Effects of enhanced sulfate and sulfide concentrations on wild rice germination and growth: results from a hydroponics experiment

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by

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Introduction

This report is one part of a larger study—the Wild Rice Sulfate Standard Study—coordinated by the Minnesota Pollution Control Agency (MPCA) on the effect of elevated sulfate concentrations on wild rice. Minnesota currently has a water quality standard of "10 mg/L sulfate - applicable to water used for production of wild rice during periods when the rice may be susceptible to damage by high sulfate levels." (Minn. R. 7050.0224, subpart 2). In 2010, the MPCA initiated a multi-year effort to clarify implementation of the state's wild rice sulfate standard, which had recently come under increased questioning and contention. Based on a review of available studies and information, the MPCA determined that additional studies were needed to evaluate the effects of sulfate on wild rice before a revision to the numeric sulfate standard could be considered. In 2011 the Minnesota Legislature provided funding to gather this additional information in the Legacy Amendment Bill (Laws of Minnesota, 2011, First Special Session, ch. 6, Art. 2, Sec. 5(j)).

Wild rice is an important aquatic plant in parts of Minnesota, particularly northern Minnesota. It provides food for waterfowl, is also a very important cultural resource to many Minnesotans, and is economically important to those who harvest and market wild rice.

The goal of the overall Wild Rice Sulfate Standard Study is to enhance understanding of the effects of sulfate on wild rice and to inform a decision as to whether a revision of the wild rice sulfate standard is warranted. The Study consists of several research efforts that have been conducted by several groups of scientists at the University of Minnesota campuses in Duluth and the Twin Cities under contract with the MPCA. The data collection phase of the study was completed in December 2013, and is documented in individual reports, along with associated data, from the researchers working on each component of the study.

The primary hypothesis driving the Study has been that it if elevated sulfate has a negative effect on the growth of wild rice it is mediated through the formation of hydrogen sulfide in the rooting zone of wild rice, and that elevated iron would mitigate the toxicity of the sulfide by forming insoluble iron sulfide compounds.

The Study components include:

- **Field study of wild rice habitats** to investigate physical and chemical conditions correlated with the presence or absence of wild rice stands, including concentrations of sulfate in surface water and sulfide in the rooting zone.

- **Controlled laboratory hydroponic experiments** to determine the effect of elevated sulfate and sulfide on early stages of wild rice growth and development.
- **Outdoor container experiments utilizing natural sediments** to determine the response of wild rice to a range of sulfate concentrations in the surface water, and associated sulfide in the rooting zone, across the growing season.
- **Collection and analysis of rooting zone depth profiles of dissolved chemicals at wild rice outdoor experiments and field sites** to characterize sulfate, sulfide, and iron in the rooting zone of wild rice.
- **Sediment incubation study** to explore the difference ambient temperature has on the rate that elevated sulfate concentrations in water enter underlying sediment, convert to sulfide, and later release sulfate back into the overlying water.

The MPCA will review the results from individual reports along with existing monitoring data, other relevant scientific studies, pertinent ecological, cultural and historical information, and the original basis for the wild rice sulfate standard to determine if a change to the current wild rice sulfate standard is warranted, and what that change might be. If change(s) are proposed, they would be adopted into Minnesota Rules via the administrative rulemaking process and subject to U.S. EPA approval before the changes could be implemented.

This report focusses on the hydroponics experiments to determine the effect of elevated sulfate and sulfide on wild rice growth and development.

Background

Northern wild rice (*Zizania palustris*) is one of four species in the genus *Zizania*, which are the only native aquatic grains in North America. The range of northern wild rice (hereafter wild rice) is centered across the Great Lakes region but is most abundant in the rivers and lakes of the watersheds of Lakes Superior and Michigan in northern Minnesota, Wisconsin, and Ontario. Because of its widespread distribution and tendency to form monotypic stands, wild rice has great potential to influence the food supply for waterfowl, muskrats, and other members of the food web. In addition, the native Ojibway people of the watersheds of Lakes Superior and Michigan teach that they were led to this region to find “the food that grows upon the water”, which is wild rice. The Ojibway identify their origins with wild rice and consider themselves

"people of the rice" (Vennum 1988). Therefore, the productivity, perpetuation, and restoration of wild rice are of great ecological and cultural significance.

Wild rice is an annual plant. It grows in both lakes and rivers in water between 0.3 and 0.67 m depth where there is some water flow. Native stands of wild rice grow in waters that are circum-neutral pH, of low conductivity and hardness, and generally low in nutrient concentrations. In lakes, the most common sediment is an organic-rich silt, but the sediment types ranges widely (Day and Lee 1990). Sediment in the riverine habitat also ranges widely and may be higher in mineral sediment in the main channels than in backwaters (Meeker 1996). Seeds germinate in the spring and first develop a mesocotyl, or primordial shoot, and a radical, or primordial root. The mesocotyl then grows above the sediment surface, where it develops into a green shoot with a primordial leaf in late spring and early summer. The plant is now at the seedling stage. When the shoot of the seedling reaches the water surface, the plant generates a long narrow leaf which floats atop the water surface; this stage is therefore called the floating leaf stage. Photosynthesis by the floating leaf is used to expand the root system and the beginnings of an aerial shoot which emerges from the leaf axil of the floating leaf and the stem below the water surface. Once the aerial stem and the first aerial leaf emerge, the floating leaf dies and the plant grows taller, putting out additional aerial leaves until late July or early August. Nutrient uptake is very rapid during this stage, and approximately 60-70% of the plant's annual requirement for nitrogen, the most limiting nutrient to both vegetative growth and seed production in most environments, is taken up then (Grava and Raisanen 1978, Sims et al. 2012a,b). In late July or early August, vegetative growth slows and the plant begins to produce a flowering shoot containing male (pollen producing) flowers above female (seed producing) flowers below. Wild rice does not self-pollinate well; instead, as for most graminoids, pollination is largely by wind although bees and flies occasionally visit the male flowers to gather pollen (J. Pastor, *personal observations*). During the seed production and ripening stage, there is another burst of nutrient uptake from the sediment and the lower vegetative leaves begin to senesce as the nutrients they contain are translocated to the ripening seeds (Grava and Raisanen 1978, Sims et al. 2012a, b). Seeds ripen in late August and through September, although the first two weeks of September are commonly the period of peak ripening. The seeds contain a long awn, which helps stabilize them vertically when they are dispersed into the water and thereby allow them to drill into the sediment (Ferren and Good 1977, J. Pastor *personal observations*). After seed

dispersal, the plant dies and its stem, leaf, and root litter are returned to the sediment. Delays in the release of nitrogen from these litters in subsequent years may be responsible for the population oscillations of 3-5 year periods often seen in wild populations (Pastor and Walker 2006, Walker et al. 2010, Hildebrandt et al. 2012).

In a survey of the distribution of aquatic plants in relation to water chemistry, Moyle (1944, 1945) found that wild rice and its abundance falls off rapidly at sulfate concentrations higher than 10 mg $\cdot$ L^{-1}. However, understanding whether sulfate itself or some other co-varying property of these lakes is responsible for the distribution and growth of wild rice requires additional experimental studies designed to test specific mechanistic hypotheses.

Sediments of wild rice lakes are almost always anoxic and consequently can have a high potential for reducing sulfate to sulfide under certain conditions. Because sulfate reduction is more thermodynamically favorable than methanogenesis (Capone and Kiene 1988), increasing concentrations of sulfate are likely to increase the rate of sulfide accumulation in sediment. High sulfide levels can inhibit root growth of wetland plants (Koch and Mendelssohn 1989, Koch et al. 1990, Lamers et al. 2002, Guerts et al. 2009), including white rice (*Oryza sativa*; Gao et al. 2003; Armstrong and Armstrong 2005). If root biomass is reduced by sulfide, then the plant's ability to take up limiting nutrients, especially nitrogen, will be impaired (Gao et al. 2003, Armstrong and Armstrong 2005). Sulfide may also directly inhibit the ability of roots to take up nitrogen (DeLaune et al. 1983), but this remains a hypothesis requiring further testing.

In order to determine whether sulfate or sulfide have toxic effects on wild rice growth, it is instructive to perform hydroponics experiments where the chemistry of the growth solution and the presence of sulfate or sulfide can be controlled precisely. Accordingly, we began a series of hydroponics experiments to test the effects of sulfate and sulfide on wild rice seed germination and juvenile growth of seedlings under highly controlled conditions in growth chambers.

Methods

In order to determine the effects of sulfate and sulfide on seed germination and plant growth in hydroponic solutions, we needed to develop methods which could be applied under both aerobic (for sulfate) and anaerobic (for sulfide) conditions. Virtually all hydroponic techniques are developed for agricultural plants which grow in aerobic soils – in fact, air is usually bubbled through the hydroponic solution to prevent anaerobic conditions from

developing. In addition, the wild rice seeds we used are from wild plants, not hybrid agricultural varieties. There are large differences between wild and agricultural plants in nutrient uptake and growth responses to nutrient gradients. Therefore, we also needed to develop a nutrient solution appropriate for healthy growth of wild rice.

Li et al. (2009) published one of the few studies of response of aquatic macrophytes (*Typha* and *Cladium*) to sulfide under anaerobic conditions. Malvick and Percich (1993) developed a simple hydroponic system to investigate effects of nutrients on germination and early growth of wild rice, but their system could only be implemented under aerobic conditions. We used these two studies as starting points for the development of our methods. We first tested wild rice growth for nutrient solutions of various concentrations. In the course of our these experiments, we found that we could not control solution pH using calcium carbonate additions to the nutrient solution as recommended by Li et al. (2009). It is important to have precise control of pH for two reasons. First, wild rice growth is observed in nature at circum-neutral pH. Second, the speciation of sulfides switches very rapidly above and below pH 7, being dominated by hydrogen sulfide at acid pH and bisulfide at alkaline pH. We therefore began additional experiments searching for a pH buffer which did not affect wild rice growth but which would allow us to control pH to within 0.5 pH units or less. Finally, in order to maintain consistent sulfide levels during trials lasting 10 or 11 days, we tested containers and septa of various materials, since both hydrogen sulfide and oxygen can diffuse through various materials at different rates.

The results of these method development experiments were reported to MPCA scientists weekly in conference calls and in various interim reports. Based on these experiments, we developed Standard Operating Procedures (SOPs) for the germination and growth of wild rice in hydroponic solutions under both aerobic and anaerobic conditions. These SOPs were approved by MPCA. Quality Assurance Project Plans (QAPPs) were developed based on these SOPs. These SOPs are presented in the Appendices 1 – 4 and the QAPPs can be found at http://www.pca.state.mn.us/index.php/view-document.html?gid=19914, but we will summarize them briefly here.

Wild rice seeds used for all experiments had been collected on 8/30/2012 from Little Round Lake (Minnesota Lake ID 03-0302, Latitude 46.97˚N, Longitude 95.74˚W). The seeds

were cold-stratified under the lake ice, then stored at 4˚C in polyethylene bottles until used in experiments.

Immediately before adding the seeds to treatment jars, approximately four times as many seeds as needed were placed on a cleaned lab tray and covered with water were selected for use based on a viable appearance (intact, absence of mold, filled kernel, not green). For tests of juvenile seedling response to sulfate or sulfide, the seedlings were allowed to germinate in deionized water until a 2 cm long mesocotyl shoot appeared. The seeds or seedlings were picked up with forceps and transferred to the appropriate hydroponic solution test in appropriate containers.

The nutrient solution used was 1/5 strength Hoagland's solution in 5 mM PIPES buffer (mean pH of final solution 6.8 ± sd 0.03). Various sulfate or sulfide solutions were added to achieve various target levels.

The general procedure for each set of experiments was first to perform a rangefinder test of seed germination or seedling growth response across a wide range of concentrations of either sulfate or sulfide. The rangefinder test was repeated regardless of whether there was a statistically significant response of wild rice to the range of sulfate or sulfide concentrations. The main effect of sulfate or sulfide concentrations on the variable of interest was tested with an analysis of variance using SigmaPlot (results of statistical analyses in the appendices are copied from SigmaPlot files). When the data were not normally distributed, the natural logarithm of the measured data was used. If there were no effects across this wide range of concentrations in both rangefinder experiments, then no further tests were done. If there were significant main effects, then pairwise comparisons were performed to determine in which part of the range of concentrations significant effects occurred. Further experiments were then conducted in this narrow range of concentrations to more precisely examine the response to the treatment. These more precise and definitive tests were also repeated. Finally, the response variable of interest in these more definitive tests was regressed against sulfate or sulfide concentrations using an appropriate statistical model.

Hydroponic techniques to test germination of wild rice seeds under aerobic conditions subject to various concentrations of sulfate. The selected seeds were placed into each of six numbered plastic cups to total fifty seeds each, then randomly assigned and transferred to each of six 1-pint Mason jars containing six sulfate treatment levels of 0, 10, 50, 100, 400, or 1600 mg

$SO_4 \cdot L^{-1}$. This seed counting and random transfer was repeated twice more to result in six treatment levels with three replicate jars per treatment. The jars were covered with plastic covers fitted with rubber stoppers to facilitate solution exchanges. Two holes in the plastic lids were left open to facilitate air exchange and to prevent the solutions from becoming anaerobic. The experiment proceeded in a growth chamber at 20 ˚C in the dark. The solutions were exchanged with fresh solution of the appropriate treatment concentration every three days. Solution pH was measured both on the initial and exchanged solutions. The germinated seedlings were harvested after 11 days. The number of successfully germinated seeds, determined as those which produced a mesocotyl shoot at least 1 cm in length, were counted. The length of the mesocotyl shoot including any leaf was measured for each seed. The germinated seeds were then dried at 65˚C for three days. Roots and shoots were then carefully separated from the remaining seed coat and weighed. The effect of sulfate concentrations on the measured properties was tested with a one-way ANOVA followed by pairwise comparisons between sulfate concentrations if the main effect of sulfate was significant. See Appendix 1 for more details.

Hydroponic techniques to test growth of juvenile wild rice seedlings under aerobic conditions subject to various concentrations of sulfate. Seedlings from germinating seeds from Little Round Lake were used. Twenty 70 mL unsealed Kimax tubes are used for each test concentration prepared. Each tube is considered a replicate for the corresponding test concentration. One seedling chosen as described above was placed with forceps into each Kimax tube, which was then filled with 1/5 Hoagland's solution and an appropriate amount of sulfate The filled tubes (solution and seed) were placed into every other opening in holding racks so that light can penetrate to all sides of each tube. A total of six 40-tube racks, each containing 20 tubes, are used to hold the test tubes. Screw caps were placed loosely on the tubes to allow for oxygen exchange across the water surface and thereby prevent the development of anaerobic conditions. The tubes were placed in an environmental growth chamber with lamps of maximum light intensity of 800 or greater μmol m^{-2} sec^{-1} (measured 6 inches below the lamps) produced by either fluorescent lamps or an LED light system. Tests were performed under a 16h:8h light:dark schedule. All racks were placed in the growth chamber so that the spaces between the racks are the same as the spaces within the racks and the tops of the tubes are within 30 cm of the bottom of the lights. The location of each rack in the growth chamber remains the same for the test duration. Test solutions in the tubes were renewed every two (2) days. Temperature was

maintained at 21˚C during lighted periods and 19˚ C during dark periods and the humidity was maintained at 85%. Plants were harvested after 10 days. Stem/leaf length (mesocotyl growth) was measured by placing the stem/leaf stretched out on a flat surface next to ruler with the zero mark aligned with the point of stem-root transition. The length from the stem-root transition to the tip of the stem/leaf was measured and recorded to the nearest millimeter. Root lengths were measured in duplicate scans of the entire root system using the program WinRhizo (©). Seedlings were weighed after drying at 100˚C for 48 hours. Tests were deemed acceptable if: 1. At least 90% of control juvenile seedlings were living at test termination; 2. Mesocotyl length of juvenile seedlings from control exposures were at least 5.0 cm at the end of the 10 day duration of growth; and 3. Control juvenile seedlings did not indicate any visible phytotoxic or developmental symptoms at any time during the test and the controls grew. See Appendix 2 for more details.

Hydroponic techniques to test germination of wild rice seeds under anaerobic conditions subject to various concentrations of sulfide. The techniques used here were the same as for the germination trials under various sulfate concentrations, except that extra care was necessary to ensure anaerobic conditions. Fifty conditioned seeds were placed in 700 mL borosilicate glass jars capped using phenolic screw caps with chlorobutyl septa 5 mm thick. The 1/5 Hoagland's nutrient solution was deoxygenated with purified nitrogen before being added to the bottles. PIPES buffer was added to the test media to maintain consistent pH levels throughout an experiment. Bottles were carefully filled to the brim with the deoxygenated nutrient solution without introducing any air bubbles, then capped with the septa. Stock sulfide solutions (20 – 30 mM) were prepared as needed by adding $Na_2S \cdot 9\ H_2O$ (sodium sulfide hydrate) to deionized and deoxygenated water. An appropriate amount of the stock solutions was added to each bottle with a Hamilton volumetric glass syringe through the septa while simultaneously withdrawing an equivalent volume of solution by means of a second syringe through the septum. Added solution volumes range between 0.2 – 3.0 mL depending on target exposure concentrations and the nominal concentration the of stock sulfide solution. The target concentrations were 0, 3, 10, 30, and 90 µM. The bottles were placed in a growth chamber in continuous darkness 20° C ± 1°C. Solutions were exchanged every two days. After 11 days, the germinated seeds were harvested and measured as described above for the experiments on effects of sulfate on germination. Each toxicity test consisted of at least four toxicologically relevant test concentrations of sulfide in the

hydroponics media (hydroponics medium + sulfide) and a negative control which consists of the deoxygenated hydroponics medium without any sulfide added. Each of the four exposure concentration levels and control are replicated three times, using 700 ml glass jars for each set of replicates. Tests were deemed acceptable using the same criteria as described above for the tests of sulfate on germination. See Appendix 3 for more details.

Hydroponic techniques to test growth of juvenile wild rice seedlings under anaerobic conditions subject to various concentrations of sulfide. Seedlings were chosen using the same techniques described above for aerobic conditions and placed in 700 mL borosilicate glass jars capped using phenolic screw caps with 5 mm thick chlorobutyl septa. For each jar, seven sprouts were selected and removed individually from a pool of conditioned seeds using a light forceps and placed into a 700 ml glass jar. Deoxygenated Hoagland's nutrient solution was added as described above. Four sulfide treatment levels and a negative control were assigned at random to three jars apiece, which served as replicates. Seedlings were grown in an environmental growth chamber at 20° C ± 1°C and light/dark intervals of 16 hours of light and 8 hours of dark. Solutions were exchanged every two days and harvested after 10 days. Harvested seedlings were measured as described above for the tests of sulfate on seedling growth. Tests were deemed acceptable using the same criteria as above for the tests of sulfate on germination. See Appendix 4 for more details.

Results

Effect of sulfate on germination of wild rice seeds under aerobic conditions. We began with a rangefinder test that examined the effect of sulfate on germination and mesocotyl growth of wild rice seeds at nominal exposure concentrations of 0, 10, 50, 100, 400, and 1,600 mg $SO_4 \cdot L^{-1}$. Between 71 and 76% of the seeds germinated at each sulfate concentration (Table 1). Sulfate concentrations did not affect germination success ($p > 0.10$, Table 1).

Table 1. Effect of sulfate concentrations on germination success of wild rice seeds

Treatment	Mean % germination	Standard deviation
0 mg $SO_4 \cdot L^{-1}$	71.3	6.4
10 mg $SO_4 \cdot L^{-1}$	74.0	8.7
50 mg $SO_4 \cdot L^{-1}$	73.3	9.0
100 mg $SO_4 \cdot L^{-1}$	76.7	7.0
400 mg $SO_4 \cdot L^{-1}$	74.7	9.0
1,600 mg $SO_4 \cdot L^{-1}$	71.3	2.3

Sulfate concentrations did not affect mesocotyl lengths ($p > 0.10$; Table 2).

Table 2. Effect of sulfate concentrations on mesocotyl lengths of germinated wild rice seeds

Treatment	Mean length (cm)	Standard deviation
0 mg $SO_4 \cdot L^{-1}$	12.5	0.89
10 mg $SO_4 \cdot L^{-1}$	12.7	0.13
50 mg $SO_4 \cdot L^{-1}$	12.7	1.7
100 mg $SO_4 \cdot L^{-1}$	12.9	0.66
400 mg $SO_4 \cdot L^{-1}$	13.5	0.77
1,600 mg $SO_4 \cdot L^{-1}$	11.8	1.6

Sulfate treatments did not affect the weights of the germinated plants (mesocotyl plus leaf and roots; $p > 0.10$; Table 3). This rangefinder test was repeated and the results were similar. See Appendix 5 for raw data and statistical analyses.

Table 3. Effect of sulfate on germinated plant weights

Treatment	Mean weight (mg)	Standard deviation
0 mg $SO_4 \cdot L^{-1}$	4.8	0.58
10 mg $SO_4 \cdot L^{-1}$	4.7	0.37
50 mg $SO_4 \cdot L^{-1}$	4.5	0.85
100 mg $SO_4 \cdot L^{-1}$	4.7	0.53
400 mg $SO_4 \cdot L^{-1}$	5.2	0.33
1,600 mg $SO_4 \cdot L^{-1}$	4.1	0.30

The pH of the solutions was stable within 0.2 pH units between the initial solution and its exchange two days later (Appendix 5). The rangefinder test was repeated, and the same results were observed. Because no effects were observed after two rangefinder tests, we did not proceed with any further tests.

Effects of sulfate on growth of juvenile seedlings under aerobic conditions. Enhanced sulfate concentrations did not inhibit the growth of juvenile stem length ($p > 0.10$, Table 4), juvenile stem weight ($p > 0.10$, Table 6) juvenile root weight ($p > 0.10$, Table 7), or total juvenile plant weight ($p > 0.10$, Table 8) in a rangefinder test at the same concentrations as for the test for effects of sulfate on germination. Sulfate decreased juvenile root length slightly ($p < 0.02$, Table 5) but only at 1,600 mg $SO_4 \cdot L^{-1}$ compared with 50 mg $SO_4 \cdot L^{-1}$. The rangefinder test was repeated, and the same results were observed. Because no effects were observed after two rangefinder tests, we did not proceed with any further tests. See Appendix 6 for raw data and statistical analyses.

Table 4. Effect of sulfate on juvenile stem length

Treatment	Mean length (cm)	Standard deviation
0 mg $SO_4 \cdot L^{-1}$	11.7	2.0
10 mg $SO_4 \cdot L^{-1}$	12.2	2.3
50 mg $SO_4 \cdot L^{-1}$	12.5	1.8
100 mg $SO_4 \cdot L^{-1}$	12.2	2.7
400 mg $SO_4 \cdot L^{-1}$	11.9	1.2
1,600 mg $SO_4 \cdot L^{-1}$	11.6	2.1

Table 5. Effect of sulfate on juvenile root length

Treatment	Mean weight (mg)	Standard deviation
0 mg $SO_4 \cdot L^{-1}$	45.8	9.7
10 mg $SO_4 \cdot L^{-1}$	41.2	10.8
50 mg $SO_4 \cdot L^{-1}$	47.8	9.6
100 mg $SO_4 \cdot L^{-1}$	44.1	9.1
400 mg $SO_4 \cdot L^{-1}$	43.3	7.1
1,600 mg $SO_4 \cdot L^{-1}$	38.5	5.2

Table 6. Effect of sulfate on juvenile stem weight

Treatment	Mean weight (mg)	Standard deviation
0 mg $SO_4 \cdot L^{-1}$	5.1	1.6
10 mg $SO_4 \cdot L^{-1}$	5.2	1.5
50 mg $SO_4 \cdot L^{-1}$	5.6	1.3
100 mg $SO_4 \cdot L^{-1}$	5.0	1.9
400 mg $SO_4 \cdot L^{-1}$	5.1	1.4
1,600 mg $SO_4 \cdot L^{-1}$	6.0	1.0

Table 7. Effect of sulfate on juvenile root weights

Treatment	Mean weight (mg)	Standard deviation
0 mg $SO_4 \cdot L^{-1}$	4.3	1.1
10 mg $SO_4 \cdot L^{-1}$	3.9	1.0
50 mg $SO_4 \cdot L^{-1}$	4.5	1.0
100 mg $SO_4 \cdot L^{-1}$	4.0	1.4
400 mg $SO_4 \cdot L^{-1}$	4.2	0.9
1,600 mg $SO_4 \cdot L^{-1}$	3.9	0.8

Table 8. Effect of sulfate on total juvenile plant weights

Treatment	Mean weight (mg)	Standard deviation
0 mg $SO_4 \cdot L^{-1}$	9.4	2.5
10 mg $SO_4 \cdot L^{-1}$	9.0	2.4
50 mg $SO_4 \cdot L^{-1}$	10.1	2.2
100 mg $SO_4 \cdot L^{-1}$	9.0	3.2
400 mg $SO_4 \cdot L^{-1}$	9.3	2.0
1,600 mg $SO_4 \cdot L^{-1}$	9.9	1.6

Effects of sulfide on germination of wild rice seeds under anaerobic conditions. Enhanced sulfide under anaerobic conditions did not affect germination of seeds ($p > 0.10$, Table 9), mesocotyl weights ($p > 0.10$, Table 10), or mesocotyl lengths ($p > 0.10$, Table 11) in a rangefinder test at nominal exposure concentrations of 0, 3, 10, 30 and 90 µM sulfide (see Appendix 7 for raw data and statistics). The rangefinder test was repeated, and because the same results were observed, so we did not proceed with any further tests.

Table 9. Effect of sulfide on germination rates

Nominal sulfide concentration	Mean germination percentage	Standard deviation
0 µM	79.0	7.9
3 µM	80.6	12.0
10 µM	87.1	10.4
30 µM	80.0	14.0
90 µM	78.0	13.0

Table 10. Effect of sulfide on mesocotyl weight

Nominal sulfide concentration	Mesocotyl weight (mg)	Standard deviation
0 µM	1.50	0.12
3 µM	1.47	0.09
10 µM	1.43	0.22
30 µM	1.39	0.31
90 µM	1.69	0.27

Table 11. Effect of sulfide on mesocotyl length

Nominal sulfide concentration	Mesocotyl length (cm)	Standard deviation
0 µM	7.9	1.0
3 µM	7.5	0.35
10 µM	8.5	1.3
30 µM	7.7	0.94
90 µM	9.3	1.7

Effects of sulfide on growth of juvenile seedlings under anaerobic conditions. Although the solutions for each of these experiments were exchanged every two days, the initial sulfide concentrations declined during those two days, perhaps because of the production of oxygen by the photosynthesizing plants. Pending confirmation of our sulfide measurements by the Minnesota Dept. of Health Laboratory, we will later report results relative to mean sulfide

concentrations during the two days between exchanges. However, here we report plant responses relative to the nominal concentrations in experimental treatments as conservative estimates of the level of sulfide producing anomalous growth responses relative to controls. Actual concentrations of sulfide producing anomalous growth may be lower than reported here.

We began with a rangefinder test, growing juvenile seedlings at nominal exposure concentrations of 0, 3, 10, 30, and 90 μM sulfide (Tables 12, 13, and 14 and Fig. 1; see Appendix 8 for data and statistical tests). All plants exposed to nominal concentrations of 0 μM sulfide grew significantly larger ($p < 0.05$) during the 10 days of the experiment, approximately doubling in size compared with initial lengths and weights. However, the growth of stem and leaf lengths and plant weight of juvenile seedlings were all significantly depressed ($p < 0.01$, $p < 0.016$, and $p < 0.001$, respectively) by more than 30% at all nominal exposure concentrations greater than 0 μM sulfide in this rangefinder test (Table 12 and 14 and Fig. 1; Appendix 8). Root lengths were not significantly affected by sulfide concentration (Table 13).

We then began additional experiments focusing on the effects of sulfide on juvenile seedling growth exposed to a range of nominal concentrations generally less than nominal exposure concentrations of 40 μM sulfide. We termed these trials “definitive” because they more precisely define the range over which sulfide affects plant growth. The first definitive trial examined growth at nominal exposure concentrations of 0, 6.25, 12.5, 25, and 50 μM sulfide (Tables 15 and 16) the second experiment examined growth at nominal exposure concentrations of 0, 5, 10, 20, and 40 μM sulfide (Tables 17 and 18).

All plants exposed to 0 μM sulfide grew significantly larger ($p < 0.05$) during the 10 days of the experiments, approximately doubling in size compared with initial lengths and weights. However, in all experiments, stem plus leaf lengths and total weights of juvenile seedlings declined significantly ($p < 0.05$) at all nominal exposure concentrations greater than 9 μM sulfide compared with those grown at nominal exposures to 0 μM sulfide (Fig. 1, Tables 15 through 18).

Table 12. Effect of sulfide on stem plus leaf lengths of juvenile seedlings: Rangefinder Test

Nominal sulfide concentration	Mean length (cm)	Standard deviation
0 µM	12.8	1.8
3 µM	12.3	1.8
10 µM	11.5	1.9
30 µM	6.9	2.5
90 µM	5.3	0.9

Table 13. Effect of sulfide on root lengths of juvenile seedlings: Rangefinder Test

Nominal sulfide concentration	Mean length (cm)	Standard deviation
0 µM	3.2	1.1
3 µM	2.8	0.8
10 µM	2.8	0.8
30 µM	2.5	0.5
90 µM	2.5	0.5

Table 14. Effect of sulfide on dry weights of juvenile seedlings: Rangefinder Test

Nominal sulfide concentration	Mean weight (mg)	Standard deviation
0 µM	7.6	1.9
3 µM	6.6	1.0
10 µM	5.8	1.1
30 µM	2.8	1.3
90 µM	1.8	0.5

Table 15. Effect of sulfide on stem plus leaf lengths of juvenile seedlings: Definitive Test, Trial 1

Nominal sulfide concentration	Mean length (cm)	Standard deviation
0 µM	14.5	0.47
6.25 µM	14.1	0.11
12.5 µM	13.5	1.1
25 µM	7.5	1.4
50 µM	5.6	1.5

Table 16. Effect of sulfide on dry weights of juvenile seedlings: Definitive Test, Trial 1

Nominal sulfide concentration	Mean weight (mg)	Standard deviation
0 µM	8.7	0.7
6.25 µM	7.5	1.2
12.5 µM	7.0	0.87
25 µM	3.4	1.0
50 µM	2.4	0.88

Table 17. Effect of sulfide on stem plus leaf lengths of juvenile seedlings: Definitive Test, Trial 2

Nominal sulfide concentration	Mean length (cm)	Standard deviation
0 µM	11.5	1.4
6.25 µM	11.4	0.25
12.5 µM	11.4	0.38
25 µM	7.9	0.84
50 µM	7.6	0.75

Table 18. Effect of sulfide on dry weights of juvenile seedlings: Definitive Test, Trial 2

Nominal sulfide concentration	Mean weight (mg)	Standard deviation
0 µM	7.2	0.90
5 µM	8.1	0.32
10 µM	7.3	0.46
20 µM	4.5	0.18
40 µM	4.5	0.32

Juvenile seedling length and weights declined exponentially with increasing sulfide concentrations ($r = 0.82$ and $r = 0.86$, respectively, $p < 0.001$) across all three experiments (Fig. 2; Appendix 10, see worksheet tab Trial Comparisons).

Conclusions

Sulfate did not affect either seed germination or seedling growth other than a slight depression of root lengths at extremely high concentrations (1,600 mg $SO_4 \cdot L^{-1}$). These high concentrations, while possible in nature, are not likely to be common. Sulfide did not affect seed germination or early mesocotyl growth. However, sulfide significantly decreased both weight and lengths of stems and roots of juvenile seedling at concentrations above 9 – 10 μM. The exponential decline in seedling weight with enhanced sulfide levels means that growth rate declines in proportion to enhanced sulfide levels.

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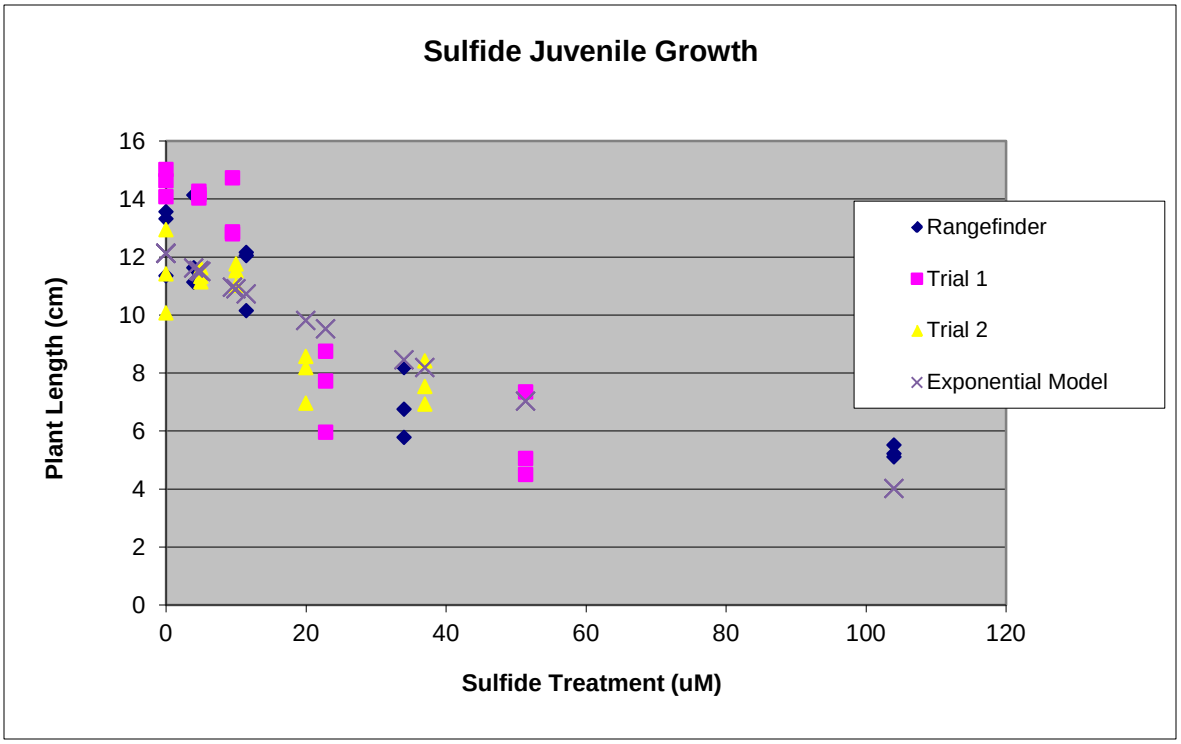
Significant decline ($p < 0.05$)

Fig. 1. Definitive test of effects of sulfide on growth of juvenile wild rice seedlings, Trial 1. Concentrations shown are measured rather than nominal concentrations.



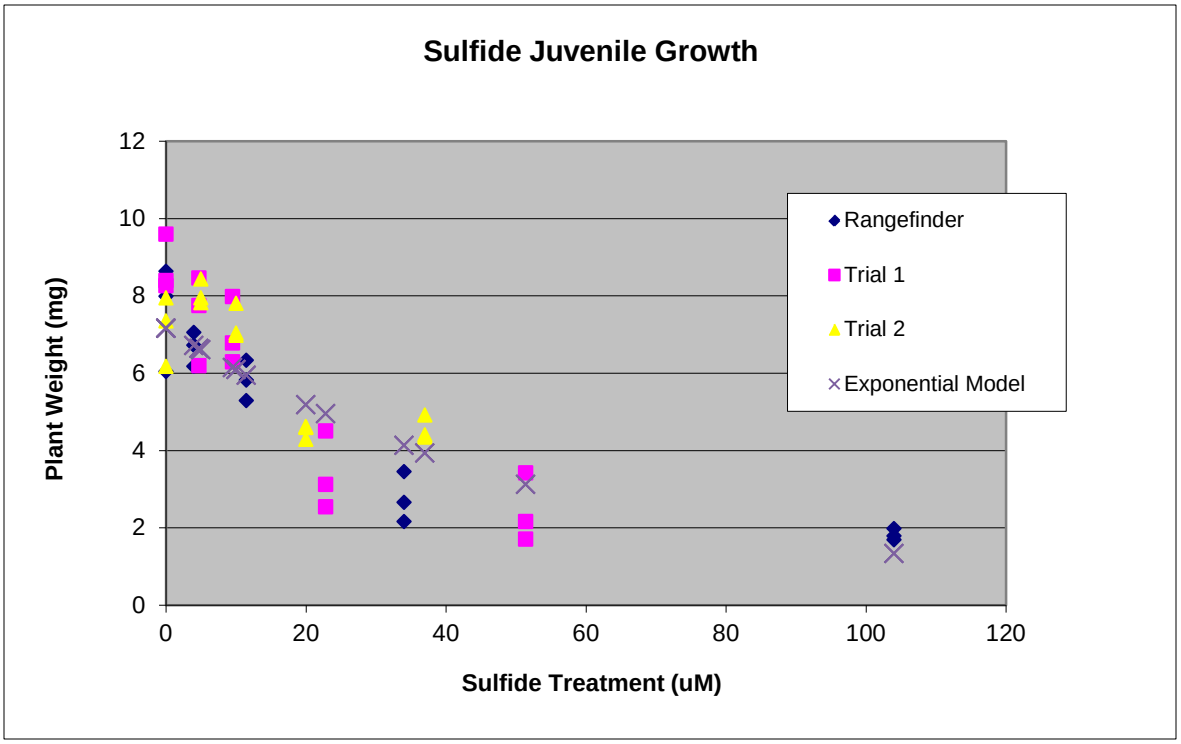


Fig. 2. Exponential decline in growth of juvenile seedlings with enhanced sulfide concentrations.