New Tier 1 Boron Guideline for Alberta: Boron Soil Ecotoxicity and Methods Development Research

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Greg Huber, (Equilibrium)

RemTech Oct 2015
Summary

Part 1
• Introduction
• Agricultural and Boreal Ecotoxicity Tests
• Boron Soil Analysis
• Chemistry vs Toxicity
• Recommendations

Part 2
• Greg Huber - Equilibrium
• Tier 1 Guideline Development

Exova
Acknowledgements

- Petroleum Technology Alliance of Canada (PTAC)
- Environment Canada
- PTAC Boron Working Group
Boron in the Environment

- Naturally present in the environment due to weathering of boron-containing minerals and decay of plant material
- Highly soluble in solution; adsorbed in soil by clay (aluminosilicates) and organic matter
- Background soils generally below current Tier 1 guideline, but in some cases background soils can be above Tier 1 (primarily clayey or organic soils)
- Essential plant micronutrient
  - Difference between plant deficiency and toxicity is small
  - Species-specific, even variant-specific
  - Concentrations which may be toxic to one species may be deficient for another
Anthropogenic Sources

- Fertilizers and herbicides
- Industrial Use/Manufacturing – glass, fiberglass insulation
- Wood Preservative
- Application of fly ash or sewage as soil amendment
- Wastewater irrigation
- Land disposal of industrial wastes
- Use of borax detergent for cleaning tanks, wellheads, equipment
- Saline produced water and drilling waste
  - Common co-contaminant with elevated salinity in Alberta
Why New Research?

• Current Tier 1 boron guideline of 2 mg/kg hot-water soluble (HWS) boron has several problems:

  ➢ Not based on a modern, risk-based approach
  ➢ Based on professional judgement and limited data in 1991
  ➢ HWS test designed to diagnose deficiency, not toxicity
  ➢ Older research often used colorimetric detection methods
  ➢ Background concentrations may exceed guidelines
  ➢ Good growth often observed above Tier 1 guideline
  ➢ Texture (clay and organic matter) influence boron sorption/toxicity
  ➢ Plant available/toxic concentrations not correlated well with HWS B measured across different soil types
Objectives

Narrow range between deficiency and toxicity demands an analytical method capable of measuring plant available B to predict ecotoxicity, regardless of soil characteristics

- Investigate the saturated paste extraction method for measuring plant available soil boron
- Conduct plant toxicity tests in a variety of boron spiked soils using agricultural and boreal test species
- Include a long-term plant growth study and an earthworm reproduction test
- Compare SatPaste B and HWS B toxicity test dose-responses
- Compile Species Sensitivity Distributions to derive new soil criteria for agricultural and boreal regions in Alberta
Reference soil collection

• Reference soils required for tox tests (boron spiking)
  – artificial soil as a benchmark
  – Agricultural Region:
    • Fine and Coarse soils
  – Boreal Region:
    • Organic and Mineral

• Soil Collection – Equilibrium: locate and collect four field reference soils using soil maps and field visits.
  – Fine reference soil (clay loam) collected from updated site near Delacour.
  – Coarse reference soil had not yet been sourced in Alberta. Suitable sandy loam reference soil collected near Vulcan.
Reference Soil Collection - Agricultural

Fine reference soil near Delacour (clay loam)

Coarse reference soil near Vulcan (sandy loam)
Reference Soil Collection - Boreal

- organic peat soils and mineral brunisol from location near Whitney Lake Provincial Park
## Soil Properties

<table>
<thead>
<tr>
<th></th>
<th>Fine</th>
<th>Coarse</th>
<th>Organic</th>
<th>Mineral</th>
<th>Artificial</th>
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<tbody>
<tr>
<td>Texture</td>
<td>Clay Loam</td>
<td>Sandy Loam</td>
<td></td>
<td>Sand</td>
<td>Sandy Loam</td>
</tr>
<tr>
<td>75 um %</td>
<td>32</td>
<td>60</td>
<td>49</td>
<td>96</td>
<td>77</td>
</tr>
<tr>
<td>Texture</td>
<td>Fine</td>
<td>Coarse</td>
<td>Fine</td>
<td>Coarse</td>
<td>Coarse</td>
</tr>
<tr>
<td>% Sand</td>
<td>33</td>
<td>62</td>
<td></td>
<td>95</td>
<td>68</td>
</tr>
<tr>
<td>% Silt</td>
<td>35</td>
<td>20</td>
<td></td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>% Clay</td>
<td>32</td>
<td>18</td>
<td></td>
<td>4</td>
<td>18</td>
</tr>
<tr>
<td>% OM</td>
<td>3.9</td>
<td>3.0</td>
<td>68</td>
<td>5.3</td>
<td>10</td>
</tr>
<tr>
<td>Saturation %</td>
<td>79</td>
<td>51</td>
<td>552</td>
<td>31</td>
<td>100</td>
</tr>
<tr>
<td>pH</td>
<td>7.4</td>
<td>5.8</td>
<td>5.5</td>
<td>4.4</td>
<td>6.8</td>
</tr>
</tbody>
</table>
Test Soils Preparation

- 800 L each of three test soils
- Field Soils air dried to < 10% Moisture, sieved < 2 mm
- Spike with boric acid solution to achieve a target range of total boron concentrations ranging from non-toxic to toxic effects based on range-finding screening tests.
  - nominal total boron concentrations from 2 to 1000 mg/kg dwb (HWS B 0 > 400 mg/kg)
  - Sufficient test concentrations at low end near criteria of 2 mg/kg HWS B to achieve good resolution.
- Bring moisture content to optimal moisture content, 80% WHC
- Age spiked soils 2 weeks
Ecotoxicity Tests

• Plant toxicity tests in soils spiked with a broad range of boron concentrations
  ➢ Six agricultural species in fine, coarse and artificial soil (18 tests)
  ➢ Five boreal species in organic, mineral and artificial soil (11 tests)
• Environment Canada Biological Test Methods:
  – Test for Growth in Contaminated Soil Using Terrestrial Plants Native to the Boreal Region. EPS 1/RM/56

• One long-term growth study with cucumber to flowering stage (3 months)
• Earthworm survival and reproduction test in coarse soil (EPS/1/RM/43)

❖ Results from boron toxicity dose-response curves used to generate direct eco-contact guideline based on CCME rank percentile methodology.
Test Design

- 13 treatments including a control; Ten replicates per treatment – 1 L test vessels
- Five to ten seeds per pot
- 2 to 3 week (ag) and 4 to 6 weeks (boreal)
- Controlled environmental conditions
Toxicity Endpoints

- Emergence
- Shoot and Root Length
- Shoot and Root Biomass
- Analytical at Test initiation and Termination
  - pH, EC Moisture, Salinity, Nutrients
  - Total Boron (SAD)
  - HWS B
  - SatPaste B as mg/kg dwb and mg/L
  - Tissue Boron
  - IC_{25} and IC_{50}
# Agricultural Plant Species

<table>
<thead>
<tr>
<th>Species</th>
<th>Classification</th>
<th>Type</th>
<th>Test Duration</th>
<th>Life Cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfalfa</td>
<td>Dicot</td>
<td>Agricultural</td>
<td>21 d</td>
<td>Perennial</td>
</tr>
<tr>
<td>Barley</td>
<td>Monocot</td>
<td>Agricultural</td>
<td>14 d</td>
<td>Annual</td>
</tr>
<tr>
<td>Durum Wheat</td>
<td>Monocot</td>
<td>Agricultural</td>
<td>14 d</td>
<td>Annual</td>
</tr>
<tr>
<td>Cucumber</td>
<td>Dicot</td>
<td>Market-garden</td>
<td>14 d</td>
<td>Annual</td>
</tr>
<tr>
<td>Carrot</td>
<td>Dicot</td>
<td>Market-garden</td>
<td>21 d</td>
<td>Biennial</td>
</tr>
<tr>
<td>Northern Wheatgrass</td>
<td>Monocot</td>
<td>Grasslands</td>
<td>21 d</td>
<td>Annual/perennial</td>
</tr>
</tbody>
</table>
## Boreal Plant Species

<table>
<thead>
<tr>
<th>Species</th>
<th>Classification</th>
<th>Type</th>
<th>Test Duration</th>
<th>Life Cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jack Pine</td>
<td>Gymnosperm</td>
<td>Tree, coniferous</td>
<td>35 d</td>
<td>Perennial</td>
</tr>
<tr>
<td>White Spruce</td>
<td>Gymnosperm</td>
<td>Tree, coniferous</td>
<td>42 d</td>
<td>Perennial</td>
</tr>
<tr>
<td>Black Spruce</td>
<td>Gymnosperm</td>
<td>Tree, coniferous</td>
<td>42 d</td>
<td>Perennial</td>
</tr>
<tr>
<td>Bluejoint Reedgrass</td>
<td>Angiosperm, monocot</td>
<td>Herb, graminoid</td>
<td>28 d</td>
<td>Perennial</td>
</tr>
<tr>
<td>Trembling Aspen</td>
<td>Angiosperm, dicot</td>
<td>Tree, deciduous</td>
<td>28 d</td>
<td>Perennial</td>
</tr>
</tbody>
</table>
Boron Analysis
Toxicity data compared to measured boron by Hot Water Soluble (HWS) and Saturated Paste (SP) soil extraction methods to determine best analytical method to predict toxicity.

Total Boron
- Strong acid digest – acid, heat, oxidizer; ICP; mg/kg dwb
- Most Tier 1 metals
- Total pool of soil boron, most largely unavailable
- Soil Ingestion Pathways

Hot Water Soluble Boron
- 2:1 water:Soil with boiling; ICP (historically colorimetric); mg/kg dwb
- Intended to measure total plant-available boron to diagnose deficiency
- Soil solution boron + substantial amount from adsorbed soil pools
Boron Analysis

Saturated Paste Boron

- Less aggressive extraction than HWS B
- Same methodology as saturated paste extractions for salinity
- Incremental water addition to a semi-fluid, saturated paste at ambient temperature.
- Boron measured in the extract (ICP) as mg/L
- May be converted to mg/kg dwb based on saturation %
  - Satn % = amount of water added to 100 g dry soil
  - correlated to soil texture
- mg/L basis advantageous – correlated to soil solution concentration
- Represents dissolved boron in soil solution, minimal adsorbed boron
Boron Soil Pools and Extraction Method

- SAD B
- HWS B
- SP B

- SAD B
- HWS B

Soil Solution B

Organic Matter B

Clay Sorption B

Fixed Mineral B

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Results: Boron Soil Concentrations - Spiked B vs HWS B

\[ y = 0.8562x - 8.2553 \quad R^2 = 0.9726 \]
\[ y = 0.9201x - 0.6354 \quad R^2 = 0.9913 \]
\[ y = 0.7974x - 0.7206 \quad R^2 = 0.9138 \]

Organic Soil
Artificial Soil
Mineral Soil
Spiked vs Satpaste B (mg/L) Agricultural Soils

\[ y = 0.0002x^2 + 1.3293x - 3.9095 \]
\[ R^2 = 0.9973 \]

\[ y = 0.0003x^2 + 0.713x - 3.7824 \]
\[ R^2 = 0.9932 \]

\[ y = 0.0006x^2 + 0.3415x - 2.4515 \]
\[ R^2 = 0.9964 \]
Spiked vs Satpaste B (mg/L) Boreal Soils

- Organic Soil
- Artificial Soil
- Mineral Soil

Equations:

1. \(y = 5 \times 10^{-5}x^2 + 0.0792x - 0.3841\)  
   \(R^2 = 0.9584\)

2. \(y = 0.0007x^2 + 0.4574x - 1.7971\)  
   \(R^2 = 0.9932\)

3. \(y = 0.0027x^2 + 1.9561x - 2.3287\)  
   \(R^2 = 0.8548\)

Sat paste boron (mg/L) vs Spiked boron (mg/kg) graph with data points and trend lines.
Satpaste B mg/L vs HWS B - Boreal Soils

Organic Soil
Artificial Soil
Mineral Soil
Ecotoxicity Test Results

- Cucumber Example – relatively sensitive species
## Cucumber in Clay Loam

<table>
<thead>
<tr>
<th>Trt #</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>HWS B mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.8</td>
<td>3.6</td>
<td>2.9</td>
<td>4.1</td>
<td>5.9</td>
<td>11</td>
<td>17</td>
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<td>45</td>
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<tr>
<td>2</td>
<td>0.08</td>
<td>1.0</td>
<td>0.9</td>
<td>1.4</td>
<td>2.3</td>
<td>4.9</td>
<td>9.3</td>
<td>16</td>
<td>28</td>
<td>51</td>
<td>Satpaste B mg/L</td>
</tr>
</tbody>
</table>

**Diagrams:**

- **Cucumber in FB:**
  - **Y-axis:** % Control
  - **X-axis:** Trt #
  - **Legend:**
    - Shoot Length
    - Root Length
    - Shoot Biomass
    - Root Biomass
    - SatPaste B mg/L
    - HWS B mg/kg

**Legend:**
- **Shoot Length**
- **Root Length**
- **Shoot Biomass**
- **Root Biomass**
- **SatPaste B mg/L**
- **HWS B mg/kg**
Barley in Clay Loam
Species Sensitivity Comparison in Clay Loam

Root Biomass in Clay Loam

- Alfalfa
- Northern Wheatgrass
- Barley
- Durum Wheat
- Carrot
- Cucumber
Example Dose Response – Cucumber Root Length

Toxicity responses for different soil types are more consistent on a saturated paste B basis (mg/L).
Boreal Species Toxicity Tests

- Similar dose response patterns and IC$_{25}$ concentrations for satpaste B mg/L
- IC$_{25}$ range from 1.42 mg/L jack pine root biomass in organic soil to 67.6 mg/L for white spruce shoot length in organic soil
- Stimulation effects at low concentrations
- Higher variability in boreal species (smaller plants, delicate roots, longer tests)
Bluejoint Reedgrass in Organic Soil

<table>
<thead>
<tr>
<th>Trt #</th>
<th>1</th>
<th>2</th>
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<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
</tr>
</thead>
<tbody>
<tr>
<td>HWS B mg/kg</td>
<td>9.9</td>
<td>19</td>
<td>28</td>
<td>41</td>
<td>56</td>
<td>76</td>
<td>108</td>
<td>141</td>
<td>213</td>
<td>282</td>
<td>413</td>
<td>547</td>
<td>859</td>
</tr>
<tr>
<td>Satpaste B mg/L</td>
<td>0.2</td>
<td>0.5</td>
<td>0.8</td>
<td>1.4</td>
<td>2.3</td>
<td>4.6</td>
<td>8.2</td>
<td>15</td>
<td>28</td>
<td>32</td>
<td>50</td>
<td>65</td>
<td>108</td>
</tr>
</tbody>
</table>

Exova
Agricultural Plant SSDs – HWS B (mg/kg)

IC$_{25}$'s in various soil types
HWS B (mg/kg)

Clay Loam
Sandy Loam
Artificial soil

25$^{\text{th}}$ percentile:
8.9 - 32 mg/kg HWS
IC$_{25}$'s in various soil types
sat paste B (mg/L)

% Rank

Clay loam
Sandy Loam
Artificial soil

25th percentile: 11.2 - 16.1 mg/L sat paste

Toxicity responses for different soil types are more consistent on a saturated paste B basis (mg/L)
Vegetation boron

- Soil texture also influences plant boron uptake
- For a given HWS, most boron taken up by plants in sandy loam, least taken up in artificial soil
- Differences in soil type reduced when using saturated paste boron (mg/L)
- Lower BCFs (bioconcentration factors) in roots than shoots
Cucumber Long-Term Growth Test

- 3 month test in clay loam
- Non-Standardized methodology
- Good growth and flowering in all treatments except high doses
- No root data obtainable
- Shoot biomass IC$_{25}$ = 4.53 mg/L SP B
- Shoot length IC$_{25}$ = 3.79 mg/L SP B
- Similar range of toxicity results to short-term test

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Earthworm Survival and Reproduction

- Earthworms exposed to boron in coarse sandy loam (13 treatments, 12 reps)
- 63 days exposure, measure adult survival, number of juveniles per adult and juvenile mass (growth).
- Juvenile # IC$_{25}$ = 5.4 mg/L satpaste B
- Juvenile mass IC$_{25}$ = 26.4 mg/L satpaste B
- High natural variability in reproductive endpoints; responsive to soil texture.
Summary

• HWS B not a good predictor of plant toxicity over different soil types
• HWS B measures primarily sorbed boron, with boron sorbed on clay and organic matter not directly toxic to plants
• Saturated paste B (mg/L) better correlated to soil solution B, plant toxicity response, and boron tissue uptake, regardless of soil characteristics
• Boreal species similar sensitivity to agricultural species;
  - cucumber, carrot, jack pine: sensitive
  - barley, alfalfa, white spruce: least sensitive
• Good growth often observed well above the current Tier 1 guideline of 2 mg/kg HWS boron, often into 4-10 mg/kg range or higher
• Growth stimulation for some species (2 to 4 mg/L SP B)
• IC$_{25}$s Agricultural: 3.2 – 53.0 mg/L; boreal: 1.6 – 29.4 mg/L
• This plant toxicity data can be combined with literature data and invert data to create overall eco-contact guideline (presentation #2)