REPORT: Potential Impact of Fly-ash Groundwater Contamination on Vegetation of Cowles Bog, Indiana Dunes National Lakeshore.

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ON THE COVER

Clockwise from top left: *Iris virginica* var. *shrevei* Cowles Bog site 5 (7/26/2010) with necrotic spotting, margin and tip burn on the leaves; *Asclepias incarnata* Cowles Bog site 5 (8/22/2010) with severe purpling of the leaf, necrotic spotting and margin burn; *Cephalanthus occidentalis* Blag Slough site 34 (8/22/2010) with pronounced chlorotic splotching; *Carex sp.* Cowles Bog site 3 (8/22/2010) with yellowing and necrotic spotting in all areas of the leaves.

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INTRODUCTION:

Fly ash is a specific concern in the Cowles Bog Wetland Community Complex (CBWC) due to its composition, which includes silica oxides, aluminum, iron, and calcium along with trace elements such as boron and molybdenum (Wilcox and Hardy, 1988; Theis et al. 1978). The research in this report focuses on the potential effects that fly ash waste discharges from the Bailly Electrical Generating Station coal-fired power plant are having on the wetland vegetation of the CWBC. The Bailly power plant came online in 1963 and deposited its fly ash waste into drying ponds, which are separated from Blag Slough in the southwest of the CBWC by a sand dike. The drying ponds were left unsealed until 1980, at which time the direct flow of fly ash into the CBWC was halted (Pavlovic, 2009).

The CBWC is a mixture of various wetland and peatland communities, which occupy approximately 80-ha of the basin between the Calumet and Tolleston dunes on the southern shore of Lake Michigan in Porter County, Indiana (Reshkin, 1981). Eight vegetative communities have been identified within the CBWC, which are: a black oak (*Quercus velutina*) woodland; red maple (*Acer rubrum*) swamp; cattail (*Typha*) marsh; *Carex/Calamagrostis* marsh, a *Thuja occidentalis* swamp; (Tamarack) *Larix laricina* swamp; *Phragmities/Typha* marsh, and a shrub swamp (Wilcox et al. 1986).

Cowles Bog, the area of primary concern, is an approximately 22-ha fen located within the CWBC and has its water source of highly mineralized, artesian flow of ground water (Wilcox et al. 1986). According to recent testing, the southwest corner, due to its proximity to the Bailly Electrical Generating Station coal-fired power plant and the sand dike that separates the fly ash drying ponds from Cowles Bog, is considered a plume area where elevated levels of aluminum, boron, and molybdenum have been detected.

Preliminary two-fold research was undertaken to determine whether there is sufficient evidence to warrant further investigation of the effects of fly ash on the vegetation in the CBWC.

The first part of the study was conducted during the 2010-growing season. Over the growing season 34 observation sites were each visited three times to look for visible symptoms of heavy metal and nutrient toxicity in the wetland vegetation of Cowles Bog. Concurrently, a greenhouse experiment was conducted to determine the effects of varying concentrations of aluminum, boron, and molybdenum, which are elements commonly present in fly ash waste in elevated concentrations, on three native wetland species *Asclepias incarnata*, *Carex aquatilis*, and *Iris virginica*.

SITE HISTORY:

In 1963, Northern Indiana Public Service Company (NIPSCO) brought online a coal-fired power plant southwest of Cowles Bog to provide electricity to a steel mill being constructed by Bethlehem Steel Corporation (Pavlovic et al. 2009). Electrostatic precipitators, scrubbers, are routinely placed in smokestacks to collect fly ash from the gas stream to prevent much of the fly ash from entering the atmosphere (Wilcox and Hardy, 1988; Theis et al. 1978). The resulting fly ash waste, however, is mixed with water to form slurry that is then piped into settling ponds to dry out before being hauled away (Wilcox and Hardy, 1988).

From 1963 to 1978, the fly ash ponds were left unsealed and leachate from these ponds seeped into Blag Slough, a wet meadow immediately west of Cowles Bog and closest to the Bailly Electrical Generating Station fly ash ponds, through the sand dike at a rate of about 7.5 million liters per day (1.97 million gallons/day for ~ 17 years) until the ponds were completely sealed in 1980 (Pavlovic et al. 2009). Throughout the mid-70s studies were conducted on the hydrology, topography, stratigraphy, and water chemistry of CBWC. A hydrologic study by Meyer and Tucci (1978) provided evidence that ground water seepage from the fly ash pond was responsible for the regular flooding of Blag Slough. This seepage increased levels of calcium, potassium, sulfate, aluminum, boron, iron, magnesium, molybdenum, nickel, strontium, and zinc in ground- and surface water down gradient from the settling ponds (reviewed by Wilcox and Hardy, 1984).

A 1986 study examined the implications of seepage from fly ash settling ponds. This study concluded the seepage raised the water levels in the wetlands of the CBWC and posed a threat of contamination from chemical constituents that leached from the fly ash (Wilcox et al. 1986). A 2009 study examined the water and soil chemistry of Blag Slough for locations of toxicities, the implications these toxicities have had on the vegetative community development over time, and concluded that natural revegetation has taken place in the 23 year period of the investigation as pH levels have increased. However, areas with elevated heavy metal concentrations remain unvegetated and areas with elevated Al and B concentrations in the soil have vegetation suggesting phytotoxicity with symptoms of vein clearing and chlorosis (Pavlovic et al. 2009).

PART 1 – FIELD OBSERVATIONS:

2010 Field Observation Methods

Prior to beginning field observations, a literature search was conducted to determine commonly reported symptoms of aluminum, boron, and molybdenum. One prevalent symptom of heavy metal toxicity is the inhibition of root growth (Wong and Bradshaw, 1982), which can damage the root system and limit nutrient and water uptake into the plant (Gregory, 2009; Poozesh et al. 2007; Zhang et al. 2007). Observing this symptom was not practical for this field study; however, it was a focus of the greenhouse experiment (part 2). Aluminum toxicity symptoms include necrosis and marginal chlorosis in leaves (Roy et al. 1988), boron toxicity symptoms include leaf tip and edge burn (Brown and Hu, 1998), necrotic spots in the leaf blade (Sotiropoulos et al. 2002), and premature leaf drop and death (Goldberg, 1993), and molybdenum toxicity symptoms include leaf burning, and yellowing of the leaves (Gupta and Gupta, 1998) and an inhibition of root/shoot growth (Kevresan et al. 2001).

ARC View/GIS version 9.3 (ESRI) was used to apply a grid of 30-meter squares over a 2005 aerial image (Indiana Spatial Data Portal) of each wetland to be visited. In Cowles Bog, the area of interest was limited to the southwestern margin, extending from the upland transition to a distance of 60 m into the wetland. Each intersection on the grid was numbered and 30 observation sites, all of which were randomly chosen with a random number generator (random.org). Four targeted sites were chosen in addition to the 30 random observation sites.

In Cowles Bog there were a total of 19 observation sites, including all four targeted sites. Two targeted sites were in the southwest corner of Cowles Bog in the plume area of concern. An additional two targeted sites were in the northeast corner of Cowles Bog, distant from potential contamination but of similar habitat. The 15 observation sites that remained were located outside of Cowles Bog. Three observation sites were in Blag Slough, seven observation sites were in the wetland between Cowles Bog and Blag Slough, one observation site was in the small wetland west of Cowles Bog, one observation site was in the small wetland to the north of Cowles Bog, and the final three observation sites were in the larger wetland, still further north. These final four observation sites along with the targeted sites from the northeast corner of Cowles Bog were used as control sites because of their distance and disconnectedness from the plume area (Figure1) (Appendix A).

ARC/GIS was used to determine the approximate latitude and longitude for the 34 observation sites. The first of three visits was made on June 16, 2010 and a GPS unit was used to locate each proposed observation site. When a site was located it was flagged for precise relocation. The lat/long location was used as the center of the site and dominant vegetation was noted. Depending on the position of an individual observation site, a radius of approximately five meters was surveyed for visible symptoms of toxicity and documented photographically, if recognized. All attempts were made to locate each randomly chosen observation site. However, a site was discarded if the observation site was located entirely in open water or in an upland position, in these instances the next randomly generated site was used.

Field Results and Discussion

Symptoms of aluminum toxicity include necrosis and marginal chlorosis in leaves (Roy, 1988); boron toxicity symptoms include leaf tip and edge burn (Brown and Hu, 1998), necrotic spots in the leaf blade (Sotiropoulos et al. 2002), and premature leaf drop

Figure 1. Observation Site Map in the Cowles Bog Wetland Community Complex

and death (Goldberg, 1993); and molybdenum toxicity symptoms include leaf malformation, golden-yellow discoloration of shoot tissue, and inhibition root and shoot growth (Hamlin, 2007; Marschner, 1995). However, there are two potential problems with regard to recognizing symptoms of toxicity *in situ*. The first problem is that any natural environment, but arguably wetland ones in particular, will exert stresses on resident plant species. As a result, even vegetation in sites with maximum biotic integrity can often exhibit at least limited leaf necrosis, chlorosis, or misshapen structures. Additionally, root inhibition is a symptom that is impractical to observe in the field. The second problem is that many plant species exhibit similar visible symptoms to multiple problems and determining if the symptom being seen is, for example, a symptom of aluminum toxicity or a symptom of calcium, phosphorous, or iron deficiency, becomes less clear. The symptoms being witnessed may be the result of a nutrient deficiency, a nutrient toxicity, or the result of other naturally occurring stressors.

With the above limitations in mind, some observed symptoms could readily be eliminated by noting that they occurred in the same species in sites both near to and far from the plume area. For example, leaf burn and necrosis in *Symplocarpus foetidus*, was seen at site 14 (near to plume area) and site 18(far from plume area) (Figures 2 & 3), *Ilex verticillata* at site 14 and site 18 (Figures 4 & 5), and *Cephalanthus occidentalis* at site 16 (near) and site 19 (far)(Figures 6 & 7). These symptoms obviously were equivocal and were discarded. However, if a symptom was observed in the plume area, without being observed in the same plant species at one of the six control sites (sites 18-23), it was assumed to be a potential symptom of toxicity.

The most frequently observed symptom in the plume area was leaf blade necrosis. At observation sites 3, 5, 6, 7, 8, and 9, which were in the plume area of Cowles Bog, necrosis was present in a variety of species. At site 5 *Scirpus pungens* (Figure 8), *Iris virginica* (Figure 9), *Epilobium coloratum* (Figure 10), *Asclepias incarnata* (Figures 11 & 12), and *Verbena hastata* (Figures 13 & 14) were recognized as exhibiting necrotic spotting, chlorosis, and leaf burn. At site 3 *Schoenoplectus tabernaemontani* (Figure 15), *Sagittaria latifolia* (Figure 16), *Alisma subcordatum* (Figure 17), *A. incarnata* (Figure 18), and *Pontederia cordata* (Figure 19), at site 6 *Rumex* sp. (Figure 20) and *Persicaria* sp. (Figure 21), at site 7 *Scirpus cyperinus* (Figure 22) and *Eupatorium perfoliatum* (Figure 23), at site 8 *Sparganium eurycarpum* (Figure 24) and at site 9 *S. tabernaemontani* (Figure 25) were observed as having symptoms of toxicity. Over the course of the three visits necrosis was persistent but did not appear, in any individual plant, to worsen over time. Outside of Cowles Bog, sites 32-34 in Blag Slough displayed a splotchy leaf chlorosis on *Cephalanthus occidentalis* (see the cover of this Report) and to a more limited extent *Lycopus sp*. and *Pilea pumila.* Although sites 25-28 are proximal to the plume area, apparent toxicity symptoms were not observed. These sites are dominated by *Phragmites australis*, a clonal invasive species. This species, as well as another clonal invasive species, *Typha latifolia*, exhibits a remarkable capacity to tolerate heavy metal contamination (Ye et al. 1997a, 1997b). This capacity may explain the lack of visible symptoms at sites 25-28.

In summary, over the three visits made during the 2010-growing season to the CBWC evidence of potential heavy metal contamination in the vegetation was observed, especially in the southwest corner of Cowles Bog. The greenhouse experiment (part 2 of this report) showed that elevated levels of aluminum, boron, and molybdenum, similar to those observed at CBWC, have significant, deleterious effects on the growth of three native wetland species *A. incarnata, C. aquatilis,* and *I. virginica*. Furthermore, the symptoms witnessed in the greenhouse were frequently witnessed in the plume area vegetation. Additional study of the vegetation of the CWBC, including whether plant tissues are accumulating elevated levels of aluminum, boron, and/or molybdenum, is warranted.

Figure 2.—*S. foetidus* (Site 13, near plume)

Figure 3.—*S. foetidus* (Site 18, far from plume)

Figure 4.—*I. verticillata* (Site 14, near plume)

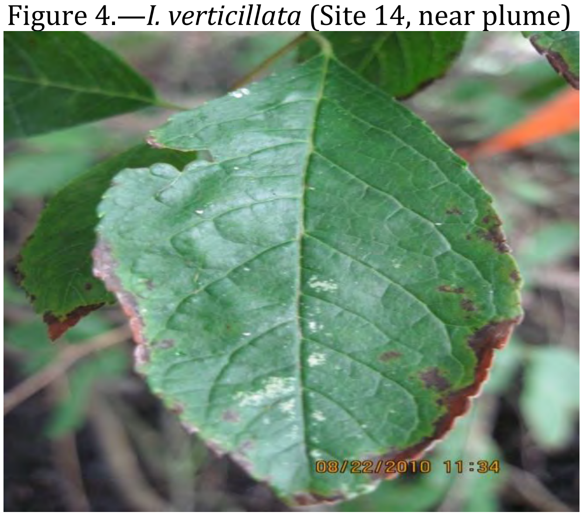


Figure 5.—*I. verticillata* (Site 18, far from plume)

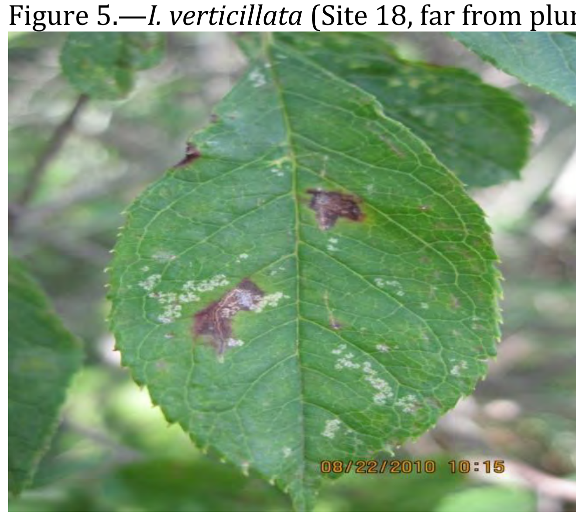


Figure 6.—*C. occidentalis* (Site 16, near plume)

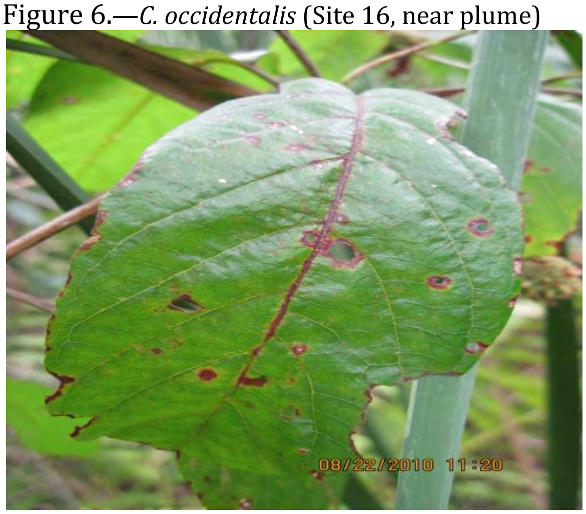


Figure 7.—*C. occidentalis* (Site 19, far from plume)

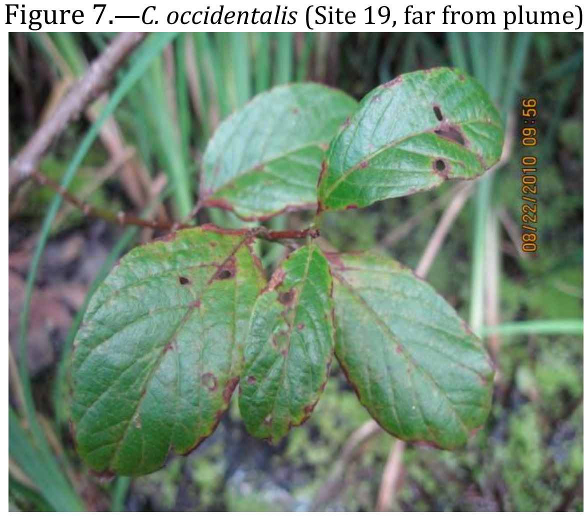


Figure 8.—*S. pungens* (Site 5)

Figure 9.—*I. virginica* (Site 5)

Figure 10.—*E. coloratum* (Site 5)

Figure 11.—*A. incarnata* (Site 5)

Figure 12.—*A. incarnata* (Site 5)

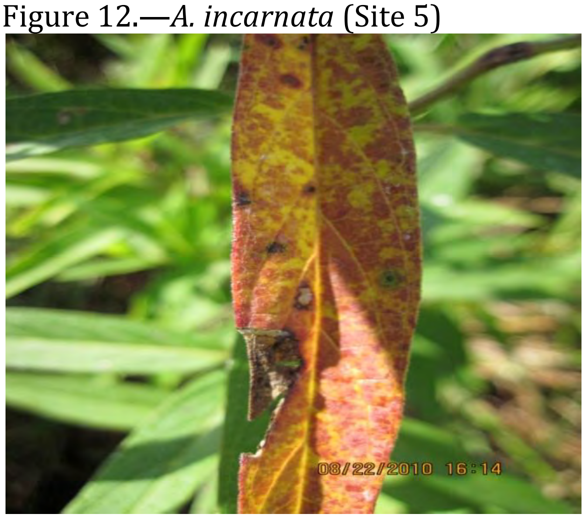


Figure 13.—*V. hastata* (Site 5)

Figure 14.—*V. hastata* (Site 5)

Figure 15.—*S. tabernaemontani* (Site 3)

Figure 16.—*S. latifolia* (Site 3)

Figure 17.—*A. subcordatum* (Site 3)

Figure 18.—*A. incarnata* (Site 3)

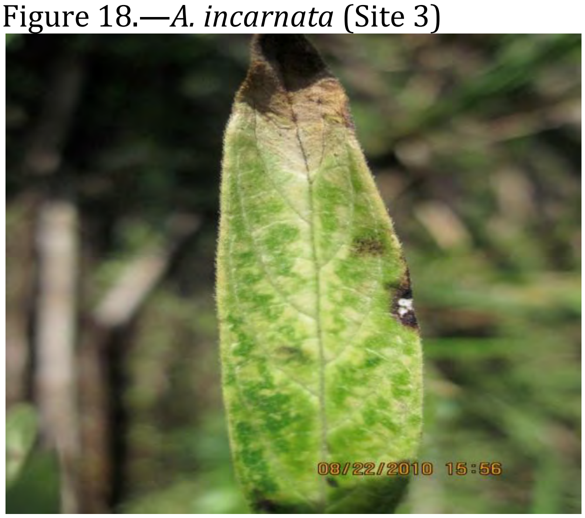


Figure 19.—*P. virginica* (Site 3)

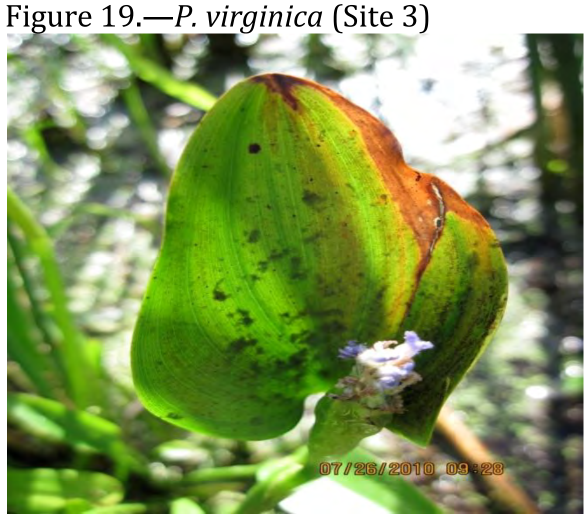


Figure 20.—*Rumex sp.* (Site 6)

Figure 21.—*Persicaria sp.* (Site 6)

Figure 22.—*S. cyperinus* (Site 7)

Figure 23.—*E. perfoliatum* (Site 7)

Figure 24.—*S. eurycarpum* (Site 8)

Figure 25.—*S. tabernaemontani* (Site 9)

PART 2 – GREENHOUSE EXPERIMENT AND EFFECTS OF Al, B, AND Mo:

Experiment Summary

Aluminum (Al), Boron (B), and Molybdenum (Mo) have been found in potentially toxic levels in the fly ash produced by coal-fired power plants. Varying concentrations of Al, B, and Mo were applied to three wetland plant species, *Asclepias incarnata, Carex aquatilis*, and *Iris virginica*. Plants were grown in a washed sand medium and received a modified Hoagland's solution every other day. Three concentration levels of Al (9 mg, 18 mg, and 27 mg/L), four concentration levels of B (14 mg, 26 mg, 47 mg, and 79 mg/L), and three concentration levels of Mo (2.5 mg, 5.0 mg, and 7.5 mg/L) were added as treatments. All three plant species showed visible symptoms of toxicity such as leaf tip necrosis and marginal leaf curl; the most severe and widespread occurring with the B treatments. Visual observations in the greenhouse revealed necrotic spotting in the leaf blade in most B treatments and highest Al concentration as early as day 15. Inductive Coupled Plasma Mass Spectrometry (ICP-MS), performed on the aboveground plant material of *A. incarnata* and *I. virginica*, indicates increasing boron uptake with concentration. While quantitative measures showed inhibition for all three species and nutrients, *Asclepias incarnata* was especially sensitive to B applications.

Literature Background

Minimal research has been conducted to determine at what concentrations constituents of fly ash may become toxic to and how they affect native wetland vegetation. Coal mines, whether active or abandoned, are significant sources of metal contamination and discharges (Batty et al. 2002). In addition, the burning of coal in electrical generation power plants can be a major source of heavy metal contamination, in particular aluminum, boron, and molybdenum (Wilcox and Hardy, 1998).

Vegetation can respond in one of three ways to increasing concentrations of heavy metals in the soil. Some are considered accumulators, which are species that accumulate and concentrate metals in the aboveground tissues. Others are called indicators, where internal concentrations reflect the external environment, a third group are excluders, which are plants that have shoot concentrations low in heavy metals and remain constant over many soil concentrations up to a critical soil level above which unrestricted transport, the point at which the plant can no longer prevent metals from entering, takes place (Baker, 1981).

Elemental uptake by wetland plants varies among species and is related to rooting depth and plant life form (Weis and Weis, 2004). In general, the inhibition of root growth is one of the most rapid responses to toxic concentrations of a heavy metal (Wong and Bradshaw, 1982). Trace elements often show an order of magnitude greater concentration in roots than in shoots. Boron is one exception. Boron is a passive mover throughout the transpiration stream and accumulates in the aboveground tissue, especially the leaves (Supanjani, 2006).

Aluminum. Soils contain, on average, 7 – 8% Aluminum (Al) and under acidic conditions Al becomes solubilized, increasing its mobility (Batty et al. 2002) and availability to plants (Miyasaka et al. 2007; Delhaize and Ryan, 1995; Rout et al. 2001; Abdalla, 2008). Runoff from coal stockpiles and coal-fired power plants are acidic and contain high levels of Al (Collins et al. 2004). High levels of Al in the soil can become a major limiting factor for plant production (Delhaize and Ryan, 1995).

Attempts have been made to establish critical Al concentrations for toxicity in plants (Foy, 1998). However, plant species respond in different ways to Al toxicity. Some plants have the ability to accumulate large amounts of Al in their foliage without any visible evidence of injury (Rout et al. 2001). However, Al toxicity also can induce deficiencies of other nutrients. Al toxicity can reduce the accumulation of calcium (Ca) in plant tissue to a level that Al toxicity resembles Ca deficiency (Rengel, 1992). Reduced Ca transport is expressed as curling of young leaves and collapse of petioles (Rout et al. 2001; Foy, 1984).

Al toxicity also can induce an iron (Fe) deficiency, which is expressed as chlorosis, and phosphorous (P) deficiency, which produces overall stunting, production of small dark green leaves and late maturity, purpling of the stem, leaf vein, and yellowing of leaf tips (Rout et al. 2001; Foy, 1998).

Al commonly accumulates in the roots in greater concentrations than in the shoots (Collins et al. 2004). The first, observable symptom of Al toxicity is the inhibition of root elongation (Miyasaka et al. 2007; Rengle, 1992; Roy et al. 1988; Delhaize and Ryan, 1995; Rout et al. 2001; Mossor-Pietraszewska, 2001). This inhibition damages the root system, limiting both nutrient and water uptake to the plant (Gregory, 2009; Poozesh et al. 2007; Zhang et al. 2007). Symptoms of Al toxicity can occur within hours of exposure (Miyasaka et al. 2007) and symptoms include stunted root growth, reduction in root hair development, and, swollen root apices (Matsumoto, 2002); in some cases, roots can become thickened and brown (Rout et al. 2001).

At the cellular level Al interferes with cytoskeleton structure and function, disrupts calcium homeostasis, phosphorous metabolism, and can cause oxidative stress (Miyasaka et al. 2007). Aluminum toxicity in leaves results in increased diffusion resistance, reduction of stomatal aperture, decreased photosynthetic activity, total decrease of leaf number and size, and a decrease of shoot biomass (Mossor-Pietraszewska, 2001). As a result, young leaves become small, curved along the margin and chlorotic and older leaves have marginal chlorosis (Roy, 1988).

Boron. Boron (B), an essential micronutrient (Gupta, 2007), is required for plant growth (Goldberg, 1993; Supanjani, 2006; Hu and Brown, 1997). Therefore, B is necessary in a continuous supply throughout the life of the plant and uptake is primarily through the roots. The species of B absorbed from the soil solution by the roots is often boric acid $B(OH)_3$ (Hu and Brown, 1997). Boric acid is a weak monobasic acid that acts as an electron acceptor (Gupta, 2007) in aqueous solution (Hu and Brown, 1997; Nable et al. 1997). Boron can become toxic in elevated concentrations (Miwa et al. 2007) and inhibit plant growth and development (Redington and Peterson, 1983).

Boron toxicity can occur when soils: 1) are naturally high in B, 2) are over-fertilized with minerals high in B, 3) receive fossil fuel combustion residues, which are produced from the burning of coal for electricity, or are used as disposal sites for waste materials containing B, such as fly ash and industrial chemicals (Nable et al. 1997) and 4) when irrigated with water high in B (Leyshon and James, 1993). Fly ash is of particular concern because of the high concentrations of B in fly ash may be readily available to plants and can prevent the establishment of vegetation on contaminated areas (Nable et al. 1997, Piha et al. 1995), especially during the first growing season (Wong and Bradshaw, 1982).

Plants vary in their B requirement, but the range of essential and toxic levels is smaller than for any other nutrient element (Goldberg, 1993; Reid et al. 2004). Research by Gupta et al. (1985) showed that boron is required in low concentrations for plant growth and becomes phytotoxic at concentrations only slightly higher than the optimal range (Sartaj and Fernandes, 2005). Boron is known to be a passive mover in plants (Supanjani, 2006). The amount of B taken up by the roots and transported to the shoots is related to the rate of transpiration (Sotiropoulos et al. 2002; Raven et al. 1980).

Studies have demonstrated that the mobility of B can vary dramatically between species (Brown and Hu, 1988). Boron enters the transpiration stream via the roots and tends to accumulate at the sites of termination in leaves (Brown and Hu, 1998; Nable et al. 1997; Reid et al. 2004; Sotiropoulos et al. 2002; Raven, 1980). In species where B is immobile toxicity symptoms always are exhibited as leaf tip and edge burn (Brown and Hu, 1998).

However, in species where B is mobile toxicity is exhibited as die back in young shoots rather than marginal leaf burn (Brown and Hu, 1998). Boron immobility is evidenced by elevated B concentrations in older leaves. Elevated B concentrations in younger leaves are an indication of B mobility because they have transpired less water than older leaves young leaves (Brown and Hu, 1998).

Boron does not accumulate evenly in leaves and typically concentrates in leaf tips of monocots and leaf margins of dicots. This is where toxicity symptoms typically first appear (Gupta, 2007; Kohl Jr. and Oertili, 1961). In general, B concentrations are lower in plant stems (Gupta, 2007). Soil pH influences the availability of B to plants and becomes less available as pH increases (Gupta, 2007; Hu and Brown, 1997). Furthermore, B toxicity can produce necrotic spots in the leaf blade (Sotiropoulos et al. 2002), marginal and tip chlorosis (Gupta, 2007), leaf burn (Nable et al. 1997), interveinal chlorosis, premature leaf drop, and plant death (Goldberg, 1993).

Molybdenum. Molybdenum (Mo) is an abundant essential micronutrient found in most plant tissues. Gupta and Lipsett (1981) concluded that the allocation of Mo throughout plant organs varies with the plant species, but generally the concentration is highest in the seeds. The concentration level considered toxic differs from plant to plant but dicots are typically more sensitive than monocots (Hamlin, 2007). However, Mo can reach toxic levels in the soil with applications of municipal sewage sludge or in soils near mining and smelting activities (Gupta, 1998).

The availability of Mo is tied to soil pH; therefore, Mo is more available at higher pH and less available at lower pH (Kaiser et al. 2005; Gupta, 1997). Under acidic soil conditions the molybdate anion is strongly adsorbed to the surface of Fe and Al oxides (Smith et al. 1997) and this adsorption is greatest at pH 4.0 (Keddy et al. 1997). Another factor in Mo availability is soil moisture. Poorly drained soils can accumulate high quantities of available MoO_4^{2-} (Gupta, 1998). However, the majority of plants tested are not particularly sensitive to excessive levels of Mo in the soil medium (Hamlin, 2007). Symptoms of Mo toxicity in plants include: burning, chlorosis and yellowing of leaves (Gupta, 1998) and an inhibition of root/shoot growth (Kevresan et al. 2001).

Greenhouse Experimental Methods

A total of 468 plants of three native species, *A. incarnata*, *C. aquatilis*, and *I. virginica*, were grown in approximately 38 cubic inch pots. Plants were organized into 13 groups, which were randomly organized on two greenhouse benches, and 12 of each species were organized randomly within each group. Washed sand was used as the growing medium. Immediately after transplanting was complete a modified Hoagland's solution was applied. The modified Hoagland's solution consisted of 940 ml of distilled water, 10 ml magnesium sulfate ($MgSO_4$) 0.14 M, 10 ml potassium nitrate (KNO_3) 0.17 M, 10 ml potassium hydrous phosphate (KH_2PO_4) 0.12 M, 10 ml iron ethylenediaminetetraacetic acid (Fe EDTA), and 10 ml calcium nitrate ($Ca(NO_3)_2 \cdot 4H_2O$) 0.10 M (Hoagland and Aron, 1950). The typical trace elements were omitted from the Hoagland's solution. The trace element solution was omitted because varying concentrations of boric acid, molybdic acid disodium salt dihydrate were applied as treatments. Aluminum chloride ($AlCl_3$) also was applied to the plants in this experiment but is not part of the Hoagland's solution.

Prior to the start of the experiment plants were treated with 50 ml of modified Hoagland's solution every other day for five weeks, daily if plants looked stressed, to ensure that the plants recovered from transplant shock and to allow time for the plants to begin regular production of new vegetative and root growth.

Plants were sorted into ten groups of 36, 12 plants of each of species. Treatment levels of Al, B, and Mo were applied every other day for 60 days. The concentrations were: Al = 9, 18, 27 mg/L; B = 14, 26, 47, 79 mg/L; Mo = 2.5, 5.0, 7.5 mg/L. There were 108 plants, (36 of each species), set aside as controls. Among the control plants, 36 (12 of each species) received Hoagland's solution and distilled water with pH reduced to 4.8. This was to provide a control group for the Al treatments where natural acidity ranged was approximately 4.8. Concentrations of Al, B, and Mo were gradually elevated to the treatment levels over a 13-day period. Due to apparently random plant loss during the course of the experimental period, final sample sizes ranged from 10-12. In addition, two individuals of *A. incarnata* were deleted from Mo concentration 2 since they were notably more robust before first measurements on day 28.

First growth measurements were taken on day 28 of the experiment. Stem height, number of leaves and branches, and total combined length of all branches were the measurements taken for *A. incarnata*. The number of shoots, number of leaves with a sheath greater than 2 cm, and length of longest leaf were measured for *C. aquatilis*. The number of leaves greater than 2 cm, length of longest leaf, number of dead leaves, and length of shortest leaf were recorded for *I. virginica*. The measurements were again recorded on day 42, and for the final time on day 58. On day 61 all of the plants were removed from their pots and root lengths, length of longest root, and photographs were taken.

After roots were measured and the plants photographed, the plants were dried at 120° F in a heated drying closet for four days and dry weights of roots and shoots, including leaves, were recorded. Data was analyzed with ANOVA for its ability to show if there are significant differences between pairs of groups. However, ANOVA cannot show which pairs are significantly different. Therefore, Tukey's Post Hoc tests were performed on measured variables against concentrations of an individual treatment to determine which groupings, if any, had significant differences. The critical p-value, or alpha, used was 0.05.

Normalization, by using the natural log, of the data was necessary on root weight, stem weight, and total weight for *C. aquatilis* and *I. virginica* with Al and Mo treatments, all three plant species for B, and stem weight only for *A. incarnata* with Mo treatments. As well, root length and length of the longest root for *C. aquatilis* with B treatments and *I. virginica* with B and Mo treatments required normalization.

Columbia Analytical Services in Seattle, WA, performed Inductive Cold Plasma Mass Spectrometry (ICP-MS) on aboveground tissue samples. ICP-MS was used to analyze boron content of 24 plant samples. Three samples were taken from the *A. incarnata* control group and three samples also were taken from each of the four boron treatment levels of *A. incarnata*. Three samples were taken from the *I. virginica* control group as well as from the B treatment levels 2 and 4 of *I. virginica*. Sample sizes of 0.5 - 1.3 grams were required from the plants above ground tissue for each sample. Tissue samples from as many as five plants were bulked within treatment type in order to obtain these sample amounts. Boron was the focus of this test because it was the nutrient whose specimens were exhibiting the majority of and most severe symptoms of toxicity and this test would provide further evidence for whether or not these symptoms could be attributed to the addition of the boron treatments.

Results from Greenhouse Experiment

Qualitative Observations: Qualitative inhibition of root growth was observed in all three plant species under all three nutrients (Figures 26-30; 38-55). The roots of *I. virginica* were particularly thickened and turned brown (Figure 31) at the lowest Al concentration. Leaf tip and edge burn were produced in *A. incarnata* and *I. virginica* (Figures 32 & 33) at the lowest concentration of B. Other symptoms of B treatment included necrotic spots on the leaf blade (Figures 34 & 35) and premature leaf drop (Figures 36 & 37).

Figure 26.—*A. incarnata* roots (Control)

Figure 27. —*A. incarnata* roots (Control pH)

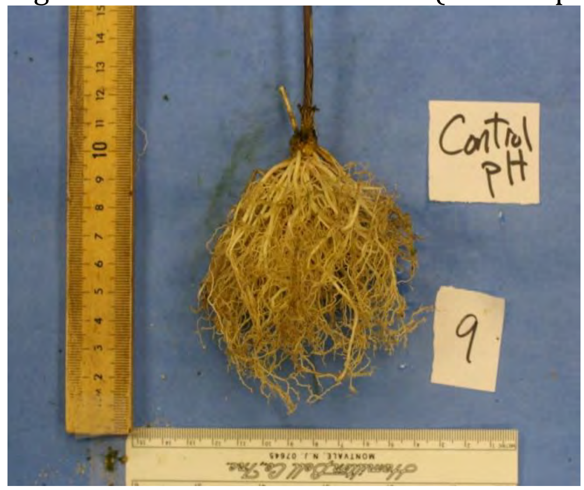


Figure 28. —*A. incarnata* roots (B1)

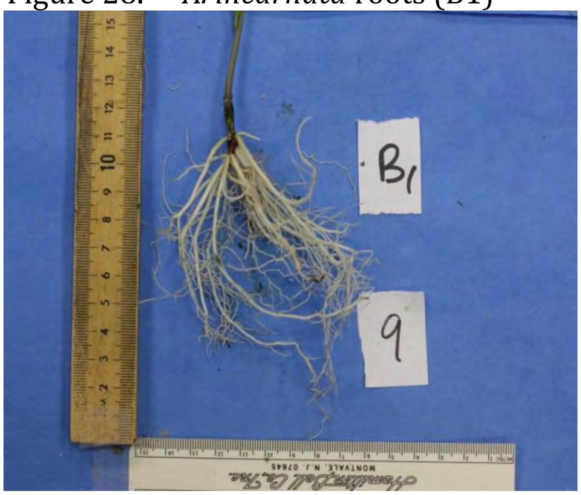


Figure 29. —*A. incarnata* roots (Mo1)

Figure 30. —*A. incarnata* roots (Al1)

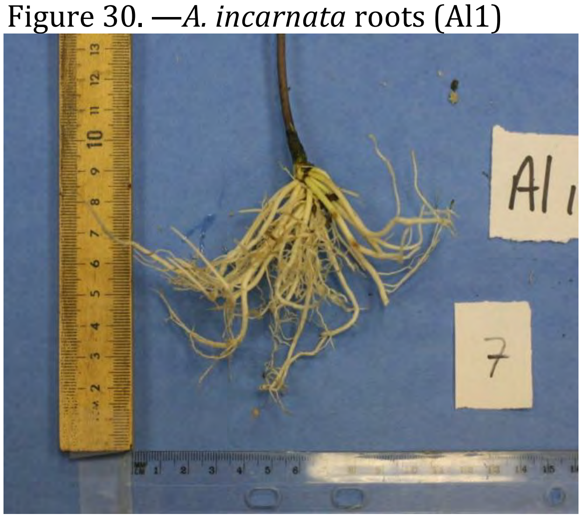
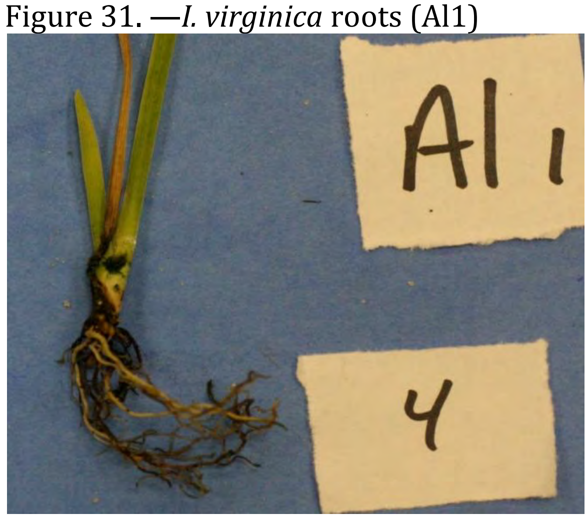


Figure 31. —*I. virginica* roots (Al1)



Figures 26-30 show the inhibition of root growth between the control and boron, molybdenum, and aluminum 1 treatments of *A. incarnata*. Figure 31 shows inhibited root growth and roots that are brittle, thickened, and brown following aluminum 1 treatment.

Figure 32. —*A. incarnata* (B1 Day 42)

Figure 33. —*I. virginica* (B1 Day 56)

Figures 32 & 33 show leaf tip and edge burn from B toxicity in *A. incarnata* and *I. virginica*.

Figure 34. —*A. incarnata* (B3 Day 33)

Figure 35. —*C. aquatilis* (B1 Day 47)

Figures 34 & 35 show boron induced necrotic spots on the leaf blades of *A. incarnata* and *C. aquatilis*.

Figure 36.—*A. incarnata* (Control)

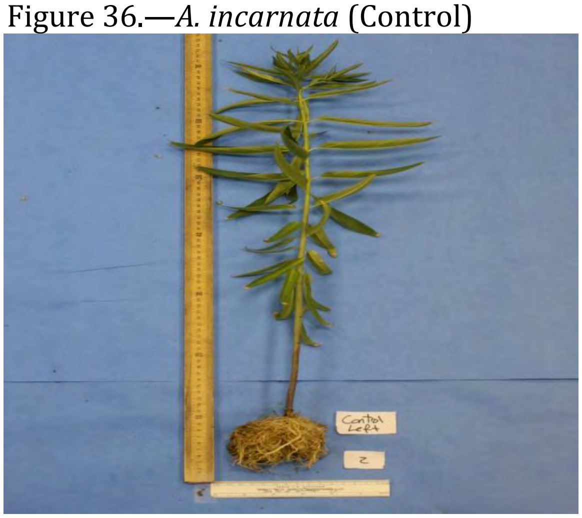


Figure 37. —*A. incarnata* (B1)

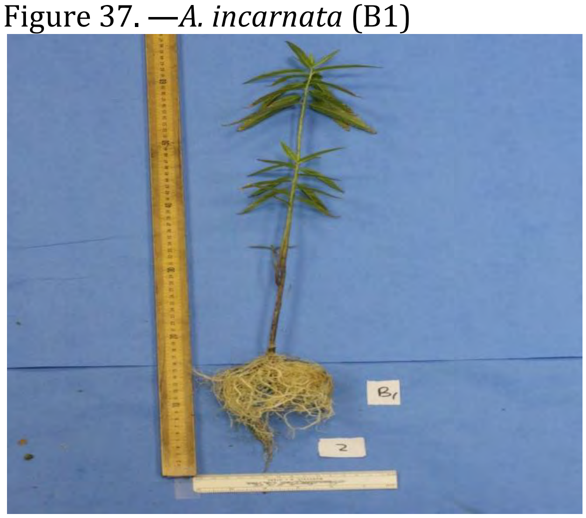


Figure 36 shows normal leaf retention; Figure 37 shows premature leaf drop.

Figure 38. —*A. incarnata* (Al Control)

Figure 39. —*A. incarnata* (Al1)

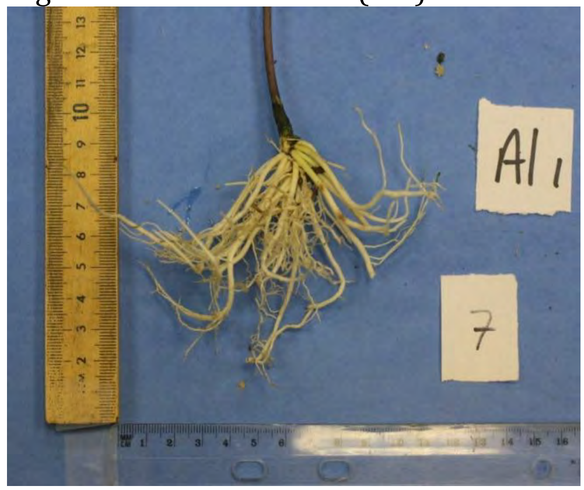


Figure 40. —*C. aquatilis* (Al Control)

Figure 41. —*C. aquatilis* (Al1)

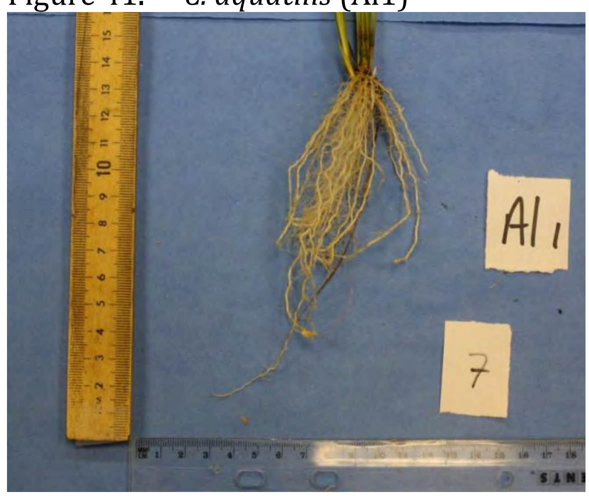


Figure 42. —*I. virginica* (Al Control)

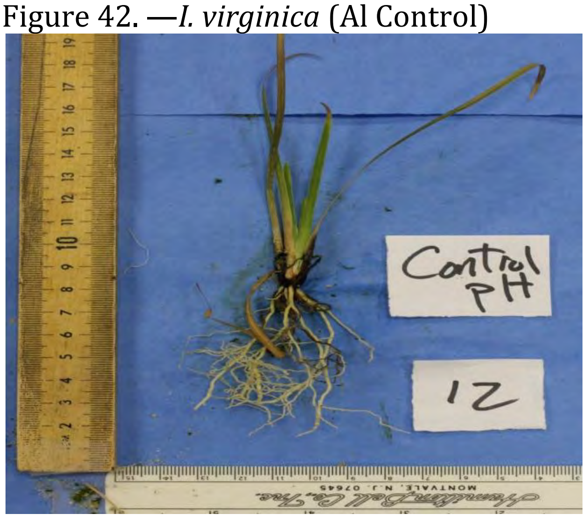
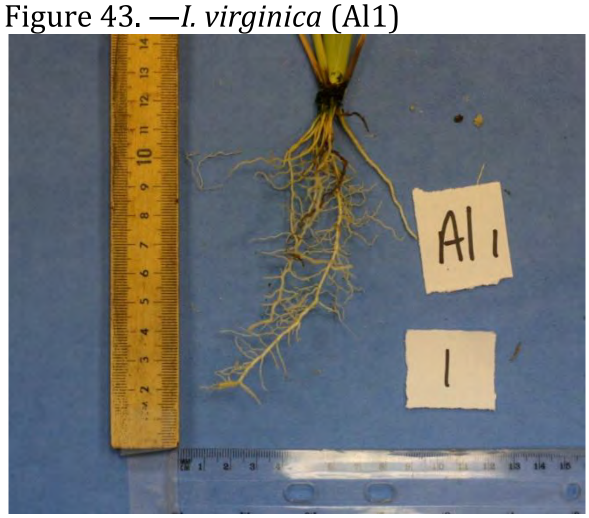


Figure 43. —*I. virginica* (Al1)



Figures 38-43 show the inhibition of root growth between Al control (low pH control) and Al treatments in all 3 plant species.

Figure 44.—*A. incarnata* (Control)

Figure 45.—*A. incarnata* (B1)

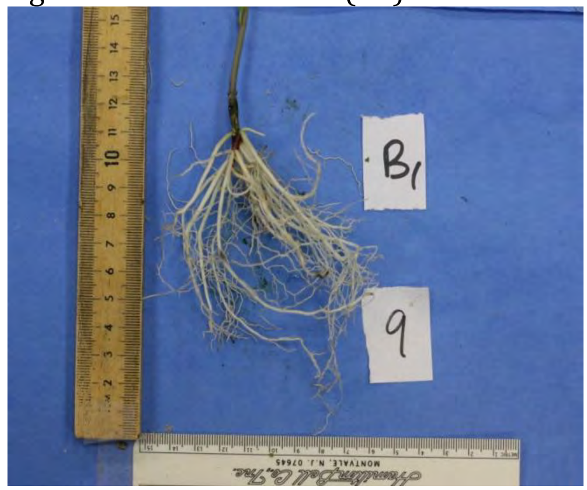


Figure 46.—*C. aquatilis* (Control)

Figure 47.—*C. aquatilis* (B1)

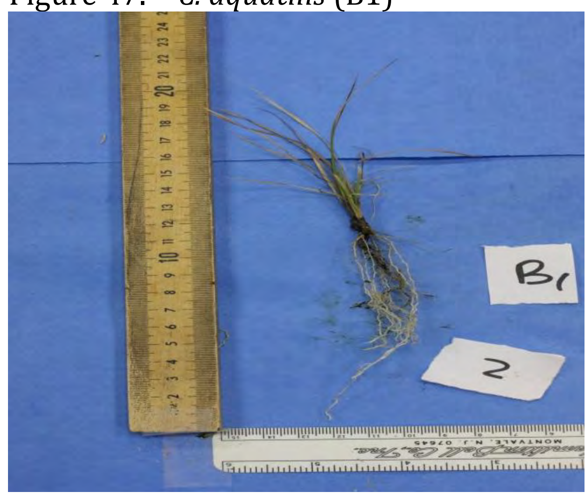


Figure 48.—*I. virginica* (Control)

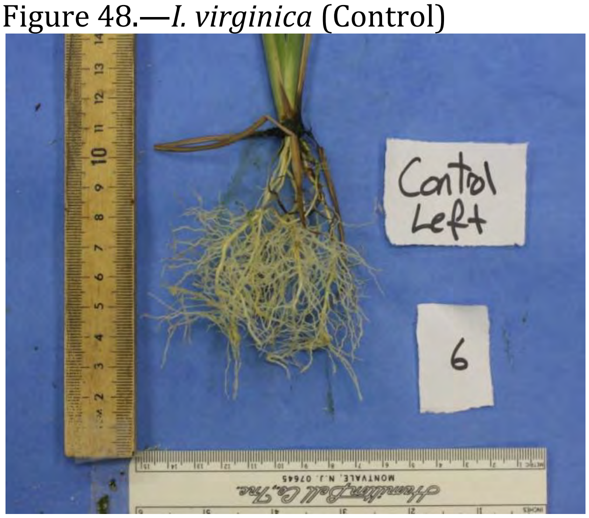
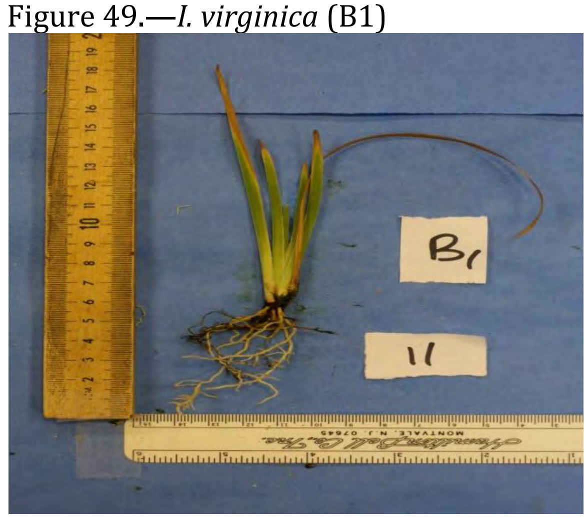


Figure 49.—*I. virginica* (B1)



Figures 44-49 show the inhibition of root growth between controls and B treatments in all 3 plant species.

Figure 50.—*A. incarnata* (Control)

Figure 51.—*A. incarnata* (Mo1)

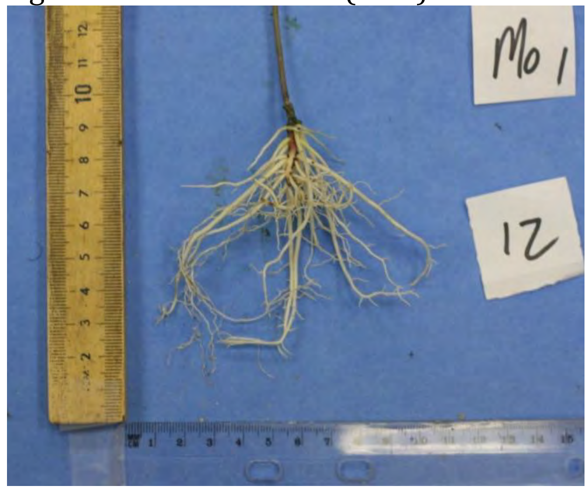


Figure 52.—*C. aquatilis* (Control)

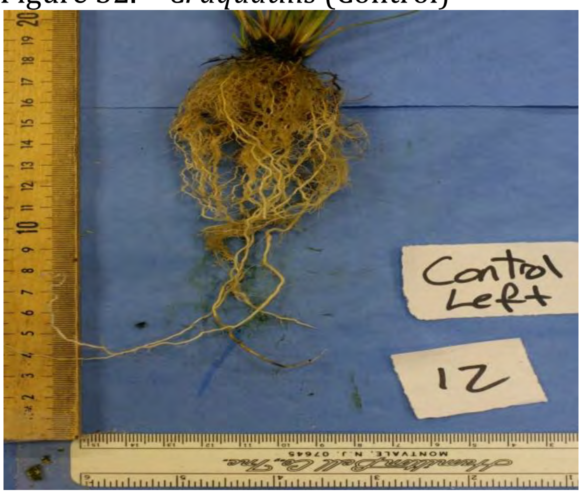


Figure 53.—*C. aquatilis* (Mo1)

Figure 54.—*I. virginica* (Control)

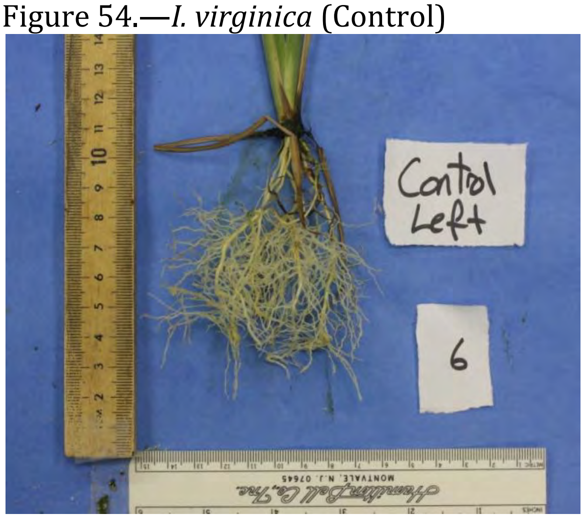
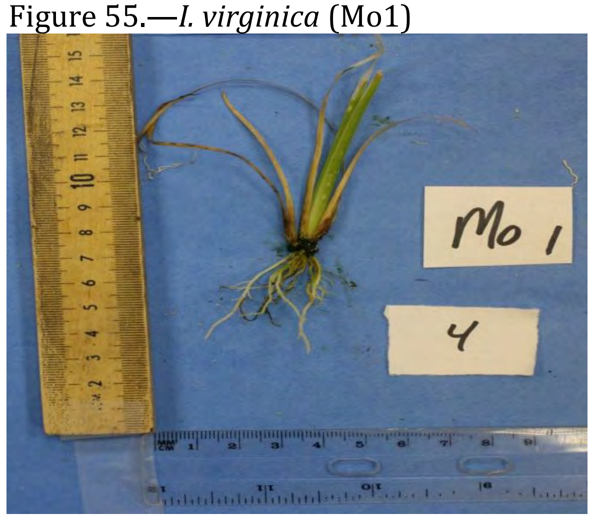


Figure 55.—*I. virginica* (Mo1)



Figures 50-55 show the inhibition of root growth between controls and Mo treatments in all 3 plant species.

Figure 56. —*A. incarnata* (Mo2 Day 56)

Necrotic spots in the leaf blade and along the margin as well as purpling of the leaf blade were witnessed by day 15 in *A. incarnata* for all nutrient treatment levels of Al, B, and Mo, except for the lowest concentration of boron. The most severe symptoms expressed were at higher concentrations of Al and B. By day 20 these symptoms became apparent in the B treatments in *C. aquatilis*. Necrosis became increasingly severe throughout all three plant species in all three nutrient concentration levels (4 in the case of boron) and by day 56 the majority of plants were expressing moderate to severe toxicity symptoms. *Asclepias incarnata* in molybdenum treatment level 2 attempted to produce an inflorescence. The inflorescence was small, dull colored, dry, and failed to open normally (Figure 53).

ANOVA Analyses. Aluminum had a statistically significant relationship on root, stem and total weight ($p \leq 0.004$), root length ($p = 0.029$), and stem length ($p = 0.040$) of *A. incarnata* and length of the longest root ($p = 0.001$), number of sheaths over 2 cm long ($p = 0.042$), and length of the longest leaf ($p = 0.024$) when applied to *C. aquatilis* (Figures 57-62; Appendix B).

Results of the Post Hoc test showed that when aluminum was applied to *A. incarnata* the root length mean of the control group was significantly less than that of Al treatment 2 (Figure 58). Likewise stem length at the lowest Al concentration was less than the mean of the next higher treatment (Figure 59). On the other hand, the various weight parameters for Al treatment 2 were not significantly different from the control (Figure 57; Appendix B). When Al was applied to *C. aquatilis* several measurements of growth, namely longest root length, number of sheaths over 2 cm, and longest leaf length, may have been enhanced at the lowest concentration (Figures 60-62) but potentially inhibited at higher concentrations.

Boron had a statistically significant relationship when applied to *A. incarnata* on root, stem, and total weight (p = 0.001), length of the longest root (p = 0.001), and the number of leaves (p = 0.002) (Figures 57, 63-65; Appendix C). The Post Hoc test showed that all concentrations of B had a significant difference in root weight, stem weight, and total weight compared with the control (Figure 57). Other indicators of growth such as length of longest root (Figure 63), number of leaves (Figure 64), and root length (Figure 65) were significantly reduced at least at the highest B concentration. While the two monocot species, *C. aquatilis* and *I. virginica*, had obvious qualitative symptoms, the quantitative measures, including total weight (Table 1), were not significantly inhibited over the course of this experiment.

Molybdenum had a statistically significant relationship on stem weight (p = 0.003), stem length (p = 0.005), and number of leaves (p = 0.001) of *A. incarnata* and on length of the longest leaf (p = 0.013) of *C. aquatilis* (Figures 57, 66-70; Appendix D). The Post Hoc test revealed that when Mo was applied to *A. incarnata* there was a significant reduction of

Asclepias incarnata

Aluminum Boron Molybdenum

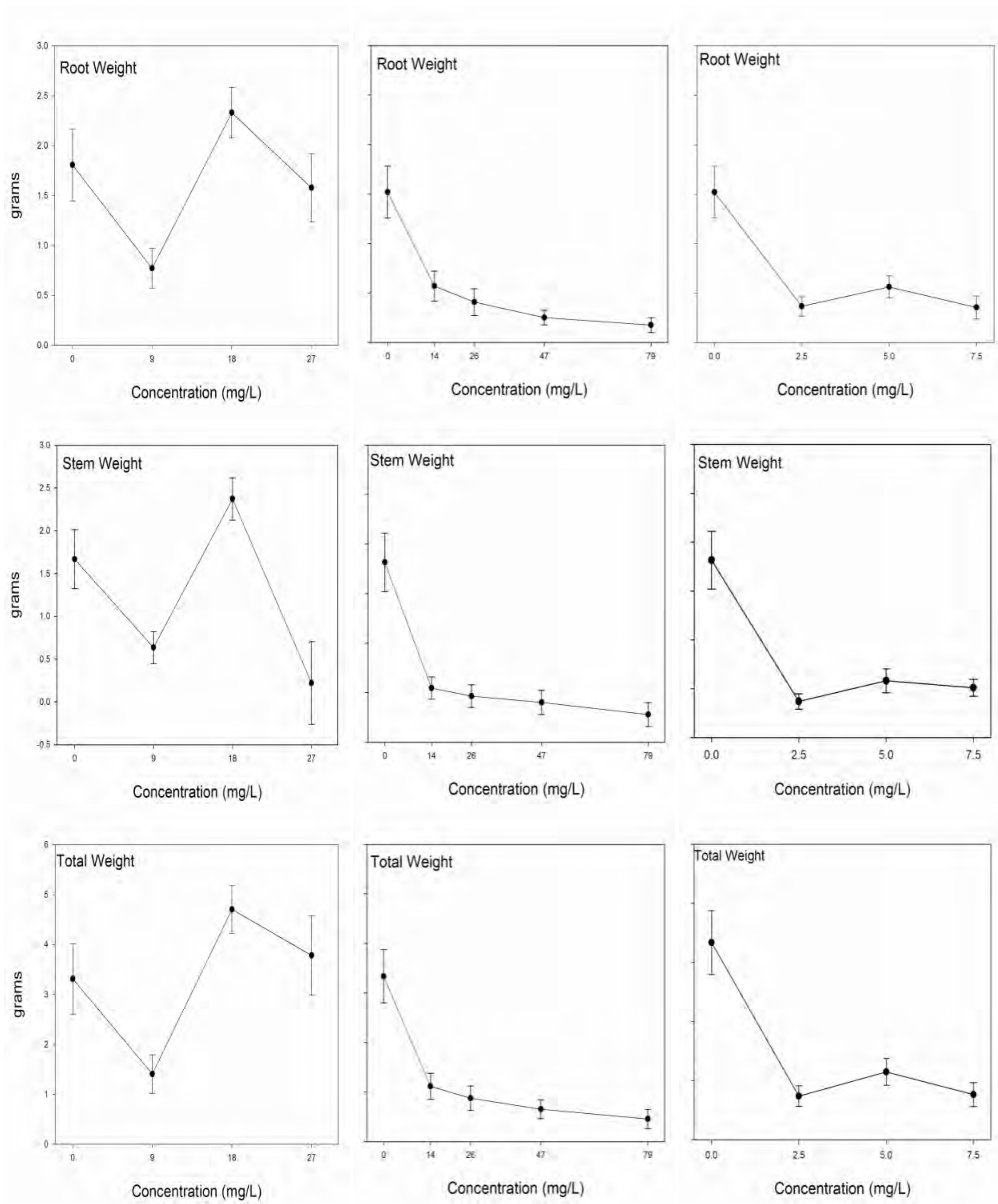


Figure 57. Response of *A. incarnata* to applications of aluminum, boron, and molybdenum. Al, B, and Mo concentrations are in mg/L; root, stem, and total weights are in grams.

Figure 58. Al: Root Length

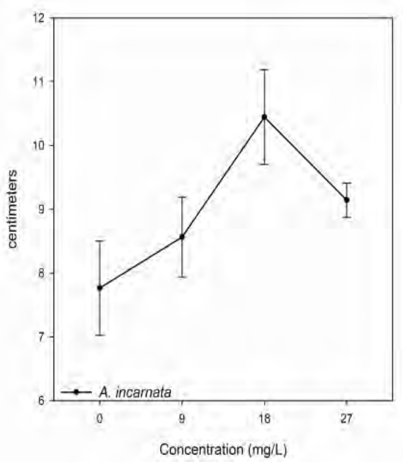


Figure 59. Al: Stem Length

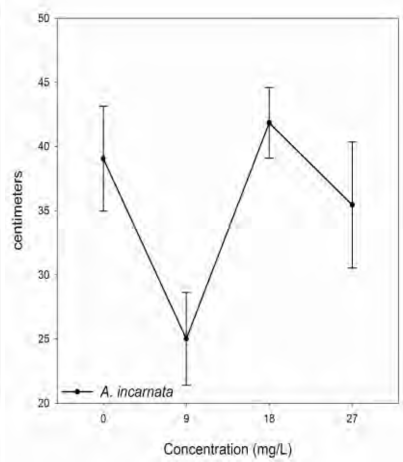


Figure 60. Al: Longest Root

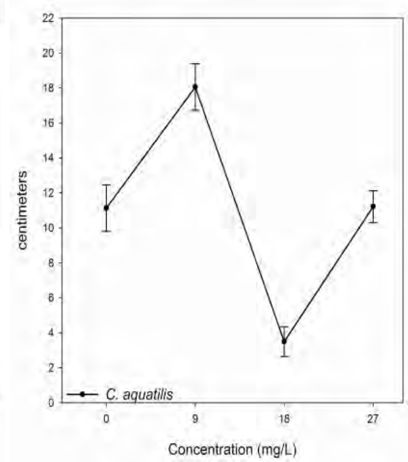


Figure 61. Al: # Sheaths > 2cm

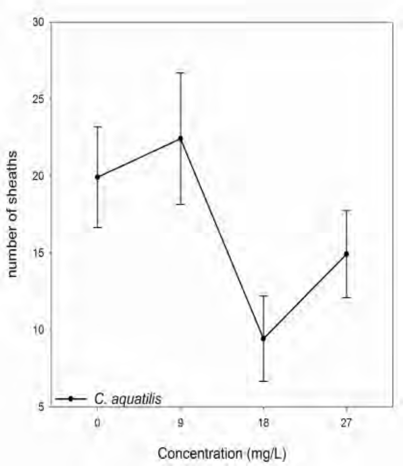


Figure 62. Al: Longest Leaf

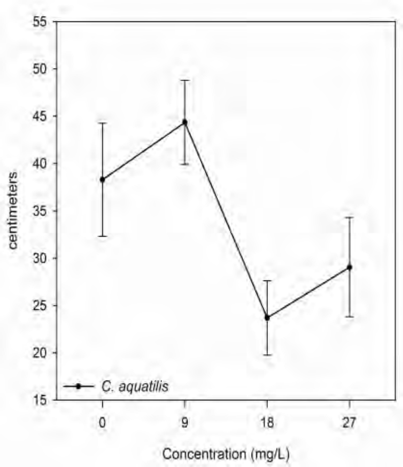


Figure 63. B: Longest Root

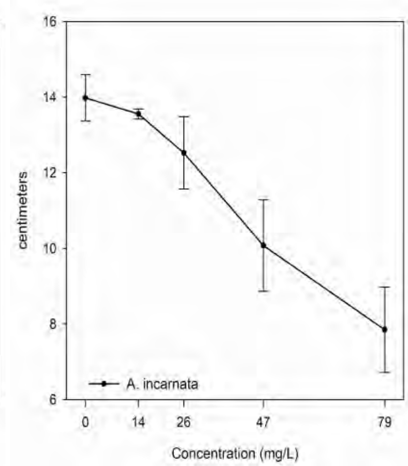


Figure 64. B: Number of Leaves

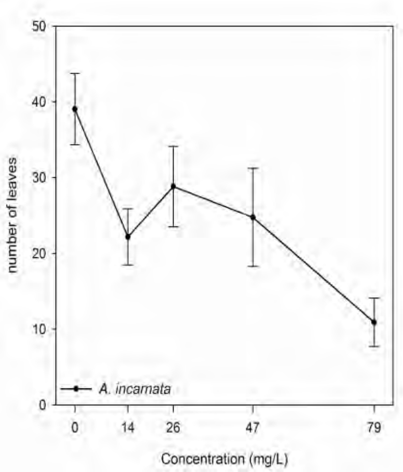


Figure 65. B: Root Length

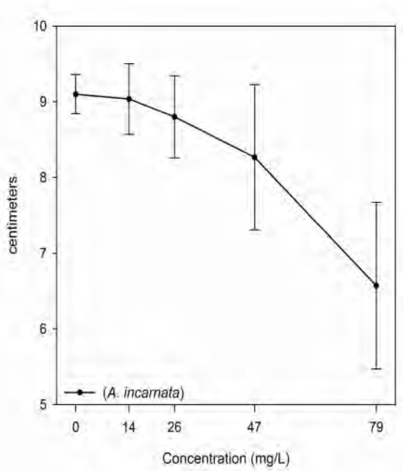


Figure 66. Mo: Number of Leaves

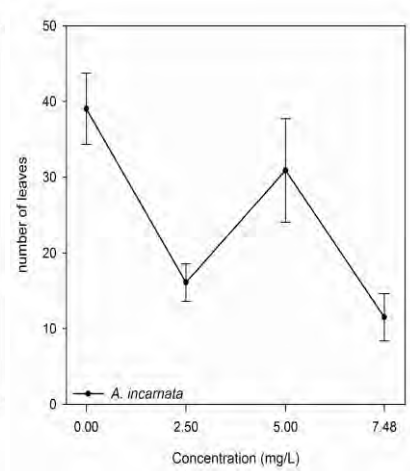


Figure 67. Mo: Stem Length

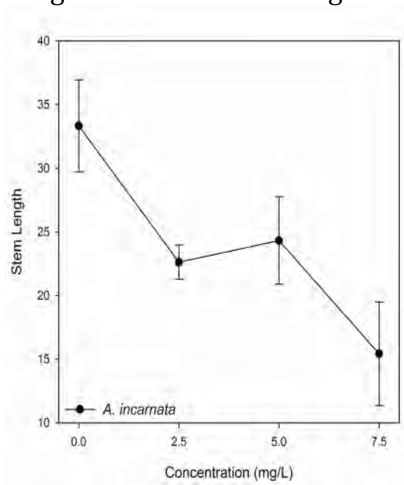


Figure 68: Mo: # Sheaths > 2cm

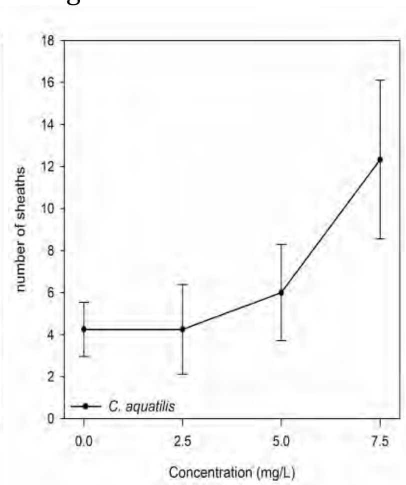


Figure 69. Mo: Longest Leaf

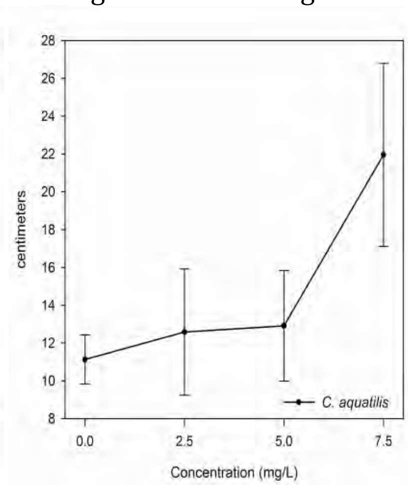
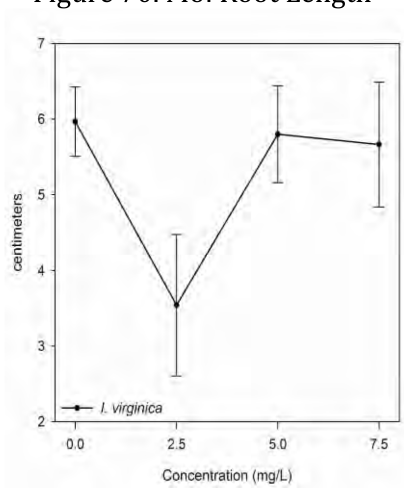


Figure 70. Mo: Root Length



stem weight between the control group and treatment levels 1 and 3, on stem length between the control group and treatment level 3, and number of leaves between the controls and treatment levels 1 and 3. A significant difference could not be demonstrated between the control and Mo treatment 2, a group with a high degree of variability among test plants (Figure 57). Not surprisingly, this same response was exhibited in another growth response, the number of leaves (Figure 66). Across the entire experiment, *A.*

incarnata at the highest Mo concentration suffered the most mortality. As a result, only ten plants had measurable root systems and eight plants with aboveground parts.

When Mo was applied to *C. aquatilis* a significant difference was found in the number of sheaths longer than 2 cm (Figure 68) and in the length of the longest leaf (Figure 69). In both instances these parameters increased with concentration of Mo, reaching a statistically significant threshold at the highest Mo concentration used in the experiment. On the other hand, root length of *I. virginica* was inhibited with low levels of Mo but no significance difference was observed at other Mo concentrations (Figure 70).

Inductive Coupled Plasma Mass Spectrometry. ICP-MS was used for this study to measure B uptake in samples of *A. incarnata* and *I. virginica*. In both *A. incarnata* and *I. virginica*, accumulation of B was evident. *Asclepias incarnata* control had a boron mean uptake of 62 ppm compared to 179 ppm, 446 ppm, 553 ppm, 427 ppm at the progressively higher concentrations of B. *Iris virginica* control treatment had a boron mean uptake of 107 ppm, while B treatment level 2 and 3 were 419 ppm and 797 ppm. The Post Hoc test revealed for *A. incarnata* that the mean of the control samples was less than the means of treatment levels 2, 3, and 4 and for *I. virginica* the mean of the control samples was lower than the mean of treatment level 4 (Appendix E).

Discussion

Visually the three native wetland plant species responded similarly to varying concentrations of Al, B, and Mo. Aluminum and B, at elevated concentrations, produced leaf purpling, necrotic spotting, and tip death in *A. incarnata* by day 15 of the experiment. By day 20 the two lowest B concentrations and the lowest Mo concentration in *A. incarnata* were affected and the necrosis expanded to include *C. aquatilis*. And by day 56 all three plant species, all treatment concentration levels of the three nutrients, had expressed pervasive necrosis and yellowing of leaves and in one specimen of *A. incarnata* incomplete flowering was expressed. Qualitatively root inhibition was apparent in *A. incarnata* in all three nutrients and concentration levels (Figures 26-30, 38-39, 44-45, 50-51). The controls of all three plant species did express some leaf wilting, leaf tip death, and purpling of the leaf margin. However, these symptoms were neither as severe nor widespread as in the plants receiving Al, B, and Mo. And plant mortalities, of which there were 52, during the experiment, appeared to be random, with the possible exception of the highest concentration of Mo, and not the result of treatments.

The symptoms observed in the greenhouse experiment are commonly noted in the literature (e.g., Miyasaka et al. 2007; Gupta, 2007; Sotiropoulos wt al. 2002; Kevresan et al. 2001). However, the prior and current literature was limited to research in vegetables and woody species, primarily. There has been little, if any, research on the responses of elevated levels of Al, B, and Mo, or other constituents of fly ash leachate, to native wetland plant species.

It was particularly instructive that the three species used in this study had some noteworthy differences in their response to elevated levels of Al, B, and Mo. The dicot *A. incarnata* seemed the most sensitive of the three species, especially to increasing concentrations of B. The applications of B to *A. incarnata* had a dramatic effect on root, stem, and total weight (Figure 57) as well as length of the longest root, number of leaves, and root length (Figures 63-65). These results were further reinforced with the ICP-MS tests. The ICP-MS tests revealed that *A. incarnata* and *I. virginica* are accumulator species, which are species that accumulate and concentrate metals in the aboveground tissues.

Although increasing concentrations of B produced progressively greater inhibitions of growth, Al treatments had unexpected variation in response (e.g., Figure 57) in the form of an unexpectedly pronounced inhibition of growth at the lowest Al concentration. Several explanations may be posited including a natural variability in plant response to elevated levels of nutrients and heavy metals in the soil medium. The fact that elemental uptake by wetland plants varies among species and is related to rooting depth and plant life form (Weis and Weis, 2004) could explain why some plants are bigger, more resilient, and appear more tolerant to varying concentration levels. This may suggest that small differences in the condition of the plants at the on-set of the treatment may lead to large differences in their ability to acclimatize over the course of the experiment.

Alternatively, because the inhibition of root growth is one of the most rapid responses to toxic concentrations of a heavy metal (Wong and Bradshaw, 1982), even a low but toxic Al concentration could have broad consequences on plant growth. One might wonder whether more extensive root damage leads to elevation of pH levels within the rhizosphere with subsequent effect on the absorption of Al (Taylor and Foy, 1985). Experimental results suggest that there are various mechanisms involving extracellular and intracellular carboxylate ion production that assist in the sequestering and detoxification of Al in plants (Panda and Matsumoto, 2007). These may act differentially over a range of concentrations.

The resilience of plant growth in response to Al and Mo was evident in several growth responses. In this experiment, toxic effects on root growth of *A. incarnata* only became apparent at and above 18 mg/ml concentration (Figure 58) and in *C. aquatilis* the number of sheaths and length of the longest leaf only decreased at Al concentrations above 9 mg/ml (Figures 61-62). And perhaps the most interesting result was the increase in the number of sheaths greater than 2 cm and leaf length of *C. aquatilis* with increased concentrations of Mo (Figures 68-69).

In summary, the above results suggest that B toxicity is uniformly expressed both in qualitative as well as quantitative measures of plant response across a range of concentrations. In contrast, the responses to Mo and especially Al, while evident and no less severe in foliage symptoms, were less uniform across the range of concentrations and quantitative measures. The latter may indicate some potential for these three plant species to acclimatize to these two fly ash constituents.

This greenhouse experiment has applications that can be translated into the field. The findings for these three common native wetland species aided in the recognition of symptoms in the field sites and corroborate observations from CBWC, especially the SW corner of Cowles Bog. The greenhouse experiment produced symptoms of incomplete flowering, leaf tip and leaf margin burn and necrosis, necrotic spotting, chlorosis of the leaf blade and veins, marginal leaf curl, and purpling of the stem and these symptoms also were observed in Cowles Bog in many species.

The long-term implications of these findings suggest that the vegetative quality of affected areas of Cowles Bog will remain low until the effects of the fly ash leachate are eliminated from the site. High levels of B, which have an especially negative impact on vegetation during the first growing season (Wong and Bradshaw, 1982), make the establishment of new native vegetation difficult. At the same time, Ye et al. (1998)

concluded that the invasive non-native *Typha latifolia* (cattail) is inherently tolerant to elevated levels of heavy metals commonly found in the leachate of fly ash. A virtual cattail monoculture is currently present in some portions of Cowles Bog and, with its sequestered B, presents a continuing risk to the establishment of more conservative, native plant species.

Further study is necessary to determine the specific levels of the constituents of fly ash, including Al, B, and Mo in Cowles Bog. The continuation of groundwater and vegetation monitoring is necessary and the testing of plant samples *in situ* by ICP-MS could be a valuable tool in determining the uptake concentrations of specific, individual fly ash constituents in the vegetation in the CBWC.

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Appendix A.

Latitude/Longitude of sites in study

Site #	Latitude	Longitude	Location
1	41° 38.229’	87° 05.816’	Cowles Bog (CB)
2	41° 38.241’	87° 05.837’	Cowles Bog
3	41° 38.219’	87° 05.858’	Cowles Bog
4	41° 38.243’	87° 05.929’	Cowles Bog
5	41° 38.231’	87° 05.953’	Cowles Bog
6	41° 38.230’	87° 06.011’	Cowles Bog
7	41° 38.236’	87° 06.052’	SW Corner Cowles Bog
8	41° 38.219’	87° 06.072’	SW Corner Cowles Bog
9	41° 38.249’	87° 06.052’	Cowles Bog
10	41° 38.314’	87° 05.990’	Cowles Bog
11	41° 38.321’	87° 06.007’	Cowles Bog
12	41° 38.345’	87° 06.000’	Cowles Bog
13	41° 38.371’	87° 05.986’	Cowles Bog
14	41° 38.390’	87° 06.006’	Cowles Bog
15	41° 38.413’	87° 05.998’	Cowles Bog
16	41° 38.438’	87° 05.964’	Cowles Bog
17	41° 38.454’	87° 05.928’	Cowles Bog
18	41° 38.685’	87° 05.514’	Cowles Bog Boardwalk
19	41° 38.691’	87° 05.340’	North Side Cowles Bog
20	41° 38.506’	87° 05.848’	Sm Wetland North of CB
21	41° 38.556’	87° 05.878’	Lrg Wetland North of CB
22	41° 38.581’	87° 05.719’	Lrg Wetland North of CB
23	41° 38.596’	87° 05.955’	Lrg Wetland North of CB
24	41° 38.405’	87° 06.170’	Sm Wetland West of CB
25	41° 38.346’	87° 06.168’	Lrg Wetland West of CB
26	41° 38.350 ‘	87° 06.217’	Lrg Wetland West of CB
27	41° 38.348’	87° 06.240’	Lrg Wetland West of CB
28	41° 38.361 ‘	87° 06.314’	Lrg Wetland West of CB
29	41° 38.380’	87° 06.216’	Lrg Wetland West of CB
30	41° 38.367’	87° 06.113’	Lrg Wetland West of CB
31	41° 38.366’	87° 06.041’	Lrg Wetland West of CB
32	41° 38.359’	87° 06.697’	Blag Slough
33	41° 38.367’	87° 06.862’	Blag Slough
34	41° 38.367’	87° 06.886’	Blag Slough

Appendix B.

ANOVA:

Mean weights in grams, lengths in centimeters, and number of sheaths ± standard error of control and three aluminum treatments (Al applied as $AlCl_3$) and representative p-values for *A. incarnata* and *C. aquatilis*.

Nutrient	Plant Species	Measurement	Control	Treatment 1 Al = 9 mg/L	Treatment 2 18 mg/L	Treatment 3 27 mg/L	P-Value
Aluminum	A. incarnata	Root Weight	1.81 ± 0.36 ab	0.77 ± 0.20 b	2.33 ± 0.25 a	1.58 ± 0.34 ab	0.006
Aluminum	A. incarnata	Stem Weight	1.67 ± 0.34 ab	0.64 ± 0.19 b	2.37 ± 0.25 a	2.20 ± 0.48 a	0.004
Aluminum	A. incarnata	Total Weight	3.31 ± 0.70 ab	1.41 ± 0.38 b	4.70 ± 0.48 a	3.78 ± 0.80 a	0.005
Aluminum	A. incarnata	Root Length	7.76 ± 0.74 b	8.56 ± 0.63 ab	10.45 ± 0.74 a	9.14 ± 0.27 ab	0.029
Aluminum	A. incarnata	Stem Length	32.54 ± 5.53 ab	18.76 ± 4.22 b	38.35 ± 4.30 a	35.44 ± 4.91 ab	0.04
Aluminum	C. aquatilis	Longest Root	11.13 ± 1.33 b	18.05 ± 1.33 a	9.49 ± 0.85 b	11.22 ± 0.91 b	0.001
Aluminum	C. aquatilis	Number of Sheaths	19.92 ± 3.27 ab	22.42 ± 4.27 a	9.42 ± 2.77 b	14.92 ± 2.83 ab	0.042
Aluminum	C. aquatilis	Length of Longest Leaf	38.28 ± 5.97 ab	44.33 ± 4.45 a	23.68 ± 3.94 b	29.04 ± 5.25 ab	0.24

Means that do not share a letter are significantly different.

APPENDIX C.

ANOVA:

Mean weights in grams, lengths in centimeters, and numbers of leaves ± standard error of control and four boron treatments (B applied as $B(OH)_3$) and representative p-values for *A. incarnata*.

Nutrient	Plant Species	Measurement	Control	Treatment 1 B = 14 mg/L	Treatment 2 26 mg/L	Treatment 3 47 mg/L	Treatment 4 79 mg/L	P-Value
Boron	A. incarnata	Root Weight	1.52 ± 0.26 a	0.57 ± 0.15 ab	0.41 ± 0.14 bc	0.25 ± .07 bc	0.18 ± .08 c	0.001
Boron	A. incarnata	Stem Weight	1.82 ± 0.30 a	0.55 ± 0.11 ab	0.47 ± 0.11 bc	0.40 ± 0.12 bc	0.28 ± 0.12 c	0.001
Boron	A. incarnata	Total Weight	3.35 ± 0.54 a	1.11 ± 0.26 ab	0.87 ± 0.25 bc	0.65 ± 0.19 bc	0.45 ± 0.20 c	0.001
Boron	A. incarnata	Root Length	9.10 ± 0.26 a	9.04 ± 0.47 ab	8.80 ± 0.54 ab	8.27 ± 0.96 ab	6.57 ±1.10 b	0.053
Boron	A. incarnata	Longest Root	13.98 ± 0.625 a	13.55 ± 1.32 ab	12.53± 0.96 ab	10.08 ± 1.21 bc	7.85 ± 1.13 c	0.001
Boron	A. incarnata	Number of Leaves	39.04 ± 4.70 a	22.17 ± 3.71 ab	28.83 ± 5.30 ab	24.75 ± 6.48 ab	10.92 ± 3.20 b	0.002

Means that do not share a letter are significantly different.

APPENDIX D.

ANOVA:

Mean weights in grams, length in centimeters, and number of leaves and sheaths ± standard error of control and three molybdenum treatments (Mo applied as molybdic acid disodium salt dihydrate) for *A. incarnata* and *C. aquatilis*.

Nutrient	Plant Species	Measurement	Control	Treatment 1 Mo = 2.5 mg/L	Treatment 2 5 mg/L	Treatment 3 7.5 mg/L	P-Value
Molybdenum	A. incarnata	Root Weight	1.53 ± 0.26 a	0.37 ± .97 bc	0.56 ± 0.11 ab	0.36 ± 0.12 c	0.001
Molybdenum	A. incarnata	Stem Weight	1.82 ± 0.30 a	0.37 ± .08 b	0.58 ± 0.12 ab	0.51 ± .09 b	0.001
Molybdenum	A. incarnata	Total Weight	3.34 ± 0.54 a	0.74 ± 0.17 b	1.15 ± 0.23 ab	0.76 ± 0.20 b	0.001
Molybdenum	A. incarnata	Stem Length	33.33±3.61 a	22.62±1.34 ab	24.32±3.43 ab	15.42±4.07 b	0.005
Molybdenum	A. incarnata	Number of Leaves	39.04 ± 4.70 a	16.08 ± 2.48 b	30.90 ± 6.84 a	11.50 ± 3.12 b	0.001
Molybdenum	C. aquatilis	Number of Sheaths	4.25 ± 1.29 b	4.25 ± 2.13 ab	6.00 ± 2.29 ab	12.33 ± 3.78 a	0.056
Molybdenum	C. aquatilis	Longest Leaf	11.13 ± 1.30 b	12.58 ± 3.34 ab	12.91 ± 2.92 ab	21.95 ± 4.85 a	0.013

Means that do not share a letter are significantly different.

Appendix E.

Mean mg/kg (ppm) of boron uptake for samples of *A. incarnata* and *I. virginica*

Sample	Asc inc Control	Asc inc B1	Asc inc B2	Asc inc B3	Asc inc B4
1	65	211	359	732	444
2	56	98	376	405	418
3	64	229	602	523	420
Mean	61.7^{c}	179.3^{bc}	445.7^{ab}	533.3^{a}	427.3^{ab}

Sample	Iris Control	Iris B2	Iris B4
1	101	430	690
2	119	408	590
3	101	na	1110
Mean	107^{b}	419^{ab}	796.7^{c}

Means that do not share a letter are significantly different.