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TOXICITY OF SULFIDE TO EARLY LIFE STAGES OF WILD RICE (*ZIZANIA PALUSTRIS*)

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Abstract: The sensitivity of wild rice (*Zizania palustris*) to sulfide is not well understood. Because sulfate in surface waters is reduced to sulfide by anaerobic bacteria in sediments and historical information indicated that 10 mg/L sulfate in Minnesota (USA) surface water reduced *Z. palustris* abundance, the Minnesota Pollution Control Agency established 10 mg/L sulfate as a water quality criterion in 1973. A 21-d daily-renewal hydroponic study was conducted to evaluate sulfide toxicity to wild rice and the potential mitigation of sulfide toxicity by iron (Fe). The hydroponic design used hypoxic test media for seed and root exposure and aerobic headspace for the vegetative portion of the plant. Test concentrations were 0.3, 1.6, 3.1, 7.8, and 12.5 mg/L sulfide in test media with 0.8, 2.8, and 10.8 mg/L total Fe used to evaluate the impact of iron on sulfide toxicity. Visual assessments (i.e., no plants harvested) of seed activation, mesocotyl emergence, seedling survival, and phytoxicity were conducted 10 d after dark-phase exposure. Each treatment was also evaluated for time to 30% emergence (ET30), total plant biomass, root and shoot lengths, and signs of phytotoxicity at study conclusion (21 d). The results indicate that exposure of developing wild rice to sulfide at $\geq$3.1 mg sulfide/L in the presence of 0.8 mg/L Fe reduced mesocotyl emergence. Sulfide toxicity was mitigated by the addition of Fe at 2.8 mg/L and 10.8 mg/L relative to the control value of 0.8 mg Fe/L, demonstrating the importance of iron in mitigating sulfide toxicity to wild rice. Ultimately, determination of site-specific sulfate criteria taking into account factors that alter toxicity, including sediment Fe and organic carbon, are necessary. *Environ Toxicol Chem* 2017;36:2217–2226. © 2017 SETAC

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INTRODUCTION

Historically, the impacts of sulfate, and thus sulfide, toxicity to wild rice (*Zizania palustris L.*) in Minnesota (USA) have been addressed by using the surface water sulfate water quality standard of 10 mg/L established by the Minnesota Pollution Control Agency [1,2]. To address the practicality of this standard, an initial 21-d hydroponic study was previously performed [3] to determine the toxicity of sulfate to wild rice seeds and seedlings. The results suggested that sulfate does not adversely affect germination and early development of wild rice at concentrations $<$5000 mg/L over a 21-d hydroponic exposure period. Some effects found at high sulfate concentrations were also observed in osmotically equivalent chloride treatments, and some sulfate-specific stimulatory effects may be attributable to the effects of sulfate as a plant nutrient. Two endpoints, shoot length and leaf number, appeared to have sulfate-specific toxic responses; however, the remainder of the observed responses were likely the result of a general conductivity-induced stress and not specifically the result of sulfate. Root length appeared to be an especially sensitive endpoint to conductivity-related stress induced by chloride-dominated salt solutions [3].

Sulfate in surface waters is reduced to sulfide by anaerobic bacteria in sediments, and sulfide is known to be much more toxic to aquatic organisms than sulfate. As an extension of the original hydroponics study [3], which examined sulfate toxicity to developing wild rice, sulfide toxicity to early life stage wild rice was evaluated under varying iron (Fe) concentrations representative of those known to be present in sediment porewaters in Minnesota. The sulfide toxicity threshold under varying Fe concentrations was determined, to facilitate a better understanding of the role of Fe in altering sulfide toxicity. The primary objective of the present study was to determine the toxicity of sulfide to wild rice seeds and seedlings from the State of Minnesota. Preliminary studies were conducted to determine the most appropriate culture media and test conditions, identify sensitive test endpoints, establish a statistically valid experimental design, and determine appropriate sulfide exposure concentrations for the range of wild rice response endpoints selected. These findings will be used to further understand the possible impact of sulfate released into the environment and subsequently reduced to sulfide under varying sediment conditions, and support the efforts to re-evaluate the State of Minnesota's wild rice sulfate water quality standard of 10 mg/L [2]. Concentration–response data, including 25% inhibitory concentrations (IC25) values, and no- and lowest-observed-effect concentrations (NOEC and LOEC) for the effects of sulfide on wild rice were determined.

MATERIALS AND METHODS

Preliminary studies

Preliminary range-finding studies were conducted to establish the testing conditions necessary to maintain a hydroponic exposure to sulfide, and to determine appropriate sulfide and Fe concentrations for the definitive study. A daily-renewal hydroponic system utilizing a modified Hoagland's solution (HS-1; [4,5]) was used to test the effects of sulfide on 10

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biological endpoints in wild rice seeds and seedlings over 21 d. A summary of the experimental design and conditions is provided in Table 1.

Hydroponic media and test materials

Modified HS-1 solution [4] contained 25% ammonium (molar basis) in a mixture of ammonium and nitrate [3], and served as the base medium and diluent for all test exposures in the definitive study. Deionized water was used to prepare all solutions, and was routinely tested to ensure the absence of various organic and inorganic contaminants. The modified HS-1 macronutrients consisted of 2.55 mM NO_3^-, 0.92 mM NH_4^+, 0.12 mM $H_2PO_4^-$, 1.10 mM K^+, and 0.75 mM Ca^{2+}, 0.50 mM Mg^{2+}, and 0.50 mM SO_4^{2-}. Micronutrients included 46.3 μM boron (B), 14.9 μM Fe, 0.76 μM zinc, 0.31 μM copper, 9 μM manganese, and 0.50 μM molybdenum. The sulfide toxicity threshold under varying iron concentrations was determined, to facilitate a better understanding of the role of iron in altering sulfide toxicity. All salts were reagent-grade materials obtained from SigmaAldrich (St. Louis, MO; >98% pure). Hydrated sodium sulfide ($Na_2S \cdot 9\ H_2O$, 99.99% pure, Sigma-Aldrich) and ferric chloride ($FeCl_3$, 98.00%, Merck) were used throughout the present study. The sulfide and Fe treatments are identified in Table 1. In addition to the HS-1 (1:4 ammonium:nitrate) negative control (0.8 mg Fe/L), and HS-1 controls containing additional iron (2.8 mg and 10.8 mg Fe/L), a 100-mg boron (B)/L treatment in HS-1 (1:4) media was included as a positive control toxicant. Boron was selected as a positive control based on use in the initial hydroponic study evaluating the toxicity of sulfate and chloride [3].

Wild rice seeds

Wild rice seeds were hand-harvested from Little Round Lake in Becker County, Minnesota (USA; 46°58′13.32″N and 95°44′44.49″W), sieved through a 4-mm mesh, and then sieved through a 2-mm mesh to remove debris. Seeds were stored at 4 °C in the dark prior to test initiation. The percentage of emergence at day 21 in preliminary studies was 47.5%, and was thus considered acceptable for use based on both preliminary studies and Fort et al. [3], as a relatively modest proportion of *Zizania palustris* germinate (criteria set at ≥30%).

Exposure system

Based on the results of preliminary testing, a sulfide exposure series of 0.3, 1.6, 3.1, 7.8, and 12.5 mg/L sulfide was utilized. Test solutions were provided using a static-renewal design in 10-L hydroponic tanks. The hydroponic tanks were plastic aquaria (~35 cm × 20 cm × 15 cm deep). Each tank was equipped with 1-L baskets with inert mesh to support the seeds and seedlings. One-liter baskets to house seeds and seedlings evaluated on day 10 (visual assessments only) and day 21 (study termination, all endpoints) were placed in each of the 4 replicate tanks per treatment or control. Exposure media were replaced daily using a 70% renewal rate. Treatment tanks were randomly assigned to a position in the exposure system to account for possible variations in temperature and light intensity. Seeds selected for study were

Table 1. Experimental conditions for hydroponic evaluation of sulfide toxicity and impact of iron in *Zizania palustris*

Test substance	Sulfide (suspected toxicant) and iron (suspected to interact with sulfide)
Test concentrations	Sulfide series: <0.1 (control), 0.3, 1.6, 3.1, 7.8, 12.5 mg/L. Each sulfide series run with either 0.8, 2.8, or 10.8 mg/L Fe
Test system (species)	*Zizania palustris* (wild rice)
Initial stage	Seed, September 8, 2014 seed lot from Little Round Lake (03-0302-00)
Exposure period	10-d (mesocotyl emergence phase in dark) and 21-d (free leaf phase). Total exposure period 21 d
Selection criteria	Seed uniformity, visual quality, and activation
Exposure system	Static-renewal (daily) in controlled environmental chambers under anaerobic aquatic phase and aerobic vegetative (shoot) phase
Exposure route	Water (hydroponics)
Test vessel	10-L chamber with 1-L sub-basket equipped with mesh bottom supports for seeds
Exchange frequency	Daily, 0.7 volumes/d
Water source	Deionized water
Media	HS-1[a] modified with 1:4 ammonia:nitrate
Replication	4/treatment
Seed density	80 seeds/replicate (320 seeds/ treatment or control)
Vessel placement	Tanks are placed randomly throughout the experimental area
Positive control	Boric acid (100 mg B/L)
Test performance criteria (control)	See Table 6
Test endpoints	
Daily	Activation, mesocotyl emergence, seedling survival, and visual inspection of development (emergence and normalcy of development)
SD 10	Activation, mesocotyl emergence (%), survival, leaf number, and signs of phytotoxicity
Conclusion (SD 21)	Activation, mesocotyl emergence (%, time to 30% emergence [ET30] if possible), survival, shoot and seminal root length and weight, leaf number, second and free leaf biomass, and signs of phytotoxicity
Feeding	
Nutrient/micronutrients	HS-1 modified with 1:4 ammonia:nitrate and either 0.8, 2.8, or 10.8 mg Fe/L
Frequency	Daily, 0.7 volumes renewed
Lighting	
Photoperiod	Dark through SD 10, then 16-h light:8-h dark
Intensity (post SD 10)	5000 ± 1000 lux (measured daily at water surface)
Temperature	In all replicates, daily, 21 ± 2 °C (day), and nightly, 12 °C ± 2 °C (night)
pH, ORP, DO, and sulfide	2×/d in all replicates prior to and following renewal
Conductivity, alkalinity, hardness, ammonia, total Fe, nitrate, sulfate, phosphate, total residual oxidants	Initiation (SD 0), SD 7, SD 14, and SD 21 (conclusion) of study in a representative test replicate of each treatment

[a]Modified Hoaglund's solution.
ORP = oxidation-reduction potential; DO = dissolved oxygen; SD = standard deviation; HS-1 = Hoagland's solution.

randomly placed in each basket such that 5 seeds were added to each insert basket in accordance with a randomized design chart until each basket contained 80 seeds/replicate (320 total per exposure condition), which was adequate to evaluate concentration–response relationships and assess significant differences in the treatments relative to their respective control (i.e., the HS-1 medium with a given Fe concentration and no sulfide). For the first 10 d of the present study, the seeds were kept in the dark to promote mesocotyl emergence and development. Following the 10-d dark-phase germination and development phase, a combination of incandescent and fluorescent plant growlights was used to provide a 16:8-h light:dark photoperiod at an intensity of 5000 ± 1000 lux (lumens/m^2) at the surface of the culture media and plants.

Water temperature was maintained at $21 \pm 2\ ^\circ C$ (day) and $12 \pm 2\ ^\circ C$ (night). Test solution pH was maintained between 6.0 and 7.5 s.u. in all exposures. Within a given replicate, variation in pH was ± 0.5 s.u. for each daily measurement at time 0 (renewal) and time 24 (immediately prior to subsequent renewal), and over the course of the study. This pH range is well within the range of conditions where wild rice grows naturally. Hypoxic (dissolved oxygen < 2.0 mg/L) conditions were maintained within the hydroponic tanks; the HS-1 test medium was deoxygenated with N_2 gas, stored in a sealed carboy until use, and checked for oxygen concentration immediately prior to use. Each hydroponic tank was equipped with a 6-inch, small-bubble air stone to deliver a constant flow of N_2 gas to the tank and ensure hypoxic conditions were maintained. For hypoxic root growth and aerobic vegetative growth, the basket was placed in the hydroponic aquaria such that the seeds resided in the culture media approximately 1 cm below the air:media interface. Seeds germinated under hypoxic conditions and mesocotyls developed in aerobic conditions under this design. Plastic wire mesh was placed inside the aquaria to provide a trellis to support vegetative growth above the hypoxic culture media. Sulfide-treated test solutions were prepared daily for use in renewal. Sulfide concentrations in the test solutions were measured prior to and following each daily media renewal using an ion-selective probe. Sulfide stability in the culture media was aided by the N_2 gas balance. A summary of the present study conditions is provided in Table 1.

Water quality analyses

In each replicate tank, temperature and light intensity (lux) were measured daily throughout the 21-d study. The dissolved oxygen (aqueous and headspace; US Environmental Protection Agency [USEPA] method 360.1 [6]), pH, oxidation-reduction potential, and sulfide were measured twice daily (i.e., prior to and following solution renewal). The dissolved oxygen, oxidation-reduction potential, and sulfide (USEPA method 9215 [7]) measurements were conducted at the same water depth as seed exposure. In addition, specific conductance (conductivity; USEPA method 120.1 [8]), total hardness (USEPA method 130.2 [9]), total alkalinity (USEPA method 310.1 [10]), total iron (USEPA method 8008 [11]), total residual oxidants (USEPA method 330.5 [12]), ammonia-nitrogen (USEPA method 350.2 [13]), sulfate (USEPA method 375.4 [14]), nitrate (USEPA method 353.2 [15]), and phosphate (USEPA method 365.2 [16]) were measured in the media in a replicate of each treatment on days 0, 7, 14, and 21 (conclusion) of the study [17]. Time-weighted average sulfide concentrations were calculated in accordance with methods of the Organisation for Economic Co-operation and Development, and accounted for the variation in instantaneous concentration over time so that the area under the time-weighted average is equal to the area under the concentration curve [18].

Data collection and biological endpoints

Visual assessments only (i.e., no plants harvested) of the following endpoints (Table 2) were conducted on day 10 following dark-phase exposure to evaluate: activation (germination), mesocotyl emergence, time to emergence (expressed as the time to 30% emergence [ET30]), seedling survival, free leaf number, and abnormal development including chlorosis (phytotoxicity). Signs of chlorosis and stem or root rot were based on observation using a dissecting microscope as needed. The use of an ET30 was based on previous studies [3] of wild rice emergence revealing that in normal-appearing seeds, between 30% and 60% of mesocotyls emerged over the course of a trial. The mesocotyl emergence acceptance frequency was set at 30% in the previous study with sulfate [3] and the present study. All subbaskets were evaluated for the endpoints mentioned, as well as the following 5 endpoints at study conclusion (day 21): shoot (mesocotyl, coleoptiles, and primary leaf) weight, shoot (mesocotyl, coleoptiles, and primary leaf) length, root (seminal and rootlets) weight, seminal root length, and free leaf biomass. All weights were expressed as dry weight recorded to the nearest 0.1 mg by drying the individual parts of each seedling together in an aluminum pan in an oven at 105 °C for 24 h.

Data analysis

The experimental unit was the replicate and $\alpha = 0.05$. For measurement endpoints (i.e., weights and lengths), replicate level data were based on the mean value for all plants measured in that replicate with the exception of the ET30 data sets, which were based on median values. The statistical tests used to compare the culture media with the sulfide and B positive control differed depending on the data type and distribution for each measurement endpoint. No outliers were identified (Grubbs's test). Data that were expressed as a percentage or proportion were transformed using the arcsine square root before further analysis. No other transformations were used. The IC25 and 95% confidence intervals for appropriate endpoints were determined by linear interpolation. Normal distribution (Shapiro–Wilks' test, $\alpha = 0.05$) and equivalence of variances (Levene's test, $\alpha = 0.05$) were performed to determine parametric data sets. For measurement endpoints, comparisons between the treatments and designated controls were performed using one-way analysis of variance (ANOVA) or a nonparametric equivalent (Kruskal–Wallis ANOVA). In all cases, sulfide treatments sharing the same Fe concentration were compared against a control condition containing that same iron concentration. When the initial test was statistically significant, post hoc tests were performed, including the Bonferroni *t* test for parametric test and Dunn's nonparametric test. Treatment median ET30 values were determined by deriving the median of replicate ET 30 values. The ET30 values for each treatment were compared with their respective controls using a Mann–Whitney *U* test.

RESULTS

Exposure conditions and sulfide concentrations

Exposure solution pH was maintained at 6.0 to 7.5 s.u. in all replicates of controls and treatments and was ± 0.5 s.u. within a given replicate for each daily measurement. The dissolved oxygen concentrations were maintained at <2.0 mg/L in all

Table 2. Effects of sulfide on hydroponic development and growth of *Zizania palustris* endpoints following 10-d exposure

Treatment		Response[a]				
Sulfide[b] (mg/L)	Iron (mg/L)	Seed activation (%)	Mesocotyl emergence (%)	Seedling survival (%)	Mean free leaf (no.)	Abnormal appearance (%)
<0.01 (negative control)	0.8[c]	100.0 (0.0)	29.1 (0.46)	100.0 (0.0)	0.0 (0.0)	0.0 (0.0)
<0.01 (positive control)[d]	0.8	100.0 (0.0)	8.4[e] (0.66)	100.0 (0.0)	0.0 (0.0)	100[f](0.0)
0.3	0.8	100.0 (0.0)	28.8 (0.47)	100.0 (0.0)	0.0 (0.0)	0.0 (0.0)
1.6	0.8	100.0 (0.0)	27.8 (0.74)	100.0 (0.0)	0.0 (0.0)	0.0 (0.0)
3.1	0.8	100.0 (0.0)	24.1 (0.46)	100.0 (0.0)	0.0 (0.0)	0.0 (0.0)
7.8	0.8	100.0 (0.0)	14.4[g] (0.63)	100.0 (0.0)	0.0 (0.0)	0.0 (0.0)
12.5	0.8	100.0 (0.0)	0.0[g] (0.00)	– (–)	– (–)	– (–)
<0.01 (negative control)	2.8	100.0 (0.0)	28.1 (0.63)	100.0 (0.0)	0.0 (0.0)	0.0 (0.0)
0.3	2.8	100.0 (0.0)	27.5 (0.67)	100.0 (0.0)	0.0 (0.0)	0.0 (0.0)
1.6	2.8	100.0 (0.0)	26.9 (0.63)	100.0 (0.0)	0.0 (0.0)	0.0 (0.0)
3.1	2.8	100.0 (0.0)	25.0 (0.47)	100.0 (0.0)	0.0 (0.0)	0.0 (0.0)
7.8	2.8	100.0 (0.0)	15.6[h] (0.63)	100.0 (0.0)	0.0 (0.0)	0.0 (0.0)
12.5	2.8	100.0 (0.0)	0.0[h] (0.00)	– (–)	– (–)	– (–)
<0.01 (negative control)	10.8	100.0 (0.0)	28.8 (0.67)	100.0 (0.0)	0.0 (0.0)	0.0 (0.0)
0.3	10.8	100.0 (0.0)	29.1 (0.46)	100.0 (0.0)	0.0 (0.0)	0.0 (0.0)
1.6	10.8	100.0 (0.0)	27.2 (0.74)	100.0 (0.0)	0.0 (0.0)	0.0 (0.0)
3.1	10.8	100.0 (0.0)	26.9 (0.63)	100.0 (0.0)	0.0 (0.0)	0.0 (0.0)
7.8	10.8	100.0 (0.0)	22.2[i] (1.00)	100.0 (0.0)	0.0 (0.0)	0.0 (0.0)
12.5	10.8	100.0 (0.0)	13.8[i] (0.47)	100.0 (0.0)	0.0 (0.0)	0.0 (0.0)

[a]Mean with standard error of the mean below. Mean of 4 replicates/treatment with 80 seeds/replicate (320 seeds/treatment).
[b]Nominal sulfide concentration.
[c]HS-1 contains 0.8 mg Fe/L. Statistical comparisons made with HS-1 with 0.8, 2.8, or 10.8 mg Fe/L controls depending on treatment set analyzed to hold the nominal Fe constant during analysis.
[d]100 mg/L boric acid (positive control).
[e]Significantly less than HS-1 with 0.8 mg Fe/L, *t* test, $p < 0.001$.
[f]Significantly greater than HS-1 with 0.8 mg Fe/L, *t* test, $p < 0.001$.
[g]Significantly less than HS-1 with 0.8 mg Fe/L, Kruskal–Wallis-analysis of variance, Dunn's test, $p < 0.05$.
[h]Significantly less than HS-1 with 2.8 mg Fe/L, Kruskal–Wallis-analysis of variance, Dunn's test, $p < 0.05$.
[i]Significantly less than HS-1 with 10.8 mg Fe/L, Kruskal–Wallis-analysis of variance, Dunn's test, $p < 0.05$.
HS-1 = Hoagland's solution.

treatments, and hydroponic chamber temperatures were maintained at $21 \pm 2\ ^{\circ}C$ (day) and $12 \pm 2\ ^{\circ}C$ (night) in all replicates of controls and treatments. A summary of sulfide concentrations based on time-weighted average values measured following test solution renewal (T0) and immediately prior to renewal (T24), along with an evaluation of 24-h sulfide losses in each treatment is presented in Table 3. Inter-replicate percentage coefficient of variation (CV) within the control or a given sulfide exposure was $\leq$6% in pre- and post-test solution renewal samples based on time-weighted average concentrations. The interreplicate CV for 24-h sulfide loss based on the time-weighted average concentration was $\leq$30%. Sulfide loss between 24-h renewals ranged from 15.2 to 23.5% in the 0.8 mg Fe/L treatments, 29.9 to 55.6% in the 2.8 mg Fe/L treatments, and 87.6 to 95.4% in the 10.8 mg Fe/L treatments. The results indicate that nominal and measured sulfide concentrations in freshly prepared test solutions were very similar, but that increased Fe reduced free sulfide concentrations in a manner that was not necessarily a linear function of iron concentrations.

Control and positive control performance

The control (HS-1) seed activation, mesocotyl emergence, and seedling survival were >95%, >30%, and >90%, respectively; on study days 10 (Table 2) and 21 (Table 4), which met validity criteria previously established for hydroponic studies [3]. The HS-1 control plants were compared against those grown in a 100 mg/L B positive control known to induce phytotoxicity. The occurrence of 100% phytotoxicity indicated compliance with the pre-established test acceptability criterion of $\geq$80% [3]. In contrast, HS-1 plants exhibited no phytotoxicity. Decreased emergence, root length and weight, and free leaf weight, an increase in the median ET30, and phytotoxicity were observed in wild rice exposed to 100 mg B/L relative to the HS-1 control with 0.8 mg Fe/L.

Sulfide toxicity with 0.8 mg Fe/L

Study day 10. Exposure of wild rice to 7.8 and 12.5 mg/L sulfide decreased emergence relative to the HS-1 control with 0.8 mg Fe/L. Free leaf number was 0 in the control and all treatments (Table 2).

Study day 21. Decreased emergence, root length and weight, and free leaf weight, an increase in the median ET 30, and phytotoxicity were observed in wild rice exposed to 100 mg B/L relative to the HS-1 control with 0.8 mg Fe/L (Table 4). Exposure of wild rice to 3.1, 7.8, and 12.5 mg/L sulfide decreased emergence at day 21 relative to the HS-1 control with 0.8 mg Fe/L. Emergence was greater in seeds exposed to 12.5 mg/L sulfide with 10.8 mg Fe/L than in treatments with 0.8 and 2.8 mg Fe/L. Seeds exposed to 12.5 mg/L sulfide exhibited 21.3% emergence in the presence of 10.8 mg/L Fe compared with no emergence occurring in this same sulfide concentration in the 2 lower Fe conditions. Root length, shoot length, root biomass, shoot biomass, secondary leaf biomass, and leaf number were 0 in seedlings exposed to 12.5 mg/L sulfide with 0.8 mg Fe/L, as a result of no emergence. The ET30 (Table 5) generally increased with increasing sulfide concentration in the 0.8 mg/L Fe series (i.e., longer emergence times indicate toxicity), ranging from a median of 10 d in the control to >21 d in the 7.8 and 12.5 mg/L sulfide treatments. The ET30 values were significantly greater in the 7.8 and 12.5 mg/L sulfide treatments than in other sulfide treatments with these Fe treatments.

Overall, mesocotyl emergence was the most sensitive endpoint, and activation, seedling survival, and phytotoxicity

Table 3. Measured sulfide concentrations in hydroponic chambers at renewal and 24-h post renewal

Treatment	Time-weighted average[a] (mg/L)				
	Post renewal (T0)[b]	CV (%)	Pre-renewal (T24)[c]	CV (%)	Loss (%)
HS-1[d]	<0.01	–	<0.01	–	–
100 mg B/L/wild rice	<0.01	–	<0.01	–	–
0.3 mg/L Sulfide	0.34	1.1	0.26	1.3	23.5
1.6 mg/L Sulfide	1.56	0.8	1.31	3.1	16.0
3.1 mg/L Sulfide	3.29	1.5	2.53	2.9	23.1
7.8 mg/L Sulfide	7.71	0.7	6.54	5.4	15.2
12.5 mg/L Sulfide	12.52	1.5	10.52	3.8	16.0
HS-1d + 2.8 mg/L Fe	<0.01	–	<0.01	–	–
0.3 mg/L Sulfide + 2.8 mg/L Fe	0.31	2.1	0.20	1.3	35.5
1.6 mg/L Sulfide + 2.8 mg/L Fe	1.48	1.8	1.00	1.4	32.4
3.1 mg/L Sulfide + 2.8 mg/L Fe	3.20	1.3	1.42	2.0	55.6
7.8 mg/L Sulfide + 2.8 mg/L Fe	7.49	1.3	4.13	1.6	44.9
12.5 mg/L Sulfide + 2.8 mg/L Fe	11.91	1.5	8.35	0.9	29.9
HS-1[d] + 10.8 mg/L Fe	<0.01	–	<0.01	–	–
0.3 mg/L Sulfide + 10.8 mg/L Fe	0.33	1.3	0.02	0.0	93.9
1.6 mg/L Sulfide + 10.8 mg/L Fe	1.52	1.2	0.07	3.4	95.4
3.1 mg/L Sulfide + 10.8 mg/L Fe	3.21	1.1	0.31	3.6	90.3
7.8 mg/L Sulfide + 10.8 mg/L Fe	7.25	2.2	0.68	1.5	90.6
12.5 mg/L Sulfide + 10.8 mg/L Fe	11.75	1.6	1.46	3.5	87.6

[a]Analysis based on Organisation for Economic Co-operation and Development method 211 [6].
[b]Time-weighted average based on analysis of fresh test solutions. Limit of detection = 0.01 mg/L.
[c]Time-weighted average based on analysis of 24 h aged test solutions at prior to renewal of fresh test solutions.
[d]Modified Hoagland's solution.
HS-1 = Hoagland's solution; CV = coefficient of variation.

were the least sensitive endpoints. No emergence occurred in the 12.5 mg/L sulfide treatment containing 0.8 mg Fe/L.

Sulfide toxicity with 2.8 or 10.8 mg Fe/L

Study day 10. Exposure of wild rice to 7.8 or 12.5 mg/L sulfide significantly decreased emergence relative to the HS-1 control in both the 2.8 mg and 10.8 mg Fe/L treatments (Table 2). Leaf number was 0 in the controls and all treatments for both the 2.8 mg and 10.8 mg Fe/L treatments.

Study day 21. Exposure of wild rice to 7.8 or 12.5 mg/L sulfide significantly decreased emergence relative to the HS-1 control in both the 2.8 and 10.8 mg Fe/L treatments (Table 4). Evaluation of the effect of iron concentration on emergence at a given sulfide concentration indicated that the addition of 10.8 mg Fe/L significantly reduced the effects of sulfide on mesocotyl emergence in the 7.8 mg/L sulfide treatments (ANOVA, Bonferroni t test, $p < 0.001$) and 400 μM (Kruskal–Wallis–ANOVA, Dunn's test, $p < 0.05$), compared with equivalent sulfide treatments with the addition of 0.8 and 2.8 mg Fe/L. In the 2.8 mg Fe/L treatment series, the median ET30 ranged from 12 d in the control to >21 d in the 12.5 mg/L sulfide treatment (Table 6). The ET30 values were significantly greater in the 7.8 and 12.5 mg/L sulfide treatments than in other sulfide treatments with these iron treatments. In terms of plants exposed to 10.8 mg Fe/L (Table 6), the median ET30 ranged from 10 d in the control to >21 d in the 12.5 mg/L sulfide treatment. The ET30 values generally increased with increasing sulfide concentrations for these iron concentrations, and the median ET30 values for 7.8 and 12.5 mg/L sulfide were significantly greater than in other sulfide treatments (Mann–Whitney U test, $p \leq 0.005$; Table 5). In addition, the ET30 decreased in the 3.1 mg/L sulfide treatment with increasing Fe concentration (Mann–Whitney U test, $p \leq 0.05$). Root length, shoot length, root biomass, shoot biomass, secondary leaf biomass, and leaf number were all 0 in seedlings exposed to 12.5 mg/L sulfide with 2.8 mg Fe/L (Table 4). This was because of the lack of emergence in the 12.5 mg/L sulfide with 2.8 mg Fe/L treatment. However, these effects were not observed in the presence of 10.8 mg Fe/L.

Overall, mesocotyl emergence was the most sensitive endpoint, whereas activation, seedling survival, and phytotoxicity were the least sensitive endpoints. No emergence occurred at 12.5 mg/L sulfide in the presence of 2.8 mg Fe/L. Mesocotyl emergence, seedling growth, and survival were recorded at 12.5 mg/L sulfide with 10.8 mg Fe/L. Thus, emergence and all root and shoot measures were greater in seeds germinated and grown in the presence of 12.5 mg/L sulfide and 10.8 mg Fe/L than in those exposed to the same amount of sulfide with either 0.8 or 2.8 mg Fe/L. The formation of a fine layer of black plaque was detected on the seminal roots of rice seedlings exposed to 7.8 mg/L sulfide with 2.8 or 10.8 mg/L Fe and 12.5 mg/L sulfide with 10.8 mg/L Fe (Figure 1). The layer of plaque when removed did not produce sufficient material to analyze or investigate further. Sulfide NOEC, LOEC, chronic values (the geometric mean of the NOEC and LOEC values), and IC25 values for each Fe concentration on day 10 and day 21 are presented in Table 6.

DISCUSSION

Mesocotyl emergence was the most sensitive endpoint at sulfide concentrations ≥3.1 mg/L with 0.8 mg/L Fe and an IC25 value of 3.9 (3.5–4.3) mg/L sulfide. However, exposure of developing wild rice to sulfide concentrations ≥7.8 mg/L (with additions of 2.8 mg and 10.8 mg Fe/L and IC25 values of 7.1 [6.5–7.7] and 9.3 [8.8–9.8] mg/L, respectively) was required to significantly reduce mesocotyl emergence. Furthermore, addition of 10.8 mg/L Fe resulted in reduction of sulfide toxicity compared with lower Fe concentration treatments, based on emergence, changes in median ET30 values, and greater percentage of emergence in seeds exposed to 12.5 mg/L sulfide.

Seed activation, seedling survival, and phytotoxicity were the least sensitive endpoints. Root and shoot growth endpoints were less sensitive than emergence endpoints. The day-21 sulfide chronic values in the 0.8 mg Fe/L series ranged from 2.2 mg/L sulfide for emergence to >12.5 mg/L sulfide for

Table 4. Effects of sulfide on hydroponic development and growth of *Zizania palustris* endpoints after 21-d exposure

Treatment		Response[a]									
Sulfide[b] (mg/L)	Iron (mg/L)	Seed activation (%)	Mesocotyl emergence (%)	Seedling survival (%)	Mean seminal root biomass (g, dry wt)	Mean seminal root length (cm)	Mean shoot biomass (g, dry wt)	Mean shoot length (cm)	Mean 2° leaf biomass (g, dry wt)	Mean free leaf (no.)	Abnormal appearance (%)
<0.01 (negative control)	0.8[c]	100.0 (0.0)	44.1 (0.46)	100.0 (0.0)	0.0016 (0.0002)	6.588 (0.301)	0.0044 (0.0003)	2.567 (0.123)	0.0088 (0.0007)	2.8 (0.2)	0.0 (0.0)
<0.01 (positive control)[d]	0.8	100.0 (0.0)	8.8[e] (0.47)	100.0 (0.0)	0.0010[f] (0.0001)	3.566[f] (0.218)	0.0039 (0.0004)	2.330 (0.150)	0.0057[g] (0.0010)	2.5 (0.4)	100[h] (0.0)
0.3	0.8	100.0 (0.0)	43.1 (0.41)	100.0 (0.0)	0.0016 (0.0001)	6.012 (0.229)	0.0038 (0.0002)	2.456 (0.116)	0.0084 (0.0011)	2.7 (0.3)	0.0 (0.0)
1.6	0.8	100.0 (0.0)	41.6 (0.66)	100.0 (0.0)	0.0020 (0.0002)	5.453 (0.238)	0.0035 (0.0002)	2.309 (0.076)	0.0068 (0.0006)	3.0 (0.2)	0.0 (0.0)
3.1	0.8	100.0 (0.0)	36.6[i] (0.66)	100.0 (0.0)	0.0016 (0.0002)	5.434 (0.345)	0.0038 (0.0002)	2.468 (0.092)	0.0075 (0.0009)	2.8 (0.1)	0.0 (0.0)
7.8	0.8	100.0 (0.0)	24.4[i] (0.41)	100.0 (0.0)	0.0014 (0.0002)	4.915 (0.386)	0.0040 (0.0003)	2.840 (0.098)	0.0081 (0.0009)	3.6 (0.3)	0.0 (0.0)
12.5	0.8	100.0 (0.0)	0.0[i] (0.00)	– (–)	– (–)	– (–)	– (–)	– (–)	– (–)	– (–)	– (–)
<0.01 (negative control)	2.8	100.0 (0.0)	45.0 (0.67)	100.0 (0.0)	0.0016 (0.0001)	4.790 (0.155)	0.0042 (0.0003)	2.511 (0.078)	0.0073 (0.0008)	3.2 (0.2)	0.0 (0.0)
0.3	2.8	100.0 (0.0)	43.4 (0.46)	100.0 (0.0)	0.0019 (0.0002)	5.315 (0.283)	0.0041 (0.0004)	2.531 (0.075)	0.0069 (0.0009)	3.1 (0.2)	0.0 (0.0)
1.6	2.8	100.0 (0.0)	40.9 (0.46)	100.0 (0.0)	0.0017 (0.0001)	5.890 (0.427)	0.0043 (0.0004)	2.571 (0.136)	0.0074 (0.0009)	3.7 (0.2)	0.0 (0.0)
3.1	2.8	100.0 (0.0)	40.0 (0.67)	100.0 (0.0)	0.0014 (0.0001)	5.506 (0.290)	0.0038 (0.0002)	2.615 (0.125)	0.0066 (0.0008)	3.1 (0.2)	0.0 (0.0)
7.8	2.8	100.0 (0.0)	32.8[j] (0.57)	100.0 (0.0)	0.0013 (0.0001)	5.127 (0.403)	0.0035 (0.0005)	2.331 (0.131)	0.0066 (0.0010)	2.6 (0.3)	0.0 (0.0)
12.5	2.8	100.0 (0.0)	0.0[k] (0.00)	– (–)	– (–)	– (–)	– (–)	– (–)	– (–)	– (–)	– (–)
<0.01 (negative control)	0.8	100.0 (0.0)	46.3 (0.47)	100.0 (0.0)	0.0016 (0.0001)	5.356 (0.299)	0.0035 (0.0002)	2.431 (0.112)	0.0072 (0.0009)	2.9 (0.2)	0.0 (0.0)
0.3	10.8	100.0 (0.0)	45.9 (0.46)	100.0 (0.0)	0.0012 (0.0001)	5.120 (0.285)	0.0034 (0.0001)	2.293 (0.124)	0.0073 (0.0005)	2.5 (0.2)	0.0 (0.0)
1.6	10.8	100.0 (0.0)	43.4 (0.66)	100.0 (0.0)	0.0014 (0.0001)	4.576 (0.221)	0.0032 (0.0002)	1.962 (0.071)	0.0061 (0.0006)	2.8 (0.3)	0.0 (0.0)
3.1	10.8	100.0 (0.0)	45.6 (0.63)	100.0 (0.0)	0.0015 (0.0001)	5.402 (0.078)	0.0041 (0.0002)	2.784 (0.080)	0.0082 (0.0004)	3.3 (0.2)	0.0 (0.0)
7.8	10.8	100.0 (0.0)	41.9[l] (0.63)	100.0 (0.0)	0.0015 (0.0001)	4.640 (0.287)	0.0038 (0.0002)	2.542 (0.065)	0.0078 (0.0005)	2.9 (0.1)	0.0 (0.0)
12.5	10.8	100.0 (0.0)	21.3[m] (0.67)	100.0 (0.0)	0.0014 (0.0001)	5.522 (0.288)	0.0038 (0.0003)	2.776 (0.120)	0.0091 (0.0007)	3.3 (0.1)	0.0 (0.0)

[a]Mean with standard error of the mean below. Mean of 4 replicates/treatment with 80 seeds/replicate (320 seeds/treatment).
[b]Nominal sulfide concentration.
[c]HS-1 contains 0.8 mg Fe/L. Statistical comparisons made without HS-1 with 0.8, 2.8, or 10.8 mg Fe/L controls depending on treatment set analyzed to hold the nominal Fe constant during analysis.
[d]100 mg/L boric acid (positive control).
[e]Significantly less than HS-1 with 0.8 mg Fe/L, *t* test, $p < 0.001$.
[f]Significantly less than HS-1 with 0.8 mg Fe/L, *t* test, $p = 0.005$.
[g]Significantly less than HS-1 with 0.8 mg Fe/L, *t* test, $p = 0.025$.
[h]Significantly greater than HS-1 with 0.8 mg Fe/L, *t* test, $p < 0.001$.
[i]Significantly less than HS-1 with 0.8 mg Fe/L, Kruskal–Wallis-analysis of variance, Dunn's test, $p < 0.05$.
[j]Significantly less than HS-1 with 2.8 mg Fe/L, Kruskal–Wallis-analysis of variance, Dunn's test, $p < 0.05$.
[k]Significantly less than HS-1 with 2.8 mg Fe/L, Kruskal–Wallis-analysis of variance, Dunn's test, $p < 0.05$.
[l]Significantly less than HS-1 with 10.8 mg Fe/L, Kruskal–Wallis-analysis of variance, Dunn's test, $p < 0.05$.
[m]Significantly less than HS-1 with 10.8 mg Fe/L, Kruskal–Wallis-analysis of variance, Dunn's test, $p < 0.05$.
HS-1 = Hoagland's solution.

seed activation, survival, and phytotoxicity endpoints. The sulfide chronic values for replicates exposed to 2.8 mg and 10.8 mg Fe/L ranged from 4.9 mg/L sulfide for emergence to >12.5 mg/L sulfide for seed activation, survival, and phytotoxicity endpoints, providing evidence of a trend toward decreased sulfide toxicity with increased Fe concentration. Historical studies of sulfide toxicity were reviewed by Lamers et al. [19]. Although no studies with wild rice were included, studies with *Oryza sativa* (Asian rice) in hydroponic culture showed reduced productivity at 5 mg/L sulfide [20] and 0.9 mg/L sulfide [21], and radial oxygen loss and reduced at nutrient uptake at 0.3 to 1.9 mg/L sulfide [22]. More recently, Pastor et al. [23] demonstrated sulfide toxicity to wild rice at 0.3 mg/L sulfide, which was markedly less than that found in the present study.

Table 5. Median emergence time endpoint in wild rice exposed to sulfide in the presence of iron on day 21[a]

	Median emergence time (d)						
Treatment iron (mg/L)	HS-1[b]	100 mg/L BA[c]	0.3 mg/L S^{2-}	1.6 mg/L S^{2-}	3.1 mg/L S^{2-}	7.8 mg/L S^{2-}	12.5 mg/L S^{2-}
0.8	10	>21[d]	11	12	15	>21[d]	>21[d]
2.8	12	–	12	12	12[e]	19[f]	>21[d]
10.8	10	–	10	12	12[e]	15[g]	>21[b]

[a]Based on time (in days) required to achieve 30% emergence.
[b]Negative control.
[c]Boric acid, positive control.
[d]Significantly greater than HS-1with 0.8 mg Fe/L, Mann–Whitney U test, $p < 0.001$.
[e]Significantly less than 3.1 mg/L sulfide with 0.8 mg Fe/L, Mann–Whitney U test, $p \leq 0.05$.
[f]Significantly greater than HS-1with 2.8 mg Fe/L, Mann–Whitney U test, $p = 0.005$.
[g]Significantly greater than HS-1with 10.8 mg Fe/L Mann–Whitney U test, $p = 0.001$.
BA = boric acid; HS-1 = Hoagland's solution.

Table 6. Summary of numerical endpoints determined on days 10 and 21[a]

	Day 10				Day 21			
Endpoint	NOEC[b] (mg/L S^{2-})	LOEC[c] (mg/L S^{2-})	ChV[d] (mg/L S^{2-})	IC25[e] (mg/L S^{2-})	NOEC (mg/L S^{2-})	LOEC (mg/L S^{2-})	ChV (mg/L S^{2-})	IC25 (mg/L S^{2-})
Sulfide + 0.8 mg Fe/L								
Activation	12.5	>12.5	>12.5	>12.5	12.5	>12.5	>12.5	>12.5
Emergence (%)[f]	3.1	7.8	4.9	3.5 (3.1–3.9)	1.6	3.1	2.2	3.9 (3.5–4.3)
Emergence (ET30)[f]	–	–	–	–	3.1	7.8	4.9	–
Survival	12.5	>12.5	>12.5	>7.8[g]	12.5	>12.5	>12.5	>7.8[g]
Shoot weight	–	–	–	–	7.8	12.5	9.8	>7.8[g]
Shoot length	–	–	–	–	7.8	12.5	9.8	>7.8[g]
Root weight	–	–	–	–	7.8	12.5	9.8	>7.8[g]
Root length	–	–	–	–	7.8	12.5	9.8	7.6 (7.1–8.1)
Leaf number	12.5	>12.5	>12.5	>7.8[g]	7.8	12.5	9.8	>7.8[g]
Leaf biomass	–	–	–	–	7.8	12.5	9.8	>7.8[g]
Phytotoxicity	12.5	>12.5	>12.5	>7.8[g]	12.5	>12.5	>12.5	>7.8[g]
Sulfide + 2.8 mg Fe/L								
Activation	12.5	>12.5	>12.5	>12.5	12.5	>12.5	>12.5	>12.5
Emergence (%)[f]	3.1	7.8	4.9	5.7 (5.3–6.1)	3.1	7.8	4.9	7.1 (6.5–7.7)
Emergence (ET30)[f]	–	–	–	–	3.1	7.8	4.9	–
Survival	12.5	>12.5	>12.5	>7.8[g]	12.5	>12.5	>12.5	>7.8[g]
Shoot weight	–	–	–	–	7.8	12.5	9.8	>7.8[g]
Shoot length	–	–	–	–	7.8	12.5	9.8	>7.8[g]
Root weight	–	–	–	–	7.8	12.5	9.8	>7.8[g]
Root length	–	–	–	–	7.8	12.5	9.8	>7.8[g]
Leaf number	12.5	>12.5	>12.5	>7.8[g]	7.8	12.5	9.8	>7.8[g]
Leaf biomass	–	–	–	–	7.8	12.5	9.8	>7.8[g]
Phytotoxicity	12.5	>12.5	>12.5	>7.8[g]	12.5	>12.5	>12.5	>7.8[g]
Sulfide + 10.8 mg Fe/L								
Activation	12.5	>12.5	>12.5	–	12.5	>12.5	>12.5	>12.5
Emergence (%)	3.1	7.8	4.9	8.5 (8.2–8.8)	3.1	7.8	4.9	9.3 (8.8–9.8)
Emergence (ET30)	–	–	–	–	3.1	7.8	4.9	–
Shoot weight	–	–	–	>12.5[g]	12.5	>12.5	>12.5	>12.5[g]
Shoot length	–	–	–	–	12.5	>12.5	>12.5	>12.5[g]
Root weight	–	–	–	–	12.5	>12.5	>12.5	>12.5[g]
Root length	–	–	–	–	12.5	>12.5	>12.5	>12.5[g]
Leaf number	12.5	>12.5	>12.5	>12.5[g]	12.5	>12.5	>12.5	>12.5[g]
Leaf biomass	–	–	–	–	12.5	>12.5	>12.5	>12.5[g]
Phytotoxicity	12.5	>12.5	>12.5	>12.5[g]	12.5	>12.5	>12.5	>12.5[g]

[a]Nominal concentrations.
[b]No-observed-effects concentration.
[c]Lowest-observed-effects concentration.
[d]Chronic value (geometric mean of NOEC and LOEC value). Represents the estimated threshold of toxicity.
[e]25% inhibitory concentration determined by linear interpolation with 95% confidence intervals in parentheses.
[f]No emergence recorded at 12.5 mg S^{2-}/L.
[g]Reported as greater than highest concentration in which mesocotyl emergence was observed. No emergence was noted in the 12.5 mg S^{2-}/L treatment with either 0.8 or 2.8 mg Fe/L.
NOEC = no-observed-effects concentration; LOEC = lowest-observed-effects concentration; ChV = chronic values; IC25 = 25% inhibitory concentration; ET30 = time to 30% emergence.

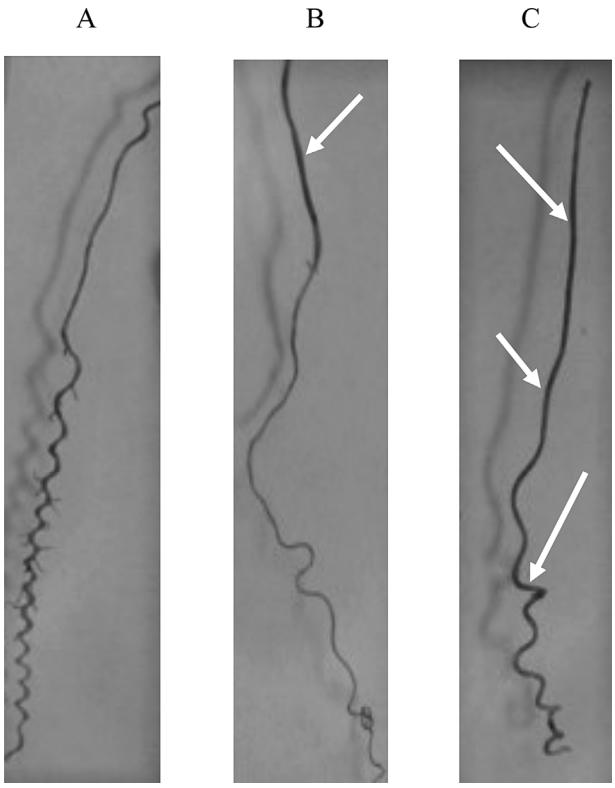

Figure 1. Representative seminal roots from (**A**) HS-1 control containing <0.01 mg/L sulfide and 0.8 mg/L Fe, (**B**) 7.8 mg/L sulfide with 2.8 mg/L Fe, and (**C**) 7.8 mg/L sulfide with 10.8 mg/L Fe. Note normal root fibers and absence of iron sulfide (FeS) plaque in seminal root from the control (**A**), increase in the formation of FeS plaque at the upper region (arrow) of the root in seminal root from the 7.8 mg/L sulfide with 2.8 mg/L Fe treatment (**B**), and more widespread FeS plaque (arrows) formed on the seminal root from the 7.8 mg/L sulfide with 10.8 mg/L Fe treatment (**C**).

However, the effects measured were on juvenile seedling growth and development using seedlings produced from seeds that were allowed to germinate and grow to 1 to 2 cm (over 5–7 d) in aerobic deionized water, whereas the present study initiated exposure in ungerminated seeds. Both studies utilized a modified Hoagland's solution [4,5], with the studies by Pastor et al. [23] containing one-fifth strength solution and 5 mM piperazine-N,N′-bis buffer and the present study using modified HS-1 solution [4] containing 25% ammonium (molar basis) in a mixture of ammonium and nitrate. The hydroponics design [24] used total hypoxia to maintain sulfide levels, but exposed the vegetative portion of the rice plants to levels of sulfide much greater than would be expected in nature. The design of the hydroponics system used in the present study allowed the seed, mesocotyl, and early primary leaf (shoot) to be exposed to the hypoxic media with sulfide, which was supported by peer review of studies supporting the re-evaluation of the State of Minnesota's surface water quality standard for sulfate [1,2]. More ecologically realistic test conditions were recommended by peer review [24], and thus the basis for the design was a scaled-down model of ponds in which wild rice grows naturally. The primary differences between the laboratory hydroponics study and rice growing naturally were the lack of sediment in the simplified, but highly controlled hydroponics and omission of the floating leaf phase. In the case of the hydroponics, allowing a floating leaf phase would have resulted in artificially greater exposure to sulfide because of the high levels of sulfide in the media, which are not generally present at the surface of pond water. Oxidation of free sulfide in the water column resulting from greater oxygen levels naturally reduces free sulfide levels exposed to the floating leaves of wild rice.

Based on measured sulfide concentrations, Fe substantially reduced free sulfide concentrations in the 10.8 mg Fe/L treatment relative to the 0.8 mg Fe/L treatment. The effect of 2.8 mg Fe/L on free sulfide concentrations fell between the 0.8 and 10.8 mg Fe/L treatments. These observations, combined with differences in wild rice responses to sulfide across different iron concentrations, demonstrate the ability of Fe to reduce sulfide toxicity to wild rice. Free sulfide loss between 24-h renewals ranged from 19.6 to 23.5% with 0.8 mg Fe/L, 32.4 to 55.6% with 2.8 mg Fe/L, and 87.6 to 95.4% with 10.8 mg Fe/L, based on time-weighted average measurements. The loss was presumably partly the result of degradation, but primarily complexation with iron. These results provide evidence that Fe reduces free sulfide concentrations, but not necessarily as a linear function of Fe concentration [25–27]. Sulfide levels in pond sediment are determined by sulfate levels, availability, temperature, oxidation-reduction potential, pH, total organic carbon, Fe^{2+} levels, and speciation [21,28]. In some cases, sediment Fe^{2+} concentration may be inadequate to detoxify the sulfide by deposition of iron sulfide (FeS), and only some sediment will exist as FeS, even with large amounts of Fe. Although less toxic than sulfide, FeS can adversely affect the root systems of aquatic plants. Sensitivity of grass species (including wild rice) to sulfide has been studied for many years. Since the late 1950s, sulfide phytotoxicity has been described historically by rotting roots, black (FeS plaque) root, leaf discoloration, and poor growth and yield [29–31] because of sulfide-induced nutritional deficiencies resulting from poor uptake and utilization of critical nutrients [20,22,29–33]. These deficiencies result in potential inhibition of various oxidases, compromising metabolic capacity, inducing oxidative stress, and reducing gas exchange [34–38] in the root systems. Detoxification of sulfide by rice requires radial oxygen loss from roots to the rhizosphere as described by Armstrong and Armstrong [29]. These investigators provided the first specific anatomical assessment of radial oxygen loss inhibition by sulfide, blockage of vascular systems, and inhibition of lateral root emergence in rice, which correspond to the toxicological impact on the rice plant. Armstrong and Armstrong [29] found that adventitious and fine lateral roots of rice exposed to sulfide had reduced radial oxygen loss to the rhizosphere atomically characterized as being thickened, resulting in inhibition of the apical cortical gas space system. More recent studies [39,40] have demonstrated mitochondria-based detoxification of sulfide primarily in the roots. Functional isoforms of O-acetylserine-(thiol)lyase C (OASTL), specifically OAS-C, detoxify sulfide primarily in the roots [41] by catalyzing the conversion of sulfide and O-acetylserine to cysteine.

In the present study, black plaque was found on the seminal roots exposed to >7.8 mg/L sulfide and 2.8 or 10.8 mg/L Fe. However, root blackening is often observed in plants growing in sulfide-laden sediment. In the present hydroponics study, limited root blackening was found, as expected, because sediment cofactors such as organic carbon and microbial flora are likely required to facilitate the process. Although it is plausible that OAS-C was responsible for detoxifying a portion of the sulfide to which the wild rice seedlings were exposed in the present study; based on the daily rate of sulfide decay (~30%), the seedlings were still exposed to a significantly high level of free sulfide during the study. Thus, enzymatic sulfide detoxification in the roots cannot explain the decreased toxicity of sulfide we observed even at

the lower Fe concentration on a physiological level. Sulfide toxicity to wild rice is also tissue dependent, with the mesocotyl and roots being less susceptible to free sulfide toxicity and the photosynthetic portion being more susceptible to sulfide. On a larger scale, to properly evaluate sulfide toxicity to wild rice, both free sulfide and complexed sulfide need to be considered, based on the appearance of black plaque on the roots of wild rice seedlings from the higher sulfide and Fe treatments and the reduction in free sulfide toxicity by Fe found in the present study.

CONCLUSIONS

The results of the present study indicate that exposure of developing wild rice (mesocotyl emergence) to sulfide-induced toxicity $\geq$3.1 mg/L sulfide in the presence of 0.8 mg Fe/L, and $\geq$7.8 mg/L sulfide in the presence of 2.8 or 10.8 mg Fe/L at study day 21. Mesocotyl emergence was the most sensitive endpoint, and growth endpoints were less sensitive. Increasing Fe concentrations reduced the toxic effects of sulfide to wild rice. Ultimately, determination of site-specific sulfate criteria considering factors that alter toxicity, including sediment Fe and organic carbon, are necessary to adequately address the potential impact of sulfate in surface waters. Additional study of the larger significance of the hydroponics study is warranted, taking into account an aquatic life cycle evaluation of sediment sulfide toxicity to wild rice using a sediment microcosm.

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Data availability—Data, associated metadata, and calculation tools are available from the corresponding author (djfort@fortlabs.com)

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