# Wild Rice Sulfate Standard Study Preliminary Analysis

## Introduction



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. This research was intended to inform an evaluation of the existing wild rice sulfate standard. In 1973 the MPCA adopted, and the U.S. Environmental Protection Agency (USEPA) approved, that standard to protect the beneficial use of "water used for production of wild rice" during periods when the rice "may be susceptible to damage by high sulfate levels." (Minn. R. 7050.0224, subp. 2).

Following the development of a detailed research protocol in 2011, in 2012 the MPCA contracted with groups of scientists at the University of Minnesota Duluth and Twin Cities to undertake a Wild Rice Sulfate Standards Study. The Study's main hypothesis is that wild rice is impacted by sulfate via the conversion of sulfate to sulfide dissolved in the water in the sediment, known as the sediment porewater. Each of the Study components has a specific purpose and associated strengths and limitations. Data collection was completed in December 2013 and is documented in individual reports from the researchers (See Appendix A for a summary of study components.)

During early 2014, MPCA staff integrated the study results; analyzed the data as a whole; gained input from the Wild Rice Standards Study Advisory Committee; and reviewed existing monitoring data, other relevant scientific studies/information, and the original basis for the wild rice sulfate standard to develop this preliminary analysis.

This document provides background on water quality standards in general and the wild rice sulfate standard in particular, and lays out the MPCA's preliminary analysis as well as the next steps in this ongoing process to further understand the effects of sulfate on wild rice and, as needed, to refine the wild rice sulfate standard. MPCA is sharing this preliminary analysis to foster ongoing dialogue and review. Continued data analysis, stakeholder input and scientific review will occur over the coming months, and the analysis will be refined as needed prior to any final recommendations and formal rulemaking.

## Background

### Wild rice and sulfate in the Minnesota environment

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

Sulfate is a natural chemical commonly found in surface and groundwater. It can be found at varying concentrations in discharges from permitted facilities such as mining operations, municipal wastewater treatment plants, and industrial facilities. In some areas, permitted facility discharges are elevated because groundwater high in sulfate is used for drinking water or industrial needs. The primary factor controlling natural concentrations of sulfate in surface water is the surface geology of Minnesota. For example, glaciation left relatively high-sulfur soils across southwestern Minnesota, which contribute sulfate to lakes and streams. Past studies have shown that wild rice is primarily found in waters with relatively low sulfate concentrations.

This recognition of the importance of wild rice in Minnesota, and the observed relationship between the presence of wild rice in waters with lower sulfate levels (and its absence in waters with elevated sulfate), led to the adoption of the wild rice sulfate standard in 1973.

## Water quality standards

Water quality standards are fundamental tools under the federal Clean Water Act (CWA) and Minnesota Statutes, designed to help protect and improve the quality of the state's waters. Minnesota water quality standards consist of three components:

1. The beneficial use(s) for which a water body is to be protected,
2. The narrative and/or numeric criteria that specify what conditions in the water are protective of the beneficial uses, and
3. Antidegradation provisions (also known in Minnesota as nondegradation) to minimize the lowering of water quality that is better than the minimum level needed to protect beneficial uses.

Under the CWA, states and federally authorized Indian Tribes are required to identify the beneficial uses for which their waters are to be protected, then to adopt criteria and antidegradation provisions to protect those beneficial uses. Explicit in the CWA is the presumption that a water body should attain healthy aquatic life and recreation uses unless a rigorous analysis finds that such uses are not attainable. Minnesota's water quality standards rules provide a framework that includes these broad uses, and also the following additional uses: domestic consumption, industrial, agriculture and wildlife, navigation and aesthetic enjoyment.

## Minnesota's wild rice sulfate standard

Minnesota's Class 4 Agriculture and Wildlife use classification covers agricultural uses as well as wildlife uses. Under the Class 4A use classification, Minnesota currently has a water quality standard of "*10 mg/L sulfate applicable to water used for production of wild rice during periods when the rice may be susceptible to damage by high sulfate levels*" (Minn. R. 7050.0224, subp. 2). MPCA is unaware of any other state with a wild rice sulfate standard.

This 10 mg/L wild rice sulfate standard was adopted into the MPCA water quality standards in 1973. Based on testimony presented at public hearings leading to the adoption of the sulfate standard, it was intended to apply both to waters with naturally occuring wild rice and to waters used for paddy rice production.

The standard was based on field observations and water chemistry correlations made by Dr. John Moyle primarily in the late 1930s and early 1940s. Dr. Moyle was a highly respected biologist with the then Minnesota Department of Conservation, and later the Minnesota Department of Natural Resources, who concluded that "No large stands of rice occur in water having sulfate content greater than 10 ppm [parts per million, or mg/L], and rice generally is absent from water with more than 50 ppm".

The existing wild rice sulfate standard was developed based on study-derived correlations of Dr. Moyle's observations and water chemistry data. However, the specific mechanism by which sulfate appears to be impacting wild rice was not the subject of Dr. Moyle's study. This, along with questions that have arisen regarding the implementation of the current standard, led to the MPCA's interest in further understanding the effects of sulfate on wild rice to inform a review of the wild rice sulfate standard.

## Wild rice sulfate standard study

As noted above, the goal of the Wild Rice Sulfate Standard Study is to enhance scientific understanding of the effects of sulfate on wild rice and to inform a decision as to whether a revision of the wild rice sulfate standard is warranted. The Study was conducted by scientists at the University of Minnesota Duluth and Twin Cities under contract with the MPCA, with input from a diverse group of interested parties and technical experts, referred to as the Wild Rice Standards Study Advisory Committee (membership listed in Appendix B).

The Study consists of parallel research efforts (Study components) that each have a specific purpose and associated strengths and limitations. 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 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 at wild rice container experiments and field sites to characterize sulfate, sulfide, and iron
- **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 http://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 this web page.

## Evaluating and integrating multiple lines of evidence

Following the completion of the Wild Rice Sulfate Standard Study research, the MPCA reviewed the data generated; integrated the results of the Study components; completed additional analysis; and reviewed existing monitoring data and other relevant scientific studies/information to develop this preliminary analysis of the effects of sulfate on wild rice. Continued data analysis, stakeholder input and scientific review will occur over the coming months, and the analysis will be refined as needed to inform recommendations and formal rulemaking regarding the wild rice sulfate standard. MPCA has the responsibility for demonstrating that any recommended changes to the standard have a scientific basis and would protect the beneficial use of "water used for production of wild rice."

The wild rice sulfate standard is intended to protect a single species from the negative effects of sulfate. This is different from the more typical toxicity standard that is based on protecting a community of organisms (such as a warm-water fishery, or a rooted plant community). Community-based standards are calculated from data about the toxic effects of the pollutant of concern on the most sensitive species in the community, and standards development guidance developed by USEPA provides that a certain percentage of the most sensitive species can be affected by the pollutant and still result in a standard that protects the community as a whole. Similar USEPA guidance does not exist for species-specific standards other than the development of human health-based standards; therefore, MPCA needs to consider the question of "what is protective" in its analysis and any future rulemaking.

The MPCA sought input from the Wild Rice Standards Study Advisory Committee on the Study. MPCA staff also conversed with the Study researchers to clarify data questions and inform the analysis, and also gained input from USEPA technical staff.

MPCA staff focused on understanding the additional scientific information about the effects of sulfate and sulfide on wild rice, and the interactions of sulfate, sulfide, and iron in the environment. The design of the overall Study relies on an approach that views each of the individual study components as providing complementary lines of evidence. When viewed together, these multiple lines of evidence provide a more complete understanding of the complex biogeochemical interactions of sulfate in the environment, and the effects of sulfate or its derivatives on wild rice. The key lines of evidence that informed the MPCA's preliminary analysis are the laboratory hydroponics experiments, the field survey data, and existing scientific literature. The information from these components was supplemented by data from the container experiments, the rooting zone depth profiles and the sediment incubation experiments.

The benefit of conducting a laboratory toxicity test is that many external factors can be controlled, which allows for a better interpretation of the effects of sulfate or sulfide on wild rice. Statistical analyses of controlled hydroponic growth tests were used to evaluate the extent to which elevated sulfate and sulfide concentrations are toxic to wild rice seed and seedlings.

Hydroponic experiments are by their nature removed from the natural environment, and the method(s) used can introduce uncertainties as well. In contrast, the field survey provides observational evidence of the environmental conditions that are supportive (or unsupportive) of wild rice. The field survey is not controlled in the sense of isolating individual variables that may be affecting wild rice, but by monitoring for the likely variables and analyzing that data an understanding of the strength of the relationships between specific variables and wild rice presence or absence can be gained. Where the hydroponics data and the field survey data showed overlap or agreement, the MPCA deemed that to be strong evidence to consider in developing this preliminary analysis, particularly where there is also scientific literature that further reinforces the agreement.

The MPCA also reviewed and analyzed the data from the outdoor container (aka "mesocosm") experiments. Those experiments showed significant effects on wild rice at sulfate concentrations of 300 mg/L and not at the lower sulfate treatment concentrations. However, MPCA technical staff are concerned that the containers may not have reached equilibrium for the sulfide, sulfate and iron reactions (meaning there may be excess iron available to "buffer" the elevated sulfate, but that once the iron is used up a toxic effect will be seen at lower sulfate concentrations). MPCA staff therefore did not directly use the results of the outdoor container experiments in this preliminary analysis; however, staff will continue to analyze the data from these studies to identify additional information that may allow the MPCA to further evaluate and refine the analysis. MPCA staff also continues to review the data from the sediment incubation experiments to enhance understanding of the sulfate-sulfide relationship in cold and warm temperatures and consider equilibrium questions.

## Summary of Preliminary Analysis

Based on information gained from the Wild Rice Sulfate Standard Study, existing data and scientific literature the MPCA presents the following summary of its preliminary analysis. Following the summary, the MPCA outlines more details of the analysis and next steps. This is not intended to exhaustively document the MPCA's analysis, but rather to highlight key elements. More details will be developed for a Technical Support Document (see Next Steps for further discussion).

1. **Although sulfate is not directly toxic to wild rice, it can be converted to sulfide which is toxic.** The MPCA Study and research commissioned by the Minnesota Chamber of Commerce (Fort, 2013) both show that sulfate is not directly toxic to wild rice. However, sulfate in the surface water can be converted by bacteria to sulfide in the sediment porewater of the rooting zone of wild rice. Sulfide dissolved in the sediment porewater has the potential to affect rooted plants. The MPCA Study demonstrated that elevated sulfide concentrations are toxic to wild rice seedlings. Sulfide effects on plants are also well established in the scientific literature. Laboratory hydroponic experiment data showed deleterious effects of sulfide on seedling plant growth when sulfide exceeded the range of 150 to 300 micrograms per liter (µg/L). Further analysis is needed to explore the potential for adopting a sediment porewater sulfide standard to replace, complement or work in conjunction with a sulfate standard.
2. **Sulfide in the sediment porewater is affected by the amount of sulfate in the water column and the amount of iron in the sediment**. Conditions at some of the field sites are more effective than others in the production of sulfide from sulfate, in part due to the availability of iron in the sediment (see Appendix C). A $75^{th}$ percentile quantile regression of the data from the field sites relates the sediment porewater sulfide concentration range of 150 to 300 µg/L to a corresponding water column sulfate range of 4 to 16 milligrams per liter (mg/L) sulfate.
3. **Site-specific standards may be needed for some waters**. Considerable data suggest that in some cases the development of a site-specific standard would be protective of wild rice production. This is most likely to occur in waters where the sediment iron is elevated. In such instances a higher sulfate water column concentration may not result in a sediment porewater sulfide concentration above the range of 150 to 300 µg/L. There are also data to suggest that a lower sulfate concentration may be needed for waters where sulfate is more efficiently converted to sulfide and/or sediment iron levels are not sufficient to mitigate sulfide concentrations.
4. **MPCA will continue to examine if characteristics of water body type affect the concentration of sulfide in sediment porewater.** The Study data do not suggest that susceptibility of wild rice to sulfide is associated with water body type (i.e. lakes, streams, paddies). At this time, it is unclear if there is a difference between lakes and streams in their production of sulfide from sulfate. The land- and water-management activities associated with paddy wild rice production may limit the potential for sulfide production in the sediment porewater.
5. **MPCA will continue to analyze data to further explore the "period of susceptibility" of wild rice to sulfate effects.** The sediment incubation experiment data show that sulfate can be converted to sulfide in both warm and cold conditions, but the conversion was slower under cold conditions. At both temperatures, rates of sulfide production decreased once sulfate concentrations in the overlying water decreased. This is a complex interaction and more data analysis is needed, including exploration of any site-specific factors that may affect this question.

## Rationale for Preliminary Analysis

The following pages systematically outline the MPCA's rationale for the preliminary analysis summarized above. The rationale presents information on the statistical techniques and data analyses used by the MPCA and also provides information about the uncertainties in the analysis.

### Sulfate in surface water has the potential to be converted into sulfide at levels toxic to wild rice

Sulfate concentrations that occur in Minnesota waters were not found in the hydroponic tests to be directly toxic to wild rice. This finding was corroborated by an independent experiment commissioned by the Minnesota Chamber of Commerce (Fort, 2013). However, sulfate in surface water can penetrate the underlying sediment and be converted by natural bacteria to sulfide in the sediment porewater. In aquatic sediment, the spaces between sediment particles are filled with water that is referred to as porewater. This so-called porewater contains the critical chemical environment that supplies nutrients and, on occasion, toxic levels of chemicals to plants. The hydroponic growth tests in the MPCA Study showed that elevated sulfide concentrations are toxic to wild rice seedlings (see next section).

It is well understood in the scientific literature that sulfide negatively impacts plants that root in saturated soils, where oxygen is excluded and bacteria can convert sulfate to sulfide. A recent review of sulfide toxicity to plants by Dutch scientists (Lamers and others, 2013) includes a long list of plant species for which sulfide toxicity has been evaluated. The hydroponic results indicate that wild rice is on the sensitive end of the spectrum when compared to the list published by the Dutch scientists.

## In the hydroponic tests, sulfide exposure level #2 significantly reduced seedling growth

A rangefinder test is often conducted to determine the appropriate concentration range for subsequent definitive tests of a toxicant. In the MPCA Study, the sulfide concentrations chosen for the rangefinder test were similar to the definitive tests. Since the methods used in the rangefinder test were the same as the methods for the two definitive tests (D1 and D2), the data from all three tests can be relied upon for assessing the effect of sulfide on wild rice growth.

Statistical analysis of the hydroponic tests (pooled analysis of variance (ANOVA) of the three tests, which included three replicates at each treatment level) showed that sulfide at exposure level #2 (target concentrations of 320 to 347 µg/L; Table 1) significantly reduced growth of wild rice seedlings relative to the control. At exposure level #1 (target concentrations of 96 to 200 µg/L), growth was not significantly different from the control. Target exposure levels sometimes differed among the three tests. Considering the observed variability in actual sulfide concentrations, the exposure levels are similar enough to pool them at each exposure level to increase the power of the statistical analysis.

## There is uncertainty in the hydroponic exposure concentrations

"Target concentrations" are referred to for the hydroponic tests rather than exact exposure concentrations because it was difficult to maintain a constant concentration of sulfide during the 10-day growth experiments. This was a limitation of the experimental method, in which photosynthesizing wild rice seedlings were immersed in an anoxic solution containing an initial concentration of sulfide, which over time was decreased by the oxygen the seedlings produced. The average reduction from the initial sulfide concentrations to each renewal (hydroponic solutions were renewed on day 2, 4, 7 and 9) ranged from about a 30% loss at the two highest sulfide concentrations (exposure levels #3 and #4) to 90% at exposure level #2, and 96% at exposure level #1. There was very little loss of sulfide in containers that did not contain wild rice seedlings, indicating that the loss of sulfide was due to the oxygen released by the seedlings.

Because of the decreases in sulfide concentrations, there are two alternative characterizations of the sulfide concentration at each exposure level. It is unknown whether the plants were most affected by 1) the initial concentration or 2) the average concentration during the exposure period. Accordingly, two alternative average sulfide concentrations are presented for each exposure level: 1) the mean of the initial concentrations and 2) the time-weighted mean of the average concentration during the exposure (Table 1).

**Table 1. Target and average sulfide concentrations for exposure levels #1 and #2 during the three tests: rangefinder (R), definitive 1 (D1), and definitive 2 (D2).**

| Sulfide Exposure Level | Target sulfide concentration for each test<br>µg/L | Measured Initial Sulfide Concentration (average of each test)<br>µg/L | Measured Initial Sulfide Concentration (average of all tests)<br>µg/L | Measured Average Sulfide Concentration (average of each test between initial and end of exposure; time-weighted average)<br>µg/L | Measured Average Sulfide Concentration (average of all tests between initial and end of exposure; time-weighted average)<br>µg/L |
|---|---|---|---|---|---|
| #1 | R: 96<br>D1: 200<br>D2: 160 | R: 109<br>D1: 140<br>D2: 153 | 134 | R: 57<br>D1: 72<br>D2: 81 | 70 |
| #2 | R: 320<br>D1: 347<br>D2: 320 | R: 333<br>D1: 291<br>D2: 302 | 309 | R: 182<br>D1: 158<br>D2: 159 | 166 |

## Hydroponic results: the protective concentration for sulfide lies in a range between 150 and 300 µg/L

Hydroponic exposure level #2 caused a statistically significant reduction in the growth of wild rice seedlings, whereas exposure level #1 did not. MPCA staff relied on the initial sulfide concentration, rather than the lower concentrations that developed between renewals, as the operative exposure concentration in this analysis. For sulfide exposure levels #1 and #2, the initial concentrations were much greater than the concentrations measured just one or two days later and therefore had the most potential to negatively affect the growth of the seedlings. The average initial concentration of exposure level #2, 309 µg/L, was toxic, whereas the average of exposure level #1, 134 µg/L, was not significantly different from the control. Therefore, the results from the hydroponic exposures suggest that the protective concentration of sulfide is most likely between 134 and 309 µg/L, or in rounder numbers, 150 and 300 µg/L.

## Effect concentrations can also be derived from the hydroponic results

The hydroponic data can also be analyzed through regression analysis, which allows for estimation of particular reductions in seedling growth due to sulfide toxicity, such as a 50% reduction in growth relative to the control (EC50) or a 20% reduction in growth relative to the control (EC20) (See Figure 1 and Table 2). An EC50 is generally interpreted to characterize a concentration that has an unquestioned deleterious effect, whereas an EC20 is sometimes used to characterize a no-effect concentration. The average EC20 estimate (257 µg/L) is between the concentrations associated with exposure levels #1 and #2, and the average EC50 estimate (383 µg/L) is higher than the concentrations associated with exposure level #2 (309 µg/L). Therefore, both the average EC20 and EC50 estimates are compatible with the conclusion that a protective concentration for sulfide is likely between 150 and 300 µg/L. The MPCA will continue to evaluate the results of these statistical tests as well as the appropriateness of using the EC20 or EC50 estimates to assist in evaluating the toxicity of sulfide to wild rice.

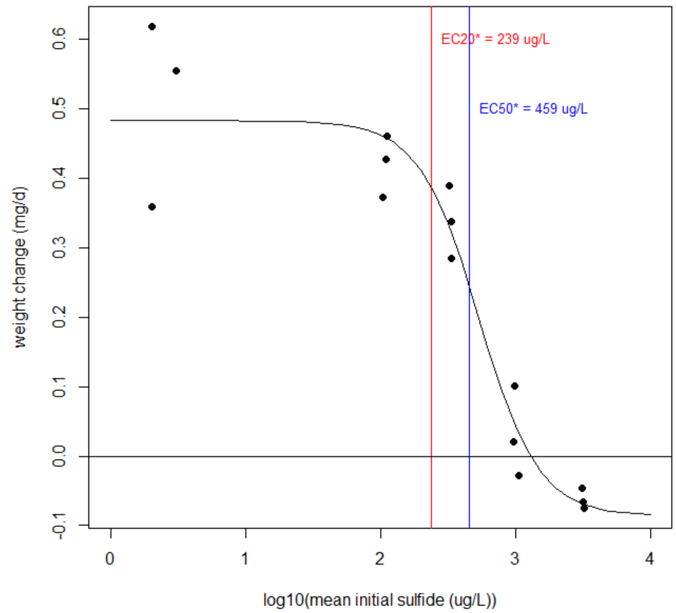


**Figure 1. The hydroponic data can be analyzed through regression analysis, by fitting the data to a logistic curve and taking estimates of EC20 and EC50 from the curve. All three hydroponic tests show similar relationships; this example is data from the rangefinder test.**

**Table 2. Sulfide concentrations estimated by regression analysis that correspond to the EC20 and EC50. The estimates are based on the initial concentrations of sulfide in the exposure tests. Growth is measured as net change in dry weight.**

| Effect Concentration Percentile | Effect Concentration for Each Test (µg/L) | Mean Effect Concentration (µg/L) |
|---|---|---|
| EC20 | R: 239<br>D1: 210<br>D2: 322 | 257 |
| EC50 | R: 459<br>D1: 326<br>D2: 365 | 383 |

## Limitations of the hydroponic experiments

The method for the sulfide seedling test involved immersing photosynthesizing seedlings in an anoxic solution containing an initial concentration of sulfide, which over time was decreased by the oxygen the seedlings produced. This led to the uncertainty in exposure concentration already addressed above. In addition, it is unknown to what degree the young stem and leaf would be exposed to sulfide in nature and if this makes a difference in the hydroponic results. This is where the investigations of multiple lines of evidence become especially important, to see if observations in one experimental approach, with any associated limitations, are reinforced by observations from other lines of evidence.

## The field survey results are compatible with the hydroponic sulfide experiment results

Over the course of the Study (2012-2013), nearly 120 individual field sites were sampled during 193 total site visits (some sites were sampled more than once), and more than 75 chemical and physical parameters were quantified at each site. The Study also benefited from a pilot field survey conducted in 2011 that involved sampling 39 individual sites. The field survey data can be examined for evidence that sediment porewater sulfide concentration influences the proportion of sites that support wild rice growth.

A principal components analysis of major non-sulfur-related habitat parameters (i.e. variables other than sulfate, sulfide, and iron) shows no consistent difference between sites that had wild rice and those that did not (Figure 2).

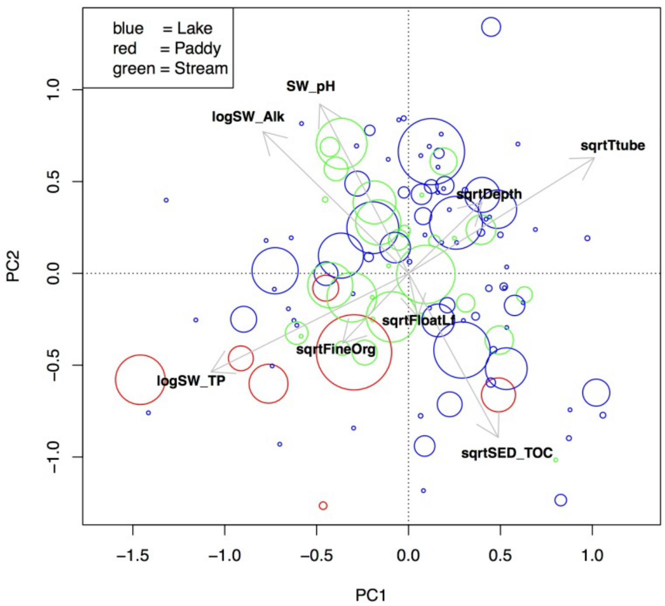


**Figure 2. Principal components analysis (PCA) of survey-site habitat parameters not related to sulfur (sulfate, sulfide, and iron). Symbols are sized in proportion to wild rice cover at the sampling site.** Sites where wild rice was found, and sites where wild rice was not found, occur in all quadrants, indicating that site selection was not biased for or against successful wild rice habitat. Streams and lakes were observed in all quadrants. Paddies occur mostly in the lower left quadrant (higher surface water total phosphorus (TP), lower transparency, shallower depth), but there are also streams and one lake in this quadrant that supported rice. (Based on 119 different field sites sampled 2012-2013.)

This preliminary analysis focuses on the primary hypothesis that wild rice is impacted by sulfate via the conversion of sulfate to sulfide in the sediment porewater. However, many other parameters were quantified for each field site. The data include extensive chemical analyses of surface water, sediment porewater, and the solids in the sediment at each site. In the coming weeks and months, these data will be further analyzed to evaluate other hypotheses presented in the 2011 research protocol.

If the field survey data are consistent with the hydroponic results, it would be expected that at concentrations above 150 to 300 µg/L of sulfide in the sediment porewater there would be a decline in the proportion of sampled sites where wild rice had been found by the field crews. A histogram of the proportion of sites with at least 5 percent wild rice cover around the sampling location, where sulfide was measured in the sediment porewater (Figure 3), shows a continuous decline above 75 µg/L (this pattern is somewhat dependent on the choice of breaks between the sulfide ranges).

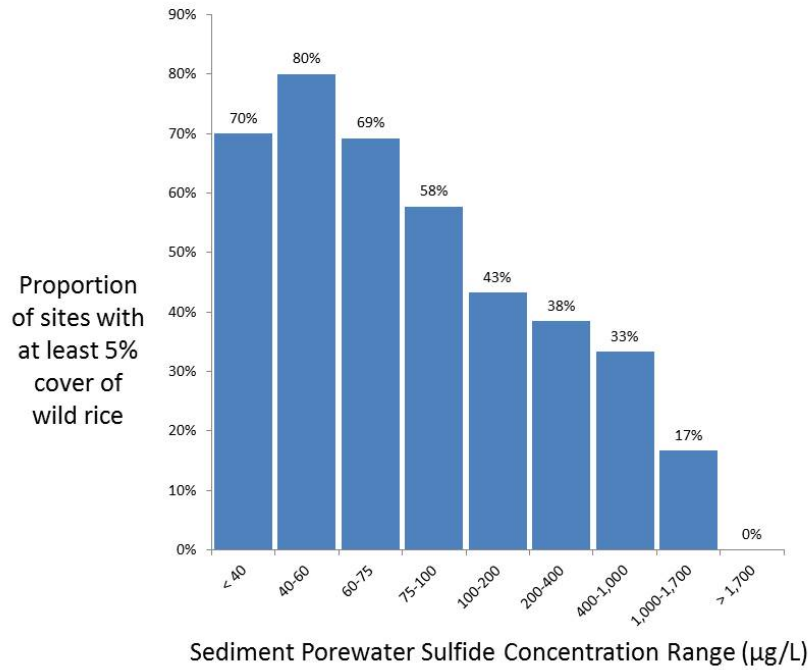


**Figure 3. As sediment porewater sulfide concentrations increase at the field survey sites, there is a decreased proportion of sites where the wild rice exceeded 5 percent cover.** (Based on 171 lake and stream samplings, where no one site was sampled more than once a year, 2011-2013).

Figure 3 illustrates that there is a negative relationship between increasing sediment porewater sulfide concentrations and sites with at least 5 percent cover of wild rice around the sampling location, an observation that is consistent with the sulfide effect observed in the hydroponic experiments. The lack of precise correspondence with the hydroponic results should not be surprising, given the nature of the field data. Sediment porewater sulfide concentrations were obtained from sediment cores from the same area where the wild rice percent cover was evaluated, but no attempt was made to core immediately adjacent to the roots of wild rice. Sometimes other rooted aquatic plants were co-located with wild rice. The primary hypothesis to be evaluated with the field data is that the sulfide in the sediment porewater can affect wild rice. However, there is published evidence that the roots of plants can affect the chemistry of the sediment porewater, including sulfide concentrations. So, the measured concentration of sulfide in sediment porewater is a function of how close the core was to plants, among other factors, and the concentration of sulfide that the wild rice was actually exposed to can only be approximated from the field data. Sediment porewater sulfide and iron were extracted from separate cores due to sample size needs, which may contribute to “noise” in the relationship between sulfide and iron.

Given the uncertainties in what concentration of sulfide the wild rice plants were actually exposed to in the field, the field data as represented in Figure 3 are broadly compatible with the likely protective sulfide concentration range of 150 to 300 µg/L derived from the hydroponic exposure experiments.

## Sediment porewater sulfide concentrations are limited by sulfate and iron

Potential sulfide production in the rooting zone of wild rice is limited by sulfate availability. Bacteria cannot produce sulfide unless sulfate is available. However, data from the field survey show that observed sulfide concentrations in the sediment porewater of the rooting zone are often not proportional to the sulfate concentration in the surface water (Figure 4A). There is a great deal of variability in the relationship. Statistical analysis of the data indicates that only 14 percent of the variability (the r-squared value) in sediment porewater sulfide concentrations is explained by the sulfate concentrations in the overlying surface water, indicating that there are other factors influencing sulfide aside from sulfate in the overlying water.

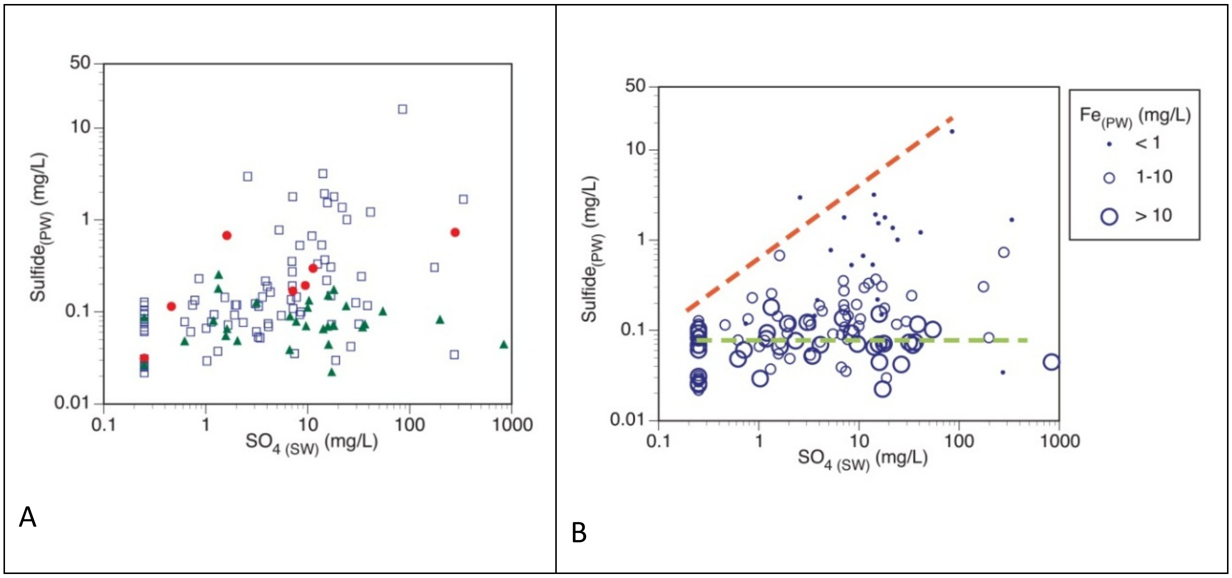


**Figure 4. A. Field survey data show that sulfide concentrations in the sediment porewater are often not proportional to the sulfate concentration in the overlying surface water ($r^2$=0.14). (Symbols: Blue=lakes; Red=cultivated paddies; Green=streams and rivers). B. Bacteria in the sediment of wild rice habitat have the potential to produce sulfide in direct proportion to surface water sulfate (dashed red line).** When elevated iron is present (greater than about 1 mg/L), sulfide is precipitated by iron, keeping sediment porewater sulfide concentrations low even when sulfate concentrations are elevated (green dashed line). (Based on 119 different field sites sampled 2012-2013.)

There are multiple mechanisms that may be responsible for the observed lack of proportionality between sulfate and sulfide: a) although we might assume that sulfate is diffusing downward into the sediment, downward diffusion may be inhibited by upwelling groundwater; b) after oxygen is consumed, bacteria may be using chemicals aside from sulfate as an electron acceptor for respiration such as nitrate, manganese, iron, or even humic acids; c) sulfate concentrations may be in excess to the needs of sulfate-reducing bacteria; and d) as sulfide is produced it may bind to iron and precipitate as a solid, effectively removing sulfide from solution.

Statistical analysis of the field survey data shows that the presence of iron in the sediment porewater has a strong role in controlling the concentration of dissolved sulfide. In general, the sediment porewater sulfide concentrations are only high if iron concentrations are low in the sediment porewater (Figure 4B, 5). If the iron supply is greater than the production of sulfide, then iron can precipitate sulfide as it is produced, yielding sulfide concentrations lower than could potentially be produced by downward diffusion of the sulfate from the overlying water. Figure 6A maps the concentration of sulfide in sediment porewater at the field survey sites; the high concentrations, in red, generally correspond to low porewater concentrations of iron, which are also shown in red in Figure 6B.

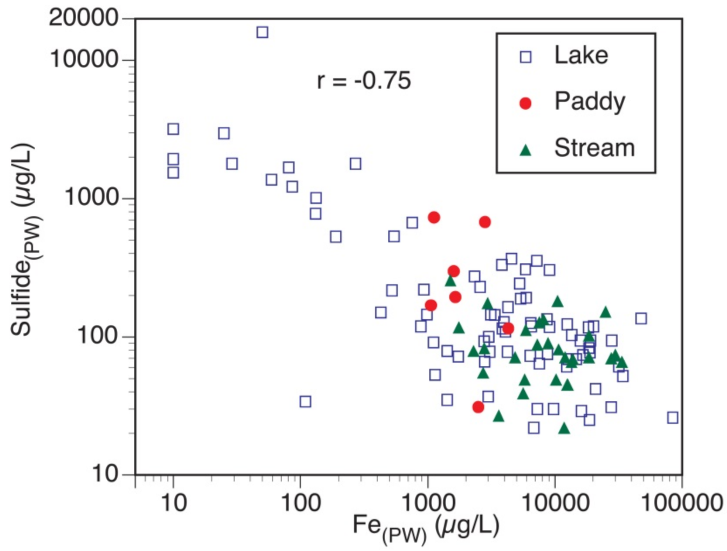


**Figure 5. The field survey data show that in general sediment porewater sulfide concentrations are only high when iron concentrations are low in the sediment porewater.** ($r^2$=0.56; Based on 119 different field sites sampled 2012-2013)

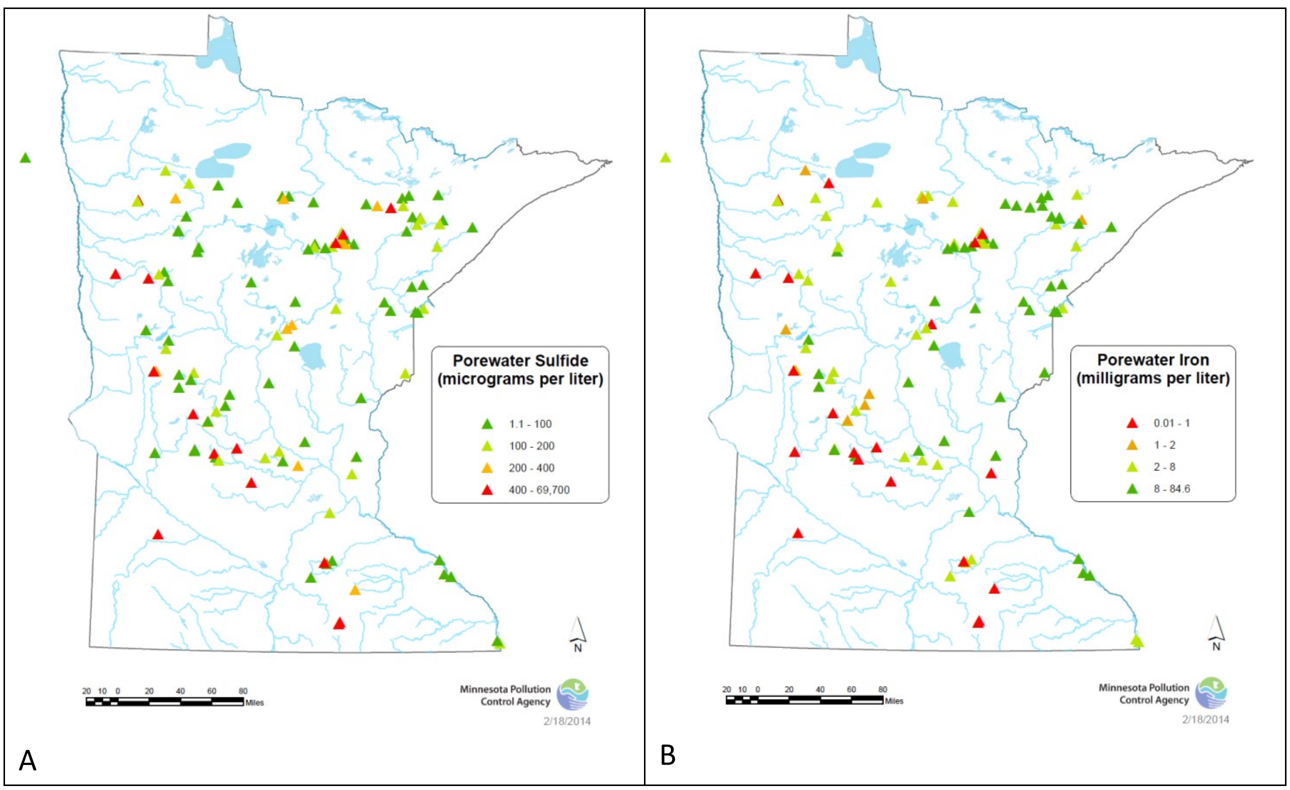


**Figure 6A. Sulfide concentrations in the sediment porewater at the sample sites. B. Iron concentrations in the sediment porewater at the sample sites.**

## Sediment porewater sulfide can be predicted from sulfate and iron

The statistical relationship between sulfate, sulfide, and iron can be expressed in an equation from a multiple regression of those variables (Figure 7). The r-squared value indicates that 58 percent of the variability in the sediment porewater sulfide concentration can be explained by considering both sediment porewater iron concentrations and sulfate concentrations in the overlying surface water, a considerable improvement over considering sulfate alone. Further data analysis may identify other variables that improve understanding of this relationship.

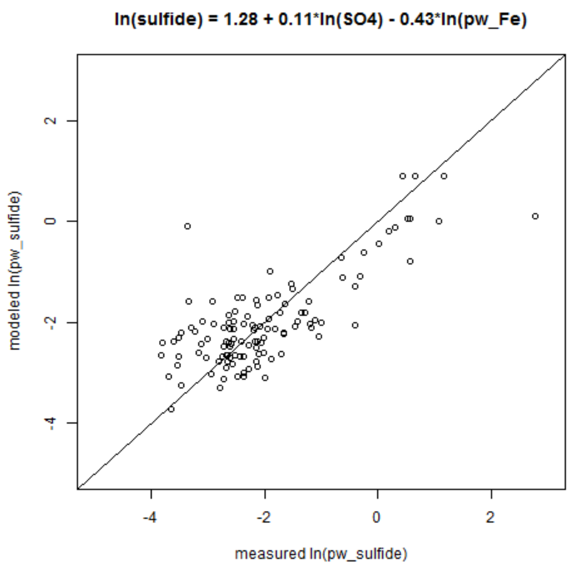


**Figure 7. A comparison between the modeled and measured sediment porewater sulfide concentrations for the surveyed lake, stream, and paddy sites.** The multiple regression model relates sediment porewater sulfide to the sulfate concentration in surface water ($SO_4$) and the sediment porewater iron concentration (pw_Fe; $r^2$=0.58; Based on 119 different field sites sampled 2012-2013, with two outliers removed, which had sulfate concentrations below the reporting limit). ln(sulfide mg S/L)=1.28 + 0.11 ln($SO_4$ mg $SO_4$/L) – 0.43 ln(pw_Fe µg Fe/L).

## Wild rice tends to grow where sediment porewater is low in sulfide and high in iron

When the relationship between sulfide and iron is presented graphically with circles that are proportional to wild rice cover at the sampling site (Figure 8), it is evident that wild rice tends to occur when sulfide sediment porewater concentrations are low and iron concentrations are high. There is a pattern of a decreasing proportion of sampled sites that had a minimal density of rice as the sulfide concentration increases in the sediment porewater (Figure 3).

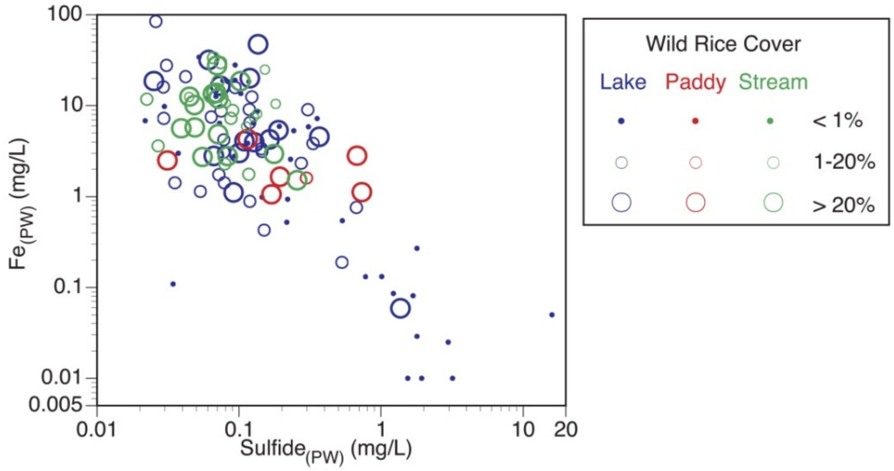


**Figure 8. The percent cover of wild rice at the sampling sites as a function of sulfide and iron in the sediment porewater**. In general, when sulfide is high, iron is low, and wild rice cover is low (Based on 119 different field sites sampled 2012-2013).

## Limitations of the field survey

It is important to note that the field survey sites were selected based on a set of targeting criteria, and are not a randomly selected (i.e. probabilistic) subset of wild rice habitat. The goal of this Study was to assess whether there was any relationship between elevated sulfate and the absence of wild rice. If wild rice habitats had been sampled probabilistically, most of the sites would have had very low sulfate concentrations, and little would have been learned about the effect of elevated sulfate. To ensure that the study included sufficient samples from potential wild rice habitat that had elevated sulfate concentrations, the survey sites (Figure 9) were intentionally not chosen in a random manner, and therefore care must be taken before extrapolating to a larger set of actual or potential wild rice habitats. With that said, the information gained from the field samples may be used to corroborate the hydroponic experiment results; to enhance understanding of the occurrence of sulfate, sulfide and iron at sites with wild rice; and to help inform evaluation of the wild rice sulfate standard.

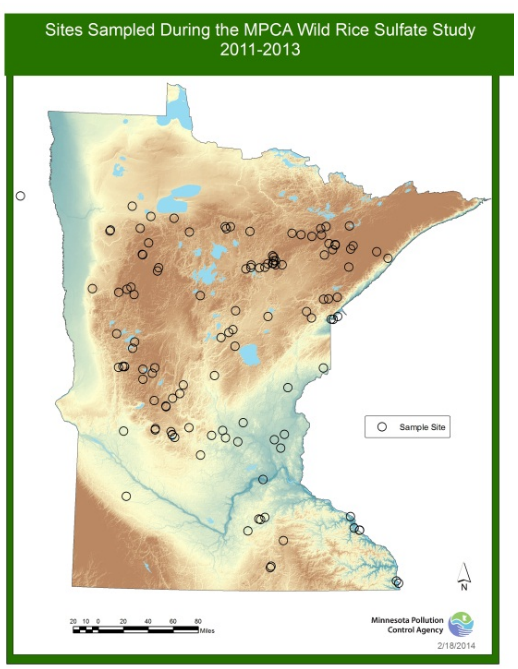


**Figure 9. Sites sampled during the Study over three field seasons, 2011-2013.**

## Further analysis is needed to explore adopting a sediment porewater sulfide standard to protect wild rice

The analysis of Study data thus far has documented: a) that sulfate is not directly toxic to wild rice, b) that sulfate can be deleterious to the growth of wild rice when bacterial activity builds up a sufficiently high sulfide concentration in the sediment porewater, and c) that the net concentration of sulfide is simultaneously controlled by bacterial conversion of sulfate and iron availability. Iron is sometimes, but not always, present in sufficient quantities to mitigate the production of sulfide from sulfate. It can also be expected that some Minnesota water bodies have sediment porewater sulfide concentrations (and overlying water sulfate concentrations) that are naturally too high to support wild rice. It is unknown at this time if any of the field sites represented in the graphs presented here fall into this category.

The Study data and existing literature support the hypothesis that wild rice is impacted by sulfate via the conversion of sulfate to sulfide in the sediment porewater, and that sediment porewater sulfide concentration is a water quality condition that can impact wild rice growth. This suggests that the MPCA should consider adopting a sediment porewater sulfide standard in the range of 150 to 300 µg/L to protect wild rice, since this is a response variable that is impacting wild rice production.

This direct approach is complicated by the fact that the primary source of elevated sulfide in the sediment is elevated sulfate in the overlying water. In other words, elevated sulfate is the causative variable that can result in the response variable that exceeds the level that is protective of the beneficial use.

This is similar to the situation with lake eutrophication standards where the response variables of excess algae and reduced transparency are usually (though not always) driven by the causative variable of elevated phosphorus. MPCA will continue to evaluate the similarities between the sulfate-sulfide and the phosphorus-algae/transparency cause-response relationships to develop recommendations regarding adopting a sediment porewater sulfide standard to replace, complement or work in conjunction with a sulfate standard in the overlying water to protect wild rice production.

## Quantile regression can be used to examine the relationship between sulfide and sulfate

It would be preferable to directly translate the observed protective concentration range of sulfide of 150 to 300 µg/L to a corresponding protective concentration range of sulfate in the overlying water. However, due to significant other variables, such as iron availability, which affect sulfate conversion to sulfide, there is a great deal of variability in the sulfate-sulfide relationship.

Although the relationship is complicated, it is generally the case that the lower the sulfate concentration in the overlying water, the potential to produce toxic levels of sulfide in the sediment porewater is diminished. Figure 10 shows that the proportion of sites that support wild rice generally declines as sulfate concentrations increase.

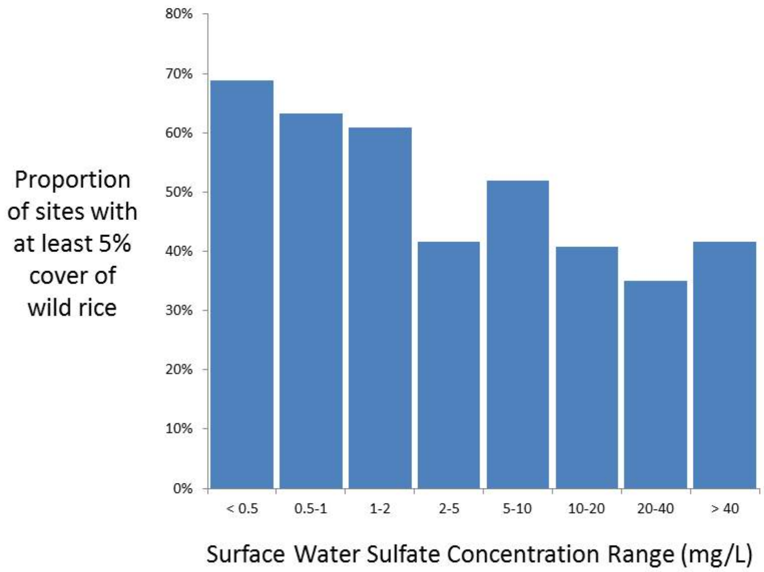


**Figure 10. As surface water sulfate concentrations increase at the field survey sites, there is a decreased proportion of sites where the wild rice exceeded 5 percent cover.** (Based on 171 lake and stream samplings, where no one site was sampled more than once a year, 2011-2013).

The field survey data include measurements of surface water sulfate and sediment porewater sulfide concentrations at each site. Quantile regression of the field data was used to analyze the wedge-shaped relationship between sulfate and sulfide (Figure 4B). By fitting the outer limits of the wedge using upper quantiles (i.e., 75$^{th}$, 80$^{th}$, 85$^{th}$, 90$^{th}$, and 95$^{th}$ percentiles), the relationship between sulfate and sulfide can be emphasized by minimizing the effects of other variables (e.g. iron). These quantile regressions further demonstrate the relationship between sulfate and sulfide (Figure 11).

A 75$^{th}$ percentile quantile regression relates the sulfide concentration range of 150 to 300 µg/L to a corresponding sulfate range of 4.3 to 16.2 mg/L, or 4 to 16 mg/L in round numbers. The current 10 mg/L sulfate standard falls within this range. Higher quantile percentages predict that sulfide concentrations of 150 to 300 µg/L relate to sulfate concentrations below 10 mg/L. However, there is more uncertainty with these extreme quantiles as the fit for each quantile is driven by fewer samples. It should also be noted that the fit of the quantile regression models is not good at high concentrations of sulfate due to a small number of samples with sulfate concentrations above 100 mg/L. Because the field survey data set is not based on a random selection of sites (see "Limitations of the field survey" section above), care must be taken to evaluate and address any gaps in the data before making predictions for sites outside of the data set. MPCA will explore ways to address this.

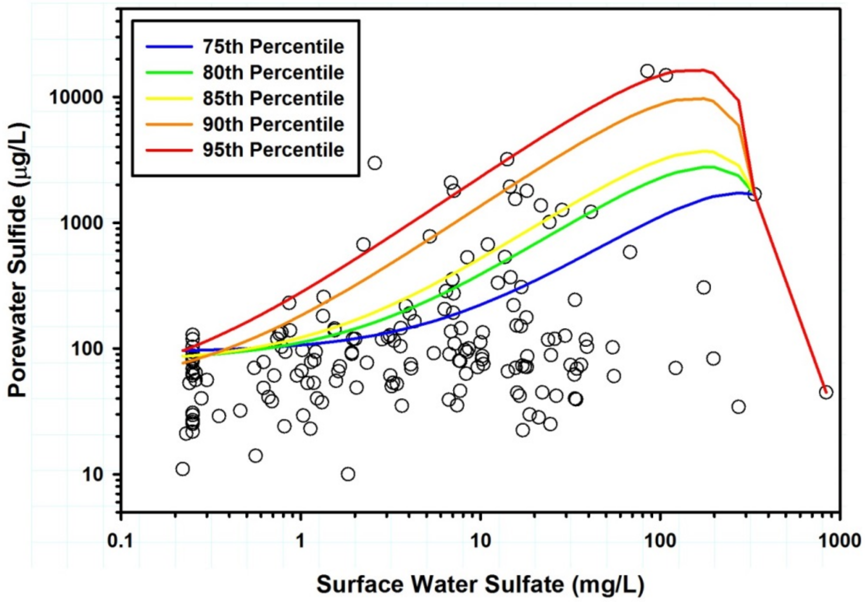


**Figure 11. Quantile regression smoothing splines for surface water sulfate versus porewater sulfide for lakes and streams (n=172). Regression fits are nonparametric quantile regressions using regression splines ("rq" in "quantreg" package; Koenker 2009 and "bs" in "splines" package; R Development Core Team 2009) which were performed in the program R ver. 3.0.0 (R Development Core Team 2013).**

## Site-specific standards for sulfate may be needed for some waters

As noted above, the field data demonstrate that some sites are less effective than others at converting sulfate to sulfide. In other words, some of the sites did not produce sediment porewater sulfide concentrations above the 150 to 300 µg/L range even with elevated sulfate concentrations in the overlying water. This pattern may be produced by: 1) sufficient iron is available to precipitate sulfide as it is produced, 2) sulfide is not produced nearly in proportion to the sulfate concentration, or 3) another mechanism that has not yet been identified.

The subset of sites that supports wild rice under elevated sulfate concentrations (but with sediment porewater that has low sulfide and high iron concentrations) includes some lakes, some streams, and some cultivated paddies. If wild rice has grown for years in surface water with sulfate concentrations greater than a concentration considered protective elsewhere, it is evidence that a relevant site-specific sulfate standard may be developed. If it can be assumed that at such a site the sediment porewater sulfide concentration is effectively in a steady state with the overlying sulfate concentration, then the observed sulfate concentration is apparently protective and could serve as the basis for developing a site-specific water quality standard.

## MPCA will continue to explore if characteristics of water body type affect the concentration of sulfide in sediment porewater

The Study data do not suggest that susceptibility of wild rice to sulfide is associated with water body type (lakes, streams, paddies). At this time it is unclear if there is a difference between lakes and streams in their production of sulfide from sulfate. In the field survey, stream sites tended to have lower average sediment porewater sulfide concentrations than lakes, although there is overlap in the distributions (Figure 4A). This observation of lower sulfide in streams may be a result of sending field crews preferentially to sites where wild rice had been observed in past years. It is unknown whether these stream sites are representative of the wild rice habitat in those streams, or whether other sites would yield sulfide concentrations that are higher on average than observed in the field survey.

Paddies do not depart from the basic relationships described here between sulfate, sulfide, and iron (Figures 4A, 5, and 8). With that said, two of the sediment porewater sulfide concentrations in paddies are among the highest observed where wild rice grew successfully (Figure 8), indicating that there may be some aspect of paddy management that allows cultivated wild rice to grow in association with sulfide near concentrations found to be toxic in the hydroponic exposures.

Water used in cultivated paddies is highly managed, as are the soils and nutrient levels. The overall result is that paddies are quite different from natural wild rice stands. Principal Components Analysis (Figure 2) demonstrates that paddies tend to cluster together, apart from most natural sites, because of differences in important ecological parameters such as phosphorus, transparency of the water, and the fine organic matter in the soil. The annual water drawdown before harvesting could have profound consequences for the biogeochemistry of paddy soils and sediment porewater, but the precise effects are poorly understood. Further analysis is needed by the MPCA to explore whether or not the sulfate standard is needed to protect paddy-grown wild rice production.

## Mesocosm and sediment incubation experiment results may help model the effect of increased sulfate concentrations on wild rice production

It may be possible to develop an estimate of the net effect of an increase in sulfate concentrations on sediment porewater sulfide concentrations. Two investigations in the Study, the outdoor container and sediment incubation experiments, looked at the effects of increased sulfate concentrations. These investigations may provide useful information after further analysis and modeling. This question is challenging, because it involves non-steady state conditions. Neither experiment included the possible effects of groundwater flow, which could be an important variable.

Minnesota's current wild rice sulfate standard of 10 mg/L applies "…during periods when the rice may be susceptible to damage by high sulfate levels." The effects of temporary increases in sulfate concentration are likely more complicated to understand and model than the effects of a permanent increase. A preliminary analysis of the experimental data shows that sulfate can be converted to sulfide in both warm and cold conditions, but the conversion was slower under cold conditions. At both temperatures, rates of sulfide production decreased once sulfate concentrations in the overlying water decreased. These experiments are complex, and need further data analysis before any recommendations can be developed. Any recommendation may also need to consider site-specific factors that may affect this question.

## Next Steps

MPCA will meet with the Wild Rice Sulfate Standards Advisory Committee and Minnesota Tribes to get their feedback on the MPCA's preliminary analysis. MPCA will also continue to seek feedback from USEPA and the Study researchers.

MPCA technical staff will continue to refine the analysis to develop a technical support document that will be used in wild rice water quality standards rulemaking.

- For example, alternatives exist for considering site-specific standards. If substantial detail on site-specific standard considerations can be extrapolated and adopted into rule (e.g. a relationship or formula can be identified that individual site data can be subsequently "plugged into"), site-specific standards could be established and used by following those rule procedures. However, if detail is unavailable for inclusion of a formula or relationship in rule, site-specific standards would need to be adopted through scientifically defensible, case-specific analysis and demonstration.

Further analysis of results and data from the overall Study and especially from the outdoor container experiments and sediment incubation study will also continue.

The MPCA is contracting for expert scientific review of the wild rice study reports and the MPCA's preliminary analysis. The expert review panel will likely be convened in 2014, and will include the opportunity for interested stakeholders/members of the public to address the panel.

MPCA will also begin exploring implementation and related policy questions including treatment options, pollution prevention opportunities, and guidelines for permitting and compliance. MPCA will seek stakeholder input and dialogue on these implementation questions, which may be addressed in the future wild rice water quality standard rulemaking.

In a parallel effort, MPCA is developing factors that will help identify specific water bodies as "water used for production of wild rice." These factors will be used in case-by-case determinations and to inform rulemaking regarding the wild rice water quality standard. Once the factors have been developed, MPCA will solicit public comments to help refine the factors. The MPCA will also explore whether to move the wild rice sulfate standard from Class 4 where it currently resides to Class 2 and create a new subclass to clarify that the standard is designed to protect the growth of wild rice grains for consumption by humans and wildlife. The MPCA will also consider revising the term "water used for production of wild rice."

Any proposed change to the wild rice water quality standard, including clarification of "water used for production of wild rice" and any changes to the use class, would be adopted into Minnesota's water quality standard rule (Minnesota Rules Chapter 7050) in accordance with the requirements of the Minnesota Administrative Procedures Act and would require the approval of the USEPA.

## References

Fort, Douglas J. 2013. Definitive Hydroponics-Based Wild Rice (*Zizania palustris*) Sulfate Toxicity Testing. Fort Environmental Laboratories, Inc. sponsored by Minnesota Chamber of Commerce.

Lamers, LPM; Govers, LL; Janssen, ICJM; et al. 2013. Sulfide as a soil phytotoxin—a review. Frontiers in Plant Science. Volume 4:268.

Koenker R. (2009) quantreg: Quantile Regression. R package.

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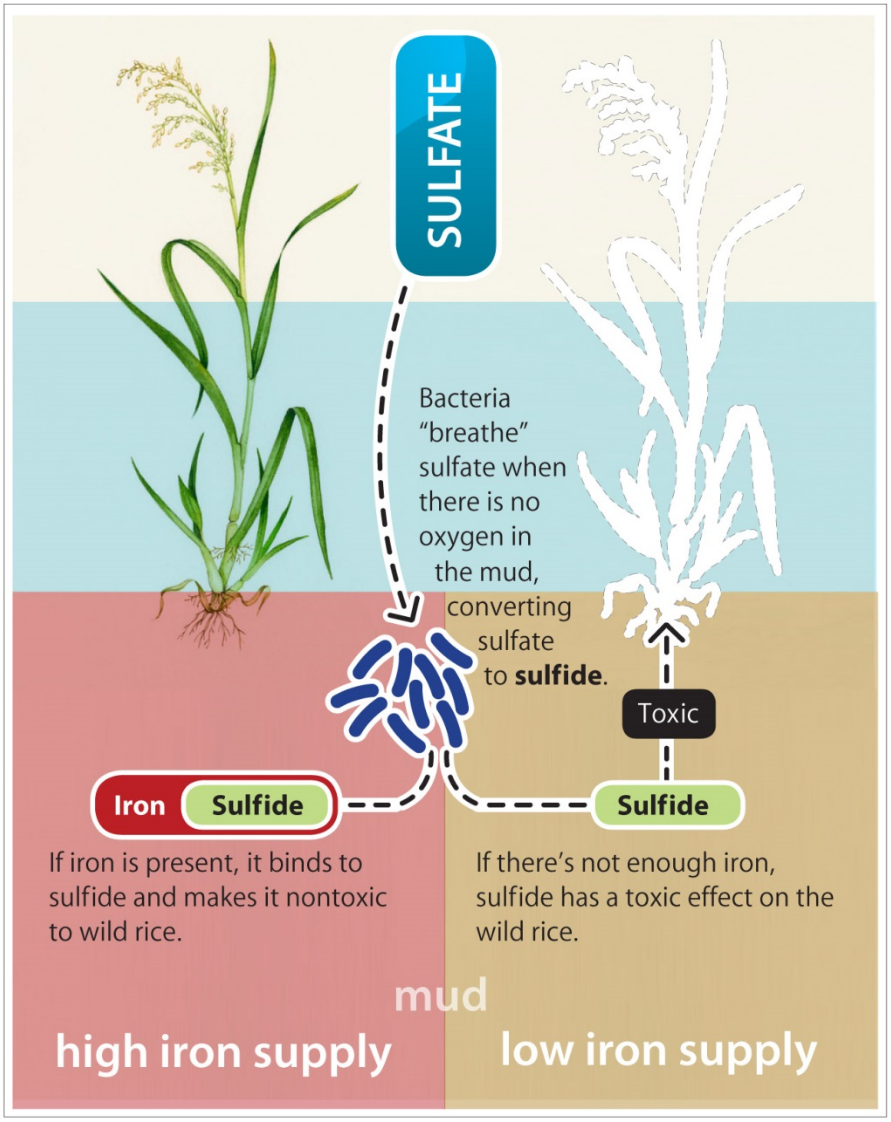
**Appendix A. Purpose, strengths, and limitations of Study components.**

| | 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, over multiple years. | Characterize sulfate, sulfide, and iron in the sediment porewater 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 & sediment porewater (e.g. $SO_4$ & $H_2S$ vs. wild rice occurrence). | Growth of wild rice sprouts (biomass, root & shoot elongation). Germination rate of seeds. | Growth of wild rice sprouts (biomass, root & shoot elongation). Germination rate of seeds. | Growth of wild rice (biomass, plus number & weight of seeds). $H_2S$ concentrations in sediment porewater. | Concentrations of sulfate, sulfide and iron in sediment porewater. | $SO_4$ in overlying water over time; $SO_4$, iron, $H_2S$, & 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 & 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 not fully transferrable to other sites. No groundwater movement. No wild rice plants grown. |

**Appendix B. Wild Rice Standards Study Advisory Committee Members**

- Kurt Anderson, Minnesota Power (ALLETE), wild rice harvester
- Leonard Anderson, wild rice harvester, citizen
- Mike Appelwick, Northeast Technical Services (NTS)
- Sara Barsel, citizen
- Rob Beranek, Cliffs Natural Resources
- David Biesboer, University of Minnesota
- Jennifer Engstrom, Minnesota Department of Natural Resources
- Tracy Ekola, Minnesota Environmental Science and Economic Review Board (MESERB)
- Ann Geisen, Minnesota Department of Natural Resources
- Craig Johnson, League of Minnesota Cities
- David Hatchett, Mesabi Mining
- Kathryn Hoffman, Minnesota Center for Environmental Advocacy
- Peter Lee, Lakehead University
- John P. Lenczewski, Minnesota Trout Unlimited
- Paula Maccabee, WaterLegacy
- Joe Mayasich, Western Lake Superior Sanitary District
- Anne Nelson, wild rice harvester, Wetlands and Water Committee of the Sierra Club
- Beth Nelson, Minnesota Cultivated Wild Rice Council
- Frank Ongaro, MiningMinnesota
- Robert Pillsbury, University of Wisconsin Oshkosh
- Raymie Porter, University of Minnesota
- Robin Richards, ENVIRON International Corporation
- Lloyd Grooms, Minnesota Chamber of Commerce
- Shane Bowe, Red Lake Nation Foods and Red Lake Department of Natural Resources
- Jon Schneider, Ducks Unlimited
- Nancy Schuldt, Fond du Lac Band of Chippewa
- Robert Shimek, wild rice harvester, member of Red Lake Band
- David Smiga, U.S. Steel
- Rod Ustipak, Minnesota Wild Rice Company, wild rice harvester
- Rachel Walker, Barr Engineering
- Darren Vogt, 1854 Treaty Authority

# When the mud has a good supply of iron, sulfate does less harm




**The iron-sulfide battle**
The amount of iron and sulfide are dynamic and one affects the other. If enough new iron is flowing into the mud (e.g. via groundwater), then even a lake or stream with high sulfate levels can support wild rice. On the other hand, enough sulfate can overwhelm the supply of iron and make sulfide levels toxic.