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Eco-Toxicity of Sulphate Relative to Chloride

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December 16, 2013**

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1. INTRODUCTION

Management of salts in the top 1.5 m of Alberta soils is currently achieved based on soil electrical conductivity (EC). Deeper soils may be managed either using EC, or based on the concentration of chloride ion, using the subsoil salinity tool (SST).

Alberta Environment (AENV, 2010) soil EC guidelines are based primarily on various research databases of plant salt tolerance including the United States Department of Agriculture (USDA) salinity databases and Howatt (2000). Plant salt tolerance studies commonly use sodium chloride as the source of salt (Howatt, 2000; Maas, 1990). However, research reported in Howatt (2000) suggests that some plants may be less sensitive to sulphate than to chloride.

Numerically the most frequent occurrences of salinity releases at oilfield sites in Alberta are related to chloride, since that is typically the anion that predominates in saline produced water. However, there are a significant number of oilfield sites in Alberta that, historically or currently, store elemental sulphur produced from sour gas sweetening operations. When these sulphur storage sites are decommissioned, environmental assessment activities may reveal areas of soil with elevated sulphate. Given the large footprint of some sulphur storage facilities, the potential size of any plume of elevated sulphate can be correspondingly large.

Currently, sulphate plumes are conservatively assessed based on EC guidelines that are in turn based on plant sensitivity to chloride. If the research reported in Howatt (2000) is correct and representative, it may be that excess sulphate in soil at former sulphur handling facilities can be managed based on sulphate-specific guidelines, which may turn out to be somewhat less stringent than the current chloride-based guidelines. This could potentially result in a significant benefit to industry in the form of cost and time savings, and additionally, reduce unnecessary environmental disruption from cleanup processes.

2. OBJECTIVES

The overall objective of this project was to determine whether sulphate has a significantly different toxicity to chloride for a representative selection of Alberta plants, and if so, to identify what further work might be needed to support a potential future sulphate-specific soil remediation guideline and/or a sulphate ecotoxicity reference value to be used with the SST.

This report describes the preliminary program of research conducted by Exova for PTAC in collaboration with Millennium EMS Solutions Inc. to develop a better understanding of the relative tolerance of plants to sulphate and chloride. An experimental program was completed to investigate the relative toxicity of chloride and sulphate to a range of plant species relevant to Alberta using the Environment Canada (2005) protocol.

3. METHODS AND MATERIALS

3.1. Study Design

Two field-collected topsoils representative of coarse and fine textured soil and an artificial soil were spiked with either sulphate or chloride over a range of concentrations expected to have no effect to strongly toxic effects to plants. Multiple concentration plant toxicity tests were conducted with these soils according to the biological test method for assessing contaminated soil with agricultural plants published by Environment Canada (2005). Test species, including barley, alfalfa and northern wheatgrass were grown in spiked soils for two to three weeks under environmental conditions as specified by the test method. Soils were analyzed in triplicate for EC, chloride and sulphate by saturated paste extraction. Biological endpoints were compared to measured EC and available chloride or sulphate concentrations and the statistically modeled dose-response relationship was used to calculate the IC_{25} and IC_{50} endpoints as EC, chloride or sulphate, with associated 95% confidence intervals.

3.2. Soils

Previous Environment Canada method development work and Alberta Environment contaminated sites soil criteria development studies had been completed using field-collected fine clay loam and coarse sandy loam collected in Alberta (Equilibrium, 2012). To be consistent, these two reference soils were also used for this study.

The fine clay loam soil was collected in 2010 near Delacour, Alberta and was classed as a well-drained Orthic Black Chernozem likely from the Delacour soil group. Laboratory chemistry and texture results are summarized in Table 1 below, with a clay content of approximately 28%, texture of fine (37.5% retained by a 75 µm sieve) and organic matter of 4.0%. Hydrocarbons, pesticides, and herbicides were all below detection limits.

The coarse soil was a sandy loam collected in 2011 near Vulcan, Alberta. Based on soil maps, the soil was likely an Orthic Dark Brown Chernozem from the Kessler or Carmangay soil series. Select parameters from this soil location are presented in Table 1. Salinity was low, with EC of 0.26 dS/m and chloride and sulphate both below 18 mg/kg. Clay content was 18.0%, with soil texture classified as a coarse sandy loam. Organic matter was 3.0%, similar to the clay loam. Hydrocarbons, pesticides, and herbicides were all below detection limits.

Full analytical results are shown in Appendix A.

Soils were prepared by homogenizing approximately 30 pails of collected soils, followed by air drying, breaking up clumps by hand, and coarse sieving through a 5 mm sieve to remove roots, rocks and other debris. The moisture, water holding capacity and optimal moisture content for plant growth was determined on both soils to be able to determine appropriate volumes of water needed for spiking salts for the toxicity tests.

Table 1. R

Parameter	Unit	Fine Soil	Coarse Soil
pH		7.4	5.8
EC	dS/m	0.90	0.26
SAR		<0.1	<0.1
Chloride	mg/kg	7	7
Sulphate	mg/kg	18	14.4
CEC	meq/100 g	19	16
Organic Matter	%	4.0	3.0
Saturation	%	57	51
Sand	%	36.6	61.6
Silt	%	35.4	20.4
Clay	%	28	18
Texture		Clay Loam	Sandy Loam
% retained 75 um sieve	%	37.5	60.1
Coarse vs. Fine		Fine	Coarse

3.3. Preparation of Sulphate and Chloride Test Concentrations

Target concentrations for plant toxicity tests were based on electrical conductivity, ranging from 0 to approximately 30 dS/m. Initial spiking tests were completed for both soils in order to determine the amount of sodium chloride or sodium sulphate required to reach a specific EC. Stock solutions of sodium chloride or sodium sulphate were added to soils at several increasing concentrations, and then additional water was added to reach the optimal water content for toxicity testing. The soils were allowed to sit for several days to hydrate, and then subsampled for salinity analysis. The resulting slope of the curve of EC vs. sulphate or chloride was used to determine approximate spiking concentrations for toxicity tests.

Soils for toxicity tests were prepared by spiking sufficient volumes of soil for each treatment with aqueous stock solutions of sodium chloride or sodium sulphate heptahydrate at a rate calculated to reach the target EC for that treatment. Additional water was then added to the soil to hydrate to the optimal water content for plant growth (approximately 35% or 44% of the water holding capacity for fine and coarse soil, respectively), and mixed well. Soils were allowed to hydrate for several days, then distributed to replicate test vessels.

3.4. Chemical Analysis

Each test treatment was subsampled in triplicate at toxicity test initiation. Soil moisture and pH (1:2 CaCl₂ extraction) were determined, then the subsamples were dried and disaggregated for chemical analysis. Saturated pastes were prepared and analyzed for EC, calcium, magnesium, potassium, sodium, chloride, sulphate and SAR by standard methods. Soils were also analyzed for total metals, including sodium and sulfur, by strong acid extraction and ICP-MS analysis.

3.5. Plant Toxicity Tests

Plant toxicity tests were completed in both the fine and coarse soil spiked with sodium chloride or sodium sulphate at multiple concentrations following the Environment Canada Biological Test Method, for a total of twelve individual tests. Tests were completed with barley (), alfalfa () and northern wheatgrass ().

In summary, eight to ten soil treatments were prepared as described previously, at target EC concentrations ranging from 0 to 30 dS/m, with specific concentrations depending on species tested. An untreated reference soil control was included for each test for comparison to treated soils. Each treatment consisted of six replicate test vessels (1 L polycarbonate cups containing approximately 500 mL moist treated soils). On day 0 soils were subsampled for chemical analysis, and then seeded with 5 or 10 seeds per replicate of the species of interest. An artificial soil control treatment was also included for each test to confirm health of test seeds and that plant growth meets the minimum requirements for a valid test (germination rate, shoot length and root length criteria specific for each species).

Toxicity tests were completed under controlled lighting using full spectrum fluorescent at $300 \pm 100 \mu\text{mol}/(\text{m}^2 \cdot \text{s})$ on a 16: h light:6 h dark cycle. Temperature was maintained at $24 \pm 3^\circ\text{C}$ daytime and $15 \pm 3^\circ\text{C}$ night. Moisture was added as required using deionized water sprayed on the soil surface in equal volumes to each test vessel. Plants were positioned randomly under light banks to minimize any minor effects of table location on growth.

Tests were terminated on day 14 for barley, or day 21 for alfalfa and northern wheatgrass. Observations on health and number of plants emerged were recorded. Each vessel was dismantled; plants were carefully removed from soil, separated into individual plants and washed. Shoot length and root length were measured, then separated for drying. Roots and shoots were dried for a minimum of 3 days at 60 °C prior to weighing for shoot and root biomass.

There were no deviations from the Environment Canada test protocol for the duration of all twelve plant toxicity tests.

3.6. Statistical Analysis

All statistical endpoint estimates were derived from the mean of triplicate soil analyses for EC, sulphate or chloride concentrations measured in each treatment at test initiation. Each mean concentration was log-transformed as appropriate for nonlinear regression procedures. In the analysis of growth endpoints, the length and weight measurements of individual shoots or roots in each replicate were pooled for each of these measurements, and the mean was used in the analysis. For dry weight measurements, the mean weight of individual shoots or roots in each replicate was calculated as the total dry weight of all the plant shoots or roots that survived in the test vessel divided by the number of plants that survived.

Nonlinear regression procedures were applied to the continuous toxicity data (shoot and root length, shoot and root biomass, Environment Canada 2005). Four nonlinear regression models and one linear regression model were applied to include IC_p estimates and their associated 95% confidence limits. The IC_p was calculated as the concentration causing a fixed percent reduction in the mean length or biomass of shoot and root growth. Residuals were examined for homogeneity of variance among treatments, and the most appropriate model fitting the concentration-response relationship with the lowest residual mean square error was selected as the final statistical IC₂₅ and IC₅₀ endpoints for the growth variables. Analysis of variance was used to examine for outliers. Statistical estimates were generated using Systat 13 (Systat Software Inc.). Endpoints for emergence were not derived due to the relative insensitivity of this endpoint compared to growth endpoints.

4. RESULTS

4.1. Chloride and Sulphate in Soil

The analytical data for measured conductivity, chloride, sulphate and sodium are presented in Appendix B. The tables include the mean, standard deviation (sd) and relative coefficient of variation (CV%) of triplicate analyses for each soil treatment for all twelve plant tests, as measured EC, saturated paste Cl or SO₄, and saturated paste Na (mg/kg dry weight basis).

Replicate variability for both chloride and sulphate in fine and coarse soil was low. Relative standard deviations between triplicate analyses for measured saturated paste EC, chloride and sulphate were generally less than 10%, except at near detection limits.

The salt spiking technique used for this study was successful as demonstrated by mass balance comparison. Recovery of chloride compared to nominal sodium chloride spiked to either fine or coarse soil ranged from 91% to 122% (mean recovery 102%). Spiking with sodium sulphate was generally more variable across tests, with recovery ranging from 64% to 165%, averaging 89% across all treatments in coarse and fine soil. High water solubility and low interaction of chloride with soil components is likely the reason for better recovery compared to sodium sulphate.

Comparison of replicate measured chloride and electrical conductivity in fine and coarse soil from each test was compiled and presented in Figure 1. Measured sulphate vs. EC in both soils is displayed in Figure 2. Addition of either sodium chloride or sodium sulphate to coarse or fine soil produced a strongly linear response ($R^2 > 0.98$) to the highest target EC of approximately 40 dS/m.

The slope of the regression differed between fine and coarse soil, with lower resulting EC for the same mass of salt added to fine soil compared to coarse soil. This observation held true for both sodium chloride and sodium sulphate. The difference between slopes is likely due to interactions of added salt occurring in the

fine soil which has higher clay content. Available sodium in pore water is possibly being removed by cation exchange with clay aluminosilicates. Additionally, higher background salt, nutrient and metal content of fine soil compared to coarse soil is possibly reducing saturated paste EC from removal of cations/anions by formation of insoluble salts and complexes.

Figure 1. Measured Chloride vs. EC (dS/m) in Fine and Coarse Soil

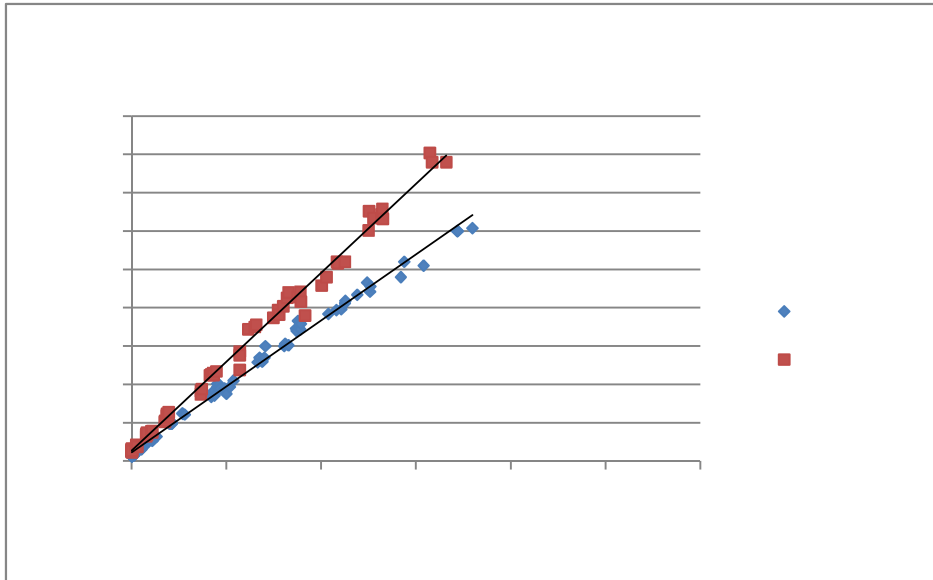
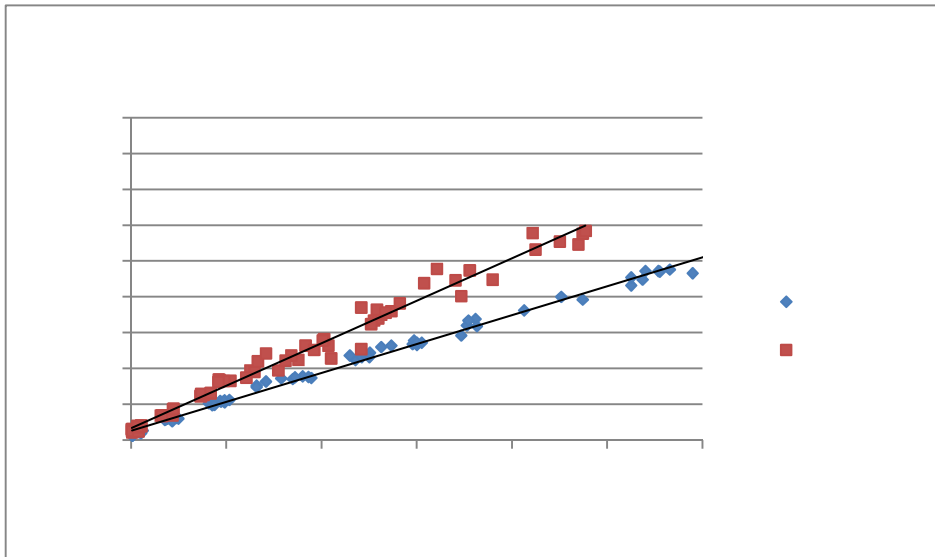


Figure 2. Measured Sulphate vs. EC (dS/m) in Fine and Coarse Soil



4.2. Plant Toxicity Test Results

Summary tables of mean results for emergence, shoot and root length, and shoot and root biomass as % effect relative to the control (untreated field soil) are presented in Appendix C. Emergence and growth endpoint data with mean, standard deviation, CV% and % effect relative to the control are presented in Appendix D. Dose-response curves for growth endpoints generated from statistical nonlinear regression against log-transformed EC, chloride or sulphate are presented in Appendix E.

All tests met the minimum emergence and growth endpoints for shoot and root length specific for each of the three species in the artificial soil control, as well as the fine and coarse untreated soil controls as specified in Environment Canada test method protocol (EC 2007).

Major reduction in seedling emergence from exposure to chloride or sulphate occurred only at relatively high salt concentrations. Barley was the least sensitive of the three species, with > 50% reduction observed at EC > 25 dS/m (> 5000 mg/kg Cl, > 9000 mg/kg SO₄). Similarly, northern wheatgrass emergence was not significantly affected until soil EC was > 17 dS/m in either soil type. Alfalfa emergence was affected at lower EC in chloride spiked soils, with > 50% reduction at EC near 10 dS/m, but not until EC > 20 dS/m in sulphate spiked soils.

Test endpoints as the IC₂₅ and IC₅₀, along with the lower and upper 95% confidence limits (LC and UC) for growth are presented in Table 3 for electrical conductivity (dS/m), and Table 4 for results as measured chloride or sulphate. The endpoints for each species are also presented graphically in figures 3 to 6 (BFCL-SL: B=Barley, F=Fine, CL=Chloride, -SL=Shoot length etc.). The IC₂₅ or IC₅₀ data point for each endpoint is graphed with the 95% confidence limits (as error bars). Individual endpoints where confidence limits overlap can be considered not to differ significantly from each other.

Barley growth endpoints as EC were quite similar regardless of soil type or whether chloride or sulphate was spiked (Figure 3). The IC₅₀ ranged from 12.56 to 19.10 dS/m in fine soil spiked with chloride, 11.59 to

17.26 in coarse soil with chloride, 10.99 to 18.58 dS/m in fine soil spiked with sulphate, and 10.47 to 18.58 in coarse soil with sulphate (Table 3). Root endpoints were more sensitive than shoot endpoints. Endpoints as IC₅₀ chloride in fine and coarse soil ranged from 2188 mg/kg to 4111 mg/kg as Cl (Table 4). The IC₅₀ for barley in sulphate spiked soil ranged from 3908 mg/kg to 8570 mg/kg as SO₄. Comparing individual endpoints (i.e shoot length) in the same soil type between soils spiked with chloride or sulphate for either the IC₅₀ (Figure 2) or the IC₂₅ (Figure 3) generally showed endpoints with overlapping confidence limits, suggesting that EC from chloride or sulphate did not make a difference on plant toxicity. However, the IC₅₀ as chloride or sulphate weight are quite different, so that the sulphate IC₅₀ was significantly higher than chloride IC₅₀.

Alfalfa was more sensitive to salt exposure than barley. The IC₅₀ ranged from 7.59 dS/m to 13.84 dS/m in fine and coarse soil with chloride, and 11.09 dS/m to 16.75 dS/m in fine and coarse soil with sulphate. Root biomass was the lowest endpoint in three of the four tests. Similar to barley, there were no discernible differences between test results in fine or coarse soil when measured as EC. However, endpoints as chloride or sulphate concentrations are significantly different with the IC₂₅ and IC₅₀ endpoints higher in fine soil compared to coarse soil, indicating greater salinity available to plants in coarse soil pore water. An exception was the root biomass endpoint in coarse-CL which was unusually high.

Unlike barley, growth endpoints as the IC₂₅ or IC₅₀ for chloride spiked soil appear to be lower as a group than sulphate spiked soil, particularly in fine soil. The geometric means of the four growth endpoints in each of the four tests, with the lowest and highest confidence limit are presented in Table 2. The mean for IC₂₅ as EC for both fine and coarse soil are lower for chloride spiked soils (5.4 and 6.29 dS/m) than sulphate spiked soils (10.45 and 9.04 dS/m) and confidence limits don't overlap, suggesting that chloride as EC is significantly more toxic than sulphate for this species.

Northern Wheatgrass sensitivity to chloride was similar to alfalfa sensitivity. The IC₅₀ ranged from 8.20 dS/m to 21.38 dS/m in fine and coarse soil with chloride, and 12.82 dS/m to 20.89 dS/m in fine and coarse soil with sulphate. Shoot length was the least sensitive endpoint. Like barley and alfalfa, there was no measurable difference between test results in fine or coarse soil as measured EC, but measured chloride

mg/kg endpoints were lower in coarse soil than fine soil. This was also the case for measured sulphate endpoints, with the exception of root length.

Table 2. Geometric Mean of Growth Endpoints with Lowest and Highest Confidence Limit

[illegible]

Comparing the individual endpoints for northern wheatgrass between chloride and sulphate in Figure 3 shows a mixed trend. The shoot and root biomass endpoints for chloride in fine soil are lower than sulphate in fine soil, but shoot and root length are similar. In coarse soil, the trend holds for all four endpoints, with the IC_{50} ranging from 8.20 to 15.17 dS/m in coarse soil with chloride, compared with 12.82 to 20.80 dS/m in coarse soil with sulphate. Similarly, the geometric mean of the IC_{25} and IC_{50} in coarse soil chloride is lower than the geometric mean of IC_{25} and IC_{50} coarse soil sulphate (7.08 vs. 11.91 dS/m and 10.83 vs. 16.07 dS/m respectively). The same trend was observed for chloride compared to sulphate EC endpoints in fine soil (IC_{25} of 6.40 vs. 10.75 dS/m; IC_{50} of 12.27 vs. 15.53 dS/m), although the confidence limits for these comparisons overlap.

As measured chloride vs. sulphate in both soils, sulphate test endpoints were significantly greater than chloride endpoints in the same soil type (geometric mean IC_{25} of 1105 mg/kg Cl vs. 4416 mg/kg SO_4 in fine soil, 940 mg/kg Cl vs 3289 mg/kg SO_4).

The results of this research indicate that chloride toxicity is greater to plant species tested than sulphate when measured as saturated paste ion concentrations, and salt availability to plants is dependent on soil texture (clay content). The similarity of toxicity endpoints by measured electrical conductivity confirms the wisdom of applying increases to soil EC above background as contaminated sites criteria limits as AESRD currently does, since it can be applicable to soils of a wide range of texture and contaminating salt source. Two of the three species results suggest that sulphate toxicity when measured as EC may occur at higher soil EC than from chloride. The two species showed a difference of 3 to 5 dS/m when compared for the same soil type. Additional plant species test would be needed to confirm if the trends hold true, and to what degree of difference for EC could be applied and still be protective of terrestrial species.

It is likely that a strong difference between the toxicity of sulphate and chloride when measured as EC was not observed since plants are not exposed directly to either ion alone, but may also be affected by the contributing sodium cation on plant toxicity and soil structure. The inherent difficulty in examining specific anion toxicity is separating the contributing effect of the accompanying cation toxicity and potential impacts on soil texture, which in turn can affect anion availability, and indirectly affect plant health by affecting water and nutrient transport and availability. A series of tests with potassium chloride and potassium sulphate would help resolve anion effects. However, since most soils contaminated with salts tend to be a result of sodium salts, further research of this nature was considered beyond the scope of this project.

Figure 3. IC₅₀ Growth Endpoints as EC (dS/m)

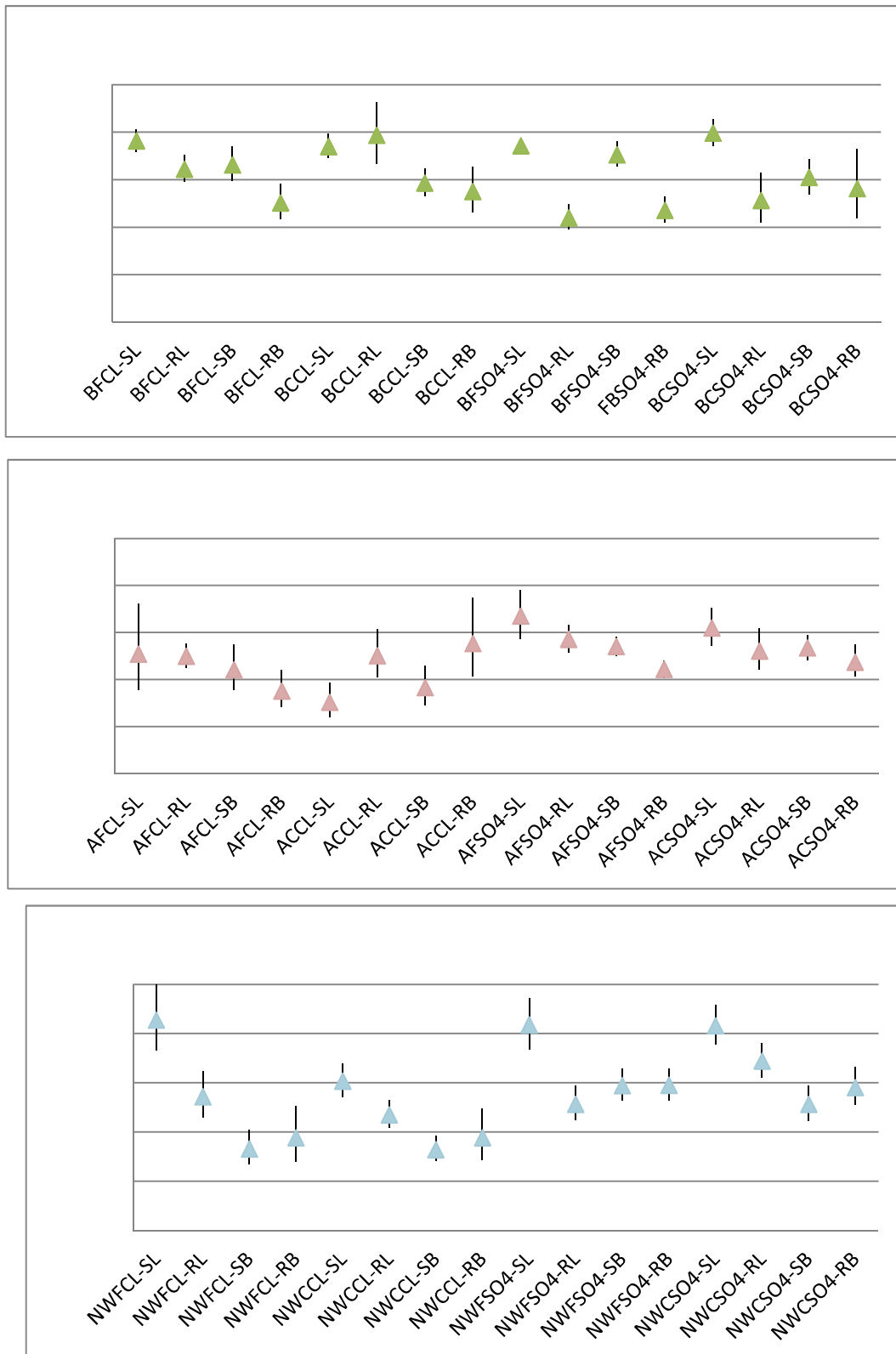


Figure 4. IC₂₅ Growth Endpoints as EC (dS/m)

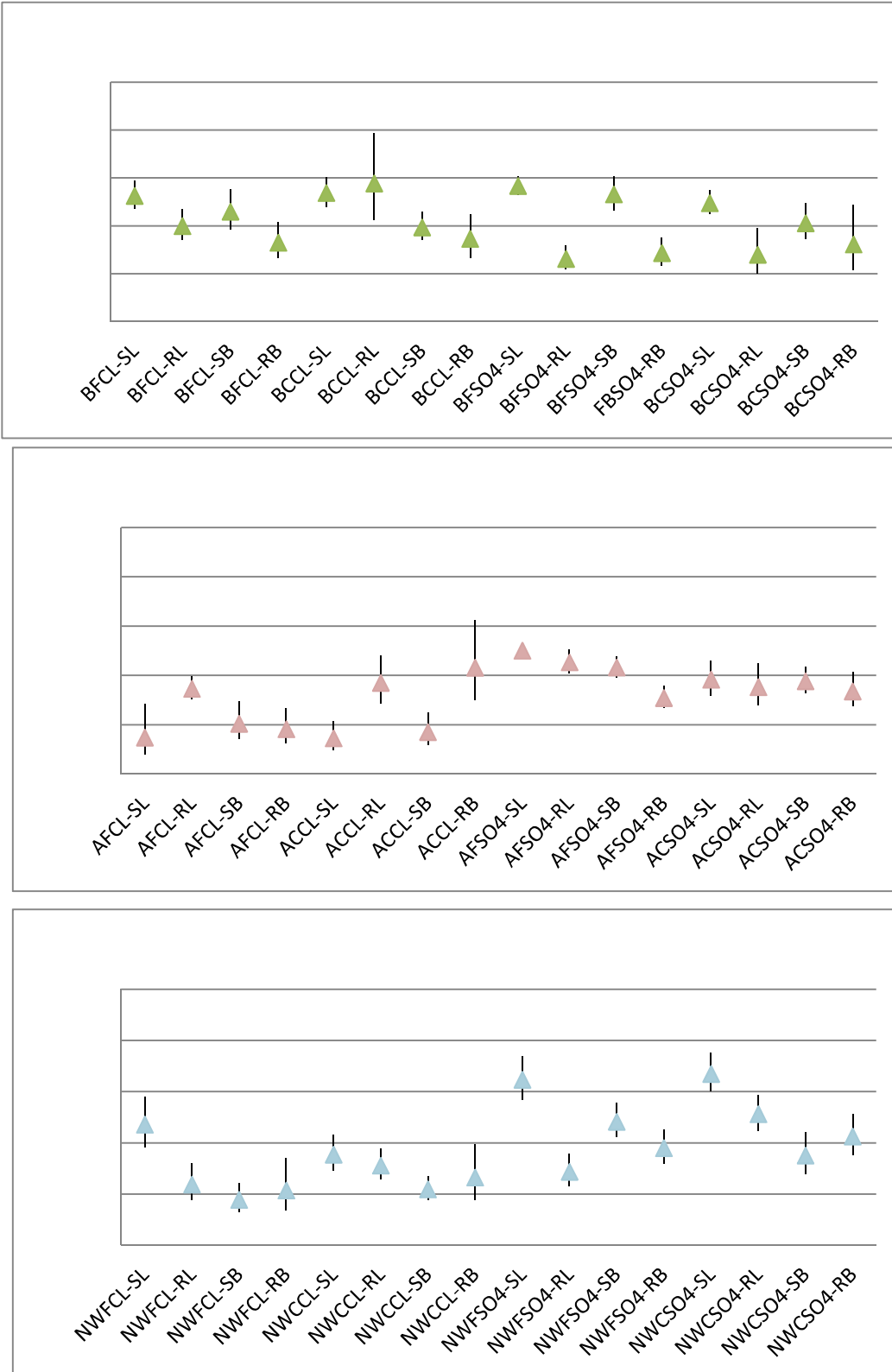


Figure 5. IC₂₅ and IC₅₀ Growth Endpoints All Tests as EC (dS/m)

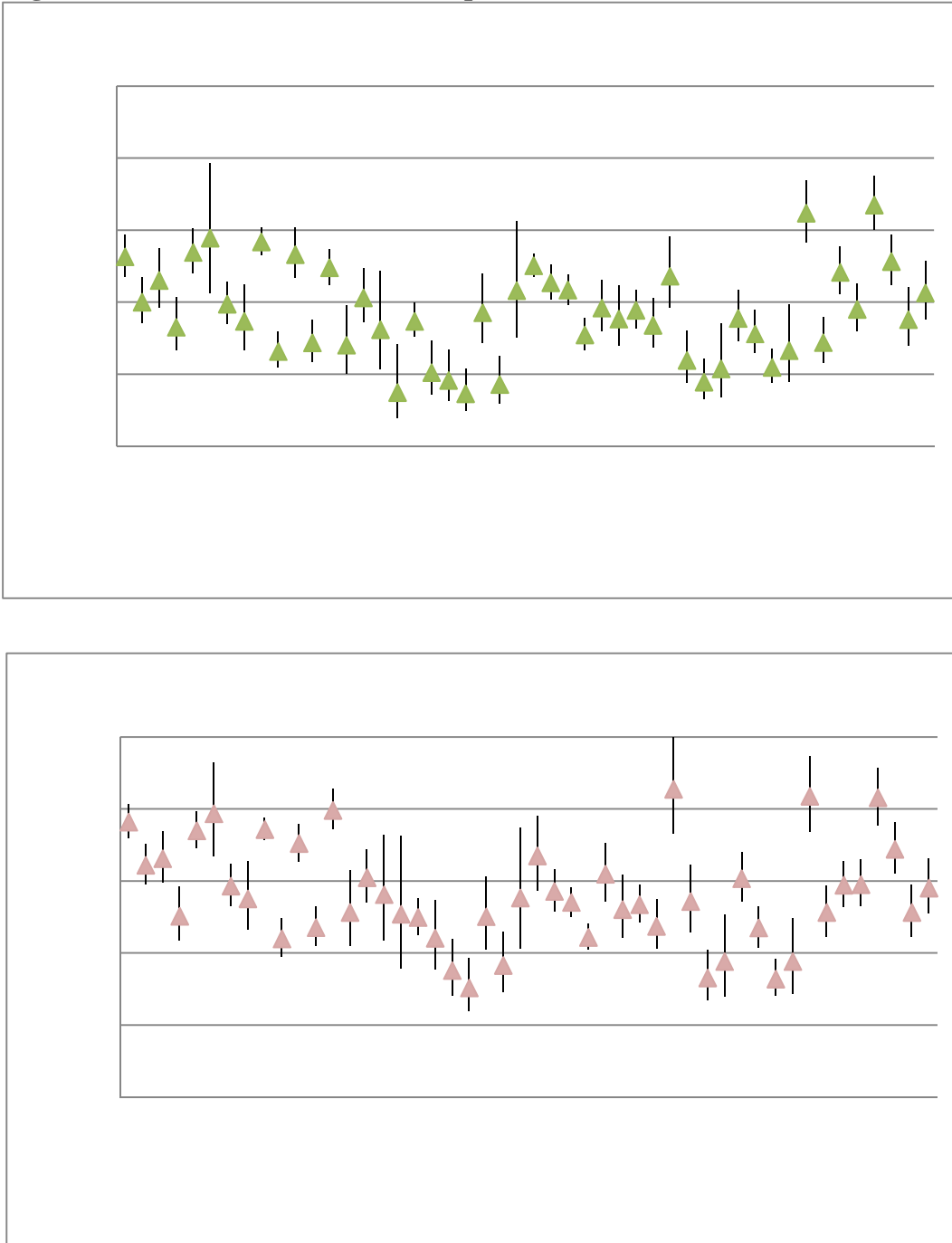


Table 3. Plant Toxicity Growth ICp Endpoints as Electrical Conductivity (dS/m)**Final Endpoints as EC (dS/m)**

	IC₂₅	LC	UC	IC₅₀	LC	UC
Shoot Length	13.15	11.75	14.69	19.10		
Root Length	10.00			16.11	14.76	17.54
Shoot Biomass	11.51	9.62	13.77	16.56		
Root Biomass	8.26			12.56	10.84	14.59

Final Endpoints as EC (dS/m)

	IC₂₅	LC	UC	IC₅₀	LC	UC
Shoot Length	13.46	11.99	15.10	18.49		
Root Length	14.45			19.68	16.71	23.17
Shoot Biomass	9.86	8.49	11.43	14.66		
Root Biomass	8.67			13.77	11.59	16.37

Barley-Fine-SO4**Final Endpoints as EC (dS/m)**

	IC₂₅	LC	UC	IC₅₀	LC	UC
Shoot Length	14.19	13.24	15.17	18.58	17.82	19.36
Root Length	6.58			10.99	9.73	12.39
Shoot Biomass	13.30	11.67	15.17	17.62	16.37	18.97
Root Biomass	7.18	5.86	8.77	11.78	10.47	13.21

Barley-Coarse-SO4**Final Endpoints as EC (dS/m)**

	IC₂₅	LC	UC	IC₅₀	LC	UC
Shoot Length	12.39	11.22	13.68	19.91	18.58	21.38
Root Length	7.01			12.82	10.47	15.74
Shoot Biomass	10.30	8.59	12.33	15.24	13.49	17.18
Root Biomass	8.09	5.36	12.19	14.06	10.89	18.20

Alfalfa-Fine-CL**Final Endpoints as EC (dS/m)**

	IC₂₅	LC	UC	IC₅₀	LC	UC
Shoot Length	3.72	1.95	7.10	12.71		
Root Length	8.67			12.47	11.25	13.80
Shoot Biomass	5.12	3.57	7.35	11.04	8.87	13.71
Root Biomass	4.57	3.13	6.67	8.79	7.05	10.96

Alfalfa-Coarse-CL**Final Endpoints as EC (dS/m)**

	IC₂₅	LC	UC	IC₅₀	LC	UC
Shoot Length	3.65	2.47	5.38	7.59	5.97	9.66
Root Length	9.27			12.53	10.23	15.31
Shoot Biomass	4.29	2.95	6.24	9.14	7.29	11.46
Root Biomass	10.81	7.50	15.60	13.84	10.28	18.66

Alfalfa-Fine-SO4**Final Endpoints as EC (dS/m)**

	IC₂₅	LC	UC	IC₅₀	LC	UC
Shoot Length	12.53	11.75	13.37	16.75	14.35	19.54
Root Length	11.35			14.29	12.88	15.81
Shoot Biomass	10.84	9.84	11.94	13.52	12.53	14.55
Root Biomass	7.73	6.68	8.93	11.09	10.21	12.02

Alfalfa-Coarse-SO4**Final Endpoints as EC (dS/m)**

	IC₂₅	LC	UC	IC₅₀	LC	UC
Shoot Length	9.59	7.98	11.51	15.49	13.58	17.62
Root Length	8.83			13.03	11.04	15.42
Shoot Biomass	9.44	8.18	10.86	13.37	12.11	14.76
Root Biomass	8.39	6.85	10.28	11.86	10.28	13.71

Northern Wheatgrass-Fine-CL**Final Endpoints as EC (dS/m)**

	IC₂₅	LC	UC	IC₅₀	LC	UC
Shoot Length	11.80	9.59	14.55	21.38	18.28	24.95
Root Length	5.96			13.58	11.46	16.14
Shoot Biomass	4.46	3.27	6.08	8.28	6.73	10.19
Root Biomass	5.37	3.39	8.49	9.42	7.00	12.68

Northern Wheatgrass-Coarse-CL**Final Endpoints as EC (dS/m)**

	IC₂₅	LC	UC	IC₅₀	LC	UC
Shoot Length	8.87	7.28	10.81	15.17	13.55	16.98
Root Length	7.80			11.75	10.40	13.24
Shoot Biomass	5.48	4.43	6.78	8.20	7.03	9.55
Root Biomass	6.64	4.47	9.84	9.42	7.14	12.42

Northern Wheatgrass-Fine-SO4**Final Endpoints as EC (dS/m)**

	IC₂₅	LC	UC	IC₅₀	LC	UC
Shoot Length	16.18	14.16	18.45	20.89	18.41	23.66
Root Length	7.19			12.82	11.17	14.69
Shoot Biomass	12.08	10.54	13.87	14.72	13.21	16.41
Root Biomass	9.51	8.00	11.27	14.76	13.24	16.48

Northern Wheatgrass-Coarse-SO4**Final Endpoints as EC (dS/m)**

	IC₂₅	LC	UC	IC₅₀	LC	UC
Shoot Length	16.75	15.00	18.75	20.80	18.88	22.86
Root Length	12.82			17.22	15.52	19.05
Shoot Biomass	8.79	7.00	11.07	12.82	11.14	14.76
Root Biomass	10.64	8.83	12.85	14.52	12.74	16.56

Table 4. Plant Toxicity Growth ICp Endpoints as Chloride or Sulphate (mg/kg dwb)**Final Endpoints as Chloride (mg/kg dwb)**

	IC₂₅	LC	UC	IC₅₀	LC	UC
Shoot Length	2618			4111	3819	4436
Root Length	1892	1556	2307	3365		
Shoot Biomass	2249			3475	3069	3936
Root Biomass	1556			2553	2148	3027

Final Endpoints as Chloride (mg/kg dwb)

	IC₂₅	LC	UC	IC₅₀	LC	UC
Shoot Length	2218			3133	2904	3381
Root Length	2455	1730	3483	3365		
Shoot Biomass	1469			2360	2099	2649
Root Biomass	1256			2188	1791	2673

Barley-Fine-SO4**Final Endpoints as Sulphate (mg/kg dwb)**

	IC₂₅	LC	UC	IC₅₀	LC	UC
Shoot Length	6012	5483	6592	8570	8091	9057
Root Length	2223	1750	2831	4365	3750	5070
Shoot Biomass	5470			7980	7211	8831
Root Biomass	2723	2133	3483	4732	4055	5508

Barley-Coarse-SO4**Final Endpoints as Sulphate (mg/kg dwb)**

	IC₂₅	LC	UC	IC₅₀	LC	UC
Shoot Length	3811	3420	4246	6412	5943	6902
Root Length	1828	1202	2786	3908	3062	5000
Shoot Biomass	3048			4764	4159	5458
Root Biomass	2218	1321	3724	4375	3221	5929

Alfalfa-Fine-CL**Final Endpoints as Chloride (mg/kg dwb)**

	IC₂₅	LC	UC	IC₅₀	LC	UC
Shoot Length	724	398	1321	2838	1995	4046
Root Length	1774	1517	2080	2655	2377	2965
Shoot Biomass	929			2301	1795	2951
Root Biomass	802	507	1268	1774	1368	2301

Alfalfa-Coarse-CL**Final Endpoints as Chloride (mg/kg dwb)**

	IC₂₅	LC	UC	IC₅₀	LC	UC
Shoot Length	455	303	685	1199	925	1560
Root Length	1538	1172	2018	2094	1710	2564
Shoot Biomass	570			1503	1167	1941
Root Biomass	1811	1222	2692	2323	1738	3105

Alfalfa-Fine-SO4**Final Endpoints as Sulphate (mg/kg dwb)**

	IC₂₅	LC	UC	IC₅₀	LC	UC
Shoot Length	5408	4989	5861	7762	6531	9247
Root Length	4055	3467	4742	5957	5321	6668
Shoot Biomass	4487			5970	5420	6577
Root Biomass	3013	2529	3597	4667	4236	5140

Alfalfa-Coarse-SO4**Final Endpoints as Sulphate (mg/kg dwb)**

	IC₂₅	LC	UC	IC₅₀	LC	UC
Shoot Length	2630	2109	3281	4550	3936	5248
Root Length	2371	1762	3192	3767	3090	4581
Shoot Biomass	2582			3890	3443	4395
Root Biomass	2193	1675	2871	3357	2805	4027

Northern Wheatgrass-Fine-CL**Final Endpoints as Chloride (mg/kg dwb)**

	IC₂₅	LC	UC	IC₅₀	LC	UC
Shoot Length	2254	1770	2864	4467	3758	5309
Root Length	1021	724	1439	2685	2218	3243
Shoot Biomass	719			1517	1197	1928
Root Biomass	899	521	1556	1754	1253	2449

Northern Wheatgrass-Coarse-CL**Final Endpoints as Chloride (mg/kg dwb)**

	IC₂₅	LC	UC	IC₅₀	LC	UC
Shoot Length	1268	1016	1585	2296	2032	2588
Root Length	1069	859	1330	1710	1503	1950
Shoot Biomass	681			1125	935	1352
Root Biomass	847	522	1374	1309	946	1816

Northern Wheatgrass-Fine-SO4**Final Endpoints as Sulphate (mg/kg dwb)**

	IC₂₅	LC	UC	IC₅₀	LC	UC
Shoot Length	7311	6223	8590	9886	8531	11455
Root Length	2710	2070	3548	2710	2070	3548
Shoot Biomass	5082			6516	5689	7464
Root Biomass	3776	3076	4645	5152	4519	5888

Northern Wheatgrass-Coarse-SO4**Final Endpoints as Sulphate (mg/kg dwb)**

	IC₂₅	LC	UC	IC₅₀	LC	UC
Shoot Length	4966	4457	5534	6266	5662	6950
Root Length	3724	3177	4365	5164	4645	5754
Shoot Biomass	1936			3436	2793	4227
Root Biomass	3266	2576	4140	4064	3451	4786

5. Summary

- Addition of increasing amounts of sodium chloride and sodium sulphate to soil results in a saturated paste electrical conductivity that is directly linear to the saturated paste concentration of chloride or

sulphate (mg/kg dwb), to an EC of 40 dS/m.

- A larger concentration of sulphate is required to reach a given EC compared to chloride.
- Soil texture has an affect on the linear relationship between measured EC and saturated paste chloride or sulphate. Fine textured soil with a higher clay content had a lower measured EC for a given measured concentration of either chloride or sulphate, compared to the same concentration in coarse textured soil with lower clay content, likely due to soil interactions of sodium with clay aluminosilicates.
- Chloride and sulphate were less toxic to barley, followed by northern wheatgrass and alfalfa.
- Emergence endpoints were relatively insensitive to salt exposure with significant reduction not observed until > 25 dS/m for barley, > 17 dS/m for northern wheatgrass, and > 10 dS/m for alfalfa. Root length and biomass were typically the most sensitive endpoint.
- Growth endpoints as the geometric mean of IC₂₅ endpoints ranged as EC from 5.24 to 11.91 dS/m, 922 to 2041 mg/kg Cl, and 2438 to 4416 mg/kg SO₄. Chloride was more toxic than sulphate for all test endpoints in both soils, and chloride or sulphate was more toxic in coarse soil than fine soil.
- Barley test endpoints were not significantly different as EC between chloride or sulphate tests.
- Alfalfa and northern wheatgrass show a small reduction in toxicity of approximately 3 to 5 dS/m in soils spiked with sulphate compared to soils spiked with chloride. Additional plant species tests would be required to confirm the difference in toxicity between the two salts.

6. REFERENCES

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