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RISK CHARACTERIZATION FOR BORON AND AQUATIC PLANTS AND ANIMALS

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RISK CHARACTERIZATION FOR BORON AND AQUATIC PLANTS AND ANIMALS

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Forestry and Natural Resources

by
Basma Damiri
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Accepted by
Dr. John H. Rodgers, Jr., Committee Chair
Dr. James Castle
Dr. Matt Huddleston

ABSTRACT

In aqueous mixtures, boron can be toxic to plants or animals at relatively low concentrations. Boron may occur at concentrations that can pose risk to plants used in constructed wetland treatment systems designed to treat constituents of concerned in a complex matrix such as flue gas desulfurization water and boron may adversely affect survival, growth, and consequently, plant performance. The three major objectives of this research are: 1) to measure responses of *Typha latifolia* (seed germination and root and shoot elongation) and *Schoenoplectus californicus* early seedlings (survival, and shoot and root elongation) to aqueous exposures of boron in diluted FGD water and moderately hard water (MHW); 2) to measure responses of *T. latifolia* and *S. californicus* (mature plants) to aqueous boron concentrations in simulated FGD water; and 3) to measure responses of *Ceriodaphnia dubia* (survival and reproduction) and *Pimephales promelas* (survival) to aqueous boron concentrations in MWW. Boron in the combination of other constituent in FGD water may adversely affect *T. latifolia* and *S. Californicus* early seedling growth and this must considered in planting CWTS for FGD water. *S. californicus* as mature plant was more sensitive, in terms of shoot elongation shoot density to boron exposures in simulated FGD water than *T. latifolia*. Similar to most elements, aqueous boron exposures elicited different responses (sensitivity) from *C. dubia* and *P. promelas. C. dubia* was more sensitive to boron exposures than *P. promelas*. Immature plants were the most sensitive species tested in this study.

DEDICATION

To the most important persons in my life: my parents, sisters, and brothers who are my source of strength and had a dream of seeing me in a graduation gown. Your understanding support and interest encouraged me throughout my journey and made this academic accomplishment possible. Without your efforts, I can not continue.

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CHAPTER 1

INTRODUCTION

Boron is an essential micronutrient for both plants and animals, with interspecies differences in concentrations required for optimum growth (Goldberg, 1997; Nielsen, 1997). However, boron is toxic at low concentrations and the difference between required and toxic concentrations is small. Reviews of current literature indicate that boron toxicity to agricultural plant species is well documented while data regarding boron toxicity to aquatic plant and animal species are limited. The research in this thesis provided a risk-based analysis of the aqueous toxicity of boron to sentinel aquatic plant species, *Typha latifolia* Linneaus (cattail) and *Schoenoplectus californicus* C. A. Meyer Palla (giant bulrush), and sentinel aquatic animal species, *Ceriodaphnia dubia* Richard (water flea) and *Pimephales promelas* Rafinesque (fathead minnow). The data of this research can provide estimates of responses of sentinel species during aqueous exposures of boron and may have the potential to predict remediation goals for waters impacted by elevated boron concentrations.

To date, Water Quality Criteria (WQC) for boron have not been established by the United States Environmental Protection Agency (U.S. EPA) and limits for releasing boron to aquatic systems have not been proposed. To protect aquatic life, WQC have been developed for a variety of elements and compounds (U.S. EPA, 1991) and have been used widely by the National Pollutant Discharge Elimination System (NPDES). WQC are based upon laboratory tests and toxicity data using sentinel plant and animal spices. In order to develop a complete comprehensive toxicity profile for a chemical, data regarding toxicity of the chemical to both sentinel aquatic plants and animals should be examined and compared. In this research, laboratory boron toxicity tests for *T. latifolia*, *S. californicus*, *C. dubia*, and *P. promelas* were conducted and the results for both plants and animals were compared.

Boron is widely distributed at low concentrations in surface waters throughout the world, but in areas associated with human activity relatively high concentrations of boron have been observed. Typical concentrations of boron in North American surface waters are below 0.1 mg L^{-1} (Coughlin, 1998). Boron concentrations in rocks and soils are typically less than 10 mg kg^{-1}. Boron occurs naturally in relatively elevated concentrations in soils (20-300 mg kg^{-1} in the USA with a mean of 30 mg kg^{-1}) and in shale (100 mg kg^{-1}) (Nable et al., 1997). Anthropogenic sources of elevated boron include household cleaners, industrial effluents, fly ash and flue gas desulfurization (FGD) waters (Nable et al., 1997; Weinthal et al., 2005). Standard water treatments are limited and ineffective in their ability to remove a significant amount of boron which results in constraints on discharge of boron-contaminated waters to watersheds or reuse for irrigation (Polat et al., 2004). Phytoremediation is an alternative option to treat contaminated waters.

Aquatic plants are essential components of healthy aquatic ecosystems (Wang, 1991). *T. latifolia* and *S. californicus* are useful sentinel aquatic plant species that have been used in previous toxicity tests (Moore et al., 1999; Muller et al., 2001). Evaluation of *T. latifolia* and *S. californicus* responses to boron is important because of the ecological and economic importance of these plants. They are common wetland species of wide geographical distribution in the continental United States. They play a major role in structural and functional aspects of wetland ecosystems including biogeochemical cycles, food webs, and physiological processes (Adriano et al., 1984; Lombardi et al., 1997). *T. latifolia* and *S. californicus* are an important biotic component of constructed wetlands and have been used to stabilize hydrosoil components and contribute organic matter (Murray-Gulde et al., 2005). They are also used in constructed wetlands to aid in the removal of different contaminants (Sinicrope et al., 1992; Powell et al., 1996; Hawkins et al., 1997; Gillespie et al., 2000; Ye et al., 2003; Murray-Gulde et al., 2005).

Constructed wetlands planted with *T. latifolia* and *S. californicus* may be a viable option for treating contaminated waters, but performance may be dependent on responses of wetland plants to aqueous boron concentrations. FGD waters are produced at coal–fired power plants to decrease sulfur dioxide emissions. This process produces relatively large volumes of water that may contain significantly elevated concentrations of boron (33 to 460 mg B L^{-1}) (Mierzejewski, 1991; Eggert, *in review*). These concentrations may prevent the establishment of vegetation especially in the first growing season. Retarded establishment may occur in wetland and aquatic systems as a result of discharging of these waters without sufficient treatment. The U.S. EPA through the NPDES requires treatment of FGD waters prior to discharge. Water quality criteria have not been established for boron, therefore, boron is not listed as a contaminant requiring monitoring or treatment.

A crucial aspect of choosing aquatic plants for toxicity tests is choosing the stage of life for testing. Some research suggested using seed germination and early seedlings to determine the effects from point and non-point source effluents (Walsh et al., 1991; Wang and Williams, 1988) while others have suggested the use of mature plants (Powell et al., 1996). Germination and the first days of seedling growth can be the most sensitive stage of plant development. Adverse effects due to chemical substances may take place during these phases. Mature plants may withstand higher concentrations of a chemical (Reid et al., 2004). Therefore, it is useful to contrast responses to boron exposures between early life stages and mature plants (Moore et al., 1999).

Ceriodaphnia dubia and *Pimephales promelas* are routinely and extensively used to evaluate the potential toxicity of effluents to receiving aquatic systems and individual chemicals by the U.S. EPA through the NPDES program (Bankston and Baer, 2005; U.S.EPA, 2002). Toxicity tests using *C. dubia* and *P. promelas* can generally provide ecotoxicity data required for environmental protection (Wang, 1991). To the author's knowledge, limited to no published data are available regarding the responses of *C. dubia* and *P. promelas* to boron (Hickey, 1989). For this thesis research, reproduction and survival of *C. dubia* as well as survival of *P. promelas* were measured, and results were compared between species.

Plants are fundamentally different from animals and likely have different sensitivities to exposures. Plants may be under-or overprotected by results from animal tests. Previous research has indicated that plants are more sensitive to some compounds than aquatic animal (Taraldsen and Norberg-King, 1989; Wang and Freemark, 1995). Therefore, toxicity tests for animals alone may not be protective for plant species and are not sufficient to assess the environmental risk of an element or a compound. To protect aquatic life, it is important to compare differences in response of aquatic plant and animal species to the same chemical exposure. The present study not only provides data about boron toxicity to sentinel aquatic plant and animal species, but also provides risk characterization of boron to these aquatic species.

1. Research Objectives

In both plant and animal experiments, boron, in the form of boric acid $B(OH)_3$, was amended with formulated moderately hard water (MHW). In plant experiments, boron was also amended with FGD water. This research had the following objectives:

1. a) to measure responses of *T. latifolia* seed germination and early seedling growth (shoot and root elongation) following 7-d exposures to boron (boric acid) in amended FGD water and formulated moderately hard water (MHW); b) to measure responses of *S. californicus* immature seedlings (survival, and shoot and root growth) following 35-d exposures to boron (boric acid) in amended FGD water and MHW; and c) to compare the responses of *T. latifolia* and *S. californicus* to boron in amended FGD water with responses to boron in MHW.

2. a) to determine responses of *T. latifolia* and *S. californicus* in terms of shoot height, shoot density, number of leaves per plant, length of brown part of the shoot (necrotic tissues) and number of inflorescences produced following 13 months of exposure to a series of boron concentrations (as boric acid) in simulated FGD water, b) to measure concentrations of boron in the hydrosoil and bioconcentration of boron in plant tissue (i.e. shoots and roots) of *T. latifolia* and *S. californicus* after 13 months of exposure to boron (boric acid) in simulated FGD water, c) to determine relationships between responses of

T. latifolia and *S. californicus* to boron exposures and concentrations of boron measured in the roots and shoots of these plants, and d) compare the responses of *T. latifolia* and *S. californicus* following 13 months of exposures to boron (as boric acid) in simulated FGD water.

3. To measure the responses of *C. dubia* and *P. promelas* to aqueous boron exposures in 7-d static/renewal tests. Reproduction and survival of *C. dubia* as well as survival of *P. promelas* were measured as endpoints. No Observed Effect Concentration (NOEC), Lowest Observed Effect Concentration (LOEC) and Lethal Concentration (LC_{50}) values were estimated. Responses of *C. dubia* and *P. promelas* were compared.

Overall, this research provided data regarding responses of sentinel aquatic species to aqueous exposures of boron. These data can contribute to development of a comprehensive profile for boron toxicity to aquatic species and aid in establishing WQC for boron.

2. Organization of the Thesis

This thesis is organized into chapters intended for publication in peer-reviewed journals. Therefore, some of the introductory material and methods are repeated. Chapter 1 introduces the risk associated with releasing boron-contaminated waters and sources of elevated boron concentrations in the environment. Chapter 2, titled "Effects of Aqueous Exposures of Boron on *Typha latifolia* and *Schoenoplectus californicus* Seed Germination and Early Seedling Growth," will be submitted for publication in *Chemosphere*. Chapter 3, titled "Effects of Aqueous Exposures of Boron on Mature Plants of *Typha latifolia* and *Schoenoplectus californicus*" will be submitted for publication in *International Journal of Phytoremediation*. Chapter 4, titled “Aqueous Boron Toxicity to Aquatic Animal Species *Ceriodaphnia dubia* Richard and *Pimephales promelas* Rafinesque,” will be submitted for publication in *Bulletin of Environmental Contamination and Toxicology*. Chapter 5 summarizes the overall results of this research.

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CHAPTER 2

RESPONSES OF *TYPHA LATIFOLIA* LINNEAUS AND *SCHOENOPLECTUS CALIFORNICUS* C. A. MEYER PALLA TO AQUEOUS BORON EXPOSURES

Abstract

Responses of *Typha latifolia* Linnaeus and *Schoenoplectus californicus* C. A. Meyer Palla to aqueous boron exposures can indicate their potential for use in constructed wetland treatment systems to treat flue gas desulfurization (FGD) waters from coal-fired power generation. The objectives of this research were: 1) to measure responses of *T. latifolia* seed germination, and shoot and root growth of early seedlings following 7-d exposures to boron in diluted FGD water and in formulated moderately hard water (MHW) as well as to measure responses of immature *S. californicus* seedlings in terms of survival, shoot and root growth following 35-d exposures to boron concentrations, 2) to compare the responses in diluted FGD water and MHW, and 3) to contrast the responses of *T. latifolia* and *S. californicus* to boron exposures. *T. latifolia* seed germination in FGD water or MHW was not sensitive to boron concentrations of 0.2-100 mg L^{-1} with seed germination of 89.6-100%. Survival of *S. californicus* immature seedlings in FGD water was ≤3.3% at boron concentrations of ≥12.6 mg L^{-1} while in MHW survival ranged from 23 to 96% at boron concentrations 12.6-49.9 mg L^{-1}. Responses of shoot and root growth to boron exposures in FGD water also differed from responses in MHW. No root emergence or elongation was observed for *T. latifolia* early seedlings at all boron concentrations ≥12.6 mg L^{-1} in diluted FGD water and shoots were yellow with lengths ≤5.36 mm. In contrast, root growth decreased with increasing concentrations of boron in MHW for *S. californicus* (≥25.8 mg B L^{-1}) and *T. latifolia* (≥12.6 mg B L^{-1}) with no root growth observed at boron concentrations ≥99.6 mgL^{-1}. The results of this study indicated that boron in conjunction with other constituents in FGD water adversely affect *T latifolia* and *S californicus* early seedling growth and this must be considered in planting constructed wetland treatment systems for FGD waters.

Key Words: Boron, *T. latifolia*, *S. californicus*, seed germination, FGD water, phytotoxicity

1. Introduction

Boron enters surface and ground waters from both natural and anthropogenic sources (Polat et al., 2004). Leaching of rocks and soils, and geothermal releases are major natural sources while release of boron containing products into water through municipal sewage disposal, mining, and electrical power generation are major anthropogenic sources (Nable et al., 1997). Boron has been of recent interest due to elevated concentrations in waters from thermal electric power generation, particularly flue gas desulfurization (FGD) waters (Changwoo and Mistch, 2001; Lamminen et al., 2001). At coal–fired power plants, FGD waters are produced to decrease sulfur dioxide emissions (Mierzejewski, 1991). The amount of water produced by an FGD scrubber can exceed 0.1 mgd at large facilities (>1,000 Mega Watt, MW) and can contain significant concentrations of boron from 33 to 460 mg L^{-1} and other constituents of concern (COC) (e.g. chlorides, mercury, selenium, arsenic and zinc) (Mierzejewski, 1991; Eggert, *in review*). These waters are semi-neutral in pH and contain high total dissolved and suspended solids (calcium, chlorides, magnesium, and sulfate) (Mierzejewski, 1991).

However, FGD waters vary significantly in their compositions (Eggert, *in review*). Treatment of FGD waters is required by the United States Environmental Protection Agency (U.S. EPA) through the National Pollutant Discharge and Elimination System (NPDES) before discharging to receiving systems. Constructed wetland treatment systems have been proposed as a novel treatment approach for efficient and effective treatment of targeted constituents (As, Hg, and Se) found in FGD waters (Ye et. al., 2003). If a treatment approach such as CWTS is considered for FGD waters, elevated chloride concentrations (≥4000 mg L^{-1}) are known to be toxic to wetland plants and must be managed (Eggert, *in review).* To maintain plant health in CWTS, FGD waters are generally diluted to achieve chloride concentrations <4000 mg L^{-1}. However, boron may also occur in the diluted FGD waters at concentrations that pose risks to plants used in CWTS designed to treat constituents of concern.

Typha latifolia Linnaeus (common cattail) and *Schoenoplectus californicus* C. A. Meyer (giant bulrush) have been used in CWTS designed to treat FGD waters (Eggert, *in review*). They play a major role in the structure and function of constructed wetlands affecting biogeochemical cycling and energy flow (Adriano et al., 1984; Lombardi et al., 1997). Wetland plants can also stabilize hydrosoil components, contribute organic matter (Murray-Gulde et al., 2005) and aid in the removal of some contaminants (Sinicrope et al., 1992; Ye et al., 2003). Due to differences in their anatomy and physiology, responses of *T. latifolia* and *S. californicus* to boron exposures may differ.

Seed germination and early seedling are sensitive stages in plant growth, and boron could adversely affect these stages (Boutin et al, 1995). *T. latifolia* propagates vegetatively via rhizomatous growth as well as by seeds (Keane et al., 1999). *T. latifolia* is tolerant of moderate soil salinity and grows rapidly in both brackish and freshwater wetlands (McNaughton, 1968; Dickerman and Wetzel, 1985). Under favorable conditions (light, temperature, and moisture), *T. latifolia* germinates quickly and grows rapidly after germination with significant responses to contaminants in seven days (Moore et al., 1999; Muller et al., 2001). In contrast, *S. californicus* seeds require a longer time to germinate (≥2 weeks). *S. californicus* spreads primarily by vegetative propagation, producing new stems from an extensive system of underground rhizomes, with limited expansion through seed dispersal. *S. californicus* has a moderate tolerance for salinity and is found in fresh and estuarine marsh areas (Howard and Rafferty, 2005). Since the *S. californicus* seedlings used for the immature plant toxicity experiment were much larger at the initiation of exposures than the *T.latifolia* seedlings were at the conclusion of seven days of exposure, and the growth of the immature *S. californicus* seedlings is slow relative to the growth of *T. latifolia* seedlings, we anticipated that immature *S. californicus* seedlings would require more than 7-d to respond to boron exposures.

Bioavailability of boron may be altered in waters with different ionic composition or strength. To date, no data are available regarding the influence of high ionic strength water (e.g. FGD water) or low ionic strength (i.e. formulated moderately hard water [MHW]) on boron toxicity to wetland plant species. The objectives of this research were: 1) to measure *T. latifolia* seed germination and early seedling growth (shoot and root elongation) following 7-d exposures to boron (boric acid) in amended FGD water and formulated MHW; 2) to measure responses of *S. californicus* immature seedlings (survival, and shoot and root growth) following 35-d exposures to boron (boric acid) in amended FGD water and MHW; and 3) to compare the responses of *T. latifolia* and *S. californicus* in boron amended FGD water with responses in MHW.

2. Materials and Methods

2.1. Experimental design

The toxicity test methods used for both *T. latifolia* and *S. californicus* in these experiments were based on previous tests developed for *T. latifolia* (Moore et al., 1999; Muller et al., 2001). In these tests, environmental parameters including 1,500-3,000 (Lux) fluorescent lighting for a 16 h light/8 h dark photoperiod and a temperature of 24 ±1 ^{o}C were used.

2.2. Exposure concentrations

2.2.1. Formulated moderately hard water (MHW)

Boron in the form of boric acid ($B(OH)_3$) (Fisher Scientific, Pittsburgh, PA) was amended to formulated MHW. Boric acid was used for these experiments because it is the dominant form of boron in natural fresh water and the available form for plants (Goldberg, 1997). MHW was prepared at Clemson University from reverse osmosis water and high purity salts of $CaCO_3$, $NaHCO_3$, $MgSO_4 \cdot 7H_2O$, $CaCl_2 \cdot 2H_2O$, KCl, KNO_3, and K_2PO_4, Cu (as CuO_2), Se (as SeO_2), Zn (as ZnO_2) with concentrations of 5.0, 101.8, 48.0, 33.0, 65.0, 2.1, 0.82, 0.02, 0.002, 0.001, 0.001 mg L^{-1}, respectively. Formulated MHW was used for these experiments in order to standardize the ionic strength and composition of the water that would be compared with the FGD water. Nominal concentrations of boron were prepared with MHW and were used for *T. latifolia* and *S. californicus* tests (0.2, 7.5, 10.0, 12.5, 25.0, 50.0, and 100.0 mg B L^{-1}).

2.2.2. Diluted flu gas desulfurization (FGD) water

FGD water was obtained from a coal-fired power plant in North Carolina with an operating wet FGD scrubber. The FGD water was diluted using MHW to maintain a chloride concentration of $\leq$4000 mg L^{-1} and a boron concentration of 12.5 mg L^{-1}. The boron concentration of 12.5 mg L^{-1} was chosen based on range finding tests with *T. latifolia* and boron. To maintain consistent concentrations of other constituents, the diluted FGD water was amended with boron to prepare other exposure concentrations (25, 50, and 100 mg B L^{-1}). Physical and chemical characteristics of boron amended waters were measured according to Standard Methods (APHA, 1998).

2.3. Typha latifolia toxicity tests

Typha latifolia seeds from mature spikes were collected from a wetland in Pickens County, South Carolina (U.S.A.) in January, 2005. Mature spikes containing seeds were placed in a plastic bag, transferred to the laboratory at Clemson University, and stored at 20 ± 1^{o}C until used. The toxicity of boron was measured in 7-d static tests. Seed germination experiments were initiated by adding 15 viable seeds (McNaughton, 1968) to a plastic container with 150 mL of testing solution with three replicates. At the experiment termination, numbers of germinating and non-germinating seeds were counted for each replicate in each testing solution. For this research, germination was defined as observation of a shoot emerging from the seed during the test period. Percent seed germination and mean shoot and root lengths of seedlings were calculated for each measured boron concentration. Growth was estimated by measuring shoot and root length for 10 germinating seeds using a Digimatic Caliper (Model CD- 8” Code No. P 500-352). The instrument repeatability was 0.01 mm and instrument error was 0.02 ± 0.001mm. Three definitive tests were performed for each point estimate (LC_{50}, LOEC, and NOEC) at three treatment concentrations plus untreated moderately hard water as a control.

2.4. Schoenoplectus californicus toxicity test

Immature seedlings of *S. californicus* (with initial shoot length 0.68-2.00 cm and root length 0.28-2.18 cm) were obtained from Horticultural Systems, Inc., Parrish, Florida. The *S. californicus* seedling test was initiated by placing one seedling in a plastic container with 150 mL test solution. Ten plants were used for each treatment. Untreated control and treatments were done in triplicate. To minimize the effect of variable shoot and root lengths at the experiment initiation, plants were selected based on similar shoot and root lengths for each treatment. The plants were initially distributed in this experiment with no significant differences in average shoot and root lengths between the treatments and the controls. The experiment was conducted in 35-d, during which daily observations of plant general health (in terms of shoot growth, and shoot browning and yellowing) were made. The duration of the experiment was based on visual observations of plant health (developing of browning and chlorosis in the shoots). Test solutions were renewed each week to minimize the effect of evapotranspiration. At test termination, numbers of surviving seedlings in each treatment were enumerated. A plant was considered to be alive if at least one green shoot was present and was considered dead if no green shoots were present and brown or chlorotic shoots were present. The number of green, brown, and chlorotic shoots was counted for each live plant. Live plants were also scanned for shoot and root lengths with a WinRhizo root scanner (Regent Instruments Inc., Quebec) to accommodate the number of roots and shoots developed during the exposure period. One toxicity test for *S. californicus* was performed due to difficulties germinating these plants in the laboratory (germination was ≤1%) and a limited number of seedlings were obtained.

2.5. Reference Toxicant Tests

Reference toxicity tests were conducted for *T. latifolia* using copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) (Fisher Scientific, Pittsburgh, PA) as the reference toxicant (U.S EPA, 2002) to provide a positive control for the boron toxicity tests. Since seedlings of *S. californicus* were difficult to obtain, reference toxicity tests were not conducted with this species. For these tests, a series of copper concentrations were prepared with MHW. The copper concentrations for testing were chosen based on previous experiments with *T. latifolia* (Muller et al., 2001)

2.6. Confirmation of Exposures

Experimental boron and copper concentrations were analyzed using Inductively Coupled Plasma Atomic Emission Spectrometer (Spectro Flame Modula; ICP-AES) according to EPA Method 200.7. (U.S. EPA, 1983). Boron and copper concentrations were measured at test initiation and after test solutions were renewed. Exposure concentrations for estimating boron or copper toxicity were calculated from the average measured concentrations of the exposure solutions for each treatment.

2.7. Statistical Analyses

Significant responses to boron exposures in terms of average seed germination and early seedling growth were determined by statistically significant differences relative to untreated controls ($p \leq 0.05$). Comparisons were made among treatments and controls to estimate No Observed Effect concentration (NOEC) and Low Observed Effect Concentration (LOEC) using Statistical Analysis System (SAS Institute Inc., Cary NC, USA). Analysis of variance (ANOVA) with Dunnett's multiple range tests for significant differences relative to controls were performed if assumptions for parametric analyses were met. One way ANOVA on ranks with a Wilcoxon test were performed if assumptions for parametric tests were not met. Statistical procedures followed recommendations of the U.S. EPA (2002) and included the trimmed Spearman-Karber method for calculating the lethal mean concentration (LC_{50}) (Hamilton et al., 1977). The 5% alpha level was used in all statistical tests.

3. Results and Discussions

3.1. *Confirmation of boron exposures in laboratory tests*

The targeted boron concentrations in the laboratory phytotoxicity tests with *T. latifolia* and *S. californicus* were confirmed through analyses using an ICP-AES (Spectro Flame Modula; ICP-AES) (U.S. EPA, 1983). Treatment concentrations for *T. latifolia* tests ranged from 0.2 to 100 mg L^{-1} and measured boron concentrations were 92-125% of nominal concentrations. Treatment concentrations in the *S. californicus* test ranged from 0.2 to 100 mg $L^{-1}$and measured boron concentrations were 97-130% of nominal concentrations.

3.2. *Responses of Typha latifolia to boron and copper exposures*

No effects of boron on *T. latifolia* seed germination in FGD water or MHW were observed under these experimental conditions with boron concentrations ranging from 0.2 to 100.0 mg L^{-1} and seed germination of 89.6-100% (Table 1). Mueller et al. (2001) and Moore et al. (1999) found germination of *T. latifolia* seeds to be relatively insensitive to copper sulfate and atrazine exposures in comparison with growth of roots and shoots of the early seedlings. The toxicity testing procedure used for these experiments involves a step to select viable seeds for initiation of the experiment (McNaughton, 1968) and these seeds germinated within three days after initiation of aqueous boron exposures. Growth of the early *T. latifolia* seedlings after germination differed in FGD water and MHW. At all boron concentrations in FGD water, no root emergence or elongation were observed and shoots were yellow with lengths $\leq$5.36 mm over the 7-d of exposures. In boron exposures in MHW, the NOEC and LOEC for *T. latifolia* shoot elongation were 25.8 and 49.9 mg B L^{-1}, respectively, and for root elongation were 10.4 and 12.6 mg B L^{-1}, respectively (Figure 1). Seedling root growth was affected by 12.6 mg B L^{-1} in MHW and root growth was completely impeded in FGD water, possibly due to boron, but likely due to interaction with other elements or constituents in FGD water (Table 2). Yellowing and depression of growth of *T. latifolia* shoots were not observed in any of the boron treatments in MHW, suggesting that adverse effects on shoot growth observed in the dilute FGD water amended with boron were also likely due to other elements or constituents. The possibility of interactive effects (e.g. synergism) cannot be ruled out by these experiments and will have to be evaluated in future experiments. These experimental waters (diluted FGD and MHW) differed significantly in terms of conductivity and hardness (Tables 3 and 4) as well as other constituents that may influence phytotoxicity (Mierzejewski, 1999; Eggert, *in review*). Results from the reference toxicity tests (Table 5) followed the same patterns previously observed for *T. latifolia* (Muller et al., 2001). Root elongation was sensitive to copper exposures with an NOEC and an LOEC of 14.7 and 46.7 µg Cu L^{-1}, respectively (Figure 2).

3.3. Responses of Schoenoplectus californicus to boron exposures

Immature seedlings of *S. californicus* showed toxicity symptoms (browning and etiolation of shoots) after the first week of exposure in boron amended FGD water and MHW. However, phytotoxicity symptoms developed earlier in *S. californicus* seedlings exposed to boron in dilute FGD water than seedlings exposed to the same boron concentrations in MHW suggesting other factors contributed to the observed toxicity in FGD water (Table 6). Seedling survival in diluted FGD water with a boron concentration of 12.6 mg L^{-1} was ≤3.3%. In MHW amended with boron seedlings survival was ranged from 23-96% and the LC_{50} for boron and *S. californicus* seedlings was 29.2 mg B L^{-1} with a 95% confidence interval of 24.7-34.4 mg B L^{-1}. In MHW, significant adverse effects on shoot growth and root growth were measured at all boron concentrations ≥25.8 mg L^{-1}(Table 7). In MHW amended with boron at a concentration of ≤12.6 mg B L^{-1}, significantly enhanced shoot and root growth of *S. californicus* seedlings was observed relative to untreated controls (0.2 mg B L^{-1}). The NOEC and LOEC were 12.6 mg and 25.8 mg B L^{-1}, respectively, for *S. californicus* response parameters (survival, shoot growth, and root growth) in boron amended MHW.

3.4. Comparison of responses of T. latifolia and S. californicus to aqueous boron exposures

Plant growth rate, boron concentrations, and duration of boron exposure may influence boron toxicity (Powell et al., 1996). In this study *T. latifolia* was more sensitive to boron exposures than *S. californicus.* This could be due to the differences in the age (size) of the two plants, but is likely due also to differences in the plants' physiology. *T. latifolia* has characteristically rapid growth (Powell et al., 1996) and thus quick responses to exposures are expected while *S. californicus* is characterized by moderate growth and a longer exposure duration (35-d) was required to observe responses to boron exposures. At a boron concentration of 12.6 mg L^{-1} and following 7-d of exposure, *T. latifolia* root elongation was adversely affected in MHW and no root elongation was observed in diluted FGD water. Root elongation was enhanced at 12.6 mg B L^{-1} in both MHW and diluted FGD water for *S. californicus*. The LOEC for *S. californicus* root elongation (25.8 mg B L^{-1}) was significantly greater than the LOEC for *T. latifolia* root elongation (12.6 mg L^{-1}). The longer exposure duration (35-d vs. 7-d) and the higher boron concentration required to observe adverse effects in root elongation (25.8 mg B L^{-1} vs. 12.6 mg B L^{-1}) indicated that *S. californicus* is less sensitive than *T. latifolia* to boron exposures even in complex matrices such as FGD waters. Based upon the results from this study, adverse effects of boron on seed germination would not be expected for *T. latifolia* in field situations uncomplicated by other sources of phytotoxicity and seed germination for *S. californicus* is so infrequent (≤1%) that effects of boron would be difficult to discern. Also in this study, *S. californicus* shoots of seedlings exhibit chlorosis and death during

35-d of boron exposures (>25.8 mg B L^{-1}) while the length of shoots of *T. latifolia* seedlings decreased proportional to boron concentrations during 7-d exposures (post germination). Importantly, root growth decreased with increasing concentrations of boron for *S. californicus* (≥25.8 mg B L^{-1}) and *T. latifolia* (12.6 mg B L^{-1}) with no root growth observed at boron concentrations ≥99.6 mg B L^{-1}. For *S. californicus* seedling mortality exceeded 60% at concentrations of boron ≥25.8 mg B L^{-1} and the LC_{50} for *S. californicus* seedlings was 29.2 mg B L^{-1} with a 95% confidence interval of 24.7–34.4 mg B L^{-1}. For crop plants and other plants exposed to boron in field studies, chlorosis, necrosis, and death are commonly reported as boron toxicity symptoms (Butterwick et al., 1989). In field observations of *T. latifolia*, symptoms in response to aqueous and sediment exposures of boron (>6 mg B L^{-1}). Powell (1997) found no correlation between boron concentrations and *T. latifolia* visual appearance during 6 months exposures. Wang (1986) reported that *Lemna minor* frond production (the most sensitive response measured) was unaffected by boron concentrations up to 60 mgL^{-1}, so *T. latifolia* and *S. californicus* root growth is more sensitive than *L. minor* to boron exposures.

Aquatic and wetland invertebrate species commonly used for toxicity testing such as *Chironomus decorus* and *Daphnia magna* had 48-h LC_{50} values of 1, 376 and 141 mg B L^{-1} (boron in the form of sodium tetraborate), respectively (Maier and Knight, 1991) while *Ceriodaphnia dubia* had a 14-d LOEC for reproduction of 18.1 mg B L^{-1} (in the form of boric acid) (Hickey, 1989). Sentinel aquatic vertebrate species such as *Pimephales promelas* had a 7-d LC_{50} value of 81.6 mg B L^{-1} (in the form of boric acid) (Damiri, *chapter 4*). The results of this research indicated that *T. latifolia* and *S.*

californicus are more sensitive to aqueous boron exposures than the relatively sensitive aquatic animal species (*D. magna* and *C. decorus*)

4. Conclusions

Boron concentrations ≥12.6 mg L^{-1} adversely affected root growth of *T. latifolia* early seedlings and *S. californicus* immature plants in MHW and FGD waters (7-d and 35-d exposures, respectively), however, other factors could have contributed to boron toxicity to these plants in FGD waters. Germination of *T. latifolia* seeds did not respond to aqueous boron exposures of 0.2 to 99.6 mg L^{-1} and germination of *S. californicus* was insufficient to measure responses of germination to boron exposures. For *T. latifolia*, shoot growth was affected at 49.9 mg B L^{-1} and at 25.8 mg L^{-1} for *S. californicus*. For *S. californicus*, seedling mortality exceeded 60% at concentrations of boron >25.8 mg L^{-1} and the LC_{50} for *S. californicus* seedlings was 29.2 mg B L^{-1} with a 95% confidence interval of 24.7–34.4 mg B L^{-1}. Seedling survival in diluted FGD water in boron concentrations ≥ 12.6 mg L^{-1} was ≤3.3%. In this study, a 7-d exposure duration was sufficient to measure significant responses in *T. latifolia* root length exposed to boron concentrations ≥12.6 mg L^{-1} in MHW, while 35-d were required for *S. californicus*, possibly due to the slower growth of *S. californicus* relative to the rapid growth of *T. latifolia*. Based on the results of this study, *T. latifolia* seedlings were more sensitive than *S. californicus* immature seedlings to boron exposures. FGD waters contain a variety of constituents that may be phytotoxic, and boron in conjunction with these constituents may adversely affect *T. latifolia* and *S californicus* in constructed wetland treatment systems designed for these waters.

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Table 1 Responses of *T. latifolia* to aqueous boron exposures

Water	B mg L^{-1} Mean (±SEM)*	Seed germination # germ/ total # of seeds	% germination	Mean root length mm (± SEM)	Mean shoot length mm (± SEM)
MHW	0.2 (0.002)	130/135	96.2	18.14 (1.20)	9.00 (0.62)
	7.8 (0.15)	132/135	97.7	19.51 (1.59)	9.29 (0.49)
	10.4 (0.24)	129/135	95.5	17.61 (1.37)	9.18 (0.44)
	12.6 (0.20)	127/135	94.1	15.61 (1.3)	9.40 (0.52)
	25.8 (0.28)	129/135	95.5	12.36 (1.04)	8.90 (0.42)
	49.9 (0.29)	130/135	96.2	5.32 (0.62)	6.12 (0.62)
	99.6 (0.55)	131/135	97	0	4.34 (0.69)
FGD	12.9 (0.24)	132/135	97.9	0	5.36 (0.63)
	25.9 (0.24)	121/135	89.6	0	4.18 (0.45)
	50.1 (0.34)	125/135	92.3	0	3.28 (0.46)
	100.0 (0.37)	124/135	91.9	0	2.91 (0.56)

*Standard Error of Mean

Table 2 Responses of *T. latifolia* to boron and copper exposures and *S. californicus* to boron exposure

Plant	Response Parameters	B mg B L^{-1}		Cu µg Cu L^{-1}	
		NOEC	LOEC	NOEC	LOEC
T. latifolia	Seed germination	NA	NA	NA	NA
	Shoot elongation	25.8	49.9	NA	NA
	Root elongation	10.4	12.6	14.7	46.7
S. californicus	Shoot elongation	12.6	25.8		
	Root elongation	12.6	25.8		
	Survival	12.6	25.8		
	LC_{50}	29.2 95% CI (24.7-34.4)			

Table 3 Chemical and physical characteristics of boron-exposure solutions in formulated moderately hard water (MHW)

Plant species	B mg L^{-1} Mean (±SE M)*	pH S.U Mean (±SE M)	D.O. mg L^{-1} Mean (±SE M)	Conductivity µs Mean (±SE M)	Hardness mg $CaCO_3$ L^{-1} Mean (±SE M)	Alkalinity mg $CaCO_3$ L^{-1} Mean (±SE M)	Temperature °C Mean (±SE M)
T. latifolia	0.2 (0.002)	7.30 (0.06)	9.2 (0.4)	313 (4)	84 (2)	61 (1)	23.4 (0.3)
	10.4 (0.24)	7.46 (0.1)	9.2 (0.1)	308 (4)	80 (2)	57 (2)	23.6 (0.4)
	12.6 (0.20)	7.55 (0.07)	8.5 (0.3)	321 (3)	86 (1)	64 (1)	23.9 (0.5)
	25.8 (0.28)	7.44 (0.07)	8.9 (0.2)	319 (3)	83 (2)	59 (2)	23.5 (0.5)
	49.9 (0.29)	7.64 (0.09)	9.1 (0.2)	318 (3)	81 (2)	61 (1)	23.4 (0.6)
	99.6 (0.55)	7.38 (0.07)	9.0 (0.3)	314 (4)	82 (1)	59 (1)	23.5 (0.3)
S. californicus	0.2 (0.03)	7.50 (0.05)	8.8 (0.2)	310 (5)	81 (1)	59 (1)	23.7 (0.5)
	12.6 (0.20)	7.65 (0.09)	9.1 (0.2)	310 (5)	82 (1)	60 (1)	23.6 (0.5)
	25.8 (0.28)	7.61 (0.08)	9.0 (0.3)	314 (4)	81 (1)	60 (1)	23.7 (0.7)
	49.9 (0.29)	7.46 (0.10)	9.0 (0.2)	308 (4)	81 (1)	60 (1)	23.7 (0.4)
	99.6 (0.55)	7.50 (0.09)	9.0 (0.2)	305 (4)	81 (1)	60 (1)	23.3 (0.4)

Table 4 Chemical and physical characteristics of boron-exposure solutions in flue gas desulfurization (FGD) water

Plant species	B mg L^{-1} Mean (±SEM)*	pH SU Mean (±SEM)	D.O. mg L^{-1} Mean (±SEM)	Conductivity µS Mean (±SEM)	Hardness mg $CaCO_3$ L^{-1} Mean (±SEM)	Alkalinity mg $CaCO_3$ L^{-1} Mean (±SEM)	Temperature °C Mean (± SEM)
T. latifolia	12.9 (0.24)	7.43 (0.06)	8.9 (0.3)	6511 (122)	3142 (27)	61 (1)	23.4 (0.5)
	25.9 (0.24)	7.65 (0.02)	9.2 (0.4)	6399 (152)	3254 (22)	61 (1)	23.5 (0.7)
	50.1 (0.34)	7.69 (0.06)	9.0 (0.3)	6299 (137)	3205 (14)	61 (1)	23.4 (0.4)
	100.0 (0.37)	7.56 (0.08)	9.0 (0.2)	6349 (152)	3301 (35)	60 (1)	23.6 (0.1)
S. californicus	12.9 (0.24)	7.72 (0.07)	8.9 (0.2)	6423 (131)	3138 (24)	61 (1)	23.7 (0.6)
	25.9 (0.24)	7.85 (0.03)	9.0 (0.2)	6433 (140)	3213 (13)	62 (1)	23.6 (0.6)
	50.1 (0.34)	7.79 (0.04)	8.9 (0.2)	6373 (119)	3188 (13)	60 (1)	23.7 (0.1)
	100.0 (0.37)	7.70 (0.06)	9.1 (0.2)	6388 (143)	3225 (25)	59 (1)	23.8 (0.1)

Table 5 Responses of *T. latifolia* to aqueous copper exposures

Cu concentrations (µg L^{-1}) Mean (±SEM)	Seed germination #germ/ total # of seeds	% seed germination	Mean root length mm (± SEM)	Mean shoot length mm (± SEM)
9.8 (1.40)	126/135	93.3	15.27 (0.16)	9.4 (0.16)
14.7 (1.30)	126/135	93.3	15.63 (0.11)	9.39 (0.12)
46.7 (3.20)	135/135	100	12.43 (0.12)	9.71 (0.15)
165.6 (10.4)	123/135	91	3.8 (0.13)	9.49 (0.13)
384 (23.5)	130/135	96.3	0.88 (0.12)	9.75 (0.09)
1060.8 (43.3)	127/135	94.1	0	9.11 (0.1)

Table 6 Visual observations during 35-d exposure of *S. californicus* to boron

Exposure Duration (Week)	Type of water	
	MHW	FGD water
First Week	New shoots and roots developed at all boron concentrations.	New shoots and roots developed at all boron concentrations.
Second Week	At 99.6 mg B L^{-1}, shoot browning observed in 12/30 plants.	At 50 mg B, L^{-1} shoot browning observed in 5/30 plants At 100 mg B/L, shoot browning observed in 17/30 plants.
Third Week	At 49.9 mg B L^{-1}, shoot browning observed in 10/30 plants. At 99.6 mg B L^{-1}, white shoots observed in 10/30 plants and brown shoots observed in 23/30 plants.	At 12.8 and 25.8 mg B L^{-1}, shoot browning observed in 5/30 plants. At 50 and100 mg B L^{-1}, white shoots observed in > 21/30 plants.
Fourth Week	At 25.8 mg B L^{-1}, shoot browning observed in 13/30 plants. At 49.9 and 99.6 mg B L^{-1}, most shoots were brown and some plants had white shoots only.	At 12.7 and 25.8 mg B L^{-1}, white shoots observed in >25/30 plants. At 50, 100mg B L^{-1},100 % of the plants had white shoots.
Fifth Week	At 25.8 and 49.9 mg B L^{-1}, 60 and 77% of plants had only white shoots. At 99.6 mg B L^{-1}, 100% of plants had only white shoots.	Plants had white shoots except one plant at boron concentration 12.7 mg B L^{-1} had green shoots.

Table 7 Responses of immature seedlings of *S. californicus* to aqueous boron exposures

Water	B mg L^{-1} Mean (± SEM)	# survived plants/Total plants	% living plants	Initial mean shoot length cm (Min-Max)	Final mean shoot length cm (Min-Max)	Initial mean root length cm (Min-Max)	Final mean root length cm (Min-Max)
MHW	0.2 (0.002)	24/29[a]	82.8	1.56 (0.98-1.953)	4.91 (2.33-9.63)	1.36 (0.44-1.98)	2.16 (1.00-4.07)
	12.6 (0.20)	29/30	96.7	1.60 (1.00-2.00)	5.52 (1.93-8.86)	1.46 (0.43-2.19)	2.77 (0.86-7.80)
	25.8 (0.28)	12/30	40	1.72 (1.13-1.98)	3.22 (1.77-4.23)	1.63 (0.34-1.98)	1.78 (1.25-4.76)
	49.9 (0.29)	7/30	23.3	1.66 (0.82-2.01)	3.40 (2.23-5.97)	1.47 (0.42-2.33)	1.21 (1.12-3.97)
	99.6 (0.55)	0/30	0	1.72 (1.3-1.98)	0	1.41 (0.36-1.96)	0
FGD	12.9 (0.24)	1/30	3.3	1.61 (1.02-1.95)	2.09	1.45 (0.51-2.07)	2.1
	25.9 (0.24)	0/30	0	1.63 (1.06-1.98)	NA	1.40 (0.14-2.41)	NA
	50.1 (0.34)	0/30	0	1.66 (0.68-1.99)	NA	1.47 (0.49-1.99)	NA
	100.0 (0.37)	0/30	0	1.72 (.0.99-1.99)	NA	1.32 (0.28-1.99)	NA

[a] One plant was broken

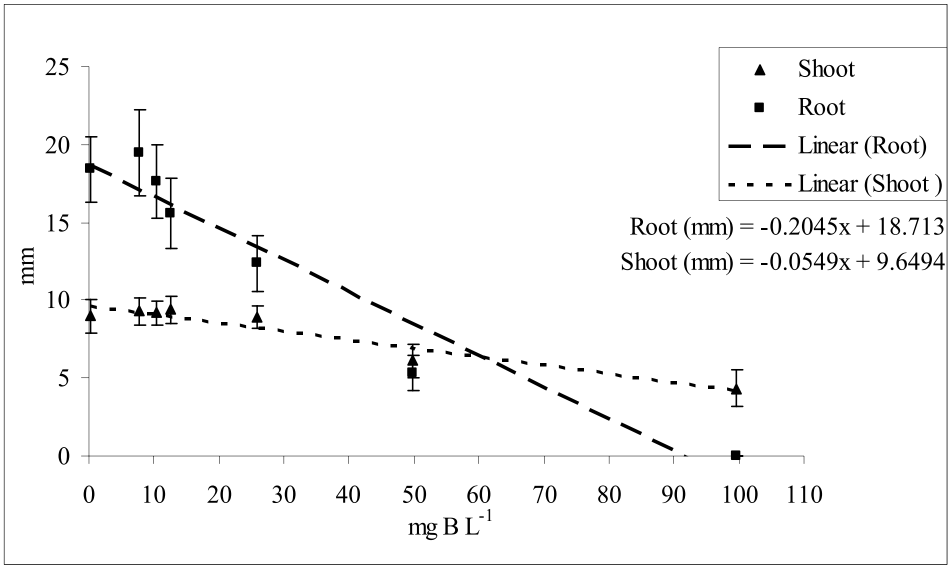

Fig. 1. Boron effects on *T. latifolia* shoot and root lengths

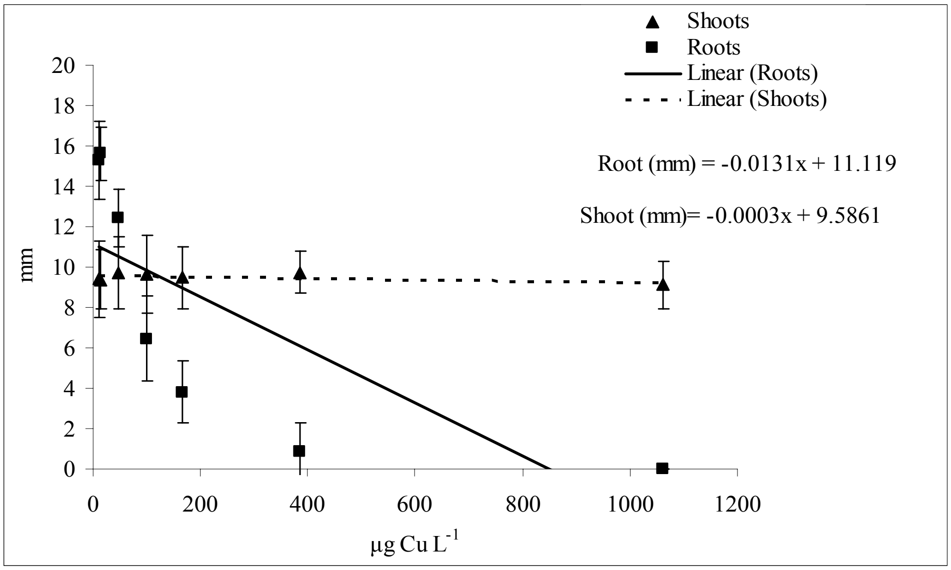

Fig. 2. Copper effects on *T. latifolia* shoot and root lengths

CHAPTER 3

EFFECTS OF AQUEOUS EXPOSURES OF BORON ON MATURE PLANTS OF *TYPHA LATIFOLIA* AND *SCHEONOPLECUTS CALIFORNICUS*

Abstract

Typha latifolia Linnaeus and *Schoenoplectus californicus* C. A. Meyer Palla are used in constructed wetland treatment systems (CWTS) to treat flue gas desulfuriztion (FGD) waters, and boron may be phytotoxic in these waters. The objectives of this research were to determine responses of *T. latifolia* and *S. californicus* to a series of concentrations of boron (as boric acid) in simulated FGD water following 13 months of exposure, to measure concentrations of boron in the hydrosoil and bioconcentration of boron in plant tissue (i.e. shoots and roots), to determine relationships between responses of *T. latifolia* and *S. californicus* to boron exposures and concentrations of boron measured in roots and shoots, and to compare the responses of *T. latifolia* and *S. californicus* to boron exposures. In this experiment, aqueous concentrations of boron greater than 25 mg L^{-1} adversely affected both *T. latifolia* and *S. californicus* with browning and yellowing of shoots and decreased shoot density and height for *S. californicus*. The shoot density of *T. latifolia* decreased by 50% at 300 mg B L^{-1}. In both the shoots and the roots of these species, concentrations of boron increased proportionally with aqueous exposure concentrations as did the concentrations of boron in the hydrosoil. Thus adverse effects of boron were related not only to the aqueous boron exposures, but also to boron concentrations in the hydrosoil, roots and shoots. *S. californicus* was more sensitive than *T. latifolia* to aqueous boron exposures in simulated FGD water with 100%

mortality of *S. californicus* at 300 mg L^{-1} , and shoot density and shoot height at 100 mg B L^{-1} were 43% and 40% less than untreated FGD water controls, respectively.

Key Words: Boron, FGD water, *T. latifolia, S. californicus.*

1. Introduction

Boron phytotoxicity to *Typha latifolia* Linnaeus and *Schoenoplectus californicus* C. A. Meyer Palla used in constructed wetland treatment systems (CWTS) to treat flue gas desulfurization (FGD) waters is a concern due to the functional importance of these wetland plant species and due to the relatively large volumes of FGD waters and elevated boron concentrations in these waters. Selection of plants used in constructed wetland treatment systems for renovation of contaminated waters is a critical step in the design process in order to achieve the targeted performance (Huddleston et al., 2000; Murray-Gulde et al., 2005). *T. latifolia* and *S. californicus* have been used in numerous CWTS designed to treat inorganics (arsenic, cadmium, copper, cyanide, lead, mercury, nitrogen, phosphorous, selenium, and zinc) (Sinicrope et al., 1992; Sobolewski et al.,1996; Hawkins et al., 1997; Ansola et al. , 2003; Huett et al., 2005; Murray-Gulde et al., 2005) and organic compounds (biochemical oxygen demand, chemical oxygen demand, and chlorinated organics) (Moore et al., 2000; Sherrard et al., 2004). In order to understand the functional role of these constructed wetland plants used in phytoremediation of contaminated waters, additional data are needed regarding their environmental requirements and tolerances for constituents contained in complex wastestreams or contaminated waters.

Often constructed wetland treatment systems are designed with emphasis on sequestering toxic constituents of concern in the hydrosoil (Murray-Gulde et al., 2005) or designed based on phytoconcentration of these constituents within plant tissues (Ye et al., 2003). Data regarding the relative partitioning of potentially toxic elements or compounds in plant tissues and to hydrosoil within CWTS can aid in design of these systems as well as understanding the potential toxicity that certain constituents may pose risks to wetland plants used in these treatment systems.

Renovation of waters associated with thermoelectric power generation is needed in order to reuse these waters (e.g. cooling waters) or discharge them into aquatic receiving systems (Mierzejewski, 1991; Eggert, *in review*) due to problematic constituents contained in these waters. With the advent of more stringent air quality standards, flue gas desulfurization technology is being implemented to decrease sulfur dioxide emissions (Lamminen et al., 2001). During coal combustion, flue gases containing sulfur dioxide (SO_2) and other gaseous elements are produced. Some of these gaseous elements and compounds are transferred and transformed into soluble species by wet scrubbing of flue gases (Solan et al., 1999). The resulting water produced by the wet scrubbing of flue gases is referred to as FGD water. The number of these scrubbing systems is increasing within the U.S. and consequently the annual production of FGD waters is increasing.

Constructed wetlands have been suggested for treatment of constituents of concern in FGD waters (Eggert, *in review*). Boron in FGD waters may be phytotoxic to wetland plant species in constructed wetland treatment systems, but potential boron toxicity is dependent on factors such as its concentration, form, frequency of occurrence, and duration of exposure in these waters. FGD waters can also contain elevated concentrations of other potentially phytotoxic constituents including arsenic, cadmium, chemical oxygen demand, chlorides, chromium, copper, lead, mercury, selenium, sulfate, zinc and hardness (e.g. calcium, iron, and magnesium), and are typically semi-neutral in pH (Mierzejewski, 1991). FGD waters vary in their composition and characteristics which are influenced by many factors such as coal type used, source water used in the scrubber, and type of flue gas scrubber employed (Eggert, *in review*). Since these waters vary widely in composition (e.g. boron concentrations), their phytotoxicity may also vary. In order to effectively use *T. latifolia* and *S. californicus* to remediate FGD waters, we need to understand the responses of these plants to exposures of boron in FGD waters.

Since full-scale constructed wetland treatment systems are costly and inefficient for experimental purposes, a smaller scale experimental system is advantageous for assessing the effects of boron in FGD waters on these wetland plants. Studies in small containers can provide crucial information and important benefits such as rigorous testing of hypotheses through replication, control of environmental conditions, and cost effective results. Since FGD waters vary based on site specific operations, simulated FGD waters can be used to control composition of the experimental water, and to provide flexibility to alter constituents of interest such as boron concentrations in these experiments. The objectives of this research were:1) to determine responses of *T. latifolia* and *S. californicus* in terms of shoot height, shoot density, number of leaves per shoot (*T. latifolia*), length of brown shoot (necrotic tissues), and number of inflorescences produced following 13 months of exposure to a series of concentrations of boron (as boric acid) in simulated FGD water, 2) to measure concentrations of boron in the hydrosoil and bioconcentration of boron in plant tissue (i.e. shoots and roots) of *T. latifolia* and *S. californicus* after 13 months of exposures to boron (boric acid) in simulated FGD water, 3) to determine relationships between responses of *T. latifolia* and *S. californicus* to boron exposures and concentrations of boron measured in the roots and shoots of these plants, and 4) to compare the responses of *T. latifolia* and *S. californicus* following 13 months of exposures to boron (as boric acid) in simulated FGD water.

2. Materials and Methods

25.1. Exposure waters

Simulated FGD water was formulated by amending city water (Clemson, SC) with high-purity salts (Fisher Scientific, Inc) containing constituents of concern (Hg, Se, and As), technical grade salts of magnesium and calcium chloride, calcium sulfate, fly ash, and dibasic acid (Table 1). Simulated FGD water was formulated based on data from chemical analyses of actual FGD waters (Eggert, *in review*). For *T. latifolia* and *S. californicus* experiments, treatments were prepared by amending simulated FGD water with boric acid salts to achieve nominal boron exposure concentrations of 15, 50, 100, and 300 mg L^{-1} and 25, 50, 100, 300, 600 mg L^{-1}, respectively. In both the *T. latifolia* and *S. californicus* tests, two controls were used: an unamended simulated FGD water [no boron added (CFGD)], and a low ionic strength control consisting of city water (C).

2.2. Experimental design

Bucket-scale experiments were conducted in controlled green house with a 16/8 light-dark photoperiod and temperature of 27 ± 3 ºC using *T. latifolia* (mature plants) and *S. californicus* plants (>1.5 years old and <126 cm). The hydrosoil used in these experiments was collected from a full-scale constructed wetland treatment system designed to treat FGD waters. Hydrosoil composition was dominated by clay (>70%) with sand, silt, and organic matter accounting for 23%, 5%, and 2%, respectively (Black, 1986). Five-gallon plastic (HDPE) buckets were filled to approximately 24 cm with hydrosoil and planted with five plants of *T. latifolia* or *S. californicus* per bucket. Three replicates were used per treatment. The plants were collected from a wetland site in Catawba County, NC. Both plant species were allowed to acclimate in the buckets and were supplied city water (8L per bucket) for a period of one and a half months before initiation of boron treatments. Osmocote ® time-release fertilizer (2.5 grams, 14-14-14) and 8-liters of simulated FGD water amended with boron were added to each bucket in the initiation of the experiment. To correct for effects of evapotranspiration and altered ionic strength of the exposure waters, city water was added to each bucket as needed. Exposure waters containing boron were renewed when the ionic strength decreased to less than 7 mS/cm. Boron concentrations in the overlying water were measured monthly and maintained at nominal treatment concentrations by addition of boric acid salts as needed. Water chemistry parameters (pH, Temperature, DO, EC, hardness, and alkalinity) were analyzed according to Standard Methods (APHA, 1998).

2.3. Response measurements

Shoot density, shoot or leaf height and shoot or leaf appearance, live (green or senescent shoot or leaf), or dead (totally brown shoot or leaf), and the length of the brown portion of the shoot (necrotic tissues), were measured 3 times during the 13 months of the experiment. For this experiment, a shoot was considered to be the emergent portion of the plant from the hydrosoil.

2.4. Plant and soil analyses

Soil samples were collected for analysis at termination of the experiment (after 13 months). Each soil sample was divided into two segments [surface (~12 cm deep) and subsurface soil (~12-24 cm deep)], and analyzed for pH and boron concentrations using a hydrochloric acid extraction method (Method 6010), (U.S.EPA, 1986). For plant analysis, one ramet was chosen randomly from each bucket and was rinsed thoroughly with Milli-Q water. The rhizome with the associated roots was separated from the shoots, and both segments were analyzed for boron using a dry ash method (Method 6010) (U.S. EPA, 1986). Boron was measured in *S. californicus* shoots that were brown for comparison with concentrations in green shoots. The targeted aqueous boron concentrations in phytotoxicity tests with *T. latifolia* and *S. californicus* were confirmed through analyses using Inductively Coupled Plasma Atomic Emission Spectrometer (Spectro Flame Modula; ICP-AES) according to EPA Method 200.7. (U.S. EPA, 1983).

2.5. Statistical Analyses

Significant responses to boron exposures in terms of length or height of shoot and number of shoots were determined by statistically significant differences relative to untreated controls ($p \leq 0.05$) using Statistical Analysis System (SAS Institute Inc., Cary NC, USA). Analysis of variance (ANOVA) with Dunnett's multiple range tests for significant differences relative to controls was performed if assumptions for parametric analyses were met. One way ANOVA on ranks with Wilcoxon test was performed if assumptions for parametric tests were not met. Linear regression analysis (General Linear Model (GLM)) used to quantify differences in boron concentrations in the soil and plant tissues of both *T. latifolia* and *S. californicus* was performed using SAS. The 5% alpha level was used in all statistical tests.

3. Results and Discussion

3.1. Confirmation of boron exposures

Boron treatment concentrations for *T. latifolia* tests ranged from 0.2 to 300 and measured boron concentrations were 92-126% of nominal concentrations. Treatment concentrations for the *S. californicus* test ranged from 0.2 to 600 and measured boron concentrations were 95-134% of nominal concentrations (Tables 2 and 3). Physical and chemical properties of exposure boron waters for *T. latifolia* and *S. californicus* are presented in Tables 4 and 5.

3.2. Plant responses

Symptoms of boron toxicity to *T. latifolia* and *S. californicus* developed progressively during the duration of the experiment (13 months) and were proportional to boron exposures. Although heights of *T. latifolia* leaves, and number of leaves were not adversely affected at boron exposure concentrations $\leq$300 mg L^{-1}, shoot density was significantly adversely affected at 300 mg B L^{-1} (Figures 1 and 2). For *T. latifolia*, necrotic tissue appeared in the tips of older leaves as spotting and yellowing during the first two weeks of exposure and progressed during the first two months in boron treatments ≥300 mg L^{-1}. However, necrotic tissue developed in all boron treatments following 4-months of exposure, but necrotic tissue increased significantly (100-200%, relative to the control) at boron concentrations of ≥50 mg L^{-1} (Table 6). These are typical boron phytotoxicity symptoms (Blevins and Lukaszewski, 1998). Following 13 months of exposure, the remaining green leaves (living tissue) were young leaves (totally green leaves <20cm) which were not exposed to boron for sufficient duration to manifest apparent adverse effects, mostly in boron treatments ≤100 mg L^{-1}. Totally brown leaves were the older leaves (≤180 cm). *T. latifolia* did not produce any inflorescences even in the controls during the 13 months of this experiment.

Schoenoplectus californicus shoot height, shoot density, and inflorescence production significantly decreased with increasing boron exposure concentrations ≥100 mg L^{-1} (Figure 3 and 4). For *S. californicus*, necrotic tissue appeared in the tips of older leaves, and spotting and yellowing progressed into the shoot. Symptoms of boron phytotoxicity developed progressively during the 13 months of the experiment and the intensity of the symptoms increased with increasing boron concentrations. The brown portion of the shoot (necrotic tissues) increased significantly (200-300%) at boron concentrations of ≥50 mg L^{-1} (Table 7). Shoots without necrosis (totally green leaves <17cm) most probably were young shoots which were not exposed to boron for sufficient duration to manifest symptoms of phytotoxicity. At boron concentrations ≥300 mg L^{-1}, only totally brown shoots (necrotic tissue) were observed. The number of inflorescences was negatively affected by increasing boron concentrations and production of inflorescences completely ceased at boron concentrations ≥300 mg L^{-1} during the13-month exposures to boron (Figure 4).

3.3. Boron concentrations in the hydrosoil and plant tissues

3.3.1. Boron concentrations in the hydrosoil

The results of this study indicated that boron concentrations in the surface soil (~12 cm) were not significantly different from boron concentrations in the subsurface soil (~12-24 cm) (Tables 2 and 3). Boron concentrations in the hydrosoil were proportional (99-105 %) to boron concentrations in exposure waters for all *T. latifolia* and *S. californicus* treatments ≤ 100mg L^{-1}. Hydrosoil boron concentrations in the *T. latifolia* and *S. californicus* treatments of 300 and 600 mg L^{-1} were not proportional to aqueous boron concentrations (74 and 42 %, respectively) indicating that the hydrosoil adsorption capacity was exceeded at boron treatments ≥300 mg L^{-1}.

3.3.2. Boron concentrations in plant tissues

Boron concentrations in *T. latifolia* and *S. californicus* roots and shoots increased with increasing aqueous boron exposure concentrations (Figures 4 and 5). However, the shoots had higher boron bioconcentrations than the roots. For instance, *T. latifolia* shoots had boron concentrations 2-4 times greater than the roots (Table 2). In *S. californicus* treatments of ≤100 mg B L^{-1}, boron concentrations in the brown shoots (necrotic tissue) were 15-39 times more than boron concentrations in the roots (Table 3), while in treatments of ≥300 mg B L^{-1}, boron concentrations were 1-3 times higher in the brown shoots than the roots (Figure 6).

3.4. Correlation between plant responses to boron exposures and boron concentrations in the hydrosoil and plant tissues

Length of shoot browning (necrotic tissues) observed in *T. latifolia* and *S. californicus* shoots significantly increased with increasing boron exposures and consequently, with increasing boron concentrations in the shoots and the roots indicating that these symptoms were developed due to boron exposures. In comparison with controls, *T. latifolia* had the greatest production of necrotic tissue and brown shoot length (3.7 times), and plant density was significantly affected (50%) at a boron concentration of 300 mg L^{-1}. For *S. californicus* treatments ≤100 mg L^{-1}, significant decreases in shoot density (≤43%), shoot height (≤40%), and inflorescence production (≤90%) were observed, however, significant boron bioconcentration was also observed in the roots and the shoots at these relatively low concentrations. For boron concentrations of ≥300 mg L^{-1}, totally brown shoots (necrotic shoots) and no production of inflorescences were observed. At these higher concentrations of boron, significant decreases (28-58%) in boron concentrations in the hydrosoil in proportional to exposure boron concentrations were also observed indicating that the hydrosoil adsorption capacity may be exceeded.

3.5. Differences between T. latifolia and S. californicus responses

Typha latifolia and *Schoenoplectus californicus* differ in their responses and tolerance of boron exposures, as well as bioconcentration potential for boron in simulated FGD water. Boron concentrations in *T. latifolia* hydrosoil and roots did not differ significantly from boron concentrations in *S. californicus* hydrosoil and roots (Figure 7 and 8). However, *S. californicus* had significantly more boron in its shoots than *T.*

latifolia (Figure 9). Therefore, differences in the responses of *T. latifolia* and *S. californicus* to boron exposures could be related to significant differences in boron bioconcentration in shoots. Necrotic tissue increased significantly with increasing boron concentrations in shoots. For the duration of this experiment, *T. latifolia* withstood (green and senesced shoots were present) boron concentrations of 300 mg B L^{-1} with significant adverse effects on shoot density, while *S. californicus* did not withstand (totally brown shoots) boron concentrations ≥300 mg L^{-1}. *T. latifolia* shoot density, leaf height, and number of leaves per shoot were not adversely affected at boron concentrations ≤100 mg B L^{-1}. In contrast, *S. californicus* shoot density and height were adversely affected at boron concentrations of ≥100 mg L^{-1}(Table 8).

The wide variance in responses of plants species to aqueous exposures of boron was reviewed by Blevins and Lakaszewski (1998). The effects of boron on a range of plants from jack pine (*Pinus banksiana*) to duckweed (*Spirodella polyrrhiza*) have been evaluated in careful laboratory studies (Apostol and Zwiazek, 2004; Davis et al., 2002). After 6 weeks of exposure, chlorosis and necrosis related to boron exposure was observed in jack pine (Apostol and Zwiazek, 2004). Duckweed was relatively sensitive with a 10-d EC_{50} of about 18-22 mg B L^{-1} (Davis et al., 2002). In the present study, mature plants of *T. latifolia* and *S. californicus* responded to concentrations of boron at ≥50 mg L^{-1}. Powell et al. (1996) reported that *T. latifolia* accumulated relatively little boron but developed chlorosis in four weeks of exposure in laboratory bioassays. In a field investigation, Powell et al. (1997) found no correlation between tissue concentrations of boron in *T. latifolia* and plant health; however boron concentrations in this study were not sufficient to cause severe adverse effects. In the present study, plant tissue concentrations of boron were strongly related to observed adverse effects for *S. californicus* but less strongly related for *T. latifolia*. In a treatment wetland microcosm study of coal gasification wastewater containing boron, Ye et al. (2003) found that most (57%) of the boron accumulated in the sediment with relatively little in the plants and growth of *T. latifolia* was not affected by this complex mixture (coal gasification wastewater) over the 54-d study. The data in the present study also indicate the need for careful selection of wetland plants for remediation of contaminated waters containing multiple constituents of concern. Prior evaluations are useful for selection of compatible plants for treatment systems for complex matrices such as FGD waters that may contain phytotoxic concentrations of boron, especially if other constituents have the capacity to interact synergistically.

4. Conclusions

The results of this study demonstrated that boron toxicity is manifested over time in *T. latifolia* and *S. californicus* with signs of toxicity increasing with the duration of exposure. These results indicated that boron in FGD waters can adversely affect *T. latifolia* and *S. californicus* (necrosis) at boron concentrations >25 mg L^{-1}. In this study, *S. californicus* shoot height and density were negatively affected at boron concentrations of ≥100 mg L^{-1} while *T. latifolia* shoot density alone was adversely affected at boron concentrations of ≥300 mg L^{-1}. For both plants, boron concentrations in hydrosoil were proportional to aqueous boron exposures and were significantly less than boron concentrations in the roots. Boron was bioconcentrated in the shoots, specifically the tips.

Boron concentrations in the shoots are correlated with plant appearance, necrosis and shoot density for both *T. latifolia* and *S. californicus* and shoot height and inflorescence production for *S. californicus*. In FGD water, boron toxicity symptoms to *T. latifolia* include necrosis and decreased shoot density and for *S. californicus*, include necrosis, decreased shoot density, shoot height, and inflorescence production. The results of this study indicated that *S. californicus* was more sensitive to boron exposures in FGD water than *T. latifolia.* In constructed wetland treatment systems used to treat FGD waters, *T. latifolia* and *S. californicus* can be used when boron concentrations in these waters are maintained at ≤ 25 mg L^{-1}.

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Table 1 Salts used to prepare simulated FGD water

Cl	4000 mg L^{-1} 1:1 $CaCl_2 : MgCl_2$
SO_4	1500 mg L^{-1} $CaSO_4$ (gypsum)
Hg	0.030 mg L^{-1} $Hg(NO_3)_2 \cdot H_2O$
Se	2 mg L^{-1} Na_2SeO_4
As	0.250 mg L^{-1} Na_2AsO_3
DBA	0.1 ml L^{-1}
Fly ash	100 mg L^{-1}
Well water is the diluted water	

Table 2 Mean boron concentrations in exposure water, hydrosoil, shoots, and roots of *T. latifolia*

Treatment	Mean B mg L^{-1} Exposure water (±SD)	Mean B mg kg^{-1} Soil surface (±SD)	Mean B mg kg^{-1} Soil subsurface (±SD)	Mean B mg kg^{-1} Roots (±SD)	Mean B mg kg^{-1} leaves (±SD)
C*	0.2 (0.06)	7.3 (6.4)	2.0 1.8	15.3 (7.0)	80.3 (16.3)
C FGD**	2.9 (0.44)	5.9 (1.1)	5.2 2.6	38.0 (39.2)	73.7 (52.6)
15	14.6 (0.91)	19.3 (1.0)	15.1 0.81	75.0 (67.4)	117.7 (62.3)
50	47.9 (3.54)	49.4 (4.3)	48.4 4.2	82.0 (11.53)	299.0 (59.9)
100	97.4 (6.71)	100.1 (6.8)	94.5 6.6	168.0 (30.5)	630.3 (110.2)
300	290.9 (16.4)	247.5 (8.6)	251.4 32.6	832.0 (565.3)	1694.7 (730.9)

- *City water control
- **FGD water control

Table 3 Mean boron concentrations exposure water, hydrosoil, shoots, and roots of *S. californicus*

Treatments	Mean B mg L^{-1} Exposure water (±SD)	Mean B mg kg^{-1} Soil Surface (±SD)	Mean B mg kg^{-1} Soil Subsurface (±SD)	Mean B mg kg^{-1} Roots (±SD)	Mean B mg kg^{-1} Shoot (brown parts (Tips)) (±SD)	Mean B mg kg^{-1} Shoot (green parts) (±SD)
C	0.1 (0.04)	1.8 (2.5)	2.3 (3.5)	8.7 (9.8)	96 (24.0)	14 7.1
CFGD	3.1 (0.64)	5.2 (1.1)	4.7 (0.8)	8 (2.6)	338.5 (12.0)	39.5 14.8
25	24.9 (1.41)	26.1 (1.3)	26.3 (3.7)	45.3 (18.9)	934 (215.0)	124 72.1
50	48.7 (2.52)	48.3 (5.4)	49.8 (2.5)	60.7 (5.5)	925 (141.4)	264 42.2
100	99.2 (5.96)	100.4 (28.5)	88.8 (8.3)	142.3 (19.5)	5594 0	560 0
300	296.5 (14.15)	218.4 (9.6)	238.2 (18.4)	696.7 (77.5)	2000.5 (450.4)	*
600	592.5 (38.1)	246.5 (41.1)	330.3 (97)	951.7 (106.7)	1748 (280.01)	*

* No green parts

Table 4 Physical and chemical properties *T. latifolia* boron treatments in simulated FGD water

Water characteristics	Boron Treatments					
	C	C FGD	15	50	100	300
pH (SU)	7.2-8.64	6.84-7.83	7.78-7.88	6.94-7.75	6.71-7.83	7.33-7.8
EC µs	499-686	6,65-9,190	9280-9880	6520-8990	6180-9190	6710-9360
DO %	45.7-120.4	77.2-80.9	93.7-95.2	73.70-83.80	47.2-77.2	59.2-73.4
Hardness mg/L as $CaCO_3$	82	5300	5700	5700	6000	6000
Alkalinity mg/L as $CaCO_3$	60	40	42	150	134	148

Table 5 Physical and chemical properties of *S. californicus* boron treatments in simulated FGD water

Water characteristics	Boron Treatments						
	NC	C FGD	25	50	100	300	600
pH (SU)	6.54-6.94	6.64-7.04	6.72-7.2	6.92-7.36	7.15-7.81	7.39-7.54	7.35-7.56
EC µs	250.6-549	6690-8990	6470-8950	6720-9200	6950-9380	6840-9710	6590-9610
DO %	57.60-84.30	60.5-81.6	68.2-81.6	68.8-84.8	71.1-78.1	65.8-73.9	44.7-67.1
Hardness mg L^{-1} as $CaCO_3$	76	6400	5600	5900	5300	6000	5700
Alkalinity mg L^{-1} as $CaCO_3$	56	38	44	94	108	122	140

Table 6 Response measurements of *T. latifolia* to boron exposures in simulated FGD water

Treatments	Mean leaf height (cm) (± SD)	Brown shoot length following 1.5 months exposure (cm) (Range)	Brown shoot length following 13 months exposure (cm) (Range)	Mean number of plants (± SD)	Mean number of leaves (± SD)
C	96 (31.7)	1 (0-9)	17 (0-23)	3.75 (0.50)	3.3 (1.45)
CFGD	81 (40.9)	3 (0-17)	28 (0-43)	4.00 (0.58)	2.3 (1.15)
15	94 (27.3)	2 (0-9)	34 (0-67)	5.00 (0.51)	3.1 (1.2)
50	98 (21.2)	1 (0-6)	45 (17-67)	6.75 (0.5)	2.5 (1.1)
100	102 (30.8)	2 (0-10)	47 (23-72)	4 (1.83)	2.5 (1.66)
300	102 (29.0)	4 (0-30)	67 (45-84)	2.00 (2.08)	2.6 (1.06)

Table 7 Response measurements of *S. californicus* to boron exposures in simulated FGD water

Boron Treatments	Mean shoot heights (cm) (± SD)	Mean brown shoot length following 1.5 months exposure (cm) (Range)	Mean brown shoot length following 13 months exposure (cm) (Range)	Mean number of shoots (± SD)	Mean number of inflorescence (± SD)
C	108 (38.4)	3 (0-11)	26 (0-72)	16 (5.7)	8 (0.7)
CFGD	104 (29.7)	8 (0-28)	26 (0-67)	22 (1.4)	10 (2.1)
25	96 (22.2)	1 (0-8)	42 (0-96)	16.5 (2.1)	7.5 (0.7)
50	71 (19.6)	2 (0-14)	65 (0-117)	20 (1.4)	9 (1.4)
100	62 (16.6)	9 (0-23)	102 (36-154)	12.5 (2.1)	1 (0.7)
300	24 (1.7)	11 (4-46)	Totally brown shoot	0	0
600	0	18 (2-84)	Totally brown shoots	0	0

Table 8 Comparison between *T. latifolia* and *S. californicus*

Treatments	Mean number of shoots (±SD)		Mean Shoot height (cm) (±SD)	
	T. latifolia	*S. californicus*	*T. latifolia*	*S. californicus*
C	3.75 (0.5)	16 (5.7)	96 (32)	108 (38)
CFGD	4 (0.6)	22 (1.4)	81 (41)	104 (30)
50	6.75 (0.5)	20 (1.4)	98 (21)	71 (22)
100	4 (1.8)	12.5 (2.1)	102 (31)	62 (13)
300	2 (2.1)	0.0 (0.0)	102 (29)	0

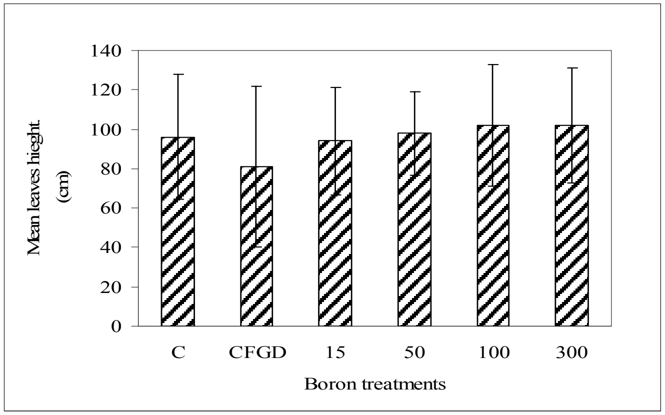

Fig 1. *T. latifolia* shoot height responses to boron exposures in simulated FGD water

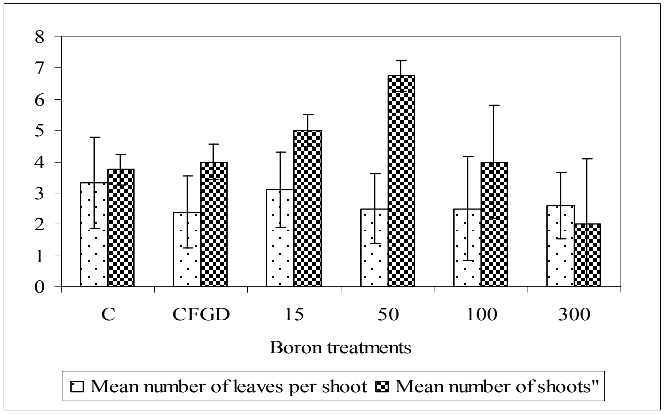

Fig. 2. *T. latifolia* shoot density and number of leaves per shoot responses to boron exposures in simulated FGD water

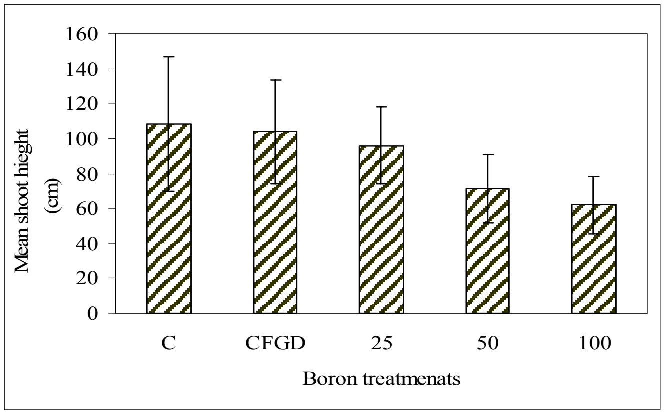

Fig. 3 *S. californicus* shoot height responses to boron exposures in simulated FGD water

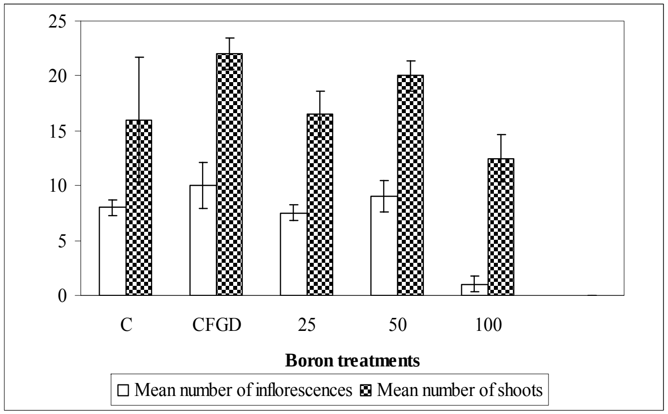

Fig. 4. *S. californicus* shoot density and inflorescence production responses to B simulated FGD water

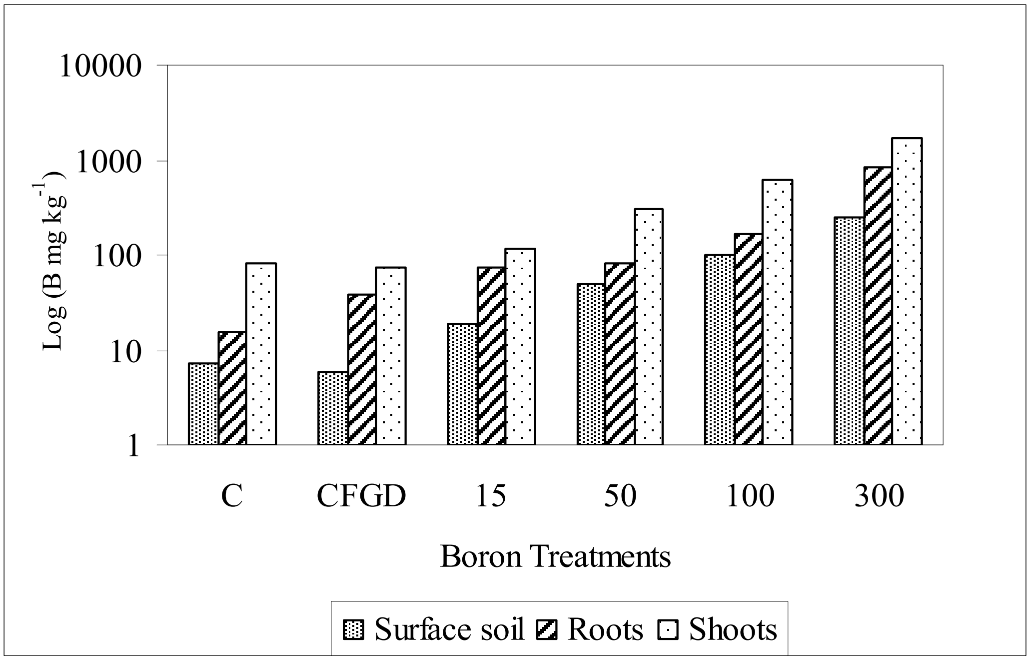

Fig. 5 Difference in boron concentrations in the soil, roots, and shoots of *T. latifolia*

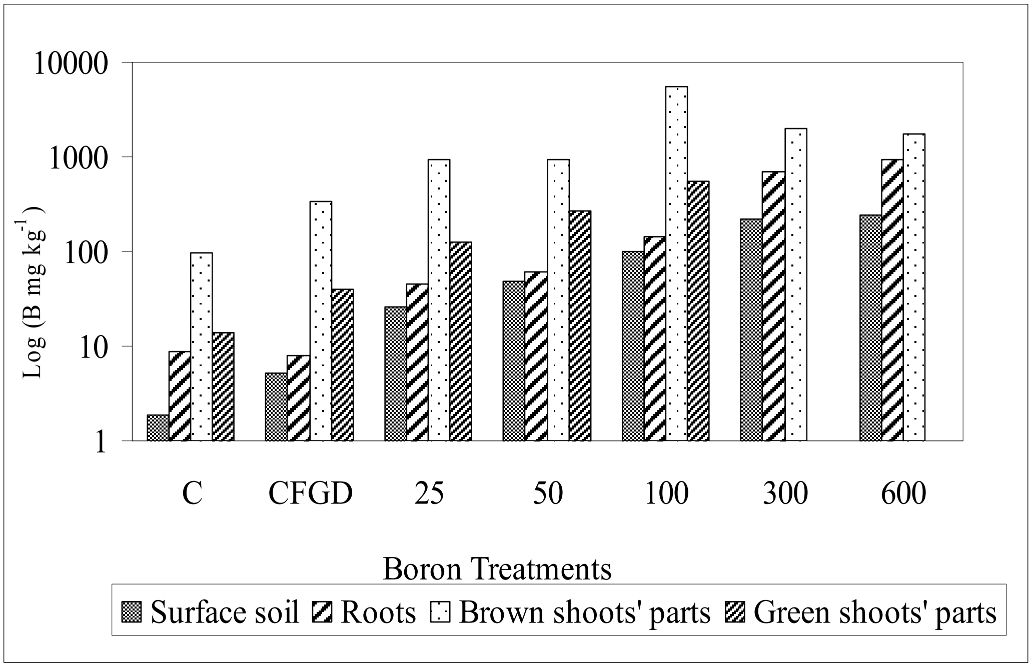

Fig. 6 Differences in boron concentrations in soil, roots, and shoots of *S. californicus*

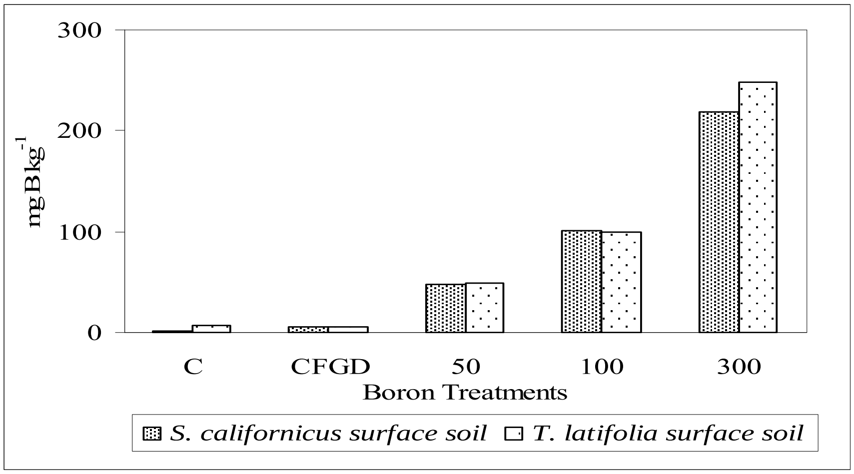

Fig. 7 Comparison between boron concentrations in *T. latifolia* and *S. californicus* hydrosoil

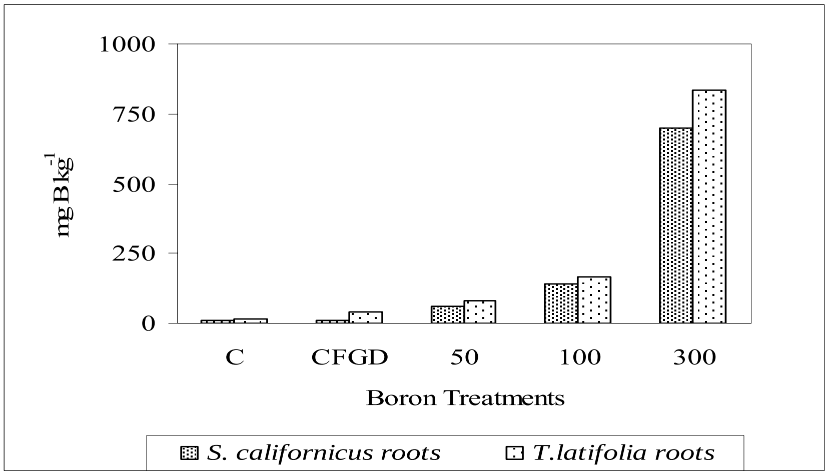

Fig. 8 Comparison between boron concentrations in *T. latifolia* and *S. californicus* roots

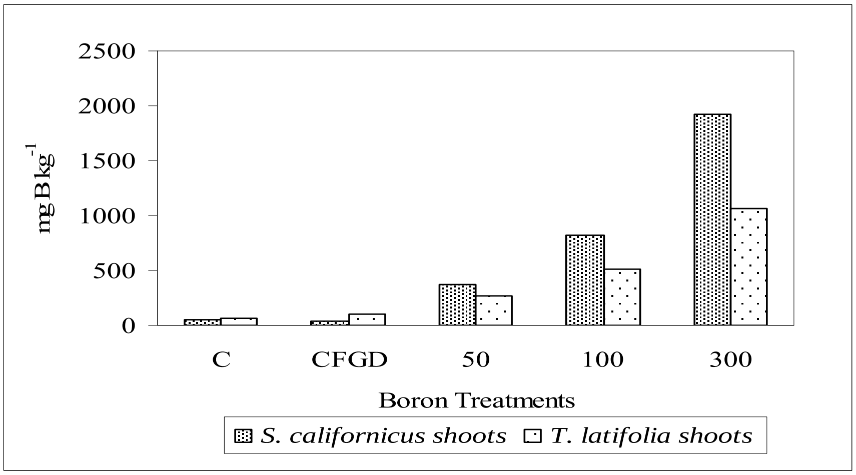

Fig. 9 Comparison between boron concentrations in *T. latifolia* and *S. californicus*

CHAPTER 4

RESPONSES OF *CERIODAPHNIA DUBIA* RICHARD AND *PIMEPHALES PROMELAS* RAFINESQUE TO AQUEOUS BORON EXPOSURES

Abstract

Boron in industrial effluents can be found at concentrations that adversely affect aquatic animals. The objectives of this research were to measure responses [potency, median lethal effect concentrations (LC_{50}s), lowest observed effect concentrations (LOECs), no observed effect concentrations (NOECs)] for *Ceriodaphnia dubia* Richard and *Pimephales promelas* Rafinesque in aqueous boron exposures (in the form of boric acid) and to compare the responses of *C. dubia* to *P. promelas*. Boron toxicity tests were conducted as 7-d static /renewal tests following U.S. testing protocols for *C. dubia* and *P. promelas* (U.S. EPA, 2002). Each test concentration was replicated three times with 30 organisms per treatment and controls. The estimated 7-d aqueous LC_{50} for *C. dubia* was 75.9 mg B L^{-1} with a 95% confidence interval of 74.9-77.0 mg B L^{-1} while the LC_{50} for *P. promelas* was 81.6 mg B L^{-1} with a 95% confidence interval of 77.8-83.7 mg B L^{-1}. NOEC and LOEC values for *C. dubia* reproduction were 16.4 and 17.2 mg B L^{-1}, respectively. No *C. dubia* reproduction was observed at boron concentrations $\geq$38.4 mg B L^{-1}. Similar to most elements, aqueous boron exposures elicited different responses (sensitivity) for *C. dubia* and *P. promelas* with potency slopes of 0.86 and 0.57 (% of mortality/mg B L^{-1}), respectively, concluding that *C. dubia* survival is more sensitive than *P. promelas* to boron.

Key words: *C. dubia*, *P. promelas*, Boron, Toxicity.

1. Introduction

Boron is widely distributed in relatively low concentrations in natural surface waters (Hu and Brown, 1997). Typical boron concentrations in North American surface waters are less than 0.1 mg L^{-1} and rarely exceed 1.0 mg L^{-1} (Coughlin, 1998). Boron enters aquatic environments from both natural and anthropogenic sources. Natural sources of boron include volcanic activity, weathering of clay-rich sedimentary rocks or soils, and intrusion from seawater (Nable et al., 1997). In the United States, U.S., natural high boron concentrations (5-15 mg B L^{-1}) can be found in southwestern U.S as a result of the weathering of boron-rich formations and deposits (Butterwick et al., 1989). Little Warm Spring Creek in California had a boron concentration of 13 mg L^{-1}, and boron concentrations exceed 20 mg B L^{-1} in subsurface agricultural drainage waters in the San Joaquin Valley of California. (Loewengart, 2001). In commerce and industry, boron minerals have several diverse applications such as mining, coal burning for power generation, and production of glass and cleaning products are major anthropogenic sources (Maier and Knight, 1991; Nable et al., 1997). Boron releases to water occur from municipal sewage containing sodium perborates from detergents and in run off from areas using boron-containing herbicide or fertilizers (Butterwick et al., 1989; Nable et al., 1997). Boron concentrations in contaminated water can vary from one source to another. Boron concentrations in flue gas desulfurization water, produced in coal-fired power plants, range from 33 to 460 mg B L^{-1} (Eggert, *in review*), while boron concentrations in typical wastewaters are in the 1.0 mg B L^{-1} (Polat et al., 2004) range. However, the discharge of boron–contaminated water during production and end use of detergents could result in accumulation of boron in waste effluents and consequently in groundwater and natural aquatic systems (Vengosh et al., 1994). Wastewater treatment systems do not effectively remove boron from contaminated water, and insufficiently treated effluents may be discharged into aquatic systems (Polat et al., 2004) which can result in potential risks of boron to aquatic species.

Boron is an essential micronutrient for plants but its essentiality for animals has not yet been established (Nielsen, 1997). Boron enhances maturation and growth of animals and regulates parathyroid function through metabolism of calcium (Nielsen, 1997). Limited studies have been performed to establish reproductive and developmental toxicity, and these studies indicated that when boron and its compounds are applied directly to laboratory animals (chickens and amphibians e.g. *Xenopus laevis* embryos), they could be potent teratogens but there is no evidence of mutagenic or carcinogenic effects (Fort et al., 1998; Rowe et al., 1998). However, the mechanisms of action are not understood.

Boron toxicity to aquatic animals is a function of its form and the species of organism and its life stage (Birge and Black, 1977). Although the predominant and most available form of boron in freshwater ecosystems (pH 6-9) is boric acid ($B(OH)_3$) (Goldberg, 1997), Birge and Black, (1997) conducted many studies to determine the toxicity of borax ($Na_2B_4O_7.10H_2O$) to a variety of organisms. These studies indicated that boric acid and borax toxicity to the same species may differ significantly (Table 1). Eisler (1990) stated that representative species of aquatic organisms (e.g. plants, invertebrates, fish and amphibians) can usually tolerate up to 10 mg B L^{-1} for an extended period (48 h–

28 d) without any adverse effects although >0.1 mg B L^{-1} may affect reproduction of rainbow trout, while >0.2 mg B L^{-1} may affect survival of other fish species (Eilser, 1990). Black et al., (1993) recommended a concentration between 0.75 and 1.0 mg B L^{-1} as an environmentally acceptable limit for boron in aquatic systems. Environmental conditions such as pH, hardness, dissolved oxygen, and temperature may also affect the responses of aquatic organisms to boron toxicity (Birge and Black, 1997).

Table 1 Boron toxicity data for fish as a function of boron form and water characteristics (Birge and Black, 1977)

Test organism	Life stages	Duration	Form of boron used in the test	LC_{50} mg B L^{-1}	
				Soft water 50 mg $CaCO_3$ L^{-1}	Hard water 200 mg $CaCO_3$ L^{-1}
Gold fish (*Carassius auratus*)	Embryos, through day 4 post hatching	7d	Boric acid	46	75
			Borax	65	59
Channel fish (*Ictalurus punctatus*)	Embryos, through day 4 post hatching	9d	Boric acid	155	22
			Borax	155	71
Rainbow trout (*Oncorhynchus kisutch*)	Embryos, through day 4 post hatching	28d	Boric acid	100	79
			Borax	27	54

To protect aquatic life, water quality standards have been developed, based primarily on laboratory toxicity tests that use relatively constant exposure concentrations.

Toxicity tests are used to estimate responses of sentinel aquatic species to contaminants in waste effluents and ambient waters (Phillips et al., 2005).

Due to the potential for species selective toxicity by contaminants, in regulated applications a minimum of two species (vertebrate fish and invertebrate) are used in effluent toxicity tests (Doherty et al., 1999). Sentinel aquatic animal species such as *Ceriodaphnia dubia* Richard and *Pimephales promelas* Rafinesque are common freshwater species required for monitoring effluents discharged to surface freshwaters by National Pollutant Discharge Elimination System (NPDES) (U.S. EPA, 1991). These studies are required by the NPDES permitting process to evaluate potential toxicity of individual chemicals as well as complex mixtures discharged to aquatic systems by industrial or domestic point sources (Bankston and Baer, 2005). *C. dubia*, commonly named 'water fleas,' are freshwater arthropods that occur in littoral areas of lakes, ponds, and marshes throughout the world. *P. promelas*, commonly named 'fathead minnow,' is widely distributed in temperate water throughout North America. *C. dubia* and *P. promelas* are recommended in toxicity tests due to their sensitivity to many contaminants, the existence of extensive published data regarding their responses to different contaminants, and ease of culture and handling in laboratory (Doherty et al., 1999). These data can provide estimates of responses of sentinel toxicity species to aqueous exposures of boron and may be useful to predict remediation goals for boron impacted waters.

This research was conducted to measure responses of *C. dubia* and *P. promelas* to aqueous exposures of boron following 7-d toxicity tests. Specific objectives were to estimate no observed effect concentrations (NOECs), lowest observed effect concentrations (LOECs), median lethal effect concentration (LC_{50}), and to compare the responses of *C. dubia* with the responses of *P. promelas* to aqueous boron exposures.

2. Materials and Methods

2.1. Exposure concentrations and experimental design

For laboratory toxicity tests, boron test solutions were prepared from boric acid ($B(OH)_3$) (Fisher Scientific, Pittsburgh, PA) and reconstituted moderately hard water (MHW), with a hardness of ≈ 80 mg L^{-1} as $CaCO_3$ and an alkalinity of ≈ 60 mg L^{-1} as $CaCO_3$. MHW was prepared at Clemson University from reverse osmosis water and high purity salts of $CaCO_3$, $NaHCO_3$, $MgSO_4 \cdot 7H_2O$, $CaCl_2 \cdot 2H_2O$, KCl, KNO_3, and K_2PO_4, Cu (as CuO_2), Se (as SeO_2), Zn (as ZnO_2) with concentrations of 5.0, 101.8, 48.0, 33.0, 65.0, 2.1, 0.82, 0.02, 0.002, 0.001, 0.001 mg L^{-1}, respectively. Temperature, pH, alkalinity, dissolved oxygen, conductivity and total hardness were measured every day in selected replicates according to Standard Methods (APHA, 1998). Boron concentrations were measured using Inductively Coupled Plasma Atomic Emission Spectrometer (Spectro Flame Modula; ICP-AES) following EPA Method 200.7 with a method detection limit of 10 µg L^{-1}. (U.S. EPA, 1983). Boron concentrations were measured when test solutions were renewed and after 24 hours from selected treatments of each concentration. The final exposure concentrations were calculated as the mean value measured for each treatment concentration. All test organisms (*C. dubia* and *P. promelas*) were cultured based on ASTM (2000) methods and tested at Clemson University. All organisms used for testing were ≤24 h old at the initiation of each experiment. The tests were conducted according to U.S. EPA wet testing guidelines protocols for *C. dubia* and *P. promelas*

(U.S. EPA, 2002). Boron toxicity tests were performed for each species as 7-d static/renewal toxicity tests. Three tests were performed for each point estimate at three concentrations plus reconstituted moderately hard water as a control. For each treatment in each test, thirty organisms were used.

Statistical Analysis

For *C. dubia* and *P. promelas* survival and reproduction endpoints (NOEC and LOEC values), Analysis of Variance (ANOVA) and Dunnett's multiple range tests were used to detect differences between control and treatment means. Each NOEC value was estimated as the highest concentration at which no significant difference was observed compared to control while LOEC was estimated as the lowest observed concentration at which significant difference was observed compared to control for each measured parameter. In both *C. dubia* and *P. promelas* surviving tests, the numbers of survived animals in each treatment were combined into one data set. The averages for each treatment were calculated and these averages were used to test for differences in control versus each treatment level with ANOVA followed by Dunnett's test. For this stage of the analysis, individual experiments were considered replicates. The analysis of reproduction data from *C. dubia* testing was accomplished in two stages. The first stage was to test for differences in control versus each treatment level with ANOVA followed by Dunnett's test. In this stage of the analysis, individual organisms were the replicates. The mean significant differences (MSD) based on Dunnett's test were calculated and shown graphically with the treatment level means. In the second stage of the analysis, the data for *C. dubia* reproduction experiments were combined into one data set. The averages for each treatment were calculated and these averages were used to test for differences in control versus each treatment level with ANOVA followed by Dunnett's test. For this stage of the analysis, individual experiments were considered replicates. Again, the MSD based on Dunnett's test was calculated and shown graphically with the treatment level means. Any mean number of offspring below the critical value for Dunnett's test considered would be significantly different from the control. All statistical calculations were performed using PROC GLM of SAS (SAS Institute Inc., Cary NC, USA). The 5% alpha level was used in all statistical tests. Linear regression analysis was used to quantify the potency of boron to *C. dubia* and *P. promelas*. Lethal mean estimates (LC_{50}) were analyzed using statistical procedures followed U.S. EPA guideline (U.S. EPA, 2002) and included the trimmed Spearman-Karber method (Hamilton et al., 1997) used for calculating median effect statistics (LC_{50}).

3. Results and Discussion

For all toxicity tests, the responses of organisms in controls met the test acceptability criterion of ≥80% survival (U.S. EPA, 2002). In *C .dubia* reproduction tests; the surviving animals reproduced at least three broods in the 7-d toxicity test. Water chemistry parameters for *C. dubia* and *P. promelas* tests (pH, dissolved oxygen, and conductivity) are presented in Table 2.

In these experiments, both *C. dubia* and *P. promelas* responses to aqueous boron exposures showed a general decrease in survival and reproduction with increasing exposure concentrations (Table 3). The 7-d NOEC and LOEC values for *C. dubia* survival were estimated at 67.5 and 71.2 mg B L^{-1}, respectively while the NOEC and

LOEC values for *P. promelas* survival were 51.5 and 61.3 mg B L^{-1}, respectively. The 7-d LC_{50} value for *C. dubia* was 75.9 mg B L^{-1} with a 95% confidence interval of 74.9-77.0 mg B L^{-1} while the *P. promelas* 7-d LC_{50} value was 81.6 mg B L^{-1} with a 95% confidence interval of 77.8-83.7 mg B L^{-1}. Similar to most elements, aqueous boron exposures elicited different responses (sensitivity) for *C. dubia* and *P. promelas* with potency slopes of 0.86 and 0.57 % mortality/ mg B L^{-1}, respectively, indicating that *C. dubia* survival is more sensitive to boron than *P. promelas* (Figure 1 and Figure 2).

The 7-d aqueous NOEC and LOEC values for *C. dubia* reproduction were 16.4 and 17.2 mg B L^{-1}, respectively (Figure 3). No reproduction was observed at boron concentrations ≥38.4 mg B L^{-1}. The estimated NOEC and LOEC for *C. dubia* mortality are approximately 4 fold more than the concentration that adversely affected reproduction, indicating that *C. dubia* reproduction is more sensitive than survival in this experiment.

The results of this study indicated that boron concentrations that adversely affected *C. dubia* and *P. promelas* reproduction and survival (≥ 17.2 mg L^{-1}) are somewhat greater than boron concentrations found in U.S. surface waters (0.01-2.0 mg B L^{-1} with a mean of 0.1mg B L^{-1}). The LOECs are also greater than the levels proposed by U.S. EPA and identified as toxic to aquatic organisms (0.75 mg B L^{-1}) (WHO, 1998). Both the *C. dubia* data and *P. promelas* data corroborated the suggestion that representative species of aquatic organisms such as fish, invertebrates and amphibians can tolerate aqueous exposures of up to 10 mg B L^{-1} without adverse effects (Eisler, 1990). In laboratory waters, *Daphnia magna* exposed to boron (boric acid) for 21 days produced NOECs ranging from 6 to 10 mg B L^{-1} and LOEC of 10-18 mg B L^{-1} (Birge and Black, 1977; Hooftman et al., 2000). In other laboratory studies, 48- hour EC_{50} values for *D. magna* (water hardness 10-170 mg $CaCO_3$ L^{-1}) were between 95 and 226 mg B L^{-1} and generally >100 mg B L^{-1} (Maier and Knight, 1991). For three species of fish exposed to boric acid, Birge and Black (1977) found the range of LC_{50} values was 22-155 mg B L^{-1} (Table 1) for embryos, through day 4 post hatching.

4. Conclusions

Boron concentrations ≥17.2 mg L^{-1} adversely affected reproduction of *C. dubia* and survival of *C. dubia* and *P. promelas*. The NOEC and LOEC values for *C. dubia* reproduction were 16.4 and 17.2 mg B L^{-1}, respectively. No *C. dubia* reproduction was observed at boron concentrations ≥38.4 mg B L^{-1}. The NOEC and LOEC values for *C. dubia* survival were 67.5 and 71.2 mg B L^{-1}, respectively. The estimated 7-d aqueous LC_{50} for *C. dubia* was 75.9 mg B L^{-1} with a 95% confidence interval of 74.9-77.0 mg B L^{-1}. The NOEC and LOEC values for *P. promelas* survival were 51.5 and 61.3 mg B L^{-1}, respectively. The 7-d LC_{50} for *P. promelas* was 81.6 mg B L^{-1} with a 95% confidence interval of 77.8-83.7 mg B L^{-1}. Similar to most elements, aqueous boron exposures elicited different responses (sensitivity) from *C. dubia* and *P. promelas* with potency slopes of 0.86 and 0.57 (% mortality/mg B $L^{-1)}$, respectively, indicating that *C. dubia* is more sensitive (in terms of survival) to boron than *P. promelas*. In the current research, all response measurements (NOEC, LOEC, and LC_{50}) are higher than concentrations of boron normally found in fresh water. Concentrations of boron of sufficient magnitude and duration to produce toxic effects in laboratory tests could be found in aquatic systems receiving industrial or domestic effluents with elevated boron concentrations. Boron toxicity tests were performed for two aquatic species in this study to provide additional data for evaluation of potential impacts of boron concentrations on aquatic organisms which may aid in establishing water quality criteria for boron.

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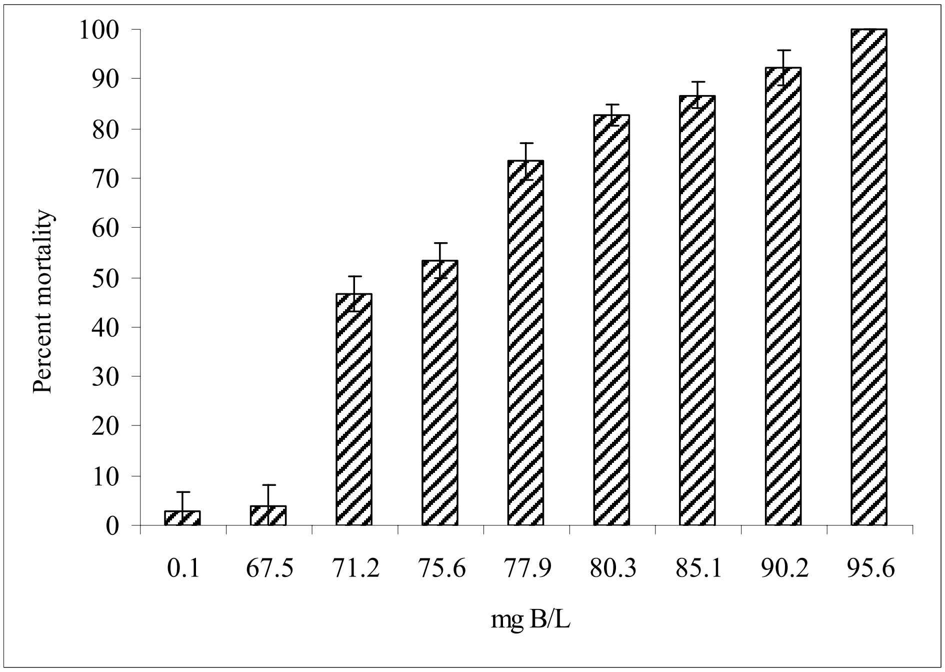
Table 2 Chemical and physical characteristics of aqueous boron-exposure concentrations to *C. dubia* and *P. promelas* during 7-d static/ renewal tests.

Test Species	B $mg\ L^{-1}$ Mean (± SEM)	pH (S.U) Mean (± SEM)	D.O. ($mg\ L^{-1}$) Mean (± SEM)	Conductivity (µS) Mean (± SEM)	Temperature (°C) Mean (± SEM)
C. dubia	0.1 (0.02)	7.38 (0.04)	7.7 (0.3)	330 (4)	24.9 (0.2)
	16.4 (0.33)	7.61 (0.05)	7.4 (0.2)	332 (3)	24.9 (0.1)
	17.2 (0.41))	7.66 (0.05)	7.5 (0.2)	328 (3)	24.9 (0.1)
	18.3 (0.32)	7.73 (0.09)	7.5 (0.2)	327 (4)	24.9 (0.1)
	67.5 (0.24)	7.45 (0.06)	7.6 (0.3)	331 (4)	24.9 (0.1)
	71.2 (0.22)	7.30 (0.09)	7.8 (0.4)	328 (4)	24.9 (0.1)
P. promelas	0.01 (0.03)	7.44 (0.08)	8.8 (0.7)	334 (3)	24.9 (0.1)
	51.5 (0.27)	7.48 (0.07)	8.6 (0.9)	314 (4)	24.9 (0.1)
	61.3 (0.34)	7.62 (0.10)	8.4 (0.9)	308 (3)	24.9 (0.2)
	184.4 (0.33)	7.62 (0.08)	9.1 (0.2)	311 (4)	24.8 (0.2)

Table 3 Effects of boron on *P. promelas* (survival) and *C. dubia* (mortality and reproduction) during 7-d static/renewal tests

		Mean Boron Concentrations mg L^{-1}			
Test Species	Test Endpoint	NOEC ± (SEM)	LOEC ± (SEM)	LC_{50} (95% CI)	EC_{100} ± (SEM)
C. dubia	Reproduction	16.4 (0.33)	17.2 (0.41)	NA	38.4 (0.27)
	Mortality	67.5 (0.24)	71.2 (0.22)	75.97 (74.91-77.04)	95.6 (0.54)
P. promelas	Mortality	51.5 (0.27)	61.3 (0.34)	81.5 (77.8-83.7)	184.2 (0.33)



Fig, 1. Boron effect on *C. dubia* mortality.

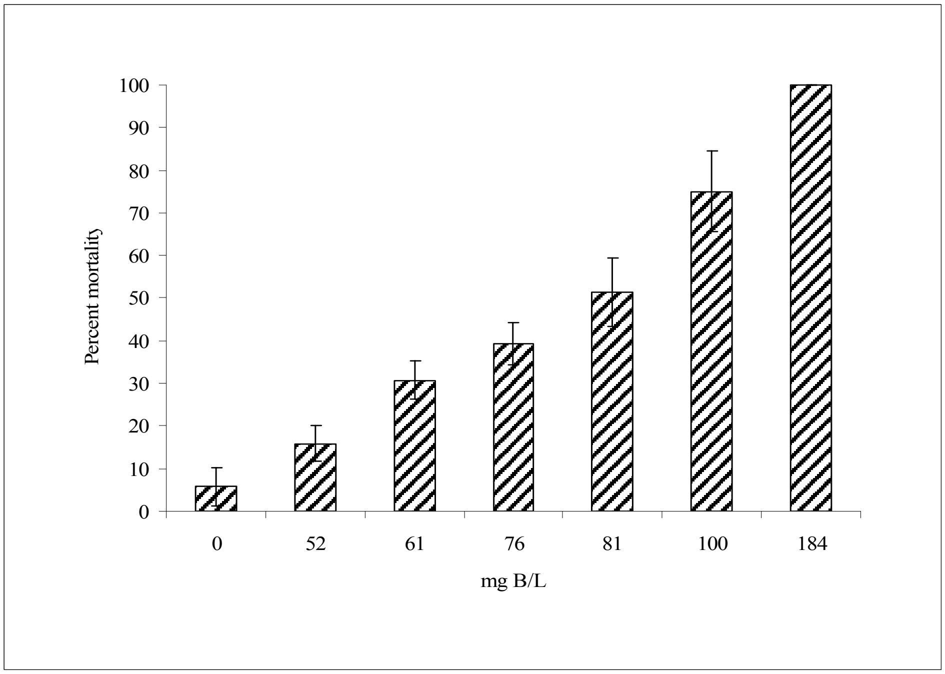


Fig. 2. Boron effect on *P. promelas* mortality.

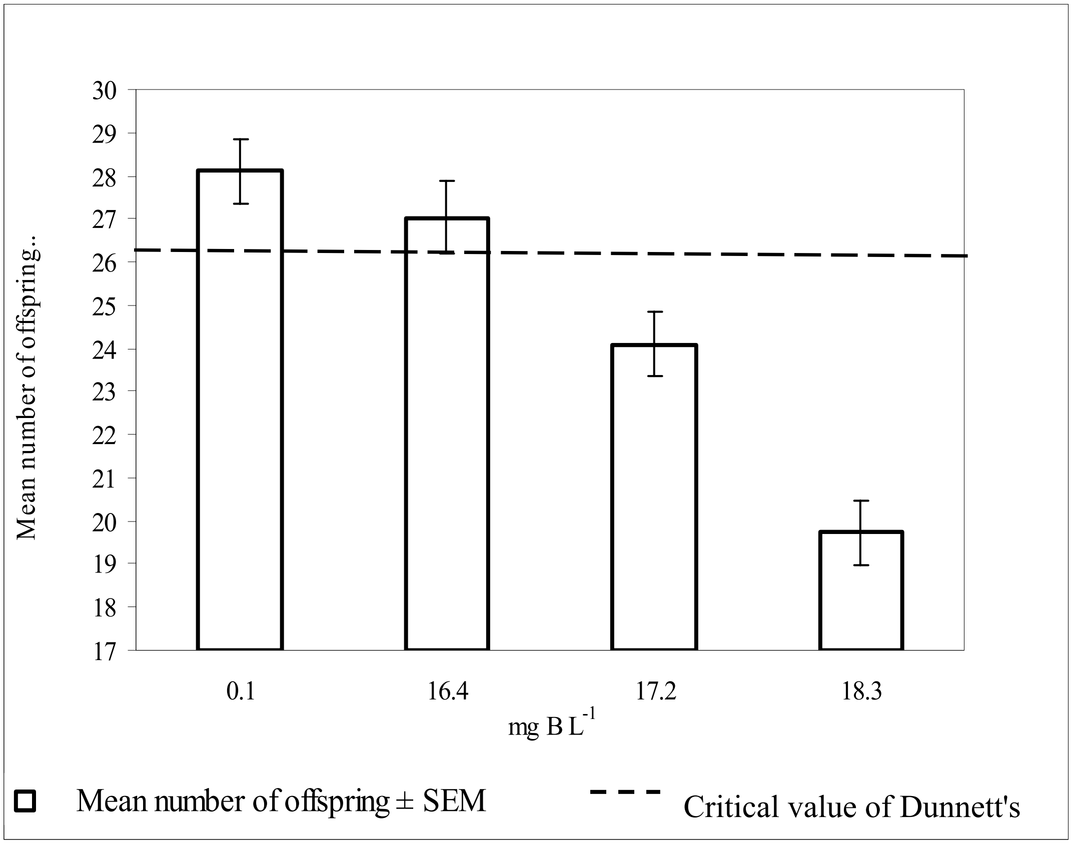


Fig. 3. Boron effect on C. *dubia* reproduction.

CHAPTER 5

SUMMARY AND CONCLUSIONS

Boron enters aquatic environments from both natural and anthropogenic sources (Polat et al., 2004). In the United States, natural high boron concentrations (5-15 mg B L^{-1}) can be found in southwestern United States because of the weathering of boron-rich formations and deposits (Butterwick et al., 1989; ECETC, 1997). In commerce and industry, boron minerals have several diverse applications and activities such as mining, coal burning for power generation, and production of glass and cleaning products. Boron releases to water occur from municipal sewage containing sodium perborates from detergents and in runoff from areas using boron-containing herbicides or fertilizers (Butterwick et al., 1989; Nable et al., 1997). Boron concentrations in flue gas desulfurization (FGD) waters, produced in coal-fired power plants, range from 33 to 460 mg B L^{-1} (Eggert *in review*), while boron concentrations in other wastewaters are typically in the 1.0 mg B L^{-1} range (Polat et al., 2004). Conventional wastewater treatment systems do not effectively remove boron from contaminated water, and insufficiently treated effluents may be discharged into aquatic systems (Polat et al., 2004) which may result in potential risks of boron to aquatic species.

Boron has been of recent interest due to elevated concentrations in waters from thermoelectric power generation, particularly flue gas desulfurization (FGD) waters (Changwoo and Mistch, 2001; Lamminen et al., 2001). With the advent of more stringent air quality standards, FGD technology is being implemented at coal-fired power plants to decrease sulfur dioxide emissions (Mierzejewski, 1991; Lammine et al., 2001). During coal combustion, flue gases containing sulfur dioxide (SO_2) and other gaseous compounds are produced. Some of these gaseous elements and compounds are transferred and transformed into soluble species by wet scrubbing of flue gases (Solan et al., 1999). The resulting water produced by the wet scrubbing of flue gases is referred to as 'FGD water'.

The amount of water produced by an FGD scrubber can exceed 0.1 mgd at large facilities (>1,000 MW) and can contain significant concentrations of boron (33-460 mg L^{-1}). FGD waters can also contain elevated concentrations of other potentially phytotoxic constituents including arsenic, cadmium, chemical oxygen demand, chlorides, chromium, copper, lead, mercury, selenium, sulfate, zinc and hardness (e.g. calcium, iron, and magnesium) (Mierzejewski, 1991; Eggert, *in review*). These waters are semi-neutral in pH and contain high total dissolved and suspended solids (calcium, chlorides, magnesium, and sulfate) (Mierzejewski, 1991). However, FGD waters vary significantly in their composition and are influenced by many factors such as coal type used, source water used in the scrubber, and type of flue gas scrubber employed (Eggert, *in review*). The number of scrubbing systems is increasing within the United States U.S. and consequently the annual production of FGD waters is increasing.

Due to problematic constituents contained in FGD waters, renovation of waters associated with thermoelectric power generation is needed in order to reuse these waters (e.g. cooling waters) or discharge them into aquatic receiving systems (Mierzejewski, 1991; Eggert, *in review*). Treatment of FGD waters is required by the United States Environmental Protection Agency (U.S. EPA) through the National Pollutant Discharge and Elimination System (NPDES) before discharging to receiving systems. Constructed wetland treatment systems have been proposed as a novel treatment approach for efficient and effective treatment of targeted constituents (As, Hg, and Se) found in FGD waters (Ye et. al., 2003). If a treatment approach such as CWTS is considered for FGD waters, elevated chloride concentrations ($\geq$4000 mg L^{-1}) are known to be toxic to wetland plants and must be managed (Eggert, *in review*). *Typha latifolia* Linnaeus and *Schoenoplectus californicus* C. A. Meyer Palla are often used in constructed wetland treatment systems for FGD waters (Eggert, *in review*). To maintain the health of plants in CWTS, FGD waters are generally diluted to achieve chloride concentrations <4000 mg L^{-1}. However, boron may also occur in the diluted FGD waters at concentrations that pose risks to plants used in CWTS designed to treat constituents of concern and boron may adversely affect survival, growth, and, consequently, plant performance.

Boron in FGD waters may be phytotoxic to wetland plant species in constructed wetland treatment systems, but potential boron toxicity is dependent on factors such as its concentration, form, frequency of occurrence, and duration of exposure in these waters. Since FGD waters vary widely in composition (e.g. boron concentrations), their phytotoxicity may also vary. In order to effectively use *T. latifolia* and *S. californicus* to remediate FGD waters, we need to understand the responses of these plants to exposures of boron in FGD waters.

Chapter Two of this thesis focused on predicting responses of *T. latifolia* and *S. californicus* to aqueous boron exposures as immature plants. Responses of *T. latifolia* and *S. californicus* can indicate their appropriateness for use in constructed wetland treatment systems to treat FGD waters. The objectives of this research were: 1) to measure responses of *T. latifolia* seed germination, and shoot and root growth of early seedlings following 7-d exposures to boron in diluted FGD water and in formulated moderately hard water (MHW) as well as to measure responses of immature *S. californicus* seedlings in terms of survival, shoot and root growth following 35-d exposures to boron concentrations, 2) to compare the responses of *T. latifolia* and *S. californicus* in diluted FGD water and MHW, and 3) to contrast the responses of *T. latifolia* and *S. californicus* to boron exposures. *T. latifolia* seed germination in FGD water or MHW was not sensitive to boron concentrations of 0.2-100 mg L^{-1} with seed germination of 92-100%. Survival of *S. californicus* immature seedlings in FGD water was ≤3.3% at boron concentrations of ≥12.6 mg L^{-1} while in MHW survival ranged from 23 to 96% at boron concentrations 12.6-49.9 mg L^{-1}. Responses of shoot and root growth to boron exposures in FGD water also differed from responses in MHW. No root emergence or elongation was observed for *T. latifolia* early seedlings at all boron concentrations ≥12.6 mg L^{-1} in diluted FGD water and shoots were yellow with lengths ≤5.36 mm. Root growth decreased with increasing concentrations of boron in MHW for *S. californicus* (≥25.8 mg B L^{-1}) and *T. latifolia* (≥12.6 mg B L^{-1}) with no root growth observed at boron concentrations ≥99.6 mg B L^{-1}. The results of this study indicated that boron in conjunction with other constituents in FGD water may adversely affect *T. latifolia* and *S. californicus* seed germination and early seedling growth and this must be considered in planting constructed wetland treatment systems for FGD waters.

Chapter 3 of this thesis focused on measuring responses of mature plants of *T. latifolia* and *S. californicus* to boron exposures in simulated wetland environments. These exposures were of sufficient duration (>1 year) to allow responses to boron to be manifested. The specific objectives of this research were: 1) to determine responses of *T. latifolia* and *S. californicus* in terms of shoot height, shoot density, number of leaves per shoots (only *T. latifolia*), necrosis and number of inflorescences produced following 13 months of exposure to a series of concentrations of boron (as boric acid) in simulated FGD water, 2) to measure concentrations of boron in the hydrosoil and bioconcentration of boron in plant tissue (i.e. shoots and roots) of *T. latifolia* and *S. californicus* after 13 months of exposure to boron (boric acid) in simulated FGD water, 3) to determine relationships between responses of *T. latifolia* and *S. californicus* to boron exposures and concentrations of boron measured in the roots and shoots of these plants, and 4) compare the responses of *T. latifolia* and *S. californicus* following 13 months of exposures to boron (as boric acid) in simulated FGD water. In this experiment, aqueous concentrations of boron greater than 25 mg L^{-1} adversely affected both *T. latifolia* and *S. californicus* with browning and yellowing of shoots, decreased shoot density and height for *S. californicus* and decreased density of *T. latifolia* at 300 mg B L^{-1}. In both the shoots and the roots of these species, concentrations of boron increased proportionally with aqueous exposure concentrations as did the concentrations of boron in hydrosoil. Thus, adverse effects of boron were related not only to the aqueous boron exposures, but also to boron concentrations in the hydrosoil, roots and shoots. *S. californicus* was more sensitive than *T. latifolia* to aqueous boron exposures in simulated FGD water with 100% mortality of

S. californicus at 300 mg L^{-1}. In exposures of 100 mg B L^{-1}, *S. californicus* shoot density and shoot height were 43% and 40% less than untreated FGD water controls, respectively. *T. latifolia* shoot density decreased to 50% of the untreated control density at a boron concentration of 300 mg L^{-1}. In constructed wetland treatment systems used to treat FGD waters, *T. latifolia* and *S. californicus* can likely tolerate boron concentrations ≤25 mg L^{-1}.

Chapter Four of this thesis focused on responses of sentinel aquatic animal species, *Ceriodaphnia dubia* Richard and *Pimephales promelas* Rafinesque, to aqueous boron exposures. Boron in industrial effluents and FGD waters may be found at concentrations that adversely affect aquatic animals. The objectives of this research were to measure responses (potency, median lethal effect concentrations (LC_{50}s), lowest observed effect concentrations (LOEC), and no observed effect concentrations (NOEC) of *C. dubia* and *P. promelas* to aqueous boron exposures (in the form of boric acid). Boron toxicity tests were conducted in 7-d static/renewal tests following U.S. EPA testing protocols for *C. dubia* and *P. promelas* (U.S.EPA, 2002). Each test concentration was replicated three times with 30 organisms per treatment and controls. The estimated 7-day aqueous LC_{50} for *C. dubia* was 75.9 mg B L^{-1} with a 95% confidence interval of 74.9-77.0 mg B L^{-1} while the 7-d LC_{50} for *P. promelas* was 81.6 mg B L^{-1} with a 95% confidence interval of 77.8-83.7 mg B L^{-1}. NOEC and LOEC values for *C. dubia* reproduction were 16.4 and 17.2 mg B L^{-1}, respectively. No *C. dubia* reproduction was observed at boron concentrations ≥38.4 mg B L^{-1}. Similar to most elements, aqueous boron exposures elicited different responses (sensitivity) from *C. dubia* and *P. promelas* with potency slopes of 0.86 and 0.57 % mortality / mg B L^{-1}, respectively, indicating that *C. dubia* is more sensitive (in terms of survival) to boron than *P. promelas*.

The primary purpose for conducting this research was to further understanding of potential risks of boron in a complex matrix such as FGD waters. Accurate characterization of both phytotoxicity and toxicity of boron to aquatic vertebrates and invertebrates is important in order to predict risks in either CWTS or receiving aquatic systems. Boron will adversely affect the health of *T. latifolia* and *S. californicus* in CWTS at concentrations ≤25 mg B L^{-1}. Boron in FGD waters could contribute to phytotoxicity in *T. latifolia* and *S. californicus.* However, other elements in FGD water may also contribute to phytotoxicity and that toxicity may be enhanced by boron. Based on the results from this research, CWTS for FGD waters should be planted with mature plants. If boron concentrations in aqueous exposures exceed 12.6 mg L^{-1} for either *T. latifolia* and *S. californicus*, survival of immature plants will be limited while mature plants can tolerate boron concentrations ≤25 mg L^{-1}. For the sensitive, sentinel aquatic animals, *C. dubia* and *P. promelas*, the NOEC for boron was 71. and 51.5 mg B L^{-1}, respectively. Based on the results from this study, immature plants were the most sensitive to aqueous boron exposures of the species tested. Water Quality Criteria for boron that are intended to protective of aquatic life in receiving systems should be supported by strategic phytotoxicity data.

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