

Wild Rice/Sulfate Protocol Development Discussion Document

For the May 9, 2011, Meeting

Minnesota Pollution Control Agency

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Introduction

This document identifies key information to enhance understanding of the effects of sulfate on wild rice, and options for obtaining data that address those needs. The goal is to obtain information that would allow the Minnesota Pollution Control Agency (MPCA) to further evaluate and, if appropriate, revise Minnesota's sulfate water quality standard for the protection of wild rice. This document is intended to inform and guide the technical discussion regarding the development of a study protocol to further investigate the effects of elevated sulfate concentrations on wild rice. Over the next few months the protocol will be developed based on information gathered through the technical discussion, in consultation with US EPA, the Minnesota Department of Natural Resources and Minnesota Tribes, and with input from interested and affected stakeholders.

Background: Minnesota's wild-rice-based sulfate water quality standard

In its water quality standards and use classification rule, Minnesota Rules Chapter 7050, the MPCA assigns a series of use classifications to all waters of the State of Minnesota. Water use classifications, and their accompanying narrative and numeric criteria and nondegradation provisions, make up the State's set of water quality standards. Aquatic life and recreation, industrial uses, agriculture and wildlife, and domestic consumption are some of the beneficial uses these standards are intended to protect.

Minnesota's Class 4 Agriculture and Wildlife use classification covers agricultural uses (crop irrigation and livestock 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 the production of wild rice during periods when the rice may be susceptible to damage by high sulfate levels."*** (Minn. R. 7050.0224, subpart 2).

This 10 mg/L sulfate wild rice standard (reported as SO₄) was adopted into the MPCA water quality standards rule in 1973. Based on testimony presented at public hearings leading to the adoption of this sulfate standard, it was intended to apply both to waters with natural wild rice stands and to waters used for paddy wild rice production. The standard was based on field observations and water chemistry correlations made by John Moyle primarily in the late 1930s and early 1940s (Appendix A). 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), and rice generally is absent from water with more than 50 ppm."

The next set of wild rice-related rule amendments occurred around 1997. It was during that time that the MPCA initiated a rulemaking proceeding that led to the adoption of new rules governing water quality standards, standards implementation, and nondegradation standards for Great Lakes Initiative (GLI) pollutants in the Lake Superior Basin. This rule was codified as Minn. R. ch. 7052 and is now informally referred to as the "Lake Superior Basin" or the "GLI" rule. The 1997 rulemaking also included two major changes to Minn. R. ch. 7050. First, the designation of a portion of the Lake Superior shoreline waters of the Grand Portage Indian Reservation as Outstanding Resource Value Waters - Prohibited in accordance with the provisions of Minn. R. 7050.0180. Second, a listing of 22 lakes and two river segments located in the Lake Superior watershed as wild rice waters (Minn. R. 7050.0470, subp. 1) as well as the inclusion of narrative language pertaining to wild rice under the Class 4 Agriculture and Wildlife use class (Minn. R. 7050.0224, subp. 1).

The 1997 rulemaking record reveals that originally there were 124 lake or river segments identified as wild rice waters within the 1854 Ceded Territory that were suggested for listing in Minn. R. ch. 7050 as wild rice waters. These waters were considered to be some of the more important existing and/or potential wild rice waters identified by the Fond du Lac, Bois Forte, and Grand Portage Bands. (The 1854 Ceded Territory covers much of the Arrowhead Region of Minnesota, and encompasses portions of the Lake Superior, Rainy River, and Mississippi River watersheds.) Since the provisions of the new GLI rule were specific to the Lake Superior Basin, in 1997 MPCA staff chose to limit assignment of the wild rice designation to those waters identified and agreed upon

that were within the Lake Superior Basin (Minn. R. 7050.0470, subp. 1). The listing of a select number of waters as wild rice waters was intended to be part of a broader process to provide greater protection for, and greater public awareness of, the ecological importance of wild rice. These listings were also viewed as an affirmation of the MPCA's commitment to work cooperatively with Tribal governments and others concerned about wild rice waters. Inclusion of the wild rice narrative language and the rule listings were considered "first steps" towards a future statewide identification and listing of wild rice waters and the development of wild rice-related best management practices.

The 1997 rulemaking post-hearing comments noted that the 10 mg/L wild rice sulfate standard was not proposed for revision during the 1997 proceedings. In addition, the 10 mg/L sulfate standard was never intended to apply only to the 24 wild rice waters that were specifically listed in Minn. R. ch. 7050.0470. Rather this numeric standard was intended to continue to have statewide applicability to those waters used for the production of wild rice.

The MPCA is currently striving to clarify current and future implementation of the wild rice sulfate standard, which recently has come under increased questioning and contention. As part of the 2012 Water Quality Standards Triennial Review¹, the MPCA intends to clarify the definition of "water used for production of wild rice." While revision of the 10 mg/L numeric sulfate standard continues to be evaluated for the 2012 Triennial Review, it is unlikely that sufficient data will be available to propose a revision to the numeric standard. Based on a review of available studies and information, MPCA believes that additional wild rice plant toxicity studies are needed to evaluate the effects of sulfate and other variables on wild rice, across the full life cycle of the plant, before a revision to the numeric standard can be considered.

Background: Water quality standards development

Efforts by states to develop new water quality standards, or to revise existing standards, consider specific guidance provided by the US EPA based on mandates of the federal Clean Water Act. This guidance has evolved over time to reflect new understandings in the science of standards development and water quality protection.

Information about the toxicity of a chemical is one category of data used to develop a water quality standard, to protect beneficial uses associated with organisms living in or consuming water. The results of toxicity tests are endpoints that describe an organism's response following an exposure to a given concentration of a chemical for a specific length of time. The response can be based on short term "acute" endpoints or long term "chronic" endpoints. The procedures for conducting and interpreting plant toxicity tests are not as well developed as they are for animals. The endpoints of tests using plants have been based on growth or reproduction, which are affected by chronic exposure to a chemical.

¹ The federal Clean Water Act (§ 303 (c) (1), requires the states, and authorized Tribes, to periodically review and amend as appropriate their water quality standards. These "triennial reviews" are intended to allow for stakeholder input and ensure recent science is incorporated into water quality standards.

Once a toxicity threshold is identified, a water quality standard can be promulgated based on avoiding exceedance of that threshold in water bodies, and the associated impact to beneficial uses. Water quality standards are implemented for a particular body of water by specifying the *magnitude*, *duration*, and *frequency* of the chemical that can be found in the water and still protect beneficial uses.

To determine if a standard is attained, concentrations are monitored and compared to the concentration, or *magnitude*, of the standard. Monitoring also supplies two other kinds of data used to determine whether a standard has been exceeded. *Duration* describes how long the concentration of the chemical has been elevated, and *frequency* describes how often both the magnitude and duration have been exceeded. For example, most aquatic life-based chronic water quality standards in Minnesota are implemented using a four-day average concentration of a chemical that cannot be exceeded more than once in a three-year period.

To further evaluate the current wild rice sulfate standard, information that augments the current understanding of the critical *magnitude*, *duration*, and *frequency* of sulfate concentration as it relates to wild rice toxicity is needed. Investigation of potential secondary effects of sulfate on the growth and reproduction of wild rice will also be important in evaluating and informing a potential revision to the sulfate standard, especially given that several current hypotheses suggest that it is not sulfate, but sulfide interactions in the sediment, that controls the toxicity of sulfate to wild rice. To acquire the necessary information to further evaluate the wild rice sulfate standard, the MPCA first needs to establish a testing protocol for additional wild rice studies.

Why are we developing a protocol?

As noted above, procedures for conducting and interpreting plant toxicity tests are not as well developed as they are for animals. In addition, the investigation of sulfate impacts on wild rice is complicated by the potential for sulfate-mediated effects on wild rice rather than direct sulfate toxicity. Given these complexities, a study protocol is appropriate to identify the key hypotheses to be tested and to document the testing methodologies and considerations for conducting such tests. If the wild rice standards study is funded, the protocol will serve as the basis for a request for proposals (RFP) for the study work.

The MPCA is in the process of developing such a protocol. To help inform the protocol development, the MPCA is consulting with the U.S. Environmental Protection Agency (EPA) and Tribal representatives and has met with interested/affected stakeholders to gain their input and perspectives on key questions to be answered. While the protocol will focus on the direct effects of sulfate and sulfide on wild rice, the impact of sulfate/sulfide on additional factors that may affect wild rice plant health and viability may also be evaluated as time and funding allows. Some of the things the MPCA thinks will be important when designing the protocol include:

- Toxicity testing of a range of sulfate concentrations to help evaluate the appropriateness of the numeric standard of 10 mg/liter.
- Evaluating how other key parameters vary with or may be affected by sulfate or sulfide (e.g., nutrients), to better understand effects on wild rice plant health and viability.

- Testing at various stages of the wild rice plant life cycle to further inform determinations of when wild rice is susceptible to high sulfate levels.
- Evaluation of various mechanisms through which elevated sulfate might result in reduced populations or reduced seed production of wild rice.
- Enhancing the understanding of natural variability of characteristics among wild rice plants to help identify the characteristics of healthy stands of wild rice. This may include mesocosm or field-scale evaluations.

Purpose of the wild rice standards study

The goal of the wild rice standards study is to provide additional information to re-evaluate the wild rice-based sulfate standard. The scientific information developed will be used by the MPCA in its decision as to whether or not a change to the existing standard is necessary, and if so, what the revised standard should be. Such a change, if warranted, would be proposed in accordance with the provisions and requirements of Minnesota's Administrative Procedure Act².

Goals for the May 9, 2011, discussion

To further inform the MPCA's development of the study protocol, technical experts from a variety of disciplines have been invited to share their expertise and perspectives on potential studies exploring the physical, chemical, and biologically-related interactions sulfate and sulfide may have on wild rice. There are three primary outcomes we hope to achieve through this gathering of technical experts:

1. A better understanding of what is currently known about the effects of sulfate and sulfide on the developmental stages of wild rice (*Zizania palustris* and *Zizania aquatica*) and/or other aquatic macrophytes;
2. Identification of the most critical "unknowns" and associated hypotheses relative to the interactions of sulfate (and related parameters) and wild rice to inform further evaluation and, if warranted, revision of the wild rice sulfate standard; and
3. Recommendations for methodologies and study designs that could test these hypotheses.

MPCA staff anticipates that follow-up conversations will be needed regarding specific design and methodology questions. The goal is to identify the key hypotheses/tests during this meeting and to have a sufficient discussion of the design and methodology

² Rulemaking activities in Minnesota are governed by the Administrative Procedure Act (Minn. Stat. ch. 14 and Minn. R. ch. 1400). When proposing rule amendments, the MPCA develops a Statement of Need and Reasonableness (SONAR) to document the factual information relating to a proposed rule amendment. As the name implies, the SONAR explains why a rule amendment is needed, and why it is reasonable. Public hearings are then held before an Administrative Law Judge where interested parties have an opportunity to voice support for, or opposition to, proposed amendments. This process also allows interested parties to bring forward alternative standards for consideration. Final decisions on rule amendments are made by the MPCA Citizens' Board and water quality standard revisions require approval of the U. S. Environmental Protection Agency.

considerations to identify follow-up needs. MPCA staff will then follow up on specific questions and develop a draft protocol, which will be shared with the technical experts and interested stakeholders for review and comment prior to being finalized. MPCA will also continue to consult with US EPA, Tribal representatives and the Minnesota Department of Natural Resources while the draft protocol is developed.

The MPCA's goal is to develop and finalize the protocol by June 30, 2011, so that if funding becomes available we are ready to publish a request for proposals this summer and begin the necessary studies as soon as possible.

Considerations for evaluating the effects of sulfate on wild rice

The effect of sulfate on wild rice in Minnesota surface waters likely involves the conversion of sulfate to sulfide—a conversion that is accomplished by anaerobic bacteria that respire sulfate instead of oxygen. During respiration, bacterial metabolism requires that the cells donate an electron to an external chemical, such as oxygen or sulfate, a chemical reaction known as *reduction*. Different groups of bacteria use different chemicals as electron acceptors. Sulfate is used as an electron acceptor by sulfate-reducing bacteria (SRB), which in principle are at an energetic disadvantage as long as certain other electron-acceptors are available. In principle, electron acceptors will be consumed in the following sequence: O₂, nitrate, oxidized manganese, oxidized iron, sulfate, and CO₂. Bacteria that respire O₂ produce H₂O as a product; respiration of nitrate produces nitrite or ammonia; respiration of oxidized manganese produces reduced manganese, which can be water soluble; respiration of oxidized iron produces reduced iron, which also can be water soluble; respiration of sulfate produces sulfide; and respiration of CO₂ produces methane.

Anaerobic bacteria are most active where there is bioavailable organic matter to decompose in aquatic systems, such as decaying plants such as algae or aquatic macrophytes. The presence of water is critical, in that water is a significant barrier to atmospheric oxygen and moisture is essential for bacterial activity. Sulfide will be produced by bacteria in aquatic systems whenever bacteria consume organic matter faster than oxygen can be supplied, and after bacteria have utilized the other electron acceptors that are energetically favored.

Sulfide is much more reactive than sulfate, and its production can alter the fundamental biogeochemical functioning of a freshwater wetland (Lamers et al. 1998, 2002). In particular, in sediment pore water, sulfide reacts with many metals, often forming insoluble precipitates. Sulfide reacts quickly with dissolved ferrous iron, forming ferrous sulfide (pyrite). The reaction of sulfide with iron is also the primary basis for the toxicity of sulfide to plants and animals; the reaction poisons iron-containing compounds that are essential to the metabolism of plants and animals.

It is conceivable that sulfide produced by SRB in organic-rich sediment in which aquatic plants such as wild rice can be directly toxic to a plant rooted in the sediment, a mechanism described by Koch et al. (1990). The increased loading of sulfate has been hypothesized to alter the aquatic plant species composition of particular wetlands (e.g. Smolders et al. 2003, Li et al. 2009).

However, the production of sulfide in sediment can conceivably have secondary and tertiary effects on aquatic plants, effects that might be mitigated by other biogeochemical reactions in the sediments. It is difficult to know which of these reactions play a significant role in any particular setting without a great deal of supporting information.

Figure 1 and the accompanying notes present a conceptual model of sulfate-mediated chemical changes in sediment. Based on this model and the above considerations, MPCA staff developed Table 1 to:

1. Identify hypotheses of potential effects of elevated sulfate concentrations to shallow-water ecosystems in Minnesota,
2. Prioritize which hypotheses are most likely to be essential to further evaluating the wild rice sulfate standard, and
3. Identify study design options for evaluating the hypotheses.

It is important to note that understanding and evaluating the hypotheses of potential effects is only one key aspect of a wild rice standards study. As noted in the background section on water quality standards development, additional information is also needed about the critical magnitude, duration, and frequency of sulfate concentration (or sulfide or other associated parameters) as it relates to wild rice toxicity.

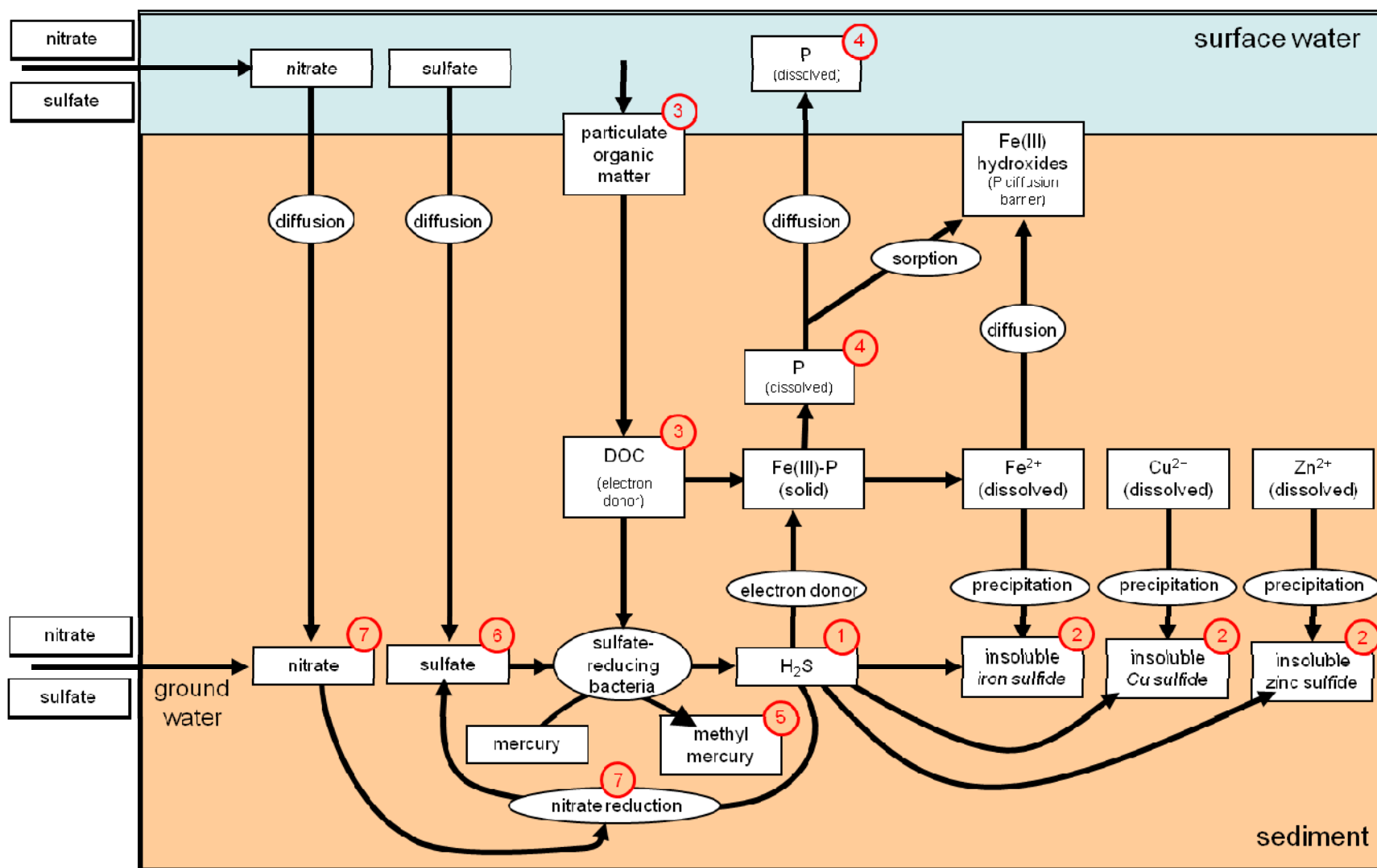


Figure 1. Conceptual model of sulfate-mediated chemical changes in sediment. Numbered notes in the diagram are below:

Notes for Figure 1.

1. H_2S (hydrogen sulfide) is produced by sulfate-reducing bacteria, and may be directly toxic to plants and animals. However, if iron concentrations are high relative to sulfide, sulfide may be precipitated (as iron sulfide) as fast as the sulfide is produced, mitigating toxicity (e.g., van der Welle et al. 2006).
2. Metal sulfides may precipitate when H_2S reacts with dissolved metals, including iron, copper, and zinc. The solid sulfides are probably less bioavailable for plant nutrition than are free metal ions, conceivably limiting plant growth due to iron, copper, or zinc limitation (these metals are essential nutrients for plant growth).
3. Organic loading controls the energy available to the system for biogeochemical reduction (electron donation, either through bacterial metabolism or inorganic chemical reaction).
4. Phosphate is mobilized and made more bioavailable when oxidized ferric iron, Fe(III) , is reduced to ferrous iron (Fe(II) or Fe^{2+}), which is initially water-soluble. Phosphate and iron that become dissolved under reducing conditions, may then diffuse toward lower-concentration waters. Phosphate may diffuse into the surface water. Reduced iron may diffuse to the oxidized water-sediment interface, where it oxidizes and precipitates as an iron hydroxide and may intercept and hold the diffusing phosphate. Alternatively, if sulfide is available in the pore water, the reduced iron would precipitate as an insoluble iron sulfide, which does not sorb, or hold, phosphate.
5. Methyl mercury is primarily formed by sulfate-reducing bacteria. The methyl group is supplied within the bacteria by methylcobalamin (vitamin B12). Mercury methylation may be limited by the availability of cobalt, an effect that has been demonstrated in the laboratory. The methylmercury diffuses into overlying water, where it can enter the food chain by sorbing to algae and other particles that are consumed by filter-feeding invertebrates.
6. Sulfate is used as an electron acceptor by sulfate-reducing bacteria, which in principle are at an energetic disadvantage as long as certain other electron-acceptors are available. In principle, electron acceptors will be consumed in the following sequence: O_2 , nitrate, oxidized manganese, oxidized iron, and sulfate. The sequence may not be followed rigorously due to heterogeneity in the sediment, groundwater flow, or differing chemical reaction speeds. Naturally occurring stable isotopes of S and O have been useful in assessing the degree of sulfate reduction.
7. Nitrate is, in principle, consumed to near zero concentrations before sulfate is consumed. But if nitrate is present simultaneously with sulfide, as has been found in some systems recently, sulfide can be oxidized to sulfate. Under such conditions, H_2S may not build up even if sulfate-reducing bacteria are active. Nitrate availability can also be responsible for the oxidation of ferrous iron (Ratering and Schnell 2001)

Table 1. Hypotheses of potential effects of elevated sulfate concentrations to shallow-water ecosystems in Minnesota.

Priority* (A-C)	No.	Specific Hypothesis Regarding the Role of Sulfate	Appropriate Options for Evaluating Hypothesis						
			Indoor Lab	Outdoor Container Mesocosm	<i>in situ</i> Mesocosm	Intensive Field Sampling	Survey Field Sampling	Empirical Model	Mechan- istic Model
	1	The sulfate ion itself is directly toxic to wild rice.							
A	1A	Wild rice is only negatively affected by elevated sulfate while it is actively growing (April through August).	X	X	X				
A	1B	From September through March sufficient sulfate diffuses into sediment, or adjacent soils, so that wild rice growth is harmed.	X	X	X			X	X
	2	The toxic agent is actually the cation associated with elevated sulfate.							
B	2A	Elevated calcium, magnesium, potassium, or sodium correlated with sulfate is the actual toxic agent.	X	X					
	3	Sulfide produced from sulfate is directly toxic.							
A	3A	Sulfide produced in the sediment is toxic to wild rice seeds, reducing germination.	X	X	X			X	X
A	3B	Sulfide produced in the sediment is toxic to the roots of wild rice.	X	X	X			X	X
	4	Sulfide produced from sulfate is sometimes not toxic to wild rice.							
A	4A	High-iron sediments reduce toxicity of sulfide (because iron precipitates sulfide as iron sulfide, reducing the toxicity).		X	X	X		X	X
A	4B	High-nitrate systems reduce sulfide toxicity (because sulfide is oxidized back to sulfate by nitrate).		X	X				
	5	Under certain circumstances, sulfide is not produced and wild rice grows in the presence of elevated sulfate.							
A	5A	Relatively high nitrate availability inhibits sulfide production.		X	X			X	X
A	5B	Relatively high oxidized manganese in the sediment inhibits sulfide production.		X	X	X		X	X
A	5C	Relatively high oxidized iron in the sediment inhibits sulfide production.		X	X	X		X	X
B	5D	Water movement carries away organic matter, limiting sulfide production.		X	X	X	X	X	X
	6	Sulfide indirectly reduces wild rice growth							
A	6A	Wild rice roots develop barrier in reaction to sulfide, which inhibits uptake of essential nutrient(s) such as iron or nitrogen.		X	X				
A	6B	Sulfide precipitates essential nutrient metal(s) such as iron, copper, or zinc, making them less bioavailable.		X	X			X	X
B	6C	Sulfide production enhances P bioavailability, increasing production of organic matter, which increases sulfide production (a positive feedback loop).			X	X	X	X	
B	6D	Sulfate, through sulfide precipitation of iron, increases P bioavailability, increasing growth of perennial plants that exclude wild rice through shading, root competition, and/or allelopathy.			X	X	X	X	
	7	External factors have increased the production of sulfide from sulfate in recent years.							
B	7A	Reduced water movement (e.g., via impoundment) increased organic matter, increasing sulfide production (e.g., residual wild rice straw).			X	X	X	X	
B	7B	Longer ice-free period, elevated temperatures have increased bacterial activity and sulfide production.		X				X	X
B	7C	External loading of phosphorus and/or nitrogen has increased production of organic matter, increasing sulfide production.					X	X	
	8	Wild rice is less sensitive to secondary effects of elevated sulfate when it is not actively growing.							
A	8A	The negative effects of elevated sulfate do not persist into the wild rice growing season.			X			X	X
A	8B	Sulfate does not have negative effects September-March due to lower temperature or other reason.			X			X	X
	9	Mercury methylation is affected by changes in sulfate concentrations.							
C	9A	Increased sulfate enhances methylation of mercury.		X	X	X	X		
C	9B	Very high sulfate concentrations inhibit methylation of mercury.		X	X	X	X		
C	9C	Factors that remove free sulfide from pore water (4A-4B) increase the production of methyl mercury.		X	X	X			
C	9D	Factors that reduce the production of sulfide (5A-5D) also reduce production of methyl mercury.		X	X	X			

*Definitions of A, B, and C:

A: Essential to evaluating the wild rice sulfate standard.

B: Potential effects, but significantly less likely to be causative than A's

C: Significant hypothesis, but does not address the wild rice issue.

Hypotheses of potential effects of elevated sulfate

From a review of the scientific literature, this document identifies numerous possible mechanisms through which elevated sulfate could have a negative effect on wild rice. Table 1 lists about 20 hypotheses as to how elevated sulfate might have negative effects on wild rice. These hypotheses that can be tested through experiment, field observation, or modeling. A well-constructed protocol should result in a mass of evidence regarding the effects of sulfate. Any revision of Minnesota's sulfate standard would be based on the overall weight-of-evidence, rather than any one test.

While it is important to recognize the diversity of hypotheses, not all of the hypotheses are focused on the key objective of informing further evaluation of the wild rice sulfate standard. Each hypothesis in Table 1 is given a preliminary rating as to the priority that it should be given in the design of data collection, given the study objective. Here they are sorted into three priority classifications, A, B, and C:

Priority “A” hypotheses. These hypotheses are essential to test in the evaluation of the wild rice sulfate standard.

- 1A Wild rice is only negatively affected by elevated sulfate while it is actively growing (April through August). While this hypothesis may not be likely, it is important to collect data so that it can be definitively excluded.
- 1B From September through March sufficient sulfate diffuses into sediment, or adjacent soils, so that wild rice growth is harmed.
- 3A Sulfide produced in the sediment is toxic to wild rice seeds, reducing germination. Wild rice seeds must overwinter in the surface sediment of wild rice stands, where they might be exposed to levels of sulfide that impede successful germination.
- 3B Sulfide produced in the sediment is toxic to the roots of wild rice. Roots release O₂ to oxidize sulfide in the rhizosphere. Sulfide toxicity therefore depends on the strength of the root oxidizing power, sulfide concentration in the pore water, and root health as affected by nutrient availability. For example, white rice is more susceptible to sulfide toxicity under poor nutrient status due to deficiencies of potassium in particular, but also P, Ca, or Mg (Dobermann and Fairhurst 2000).
- 4A High-iron sediments reduce toxicity of sulfide (because iron precipitates sulfide as iron sulfide, reducing the toxicity). Dutch researchers have extensively investigated the role of sulfate reduction in wetlands and have documented that iron can reduce the toxicity of sulfide (van der Welle et al. 2006).
- 4B High-nitrate systems reduce sulfide toxicity (because sulfide is oxidized back to sulfate by nitrate). Research has shown that there are many microbial pathways for nitrate, including the oxidation of sulfide to sulfate (Haaiker et al. 2006, Burgin and Hamilton, 2008).
- 5A Relatively high nitrate availability inhibits sulfide production. Research in the Netherlands has demonstrated that nitrate can inhibit the production of sulfide in wetlands, even when sulfate concentrations are elevated (Lucassen et al. 2004).

- 5B Relatively high oxidized manganese in the sediment inhibits sulfide production. In principle, oxidized manganese (and oxidized iron) will be utilized as an electron acceptor before sulfate is respired (Kirk 2004).
- 5C Relatively high oxidized iron in the sediment inhibits sulfide production. In saturated soils, oxidized iron can be the dominant electron acceptor for respiration of organic matter (e.g., Frenzel et al. 1999).
- 6A Wild rice roots develop a barrier in reaction to sulfide, which inhibits uptake of essential nutrient(s) such as iron or nitrogen.
- 6B Sulfide precipitates essential nutrient metal(s) such as iron, copper, or zinc, making them less bioavailable (Kirk 2004).
- 8A The negative effects of elevated sulfate do not persist into the wild rice growing season. It is hypothesized that there may be potential negative effects of elevated sulfate on wild rice--elevated sulfide, for instance—but those potential negative effects are not realized because they are not retained within the wild rice stand long enough to affect the growth of wild rice. For example, sulfide developed during the winter is dissipated or detoxified before the start of the growing season.
- 8B Sulfate does not have negative effects during some months, due to lower temperature or other reason. It is hypothesized that sulfate might have negative effects during the growing season, but the negative effects do not occur during certain times of year because of environmental factors such as reduced temperature.

Priority “B” hypotheses. These hypotheses potentially occur in the Minnesota environment, but are significantly less likely to be causative than A's.

- 2A Elevated calcium, magnesium, potassium, or sodium correlated with sulfate is the actual toxic agent. Correlations can be misleading. For instance, Lehman (1976) showed that experiments supporting the hypothesis that phosphate is toxic to the alga *Dinobryon* failed to control for the cations associated with phosphate; he showed that potassium was the actual toxic agent when phosphate was added as potassium phosphate.
- 5D Water movement carries away organic matter, limiting sulfide production. Because sulfide production is dependent on the availability of organic matter, removing organic matter could decrease sulfide production, just as adding organic matter may increase sulfide production and associated toxicity (e.g. Gao et al. 2003).
- 6C Sulfide production enhances P bioavailability, increasing production of organic matter, which increases sulfide production (a positive feedback loop). This positive feedback loop is well documented for the pelagic zone of eutrophic lakes, and, more recently, for wetlands (Lamers et al. 1998, Geurts et al. 2009).
- 6D Sulfate, through sulfide precipitation of iron, increases P bioavailability, increasing growth of perennial plants that exclude wild rice through shading, root competition, and/or allelopathy. Competition from perennial aquatic plants (DNR 2008) has been hypothesized as a cause of decreased wild rice populations, an effect that may involve allelopathy (Quayyum et al. 1999). Whether this

competition results from increased P bioavailability is speculative. Increased phytoplankton could also be competing with wild rice for light.

- 7A Reduced water movement (e.g., via impoundment) increased organic matter, increasing sulfide production (e.g., residual wild rice straw). Gao et al. (2003) have shown that residual straw from white rice production can cause sulfide toxicity, so it can be hypothesized that any action that decreases removal of organic matter from wild rice stands could increase sulfide production.
- 7B Longer ice-free period, elevated temperatures have increased bacterial activity and sulfide. If sulfide production is limited by the temperature of surface sediment, recent increases in ambient temperature could be responsible for increased sulfide production.
- 7C External loading of phosphorus and/or nitrogen has increased production of organic matter, increasing sulfide production. If organic production has increased due to external nutrient loading, then sulfide production could have increased— independent of any change in sulfate loading.

Priority “C” hypotheses. These hypotheses concern potentially significant impacts of sulfate in the Minnesota environment, but they do not address the wild rice issue.

- 9A Increased sulfate enhances methylation of mercury. There is evidence that the addition of sulfate to low-sulfate wetlands in Minnesota enhances the methylation of mercury (Jeremiason et al. 2006), most likely by stimulating the metabolism of sulfate-reducing bacteria, which are known to methylate mercury.
- 9B Very high sulfate concentrations inhibit methylation of mercury. It has been hypothesized that high activity rates of sulfate-reducing bacteria can produce sufficient sulfide to result in a negative feedback loop in regards to mercury methylation (Benoit et al. 1999). Higher concentrations of sulfide are thought to result in reduced bioavailability of mercury to the sulfate-reducing bacteria that have the potential to methylate the mercury.
- 9C Factors that remove sulfide from pore water (hypotheses 4A and 4B) increase the production of methyl mercury. If elevated sulfide concentrations inhibit mercury methylation (Benoit et al. 1999), then it is reasonable to hypothesize that removal of sulfide might enhance the methylation of mercury.
- 9D Factors that reduce the production of sulfide (hypotheses 5A and 5D) reduce the production of methyl mercury. Inhibition of the activity of sulfate-reducing bacteria can reasonably be thought to result in reduced methyl mercury, as observed in Lake Onodonga (Todorova et al. 2009).

Options for testing hypotheses

It is anticipated that the majority of the data collection described here would occur over two field seasons. It is proposed that those two field seasons be preceded by one field season in which a survey is conducted of about 50 wild rice stands. The field survey (see Appendix B) would enhance the quality of the larger protocol study by getting a jump on identifying field sites, obtaining preliminary data, and gaining experience that will make the protocol study more efficient.

Table 1 identifies the specific hypotheses that could be tested associated with the mechanism by which elevated sulfate concentrations affect wild rice in Minnesota. Each specific hypothesis is described, and appropriate options for testing that hypothesis are identified as follows:

The “Indoor Lab” option could be used to assess the germination and initial growth of wild rice seedlings (e.g., Nimmo et al. 2003).

The “Outdoor Container Mesocosm” option might be appropriate to compare growth of wild rice plants under various defined conditions (e.g. Walker et al. 2006).

The “in situ Mesocosm” option, while suitable for many of the hypotheses addressable in containers, may be appropriate for assessing hypotheses requiring undisturbed sediments and ecological interactions such as interspecific competition among aquatic plants.

“Intensive Field Sampling,” while lacking the control over variables that laboratory experiments and mesocosms offer, offers the promise of environmental data that may support or contradict a hypothesis.

“Survey Field Sampling” provides data that inform the choice of intensive field sites and representivity of sediment and water conditions during experiments. The preliminary field survey (Appendix B) will provide information on the range and variability of sediment and water parameters among wild rice stands, both constraining hypotheses and perhaps generating new hypotheses. Field data may be augmented during succeeding field seasons.

“Empirical Model” is an option to generalize the data obtained from experiments and field observations, toward the end of understanding the response of wild rice to different concentrations of sulfate.

“Mechanistic Model” is an option to apply fundamental scientific understandings to the wild rice-sulfate question. For instance, it may be possible to model the geochemical reactions that occur in sediment under various scenarios.

Some questions concerning study design

Note: this is an incomplete list, to be further added to, and addressed, during and following the technical meeting.

- A. To what depth should sediments be sampled to a) characterize the root environment, b) understand chemistry that may be deleterious to roots to inform standard evaluation and potential refinement?

- B. What are the appropriate endpoints to measure for wild rice, as individuals or as a population? Options include:
- Density of individuals per square meter
 - Dry weight of plant tissue (roots, stem, leaves, seeds)
 - Recommended drying temperature and time?
 - Number of seeds per plant
 - Germination success of seeds
 - Elemental concentrations in plants (P, N, K, Fe, Zn, Cu)
 - Protein content of seeds.
 - Plant DNA, as an index of inbreeding or selection pressure.
 - Other?
- C. How can the multi-year cycle of wild rice growth be controlled for during hypothesis testing?
- D. How important is understanding the within-species variability among wild rice stands to further evaluation of the wild rice sulfate standard?
- E. Should experiments be designed, if possible, incorporating the recommendation of Cottingham et al. (2005)?
- Cottingham et al. (2005) recommend employing replicated regression, in contrast to ANOVA, when designing experiments that address with a continuous factor. They argue that regression is generally a more powerful approach than ANOVA and provides quantitative output that can be incorporated into models more effectively than ANOVA output.
- F. How would the duration and frequency components of a water quality standard for either sulfate or sulfide be best addressed and factored into the protocol study design in order to insure adequate short and long-term protection of wild rice?
- G. Could elevated sulfate affect the dormancy or germination rate of wild rice seed?

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Appendix A. The environmental setting of wild rice in Minnesota

John Moyle published his observations on the correlation between wild rice occurrence and the chemistry of surface waters (Figure 3 A-C). Moyle recognized that Minnesota exhibits a strong gradient in the chemistry of surface water from soft water in the northeastern part of the state, to hard water in central Minnesota, to alkaline water in the west and southwest (Fig. 3C).

Moyle (1956) stated that wild rice is a species that requires hard water, but low sulfate concentrations: "...no large stands are known from waters where the sulphate ions exceed 10 ppm. Plantings of wild rice in the high-sulphate waters area have generally failed. The cause-and-effect relationship between sulphates and the distribution of plants is not known, but may be related to sulphur demands in plant nutrition, osmotic pressure of the water solution, or the toxicity of magnesium usually associated with sulphates."

The correlation observed by Moyle between wild rice occurrence and the broad trends in the chemistry of surface water has held up over time (compare contemporary wild rice occurrence in Fig. 3D to sulfate in lakes (Fig. 2A), depressional wetlands (Fig. 4A), and groundwater (Fig. 4B). Interestingly, terrestrial crops benefit from the application of sulfur fertilizer in the low-sulfate area of central Minnesota (Fig. 4C) where wild rice stands are common, indicating that soils in that area contain low levels of sulfur.

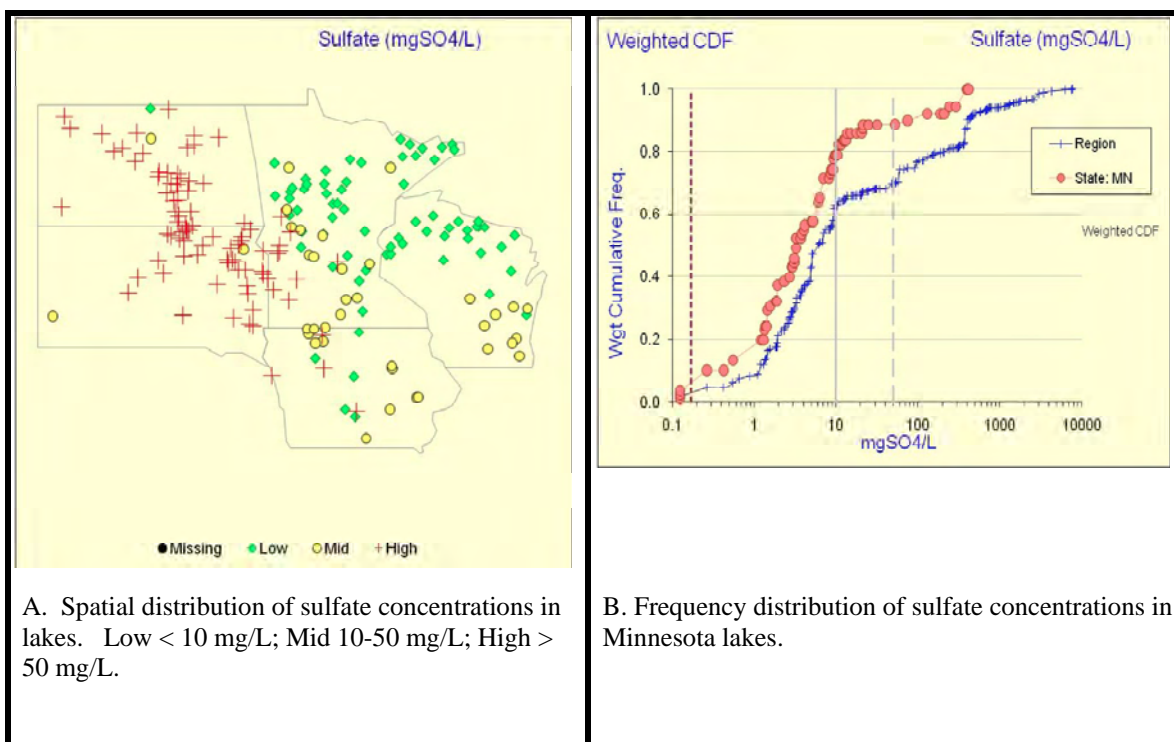
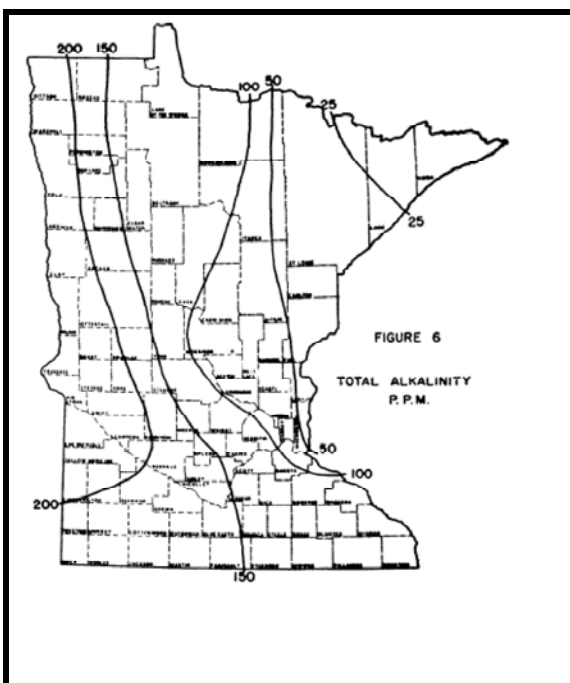
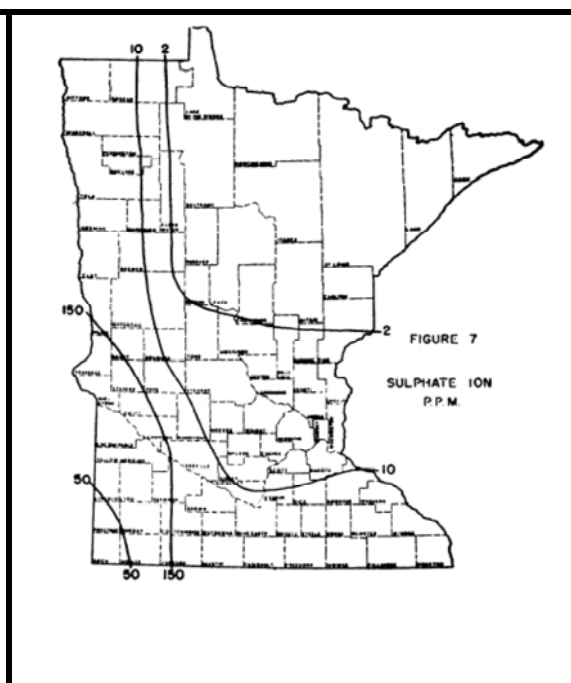


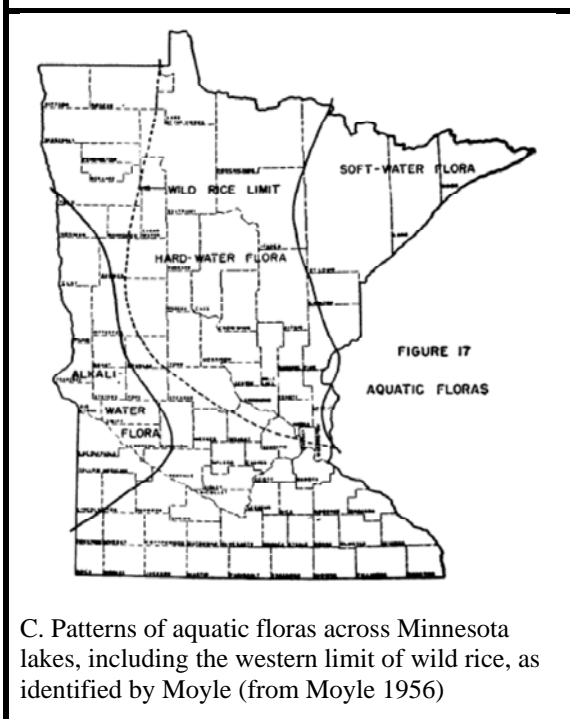
Figure 2. Sulfate concentrations in lakes randomly selected as part of the USEPA National Lakes Assessment in 2007.



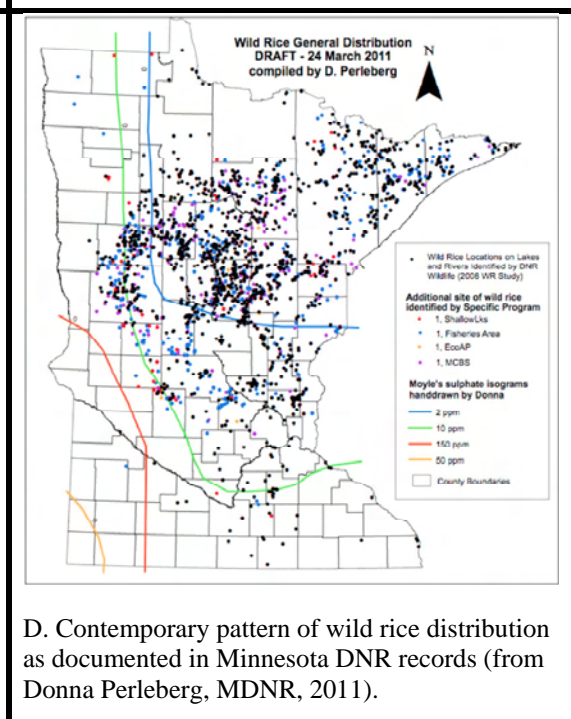
A. Alkalinity patterns in Minnesota lakes (from Moyle 1956)



B. Sulfate patterns across Minnesota lakes (from Moyle 1956).



C. Patterns of aquatic floras across Minnesota lakes, including the western limit of wild rice, as identified by Moyle (from Moyle 1956)



D. Contemporary pattern of wild rice distribution as documented in Minnesota DNR records (from Donna Perleberg, MDNR, 2011).

Figure 3. Patterns across Minnesota of lake chemistry (A & B) and aquatic floras (C), as published by Moyle (1956), compared to the contemporary pattern of wild rice distribution (D).

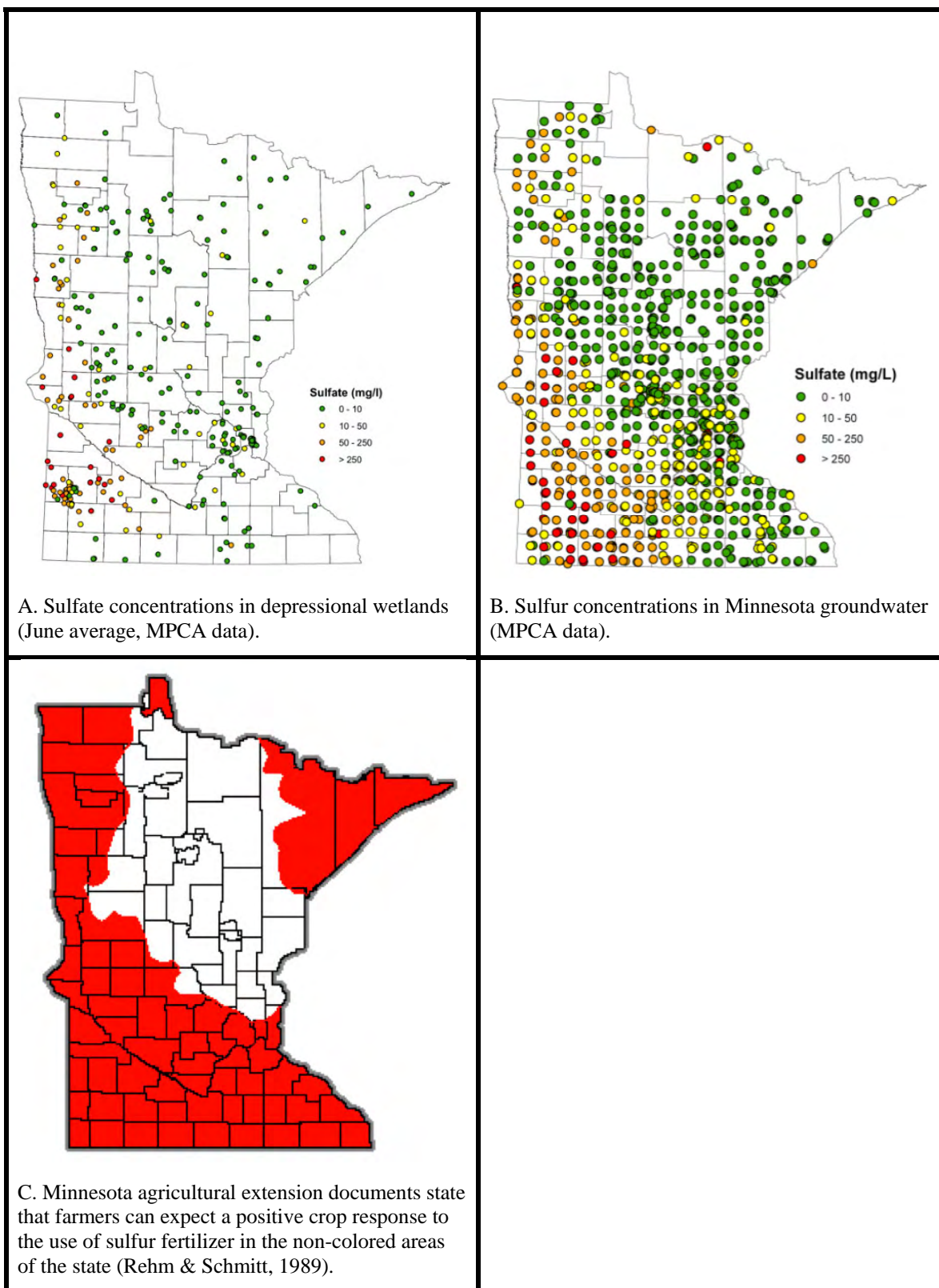


Figure 4. Patterns of sulfate availability across Minnesota in depressional wetlands (A), groundwater (B), and soil (C).

Appendix B. Proposed field study for 2011.

Wild Rice-Sulfate 2011 Preliminary Field Study

Minnesota Pollution Control Agency

April 3, 2011

Introduction

This document describes a proposed preliminary field study to collect data to aid in the development of a protocol that will inform a potential revision to the Minnesota sulfate water quality standard for the protection of wild rice. The MPCA is working cooperatively with the U.S. Environmental Protection Agency, the Minnesota Department of Natural Resources (DNR), Minnesota and Wisconsin tribes, universities, and other stakeholders to design a protocol to investigate the effects of elevated sulfate concentrations on wild rice. The protocol will describe a comprehensive investigation into possible seasonal sensitivity of wild rice to elevated sulfate and any effects on the health of wild rice stands. The protocol will undoubtedly incorporate both field observation and experimental evaluation of elevated sulfate. If a protocol study is funded by Minnesota's legislature during the current session, a budget would most likely be available for two field seasons. This field study would enhance the quality of the larger protocol study by getting a jump on identifying field sites, obtaining preliminary data, and gaining experience that will make the protocol study more efficient.

While the protocol is being designed, this preliminary field study will be conducted. The objectives of a preliminary field study are to:

1. Better understand and document the range of water column and sediment chemistries at sites with robust, declining, and absent wild rice stands—data that will inform the selection of sites and/or sediment for more detailed study under the protocol through, for example, seasonal sampling or experimentation;
2. Gather ambient (*in situ*) data on sulfate and related parameters to supplement the field study anticipated to occur under the protocol; and
3. Test and perfect the method(s) for sampling sediment pore water in lakes and streams.

Overall Field Study Approach

During the summer of 2011 it is proposed to sample approximately 50 shallow-water ecosystems so that the range of sulfate concentrations and other factors in the water column and sediment that might correlate with the presence or absence of wild rice can be better understood. A relatively large number of samples are needed to adequately describe the range. Sampling a relatively large number of sites also increases the likelihood that some of the sites sampled under the protocol study will already have been sampled, which will extend the data set for those lakes and streams to three years. In

addition, sediment sampling techniques will be tested at one or two sites, and a few conveniently accessed sites will be sampled once a month to assess the variability of sediment chemistry over time.

Site Selection

Study sites will be selected to represent various categories in an effort to widely characterize wild rice habitats in Minnesota (Table 1), including sites that are known to be productive wild rice habitats, apparently good wild rice habitat that nevertheless do not support wild rice (if they can be identified), wild rice sites that John Moyle sampled 50 to 70 years ago, low-sulfate sites, sites naturally high in sulfate, sites enriched with sulfate due to human activity, and paddies used for the commercial production of wild rice. It is important to sample commercial paddies because a) the current sulfate standard applies to these sites, and b) constructed wild rice paddies may respond to elevated sulfate in a manner that is different than natural wild rice stands because of differences in soil substrate, nutrient levels, background water chemistry, hydrologic environment, or wild rice strain.

The number of sites to be sampled is approximate because a) the MPCA is in the process of compiling a database of the potential sites to sample, b) consultations need to be held with knowledgeable individuals as to the logistical challenges involved in obtaining the required samples, and c) there may be opportunities to sample sites that are being studied as part of studies being conducted for other reasons by the DNR, MPCA, or Tribes—such opportunities would likely allow more sites to be sampled within a given budget.

Table 1. Classes of sites to be sampled in 2011 to characterize the variability of wild rice habitats in Minnesota.

Site Class	Approximate Number of sites	Information Sources
Productive wild rice sites (in naturally low- and high-sulfate areas)	10 to 20	DNR, MPCA, Tribes
Historically productive wild rice sites, but little wild rice recently.	5 to 10	John Moyle reports, Tribes
Sites receiving elevated sulfate loading from human activity	5 to 10	MPCA, DNR
Apparently good wild rice habitat, but without wild rice	5 to 10	DNR, MPCA, Tribes
Commercial wild rice paddies	5 to 10	Commercial Growers

Field Work

Field crews will travel to designated sites and will use a canoe or other small boat to carry out their tasks (Table 2). Locations will be recorded on maps and by GPS. Dominant aquatic plants will be identified, coverage estimated, and representative digital photographs taken. If wild rice is present, its growth stage will be recorded, and leaves from 5 plants will be sampled for possible future genetic analysis, which could be important for understanding differential responses to sulfate concentrations. One grab sample of water will be obtained from below the surface, but taking care to minimize suspended sediment and dislodged periphyton. Sediment samples will be taken from five locations, composited in a mixing bowl, and subsampled into a number of different sample containers for the various analyses. In a subset of locations, five subsamples will

be preserved separately in order to perform separate analyses, in an effort to understand variability in the sediment matrix.

Table 2. Data to be collected during field work. Analyses preceded by “*” would enrich the study, but are unlikely to be obtained at all sites, and may depend on the limits of the project budget.

Analysis	Method	Reference
Water depth	Sounding line /stage if available	
Water temperature	Sonde	
Plant species/abundance, including wild rice	Visual	MPCA and DNR guidance
*Water: pH, oxygen, conductivity, Eh	Sonde	MPCA guidance
*Sediment: pH, Eh	Sonde	MPCA guidance
GPS		MPCA guidance
Transparency	T-tubes	MPCA guidance
Photo points	4-cardinal directions	MPCA guidance

Sediment will be obtained by inserting a polycarbonate tube into the top 10 to 20 cm of sediment, and bringing the sediment up to the surface. The uppermost 5 cm of sediment will be retained after discarding the deeper sediment and tilting the tube to pour off the overlying water. A small subset of sites (1-2 lake and stream sites) will be chosen to evaluate and refine method(s) for extracting pore water from the sediment, a type of sample that is thought to be critical for the understanding of the chemistry that affects the roots of wild rice. One such method of sampling pore water is to deploy a so-called “peeper,” which achieves equilibration of distilled water with pore water across a membrane.

Samples will be preserved appropriately and transported in a timely manner to the laboratory, the identity of which is to be determined. A quality assurance plan will be developed that will specify details for replicate samples, field blanks, laboratory blanks, and related issues.

Sample Analyses

The water analyses are normal water quality parameters (Table 3). The sediment analyses are much more specialized, and special care is needed in selecting a laboratory capable of these analyses.

Table 3. Data to be collected as part of the 2011 preliminary field survey. Analyses preceded by “*” would enrich the study, but are unlikely to be obtained at all sites, and may depend on the limits of the project budget.

Analysis	Suggested Method
Water Analyses	
pH	Probe
Conductivity	Probe
Alkalinity	Gran Titration
Major cations and trace metals (Na, K, Mg, Ca, K, Fe, Mn, Cu, Zn, Co, Ni, As)	ICP-Mass spectrometry
Anions (SO ₄ , Cl, F)	Ion chromatography
Total P / Total N	Dual alkaline/ persulfate digestion & autoanalyzer
Nitrate, Ammonia	Autoanalyzer
DOC (dissolved organic carbon)	UV / persulfate digestion & carbon analyzer
SUVA (Specific UV Absorbance—water color)	UV-Vis spectrometer
Chlorophyll	Spectrometer
Sediment Analyses	
Inorganic grain size	Wet oxidation & Horiba laser-diffraction grain-size analyzer
Organic grain size	Wet sieve & combustion weight loss
Carbonate	Carbon coulometry
TOC (total organic carbon)	Carbon analyzer
TN (total nitrogen)	Nitrogen analyzer
TS (total sulfur)	Sulfur analyzer
Inorganic S (sulfides)	CRS (chromium-reducible sulfur)
TP (total phosphorus)	Wet oxidation/dilute HCl & autoanalyzer
P-fractionation (NaOH-P, HCl-P, Organic-P)	Selective extraction w/ dilute NaOH, HCl, & autoanalyzer
Extractable major cations & metals (Fe, Mn, Ca, K, Mg, Ca, Cu, Zn, Co, Ni, As)	Dilute HCl-extraction & ICP-Mass spectrometry
*Total mercury ¹	CV-AFS by DMA (Direct Mercury Analyzer)
*Methylmercury ¹	GC-ICPMS (with stable Hg isotope dilution of each sample)

Notes:

1. Measuring mercury during this field survey would likely not illuminate any hypotheses concerning wild rice, but, rather, would capitalize on the investment in sampling and supporting analyses to better understand the hypothesis that elevated sulfate might enhance the production of methylmercury in highly organic sediments.

Desired Detection Limits for Laboratory Analyses

Table 4. Water Analyses.

Analyses	Suggested Method	Desired Detection Limit
pH	Probe	n/a
Conductivity	Probe	n/a
Alkalinity	Gran titration	1 µeq/L
Chlorophyll	MPCA Guidance	
Major cations/ trace metals (Na, K, Mg, Ca, Fe, Mn Cu, Zn, Co, Ni, As)	ICP-mass spectrometry	1 ppb
Anions (SO ₄ , Cl, F)	Ion chromatography	10 ppb
Total P/Total N	Dual alkaline/persulfate digestion & autoanalyzer	5, 20 ppb
Nitrate/Ammonia	Autoanalyzer	10 ppb
DOC (dissolved organic carbon)	UV/persulfate digestion & carbon analyzer	100 ppb
SUVA (specific UV absorbance)	UV-Vis spectrometer	n/a

Table 5. Sediment Analyses.

Analyses	Suggested Method	Desired Detection Limit*
Freeze drying & subsampling	Bulk Freeze Dryer	n/a
Inorganic grain size	Wet oxidation & Horiba laser-diffraction grain-size analyzer	n/a
Organic grain size	Wet sieve & combustion weight loss	n/a
Carbonates	Carbon coulometry	0.10%
Total organic carbon	Carbon Analyzer	0.02% (200ppm)
Total nitrogen	Nitrogen Analyzer	0.02% (200ppm)
Total sulfur	Sulfur Analyzer	0.05% (500ppm)
Inorganic S (sulfides)	CRS (chromium-reducible sulfur)	30 ppb S
Total phosphorus	Wet oxidation/dilute HCl & autoanalyzer	65 ppm
P-fractions (NaOH-P, HCl-P, Organic-P)	Selective extraction w/dilute NaOH, HCl & autoanalyzer	12 ppm
Extractable major cations & metals (Fe, Mn, Ca, K, Mg, Ca, Cu, Zn, Co, Ni, As)	Dilute HCl-extraction & ICP-MS	0.5 ppm
Total mercury	CV-AFS by DMA (direct mercury analyzer)	2 ppb
Methylmercury	GC-ICPMS (with isotope dilution)	0.6 ppb
*Detection limits for sediments reported as concentrations in dry sediment (0.1 g sample mass).		

Appendix: Rationale for choice of analyses during this preliminary field study

The simplest hypothesis for why elevated sulfate may have negative effects on the growth of wild rice is that some bacteria produce sulfide from sulfate, and that wild rice is relatively sensitive to sulfide. Review of the scientific literature indicates that the net effect of sulfate may be controlled by iron, pH, Eh (redox state), organic matter, nitrate, calcium carbonate, and perhaps other variables (see Figure 1). For instance, published studies have hypothesized that relatively high concentrations of iron can detoxify sulfide by forming solid iron sulfide. However, the presence of iron may be irrelevant if background nitrate concentrations are elevated, since nitrate is thought to inhibit the production of sulfide, even under elevated sulfate concentrations. The production of sulfide in an aquatic ecosystem has many consequences that have the potential to affect the ability of wild rice to compete and grow successfully. For instance, sulfide production is known to increase the bioavailability of phosphorus, which may alter the outcome of competition among aquatic plant species. Sulfide production can reduce the bioavailability of other essential plant nutrients, notably iron, copper, and zinc. Consequently, elevated sulfate has the potential to affect many factors that control plant growth, effects that are likely to vary according to background environmental chemistry. Implementation of the study protocol may benefit from knowledge of both the range of these non-sulfate environmental variables, and from knowledge of concentrations at specific sites that may be studied in more detail.

This preliminary field study proposes to sample and homogenize the top 5 cm of sediment, freeze-dry it, extract with a weak acid, and to analyze the biologically active metals (in particular, iron, manganese, copper, and zinc). Such data may be satisfactory for an assessment of the range of concentrations across the range of wild rice in Minnesota, but likely would provide inadequate data for the protocol, which may pursue 1) a mechanistic modeling of the important chemical reactions in the sediment, and 2) an understanding of progressive seasonal changes in sulfide production. Sufficient data for those purposes would require a vertical profile of chemical parameters, including sampling and analysis of the pore water in the sediment. Data on the chemistry of pore water is valuable, but is difficult to obtain, and so its collection will largely be deferred until this preliminary survey has been completed and representative sites can be more thoughtfully chosen. However, a small part of this preliminary field study will be directed to development and testing of a method for sampling stratigraphic pore water in the field.

Water color and chlorophyll will be analyzed as indices of relative light penetration, which could be a factor limiting early growth of wild rice.

Methylmercury is difficult to extract quantitatively from sediment, so it is essential to employ isotope dilution to correct for extraction recovery. We propose to measure methylmercury concentrations in the sediment rather than the overlying water because the sediment is most likely the methylation site and one measurement of the concentration in the sediment is a better metric of average production than one measurement in the water, which is subject to variable dilution. Percent methylmercury of total mercury in the sediment has been used as a measure of methylation efficiency. Cobalt (Co) is measured as one of the metals in the sediment analysis because its abundance has been shown to sometimes limit methylation rates, as the methyl group is transferred to mercury by a cobalt-containing molecule (vitamin B12). Adding cobalt

would not raise the cost of analyses appreciably because it is possible to measure multiple metals simultaneously by ICP-Mass spectrometry.

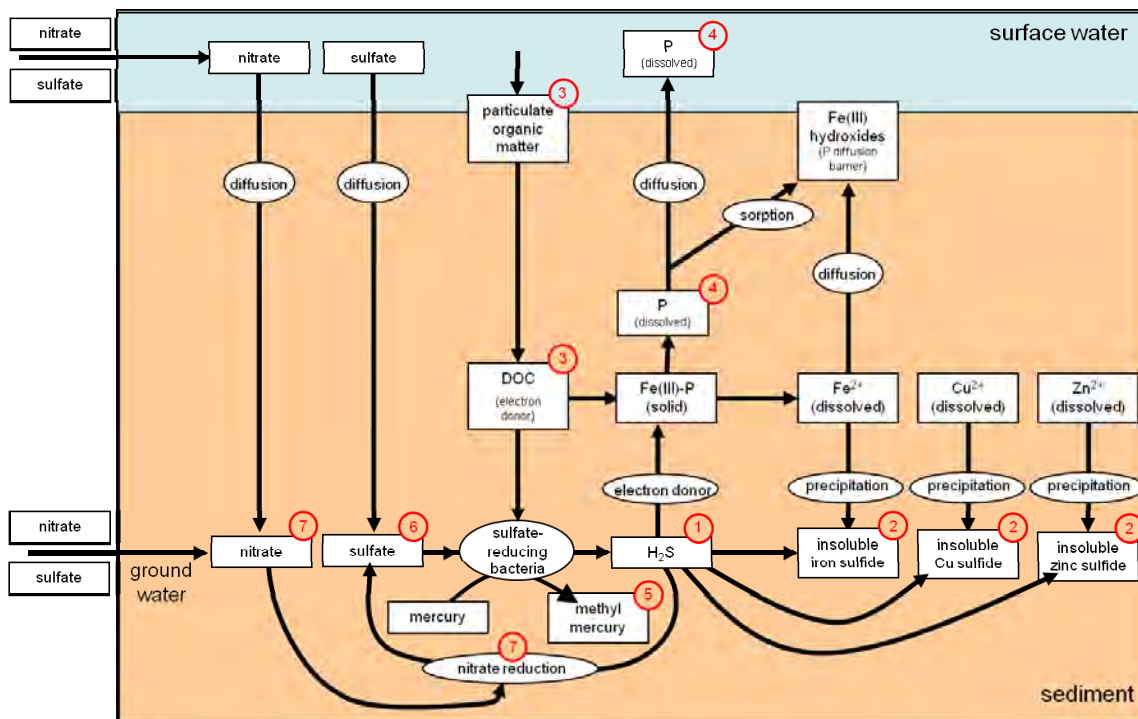


Figure 1. Conceptual model of potential sulfate-mediated chemical changes in the sediment of wild rice beds.

Notes for Figure 1.

1. H_2S (hydrogen sulfide) is produced by sulfate-reducing bacteria that live in environments that are high in organic matter and have zero oxygen. H_2S may be directly toxic to plants. However, if iron concentrations are high relative to sulfide, sulfide may be precipitated (as iron sulfide) as fast as the sulfide is produced, mitigating toxicity.
2. Metal sulfides may precipitate when H_2S reacts with dissolved metals, including iron, copper, and zinc. The solid sulfides are probably less bioavailable for plant nutrition than are free metal ions, conceivably limiting plant growth due to iron, copper, or zinc limitation (these metals are essential nutrients for plant growth).
- 3 Organic loading controls the energy available to the system for biogeochemical reduction (electron donation, either through bacterial metabolism or inorganic chemical reaction). The organic load normally consists of plant material; in a shallow-water system this might be aquatic plant material such as the straw from the previous years' wild rice growth.

4. Phosphate is mobilized and made more bioavailable when oxidized iron (Fe(III)) is reduced to ferrous iron (Fe²⁺), which can occur where high organic production results in low-oxygen conditions in the sediment. Fe²⁺ does not sorb phosphate after iron precipitates as a sulfide, unlike the solid form of Fe(III).
5. Methyl mercury is primarily formed by sulfate-reducing bacteria. The methyl group is supplied within the bacteria by methylcobalamin (vitamin B12), a cobalt-containing molecule. Mercury methylation may be limited by the availability of cobalt, an effect that has been demonstrated in the laboratory. The methylmercury diffuses into overlying water, where it can enter the food chain by sorbing to algae and other particles that are consumed by filter-feeding invertebrates.
6. Sulfate can be used as an electron acceptor by sulfate-reducing bacteria, which in principle are at an energetic disadvantage as long as certain other electron-acceptors are available. In principle, electron acceptors will be consumed in the following sequence: O₂, nitrate, oxidized manganese, oxidized iron, and sulfate. The sequence may not be followed rigorously due to heterogeneity in the sediment, groundwater flow, or differing chemical reaction speeds. Naturally-occurring stable isotopes of S and O have been useful in assessing the degree of sulfate reduction.
7. Nitrate is, in principle, consumed to near zero concentrations before sulfate is consumed. But if nitrate is present simultaneously with sulfide, as has been found in some systems recently, the sulfide can be oxidized to sulfate. Under such conditions, sulfide may not build up even if sulfate-reducing bacteria are active.