

March 2015 proposed approach for Minnesota's sulfate standard to protect wild rice

Minnesota Pollution Control Agency

March 24, 2015

Introduction

Wild rice is an important plant species in Minnesota. Wild rice provides food for waterfowl, is economically important to those who harvest and market it, and is also an important cultural resource to many Minnesotans.

Sulfate is a natural chemical commonly found in surface and groundwater. It can also be found at varying concentrations in discharges from permitted facilities such as mining operations, municipal wastewater treatment plants, and industrial facilities.

This recognition of the importance of wild rice in Minnesota, and the observed positive relationship between wild rice and water bodies with lower sulfate levels, led to the adoption of a wild rice sulfate standard in 1973. Recent questions about implementation of the standard led to interest in further understanding the effects of sulfate on wild rice to inform a review of the wild rice sulfate standard.

In 2011, the Minnesota Legislature directed the Minnesota Pollution Control Agency (MPCA) to conduct research on the effects of sulfate and other substances on the growth of wild rice. The study began in 2012.

This document summarizes the MPCA's analysis of the study results and other available information, and outlines a proposed approach for protecting wild rice from sulfate pollution. In addition, this document discusses implementation questions related to the current standard, and a proposed approach to clarify where the standard applies.

Summary

Following the development of a detailed research protocol in 2011, in 2012 the MPCA contracted with groups of scientists at the University of Minnesota's Duluth and Twin Cities campuses to undertake a Wild Rice Sulfate Standard Study.

The study's main hypothesis was that wild rice is affected by sulfate via the conversion of sulfate to sulfide dissolved in the water in the sediment, known as the sediment porewater. The study focused on collecting data on the relationship between sulfate, sulfide, and wild rice through multiple study components: field surveys, laboratory hydroponic experiments, and outdoor mesocosm experiments in containers.

The parallel study components each have a specific purpose and associated strengths and limitations (Table 1). The study was designed so that the individual components together provide a better understanding of the effects of sulfate on wild rice. The study components are:

- Field survey of wild rice habitats to investigate physical and chemical conditions correlated with the presence or absence of wild rice, including sulfate in surface water and sulfide in the sediment porewater of 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 mesocosm experiments using natural sediments to determine the response of wild rice to a range of sulfate concentrations in the surface water, and

associated sediment porewater sulfide concentrations in the rooting zone, across the growing season.

- Collection and analysis of rooting zone depth profiles of dissolved chemicals in wild rice container experiments and field sites to characterize sulfate, sulfide, iron and other constituents.
- Sediment incubation laboratory experiments to explore the difference ambient temperature has on the rate that elevated sulfate concentrations in water enter underlying sediment and convert to sulfide, and to what degree sulfate is later released back into the overlying water.

Each of the reports for the study components may be accessed via the MPCA's wild rice sulfate standard web page at www.pca.state.mn.us/ktqh1083 (a link to an FTP site with all the reports and data is available on this page). A brief summary of the study is also available on the web page.

During the first half of 2014, MPCA staff integrated the study results, analyzed the data as a whole, received input from MPCA's Wild Rice Standards Study Advisory Committee, and reviewed existing monitoring data and other relevant scientific studies/information. Based on these inputs, MPCA developed a draft analysis that was the subject of a scientific peer review conducted during summer 2014 (ERG 2014, available at www.pca.state.mn.us/kppq38rq).

Following the peer review, MPCA scientists completed additional analyses based on the recommendations of the peer reviewers and feedback received from the Wild Rice Standards Study Advisory Committee (a list of committee members is available at www.pca.state.mn.us/u3uw4ya).

Through the study and subsequent analysis, MPCA has learned the following:

- Wild rice is vulnerable to elevated sulfate concentrations because sulfate can be converted to sulfide by bacteria in the sediment where wild rice is rooted. Sulfide's toxicity to animals and plants, including wild rice, varies with its concentration.
- The protective threshold of sulfide in porewater is 0.165 milligrams per liter, or 165 micrograms per liter; the likelihood of wild rice being present in a lake or stream declines as sediment porewater sulfide levels increase above that concentration.
- Data from the mesocosm container studies demonstrate that as sulfate concentrations in surface water increase, the sulfide concentrations in the sediment also increase.
- In Minnesota lakes and streams, the efficiency of this conversion of sulfate to sulfide is highly variable due to environmental differences. The most important of these differences are the concentrations of iron and organic carbon in the sediment.
- Elevated sulfate has the potential to negatively impact wild rice. However, no single concentration of sulfate is protective of wild rice in all water bodies; sulfate is converted to sulfide at a different efficiency in each water body, depending on iron and total organic carbon concentrations in the sediment.
- The different efficiency of sulfate conversion among water bodies can be characterized by an equation. The equation can then be used to calculate a protective sulfate concentration for a water body that will still allow the wild rice population to self-perpetuate over the long term.
- The equation is founded in a model that considers the variability in sediment iron and total organic carbon levels among wild rice water bodies. The inputs for the equation are the concentrations of iron and organic carbon in sediment (Equation 1).

$$\text{Sulfate} = 0.0000136 \text{ Organic Carbon}^{-1.410} \text{ Sediment Iron}^{1.956} \quad (\text{Equation 1})$$

(Sulfate is expressed in milligrams per liter; organic carbon is percent total organic carbon in the sediment; sediment iron is micrograms extractable iron per gram sediment.)

Equation-based approaches for identifying a protective value of a chemical have been incorporated into water quality standards for other chemicals of concern, including ammonia and some metals. A general process to calculate the protective sulfate threshold is shown in Figure 1.

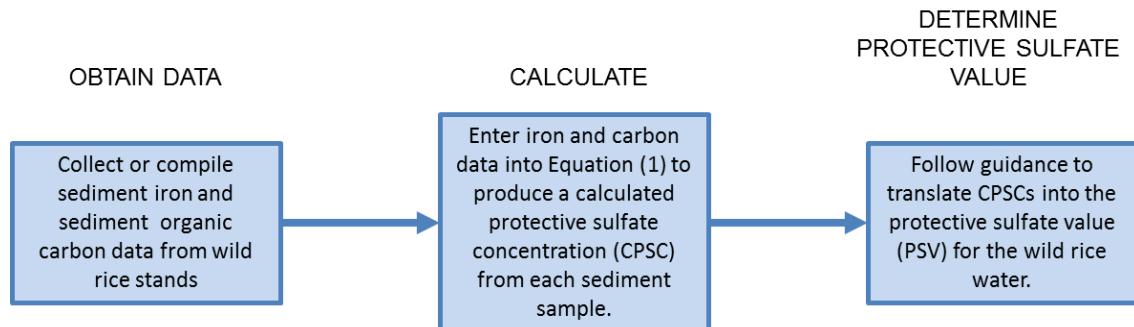


Figure 1. Flowchart of a process to calculate the protective sulfate value (PSV) for each wild rice water. This proposed approach to determining a PSV will be further discussed and refined prior to, and during, rulemaking.

Table 1. Purpose, strengths, and limitations of study components.

	Field survey	Laboratory hydroponic experiments		Outdoor container experiments	Collection and analysis of rooting zone depth profiles	Sediment incubation laboratory experiments
		Sulfate (SO_4)	Sulfide (H_2S)			
Main purpose	Expand understanding of environmental conditions correlated with presence/absence of wild rice	Evaluate effects of sulfate on wild rice seed germination and growth of sprouts	Evaluate effects of sulfide on wild rice seed germination and growth of sprouts	Evaluate effects of sulfate on wild rice plants over full life cycle, and multiple years	Characterize sulfate, sulfide, and iron in the rooting zone of wild rice container experiments and field sites	Evaluate effect of temperature on movement of sulfate into and out of underlying sediment
Endpoints	Concentrations of chemicals in surface water & rooting zone (e.g. SO_4 & H_2S vs. wild rice occurrence)	Growth of wild rice sprouts (biomass, root and shoot elongation); germination rate of seeds	Growth of wild rice sprouts (biomass, root and shoot elongation); germination rate of seeds.	Growth of wild rice (biomass, plus number and weight of seeds); sulfide concentrations in rooting zone	Concentrations of sulfate, sulfide, and iron in porewater	Sulfate concentrations in overlying water over time; SO_4 , iron, H_2S , and anion tracers in sediment porewater; simple model
Key strengths	Most reflective of actual environmental conditions; multiple wild rice stands and breadth of characteristics sampled	Controlled dose-response experiment; controlled exposure to known concentrations of SO_4	Controlled dose-response experiment; controlled exposure to known concentrations of H_2S	Controlled dose-response experiment. Includes natural sediment matrix as rooting environment; involves entire growth cycle, multiple years	Provides additional data to understand and interpret container experiments and field sites	Controlled experiment with natural sediment and water
Key limitations	Least controlled; annual visit for most sites, 3x/year for a subset; not definitive on cause and effect	Only evaluates early growth stages; leading hypothesis is that sulfate is converted to sulfide, which is directly toxic	Only evaluates early growth stages; unable to simultaneously keep roots anaerobic and shoots aerobic	Full effect of sulfate may take longer than several years to realize; no groundwater movement	Utility lies in the integration of this data with the other study components, not in this data set alone	Provides preliminary assessment of sediment from two sites that may inform but is not fully transferrable to other sites; no groundwater movement; no wild rice plants grown

Supporting scientific information

Definitions

Sulfate always refers to sulfate in surface water, which can diffuse into sediment and be converted to sulfide by bacteria.

Sulfide always refers to sulfide in porewater (with the minor exception of acid-volatile sulfide, or AVS, which is mentioned once in this report). Sulfide refers to the sum of several different forms that vary according to pH: Below pH 7.0 hydrogen sulfide (H_2S) is dominant, and above pH 7.0 bisulfide (HS^-) is dominant. It is thought that hydrogen sulfide is the more toxic form.

Two forms of **iron** in the sediment of wild rice waters are discussed in this report: iron in the porewater of sediment (which is always called porewater iron) and sediment iron. Sediment iron, which includes porewater iron, is the iron that is thought to potentially interact with sulfide (it is the iron that goes into solution when a sediment sample is extracted with 0.5 N hydrochloric acid for half an hour at 80 degrees C). Porewater iron is a very small proportion—less than 3%—of extractable iron, which is the main reservoir of iron that can interact with sulfide.

AVS (acid-volatile sulfide) is liberated from a sediment sample when 6 N hydrochloric acid is added to a sediment sample.

Sediment Total S is the concentration of sulfur in a sediment sample.

Total Organic Carbon (TOC) is a measure of the organic matter in the sediment that is available for decomposition by bacteria.

1) The dose-response relationship between sulfide and wild rice

Wild rice grows in aquatic systems (lakes and rivers) where it germinates and roots itself on the bottom substrate (otherwise known as sediment) that is perpetually saturated with water.

Water saturation of soil has profound consequences for plants; the lack of oxygen greatly alters the chemistry and presents a challenging environment. When oxygen is not available, natural bacteria respire other chemicals. When bacteria respire sulfate, they release sulfide into the sediment porewater, which is potentially toxic to virtually all plants and animals. In a review article, Lamers et al. (2013) found that, in general, freshwater plants are more sensitive to sulfide than marine plants and that white rice experiences sulfide toxicity at sulfide concentrations of 10 micromolar sulfide (320 micrograms of sulfide per liter). If a protective sulfide concentration were developed for white rice, it would therefore have to be lower than 320 micrograms per liter, and would perhaps be similar to MPCA's proposed protective concentration for wild rice of 165 micrograms per liter.

It's therefore expected that at some concentration, sulfide is toxic to wild rice. The question is, what is the critical concentration of porewater sulfide that allows wild rice to germinate, grow, reproduce, and perpetuate over many generations?

The relationship between the concentration of a chemical and the effects of that chemical on the growth or survival of an organism is termed by toxicologists to be the *dose-response relationship*, which is generically represented in Figure 2 as a logistic curve. Biological response data are fitted to curves and these curves may be used to represent the response of an organism to a chemical. Curves are routinely used to derive the concentration associated with a particular level of negative effect on the organism. For instance "Effect Concentration_{percent}" values can be derived from such curves. EC₁₀, for example, is the concentration estimated to reduce the response (expressed as an endpoint such as growth, survival, reproduction, presence, etc.) of the organism by 10% relative to the control. Effect concentrations can be

calculated for a variety of response variables. Different response variables, as quantified by effect concentrations, will not necessarily produce similar thresholds. For instance, reproduction (e.g., seed production) may be more sensitive to impacts from a chemical than growth (e.g., change in biomass), so that for a given chemical the EC₁₀ may be lower for reproduction than for growth.

The MPCA uses the calculation of EC₁₀ values as a way to establish the proposed protective concentration of sulfide for wild rice. The agency had originally calculated this concentration based on the EC₂₀, but the peer reviewers suggested that using EC₂₀ was not adequately protective.

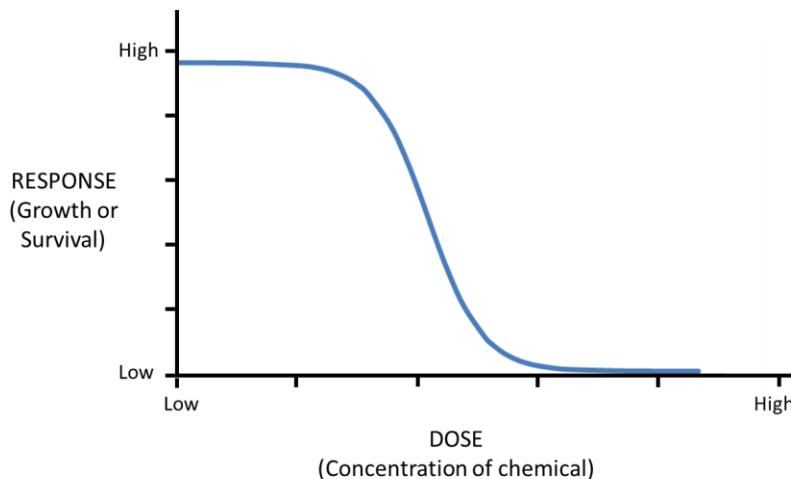


Figure 2. Generic schematic representation of a dose-response relationship for the concentration of a chemical on the measured effect (response) of an organism. At low chemical concentrations, the organism can tolerate the chemical, but toxicity (as measured by the response) increases as chemical concentrations rise.

The MPCA wild rice sulfate study collected data from three different study components, laboratory hydroponic experiments, outdoor mesocosm experiments, and field survey data. EC₁₀ sulfide concentrations can be estimated for different responses from all three components. MPCA relies on the EC₁₀ from the field survey to determine the protective concentration of sulfide, because the field data best characterize the conditions under which wild rice populations are self-perpetuating over many generations. The hydroponic and mesocosm experiments examined specific response variables, for which EC₁₀ sulfide values can be estimated. The EC₁₀ values from all three study components can be compared to each other, but are not estimates of the same responses, and therefore would not necessarily be expected to converge on a common concentration.

In the field survey, the biological response measured was the density of wild rice stems around the boat at the location where sediment was sampled to measure porewater sulfide. For the logistic curve fitting, the response variable was the presence or absence of wild rice at sites, with presence defined as at least 2 stems per square meter. The dose variable was the concentration of sulfide in the sediment porewater at the site. The EC₁₀ for the field survey is the porewater sulfide concentration at which there is a 10% reduction in the probability that wild rice would be observed at any given site (Figure 3). The field survey data EC₁₀ is 165 micrograms sulfide per liter, which MPCA proposes as the porewater sulfide concentration that is protective of growth and reproduction for wild rice populations.

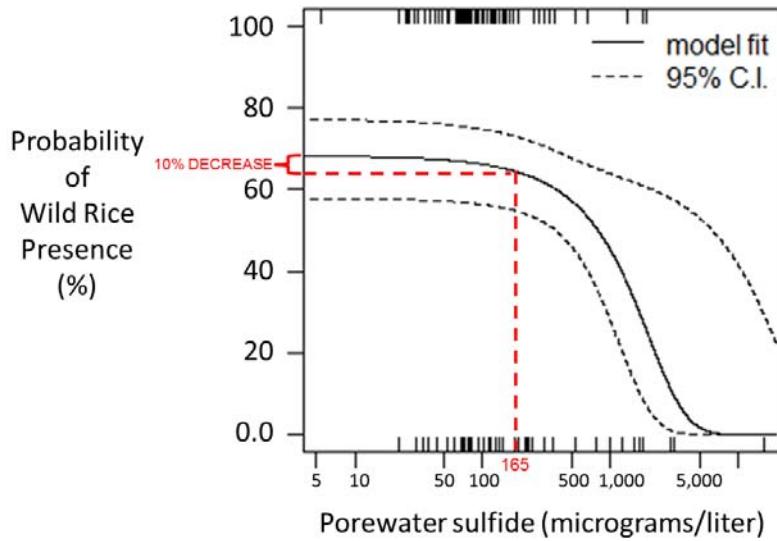


Figure 3. Logistic regression of wild rice presence (at a minimum of 2 stems per square meter) for 112 different field sites (cultivated paddies are not included). A sulfide concentration of 165 micrograms per liter is associated with a 10% reduction in the probability of wild rice presence, compared to the maximum observed probability. The 95% confidence interval is larger at higher sulfide concentrations because there are fewer data points above 500 micrograms per liter.

Estimates of effect concentrations are described individually for the mesocosm, hydroponic, and field study components because each are unique in their measured responses. Figure 4 shows estimated EC₁₀ values for each of the three study components plotted in comparison to the 165 micrograms per liter sulfide concentration. While they are quantifying different biological responses and therefore show different results, all the results are relevant to wild rice. It is notable that all of the EC₁₀ values are of similar magnitude (Table 2). The field survey EC₁₀ value is relied upon for the MPCA proposal because it best represents the full lifecycle of wild rice.

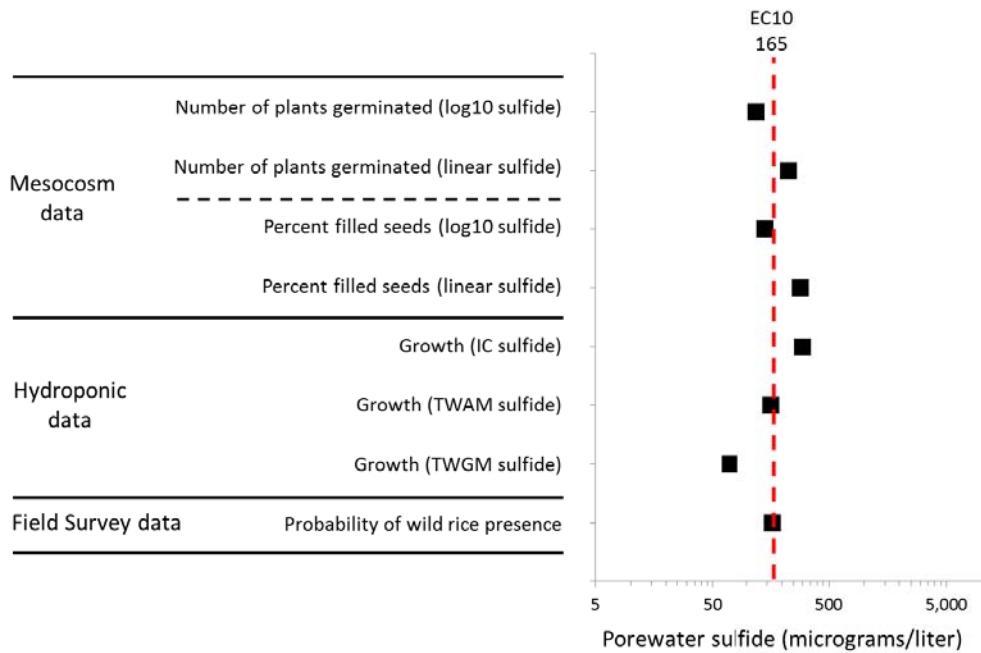


Figure 4. Comparison of EC₁₀ values from the field survey (165 micrograms sulfide per liter) to EC₁₀ values produced by other study components. The mesocosm EC₁₀ values were derived from linear regressions of 2013 biological data against sulfide data (either linear or log10-transformed) obtained August 28, 2013 (all p< 0.05). The three hydroponic EC₁₀ values were produced by combining normalized growth data from multiple experiments and performing logistic regressions using three different measures of sulfide exposure concentrations (IC = initial concentration; TWAM = time weighted arithmetic mean; TWGM = time weighted geometric mean).

The regression analysis of the mesocosm data with log-transformed or non-transformed sulfide data were of similar statistical quality, so EC₁₀ values are presented from both regressions (Figure 4). For the two biological response variables (number of plants germinated and percent filled seeds), the two regression methods bracket the field survey EC₁₀, the log transformation is less than 165 micrograms/liter, and the non-transformed regression is greater than 165 micrograms/liter.

Multiple estimates of the EC₁₀ for seedling growth were also calculated from the hydroponic experiment. During the 11-day experiment, the sulfide concentration declined during the 2 to 3 days of exposure between sulfide renewals, probably due to oxygen released by the photosynthesizing seedlings (details are presented in MPCA, 2014). Figure 4 and Table 2 present effect concentrations based on three different assumptions about the sulfide concentration that affected the biological response. The issue is, what sulfide concentration should be used to calculate the regression curves for the hydroponic experiment? There are three choices:

- Initial sulfide concentration (which the peer reviewers concluded was inappropriate because it would underestimate the toxicity of sulfide)
- Time-weighted arithmetic mean sulfide concentration
- Time-weighted geometric mean sulfide concentration

The reviewers were divided on which of the latter two methods would be the most appropriate estimate of sulfide exposure.

Table 2. Calculated 10% effect concentrations (EC_{10}) for the wild rice study components.

Study component	Biological endpoint	Sulfide concentration (micrograms per liter)
		EC_{10}
Mesocosm	Number of plants germinated (log10)	121
Mesocosm	Number of plants germinated (linear)	226
Mesocosm	Percent filled seeds (log10)	143
Mesocosm	Percent filled seeds (linear)	288
Hydroponic	Growth (initial concentration)	299
Hydroponic	Growth (time-weighted arithmetic mean)	160
Hydroponic	Growth (time-weighted geometric mean)	71
Field Survey	Probability of wild rice presence	165

2) The relationship between sulfide and sulfate

Sulfide found in the sediment porewater of lakes, streams, and wetlands is primarily generated from sulfate that diffuses into the sediment from the surface water. The relationship between elevated sulfate in a water body and porewater sulfide impacts on wild rice is affected primarily by two factors:

- The rate of conversion of sulfate to sulfide by bacteria in the sediment varies from one water body to the next. This variability is primarily due to different levels of total organic carbon that occur in the sediment of each site; total organic carbon is the food for the bacteria.
- As sulfide is produced from sulfate, if iron is available in the sediment it can interact with the sulfide and form a solid that does not affect wild rice.

In other words, higher levels of organic carbon in the sediment can lead to more sulfide being produced from a given amount of sulfate in the overlying water; conversely, higher levels of iron in the sediment can lead to less sulfide being available to impact the wild rice plants at a given sulfate level.

MPCA developed a structural equation model (SEM) based on the relationships in Figure 5. The model is used to predict the sulfide concentration in sediment porewater, from the concentrations of surface water sulfate, sediment iron, and sediment organic carbon, which can be expressed as:

$$Sulfide = 7.873 \cdot Sulfate^{0.345} \cdot Organic\ Carbon^{0.486} \cdot Sediment\ Iron^{-0.675} \quad (Equation\ 2)$$

(Sulfate and sulfide are expressed in mg/L; organic carbon is percent total organic carbon in the sediment; iron is micrograms extractable iron per gram sediment).

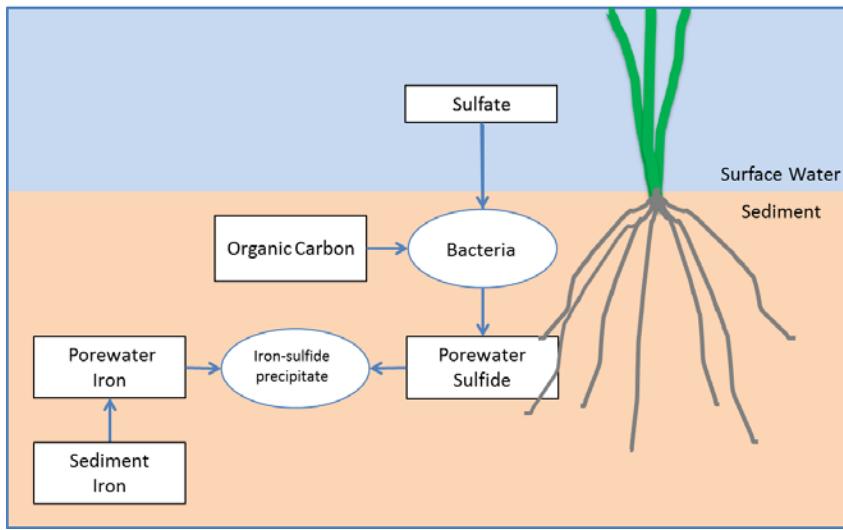


Figure 5. A simplified diagram of the ways sulfate, sulfide, iron, and organic carbon interact in the sediment of wild rice waters. Sulfate in surface water diffuses into sediment, where wild rice grows. Bacteria use organic carbon in the sediment as food and simultaneously respire sulfate, which is released into sediment porewater as sulfide. Sulfide may be toxic to wild rice if the roots, stems, or leaves are in contact with elevated concentrations. However, porewater sulfide may be reduced to tolerable concentrations if it combines with iron in the sediment to form a solid iron sulfide compound (a precipitate) that ties up the sulfide.

For purposes of model development and use of the field data, it is assumed that the concentrations of these variables are relatively stable over the long term, representing at least one-year averages. SEM is useful for modeling processes that involve indirect effects and feedback between variables like dissolved iron and sulfide. The use of SEM was recommended by the scientific peer review.

The model predicts that a given concentration of surface water sulfate can produce different concentrations of porewater sulfide depending on the concentrations of iron and total organic carbon in the sediment at a given site. For instance, relatively high concentrations of porewater sulfide will be produced when iron concentrations are low and organic carbon is high—the bacteria make a high concentration of porewater sulfide, which is potentially toxic to wild rice, and there is little iron available to convert it to a solid iron sulfide compound.

Given the strong predictive relationship between sulfate, sulfide, iron, and total organic carbon, the model can be used to derive an equation that identifies a sulfate threshold based on the sediment porewater sulfide level that is protective of wild rice, and the iron and total organic carbon measured in the sediment at a site.

The relationship between sulfate in surface water and sulfide in the porewater of underlying sediment is complicated. Sulfide is primarily produced by bacterial respiration of sulfate in their metabolism of organic carbon in anaerobic sediments, which can only occur if there is a source of sulfate. In the absence of groundwater movement upward through the sediment, the only significant source of sulfate is the overlying water.

The working conceptual model is that sulfate in surface water diffuses into anoxic sediment, where bacteria use organic carbon as food and simultaneously respire sulfate, releasing sulfide (Figure 5). The bacterial activity may be limited by the concentration of organic carbon in the sediment. So, sulfide production may be lower in sediment poor in organic carbon, and higher in sediment high in organic carbon. Sulfide may accumulate in the sediment porewater to concentrations that affect wild rice. However, if sufficient iron is present in the sediment, much

of the sulfide may react with the iron, forming an insoluble precipitate that is not toxic. Therefore, even though sulfide is made from sulfate, because of variation among field sites in the concentrations of organic carbon and iron, one would not expect a strong correlation between sulfate and sulfide in the field data.

However, if the carbon and iron are held relatively constant, such as would be the case in a particular lake, then one would expect a correlation between the concentration of sulfate in the surface water and the concentration of sulfide in the porewater. Just such an experiment was conducted as the mesocosm component of the study (MPCA 2014). Sulfate was added to the mesocosms at five different concentrations, with six replicates at each concentration. As sulfate concentrations increased, more sulfide accumulated in the porewater (Figure 6A). And, consistent with the conceptual model (Figure 5), the concentration of iron in the porewater decreases as the porewater sulfide increases (Figure 6B).

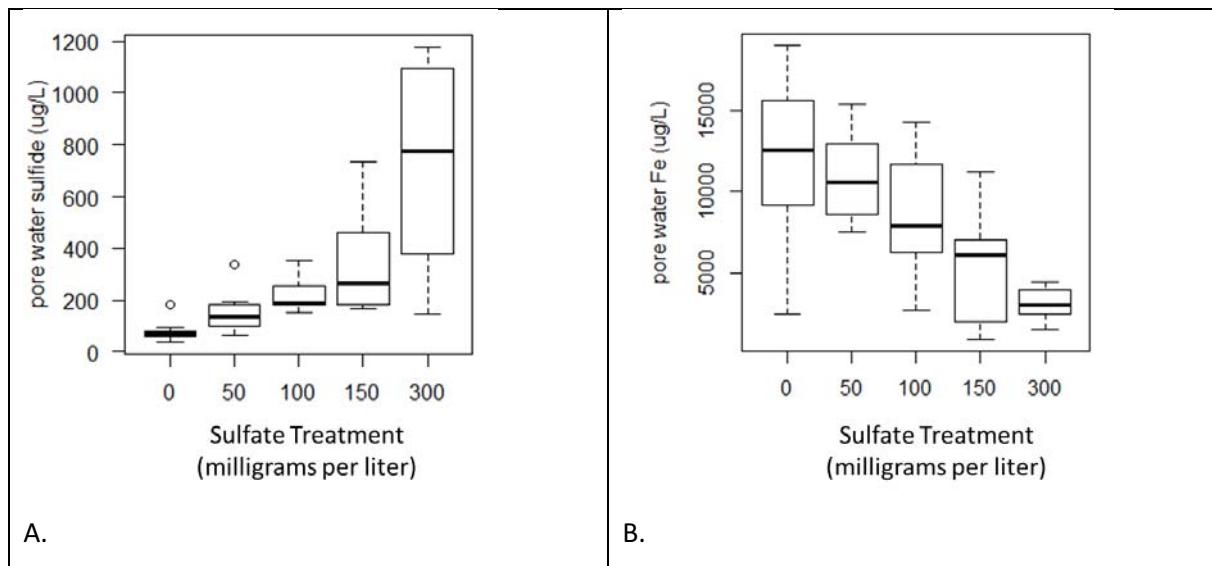


Figure 6. Response of (A) porewater sulfide and (B) porewater iron to sulfate treatments in the mesocosm experiment, as assessed in the sampling carried out August 28, 2013, when all 30 mesocosms were sampled.

Because of the complicated relationship between sulfate and sulfide, the scientific peer review recommended that MPCA investigate the factors that control sulfide concentrations by using a statistical tool that can simultaneously evaluate an expanded set of variables (e.g. several forms of iron, sulfur, and organic carbon) from the field survey, such as structural equation modeling (SEM). SEM is essentially linked linear regressions between variables, where predicted variables can in turn become variables that predict a third variable.

SEM is appropriate for modeling processes that involve indirect effects and feedback between variables. For instance, dissolved iron and sulfide affect each other, because they precipitate each other. SEM evaluates complex hypotheses of multivariate relationships that can be statistically compared to field data (Gough and Grace 1999). To statistically evaluate models that contain many variables requires relatively large data sets. In this case, where six or more variables were included in proposed models, the field survey of 184 site visits (not including cultivated wild rice paddies) was adequate for the task.

Using the field survey data, multiple structural equation models were developed and evaluated using the Stata Data Analysis and Statistical Software package ([Stata.com](http://www.Stata.com)). The Stata output for the best model is given in Appendix 1, and presented as a path diagram in Figure 7, where the connections between variables are indicated by arrows. Other SEM models were evaluated

that included additional variables that are potentially involved in the relationship between sulfate and sulfide, including acid volatile sulfide (AVS), dissolved organic carbon (DOC), phosphorus, and nitrogen. The adopted model (Figure 7) was chosen for its goodness of fit, simplicity, and ease of practical application. Aside from sulfate, the other statistically important variables affecting the net concentration of porewater sulfide in the model are the concentration of sediment organic carbon, the concentrations of iron in both sediment and porewater, and the total concentration of sulfur in the sediment. The model can be reduced to a single equation, where sulfide can be predicted by inputting sulfate, sediment iron, and sediment organic carbon, as in Equation 2.

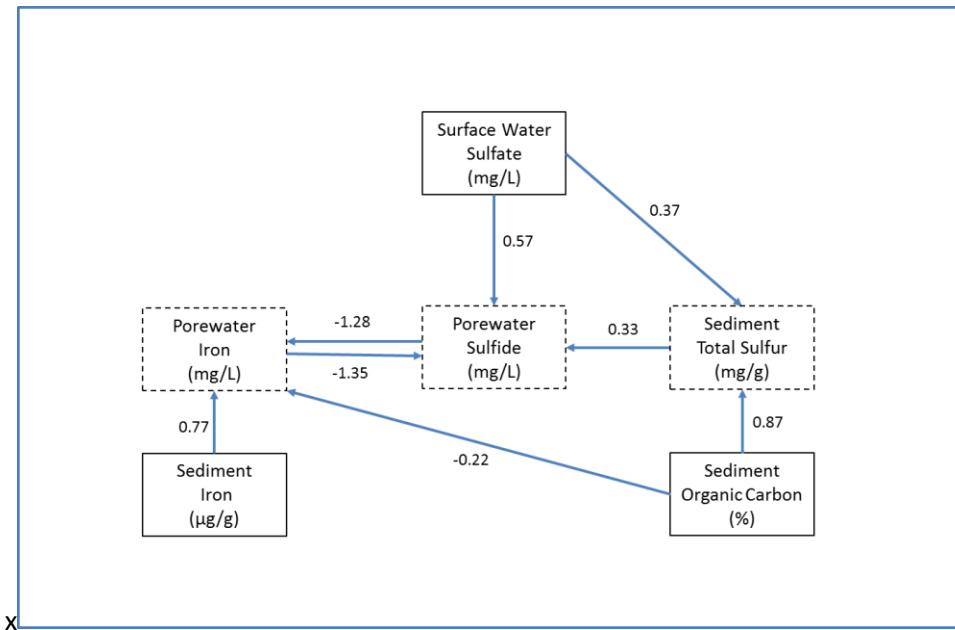


Figure 7. The structural equation model (SEM) that was used to develop Equation 1 is based on the relationships shown. Standardized coefficients, which represent the relative strength of the variables, are given. The model was calibrated with data from 184 sites sampled during the MPCA field survey. All coefficients are significant at the $p < 0.001$ level. Field data for each variable was used to calibrate the model, but the model is implemented by inputting sulfate, sediment iron, and sediment organic carbon (exogenous variables, in solid boxes). The variables in the dashed boxes are predicted in the model (endogenous variables). All variables were transformed by log base 10 to approach normality. Option ADF in the Stata SEM package was employed, which does not require variables to be normally distributed.

The SEM-derived Equation 2 can be compared to predictions produced by a multiple linear regression (MLR) model of the same variables, using the same data set. The MLR model (Table 3) yields the following equation:

$$\text{Sulfide} = 7.777 \text{ Sulfate}^{0.3464} \text{ Organic Carbon}^{0.5295} \text{ Sediment Iron}^{-0.6841} \quad (\text{Equation 3})$$

(units as in Equation 2)

Although the coefficients of the MLR model (Equation 3) are different from the coefficients of the SEM model (Equation 2), the predictions are very similar (Figure 8). The slope is close to one, the intercept is close to zero, and the R^2 value is 0.996.

Table 3. Results of multiple linear regression modeling.

Porewater sulfide ←	Coefficient	P value
Sulfate	0.3464982	<0.001
Sediment Iron	-0.6841846	<0.001
Sediment TOC	0.5295977	<0.001
Constant	0.8908034	<0.001

N = 184

F (3, 180) = 49.09

Prob > F = 0.0000

R² = 0.4500

Adjusted R² = 0.4408

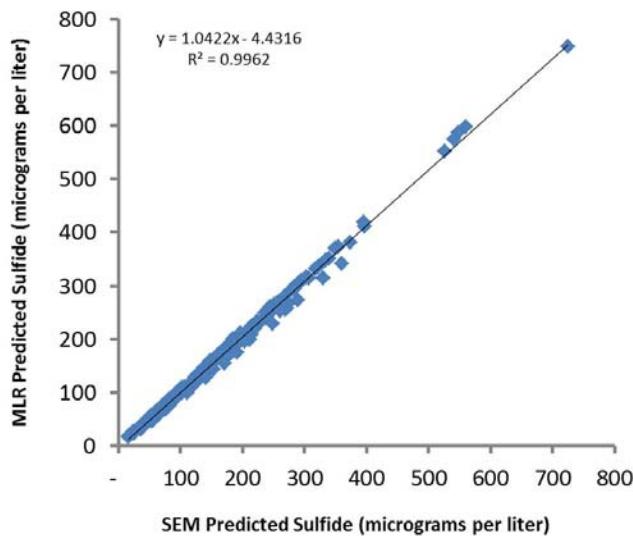


Figure 8. Comparison between porewater sulfide predicted by the structural equation model (SEM) and the multiple linear regression model (MLR).

It is important to understand the degree to which models are accurate in predicting the conversion of sulfate to sulfide. First, all of the SEM models, and the MLR model, tend to over-predict porewater sulfide at observed concentrations below about the proposed protective concentration of sulfide of 165 micrograms per liter (Figure 9). This over-prediction can be viewed as a safety factor that is incorporated into the calculation of protective sulfate concentrations. Such a safety factor is welcome because use of any model to translate porewater sulfide to surface water sulfate works on the central tendency of the data, introducing the possibility that a predicted protective sulfate concentration is under-predicted.

Second, all of the models under-predict sulfide concentrations for sites with high sulfide levels (above about 500 micrograms per liter of sulfide, Figure 9). However, this under-prediction does not affect the utility of the model, since the central tendency of the predictions region of goal-setting—around 165 microgram per liter—is the most accurate region. Under-prediction above 500 micrograms per liter does not affect the determination that the sulfide would be too high for wild rice.

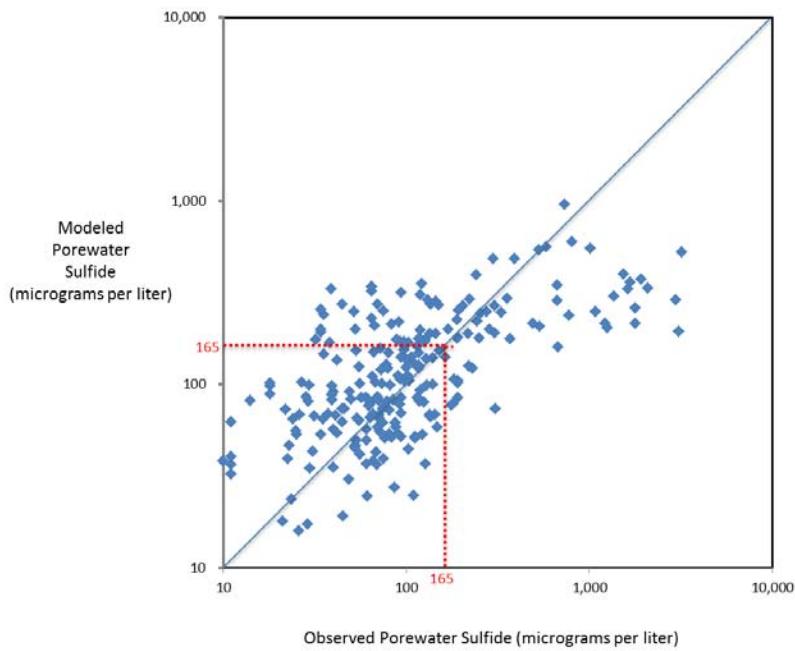


Figure 9. Modeled porewater sulfide concentrations (based on Equation 2) compared to observed porewater sulfide for the data that was used to calibrate the model. (Excluded data were commercial wild rice paddies and two samples with unusually high sulfide concentrations: 14,840 and 16,000 micrograms sulfide per liter. The next highest observed sulfide concentration was 3,190 micrograms sulfide per liter.) The dashed red lines correspond to the protective sulfide concentration, 165 micrograms per liter.

3) Calculation of a sulfate concentration that is protective of wild rice

The information from the above two sections can be combined to produce an equation that allows the calculation of a surface water sulfate concentration that is protective of wild rice. Sulfate concentration above the protective concentration are predicted to produce porewater sulfide concentrations above 165 micrograms per liter (the protective sulfide concentration), given the sediment organic carbon and iron concentrations of a given wild rice water.

First, Equation 2 is solved for sulfate:

$$\text{Sulfate} = 0.002525 \text{ Sulfide}^{2.899} \text{ Organic Carbon}^{-1.410} \text{ Sediment Iron}^{1.956} \quad (\text{Equation 4})$$

Since the goal is to calculate the sulfate concentration that is associated with a sulfide concentration of 165 micrograms per liter, that sulfide concentration is substituted into Equation 4, producing Equation 1:

$$\text{Sulfate} = 0.0000136 \text{ Organic Carbon}^{-1.410} \text{ Sediment Iron}^{1.956} \quad (\text{Equation 1})$$

A calculated sulfate concentration that would be protective of wild rice can be calculated from every sediment sample that is analyzed for organic carbon and sediment iron. Figure 10 plots all of the sediment organic carbon and sediment iron data pairs from the field survey sites against a background of isopleths of surface water sulfate concentrations that would be required for that site to produce a protective porewater sulfide concentration. An important conclusion to be drawn from Figure 10 is that there is a large range in protective surface water sulfate concentrations.

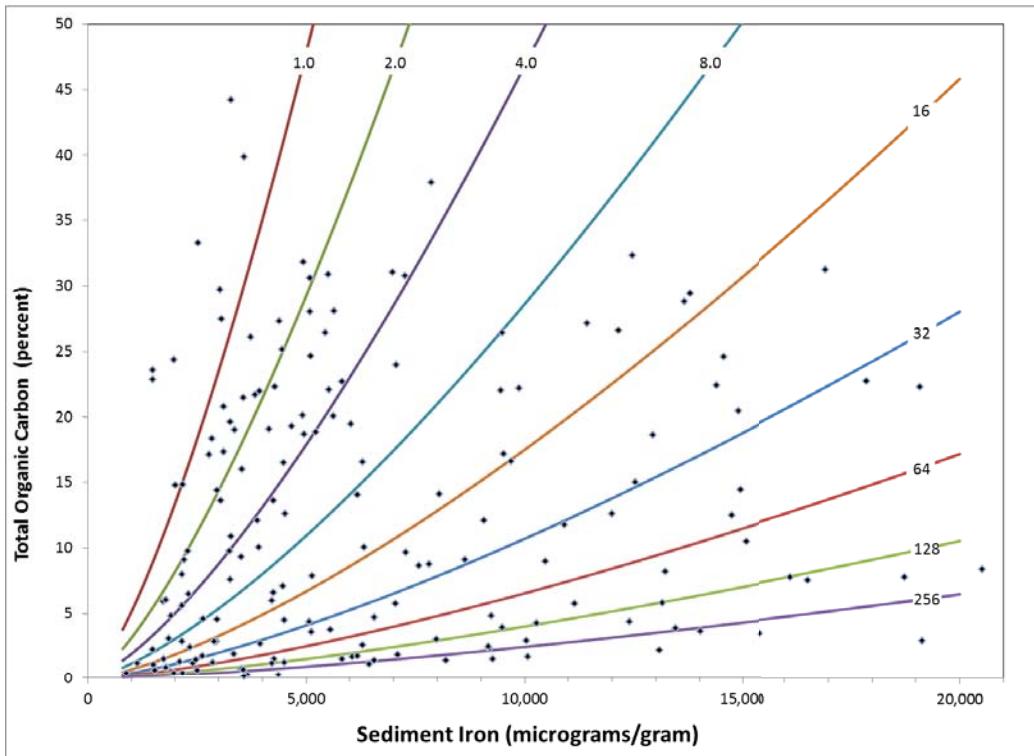


Figure 10. Isopleths (lines of combinations of iron and organic carbon that describe a constant sulfate-to-sulfide conversion efficiency) of surface water sulfate concentrations (1 to 256 mg/L) that would produce porewater sulfide concentrations of 165 micrograms per liter (calculated using Equation 1). An isopleth is the calculated protective sulfate concentration for the combination of iron and organic carbon concentrations that it traces. The individual data points show the observed sediment iron and organic carbon at each of the surveyed sites. The vulnerability of a site to elevated sulfate increases as one moves upward and leftward on this graph (towards higher sediment organic carbon and lower sediment iron). A site can be considered protected if its observed sulfate concentration is less than the sulfate value for the isopleth that goes through its position on this graph.

Figure 11 shows the importance of quantifying the concentrations of sediment iron and organic carbon present at a given site to determine its vulnerability to a particular concentration of sulfate. The concentration of sulfate that would be protective of wild rice at that site can be calculated with Equation 1.

If sediment organic carbon and sediment iron data are available from a wild rice site, the protective concentration of surface water sulfate can be calculated. Given that the field data show that iron and total organic carbon vary among sites and are independent of each other, calculating the protective level of sulfate based on these variables is a more appropriate approach to protecting wild rice from the impacts of elevated sulfate than relying upon a single sulfate concentration as a protective threshold for all sites. Because of differences in iron and carbon in sediments, any given concentration of sulfate can produce vastly different concentrations of porewater sulfide (Figure 11).

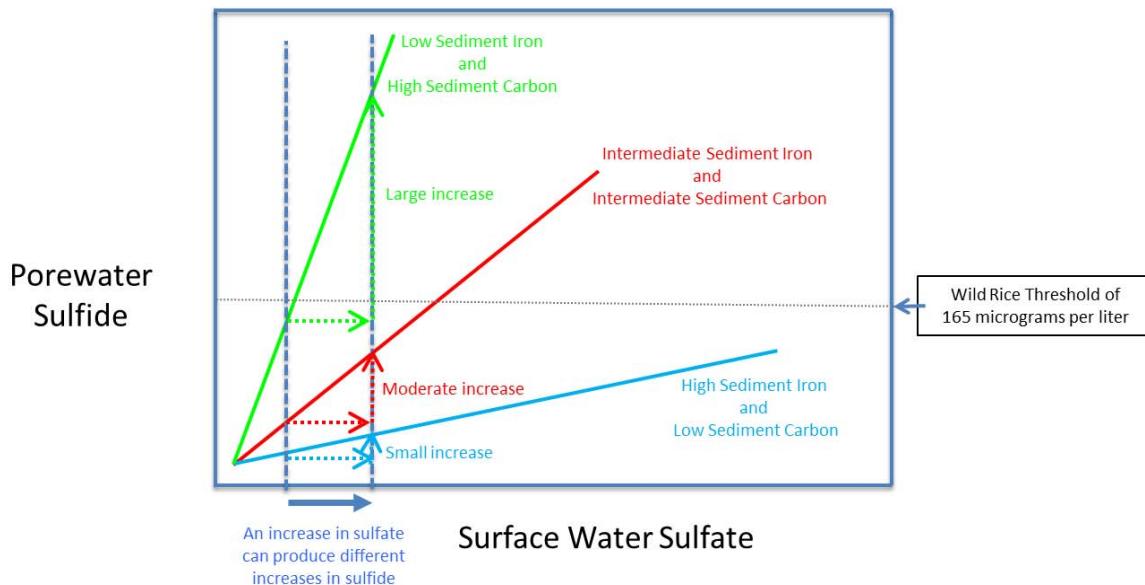


Figure 11. The relationship between sulfate in surface water and sulfide in porewater, as influenced by sediment concentrations of iron and organic carbon. Because of differences in iron and carbon, any given concentration of sulfate (the vertical dashed line, for example) can produce vastly different concentrations of sulfide. The solid red line represents a site that is relatively efficient in producing porewater sulfide from sulfate, and the solid blue line represents a site that is relatively inefficient in producing porewater sulfide from sulfate.

The large range in protective sulfate concentrations can be illustrated by calculating the sulfate thresholds for the sites sampled during the MPCA wild rice field survey. For instance, 58% of the field sites have a predicted sulfide concentration less than 165 micrograms per liter at a surface water sulfate concentration of 10 mg/L (Table 4). However, 42% of the field sites have a predicted sulfide concentration greater than 165 micrograms at a sulfate concentration of 10 mg/L in the overlying water, and the wild rice populations in those waters would have a greater likelihood to decline over time if the sulfate concentration were 10 mg/L. Even a concentration as low as 2.5 mg/L would leave some sites unprotected, while other sites — those high in iron and low in organic carbon — would have tolerable porewater sulfide concentrations even at a sulfate concentration of 200 mg/L (Table 4). In other words, any given sulfate concentration would be protective for some sites while not protective for other sites, depending on the total organic carbon and iron concentrations in the sediment.

Table 4. Proportion of sites that would be protected and under-protected by various potential sulfate values based on using Equation 1 for the 112 different sites that were surveyed. Note that the calculations do not incorporate the observed sulfate concentration in the surface water.

Sulfate Concentration (mg/L)	Proportion of sites protected	Proportion of sites under-protected
2.5	77%	23%
10	58%	42%
20	40%	60%
50	26%	74%
200	11%	89%

It is possible to use Equation 1 and the sediment data from the field survey to calculate potential protective sulfate values at each of the field survey sites (Figure 12). By doing so, we can look for regional patterns. The wide variability of values across the state further demonstrates that no one statewide, or even regional, sulfate concentration would be

protective due to the variability of sediment iron and total organic carbon among sites. An appropriate level of protection requires sediment sampling and analysis.

Because a single concentration of sulfate is not an appropriate tool for protection of wild rice in different water bodies, MPCA proposes that whenever a protective sulfate concentration needs to be determined for a given wild rice water, sediment concentrations of iron and organic carbon be measured. The data, through Equation 1, will then be used to determine the protective sulfate value for that wild rice water.

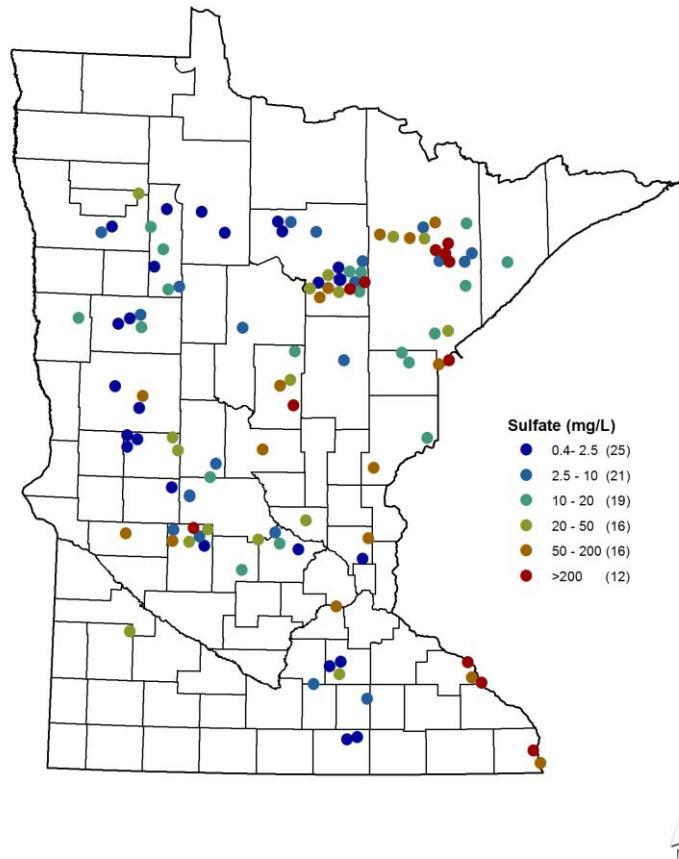


Figure 12. Protective sulfate concentrations calculated with Equation 1 for the water bodies in the MPCA field survey. Numbers in parentheses are the number of sites in the indicated sulfate range. Overlapping point symbols have been moved slightly from their true position so as to be visible. Point values were averaged when multiple values were available from a water body, an approach that requires further review.

To further illustrate the calculation of protective sulfate concentrations, Equation 1 was applied to three different MPCA field survey sites where total organic carbon and iron data are available: Little Round Lake, Elk Lake, and Rice Lake. These sites are minimally disturbed watersheds, with widely varying calculated protective sulfate concentrations (Table 5).

Table 5. Example calculations of protective surface water sulfate concentrations for three study sites: Little Round Lake, Elk Lake, and Rice Lake, using Equation 1. The sediment data for these three lakes can be compared to the 82 lakes of the field survey: The 25th, 50th, and 75th percentiles of the field survey of organic carbon were, respectively, 5.8, 13.9, and 22.0 percent, and the corresponding percentiles of sediment iron were 2,300, 4,300, and 9,500 micrograms per gram. Note that these examples are for illustrative purposes only.

Study Site	State ID	Sediment Total Organic Carbon (%)	Sediment Iron (micrograms/gram)	Calculated Protective Sulfate Concentration (mg/L)
Little Round Lake	03-0302	27.5	3,069	0.8
Elk Lake	15-0010	10.2	8,480	25
Rice Lake	18-0053	35.6	50,389	140

The three lakes had medium-to-high total organic carbon to support bacteria that convert sulfate to sulfide (Table 5). Of the three lakes, Little Round had the lowest sediment iron at 3,069 micrograms per gram and is calculated to be the most sensitive to sulfate. Rice Lake had the highest sediment iron at 50,389 micrograms per gram and is calculated to be the least sensitive to sulfate. Elk Lake had an intermediate sediment iron concentration of 8,480 micrograms per gram and is calculated to be intermediate in sensitivity to sulfate.

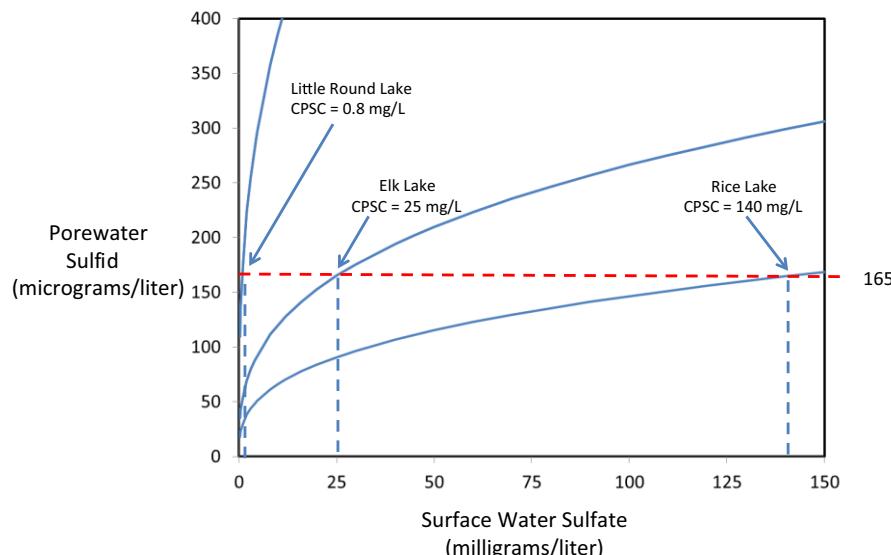


Figure 13. Three wild rice lakes of contrasting sensitivity to sulfate in surface water, showing the modeled increase in porewater sulfide as sulfate increases in the surface water, using Equation 2. The vertical dashed lines show the calculated protective sulfate concentrations of 0.8, 25, and 140 milligrams per liter. The horizontal dashed line is the protective concentration of sulfide of wild rice (165 micrograms/liter), and the solid lines show the calculated increase in porewater sulfide associated with theoretical increases in sulfate in each of the three lakes. All three lakes had wild rice populations, and observed sulfate in the surface water of less than 0.5 mg/L. Note that these examples are for illustrative purposes only.

Figure 13 shows the calculated change in sediment porewater sulfide in the three lakes given theoretical increases in surface water sulfate concentrations. For instance, the curve representing the most sensitive lake (Little Round) is nearly vertical and a very small increase in sulfate is predicted to result in relatively high concentrations of sulfide. In contrast the curve representing the least sensitive lake (Rice Lake) has a much lower slope, and it is predicted that sulfate concentrations could increase to 140 mg/L before sulfide concentrations reach 165 micrograms per liter. The curve for the lake of intermediate sensitivity (Elk Lake) has a medium slope and it is predicted that sulfate concentrations could increase to 25 mg/L before sulfide concentrations reach 165 micrograms per liter.

Fifteen of the wild rice waters were sampled three or more times during the field survey. This allowed an examination of the range in calculated protective sulfate concentrations that is produced by analyzing multiple sediment samples from a water body (Figure 14, Table 6). Figure 14, which contains a subset of the data in Figure 10, is similar except that it is on a log-log scale which makes it easier to see the data point clusters. It is clear that there is variability within wild rice waters, but the difference *between* waters is much greater than the variability *within* a specific wild rice water.

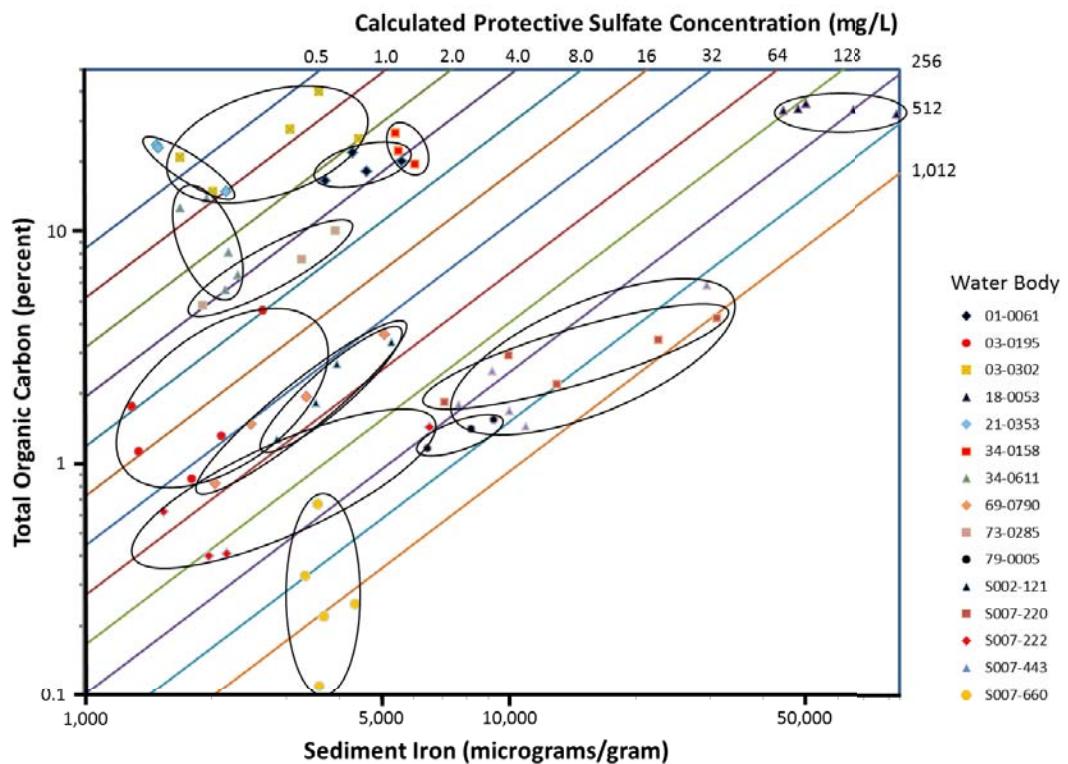


Figure 14. Data from water bodies with wild rice where three or more sediment samples were taken within 1,000 feet of each other. Ellipses encompass the range in calculated protective sulfate concentrations, which are based on sediment iron and total organic carbon data as modeled with Equation 1. The log-log display allows both a greater range of sediment concentrations and better separation of the sites than in Figure 10. Additional water body information is given in Table 6.

Table 6. Information about the sites displayed in Figure 14.

State ID	Site name	Latitude	Longitude
01-0061	Flowage	46.6895	-93.3380
03-0195	Height of Land	46.9129	-95.6095
03-0302	Little Round	46.9759	-95.7404
18-0053	Rice	46.3392	-93.8918
21-0353	Anka	46.0769	-95.7292
34-0158	Monongalia	45.3334	-94.9293
34-0611	Unnamed	45.2675	-94.8650
69-0790	Dark	47.6389	-92.7781
73-0285	Raymond	45.6286	-95.0225
79-0005	Mississippi Pool 4/Robinson Lake	44.3593	-91.9881
S007-220	Second Creek	47.5205	-92.1925
S002-121	Clearwater River	47.9372	-95.6907
S007-222	Mississippi Pool 8 at Genoa	43.5766	-91.2341
S007-443	Partridge River	47.5212	-92.1901
S007-660	Mississippi Pool 5 / Spring Lake	44.2018	-91.8444

Sediment sample considerations

Protecting a wild rice water from elevated porewater sulfide depends on calculating the protective value for that water body's sulfate based on iron and organic carbon sediment data collected where the wild rice is growing. If iron and organic carbon concentrations were relatively constant in wild rice waters, the protective concentration would also be relatively constant. However, iron and organic carbon vary dramatically among wild rice waters, and as a result the calculated protective concentrations of sulfate also vary a great deal (Figure 10).

Prior to formally proposing changes to the existing wild rice sulfate standard through rulemaking, MPCA intends to define the number of organic carbon and iron sediment samples needed to characterize a water body, and to adequately calculate the protective sulfate value. Protocols to collect and analyze sediment samples for iron and organic carbon are being prepared and will be made available on MPCA's web site in coming months, as well as included in a Technical Support Document prior to formal rulemaking.

Consideration of commercial wild rice paddies

The human management of the water environment in wild rice paddies likely produces conditions in the sediment that are rarely seen in natural wild rice waters. In particular, sediment dewatering from July through September may oxidize the sediment, potentially reducing conversion of sulfate to sulfide or oxidizing sulfide that has been produced. Both of these conditions may reduce the production of sulfide from sulfate, or the impact of sulfide on plants. Therefore, MPCA proposes that a sulfate standard is not needed to prevent sulfide impacts to commercial wild rice paddies. Data from commercial wild rice paddies were not used to calibrate the model and develop the equation discussed above.

Wild rice waters – Where the standard applies

The 2011 Minnesota Legislature directed the MPCA to identify the specific surface waters "to which the wild rice water quality standards apply" (Laws of Minnesota 2011, 1st Special Session, Chapter 2, Article 4. Section 32). A draft list of wild rice waters has been developed, and they are identified on a map on the MPCA web site at www.pca.state.mn.us/r6wxpf9. This

draft list of 1,268 wild rice waters was developed using information from various wild rice water inventories. MPCA also sought and received input from the Minnesota Department of Natural Resources (DNR) and Minnesota Indian Tribes while developing the draft list. MPCA considered acreage, density, and the importance of wild rice to humans and waterfowl when developing the proposed approach to identifying and listing specific Minnesota water bodies as wild rice waters. The sources of wild rice information considered included wild rice harvester and field survey databases and responses to a 2013 formal call-for-data completed by MPCA. Short descriptions of the information resources used by MPCA to formulate the draft list of wild rice waters are available on the MPCA web site. MPCA also intends to replace the existing rule language of “water used for the production of wild rice” with “wild rice waters” as the term describing the beneficial use that the standard is intended to protect.

Updating the list of wild rice waters

Given that a comprehensive inventory of Minnesota’s natural wild rice stands does not exist, and that both monitoring and restoration projects are underway in some water bodies, MPCA recognizes that the list of wild rice waters will require periodic update as new information on density and acreage of wild rice stands become available. After the initial rulemaking to identify and list wild rice waters, MPCA intends to consider listing additional waters in Minnesota Rules as wild rice waters approximately every five years. Through the public rulemaking process, a new water body will be added for which there is information demonstrating that the water meets the following proposed definition of “wild rice water.”

“Wild Rice Water” means a surface water of the state that contains a self-perpetuating population of wild rice plants, either currently present or that have been present in the given water body since November 28, 1975. The self-perpetuating wild rice population must be represented by a minimum of 8,000 wild rice stems over the surface of a lake, wetland, or reservoir water body or a minimum of 800 wild rice stems over a river-mile reach for a riverine water body. Waters designated as wild rice waters are specifically listed as such in Minn. R. 7050.0470 and are identified with the symbol [WR] preceding the name of the water body.

To provide some context to the proposed definition of wild rice waters, 8,000 stems in a lake roughly equate to 2 stems per square meter over one acre (see Figure 15). Seed from 8,000 stems theoretically has the potential to feed approximately 12 ducks during a one week migratory stop.



Figure 15. A wild rice water with about 2 stems per square meter, or about 8,000 stems in one acre.

Implementation questions and considerations

The MPCA intends to begin rulemaking beginning in 2015 to refine the existing wild rice sulfate water quality standard to reflect the most up-to-date science on the understanding of how sulfate and sulfide impact wild rice. Rule revision would also add wild rice waters to those currently listed in rule.

Prior to rulemaking, MPCA will consider how to use this updated understanding to inform regulatory decisions. MPCA believes it is important to consider this new science when making regulatory decisions to more precisely protect wild rice waters from potential sulfate impacts and better ensure efficient and effective use of resources.

The MPCA will engage with interested stakeholders to discuss:

- The proposed approach for revising the sulfate standard, to protect wild rice
- Approaches to monitor for wild rice and sediment conditions
- Consideration of the new science for the purposes of making regulatory decisions

Toward that end, MPCA will schedule meetings with Minnesota Tribes, the Wild Rice Standards Advisory Committee, and EPA in April and May of 2015 to further describe and get input on the proposed approach. MPCA also welcomes opportunities to meet with other interested parties about this proposal.

The MPCA will continue to refine this proposal based on feedback and any new information received, including an examination of wild rice population dynamics. At the same time, MPCA technical staff will continue to develop the more detailed technical documentation needed for the formal rulemaking proposal.

As noted above, the MPCA will be developing further details about the data collection protocol to calculate a protective level of sulfate, per the proposed approach to the standard. Additional field data is expected to be collected in the summer of 2015 to help in developing those details.

The MPCA anticipates having a formal rulemaking package, including draft rule language and the detailed Statement of Need and Reasonableness, ready for discussion toward the end of 2015. The rulemaking process is governed by the Minnesota Administrative Procedures Act and includes multiple opportunities for informal and formal public comment.

Literature cited

ERG. 2014. Summary Report of the Meeting to Peer Review MPCA's *Draft Analysis of the Wild Rice Sulfate Standard Study*. Eastern Research Group, Inc., Lexington Massachusetts. <http://www.pca.state.mn.us/kppq38rq>

Gough, L and JB Grace. 1999. Effects of environmental change on plant species density: comparing predictions with experiments. *Ecology*. 80(3):882-890.

Lamers, LPM, LL Govers, ICJM Janssen, JJM Geurts, MEW Van der Welle, MM Van Katwijk, T Van der Heide, JGM Roelofs, and AJP Smolders. 2013. Sulfide as a soil phytotoxin - a review. *Frontiers in Plant Science* 4:1-14.

MPCA. 2014. Analysis of the Wild Rice Sulfate Standard Study: Draft for Scientific Peer Review. Minnesota Pollution Control Agency. June 9, 2014.
<http://www.pca.state.mn.us/kppq38rq>

Appendix 1. Coefficients and characteristics of the SEM model that was used to develop Equation 1.

Model Variable	Coefficient, Unstandardized	Coefficient, Standardized	P value
Porewater Sulfide ←			
Porewater Iron	-0.4504578	-0.7085618	<0.001
Sediment Total S	0.147297	0.1720401	<0.001
Sulfate	0.1421145	0.2345237	<0.001
Constant	0.5129075	1.050636	<0.001
Porewater Iron ←			
Porewater Sulfide	-1.055696	-0.6711435	<0.001
Sediment Iron	0.7855547	0.4020308	<0.001
Sediment TOC	-0.2655787	-0.1868744	<0.001
Constant	-0.089077	-0.1159993	0.795
Sediment Total S ←			
Sulfate	0.2634474	0.3722258	<0.001
Sediment TOC	0.9197721	0.8716147	<0.001
Constant	-0.5637775	-0.9887471	<0.001

Model characteristics and goodness of fit

N	184
Degrees of freedom	3
χ^2	3.23
$p > \chi^2$	0.3572
RMSEA	0.020
Lower bound	0.000
Upper bound	0.128
pclose	0.544
CFI	0.999
TLI	0.996
SRMR	0.020
CD	0.961

Total Effects

Model Variable	Coefficient, Unstandardized	Coefficient, Standardized	P value
Porewater Sulfide ←			
Porewater Sulfide	0.9067471	0.9067471	<0.001
Porewater Iron	-0.8589092	-1.351048	<0.001
Sediment Total S	0.2808581	0.3280369	<0.001
Sulfate	0.3449677	0.5692812	<0.001
Sediment Iron	-0.6747201	-0.5431629	<0.001
Sediment TOC	0.4864334	0.5383982	<0.001
Porewater Iron ←			
Porewater Sulfide	-2.012946	-1.279701	<0.001
Porewater Iron	0.9067471	0.9067471	<0.001
Sediment Total S	-0.2965008	-0.2201598	<0.001
Sulfate	-0.3641811	-0.3820693	<0.001
Sediment Iron	1.497854	0.766571	<0.001
Sediment TOC	-0.7791045	-0.5482168	<0.001
Sediment Total S ←			
Sulfate	0.2634474	0.3722258	<0.001
Sediment TOC	0.9197721	0.8716147	<0.001