appendix B). The IC50s ranged from a nominal concentration of 330 mg ethylbenzene/kg soil d.w. for root wet mass to 12,231 mg benzene/kg soil d.w. for shoot length. The corresponding EECs derived from the regression equations (Figure C.1; Appendix C) ranged from 5.25 mg benzene/kg soil d.w. for root dry mass to 1047.37 mg ethylbenzene/kg soil d.w. for shoot length. When the toxicity estimates for all growth endpoints were averaged, the ranges of the nominal IC50s and IC20s were 1808 – 6802 and 601 – 2524 mg/kg soil d.w. (Table B.3; Appendix B). The corresponding converted values (i.e., the EECs for IC50s and IC20s) were 13.3 – 377 and 3.5 – 61.8 mg/kg soil d.w. (Table B.4; Appendix B). Root dry and wet mass metrics tended to be the most sensitive endpoints for each of the four compounds (Table B.5; Appendix B).

The pH and electrical conductivity of the sandy loam soil at the beginning of the tests were 6.19 – 8.08 and 103-224 μS/cm, respectively, and at the end of the test values ranged across treatments from 6.70 to 7.53 and from 782 to 1535 μS/cm, respectively. The increase of the electrical conductivity of the test soils over the test duration is the result of watering the test units with a weak nutrient solution.

3.2.2 Clay Loam Soil (RS)

Northern wheatgrass seedling emergence was insensitive to the range of exposure concentrations used for both benzene and toluene in the clay loam soil (Figure B.6; Appendix B). However, seedling emergence was inhibited by ethylbenzene at nominal exposure concentrations of 8000 and 12,000 mg/kg soil d.w., which corresponded to EECs of 1990.18 and 3317.17 mg/kg soil d.w., respectively (Figure B.6; Appendix B). Northern wheatgrass seedling emergence was significantly inhibited by xylenes at the highest concentration of 12,000 mg/kg soil d.w., which corresponded to an EEC of 6365.34 mg/kg soil d.w. (Figure B.6; Appendix B).

The results of the acute toxicity tests with northern wheatgrass exposed to nominal concentrations of benzene, toluene, ethylbenzene, and xylenes in the clay loam soil are summarized in Table B.1 (Appendix B) with the corresponding EECs summarized in Table B.2 (Appendix B). The concentration-response relationships are presented in Figures B.7 to B.10 (Appendix B). The IC50s ranged from a nominal concentration of 1820 mg ethylbenzene/kg soil d.w. for root wet mass to 38,113 mg benzene/kg soil d.w. for root length. The corresponding EECs derived with the regression equations (Figure C.2; Appendix C) ranged from 65.85 mg benzene/kg soil d.w. for root wet mass to 3498.60 mg xylenes/kg soil d.w. for shoot length. Root endpoints, particularly root dry and wet mass, tended to be the most sensitive endpoints for each of the four compounds (Table B.5; Appendix B). The average IC50s and IC20s for all growth endpoints for nominal and adjusted estimates of toxicity are summarized for each compound in Tables B.3 and B.4 (Appendix B), respectively.

While toxicity trends within a compound among endpoints were similar when comparing between nominal and measured concentrations, toxicity among the four BTEX compounds cannot be compared directly. Each compound should be considered independent of the other since each volatilizes or degrades at different rates (Table C.2; Appendix C). Therefore there are differences between nominal and measured concentrations that are not consistent among the four compounds.

The pH and electrical conductivity of the clay loam soil at the beginning of the tests were 5.99 - 6.23 and 397 μS/cm, respectively, and at the end of the test values ranged across treatments from 5.98 to 6.44 and from 235 to 1007 μS/cm, respectively. The increase of the electrical conductivity of the test soils over the test duration is the result of watering the test units with de-chlorinated municipal tap water.

3.2.3 Sandy Loam vs. Clay Loam Soil

Northern wheatgrass was more sensitive to all four BTEX compounds in sandy loam soil than in the clay loam soil. The effect of soil type was the same for the IC50s and IC20s, averaged among endpoints, regardless of whether they were expressed as nominal concentrations or as EECs (Tables B.3 and B.4; Appendix B).

3.2.4 Chemical Analysis

There were large differences between the nominal (i.e., the desired exposure concentrations) and the measured (i.e., those determined analytically) concentrations of BTEX in both types of soil. In the sandy loam soil, the percent of the nominal concentrations in the soil varied from 0.01% for benzene (3,000 mg/kg soil d.w.) to 49.75% for ethylbenzene (12,000 mg/kg soil d.w.) (Table C.1; Appendix C). In general, the loss from sandy loam-spiked soil was the greatest for benzene, less for toluene and xylenes, and the least for ethylbenzene. In the clay loam soil, the percent of the nominal concentrations in the soil varied from 0.21% for benzene (12,000 mg/kg soil d.w.) to 44.75% for xylenes (12,000 mg/kg soil d.w.) (Table C.2; Appendix C). In clay loam soil, the loss was the greatest, in general, for benzene, less for toluene and ethylbenzene, and greatest for xylenes. The loss of each substance from both soil types was concentration-dependent with greater losses observed for lower concentrations. In general, losses of BTEX were greater in the artificial sandy loam when compared to the reference clay loam soil.

The measured exposure concentrations of the individual BTEX compounds in each test soil were regressed, with the use of logarithmic scales, against the nominal exposure concentrations (Figures C.1 and C.2; Appendix C). A linear relationship (i.e. the regression equation of the line) describing the interaction between nominal and measured concentrations was generated for each chemical, in each soil type. These regression equations are shown in Figures C.1 and C.2 (Appendix C) for sandy loam and clay loam soils, respectively. With regard to benzene, the data describing the relationship between nominal and measured soil concentrations in the first test with clay loam soil were not amenable to linear regression. However the analytical results from all of the tests with benzene in the artificial soil and the reference soil were assessed independently (i.e., by soil type) and were regressed to determine the estimated effect concentrations described within this report (Figures C.1 and C.2; Appendix C). In the case of the analytical results from all of the tests with benzene in artificial soil, a statistical outlier was observed (Figure C.3; Appendix C). This outlier was removed and the data were re-evaluated resulting in a better regression (Figure C.1 and C.4; Appendix C), however, the regression equation used was that generated with the statistical outlier. The analytical results derived from both first and second tests with benzene in the artificial and reference soils were also examined separately (Figures C.5, C.6, C.7, C.8, and C.9; Appendix C).

4. DISCUSSION

4.1 Toxicity of BTEX to Earthworms

The selected exposure concentrations used in these tests with *Eisenia andrei* were based on the results of similar tests conducted with *E. andrei* exposed to mogas in an artificial soil (a formulated sandy loam soil) and a field-collected sandy loam reference soil (ESG, 2000a). Although the range of exposure concentrations was adequate to describe the exposure concentration-response relationships for toluene, ethylbenzene, and xylene, the benzene concentrations in AS to which the earthworms were exposed were not sufficiently high to determine toxic effects. Therefore, the test with benzene was repeated with higher exposure concentrations and the concentration-response relationships in the second test was adequately described. The exposure concentrations in AS were measured again, however the results of the chemical analyses were inconclusive. The loss of benzene from the soils was greater than 98 % and, from past experience, the expected loss should have been in the range of 70 to 90 %, depending on the concentration (ESG, 2000a).

The 7-d and 14-d LC50s for *Eisenia andrei* exposed to benzene, toluene, ethylbenzene, and xylene in AS were 1.79, 5.13, 10.65, and 8.83 mg/kg soil d.w., as estimated effect concentrations. The LC50s did not differ between 7 and 14 days. This suggests that the incipient lethal level (ILL) or acute toxicity threshold for earthworms was reached within the 7 day exposure period. It also suggests that the compounds in soil have either volatilized and/or been degraded to below the no effect levels of <1.4, 2.8, 2.29, and 2.66 mg/kg soil d.w. for benzene, toluene, ethylbenzene, and xylene, respectively. Therefore, an acceptable duration for acute toxicity tests with these highly volatile compounds is 7 days.

The estimates of toxicity for the BTEX compounds in RS did not differ between the two exposure periods (e.g., 7 and 14 d). The influence of the nature of the test soils on toxicity of the BTEX compounds was apparent when the 7- and 14-d LC50s for these compounds in the clay loam soil (RS) were compared to those for the sandy loam soil (AS). The actual 7-d LC50s for BTEX in RS were 43.84 (Test 1 benzene), 41.87 (Test 2 benzene), 65.43, 29.40, and 42.32 mg/kg soil d.w., respectively, which are about 23, 10, 3, and 6 times higher, respectively, than the corresponding 7-d LC50s in AS. The physico-chemical characteristics in the RS that most probably influenced bioavailability of BTEX were the cation exchange capacity, organic matter content, and clay mineralogy (Table A.1; Appendix A).

The pH and electrical conductivity of the different exposure concentrations of the BTEX compounds in both types of soil did not change substantially over the test period. These data also suggest that these physico-chemical characteristics of the soils were not influenced to any large extent by the nature of the compounds themselves because the ranges were comparable among tests. Moisture content was also comparable among and within (i.e., start versus end of test) tests.

4.2 Toxicity of BTEX to Plants

4.2.1 Seedling Emergence

It has been established that toxicity of contaminants in soil to plants can be species-, chemical-, soil-, and endpoint-dependent (ESG, 2000a and 2000b). Seedling emergence, as a toxicity endpoint in acute tests with plants, is generally less sensitive than seedling growth endpoints; however, with the TEX compounds at the nominal exposure concentration in AS of 12,000 mg/kg soil d.w., complete inhibition of seedling emergence was observed (Figure B.1; Appendix A). The results indicate that concentrations of 12,000, 6,000 and 3,000 mg/kg soil d.w. significantly ($p \leq 0.05$) inhibited seedling emergence in toluene, ethylbenzene, and xylene, respectively. When these nominal exposure concentrations were adjusted to the estimated effect concentrations they were 730.5, 1616.6 and 418.3 mg/kg soil d.w., respectively.

In RS with the same nominal concentration of 12,000 mg/kg soil d.w., complete inhibition of seedling emergence occurred for seeds exposed to ethylebenzene and xylene and 50 % inhibition of emergence was observed for toluene. Seedling emergence was not affected by benzene at any of the exposure concentrations that ranged from 0.5 to 12,000 mg/kg soil d.w. , regardless of soil type. Generally, seedling emergence was less affected by TEX when northern wheatgrass was exposed to these compounds in RS relative to AS. Similar to the results for *E. andrei*, BTEX were generally less toxic to plants when in clay loam soils opposed to sandy loam soils.

4.2.2 Seedling Growth

The exposure concentration-response relationships for the different growth endpoints (6) for northern wheatgrass seedlings were variable but quantifiable using non-linear regression procedures. When the IC50s as EECs for all growth endpoints were combined for a given compound, the mean AS-IC50s were 12.0, 137.9, 377.1 and 215.5 mg/kg soil d.w. for BTEX, respectively, and the mean AS-IC20s were 4.0, 18.4, 61.8, and 43.82 mg/kg soil d.w., respectively. Given these data, it would appear that the order of increasing toxicity to northern wheat grass of the BTEX compounds was ethylbenzene < xylene < toluene < benzene.

A similar pattern was apparent in the data for BTEX in RS; however, the estimates of toxicity were simply not as high because these substances in clay loam soil were not as readily bioavailable to the organisms. When the IC50s as EECs for all growth endpoints were combined for a given compound, the mean RS-IC50s were 182.7, 493.1, 982.1, and 1784.1 mg/kg soil d.w. for BTEX, respectively, and the mean RS-IC20s were 76.3, 167.5, 310.8, and 757.6 mg/kg soil d.w., respectively. Given these data, it would appear that the order of increasing toxicity to northern wheatgrass of the BTEX compounds was xylene < ethylbenzene < toluene < benzene.

In terms of the sensitivity of the different growth endpoints (IC50s), patterns within soil types are apparent. In the sandy loam soil, shoot length is generally the least sensitive variable, for each of the compounds. Root wet mass was the most sensitive endpoint for northern wheatgrass exposed to TEX in the sandy loam soil; however, root dry mass was the most sensitive endpoint for plants exposed to benzene. In the clay loam soil, shoot length remained the least sensitive variable to TEX, but root length was the least sensitive variable for plants exposed to benzene in RS. Root wet mass was the most sensitive indicator of toxicity of benzene, toluene, and ethylbenzene but, for xylene, it was root dry mass.

4.3 BTEX Chemistry

Although the data are limited, the results of the chemical analyses establish that: 1) the organisms were exposed, for the most part, to a series of increasing exposure concentrations; 2) the rate of loss from soils was chemical dependent in that ethylbenzene and xylene behaved in a similar manner, but the loss of toluene and benzene from soils was greater; 3) the amount of material lost from the soils was concentration dependent; and, 4) the loss was also soil dependent, in that less was lost from clay loam soils than from sandy loam soils. However, given the volatility of these compounds and the probability that volatilizaton and degradation likely continues to some extent while the soil samples are in transit, use of the EECs as indicators of effect is, in all probability, likely to overestimate the toxicity. In other words, the organisms were probably exposed to concentrations in soil that were higher than those that were measured but not as high as the nominal values.

4.4 Comparison with Existing Standards

For comparative purposes, the average EECs for each compound has been presented for both test organisms in both types of soil along with the Alberta Tier 1 BTEX standards and the CCME BTEX standards. For agricultural land uses, the new CWS for fraction 1 (not including BTEX) is 260 mg/kg soil d.w. for soil contact exposures in surface, fine-grained soils and 130 mg/kg soil d.w. for soil contact exposures in surface, course-grained soils.

	Estimated Effect Concentrations (mg/kg)		Alberta Tier I Criteria[3] (mg/kg)	CCME Soil Quality Guidelines[4]: Environmental Health (mg/kg)
	AS	RS		
Earthworms[1]				
Benzene	1.79	42.86[5]	0.05	0.05
Toluene	5.13	65.43	1.0	0.1
Ethylbenzene	10.65	29.40	0.5	0.1
Xylene	8.83	42.32	1.0	0.1
Plants[2]				
Benzene	3.50	76.34	0.05	0.05
Toluene	18.44	167.49	1.0	0.1
Ethylbenzene	61.78	310.84	0.5	0.1
Xylene	43.82	757.59	1.0	0.1

[1] EECs for LC50s ; [2] EECs for IC20s; [3] EC (1991); [4] CCME (1997); [5] mean of Test 1 and 2 results

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APPENDIX A

ACUTE TOXICITY TESTS WITH *EISENIA ANDREI*

Table A.1. Physico-chemical characteristics of the artificial and the reference control soils.

Parameter	Artificial Soil (AS)	Alberta Clay Loam (RS)	Analytical Method
Phosphorous (mg/kg)	23	12	Nitric/perchloric acid digestion
Potassium (mg/kg)	22	748	NH_4Ac extractable
Magnesium (mg/kg)	149	553	NH_4Ac extractable
Calcium (mg/kg)	1848	5127	NH_4Ac extractable
Sodium (mg/kg)	67	57	NH_4Ac extractable
Sodium Absorption	0.57	0.42	
Total Carbon (%)	4.46	6.83	Leco furnace method
Total Nitrogen (%)	0.05	0.59	Kjeldahl method
C.E.C. (Cmol+/kg)	18.5	34.5	Barium chloride method
Soil Texture	Fine Sandy Loam	Clay Loam	Gravimetric grain size distribution
Sand (%)	77.3	26.6	
Silt (%)	7.8	43.3	
Clay (%)	14.9	30.1	
Organic Matter (%)	9	12.8	Dichromate oxidation
Bulk Density (g/cm^3)	0.98	0.83	Clod method
pH (units)	6.09	6.05	Water method (1:2)
Conductivity (mS/cm)	0.3	1.52	Saturated paste method
Source	Formulated from constituents	Field-collected from Alberta	

C.E.C. Cation Exchange Capacity

Table A.2. Physico-chemical characteristics of the types of water used to irrigate test units.

Parameter	Tap Water	Nutrient Solution
Total salts (mmho/cm)	0.84	2.96
pH (units)	7.60	5.90
Total hardness (mg/L)	77.66	412.01
Total solids (%)	0.06	0.16
Nitrate N (mg/L)	1.00	221.00
Phosphorous (mg/L)	< 0.10	191.50
Potassium (mg/L)	0.64	259.50
Calcium (mg/L)	21.71	93.79
Magnesium (mg/L)	5.68	41.98
Zinc (mg/L)	0.02	0.80
Manganese (mg/L)	0.01	0.66
Copper (mg/L)	0.03	0.85
Iron (mg/L)	0.01	1.45
Boron (mg/L)	0.04	0.33
Silicon (mg/L)	3.60	5.50
Sodium (mg/L)	146.80	41.79
Chloride (mg/L)	49.00	93.00
Sulphates (mg/L)	85.90	108.80
Bicarbonate (mg/L)	344.00	104.00

Table A.3. The effect of BTEX compounds on *Eisenia andrei* survival expressed as the nominal LC50 with corresponding 95% confidence limits (mg/kg soil d.w.). Analysis of variance was used to determine the lowest observed adverse effect concentration (LOAEC) and the no observed adverse effect concentration (NOAEC).

Species	Chemical Compound	Soil Type	7-d LC50 (mg/kg soil d.w.)	LCL	UCL	7-d LOAEC	7-d NOAEC	14-d LC50 (mg/kg soil d.w.)	LCL	UCL	14-d LOAEC	14-d NOAEC
Eisenia andrei	Benzene test 1	AS	N/A	N/A	N/A	No effect	No effect	N/A	N/A	N/A	No effect	No effect
Eisenia andrei	Benzene test 2	AS	1247	-	-	1000	0	1247	-	-	1000	0
Eisenia andrei	Toluene	AS	707	-	-	1000	500	707	-	-	1000	500
Eisenia andrei	Ethylbenzene	AS	261	213	319	500	100	261	213	319	500	100
Eisenia andrei	Xylene	AS	224	-	-	500	100	224	-	-	500	100
Eisenia andrei	Benzene test 1	RS	2603	2369	2861	>3000*	3000	2603	2369	2861	>3000*	3000
Eisenia andrei	Benzene test 2	RS	2449	-	-	3000	2000	2449	-	-	3000	2000
Eisenia andrei	Toluene	RS	1414	-	-	2000	1000	1414	-	-	2000	1000
Eisenia andrei	Ethylbenzene	RS	282	222	357	500	100	282	222	357	500	100
Eisenia andrei	Xylene	RS	413	307	556	500	100	413	307	556	500	100

N/A Not available – Spearman-Karber Probit Procedure could not be applied.
- Confidence limits are unreliable.
* p=0.07
AS Artificial Soil
LCL Lower 95% Confidence Limit
UCL Upper 95% Confidence Limit
LOAEC Lowest Observable Adverse Effect Concentration (values of the lowest concentration significantly different from those of zero)
NOAEC No Observed Adverse Effect Concentration (highest concentration of which the values are not significantly different from zero)

Table A.4. The effect of BTEX compounds on *Eisenia andrei* survival expressed as the EECs with corresponding 95% confidence limits (mg/kg soil d.w.). Analysis of variance was used to determine the lowest observed adverse effect concentration (LOAEC) and the no observed adverse effect concentration (NOAEC). The EECs were derived using the associated regression equations derived for each chemical in each type of soil.

Species	Chemical Compound	Soil Type	7-d LC50 (mg/kg soil d.w.)	LCL	UCL	7-d LOAEC	7-d NOAEC	14-d LC50 (mg/kg soil d.w.)	LCL	UCL	14-d LOAEC	14-d NOAEC
Eisenia andrei	Benzene test 1	AS	N/A	N/A	N/A	No effect	No effect	N/A	N/A	N/A	No effect	No effect
Eisenia andrei	Benzene test 2	AS	1.79	-	-	1.40	0	1.79	-	-	1.40	0
Eisenia andrei	Toluene	AS	5.13	-	-	9.42	2.80	5.13	-	-	9.42	2.80
Eisenia andrei	Ethylbenzene	AS	10.65	7.69	14.69	30.18	2.29	10.65	7.69	14.69	30.18	2.29
Eisenia andrei	Xylene	AS	8.83	0.00	0.00	29.13	2.66	8.83	0.00	0.00	29.13	2.66
Eisenia andrei	Benzene test 1	RS	43.84	40.84	47.07	> 48.78*	48.78	43.84	40.84	47.07	> 48.78*	48.78
Eisenia andrei	Benzene test 2	RS	41.87	-	-	48.78	35.95	41.87	-	-	48.78	35.95
Eisenia andrei	Toluene	RS	65.43	-	-	110.60	38.73	65.43	-	-	110.60	38.73
Eisenia andrei	Ethylbenzene	RS	29.40	21.75	39.57	60.49	7.96	29.40	21.75	39.57	60.49	7.96
Eisenia andrei	Xylene	RS	42.32	27.22	65.87	56.24	5.13	42.32	27.22	65.87	56.24	5.13

N/A Not Available – Spearman-Karber Probit Procedure could not be applied.
\- Confidence limits are unreliable.
AS Artificial Soil
LCL Lower 95% Confidence Limit
UCL Upper 95% Confidence Limit
LOAEC Lowest Observable Adverse Effect Concentration (values of the lowest concentration significantly different from those of zero)
NOAEC No Observed Adverse Effect Concentration (highest concentration of which the values are not significantly different from zero)
EEC Estimated effect concentrations – Nominal LC50s adjusted for measured concentrations (Figures C.1 or C.2; Appendix C)

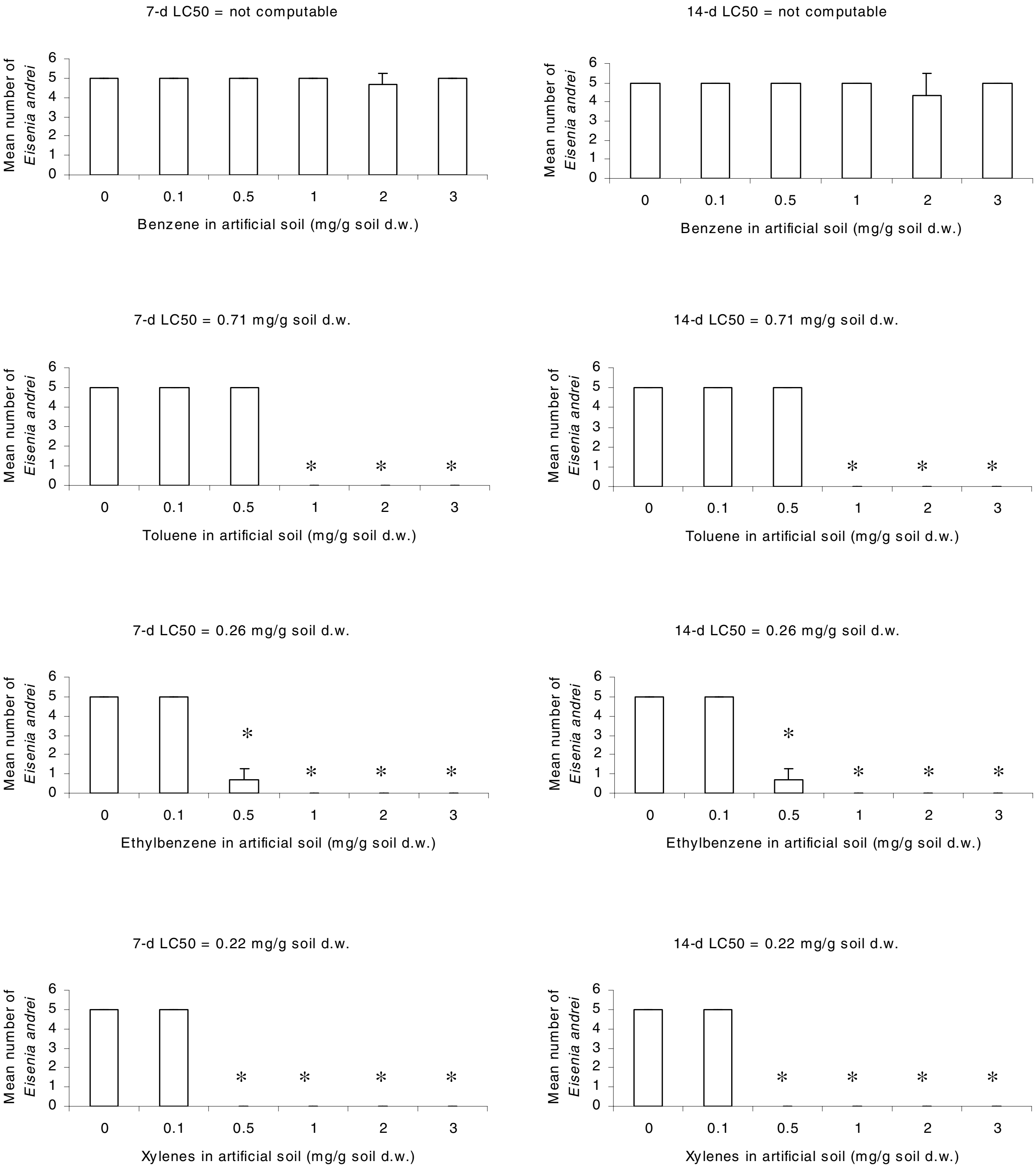
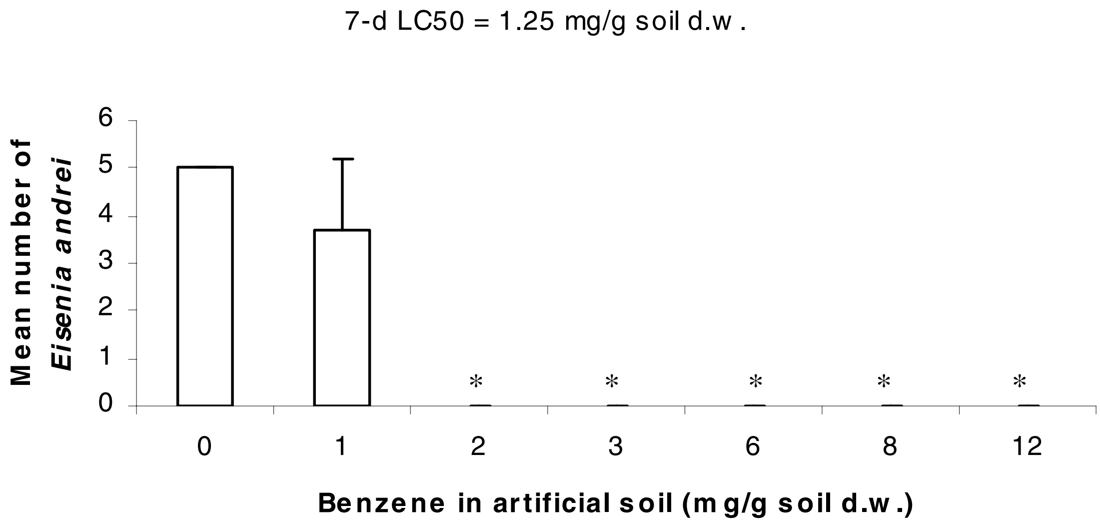


Figure A.1. Survivorship of *Eisenia andrei* exposed to nominal concentrations of benzene (test 1), toluene, ethylbenzene, or xylenes in artificial soil (mg/g soil d.w.) at 7 and 14 days. Bars indicate standard deviation for survivorship in the treatment. Stars denote a significant difference (α=0.05) from the controls.



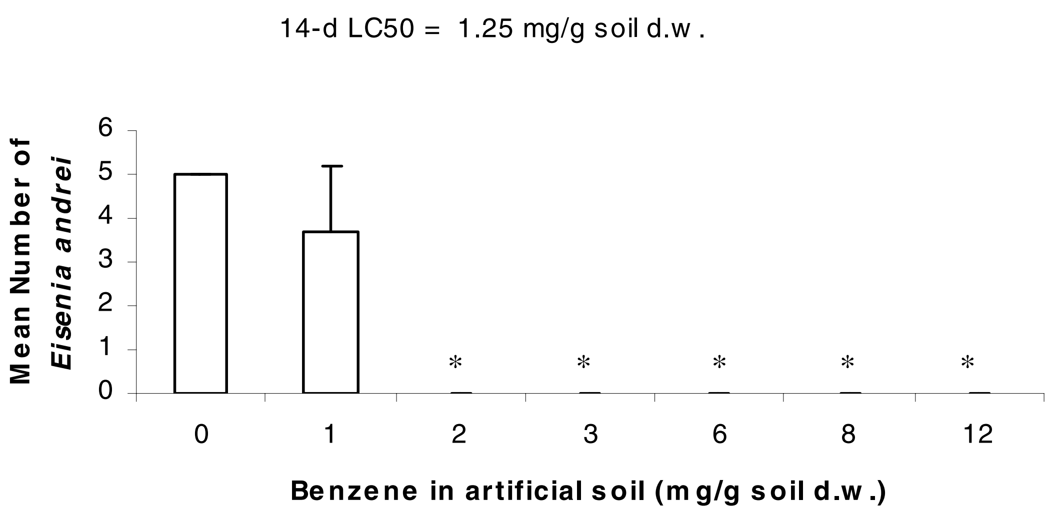


Figure A.2. Survivorship of *Eisenia andrei* exposed to nominal concentrations of benzene in artificial soil (test 2) (mg/g soil d.w.) at 7 and 14 days. Bars indicate standard deviation for survivorship in the treatment. Stars denote a significant difference ($\alpha = 0.05$) from the controls.

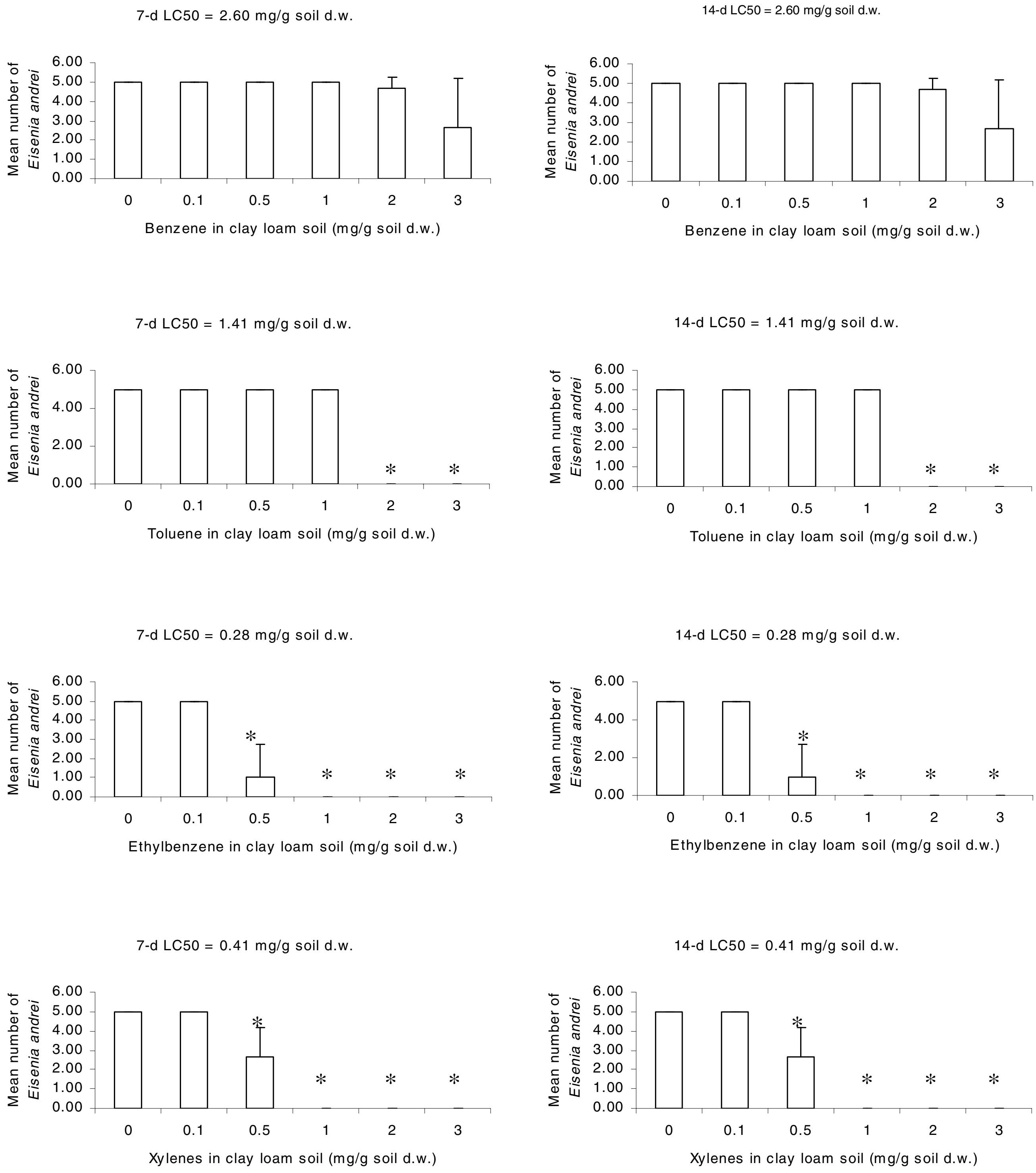


Figure A.3. Survivorship of *Eisenia andrei* exposed to nominal concentrations of benzene (test 1), toluene, ethylbenzene, or xylenes in the Alberta clay loam soil (mg/g soil d.w.) at 7 and 14 days. Bars indicate standard deviation for survivorship in the treatment. Stars denote a significant difference (α=0.05) from the controls.

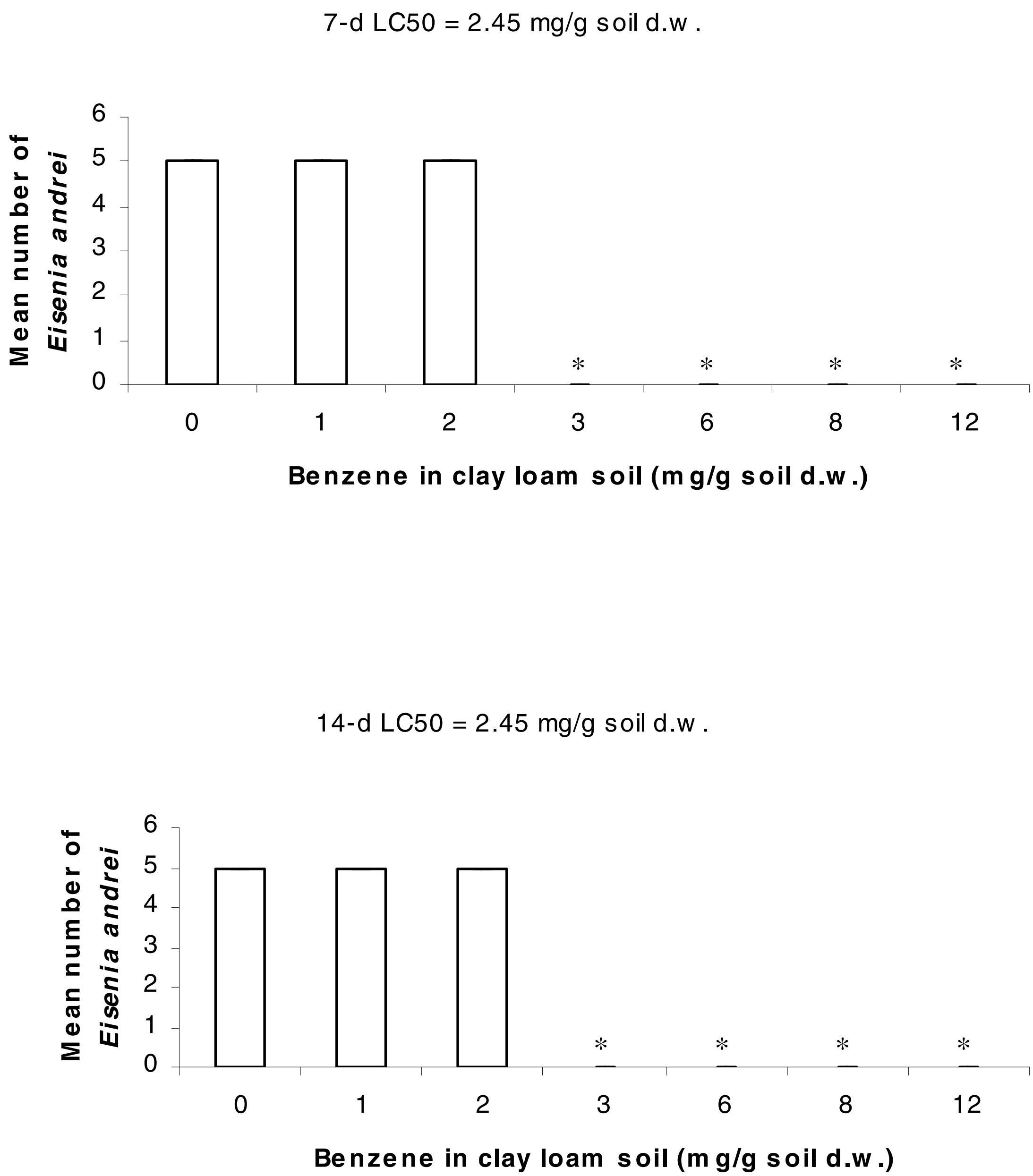


Figure A.4. Survivorship of *Eisenia andrei* exposed to nominal concentrations of benzene in the reference clay loam soil (test 2) (mg/g soil d.w.) at 7 and 14 days. Bars indicate standard deviation for survivorship in the treatment. Stars denote a significant difference ($\alpha = 0.05$) from the controls.

Appendix B

Effects of Benzene, Toluene, Ethylbenzene, and Xylenes on Early Northern Wheatgrass Growth

Table B.1. Summary of the nominal IC50s and IC20s (mg contaminant / kg soil d.w.) for six endpoints for northern wheatgrass grown in soil spiked with each chemical compound independently.

Species	Soil	Contaminant	Root/Shoot	Parameter	Model	IC50 (mg/kg soil d.w.)	LCL	UCL	IC20 (mg/kg soil d.w.)	LCL	UCL	W?
Northern Wheatgrass	AS	Benzene	Shoot	Length	Logistic	12231	7827	16635	4272	2117	6427	N
			Shoot	Wet Mass	Linear	7098	5698	8497	2839	2279	3399	N
			Shoot	Dry Mass	Gompertz	4530	2910	6151	1189	196	2182	N
			Root	Length	Gompertz	8033	5760	10306	2607	897	4317	N
			Root	Wet Mass	Logistic	5412	4014	6810	3520	1770	5270	N
			Root	Dry Mass	Exponential	3508	2465	4552	717	-556	1989	N
Northern Wheatgrass	AS	Toluene	Shoot	Length	Hormesis	9856	5287	14424	2463	1672	3253	N
			Shoot	Wet Mass	Hormesis	3258	2219	4296	1709	822	2596	N
			Shoot	Dry Mass	Logistic	3105	2234	3976	1622	787	2457	N
			Root	Length	Logistic	4265	3443	5087	1059	632	1486	N
			Root	Wet Mass	Logistic	1504	952	2056	712	268	1156	N
			Root	Dry Mass	Gompertz	1875	1055	2695	554	49	1059	N
Northern Wheatgrass	AS	Ethylbenzene	Shoot	Length	Gompertz	4576	3656	5496	1894	958	2829	N
			Shoot	Wet Mass	Gompertz	1453	652	2254	311	-73	696	N
			Shoot	Dry Mass	Gompertz	1689	1009	2369	491	71	912	N
			Root	Length	Gompertz	3377	2419	4336	704	209	1199	N
			Root	Wet Mass	Gompertz	330	-133	793	22	-50	93	N
			Root	Dry Mass	Gompertz	1509	945	2074	386	74	698	N
Northern Wheatgrass	AS	Xylenes	Shoot	Length	Gompertz	3669	2784	4554	808	329	1286	N
			Shoot	Wet Mass	Gompertz	1325	779	1871	319	37	602	N
			Shoot	Dry Mass	Gompertz	1399	971	1827	388	139	637	N
			Root	Length	Exponential	2349	1702	2997	1377	970	1784	N
			Root	Wet Mass	Gompertz	861	429	1294	230	-17	477	N
			Root	Dry Mass	Exponential	1250	862	1639	486	322	651	N
Northern Wheatgrass	RS	Benzene	Shoot[1]	Length	Gompertz	19503	5778	33227	9877	6396	13358	N
			Shoot[2]	Wet Mass	Hormesis	20380	-8376	49135	6088	1280	10896	N
			Shoot[2]	Dry Mass	Hormesis	17112	-3386	37610	5430	1679	9181	N
			Root	Length	Hormesis	38113	-15532	91758	5235	2393	8076	N
			Root	Wet Mass	Hormesis	4468	2849	6087	2552	1556	3549	N
			Root	Dry Mass	Hormesis	9082	3239	14295	4090	2119	6060	N

Table B.1. Summary of the nominal IC50s and IC20s (mg contaminant / kg soil d.w.) for six endpoints for northern wheatgrass grown in soil spiked with each chemical compound independently.

Species	Soil	Contaminant	Root/Shoot	Parameter	Model	IC50 (mg/kg soil d.w.)	LCL	UCL	IC20 (mg/kg soil d.w.)	LCL	UCL	W?
Northern Wheatgrass	RS	Toluene	Shoot	Length	Hormesis	8323	6498	10148	3958	2823	5093	N
			Shoot	Wet Mass	Hormesis	5178	3856	6500	2947	1781	4113	N
			Shoot	Dry Mass	Hormesis	4860	3874	5846	3101	2165	4037	N
			Root	Length	Gompertz	5460	4002	6918	1331	478	2185	N
			Root	Wet Mass	Exponential	3149	1421	4878	821	-685	2326	N
			Root	Dry Mass	Hormesis	4549	3490	5607	2878	1700	4055	N
Northern Wheatgrass	RS	Ethylbenzene	Shoot	Length	Hormesis	9572	7569	11576	4160	3432	4888	N
			Shoot	Wet Mass	Hormesis	3263	2188	4338	1270	758	1781	N
			Shoot	Dry Mass	Gompertz	3604	2878	4331	1578	887	2269	N
			Root	Length	Logistic	5126	4194	6057	1439	894	1984	N
			Root	Wet Mass	Logistic	1820	1015	2624	695	149	1242	N
			Root	Dry Mass	Gompertz	3015	2237	3793	1383	642	2123	N
Northern Wheatgrass	RS	Xylenes	Shoot	Length	Logistic	8026	6505	9548	3346	2251	4441	N
			Shoot	Wet Mass	Logistic	4903	3806	6000	3313	1878	4747	N
			Shoot	Dry Mass	Logistic	4309	3398	5220	3122	1972	4271	N
			Root	Length	Gompertz	5075	4144	6006	1739	974	2504	N
			Root	Wet Mass	Logistic	4278	3064	5492	3131	1599	4663	N
			Root	Dry Mass	Logistic	3455	2694	4217	2388	1557	3219	N

1 There was no significant difference in growth for all concentrations relative to the controls with the exception of 12 mg benzene/g RS d.w. whereby a significant decrease in growth was observed.

2 There was no significant difference in growth for all concentrations relative to the controls with the exception of 1 mg benzene/g RS d.w. whereby a significant increase in growth was observed.

W? Indicates if data has been weighted; LCL Lower 95% Confidence Limit; RS Alberta clay loam

N No; UCL Upper 95% Confidence Limit

Y Yes; AS Artificial soil

Table B.2. Summary of estimated effect IC50s and IC20s (mg contaminant / kg soil d.w.) for six endpoints for northern wheatgrass grown in soil spiked with each chemical compound independently. The estimated effect concentrations were derived using the associated regression equations derived for each chemical in each type of soil.

Species	Soil	Contaminant	Root/Shoot	Parameter	Model	IC50 (mg/kg soil d.w.)	LCL	UCL	IC20 (mg/kg soil d.w.)	LCL	UCL	W?
Northern Wheatgrass	AS	Benzene	Shoot	Length	Logistic	22.72	13.82	31.99	7.05	3.22	11.10	N
			Shoot	Wet Mass	Linear	12.40	9.71	15.15	4.47	3.50	5.46	N
			Shoot	Dry Mass	Gompertz	7.52	4.60	10.57	1.70	0.23	3.34	N
			Root	Length	Gompertz	14.23	9.83	18.77	4.07	1.24	7.13	N
			Root	Wet Mass	Logistic	9.17	6.57	11.84	5.68	2.64	8.90	N
			Root	Dry Mass	Exponential	5.66	3.82	7.56	0.97	-0.73	3.01	N
Northern Wheatgrass	AS	Toluene	Shoot	Length	Hormesis	517.57	173.92	1008.23	45.65	23.17	74.30	N
			Shoot	Wet Mass	Hormesis	74.50	38.03	120.92	24.07	6.68	50.06	N
			Shoot	Dry Mass	Logistic	68.49	38.48	105.59	21.97	6.19	45.46	N
			Root	Length	Logistic	119.40	82.07	162.56	10.41	4.22	18.85	N
			Root	Wet Mass	Logistic	19.25	8.64	33.27	5.20	0.94	12.14	N
			Root	Dry Mass	Gompertz	28.32	10.34	53.45	3.35	0.05	10.41	N
Northern Wheatgrass	AS	Ethylbenzene	Shoot	Length	Gompertz	1047.37	731.03	1404.61	254.90	85.54	484.75	N
			Shoot	Wet Mass	Gompertz	166.71	46.18	336.85	14.11	-	51.27	N
			Shoot	Dry Mass	Gompertz	212.16	92.95	364.80	29.32	1.32	79.05	N
			Root	Length	Gompertz	643.73	377.21	960.76	52.22	7.46	122.54	N
			Root	Wet Mass	Gompertz	15.51	-	63.19	0.20	-	2.04	N
			Root	Dry Mass	Gompertz	177.12	83.68	294.80	19.94	1.41	51.51	N
Northern Wheatgrass	AS	Xylenes	Shoot	Length	Gompertz	564.26	374.30	778.09	59.47	15.63	118.70	N
			Shoot	Wet Mass	Gompertz	124.09	56.33	207.29	14.93	0.61	38.39	N
			Shoot	Dry Mass	Gompertz	134.53	78.16	200.08	19.98	4.34	41.76	N
			Root	Length	Exponential	290.74	180.07	417.67	131.40	78.04	193.12	N
			Root	Wet Mass	Gompertz	65.37	23.20	119.80	9.18	-	27.16	N
			Root	Dry Mass	Exponential	113.79	65.48	170.25	27.93	15.14	43.13	N
Northern Wheatgrass	RS	Benzene	Shoot[1]	Length	Gompertz	199.73	79.91	298.32	119.66	86.27	150.20	N
			Shoot[1]	Wet Mass	Hormesis	206.46	-105.69	400.51	83.12	25.69	128.84	N
			Shoot[2]	Dry Mass	Hormesis	181.00	-53.44	327.49	76.26	31.51	113.25	N
			Root	Length	Hormesis	330.79	-168.27	641.02	74.19	41.15	102.83	N
			Root	Wet Mass	Hormesis	65.85	46.92	83.11	43.19	29.76	55.36	N

Table B.2. Summary of estimated effect IC50s and IC20s (mg contaminant / kg soil d.w.) for six endpoints for northern wheatgrass grown in soil spiked with each chemical compound independently. The estimated effect concentrations were derived using the associated regression equations derived for each chemical in each type of soil.

Species	Soil	Contaminant	Root/Shoot	Parameter	Model	IC50 (mg/kg soil d.w.)	LCL	UCL	IC20 (mg/kg soil d.w.)	LCL	UCL	W?
			Root	Dry Mass	Hormesis	112.33	51.68	158.07	61.61	37.55	82.83	N
Northern Wheatgrass	RS	Toluene	Shoot	Length	Hormesis	957.86	658.49	1293.18	310.87	186.37	455.36	N
			Shoot	Wet Mass	Hormesis	466.92	298.82	658.80	198.90	92.79	329.49	N
			Shoot	Dry Mass	Hormesis	424.20	300.94	561.08	214.85	124.70	320.31	N
			Root	Length	Gompertz	505.95	316.12	723.99	59.70	12.67	126.45	N
			Root	Wet Mass	Exponential	219.90	65.92	426.58	28.73	-	139.01	N
			Root	Dry Mass	Hormesis	383.78	256.94	526.72	191.89	86.48	322.48	N
Northern Wheatgrass	RS	Ethylbenzene	Shoot	Length	Hormesis	2494.96	1856.05	3170.18	873.09	685.16	1069.81	N
			Shoot	Wet Mass	Hormesis	642.92	388.56	920.42	195.79	102.18	299.80	N
			Shoot	Dry Mass	Gompertz	728.70	548.85	918.55	257.40	124.56	406.77	N
			Root	Length	Logistic	1135.85	882.09	1401.67	229.17	125.80	343.48	N
			Root	Wet Mass	Logistic	308.10	147.62	488.53	91.60	13.16	190.37	N
			Root	Dry Mass	Gompertz	581.97	399.56	777.17	217.99	82.89	374.07	N
Northern Wheatgrass	RS	Xylenes	Shoot	Length	Logistic	3498.60	2559.25	4530.12	951.69	527.64	1450.28	N
			Shoot	Wet Mass	Logistic	1680.38	1152.76	2269.29	937.76	402.96	1601.44	N
			Shoot	Dry Mass	Logistic	1386.60	973.78	1844.56	858.45	433.33	1368.45	N
			Root	Length	Gompertz	1768.84	1308.34	2272.66	359.39	151.70	618.26	N
			Root	Wet Mass	Logistic	1371.79	834.83	1989.39	862.14	317.20	1559.46	N
			Root	Dry Mass	Logistic	998.18	689.34	1342.78	576.12	304.88	898.44	N

1 There was no significant difference in growth for all concentrations relative to the controls with the exception of 12 mg benzene/g RS d.w. whereby a significant decrease in growth was observed.

2 There was no significant difference in growth for all concentrations relative to the controls with the exception of 1 mg benzene/g RS d.w. whereby a significant increase in growth was observed.

\- 95% confidence limit was less than zero.

EEC Estimated effect concentrations – Nominal LC50s adjusted for measured concentrations (Figures C.1 or C.2; Appendix C)

W? Indicates if data has been weighted; LCL Lower 95% Confidence Limit; RS Alberta clay loam

N No; UCL Upper 95% Confidence Limit

Y Yes; AS Artificial soil

Table B.3. The effect of soil type on the toxicity of benzene, toluene, ethylbenzene, and xylenes to northern wheatgrass seedlings, according to the nominal average IC50s and IC20s of all endpoints, in artificial (AS) and clay loam (RS) soils.

	IC50 (mg/kg soil d.w.)				IC20 (mg/g soil d.w.)			
Chemical	AS	Standard Deviation (mg/kg AS d.w.)	RS	Standard Deviation (mg/kg RS d.w.)	AS	Standard Deviation (mg/kg AS d.w.)	RS	Standard Deviation (mg/kg RS d.w.)
Benzene	6802.00	3131.18	18109.67	11625.99	2524.00	1356.55	5545.33	2459.49
Toluene	3977.17	3048.18	5253.17	1705.89	1353.17	716.51	2506.00	1184.49
Ethylbenzene	2155.67	1537.04	4400.00	2748.73	634.67	656.25	1754.17	1277.57
Xylene	1808.83	1035.83	5007.67	1584.75	601.33	429.13	2839.83	641.95

Table B.4. The effect of soil type on the toxicity of benzene, toluene, ethylbenzene, and xylenes to northern wheatgrass seedlings, according to the average IC50s and IC20s of all endpoints (EECs), in artificial (AS) and clay loam (RS) soils.

	IC50 (mg/kg soil d.w.)				IC20 (mg/g soil d.w.)			
Chemical	AS	Standard Deviation (mg/kg AS d.w.)	RS	Standard Deviation (mg/kg RS d.w.)	AS	Standard Deviation (mg/kg AS d.w.)	RS	Standard Deviation (mg/kg RS d.w.)
Benzene	11.95	6.14	182.69	90.99	3.99	2.32	76.34	25.47
Toluene	137.92	189.43	493.10	248.27	18.44	15.83	167.49	105.12
Ethylbenzene	377.10	390.53	982.08	788.16	61.78	96.18	310.84	281.27
Xylene	215.46	187.13	1784.07	882.74	43.82	46.41	757.59	237.79

EEC Estimated effect concentrations – Nominal IC50s adjusted for measured concentrations (Figures C.1 or C.2; Appendix C)

Table B.5. Rank of the most sensitive endpoints of northern wheatgrass upon exposure to benzene, toluene, ethylbenzene, and xylenes in artificial (AS) and clay loam (RS) soils.

	Mean IC50 (mg/kg soil d.w.)		Mean IC20 (mg/kg soil d.w.)	
	AS	RS	AS	RS
Benzene				
Most sensitive	Root Dry Mass	Root Wet Mass	Root Dry Mass	Root Wet Mass
	Shoot Dry Mass	Root Dry Mass	Shoot Dry Mass	Root Dry Mass
	Root Wet Mass	Shoot Dry Mass	Root Length	Root Length
	Shoot Wet Mass	Shoot Length	Shoot Wet Mass	Shoot Dry Mass
	Root Length	Shoot Wet Mass	Root Wet Mass	Shoot Wet Mass
Least sensitive	Shoot Length	Root Length	Shoot Length	Shoot Length
Toluene				
Most sensitive	Root Wet Mass	Root Wet Mass	Root Dry Mass	Root Wet Mass
	Root Dry Mass	Root Dry Mass	Root Wet Mass	Root Length
	Shoot Dry Mass	Shoot Dry Mass	Root Length	Root Dry Mass
	Shoot Wet Mass	Shoot Wet Mass	Shoot Dry Mass	Shoot Wet Mass
	Root Length	Root Length	Shoot Wet Mass	Shoot Dry Mass
Least sensitive	Shoot Length	Shoot Length	Shoot Length	Shoot Length
Ethylbenzene				
Most sensitive	Root Wet Mass	Root Wet Mass	Root Wet Mass	Root Wet Mass
	Shoot Wet Mass	Root Dry Mass	Shoot Wet Mass	Shoot Wet Mass
	Root Dry Mass	Shoot Wet Mass	Root Dry Mass	Root Dry Mass
	Shoot Dry Mass	Shoot Dry Mass	Shoot Dry Mass	Root Length
	Root Length	Root Length	Root Length	Shoot Dry Mass
Least sensitive	Shoot Length	Shoot Length	Shoot Length	Shoot Length
Xylene				
Most sensitive	Root Wet Mass	Root Dry Mass	Root Wet Mass	Root Length
	Root Dry Mass	Root Wet Mass	Shoot Wet Mass	Root Dry Mass
	Shoot Wet Mass	Shoot Dry Mass	Shoot Dry Mass	Shoot Dry Mass
	Shoot Dry Mass	Shoot Wet Mass	Root Dry Mass	Root Wet Mass
	Root Length	Root Length	Shoot Length	Shoot Wet Mass
Least sensitive	Shoot Length	Shoot Length	Root Length	Shoot Length

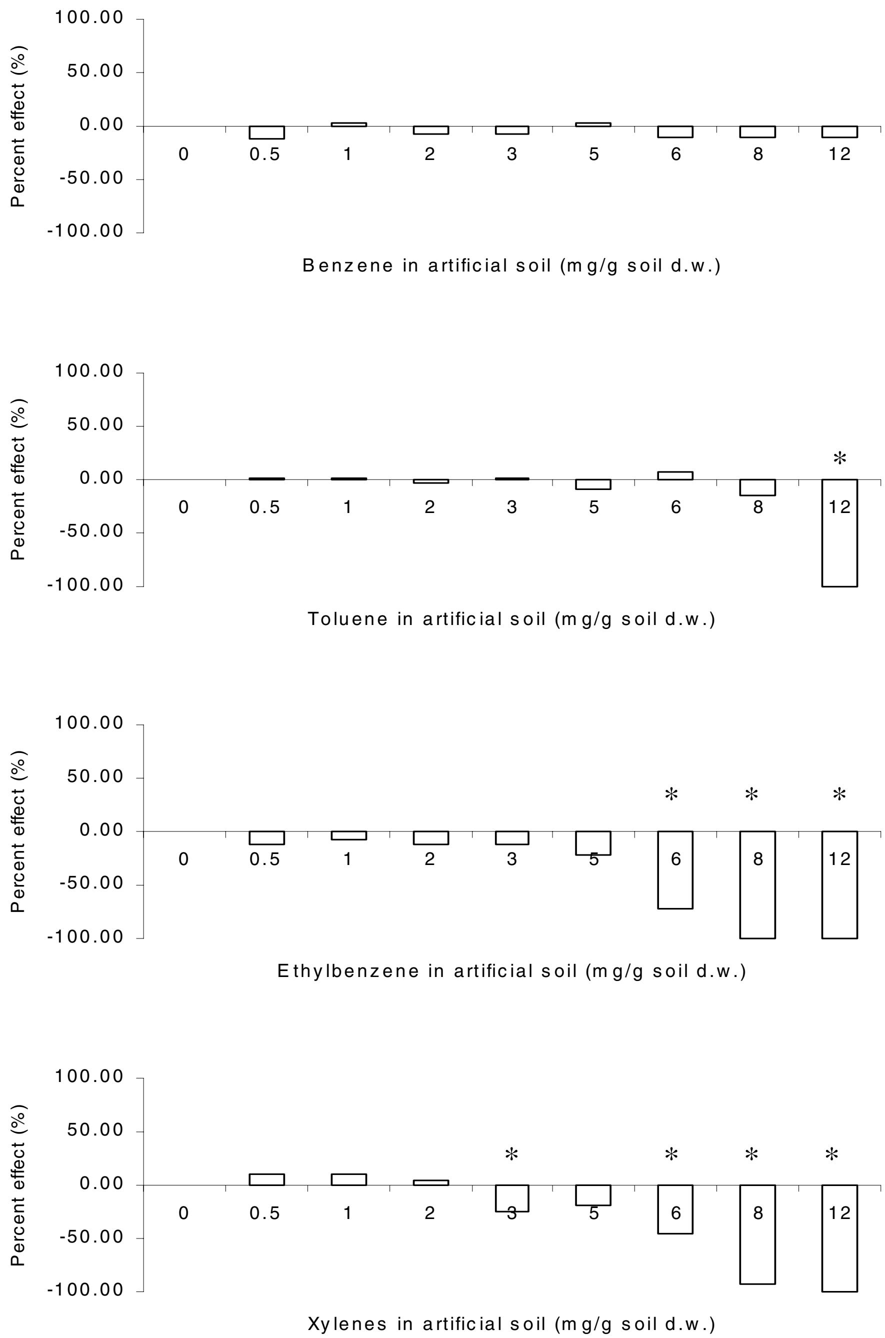


Figure B.1. Seedling emergence expressed as a percent of the control emergence upon exposure to nominal concentrations of benzene, toluene, ethylbenzene, or xylene in artificial soil (AS). Stars denote significant differences ($p \leq 0.05$) from the controls.

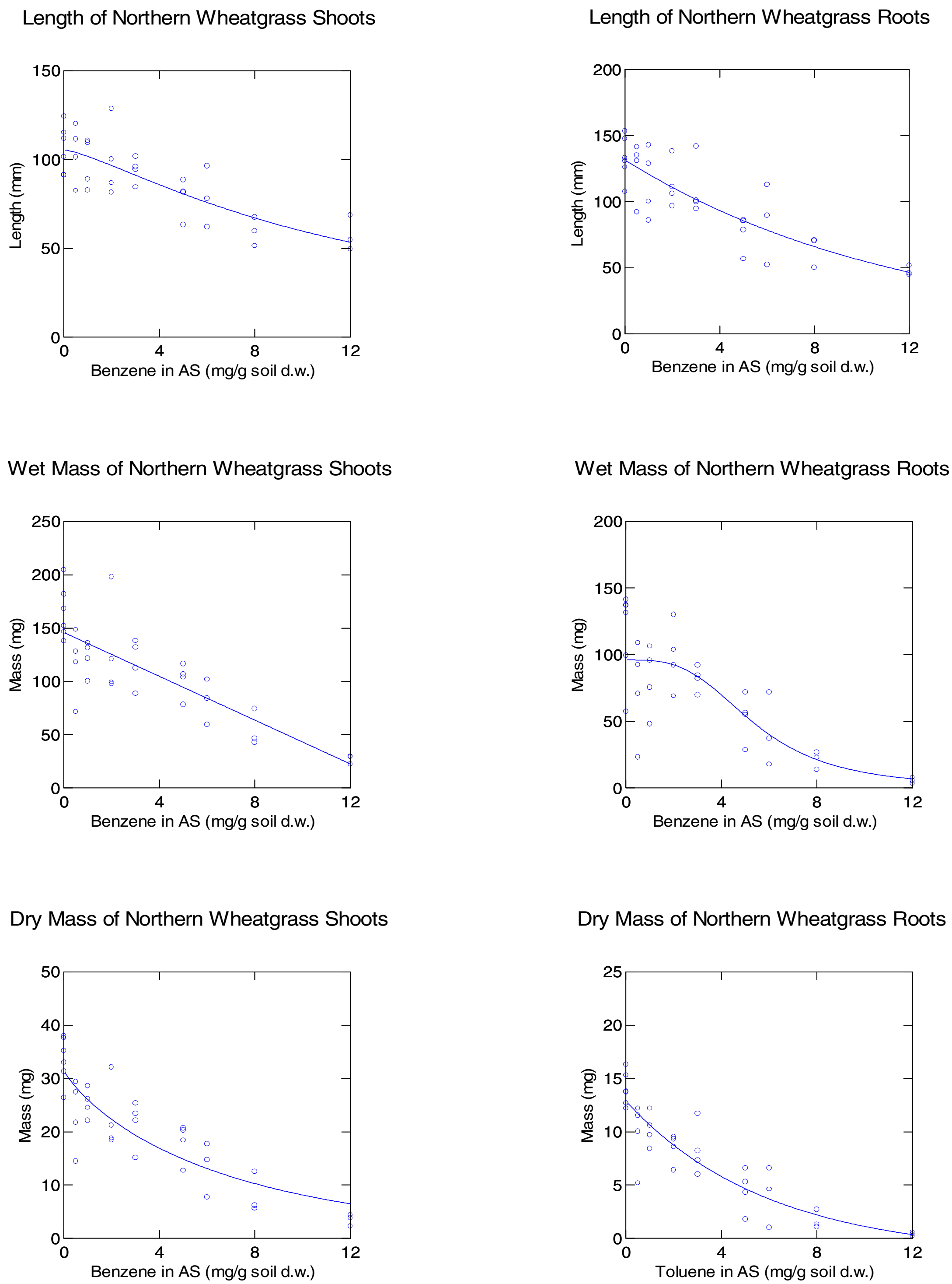


Figure B.2. The effect of short-term (acute) exposure of northern wheatgrass grown in artificial soil (AS) amended with nominal concentrations of benzene (mg benzene/g soil d.w.).

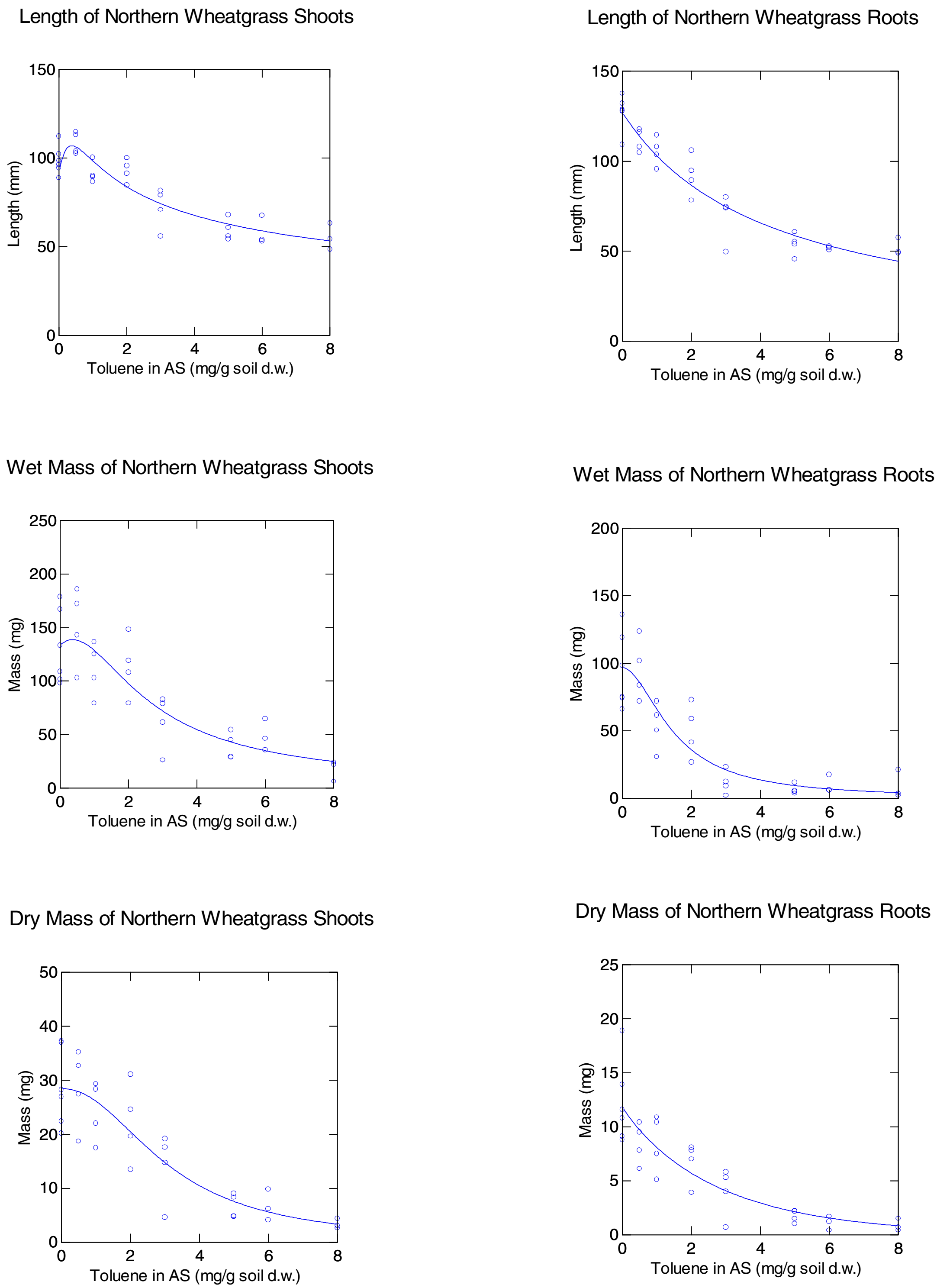


Figure B.3. The effect of short-term (acute) exposure of northern wheatgrass grown in artificial soil (AS) amended with nominal concentrations of toluene (mg toluene/g soil d.w.).

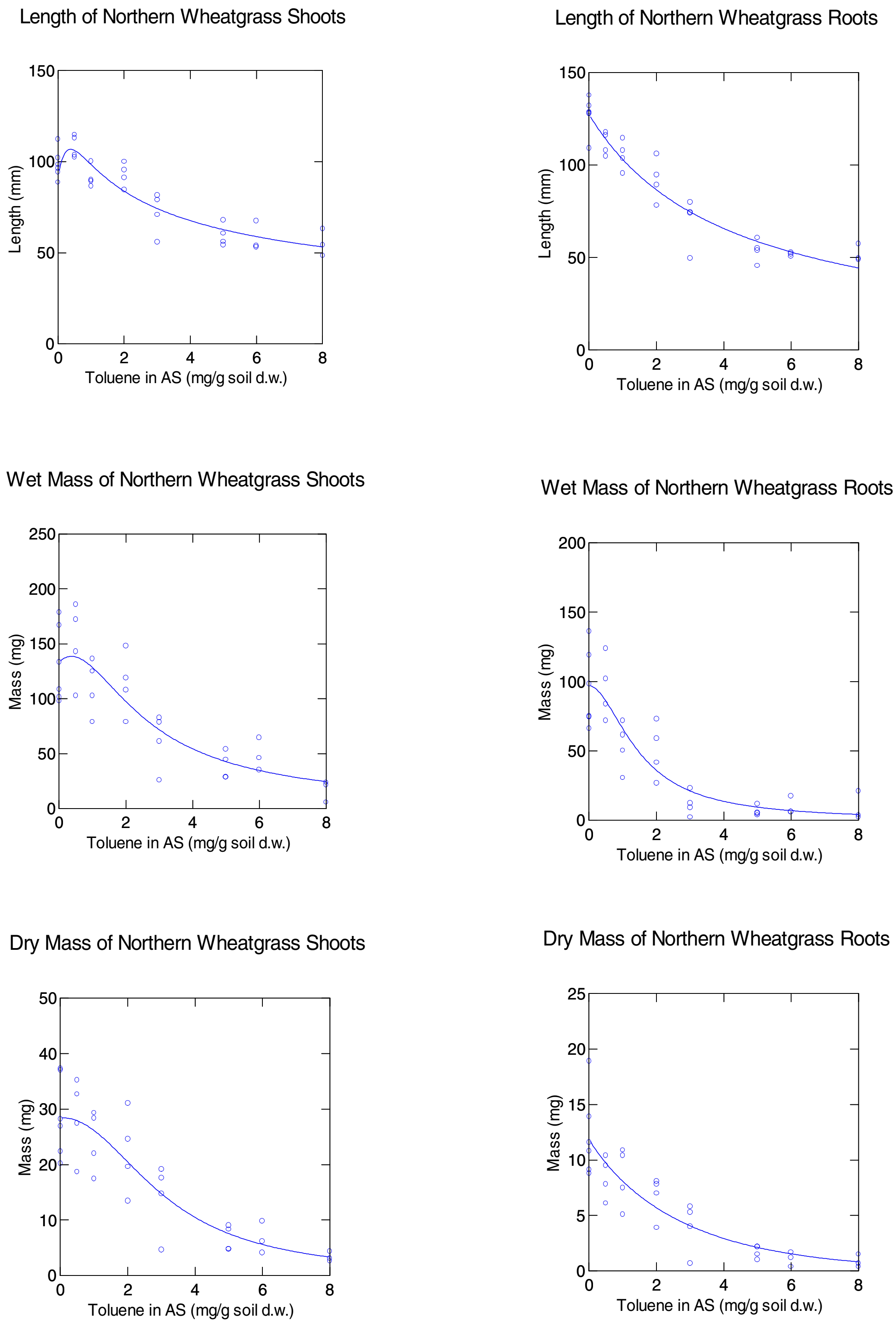


Figure B.4. The effect of short-term (acute) exposure of northern wheatgrass grown in artificial soil (AS) amended with nominal concentrations of ethylbenzene (mg ethylbenzene/g soil d.w.).

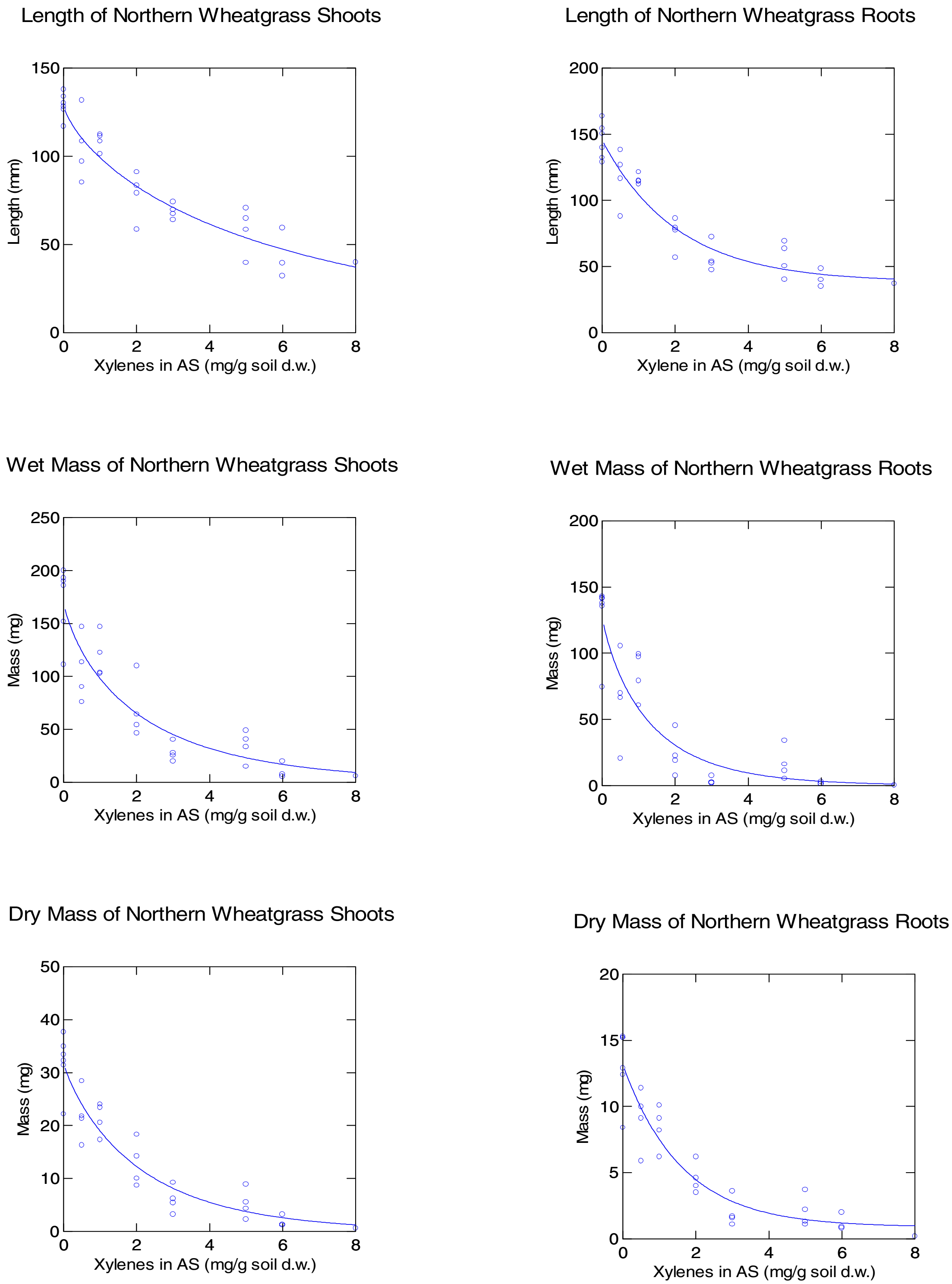


Figure B.5. The effect of short-term (acute) exposure of northern wheatgrass grown in artificial soil (AS) amended with nominal concentrations of xylenes (mg xylenes/g soil d.w.).

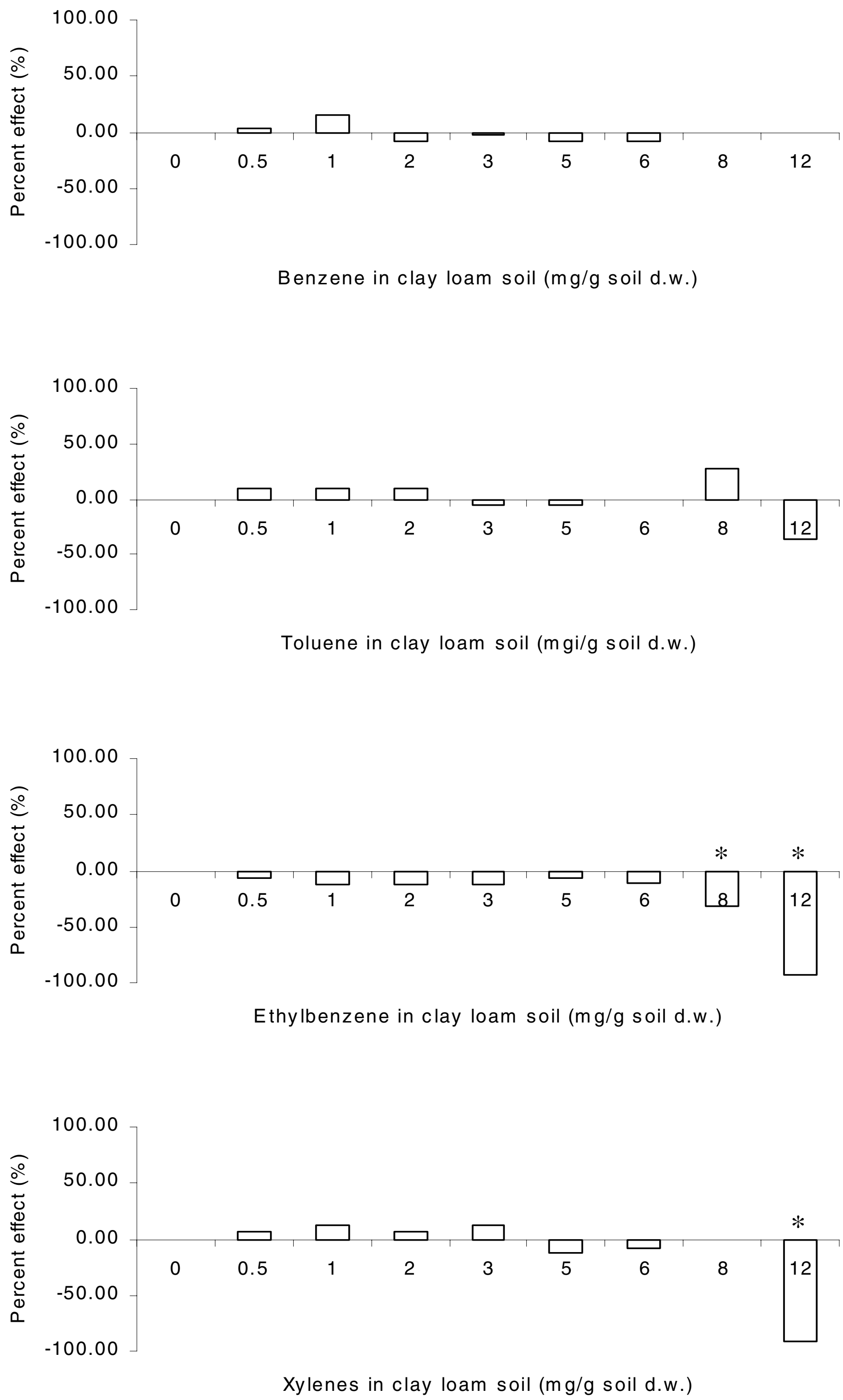


Figure B.6. Seedling emergence expressed as a percent of the control emergence upon exposure to nominal concentrations of benzene, toluene, ethylbenzene, or xylene in the Alberta clay loam soil (RS). Stars denote significant differences ($p \leq 0.05$) from the controls.

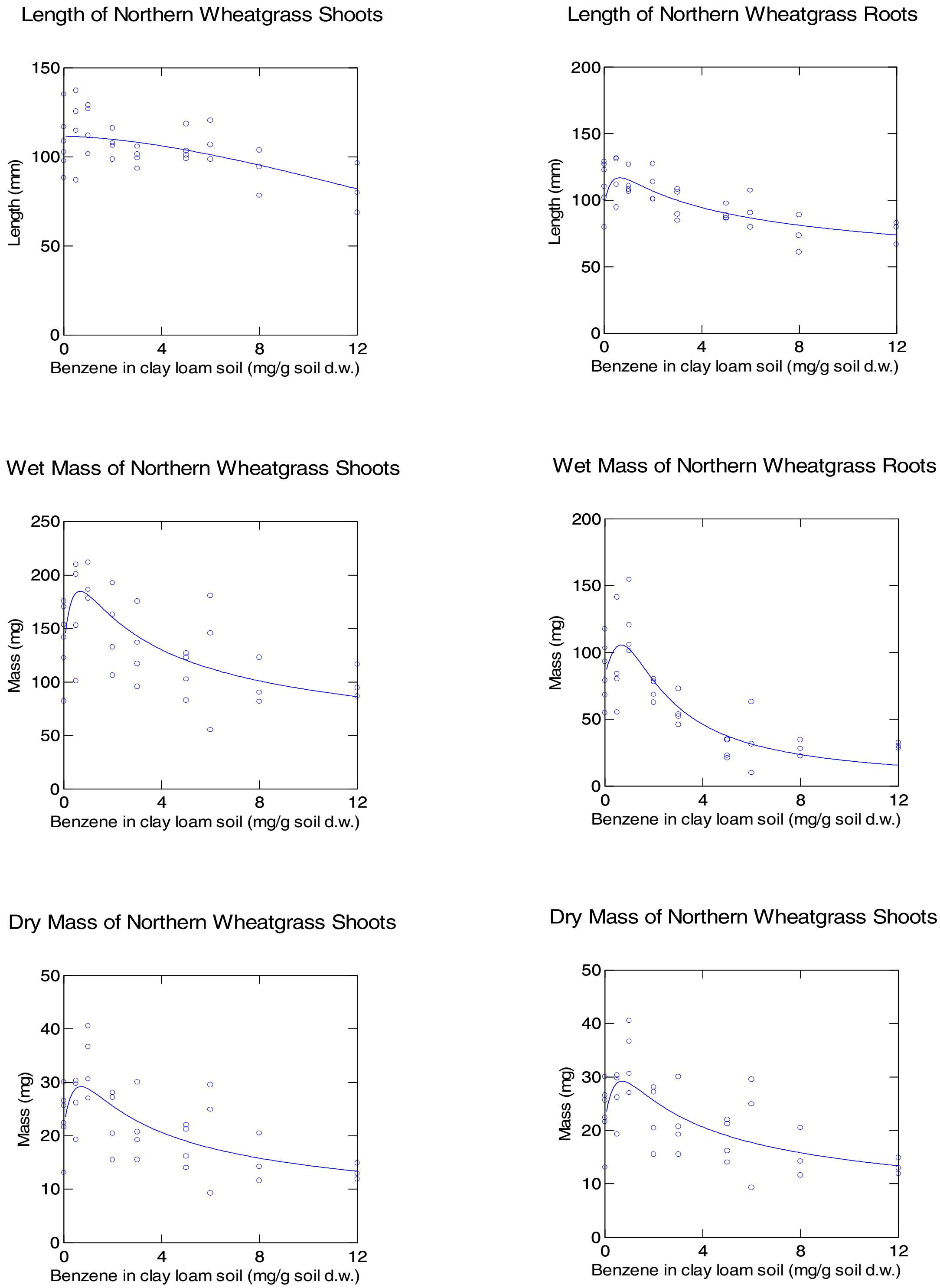


Figure B.7. The effect of short-term (acute) exposure of northern wheatgrass grown in Alberta clay loam soil (RS) amended with nominal concentrations of benzene (mg benzene/g soil d.w.).

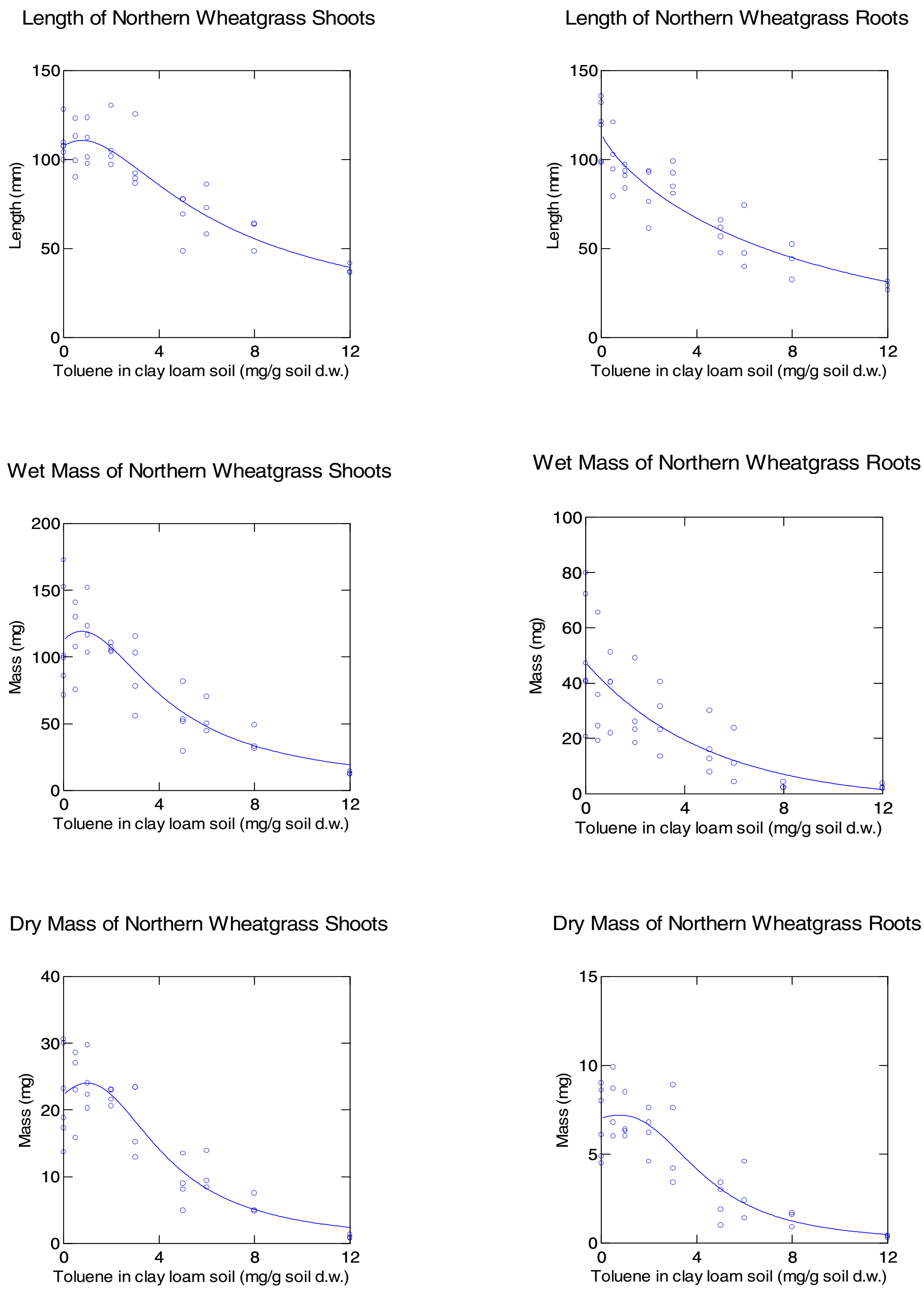


Figure B.8. The effect of short-term (acute) exposure of northern wheatgrass grown in Alberta clay loam soil (RS) amended with nominal concentrations of toluene (mg toluene/g soil d.w.).

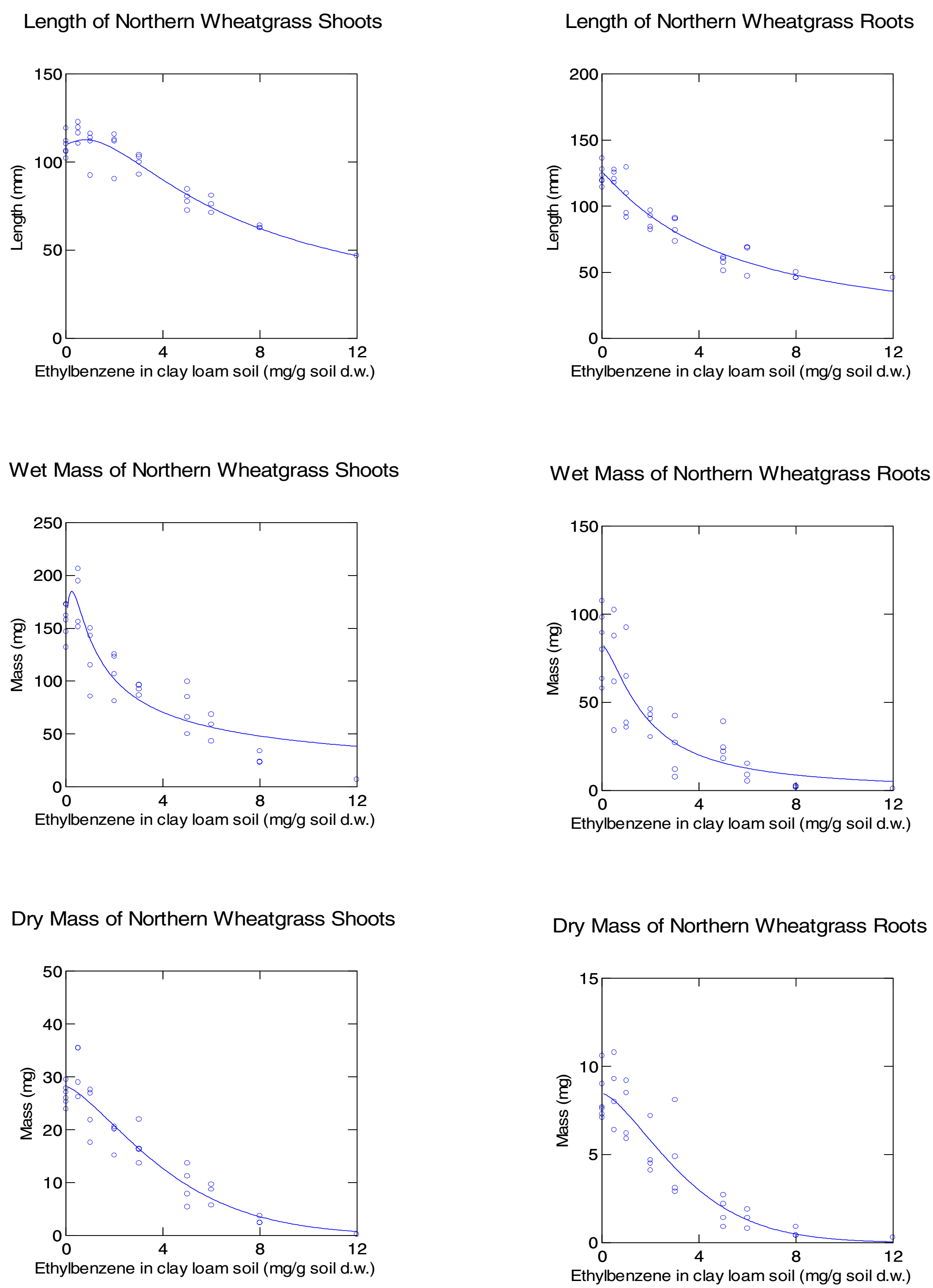


Figure B.9. The effect of short-term (acute) exposure of northern wheatgrass grown in Alberta clay loam soil (RS) amended with nominal concentrations of ethylbenzene (mg ethylbenzene/g soil d.w.).

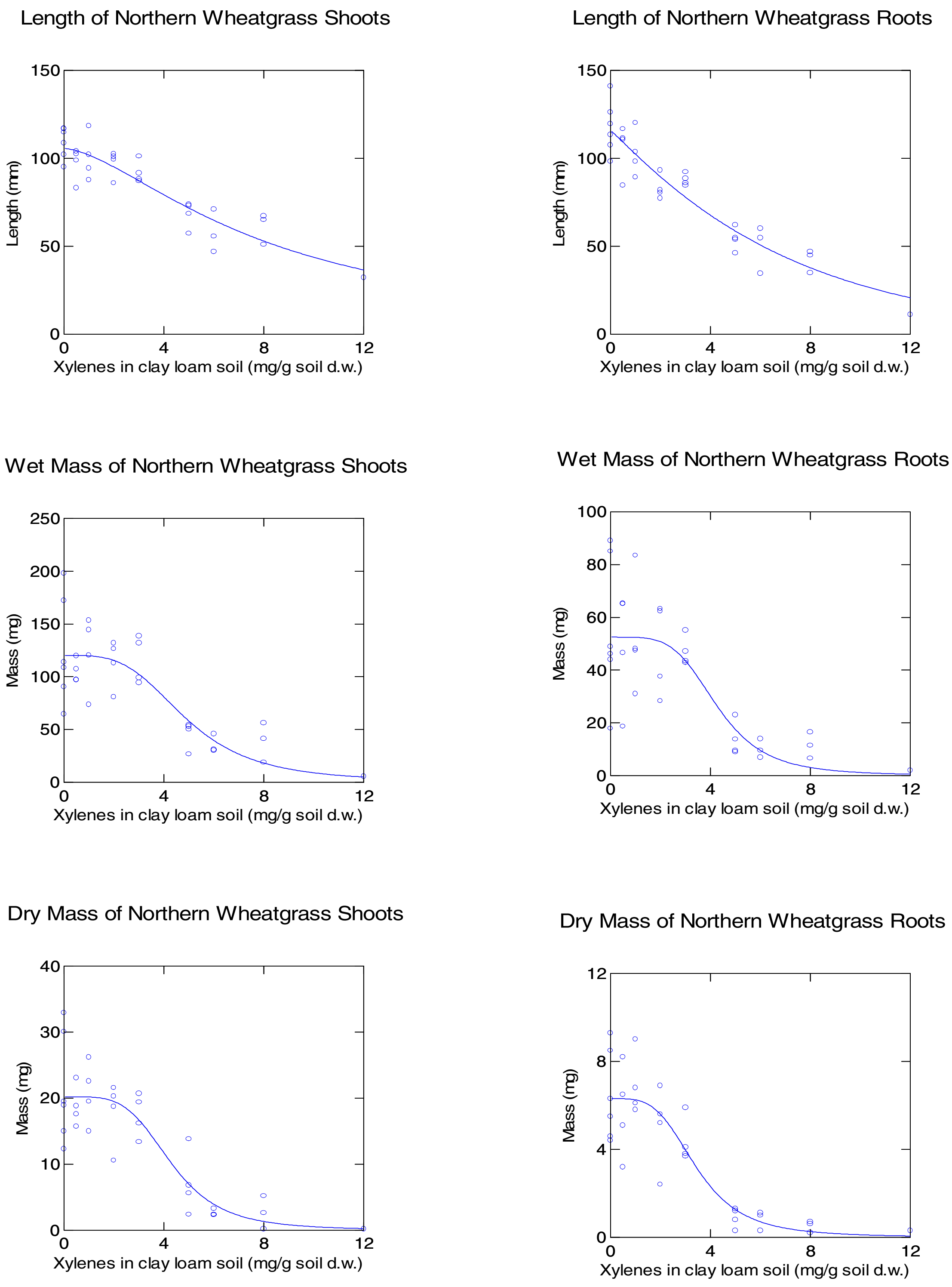


Figure B.10. The effect of short-term (acute) exposure of northern wheatgrass grown in Alberta clay loam soil (RS) amended with nominal concentrations of xylenes (mg xylenes/g soil d.w.).

APPENDIX C

THE QUANTIFICATION OF BENZENE, TOLUENE, ETHYLBENZENE, AND XYLENES AS DETERMINED THROUGH CHEMICAL ANALYSES

Table C.1. Comparison of the measured initial exposure concentrations (t=0) of benzene, toluene, ethylbenzene, and xylenes in sandy loam soil (AS) prepared by spiking the soils at 500, 3,000, and 12,000 mg/kg soil d.w.

Chemical	Nominal Concentration (mg/kg soil d.w.)	Measured* Concentration (mg/kg soil d.w.)	% of Nominal
Sandy Loam Soil			
Benzene	500	0.37	0.07
	500	0.26	0.05
	500**	1.98	0.40
	500	13.82	2.76
	3,000	1.74	0.06
	3,000	5.31	0.18
	3,000	5.59	0.19
	3,000	0.02	0.0067
	12,000	44.81	0.37
	12,000	36.11	0.30
	12,000	119.82	1.00
	12,000	41.82	0.35
Toluene	500	1.485	0.30
	500	5.795	1.16
	3,000	41.125	1.37
	3,000	81.525	2.72
	12,000	2569.725	21.41
	12,000	234.725	1.96
Ethylbenzene	500	21.735	4.35
	500	50.235	10.05
	3,000	429.635	14.32
	3,000	434.635	14.49
	12,000	5969.635	49.75
	12,000	5099.635	42.50
Xylenes***	500	37.4	7.48
	500	21.38	4.28
	3,000	209.8	6.99
	3,000	945	31.50
	12,000	3473	28.94
	12,000	2877	23.98

*Values have been corrected for background concentrations:

Benzene - 0.09 (AS), 0.95 (RS) mg/kg

Toluene - 0.30 (AS), 0.87 (RS) mg/kg

Ethylbenzene - 0.37 (AS), 0.27 (RS) mg/kg

Xylenes - <0.06 (AS), <0.09 (RS) mg/kg

**Shaded boxes denote values obtained from the second set of earthworm toxicity tests with benzene

***Includes m-, p- & o-xylenes

Table C.2. Comparison of the measured initial exposure concentrations (t=0) of benzene, toluene, ethylbenzene, and xylenes in the Alberta clay loam test soils prepared by spiking the soils at 500, 3,000, and 12,000 mg/g soil d.w.

Chemical	Nominal Concentration (mg/kg soil d.w.)	Measured* Concentration (mg/kg soil d.w.)	% of Nominal
Alberta Clay Loam Soil			
Benzene	500	10.65	2.13
	500	21.95	4.39
	500**	30.74	6.15
	500	6.94	1.39
	3,000	13.75	0.46
	3,000	6.31	0.21
	3,000	228.74	7.62
	3,000	63.74	2.12
	12,000	502.05	4.18
	12,000	84.75	0.71
	12,000	128.74	1.07
	12,000	158.74	1.32
Toluene	500	19.03	3.81
	500	19.53	3.91
	500	11.13	2.23
	3,000	141.13	4.70
	3,000	92.83	3.09
	12,000	2099.13	17.49
	12,000	1969.13	16.41
	12,000	2159.13	17.99
Ethylbenzene	500	73.63	14.73
	500	73.03	14.61
	3,000	408.73	13.62
	3,000	339.73	11.32
	12,000	3909.73	32.58
	12,000	4629.73	38.58
Xylenes***	500	41.13	8.23
	500	44.83	8.97
	500	69.13	13.83
	3,000	1023.93	34.13
	3,000	1339.93	44.66
	12,000	4919.93	41.00
	12,000	5369.93	44.75

*Values have been corrected for background levels:

benzene - 0.09 (AS), 0.95 (RS) ug/g

toluene - 0.30 (AS), 0.87 (RS) ug/g

ethylbenzene - 0.37 (AS), 0.27 (RS) ug/g

xylenes - <0.06 (AS), <0.09 (RS) ug/g

**Shaded boxes denote values obtained from the second set of earthworm toxicity tests with benzene ***Includes m-, p- & o-xylenes

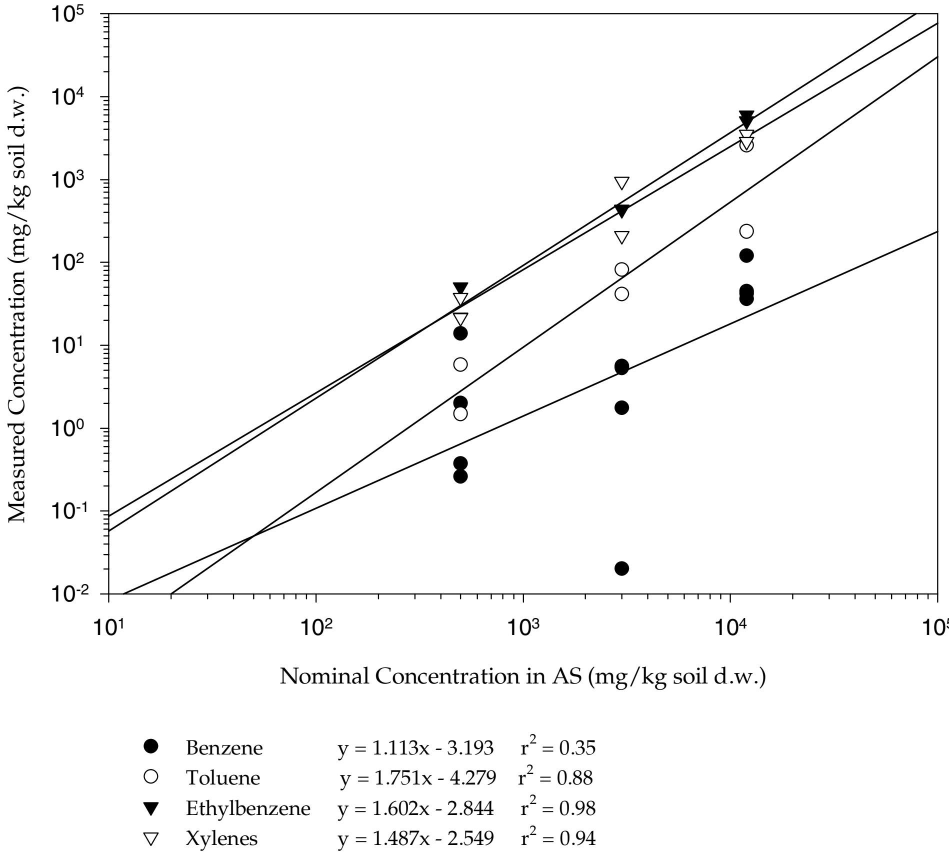


Figure C.1. Regression of the initial (t=0) acute nominal exposure concentrations of benzene, toluene, ethylbenzene, and xylenes in the sandy loam soil (AS) versus the measured concentrations of these constituents in the artificial soil.

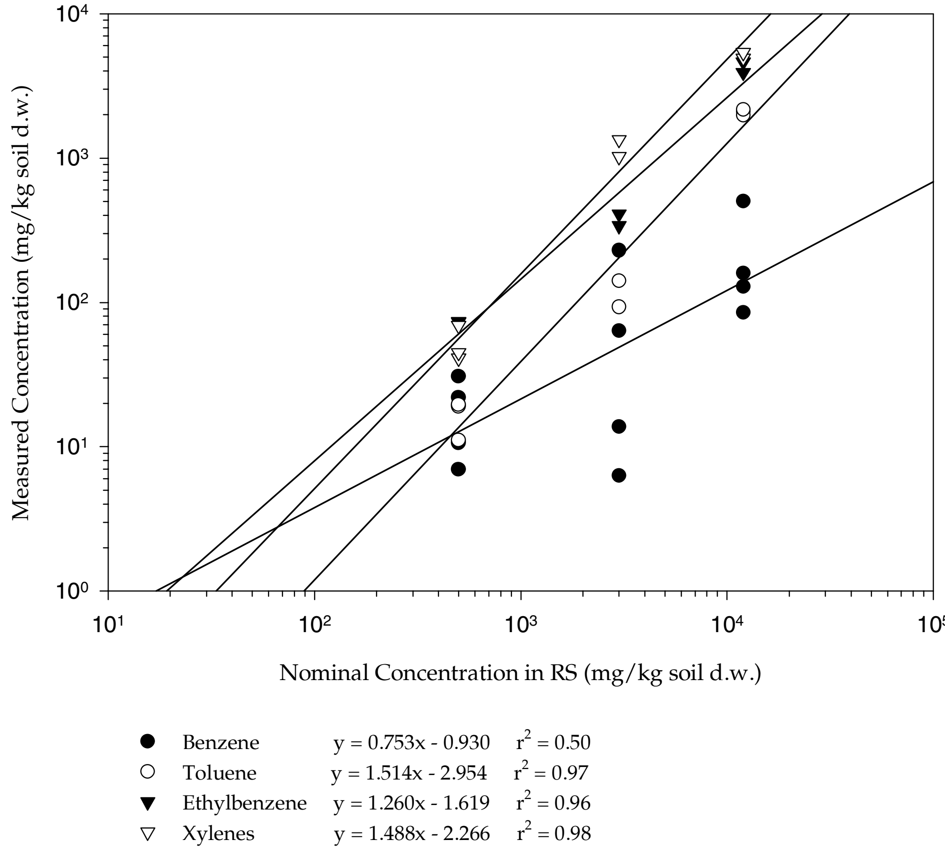


Figure C.2. Regression of the initial (t=0) acute nominal exposure concentrations of benzene, toluene, ethylbenzene, and xylenes in the Alberta clay loam (RS) versus the measured concentrations of these constituents in the clay loam soil.

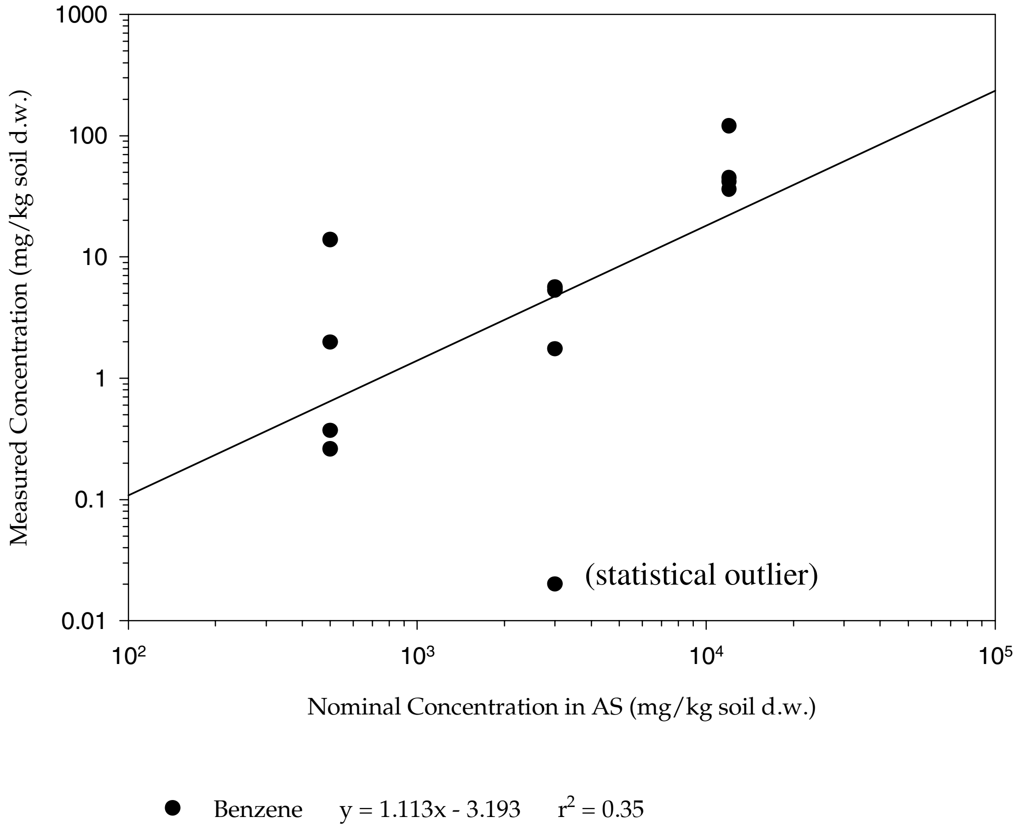


Figure C.3. Regression of the initial (t=0) acute nominal exposure concentrations of benzene versus the measured concentrations of benzene in the artificial sandy loam soil (AS). The regression incorporated data from all of the tests performed with benzene in the artificial soil.

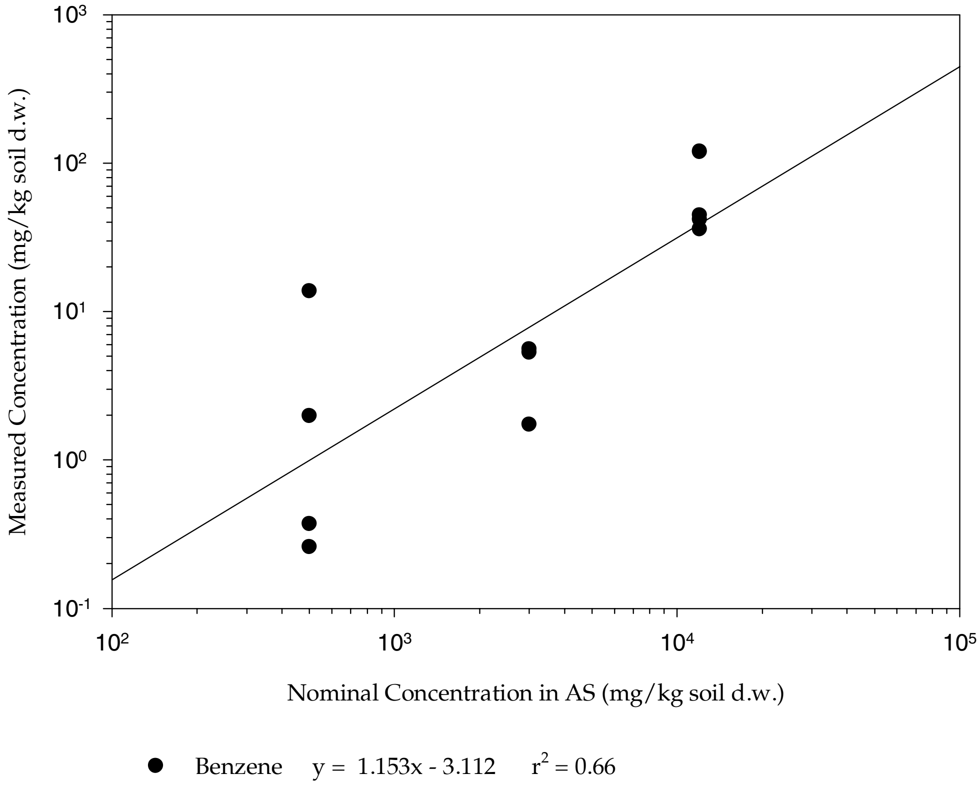


Figure C.4. Regression of the initial (t=0) acute nominal exposure concentrations of benzene versus the measured concentrations of benzene in the artificial sandy loam soil (AS). The regression incorporated data from all of the tests performed with benzene in the artificial soil. The statistical outlier has been removed.

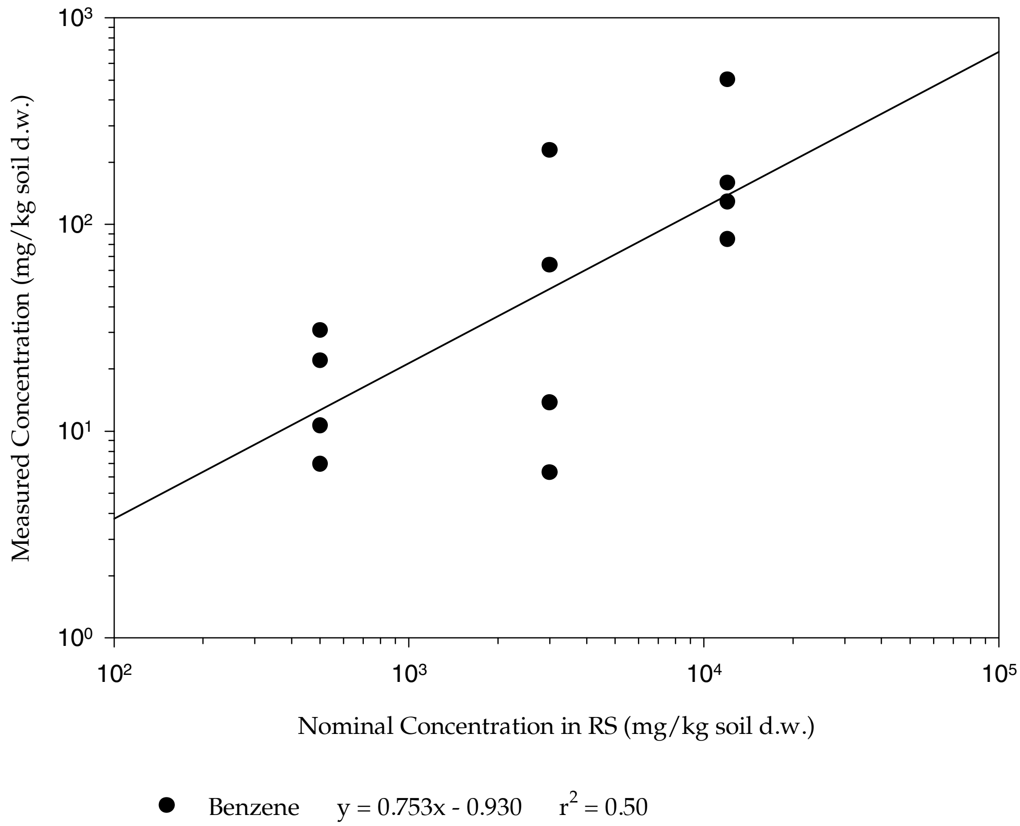


Figure C.5. Regression of the initial (t=0) acute nominal exposure concentrations of benzene versus the measured concentrations of benzene in the reference clay loam soil (RS). The regression incorporated data from all of the tests performed with benzene in the reference soil.

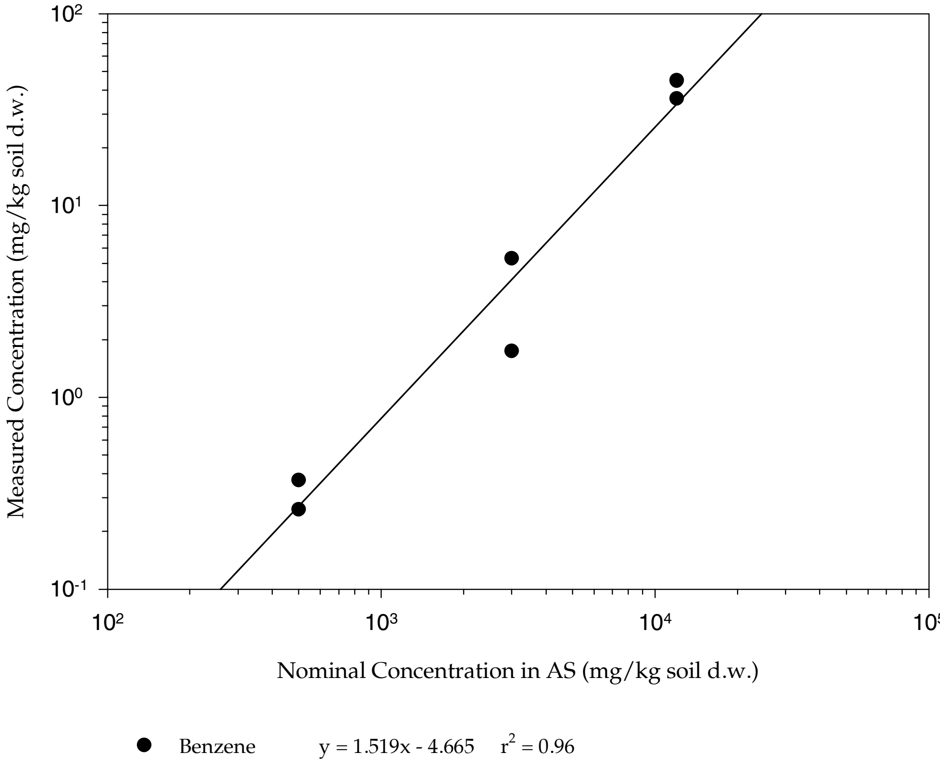


Figure C.6. Regression of the initial (t=0) acute nominal exposure concentrations of benzene versus the measured concentrations of benzene in the artificial sandy loam soil (AS). Data are derived from the first acute earthworm test with benzene.

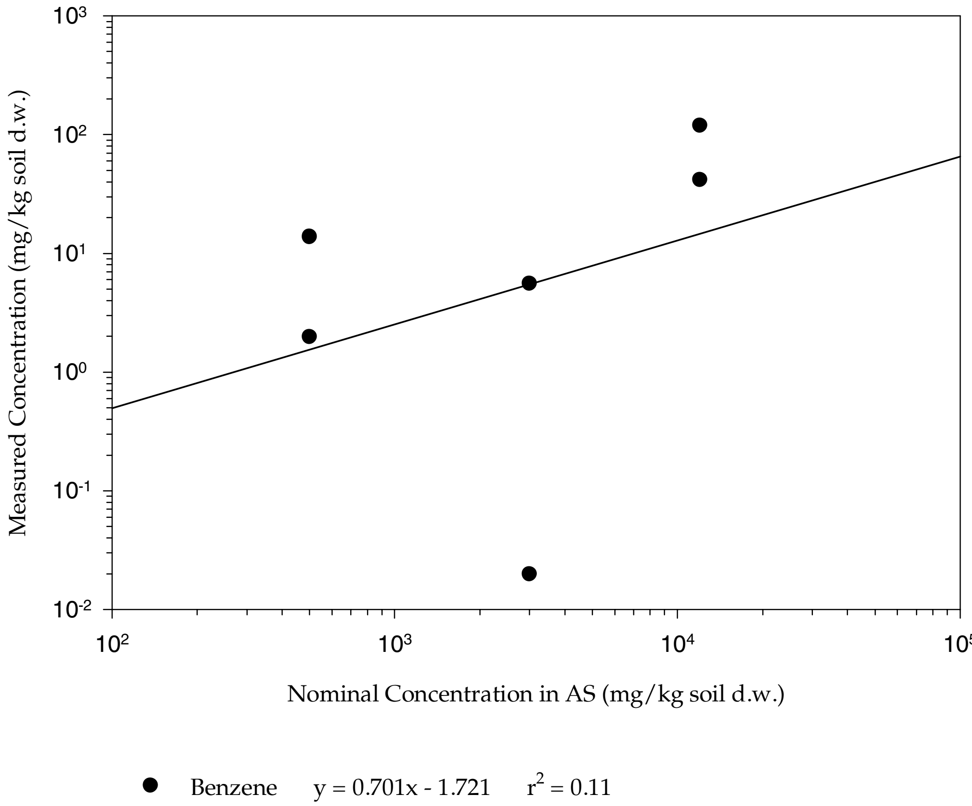


Figure C.7. Regression of the initial (t=0) acute nominal exposure concentrations of benzene versus the measured concentrations of benzene in the artificial sandy loam soil (AS). Data are derived from the second acute earthworm test with benzene.

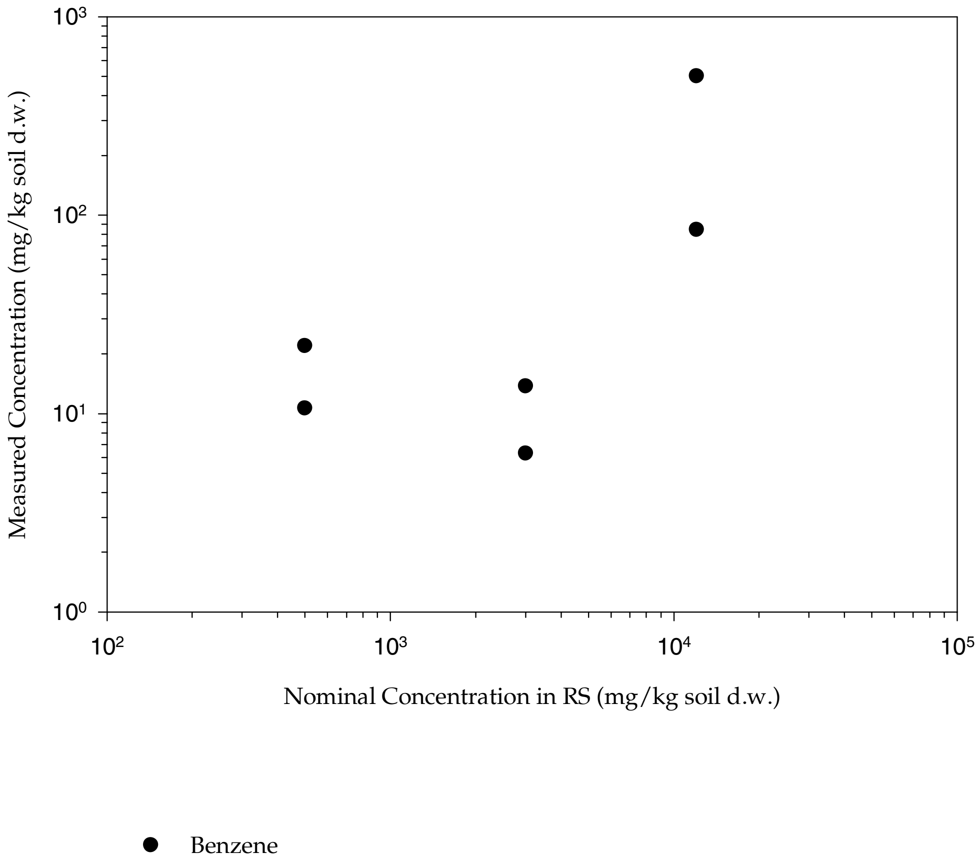
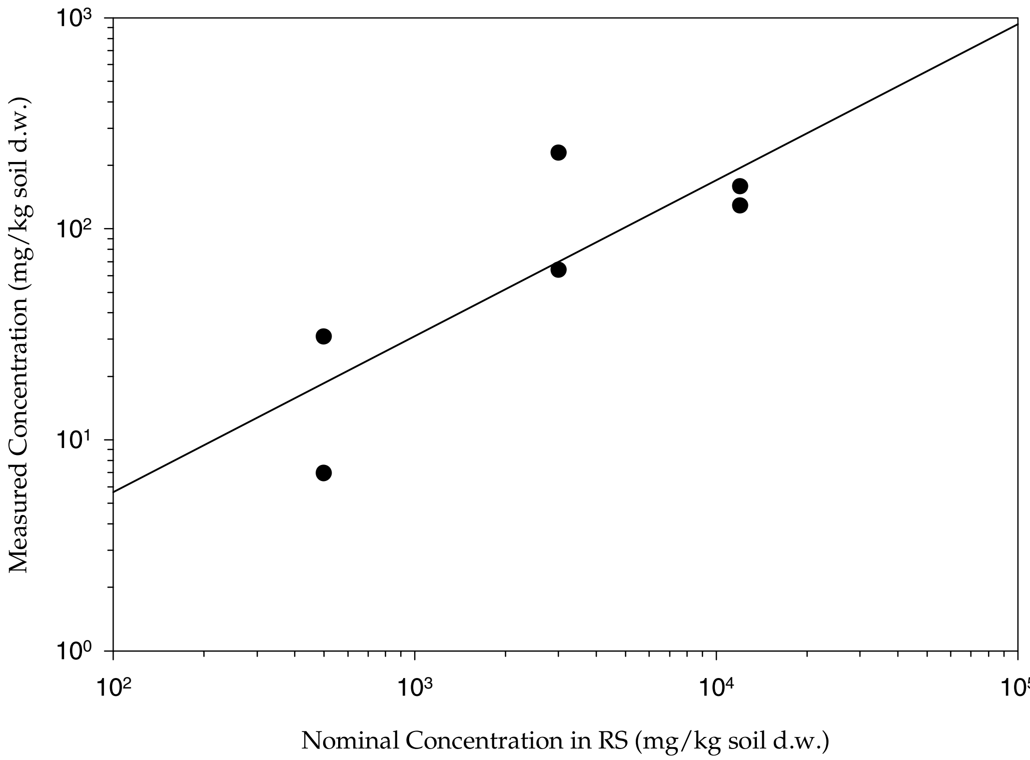


Figure C.8. Regression of the initial (t=0) acute nominal exposure concentrations of benzene versus the measured concentrations of benzene in the reference clay loam soil (RS). Data are derived from the first earthworm test with benzene.



● Benzene   y = 0.740x - 0.728 $r^2 = 0.66$

Figure C.9. Regression of the initial (t=0) acute nominal exposure concentrations of benzene versus the measured concentrations of benzene in the reference clay loam soil (RS). Data are derived from the second earthworm test with benzene.