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Soil Properties That Influence the Occurrence of Hydrogen Sulfide Toxicity in Rice Fields

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Soil Properties That Influence the Occurrence of Hydrogen Sulfide Toxicity in Rice Fields

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ABSTRACT

Rice (*Oryza sativa* L.) producers face many challenges throughout each growing season. Hydrogen sulfide (H_2S) toxicity is a physiological disorder where sulfate (SO_4^{2-}) is excessively reduced to the toxic gas, H_2S. This can reduce yield and, in severe cases, result in crop death. The main research objectives were to: i) understand chemical and physical characteristics in soils prone to H_2S toxicity, ii) determine influential soil characteristics on the incidence of H_2S toxicity, iii) determine ammonium sulfate ($(NH_4)_2SO_4$) fertilizer additions influence on H_2S toxicity, and iv) predict when and where H_2S will occur. Three greenhouse experiments were conducted using Arkansas field soils with varying degrees of H_2S toxicity history. Half of the soils were sterilized in the first experiment, cultivar CL151 planted in the last two experiments, $(NH_4)_2SO_4$ treatments added to the last experiment, and soil solution samples and redox potential (Eh) monitored in all experiments. Soluble SO_4^{2-} concentrations were greater ($p=0.0231$ to 0.0005) and Eh declined slower in sterilized soils, indicating microorganisms important role in reduction. Significant differences in soluble SO_4^{2-} concentrations between locations on day 21 ($p = 0.0310$) occurred, however the most and least prone soils were not statistically different, indicating this measure may not be the best indicator for H_2S toxicity. A significant interaction between sterilization and location reaffirmed this and the influence of microbes. As rice grew, differences between locations ($p = 0.0183$ to <0.0001) and fertilizer treatments ($p = 0.0275$ to < 0.0001) occurred, and SO_4^{2-} concentrations in the most and least prone soils were different ($p = 0.0405$ to 0.0106), indicating multiple influential factors. Highest soluble SO_4^{2-} concentration occurred in a soil not prone to H_2S toxicity, indicating that the cause of H_2S toxicity is independent of SO_4^{2-} concentrations. Concentration of SO_4^{2-} followed fertilizer rate, yet H_2S toxicity symptoms did not occur. Though results from these studies did not determine the cause

of H_2S toxicity, evidence of multiple influential factors was apparent. Further work focused on the interaction of soil microbes and the quantity of terminal electron acceptors in the soil may shed light on the variable severity of H_2S toxicity across soils.

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Literature Review

Introduction

Rice (*Oryza sativa L.*) production is a vital part of Arkansas's economy. Unfortunately, farmers must combat harsh environmental conditions and disease each year which can lead to severe yield loss. Autumn decline and hydrogen sulfide (H_2S) toxicity, also frequently termed 'Akiochi disease', are often referred to as the same phenomenon even though they appear to be two separate disorders. These phenomena are not well understood conditions and can be problematic to overcome (Hardke et al., 2013; Wamishe et al., 2013; Wamishe, 2015). Autumn decline and H_2S toxicity both appear to be caused by excessive S and Fe in the root zone, though there are many factors that influence these disorders. Sulfate (SO_4^{2-}) is reduced to H_2S which is toxic to the roots limiting their ability to take up nutrients and resulting in root blacken and eventually death and rot (Hardke and Wamishe, 2015). Though this disorder has not been a major problem in the past, more cases of H_2S toxicity and autumn decline in Arkansas have been reported over the last few years.

Since rice production is a large portion of the economy in Arkansas, understanding and managing problems is an important task for researchers and producers. Although H_2S toxicity is not new to the rice producing regions of Arkansas, the relative abundance or reports of the disease are increasing. However, this disease is not well understood and can be easily mistaken for nutrient deficiencies and other diseases. In Arkansas, H_2S toxicity has become a more common problem over the past few years. This may be due to better identification of the symptoms, changes in cultivar tolerance or a chemical and physical change occurring in the soil over time causing the symptoms to appear more frequently. The occurrence of H_2S toxicity is inconsistent from year to year, and appears patchy throughout the field (Groth and Lee, 2003;

Wamishe, 2015). A clear connection between the disease, environment, and soil conditions has not been found though is suspected (Tanaka and Yoshida, 1966; Wamishe, 2015). However, scientists do agree that H_2S toxicity is caused by the production of H_2S in the soil causing toxicity to rice roots, but why this H_2S is being produced in toxic quantities is still a mystery (Baba et al., 1964; Tanaka and Yoshida, 1966; Parks, 1971; Joshi, 1975; Hardke et al., 2013). The production of H_2S and the occurrence of autumn decline depend on the right combination of soil physical properties, such as soil texture and presence of organic matter (carbon source for microbial growth), and soil chemical properties, such as redox potential and pH.

The goal of the following literature review is to summarize information gathered about autumn decline and H_2S toxicity and the significance of these problems in Arkansas as well as to attempt to determine possible causes specifically of H_2S toxicity.

Rice production in Arkansas

Since 1973, Arkansas has been the top producer of rice (*Oryza sativa L.*) in the United States, accounting for 50.7% of rice produced in the U.S. in 2014 (Hardke, 2015). Rice, particularly long grain cultivars, are well adapted to the Arkansas Grand Prairie and the Mississippi Delta regions in Arkansas (USDA-ERS, 2012). Arkansas holds the record land area planted and kilograms per hectare produced within the U.S. (Hardke, 2013). Rice production is a vital component of Arkansas's economy with sales reaching nearly one billion dollars in 2016 (USDA-NASS, 2017). In 2014, over 600,000 hectares of land in Arkansas was devoted to rice production (Hardke, 2015) and rice production supplies over 25,000 jobs in the state (Richardson and Outlaw, 2010). In 2014, approximately 566,000 hectares of rice was harvested in Arkansas, valued at $1.4 billion (USDA-NASS, 2015). Yield typically ranges from 7,566-10,088 kg ha^{-1}, with the state average yield topping out in 2014 at 84.67 kg ha^{-1} (Hardke, 2013).

Over half of the rice grown is produced on silt loam soils while approximately 20% grown on clay and 20% on clay loams which require different management strategies than silt loam soils. Rice is most commonly grown in rotation following soybean (*Glycine max).* The majority of rice is planted using conventional tillage methods, tilling once in the fall and once in the spring to prepare seedbeds. Rice is commonly planted with a dry-seeded, delayed-flood system and is drill-seeded. Irrigation is primarily sourced from groundwater, though water conservation is becoming an important issue. To conserve water, some producers have constructed water reservoirs to capture run-off in order to re-use any available water. Approximately 20% of producers use water captured in reservoirs or other surface water supplies to irrigate their farm. Pest and disease management are managed through foliar applications of fungicides and pesticides to help reduce damage from common diseases and pests. Additionally, over 70% of producers used insecticide seed treatments to improve pest control and increase plant vigor and growth (Hardke, 2015).

One factor that greatly affects the yield production of rice is disease pressure and management. Disease reduces the quality of the crop and yield along with increasing production cost for fungicides to combat the problem (Wamishe et al., 2013). Crop damage from disease occurs in a number of ways. Poor stand establishment, poor plant vigor and nutrient utilization, yield loss, degraded quality, and premature plant death are all common ramifications from disease infestation (Groth and Lee, 2003). Disease is caused by a combination of the presence of susceptible plants, the presence of a virulent pathogen, and a favorable environment for disease development (Groth and Lee, 2003). According to the International Rice Research Institute (IRRI), farmers lose 37% of their rice yield due to pests and diseases each year (Sparks et al., 2012). Loss caused by disease alone is difficult to quantify due to numerous factors that

influence disease. Underground damage from root disease, lack of data on diseases affecting rice, and severity of diseases in commercial fields makes quantifying damage difficult (Groth and Lee, 2003). Since rice is commonly grown in flooded paddies, a humid microclimate is created that is ideal for disease development (Damicone et al., 2001). Understanding the interaction between the susceptible plant, virulent pathogen, and environmental conditions is key in developing a management plan (Groth and Lee, 2003). Though disease pressure is relatively high in the Mid-South region of the U.S., devastating viral diseases and nematodes are not as prevalent in the U.S. as other rice producing regions of the world (Groth and Lee, 2003). The majority of the problematic pathogens in the U.S. are fungal (Groth and Lee. 2003). In 2014, over half of the rice hectares in Arkansas had foliar fungicide applications to protect against common diseases such as sheath blight (*Rhizoctonia solani*) and rice blast (*Magnaporthe grisea*) (Hardke, 2015). High-yielding cultivars that require more N inputs are commonly grown, but these cultivars are less disease resistant. Along with planting more susceptible cultivars, rice is now commonly grown on soil with decreasing fertility, in a monoculture or in short rotations, and irrigation is becoming a constraint. These conditions all increase the likelihood of disease development (Wamishe et al., 2013). Major rice diseases that are prevalent in Arkansas include sheath blight, rice blast, stem rot (*Sclerotium oryzae*), crown (black) sheath rot (*Gaeumannomyces graminis* var. *graminis*), and straighthead (Wamishe et al., 2013).

There are many methods used to control disease. Plant quarantines, cultural controls, chemical control, and biological controls are all methods that are integrated together for the best disease management (Groth and Lee, 2003). Cultural practices include crop rotations, thinning plant stands to reduce canopy moisture, and removing plant debris to reduce pathogen survival (Groth and Lee, 2003). Chemical control through the use of fungicides has become a common

practice to fight many major diseases despite the increasingly negative consumer attitude toward the use of chemicals (De Waard et al., 1993). Biological control methods of disease management include the use of microorganisms in the soil to restrict or reduce pathogen presence in the soil through parasitism, competition, and antibiotic production (Groth and Lee, 2003). The most common and easiest method for disease management is by choosing to plant a high-yielding and disease resistant cultivar (Parsons et al., 2004). Learning to manage and control diseases through a combination of practices is essential for farmers in order to protect their crop and livelihood.

Hydrogen Sulfide Toxicity Versus Autumn Decline

Though autumn decline and H_2S toxicity have been combined into one topic throughout literature, these two phenomena appear to be separate disorders, though their symptomology is very similar. H_2S toxicity generally occurs early in the season, a few weeks after flooding. The H_2S produced in the soil is toxic to rice roots, limits root respiration and causes stunted growth. Autumn decline generally occurs late in the season and is characterized by the invasion of an opportunistic fungi that invades the crown, causing crown rot and plant death. However, one contributor of autumn decline is the production of H_2S in the soil. Since these disorders are often referred to as the same phenomenon, autumn decline and H_2S toxicity will be used interchangeable for the purposes of this literature review. For the research conducted, H_2S toxicity will specifically be investigated.

Autumn decline appears to be caused by the accumulation of H_2S in the soil (Groth and Lee, 2003). This disorder causes black crown and root rot in rice, which can kill the plant (Wamishe, 2015). Though this phenomenon is not fully understood, a variety of factors may influence the occurrences and severity of this disorder. Autumn decline occurs in anaerobic soil systems and the main cause is the overproduction of H_2S in the soil. The main symptom of

autumn decline is the blackening of the roots from the buildup of reduced Fe^{2+} (Groth and Lee, 2003). Other above-ground symptoms of this physiological disorder include wilting, stunting, yellowing (Wamishe et al., 2013), a reduction in tillering, shorter culms and panicles, fewer spikelets, some unfilled grain, dark brown spots on the grain, and leaf spots (Tanaka and Yoshida, 1966). The leaves turn brown at the tip and die, and the plants appear to be under drought stress (Groth and Lee, 2003). Autumn decline weakens the plant which predisposes the plant for the invasion of other opportunistic organisms. Brown Leaf Spot (previously referred to as *Helminthosporium* leaf spot), rice blast, and crown rot have all been found on plants with autumn decline (Tanaka and Yoshida, 1966). If the condition is severe enough, crown rot can progress into the root crown which leads to plant death.

Hydrogen sulfide toxicity was first recognized in Japan where it was termed 'Akiochi' meaning 'autumn-decline' (Baba et al., 1964; Tanaka and Yoshida, 1966). Autumn decline was predominately seen on sandy, degraded soils low in active Fe and on soils with high organic matter (Fairhurst et al., 2007; Ponnamperuma, 1965; Tanaka and Yoshida, 1966). These degraded soils were all low in free Fe, reducible Mn, available Si, and cation exchange capacity (CEC). Plant tissue analysis showed that plants were low in Si and K but high in Fe (Tanaka and Yoshida, 1966). In Arkansas, cases of autumn decline occurs on different textures than those reported from Japan - silt loam to clay loam (Wamishe, 2012).

The buildup of H_2S on rice roots inhibits respiration, inhibiting the uptake of nutrients due to the lack of energy supplied from respiration (Tanaka and Yoshida, 1966). Rice roots can oxidize several compounds on the root surface and in the rhizosphere, including H_2S, to protect the plants from toxic substances (Ando et al., 1983). Thus, the build-up of H_2S to the point of toxicity depends on the oxidizing strength of the roots (Fairhurst et al., 2007).

Current Management Strategies

Though autumn decline is not well understood, there are several effective cultural control methods to avoid the disease or rescue affected plants. Properly managing soil fertility and irrigation, post-harvest management, selecting known straighthead resistant cultivars, scouting, and performing emergency rescue techniques are all used in avoiding and managing autumn decline.

Maintaining proper soil nutrient levels is vital for plants to avoid or resist disease pressure. If a plant is already stressed from lack of nutrition, the plant is more susceptible to disease (Huber and Arny, 1985). Many plants affected by autumn decline are low in various plant macro- and micronutrients such as Si, K, Ca, and Mg (Tanaka and Yoshida, 1966). Poor nutrition in plants, especially deficiencies in K, can reduce the oxidizing ability of roots which allows an increase in the concentration of H_2S in the rhizosphere (Fairhurst et al., 2007). Having sufficient nutrients, particularly K, will also help plants resist other diseases that autumn decline predisposes plants to, such as Brown Leaf Spot (Tanaka and Yoshida, 1966). However, sufficient applications of K is not guaranteed to avoid or correct autumn decline. Potassium availability is effected by several factors including soil and environmental conditions, as well as features of the host and pathogen (Huber and Arny, 1985). Fertilizers containing Si appear to have a positive effect on rice grown in soils prone to autumn decline, though the reason for the positive response is unclear (Park and Tanaka, 1968). However, SO_4^{2-} containing fertilizers, such as ammonium SO_4^{2-}, should be avoided in fields with a history of autumn decline (Hardke and Wamishe, 2015). Park and Tanaka (1968) also concluded that urea was a better N source for rice grown on soils prone to autumn decline.

Irrigation water can also play a role in managing H_2S toxicity. Cold irrigation water and water that contains Fe is suspected to aggravate the problem (Hardke et al., 2013). If irrigation water contains Fe and the field has a history of autumn decline, using an alternative water source, if possible, that does not contain Fe is ideal to lessen the chance for the occurrence of autumn decline. Hydrogen sulfide toxicity becomes highly likely when SO_4^{2-} levels in irrigation water reach 10 mg kg^{-1} (Parks, 1971).

Post-harvest management is also important in managing autumn decline. Management affects the chemical and physical properties of the soil. After harvest, stubble must be managed for various reasons. Nutrient management should be considered since N, P, K, and S remain in the residue and may be returned to the soil to some degree through decomposition (Fairhurst et al., 2007). Common practices for rice residue management are burning or tillage (Hardke, 2013). Burning rice stubble after harvest is a common practice. However, burning residue results in significant loss of N, P, K, and S that would have otherwise been incorporated into the soil through decomposition (Fairhurst et al., 2007). In some states, burning rice stubble has been banned, so producers have turned to tilling residue into their fields (Gao et al., 2004). Tillage during the fallow period is another technique used that promotes the oxidation of S and Fe in the soil to limit the formation of H_2S the forthcoming year (Fairhurst et al., 2007). For fields with a history of autumn decline, draining the field after harvest to increase oxygen levels throughout the soil profile may increase redox potential. Since organic matter contributes to reducing conditions, removing the organic matter may negate the occurrence of H_2S toxicity. However, the incorporation of residue in the soil appears to be expediting reducing conditions and subsequently inducing H_2S toxicity under certain environmental conditions (Gao et al., 2004). The addition of organic matter promotes reducing conditions by supplying microorganisms with

a carbon source to oxidize for anaerobic respiration, resulting in reduced byproducts such as H_2S (Gao et al., 2004).

Cultivar selection is another simple method for controlling any disease. For fields with a history of H_2S toxicity, cultivars tolerant to H_2S that have strong oxidizing power are recommended (Fairhurst et al., 2007). Many of the straighthead resistant cultivars have better oxidizing power and, thus, autumn decline is less likely to occur when those cultivars are planted (Joshi et al., 1975). According to the Arkansas rice performance trials conducted by the University of Arkansas in 2014, the most straighthead resistant cultivars are 'Taggart' and 'CLXL745', with 'Francis' scoring at moderately resistant. From field observation in Arkansas in 2004 by the University of Arkansas Division of Agriculture Cooperative Extension Service, 'Cocodrie', 'Wells', and 'CL161' cultivars were all affected by autumn decline and are likely susceptible to the disorder (Wamishe, 2012). Planting short-season varieties may also help avoid damage (Wamishe et al., 2013). Using seeds treated with oxidants, such as calcium peroxide, can increase the supply of oxygen to rice seedlings to aid the young plant with root oxidizing power to prevent the buildup of H_2S in the rhizosphere (Fairhurst et al., 2007).

Regular scouting is recommended in order to identify autumn decline during the growing season. The first symptom to appear and the easiest to identify is the blackening of the rice roots. This black root color is due to the reduced Fe in the soil coating the roots (Groth and Lee, 2003). The easiest way to know if the blackened roots is due to H_2S toxicity is to expose the roots to air. After approximately an hour of exposing roots to oxygen, the roots will turn either red or white (Hardke and Wamishe, 2015). Symptoms of autumn decline may begin to appear a few weeks after the permanent flood has been established (Wamishe, 2015) though the most notable decline occurs around tillering through maturity (Groth and Lee, 2003). The lower leaves begin to

yellow and growth slows. In severe cases, opportunistic fungi attacks the roots preventing nutrient uptake. If the disease is not addressed early, plant death will occur (Wamishe, 2015). Another highly noticeable sign that H_2S toxicity is present is a rotten egg odor emitting from the field (Groth and Lee, 2003; Gao et al., 2004; Wamishe et al., 2013). Autumn decline often appears in patches within the field and the entire field is rarely affected by this disease (Groth and Lee, 2003). The main technique to reverse autumn decline is to drain the field to allow oxygen back into the root zone to re-aerate the soil. This does not mean drying the field completely, but rather pulling the flood back to leave the soil muddy where the upper roots are exposed to allow oxygen to reenter the root zone (Hardke and Wamishe, 2015). Once new roots begin to form, the field should be flooded again (Wamishe, 2015). Removing the flood and allowing oxygen back into the root zone stopping the reduction reaction is the best "rescue" technique for plants affected by autumn decline (Hardke et al., 2015; Wamishe, 2015). If done early enough, the majority of rice is likely to recover, though yield loss may still occur (Hardke, 2015). However, rice is not a drought tolerant plant, and aerobic conditions increase the potential for other disease to take over, such as blast, and may influence yield (Wamishe et al., 2013). In Arkansas, if autumn decline is found, extension specialists recommend that producers drain the fields at the same time they would drain them for straighthead using the DD-50 program, a database used to predict management timing (Wamishe et al., 2013).

Physical Soil Characteristics

Autumn decline occurs under many different soil conditions though it typically occurs in soils with a more sandy texture with low CEC, low active Fe, high organic matter, and high soluble SO_4^{2-} content (Groth and Lee, 2003). In Arkansas, a clear pattern in soil properties and the occurrence of autumn decline has not been discovered. From field observations in 2004 by

the University of Arkansas Cooperative Extension Service, autumn decline occurred in fields with a high soil pH and in soil textures from silt loam to clay loam (Wamishe, 2012).

However, autumn decline has been a major problem in Japan where it was named 'Akiochi' literally meaning 'autumn decline' (Tanaka and Yoshida, 1966). In Japan, autumn decline typically occurred under two specific soil conditions. The first combination of properties is a well-drained, sandy textured, degraded paddy soil. In anaerobic conditions, this soil did not have free Fe, which allowed the production of H_2S resulting in the blackening of the roots and the development of root rot. The second combination is a poorly-drained paddy soil rich in organic matter. In this soil, the decomposition of organic matter during the summer produced organic acids, ferrous Fe, and H_2S – each of which is harmful and can result in root rot (Baba et al., 1965).

Organic matter plays an important role in microbial activity and the reduction of SO_4^{2-} to H_2S. This reduction process is paired with the oxidation of organic matter to complete the reaction (Reddy et al., 1986). Reddy et al. (1986) also notes that SO_4^{2-} reduction to H_2S naturally occurs when there is a sufficient amount of easily decomposable organic matter in anaerobic soil that is also void of oxygen, nitrate, Mn and Fe. Rice straw is considered an easily decomposable organic matter (Gao et al., 2004) thus promoting the reduction of SO_4^{2-} to H_2S.

Chemical Soil Characteristics

Sulfur, an essential element in all life, is an important component of rice nutrition, as it is a necessary component for amino acid and protein synthesis (Lefroy et al., 1992). There are five possible transformations of S in soil: immobilization into organic compounds, mineralization from organic-S, production of sulfides, production of volatile S compounds such as H_2S, and

oxidation of both organic-S and inorganic-S (Freney et al., 1982). Immobilization, mineralization, sulfide production, and the production of volatile S compounds are common fates of S in anaerobic soil conditions (Lefroy et al., 1992). In the soil, S occurs in organic and inorganic forms. Though organic-S is the most abundant form in soils, organic-S is not plant available. Organic-S must be mineralized to an inorganic form, SO_4^{2-}, in order to be plant available (Lefroy et al., 1992). Sulfate occurs in soils as soluble salts, absorbed onto soil colloids, or in insoluble forms (Tabatabai, 1992). The transformation of S in the soil is dynamic and changes as the soil environment changes.

Sulfur is used and transformed by many different microorganisms in the soil (Starkey, 1950). Soil microbes drive oxidation and reduction reactions as they decompose organic materials. Specifically, reduction occurs by facultative and obligate anaerobes (Gao et al., 2004). Two genera of microbes in particular are responsible for the reduction of SO_4^{2-}: *Desulfovibrio* and *Desulfotomacuclum* (Ponnamperuma, 1984; Reddy et al., 1986). In flooded soils, S is present in many different states – in solution, sorbed onto charged surfaces as SO_4^{2-}, bound in organic matter, and bound to other elements as sulfides, such as Fe and Mn sulfides (Bell, 2008).

To understand the transformations of sulfate, redox potential (Eh) of the soil must be understood. Redox potential is defined by Fuhrmann (1999) as the "inherent tendency of a compound to act as an electron donor or electron acceptor." Typically, Eh, which is measured in volts, ranges between -300 to 700 millivolts (mV) depending on soil pH (Strawn et al., 2015). When soils are subjected to regular flooding for rice production, soil chemical properties are constantly changing as the soil fluctuates between aerobic and anaerobic conditions. While redox reactions can be driven by both biotic and abiotic factors, Eh is influenced more by biotic factors (Strawn et al., 2015). In microbial respiration, oxygen acts as the primary electron acceptor, but

once depleted, microbes use the oxidized states of N, Mn oxides, Fe, and SO_4^{2-} as secondary terminal electron acceptors. Microbes that can utilize the secondary terminal electron acceptors are facultative anaerobes or strict anaerobes (Strawn et al., 2015). After a field is flooded, due to the rapid depletion of oxygen, Eh of a soil changes quickly. Under optimal condition, Eh can decrease to -250 mV within two weeks (Ponnamperuma, 1981). During anaerobic conditions, Eh decreases; pH changes; denitrification occurs; Mn (IV), Fe (III), and SO_4^{2-} are reduced; organic acids are produced; and the accumulation of carbon dioxide occurs as well as other chemical and physiochemical processes (Ponnamperuma, 1981).

Sulfate is unstable at low Eh which favors the reduction of SO_4^{2-} with an end result of H_2S (Ponnamperuma, 1981).

$$\frac{1}{8} SO_4^{2-} + \frac{5}{4} H^+ + e^- \rightarrow \frac{1}{8} H_2S + \frac{1}{2} H_2O \quad \log K = 4.25$$

$$pE = 5.13 - \frac{5}{4} pH$$

Over years of data collection, researchers have found that reduction to sulfide occurs over a wide range of Eh with pH playing a role in when sulfide is produced (Bell, 2008). However, in some soils under reduced conditions, H_2S does not always accumulate and cause problems with plant growth. As SO_4^{2-} is reduced to H_2S, H_2S can build up due to a lack of reducible Fe. This form of Fe can be easily reduced (usually Fe III). In the presence of active Fe, H_2S reacts with the Fe and precipitates into ferrous sulfide (FeS) (Bell, 2008; Parks, 1971).

$$Fe^{2+} + S^{2-} = FeS \qquad pKs = 18.4$$

Between 5-50% of reducible Fe is reduced to ferrous Fe (Fe II) in a few weeks after flooding (Ponnamperuma, 1984). However, if reducible Fe is not present to precipitate H_2S into FeS, H_2S

can accumulate in the soil which can be toxic to plant roots even at low concentrations (Allam and Hollis, 1972; Wamishe, 2015). According to Ponnamperuma (1984), H_2S becomes toxic at 0.1 g m^{-3}. Though the aerenchyma allows the roots to oxidize the rhizosphere to a certain degree, the toxicity of H_2S diminishes root respiration (Bell, 2008) resulting in root damage limiting the ability to take in nutrients (Hardke et al., 2013; Wamishe, 2015).

Since Eh is driven primarily my microorganisms, the question that rises is: what happens to Eh in a sterilized soil? During anaerobic conditions, the S cycle is completely microbial (Reddy et al., 1686) which could indicate that S would not be reduced in anaerobic sterilized soil. Without the presence of *Desulfovibrio* and *Desulfotomacuclum*, reduction of SO_4^{2-} to H_2S may not occur. The three main influential factors of oxidation-reduction reactions occurring are: amount of available oxygen, the presence of microorganisms, and the presence of organic matter. By eliminating one component, redox reactions may not occur or will be significantly reduced.

Soil pH also affects the availability and transformations of chemical substances. Once a flood has been applied to soil, pH moves towards neutrality. Acidic soils increase in pH and the pH of alkaline soils decreases (Ponnamperuma, 1981). In acidic soils, Fe reduces under anaerobic conditions which increases the pH, whereas the accumulation of carbon dioxide decreases the pH of sodic and calcareous soils (Ponnamperuma, 1984). H_2S is produced when pH ranges from 5.5-7.0, according to a study by Bromfield (1953). The amount of free S and S adsorbed onto soil surfaces is also influenced by pH. In a study by Kamprath et al. (1956), they found that SO_4^{2-} adsorbed to soil at pH less than 5, but very little SO_4^{2-} adsorbed when pH was near neutrality. With less SO_4^{2-} bound to soil, the reduction of SO_4^{2-} to H_2S by microbes is more likely.

Availability of other essential nutrients may influence the occurrence or severity of autumn decline. Nutrition of a plant influences the resistance or susceptibility of the plant to diseases (Huber and Arny, 1985). In the early growth stages of rice, nutrient supply is particularly important. During midtillering, healthy, well-nourished leaf tissue should contain 2.8-3.6% N, 0.14-0.27%P, 1.5-2.7% K, ≥0.17% S with N:S ratio of less than 10, and 90-190 mg kg^{-1} Fe (Bell and Kovar, 2000). In typical "Akiochi soils" in Japan, top soil has been found low in Fe, Mg, P, K, and Mn (Tanaka and Yoshida, 1966). As noted previously, low active Fe increases the production of H_2S toxicity causing autumn decline to be more severe (Ponnamperuma, 1981). However, plants affected by autumn decline generally have elevated levels of Fe in the plant tissue compared to healthy plant tissue, which has not been thoroughly explained (Park & Tanaka, 1968). Tanaka and Yoshida (1966) noted that plants affected by autumn decline in Korea had elevated tissue levels of Fe and S but were low in P, Mn, Mg, and Si. A healthy balance of nutrients may be more beneficial in disease resistance rather than the level of any one nutrient (Huber and Arny, 1985).

Though well balanced nutrition may be the best scenario for disease resistance, extensive research indicates that K nutrition is highly linked to disease resistance since K is essential in regulating enzyme activity in plant cells which influences disease intensity (Huber and Arny, 1985). Brown leaf spot (*Bipolaris oryzae*) and stem rot (*Sclerotium oryzae*) have both been significant disease problems in K deficient rice (Slaton et al., 1995). This is of particular interest since brown leaf spot and crown rot are known ramifications of autumn decline (Tannaka and Yoshida, 1966). In a study performed by Park and Tanaka (1968), plants that were deficient in K showed symptoms of autumn decline, however, brown leaf spot (referred to as *Helminthosporium*) did not appear which indicated that K deficiency is associated with autumn

decline but is not the main cause. A study by Maschmann et al. (2010) compared stem rot severity to K fertilization rate and found that the incidence and severity of stem rot decreased as K-fertilizer rate increased.

Summary

Cases of H_2S toxicity have increased over the past few years in Arkansas causing yield loss, and yet there is not a clear understanding of why this disorder occurs. With rice as a major contributor to the Arkansas economy, proactive research to correct problems is vital. Since H_2S toxicity is currently poorly understood, many aspects of soil chemistry, microbial life, and the physical soil must be examined in order to shed light on the cause of this disorder. The goal of this research is to understand the causes of H_2S toxicity in rice and to develop a solution to this disorder. More specifically, the objectives are to determine the primary causes of H_2S toxicity, understand the chemical and physical soil characteristics that influence the occurrence and severity of H_2S toxicity, and be able to predict when and where H_2S toxicity will occur.

The hypotheses of this project are:

H_2S toxicity is caused by a soil chemical reaction rather than soil physical properties.
H_2S toxicity is driven by soils in a low soil redox potential (pE) for extended time periods.
If soil has been sterilized, Eh will not decrease.

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CHAPTER 2

Soil Physical and Chemical Characteristics Influencing Hydrogen Sulfide Toxicity

ABSTRACT

Hydrogen sulfide (H_2S) toxicity is a poorly understood physiological disorder that occurs under anaerobic conditions and can cause substantial yield loss in rice (*Oryza sativa* L.). Though high concentrations of sulfur (S) and the reduction of sulfate (SO_4^{2-}) to H_2S are the causes of toxicity, there are many factors that influence the extent to which SO_4^{2-} is reduced to H_2S. Two greenhouse studies were designed to investigate the chemical and physical characteristics of four soils in Arkansas where this disorder occurs regularly (H and HR-W), sometimes occurs (HR-E), and has never been reported (PTRS). The three soils that have had this disorder (H, HR-W, and HR-E) contained approximately 30% more silt than PTRS. Mehlich 3 extractable SO_4^{2-} and Fe concentrations were significantly different among soils. In the first study, the effect of soil sterilization on SO_4^{2-} reduction was examined. This study showed that SO_4^{2-} concentrations over time were significantly greater in the sterilized soils from day 7-77 ($p=0.0231$ to <0.0001) indicating that microbes play a key role in the disappearance of SO_4^{2-}. Sulfate concentrations were significantly among locations different from day 21-77 ($p=0.0310$ to <0.0001), however H and PTRS were not statistically different. Redox potential dropped more rapidly in H than PTRS, suggesting that redox potential greatly influences the occurrence of H_2S toxicity regardless of the amount of SO_4^{2-} being reduced. When rice was grown, there was again a statistical difference between locations ($p=0.0405$ to 0.0095), however H contained the most SO_4^{2-} and PTRS the least SO_4^{2-}. The most rapid decline in SO_4^{2-} occurred after two weeks of flooding, which coincides with the onset of symptoms in the field. Within four weeks after flooding, H lost 20.7 mg SO_4^{2-} kg^{-1} soil solution whereas PTRS lost 13.5 SO_4^{2-} kg^{-1} soil solution. These results indicate that the rate of SO_4^{2-} reduction, decline in redox potential, and activity of microorganisms all play a role in the occurrence of H_2S toxicity.

INTRODUCTION

With the value of rice sales in Arkansas reaching nearly one billion dollars (USDA-NASS, 2017), rice production is a vital component of the economy in Arkansas. For the past 44 years, Arkansas has been the leader in rice production in the United States, responsible for producing over half of the rice in the country (Hardke, 2015). In order to produce this quantity of rice, over 25,000 jobs in Arkansas are associated with rice production (Richardson and Outlaw, 2010). Because rice production provides a major food staple, creates thousands of jobs, and contributes billions to the economy, researchers must continually address challenges and develop solutions and advancements for producing quality rice crops.

In recent years, reports of H_2S toxicity have increased in Arkansas. Hydrogen sulfide toxicity appears to be caused by excessive S and Fe in the root zone, though there are likely many contributing factors. Soils prone to H_2S toxicity have been termed "akiochi" soils, which is Japanese for "autumn decline". This disorder was first identified in Japan before the 1950s and was classified as a "serious physiological disease" (Baba et al., 1964). Yoshida (1981) later referred to this problem as a "nutritional disorder", and this may, indeed, be a more accurate term. When soils are under anaerobic conditions, SO_4^{2-} reduces to H_2S, a gas toxic to plant roots. However, H_2S typically reacts with reducible Fe^{3+} in the soil and precipitates out as insoluble FeS, preventing the buildup and toxicity of H_2S (Yoshida, 1981). However, in soils prone to this disorder, H_2S does not precipitate out but builds up in the rhizosphere, inhibiting root respiration and nutrient and water uptake due to the lack of energy supplied from respiration (Tanaka and Yoshida, 1966). If root exposure to H_2S is prolonged, roots will eventually die and rot (Hardke and Wamishe, 2015).

Hydrogen sulfide toxicity weakens plants causing them to be more prone to invasion by opportunistic disease organisms. Brown spot (historically referred to as *Helminthosporium* leaf spot), rice blast, and crown rot have all been found in increased severity on plants affected by H_2S toxicity (Tanaka and Yoshida, 1966). Under severe conditions, opportunistic fungi causing crown rot can invade the root crown, resulting in plant death.

The H_2S toxicity phenomenon is not well understood and has been problematic to overcome. Little conclusive information on soil physical and chemical characteristics has been confirmed regarding when this disorder will occur and to what level of severity (Hardke et al., 2013; Wamishe et al., 2013; Wamishe, 2015).

Physical characteristics of akiochi soils vary. In Japan and Korea, H_2S toxicity is typically reported in sandy soils with low cation exchange capacity (CEC), low active Fe, high organic matter, and high soluble SO_4^{2-} content (Fairhurst et al., 2007; Groth and Lee, 2003; Ponnamperuma, 1965; Tanaka and Yoshida, 1966). In Arkansas, however, based on field observations in 2004 by the University of Arkansas Cooperative Extension Service, H_2S toxicity occurred in fields with a high soil pH and in soil textures from silt loam to clay loam (Wamishe, 2012).

Additionally, organic matter (OM) is an important physical characteristic for identifying when and where H_2S toxicity will occur. The majority of S in soil comes from OM as microbes mineralize organic-S to SO_4^{2-} (Germida, 1999). Reddy et al. (1986) notes that SO_4^{2-} reduction to H_2S naturally occurs when sufficient easily decomposable OM is present in flooded soil devoid of oxygen (O), NO^{-3}, manganese (Mn) and Fe. Rice straw is considered easily decomposable OM (Gao et al., 2004) and would thus promote the reduction of SO_4^{2-} to H_2S.

The chemical transformations of S in the soil are dynamic and change with the environment. Immobilization, mineralization, sulfide production, and the production of volatile S compounds are common fates of S in anaerobic soil conditions (Lefroy et al., 1992). With approximately 90% of the total S under soil found as organic-S (Germida, 1999), the fate of S depends principally on the activity of soil microorganisms (Starkey, 1950), particularly *Desulfovibrio* and *Desulfotomacuclum* (Ponnamperuma, 1984; Reddy et al., 1986). Soil microbes catalyze oxidation and reduction reactions as they decompose organic materials.

Redox potential (Eh), the measure of the tendency of chemical species to gain electrons, is a useful measure of what is happening chemically in soils. Microorganisms along with abiotic factors influence the Eh of soil. Typically, Eh ranges from 700 to -300 millivolts (mV) depending on the soil pH, though may be even lower than -300 mV (Strawn et al., 2015b). Though abiotic factors can influence Eh, Eh is mainly influenced by biotic factors (Strawn et al., 2015b). Under flooded conditions, microbes quickly deplete the soil of dissolved O_2 then move on to using the oxidized states of N, Mn oxides, Fe, and SO_4^{2-} as secondary terminal electron acceptors for respiration. During anaerobic conditions, Eh decreases; pH changes; denitrification occurs; and Mn (IV), Fe (III), and SO_4^{2-} are reduced. In addition, organic acids are produced and carbon dioxide (CO_2) accumulates in the soil (Ponnamperuma, 1981). Under anaerobic conditions, the S cycle is completely microbial (Reddy et al., 1986) which suggests that SO_4^{2-} would not be reduced and Eh would not rapidly decline if the soil were to be sterilized prior to flooding.

The goal of this research was to investigate differences in physiochemical properties among soils in Arkansas that exhibit varying degrees of this disorder. Reduction of SO_4^{2-},

changes in Eh, and the effects of soil sterilization were evaluated in an attempt to link the occurrence of H_2S toxicity to specific soil physical and chemical characteristics.

MATERIALS AND METHODS

Soil Description

Soil was collected from three locations in Arkansas during 2015. Surface soil was collected from fields in Hunter, AR; Hickory Ridge, AR; and the Pine Tree Research Station in Colt, AR. The field in Hunter, AR (H) was reported to have symptoms of H_2S toxicity every year in which rice was planted (Y. Wamishe, personal communication, 2015). Soil was collected separately from the east and west ends of the Hickory Ridge field, where H_2S toxicity was reported to occur every year when rice was planted in the west end of the field (HR-W), and H_2S toxicity occurred approximately half the time when planted to rice on the east end of the field (HR-E). Hydrogen sulfide toxicity had never been reported in rice growing on the soil collected from the Pine Tree Research Station (PTRS).

A preliminary soil test was conducted to assess pH (1:2 v:v soil:water ratio) (Thomas, 1996), soil texture (Gavlak et al., 2003), total nitrogen (TN) (Bremner, 1996), total carbon (TC) (Nelson and Sommers, 1996), soil OM via loss on ignition (LOI) (Schulte and Hopkins, 1996), and Mehlich 3 extractable nutrients, P, K, and S (Helmke and Sparks, 1996). Detailed soil chemical and physical information is listed in Table 2.1.

Sterilization

Soil from each location was sterilized using an autoclave. Fifteen liters of soil from each location was brought to approximately field capacity using deionized water and covered with aluminum foil. After allowing soil to sit at room temperature for three days, each location was separated into four polyethylene biohazard autoclave bags, one gallon of soil per bag. Bags were

then placed onto cookie sheets and soil was spread as thin as possible to maximize surface area exposure. The soil was then autoclaved for one hour at 122°C and a pressure of 1.1 kg cm^{-1}. Soil was then removed from the autoclave and allowed to sit at room temperature for three days to allow for any dormant microorganisms to become active. The sterilization process was repeated two more times.

Sixteen two-gallon buckets were sterilized using a dilute bleach solution. After soil was sterilized three times, each bag was emptied into a sterilized pot and covered with plastic wrap until the beginning of the experiment.

Greenhouse Experiment - One

Prior to sterilization, all soil was sieved using a one cm screen to remove clods and large pieces of organic matter. After soils had been obtained and sterilized, four gallons of non-sterilized soil from each location that had not been sterilized were divided into four 7.57-liter buckets, 3.79 liters of soil per bucket, yielding 16 buckets. Buckets were used to ensure that water would not leave the system via drainage. Each location and sterilization treatment was replicated four times, giving a total of 32 buckets. All treatments were randomized and blocked with one replication occurring in each block. The four blocks were divided over two greenhouse benches.

Platinum electrode redox sensors (Sensorex® electrochemical ORP sensor) were inserted approximately 8 cm deep in the soil in two buckets from each treatment, totaling 16 redox sensors (Patrick et al., 1993). Porous ceramic cup samplers (IRROMETER® Soil Solution Access Tube – Model SSAT, Riverside, CA) were placed in each pot. Each bucket was flooded with deionized water 10 cm above the soil surface and maintained for the duration of the experiment. Redox was continuously monitored by the electrodes and logged into a data logger.

Before extracting soil solution, a 60 cc syringe was used to extract and discard all fluid in the porous ceramic cup sampler. To extract soil solution samples, pressure was drawn to 60 cbar to create a vacuum in each porous ceramic cup sampler using a hand pump vacuum. The vacuum was maintained for 3 hours before the solution was collected. A clean 60 cc syringe was then double rinsed with deionized water, and used to extract soil solution, which was then placed in a scintillation vial containing two drops of concentrated HCl (37%) to acidify the solution to prevent precipitation of solution constituents and to reduce microbial activity. The syringe was double rinsed with deionized water between each sample. Samples were stored at room temperature until analyzed for P, K, Ca, Mg, Na, S, Fe (I), Fe (II), Mn, Zn, Cu, and B using an inductively coupled argon plasma (ICAP) spectrophotometry. Following the protocol of Gao et al. (2002), soil solution samples were extracted and analyzed 1, 2, 7, 14, 21, 28, 35, 42, 49, 63, 77, and 91 days after flooding.

Greenhouse Experiment – Two

After termination of study one, new soil samples from H, HR-E, HR-W, and PTRS were obtained. Quadruplicate 3.79 liters samples of each soil were placed in 7.57 liter buckets. Sterilized soil was not included in this study. Cultivar "CL 151" seeds were germinated in a damp paper towel to ensure viable seeds were used. Fertilizer was incorporated in the top few cm of the soil at rates of 45 kg P_2O_5 ha^{-1} and 67 kg K_2O ha^{-1}. Soil was wetted with deionized water and left to sit overnight in the greenhouse. The following day, ten germinated CL 151 rice seeds were planted at a depth of 1.5 cm in each bucket. Soil was misted with deionized water and buckets were then covered with plastic wrap to retain moisture. The plastic wrap was temporarily removed each day to mist soil with deionized water. Misting with water reduced the potential for soil crusting which could interfere with emergence. After plants were established, each pot was

thinned to five uniform plants. One day prior to flooding, the equivalent of 692 kg urea ha^{-1} was added to each pot which provided the equivalent of 318 kg N ha^{-1}. The rice was flooded at the V5 growth stage with deionized water and platinum electrode redox sensors and ceramic cup samplers were inserted approximately 8 cm into the soil in each bucket. Continuous redox measurements were taken for the duration of the experiment. The flood was maintained approximately 10 cm above the soil surface. Soil solution samples from each pot were collected 1, 2, 7, 14, 21, 28, 35, 42, 49, 63, and 77 days after flooding and analyzed as previously described. Soil solution samples were analyzed for P, K, Ca, Mg, Na, S, Fe (I), Fe (II), Mn, Zn, Cu, and B. Plants were monitored for signs of H_2S toxicity throughout the experiment. This experiment was carried out for 77 days. At the termination of this experiment, all plant roots were washed and examined for blackening of the roots. Above ground biomass was collected, dried, and ground to pass a one mm sieve. Acid digests of plant material in concentrated HNO_3 and 30% H_2O_2 were analyzed for S by ICAP spectrophotometry (Jones and Case, 1990).

Statistical Analysis

The first greenhouse experiment consisted of four locations and two treatments, unsterilized and sterilized soil. Each treatment was replicated four times totaling 32 individual buckets. Buckets were arranged in a randomized complete block design with replications blocked. Redox sensors were placed at random in two replications across all replications. Soil solution samples were collected from each individual pot. An analysis of variance (ANOVA) was conducted by day for soil solution data using JMP® Pro 12 (SAS Institute, Inc., Cary, NC). Comparisons were made at the $p \leq 0.05$ significance level to evaluate if sterilization impacted SO_4^{2-} reduction and if SO_4^{2-} reduction differed between locations. Student's T Test was used to separate significant means.

Our statistical hypotheses were that SO_4^{2-} levels would decrease more rapidly in H and HR-W soils, HR-E soil should decrease less rapidly, and PTRS soil should have the slowest decline. Within the sterilization treatment, our statistical hypothesis was that the SO_4^{2-} level in sterilized soils would not decline whereas the non-sterilized soils would decline in an exponential manner. Soil solution data were analyzed by an ANOVA. Redox data were used to support the findings of the soil solution ANOVA.

For the second greenhouse study, our experiment was a one factor complete randomized block design, with one replication blocked. An ANOVA was conducted by day for soil solution data using JMP® Pro 12 (SAS Institute, Inc., Cary, NC). Comparisons were made at the $\alpha = 0.05$ significance level to evaluate if there were differences in SO_4^{2-} reduction between locations while rice was grown. Student's T Test was used to separate significant means. Our statistical hypothesis was that SO_4^{2-} would be reduced more rapidly in H_2S toxicity prone soils than soils where this is not a known problem. Additionally, we hypothesized that rice growth would be affected by the extent of SO_4^{2-} reduction in soils prone to H_2S toxicity. Redox data were used to support soil solution findings.

RESULTS AND DISSUCUSION

Physical and Chemical Soil Characteristics

Before the experiment took place, a soil test was conducted for soil extractable plant nutrients, soil characterization, and soil organic matter. Due to the dynamic availability of nutrients in flooded systems, dried soil used in tests may not accurately represent the nutrient status after the soil is flooded (Dobermann et al., 1998). Based on the soil tests performed on the soils used in this experiment, some differences were observed (Table 2.1).

Mehlich 3 extractable S ranged from 9 to 16, with H containing the highest (16 mg S kg soil^{-1}) and PTRS containing the lowest (9 mg S kg soil^{-1}). Concentrations of extractable SO_4^{2}-S are considered low when less than or equal to 10 mg kg^{-1} (Espinoza et al., 2007). Due to the majority of S being contained in the organic-S pool, extractable soil nutrient analyses are not the most accurate representation of available S in the soil (Dobermann et al., 1998). This organic-S pool accounts for nearly 90% of the total soil S (Strawn et al., 2015a). One pathway for the release of plant available SO_4^{2-} from organic-S is mineralization by microorganisms (Zhou et al., 1999). Unfortunately, there is currently no direct method to evaluate total mineralizable organic-S (Freney, 1986).

Organic matter in each of the soils was assessed by loss on ignition (LOI) in a muffle furnace at 360°C (Combs et al., 1998). Organic matter estimates in these soils were fairly consistent between locations, ranging from 2.01-2.69%. Though this is an important source of SO_4^{2-} as organic-S is mineralized, decomposition and release of SO_4^{2-} slows considerably under anaerobic conditions (De Datta, 1981).

Iron also impacts whether or not H_2S toxicity occurs. Soluble Fe was measured using the Mehlich 3 soil test. Though a clear trend of soluble Fe concentration by location is not apparent, PTRS contained the highest concentration of 464 mg Fe kg soil^{-1}, with HR-E and H containing 383 and 385 mg Fe kg soil^{-1}, respectively. Sulfate, OM and soluble Fe all seem to interact to influence the production and toxicity of H_2S. When Fe is in the insoluble form of Fe^{2+}, formation of FeS may not occur as microbes utilize SO_4^{2-} in respiration. In this situation, H_2S is likely to be formed and become problematic in these soils (De Datta, 1981).

In addition to concentrations of reducible elements such as N, Fe, and Mn, soil texture may also influence H_2S toxicity. In all three locations where H_2S toxicity has been known to

occur, the percentage of silt ranged from 72.9-79.3% whereas PTRS only contained 47.7% silt. These data bring up the question of how soil texture may influence H_2S toxicity. In Japanese paddy soils, H_2S toxicity generally appear on sandy soils (Fairhurst et al., 2007; Ponnamperuma, 1965; Tanaka and Yoshida, 1966), whereas the textures of these soils were all silt loams.

Flooding Effect on Sulfate Concentration and Redox Potential

Days 7-14

A significant difference in concentrations of solution SO_4^{2-} between the sterilized and non-sterilized soils was found on days 7 and 14 after flooding ($p = 0.0231$ and 0.0005 respectively). Despite this significant difference, we believe that the sterilization was not completely effective for several reasons. In a soil completely devoid of soil microbes, neither SO_4^{2-} concentration nor Eh would decline to the same degree as in non-sterilized soils. In order for reducing conditions to develop in soils, it is necessary to have anaerobiosis, mineralizable OM and sufficient numbers of viable anaerobic bacteria. Reduction requires three features: anaerobic conditions, presence of OM, and activity of anaerobic bacteria (Ponnamperuma, 1972). Without microbes, SO_4^{2-} concentrations would not decline. While abiotic forces can drive Eh, the most common driving force behind Eh changes are biotic (Strawn et al., 2015b). Redox potential and SO_4^{2-} concentration steadily declined from day one in both the sterilized and non-sterilized soils indicating the presence of at least a limited population of soil microbes in both treatments.

Another reason for suspecting incomplete sterilization was the germination of weeds in the sterilized soils. Sterilization may have been compromised by air contamination or the presence of highly resistant spores that withstood the sterilization process. Also, steam sterilization may not be the most effective long term sterilization method. Tanaka et al. (2003)

found that bacteria counts were relatively unaffected after steam sterilization. Eno and Popenoe (1964) were able to obtain near complete sterilization through steam, though some fungi and bacteria were still detected in the muck soil. The incomplete sterilization in our study may be due to the nature of the sterilization technique used.

Though the soils were apparently not completely sterilized, sterilization was effective to some degree. Soil solution from sterilized soil consistently contained significantly higher concentrations of SO_4^{2-} than soil solution from the non-sterilized soils. This difference was statistically significant on days 7, 14 and 21 ($p = 0.0231$, 0.0005, and < 0.0001 respectively). On day 7, the sterilized soils contained 7.49 mg L^{-1} more sulfate than the non-sterilized soils. Since dead microbial cells contribute to OM residue, this elevated concentration of sulfate in the sterilized soil is likely due to the addition of microorganism detritus as organic-C and S as a result of sterilization (Reddy et al., 1986). Surviving microbes then had more organic-S to mineralize into SO_4^{2-}. This may have occurred to some degree, however mineralization of OM slows greatly once submergence occurs (Ponnamperuma, 1984).

Initial sterilization occurred 7.5 weeks before the flooding occurred but was sterilized again 12 days before flooding. This time gap allowed for aerobic mineralization to occur by any surviving microbes. The amount of organic-S mineralized under aerobic conditions varies from soil to soil, but according to a study by Zhou et al. (1999), 4.4-7.2% organic S can be mineralized over a 28 week period. Another theory as to why the sterilized soils contained more sulfate than the non-sterilized soils in the beginning is that autoclaving resulted in a chemical change in the soil. According to Eno and Popenoe (1964), steam sterilization changes the soil chemistry by increasing extractable N, P, and S. This could be the case in our soils and thus account for the additional SO_4^{2-} in solution. However, Eh for sterilized soils was greater than 300

mV on day 7, indicating that the majority of the soils were still mainly aerobic with O_2 as the main terminal electron acceptor (TEA) (Reddy et al., 1986). Redox potential for the non-sterilized soils was 250 mV indicating that NO_3^- and Mn^{4+} were the primary TEAs (Reddy et al., 1986).

Two weeks after flooding, sterilized soil contained 13.91 mg L^{-1} more SO_4^{2-} than the non-sterilized soils. Sterilized soil lost a mere 2.23 mg L^{-1} over one week whereas non-sterilized soils lost just over half of the SO_4^{2-} (8.64 mg L^{-1}). The Eh declined for both sterilization treatments, however Eh for the non-sterilized soils dropped below -20 mV while sterilized soils only declined to 220 mV, a 240 mV difference (Figure 2.2). With an Eh of 220 mV and a limited microbial presence, the sterilized soils appear to be utilizing other TEAs such as NO_3^- and Fe^{3+} (Harter and McLean, 1965; Reddy et al., 1986), explaining the very small reduction of SO_4^{2-}. Sulfate concentration and Eh declined in both sterilization treatments, but the non-sterilized soils experienced a more extreme decline than the sterilized soils. This supports the hypothesis that microbes catalyze in the reduction of SO_4^{2-} to S^{2-} and thus are important causative agents in the onset of H_2S toxicity.

Day 21

On day 21, sterilization was again a significant main effect but location also became a significant main effect. For the sterilization main effect, SO_4^{2-} concentration in the non-sterilized soils only declined by 27% from the previous week whereas 55% of the SO_4^{2-} was lost between days 7 and 14. The Eh for the non-sterilized soils continued to decline to below -200 mV, well below the Eh where SO_4^{2-} reduction is expected to occur. Sulfate concentration in the sterilized soils was unchanged from day 14 to day 21, but the Eh declined to approximately -60 mV. In contrast, as the non-sterilized soils approached this Eh, sulfate reduction rapidly took place.

There are several possible explanations for the observed differences in Eh between the sterilized and non-sterilized soils. Several reports in the literature indicate that measuring redox with platinum electrodes may not accurately reflect chemical changes taking place under anoxic conditions (Bohn, 1971; Ponnamperuma, 1972). Additionally, Eh varies throughout the bulk soil both vertically and horizontally (Aomine, 1962). Taking continuous redox measurements in one spot in our soils could explain the variation in Eh between replications.

Another factor influencing Eh measurements are microorganisms. The difference in Eh as well as SO_4^{2-} concentration between sterilized and non-sterilized soils may indicate differences in microbial populations. The greatest microbial diversity appears in soils with a near neutral pH (Fierer and Jackson, 2006). The soils used in this study ranged in pH from 7.6-8.1 (Table 2.1). Once a soil is flooded, pH approaches neutral regardless of whether the pH was previously acidic or alkaline (Ponnamperuma, 1972). This research supports the assumption that microbial diversity is likely very high in these four Arkansas soils, however we do not know what species are present. With sterilization limiting microbe species diversity as well as density, the lack of sulfate reduction could indicate that fewer facultative anaerobes are present. Over two weeks (days 7-21), the non-sterilized soils lost approximately 70% of the SO_4^{2-} in solution while the sterilized soils only lost 10%. While soils did not appear to be completely sterilized, the difference in SO_4^{2-} concentration between the sterilized and non-sterilized soils indicated that the elimination of microbes greatly impacts SO_4^{2-} reduction, thus indicating the importance of microorganisms in the H_2S toxicity phenomenon.

For the first three weeks after flooding, there were no statistical differences in SO_4^{2-} concentrations in solution between any of the soil locations. On day 21, SO_4^{2-} concentrations in solution were significantly different between locations, regardless of sterilization treatment

effects. This day is of particular interest since symptoms of H_2S toxicity typically appear in the field two to three weeks after flooding (Hardke et al., 2015). Interestingly, soluble SO_4^{2-} concentrations in H, PTRS, and HR-W were not significantly different from each other. These three locations contained higher concentrations of soluble SO_4^{2-} than HR-E, but SO_4^{2-} concentrations in soils from HR-E and HR-W were also not statistically different (Table 2.2). These results indicate that the concentration of SO_4^{2-} in solution on a given day after flooding may not be the best indicator of likelihood of H_2S toxicity. Chemical reactions prior to SO_4^{2-} reduction are likely a better indicator of when and where H_2S toxicity will occur. Microbes transform organic-S to H_2S under anaerobic conditions (De Datta, 1981), but H_2S reacts with Fe^{2+}, Mn^{2+}, Cu^{2+}, Cu^{+}, and Zn^{2+} to form insoluble sulfides (Ponnamperuma, 1981). Typically, any H_2S formed in the soil would react with Fe^{2+} present in solution to form insoluble FeS. Without ample amounts of Fe^{2+} in the soil, H_2S can build up and cause toxicity to rice (De Datta, 1981).

As expected, water-soluble Fe increased over time in each location after soils were flooded (Ponnamperuma, 1981) (Table 2.3). On day 21, PTRS contained nearly 6.5 times higher concentrations of Fe than all other soil locations. According to the Mehlich 3 soil extraction, PTRS did contain the highest concentration of extractable Fe, though it is unclear as to why soluble Fe was so much higher in PTRS than the other soils (Table 2.3). Based on the elevated soluble Fe concentration and the higher Eh of 60 mV, Fe^{3+} was likely acting as the primary TEA and SO_4^{2-} reduction was minimal on this day. With high soluble SO_4^{2-}, high soluble Fe, and high Eh, the H_2S produced once SO_4^{2-} became the TEA would likely react with the plentiful soluble Fe and precipitate as insoluble FeS, therefore preventing H_2S buildup and toxicity (Table 2.3).

In contrast, on day 21 the H location contained high concentrations of SO_4^{2-}, low Fe, and a low Eh. Sulfate was likely the TEA on this day with the low Eh (-131 mV). Since soluble Fe concentrations were relatively low (6.8 mg L^{-1}), H_2S formed during SO_4^{2-} reduction would not have enough Fe^{2+} to react with to precipitate out, meaning that H_2S would likely build up in the soil to the point of toxicity.

Days 28-77

From days 28 to 77, a significant interaction between sterilization and location occurred for soluble SO_4^{2-} concentration over time (p values ranged from 0.0105 to <0.0001). Sterilized soils consistently contained higher concentrations of SO_4^{2-} than the non-sterilized soils, regardless of location. Non-sterilized soils had the lowest concentrations of SO_4^{2-} but were not statistically different between locations until day 63. However, on days 63 and 77, numerical differences in SO_4^{2-} concentration were so small that statistical differences were negligible in practical application. On day 77, the difference in SO_4^{2-} concentration between the highest sterilized soil and lowest non-sterilized soil was only 2.76 mg L^{-1} which is a minimal difference in a practical sense.

For all non-sterilized soils from each location, the majority of the SO_4^{2-} in solution was reduced by day 28 with little numerical difference in concentrations from days 28-77. In contrast, SO_4^{2-} concentration in sterilized soils continued to be reduced from days 28-42. The majority of the SO_4^{2-} in the sterilized soils was reduced by day 42, but each location continued to decrease in SO_4^{2-} concentration for the remaining days with minimal differences in concentration. The difference in SO_4^{2-} concentration between sterilized and non-sterilized soils was likely due to larger microbial populations in the non-sterilized soils which used more TEAs more rapidly than

the limited number of microbes in the sterilized soils. This resulted in more SO_4^{2-} reduction at a faster rate in the non-sterilized soils for each location.

The magnitude of difference in soluble SO_4^{2-} concentration between the sterilized and non-sterilized soils of each location is notable (Figure 2.1). At the beginning of the statistical interaction, SO_4^{2-} concentration in sterilized soils from PTRS were 6.5 times higher than the SO_4^{2-} concentrations in the non-sterilized PTRS soils. On this same day, sterilized soils from H contained five times more soluble SO_4^{2-} than the non-sterilized H soils. Both Hickory Ridge locations had differences in SO_4^{2-} concentration between the sterilized and non-sterilized soils, however the 3.5 and 2.5 fold differences were not as extreme as the differences in H and PTRS. The difference between the Hickory Ridge locations and PTRS and H could be due to differences in microorganism population sizes and diversity, amounts of organic substrates in the soils, as well as Eh and pH. Fierer and Jackson (2006) found that soil pH affected microbial diversity on a local scale with more diversity and richness occurring around a neutral pH. Several studies throughout the literature show that steam sterilization using an autoclave can alter soil chemical properties including pH and extractable S (Eno and Popenoe, 1964; Skipper and Westermann, 1973; Wolf et al., 1989). Comparing and contrasting chemical changes in the sterilized and non-sterilized soils by location brought some understanding to these extreme differences in soluble SO_4^{2-} concentrations between the sterilized and non-sterilized soils.

As previously noted, sterilization likely caused an increase in soluble SO_4^{2-} by adding more mineralizable S to the system by killing microorganisms. Samples from 24 hours after flooding showed that soluble SO_4^{2-} concentrations in the sterilized soils were higher by 9.45, 11.95, 12.61, and 2.88 mg L^{-1} for H, PTRS, HR-E, and HR-W respectively compared to the non-sterilized soils of each location. Though this was not of statistical interest ($p = 0.8879$), elevated

SO_4^{2-} concentrations on day one is notable. However, by day 28 this difference became statistically significant as an interaction with location ($p = 0.0014$). This elevated SO_4^{2-} concentration in the sterilized soils and the interaction with location may be of importance in understanding under what conditions H_2S toxicity is likely to occur.

By day 28, over half of soluble SO_4^{2-} was reduced in the sterilized soils of each location. From days 28 to 77, SO_4^{2-} concentration declined sharply for one week then gradually declined approaching a minimum value asymptotically in the sterilized soils (Figure 2.1). Redox potential varied by location during this time period.

Both sterilized Hickory Ridge locations experienced rapid SO_4^{2-} reduction from days 28 to 35. In the sterilized HR-E soils, Eh reached the SO_4^{2-} reducing potential, -100 mV, shortly before day 28 and declined to -230 mV by day 28. Redox potential continued to decline until day 42, reaching and maintaining a minimum value around -320 mV with small fluctuations over time but never rising above -300 mV. However for sterilized HR-W soils, Eh reached the SO_4^{2-} reducing potential one week before HR-E and was 50 mV lower on day 28. Redox potential gradually declined until day 49 then remained around -300 mV for the remainder of the experiment.

Interestingly, on the first day of Eh data (72 hours after flooding), HR-W was 150 mV higher than HR-E and declined more rapidly reaching the SO_4^{2-} reducing potential and the maximum negative value before HR-E. Differences in Eh between locations and sterilization treatments shortly after flooding could be due to the quantity of OM present as was seen in Gao et al. (2004). On day one of sampling, HR-W contained 6.2 mg L^{-1} less soluble SO_4^{2-} than HR-E. One possible explanation for the faster decline in Eh and lower starting concentration of SO_4^{2-} in sterilized HR-W soils is that fewer microbes were killed during sterilization compared to the

amount killed in sterilized HR-E soils. The presence of highly resistant spores in one location could account for differences in sterilization effectiveness (Skipper and Westermann, 1973). A faster decline in Eh would be more likely with more microorganisms present, less organic-S added through dead microbes, and less soluble SO_4^{2-} in solution. With potentially fewer microbes eliminated during sterilization, this could possibly explain differences in SO_4^{2-} concentrations between the sterilized and non-sterilized soils in the HR-W soil were greater than the corresponding difference as the other two locations. The differences in effectiveness of sterilization between HR-E and HR-W may indicate differences in microbial populations. With similar concentrations of SO_4^{2-} but slightly different Eh trends and different microbial populations, this could explain why H_2S toxicity occurs regularly in half of the field and only occurs occasionally in the other half.

Sterilized PTRS soils also experienced rapid reduction of SO_4^{2-} from days 28-35 but reduced the greatest quantity of SO_4^{2-} out of all sterilized locations. For the duration of the significant interaction, sterilized PTRS soils contained the most SO_4^{2-} out of all locations, sterilized and non-sterilized, except for the sterilized H soil which was not statistically different. Additionally, sterilized PTRS soil contained numerically the highest concentration of soluble SO_4^{2-} on day one though this was not statistically significant.

Redox potential was below SO_4^{2-} reducing potential on day 28 at -163 mV, but was over 100 mV higher than both Hickory Ridge locations and 68 mV higher than H. Redox potential continued to rapidly decline until day 42 at which time Eh settled around -340 mV for the remainder of the experiment. This was the lowest Eh of all sterilized and non-sterilized soil locations, though only by a few mV. This decline in Eh again supports the suspicion that sterilization was not entirely effective. Despite having the lowest Eh, sterilized PTRS soils

contained the most SO_4^{2-} along with sterilized H soils for the duration of the experiment. This research, along with others, does indicate that Eh is difficult to use as an indicator of the progression of reducing conditions in the soil, though does identify oxic and anoxic conditions well (Gao et al., 2002). Redox potential alone may not be the best indicator of whether H_2S toxicity is likely to occur or not, though it may still be a useful tool in understanding this complex disorder.

Another unique attribute of the sterilized PTRS soils compared to all other locations and sterilization treatments was the abundant production of soluble Fe. While Fe concentration increased logarithmically for all other sterilized soils, Fe increased in the sterilized PTRS soils following a quadratic trend (Table 2.3). At the peak of soluble Fe in solution, sterilized PTRS soils contained nearly 6.5 times more Fe than the other sterilized soil locations. On day 35, Fe began to decline indicating the possibility of FeS precipitation due to the increase of H_2S from the rapid reduction of SO_4^{2-} the week before. Though sterilization can affect soil chemistry, this drastic change in soluble Fe in sterilized PTRS soils is difficult to explain. No other sterilized soil experienced increased Fe to this extent. However, all other soils, both sterilized and non-sterilized soil, did increase in Fe concentration and plateaued as expected (Ponnamperuma, 1981). Non-sterilized PTRS soils contained the lowest concentration of soluble Fe which could indicate that the Fe had been reacting with any H_2S produced, therefore preventing H_2S toxicity.

As with sterilized PTRS soils, a rapid decline in SO_4^{2-} concentration occurred between days 28 and 35 for sterilized H soils. Sulfate concentration in sterilized H soils followed the same trend as the sterilized PTRS soils and the two were not statistically different for the duration of the significant interaction. Unlike PTRS, Eh had already declined to and plateaued at a relatively high Eh of approximately -270 mV for the remainder of the experiment. Though not

statistically significant, Eh in sterilized H soils was numerically higher than all other locations once each soil reached a minimum Eh value. The non-sterilized H soils followed a similar Eh trend. Eh fell rapidly after flooding and reached a minimum value by day 28. However, non-sterilized H soil was around 80 mV lower than the sterilized soil, and the Eh of the non-sterilized soils reached similar values in all other non-sterilized soil locations.

While some interesting differences were observed between locations for the sterilized soils, the importance of this data becomes apparent when compared to their non-sterilized soil counterparts. Two major general differences were observed between the sterilized and non-sterilized soils for all locations. One, SO_4^{2-} was reduced to the reached value for all non-sterilized soils by day 35, while SO_4^{2-} concentrations were much higher and were still being reduced in all the sterilized soils. There are likely more SO_4^{2-} reducing bacteria present in the non-sterilized soils which explains why sulfate was reduced to the minimum value more rapidly. Other measurements could have been useful to explain this such as pH and temperature (Ponnamperuma, 1981). Secondly, the minimum Eh eventually reached by all soils had a larger spread in the sterilized soils while the non-sterilized soils all reached similar values. This is interesting because H_2S toxicity occurs naturally in some of these field soils (non-sterilized), but we do not see much difference in Eh between locations. Since sulfate concentrations in non-sterilized PTRS and H were not statistically different from each other and Eh for both were very similar, we can conclude that sulfate reduction and Eh are not strong indicators of whether or not H_2S toxicity will occur. However, this conclusion may not hold in the presence of growing rice.

Rice Experiment

Due to equipment malfunction, half of redox data were not able to be used to support soil solution findings. Unfortunately, consistency between the two successfully recorded replications

was poor during certain time periods yet fairly similar during other times, so accuracy of the data is questionable. This data will be used to supplement findings of the soil solution data when appropriate.

Poor results with redox data could be attributed equipment. The redox sensors were potentially faulty as was the battery on one data logger. Another possibility for the inconsistency in data from two replications could be due to oxygenation of the system when water was added to each bucket throughout the growing period to maintain the flood. Fresh deionized water was used which likely contained plentiful dissolved oxygen. Then, the deionized water was poured into each bucket using another bucket which stirred the system allowing more oxygen into the system. Another possibility for differences between replications is that Eh varies greatly throughout soil (Aomine, 1962). Oxygen could potentially be trapped in different micro and macropores at the tip of the electrode causing higher Eh readings than we would see in other areas within that soil profile. Mosaics of high and low Eh throughout a soil are likely (Aomine, 1962). Proof is lacking that any of these potential reasons were responsible for lost and inconsistent data, however precautions against these possibilities were made in the third experiment.

Days 1-28

During rice growth, sulfate concentrations in soil solution were significantly different between locations from days 1-28 and 63-77 (p values ranged from 0.0405 to 0.0095) (Figure 2.3). However, on days 63 and 77, differences in SO_4^{2-} were 1.4-2 mg L^{-1} different between the location with the highest concentration and the location with the lowest concentration and are therefore not of practical interest.

The overall trend in SO_4^{2-} loss over time greatly differed from the first experiment. Rather than SO_4^{2-} concentration immediately declining, SO_4^{2-} concentrations decreased very gradually for approximately one week after flooding before rapidly declining. Sulfate concentration actually rose over the first week post flooding in the H soils. Though SO_4^{2-} increased just over one mg L^{-1}, this is the only location that had a slight increase rather than a slight decrease over the first week.

The delayed decline in SO_4^{2-} concentration was likely due to the rice growing in the soil. Rice roots released O_2 into the rhizosphere through the aerenchyma, oxidizing many compounds near the root to allow plant uptake (Ando et al., 1983; Joshi et al., 1975). Small aerobic zones were likely created in the rhizosphere allowing microbes to continue utilizing O_2 as the terminal electron acceptor and delayed the reduction of SO_4^{2-} due to the diffusion of O_2 into the soil from rice roots (Yoshida, 1981). Though O_2 is depleted within a few hours of flooding (De Datta, 1981), O_2 release from the roots may have been enough to delay SO_4^{2-} reduction for a week. The first week of redox data supports the delayed reduction with Eh values all remaining near 500 mV for each location except HR-E which was near 350 mV 24 hours after flooding then increased to nearly 500 mV the next day (Figure 2.4). Since the majority of the redox data for HR-E was very inconsistent between the two replications, this could likely be due to equipment error.

Sulfate rapidly declined two weeks after flooding (Figure 2.3), which again is consistent with when symptoms typically appear in the field. However, above ground symptoms of H_2S toxicity did not appear in any treatment. At the termination of the experiment, roots were removed, washed, and examined for accumulation of black iron sulfides, but no signs of H_2S

toxicity were present. This was likely due to the volume of roots in each bucket. Root respiration in a small, closed system may account for lack of H_2S toxicity.

By day 49, all locations began to asymptotically approach a minimum value of SO_4^{2-}, but no statistical difference was found between locations as the decline of SO_4^{2-} concentration slowed. By day 77, SO_4^{2-} concentrations of all locations were less than 3 mg L^{-1}. Unlike the first experiment, H and PTRS were significantly different with H containing substantially more SO_4^{2-} than PTRS. Concentrations of SO_4^{2-} in solution 24 hours after flooding differed greatly between these two locations, with soluble SO_4^{2-} concentrations of 39.3 mg L^{-1} and 16.8 mg L^{-1} for H and PTRS, respectively. These value were much higher than the Mehlich 3 extractable S from the initial bulk soil samples and the SO_4^{2-} concentration of the first experiment 24 hours after flooding. One contributing factor was likely microbial activity mineralizing organic-S to SO_4^{2-} for six months between the initial bulk soil test and the beginning of the second experiment. Storage in the warm greenhouse environment along with slowly air drying likely promoted mineralization (Williams, 1967). However, the Mehlich 3 soil test results represent the nutrient index of soils before flooding (Slaton et al., 2006). Once soil has been flooded, the chemistry changes substantially which likely caused the differences between the Mehlich 3 results and the SO_4^{2-} in solution 24 hours after flooding.

Between 24 hours and 28 days after flooding, approximately 7 mg L^{-1} more SO_4^{2-} was reduced in H than PTRS (Figure 2.3). However, H still contained 15.4 mg L^{-1} more soluble SO_4^{2-} as PTRS which had reduced to 3.26 mg L^{-1}. By the termination of the experiment, H reduced twice as much SO_4^{2-} than PTRS with a total of 36.5 mg L^{-1} reduced, whereas PTRS reduced 15.4 mg L^{-1}. Unfortunately, Eh data was not consistent between replications for H after one week of flooding. However, Eh data for PTRS was consistent between replications for five weeks after

flooding and can be used to support solution data (Figure 2.4). For the first 28 days of flooding, Eh of PTRS remained fairly steady, around 500 mV. In comparison, during the first week of flooding, Eh of H declined from 500 mV to 450 mV. Redox potential for H continued to decline whereas Eh of PTRS remained fairly steady for 4 weeks (Figure 2.4). If this was true, the decline in Eh in H soils would have reached the SO_4^{2-} reducing potential weeks before PTRS which would have promoted more SO_4^{2-} reduction, accounting for the rapid reduction of SO_4^{2-} .

Though H and PTRS locations were statistically different during the first four weeks after flooding and were the highest and lowest concentrations in this experiment, soluble SO_4^{2-} concentrations in the Hickory Ridge locations fell in between those two extremes. While HR-E and HR-W were not statistically different from each other, SO_4^{2-} concentration in HR-E was also not statistically different from H during the first four weeks after flooding. A sharp decline in soluble SO_4^{2-} occurred in both of these locations from days 14 to 28, and both likely experienced similar rapid declines in Eh. Replications of Eh data for HR-E were consistent from the beginning of the experiment until day 21 and showed a sharp decline between days 14 and 21 with Eh dropping from nearly 500 mV to 150 mV. If this trend continued, SO_4^{2-} reducing potential would be obtained weeks before PTRS and HR-W (Figure 2.3).

While soluble SO_4^{2-} concentrations in HR-E and H were not statistically different during the first four weeks after flooding, the same was true of soluble SO_4^{2-} concentration in HR-W and PTRS. Though there was nearly a 10 mg SO_4^{2-} L^{-1} difference in these two locations, reduction occurred at the same rate (Figure 2.3). In both locations, SO_4^{2-} reduced steadily for five weeks before approaching the minimum content asymptotically. From what was able to be interpreted from Eh data, PTRS and HR-W followed similar Eh patterns of maintaining a high, aerobic Eh

for the first 5 weeks after flooding then ending with a low Eh near -300 mV by the termination of the experiment.

Though the frequency of H_2S toxicity occurring in the field is different between HR-E and HR-W, these two soils were not statically different from each other for the duration of the experiment. Despite having the same pH, according to the Mehlich 3 soil report HR-W contained higher concentrations of nearly every nutrient as well as higher LOI, %N and %C (Table 2.1). Additionally, the Eh reacted differently between these soil locations. Redox potential in the HR-W soil maintained near 500 mV for the first 6 weeks after flooding before rapidly declining to below -100 mV by the end of the experiment. Redox potential for HR-E, however, increased during the first week after flooding, maintained near 500 mV for two weeks, then rapidly declined. Unfortunately, data between replications of HR-E was inconsistent after three weeks so Eh for the rest of the experiment is unknown. However, based on the previous experiment and data from the literature, a reasonable conclusion is that Eh declined to anaerobic levels at least by six weeks after flooding (Takai and Kamura, 1966; Ponnamperuma, 1981; Goa et al., 2004; Rogers et al., 2011).

Differences in Eh between locations are particularly interesting when compared to the results of the first experiment. With an immediate decline in Eh during the first experiment, the most likely explanation for the delay in Eh decline was the diffusion of O_2 into the rhizosphere from the rice roots (Yoshida, 1981; Ando et al., 1983). White there is no definitive answer as to why Eh declined more rapidly in H and HR-E than PTRS and HR-W, there are several possibilities. First, microbial populations may indeed be different, especially between H and PTRS. Reduction is driven by anaerobic respiration (Ponnamperuma, 1972), so the differences in these two flooded soils may be due to the presence of different species of anaerobic microbes.

Bacterial community structure is believed to be strongly correlated with soil pH (Fierer and Jackson, 2006), and soil pH differed between H, PTRS, and the Hickory Ridge field. However, since HR-E and HR-W have the same soil pH yet different Eh trends, microbial populations alone may not be the driving factor. Another possible factor influencing Eh was nutrient concentration differences, particularly Fe content. Decline in Eh was likely resisted by PTRS soil due to the elevated reducible Fe content which can help prevent a decline in Eh (Yoshida, 1981). This would also explain why Eh in HR-W declined two weeks after HR-E since Fe concentrations were higher in HR-W.

Though root blackening symptoms of H_2S toxicity did not appear during this experiment, leaf tissue was analyzed for nutrient content since H_2S toxicity damages roots and impedes nutrient and water uptake. Visual symptoms of potassium (K) deficiencies appeared in several plants and, according to the leaf tissue report, K levels were below optimum in each location (Dobermann and Fairhurst, 2000; Slaton et al., 2006). H, HR-W, and HR-E were all below the critical level for deficiency for K (Dobermann and Fairhurst, 2000). Sulfur concentration was also below the critical level for deficiency in HR-W and PTRS (Dobermann and Fairhurst, 2000). Though there were nutrient deficiency problems throughout all the soil locations, H_2S was not likely the cause. In cases of H_2S toxicity, P is the greatest and most common deficiency followed by K (Yoshida, 1981). However, P concentrations in leaf tissue were above optimum in all locations (Dobermann and Fairhurst, 2000). The K and S deficiencies were likely due to low levels in the soil and an insufficient K_2O fertilizer application rate.

With rice growing in these soils, soluble SO_4^{2-} concentrations and Eh reacted differently than when soil alone was submerged. While symptoms of H_2S toxicity did not occur during the second experiment, some differences between soil locations were identified as potentially

influencing factors to this nutritional disorder. Further research is necessary to investigate the physical and chemical factors that may be influencing the occurrence of H_2S toxicity.

SUMMARY

The primary objectives of these two studies were to investigate the chemical and physical characteristics of a variety of soils that have experienced H_2S toxicity to varying degrees compared to a soil where H_2S toxicity has never been reported. Soluble SO_4^{2-} concentrations in solution, Eh, and sterilization were all examined in each of these soils both with and without rice growing. When comparing soil test results of the four locations, Mehlich 3 extractable SO_4^{2-} and percent silt were greater in all three soils that had experience H_2S toxicity. The amount of these substrates present in the soil likely influence the chemical reactions that took place once the soils were submerged. However, Mehlich 3 extractable SO_4^{2-} is not an exact representation of nutrient availability once the soil is anaerobic. Additionally, plant available S is difficult to accurately assess since the majority of S is located in the organic-S pool which cannot be quantified accurately. In these Arkansas soils, silt was the dominant texture whereas sand predominates in Japanese soils prone to H_2S toxicity. Though texture may not be a critical factor in H_2S toxicity, it may influence the occurrence to some degree. However, this information does indicate differences that likely alter the environment and favor the production of H_2S to the point of toxicity.

By sterilizing the soils, we were able to determine that the reduction of sulfate and decline in redox potential is primarily driven by microorganisms. With reduced populations, SO_4^{2-} concentrations remained greater in all location compared to the all non-sterilized soils ($p=0.0231$ to <0.0001). Redox potential declined over time in both treatments though at a slower rate and with more variation between locations in the sterilized soils. Fourteen to 42 days passed

before Eh dropped below -100 mV in the sterilized soils but only between 14 to 28 days passed for all non-sterilized soils to reach this redox potential.

Soluble SO_4^{2-} concentrations immediately began to decline in all soil locations regardless of sterilization treatment in the first experiment. Unexpectedly, there was not a significant difference between the non-sterilized H and PTRS soils for the first 28 days after flooding. However, from days 28-42 after flooding, there was a significant interaction between location and sterilization treatment. Again, sterilized soils all contained greater concentrations of soluble SO_4^{2-} than non-sterilized soil from each location. The magnitudes of the differences between the sterilized and non-sterilized soil treatments for each individual soil location were significantly different. In PTRS soil, sterilized soil contained 6.5 times more soluble SO_4^{2-} than the non-sterilized, and sterilized H soil contained 5 times more soluble SO_4^{2-} than the non-sterilized H soil. However, sterilized HR-W and HR-E soils only contained 2.5 and 3.5 times more than their non-sterilized counterparts, respectively. As time progressed, differences between soluble SO_4^{2-} concentrations in the sterilized and non-sterilized soils reduced but were still 1.5-3 times different by day 77 depending on location. These differences were likely due to microbial population density and diversity differences between soil locations as well as between the sterilization treatments. Other influential factors which may cause such different soluble SO_4^{2-} concentrations were total initial amounts of SO_4^{2-}, Fe, and OM in the soil, Eh, and soil pH.

The goal of the second experiment was to evaluate the rate and degree of SO_4^{2-} and Eh reduction after flooding during rice growth. Soil from the same locations as the first experiment were used. In the presence of rice, soluble SO_4^{2-} and Eh reacted differently. Instead of soluble SO_4^{2-} immediately declining, SO_4^{2-} concentrations remained fairly steady in each location for the first week after flooding before beginning to decline. From the data that was able to be

interpreted from, decline in Eh also delayed in this study compared to the first study. These differences from the first study were attributed to the diffusion of oxygen from the rice roots into the rhizosphere. Though symptoms of H_2S toxicity did not appear in any of the plants, we were able to determine from information from both studies that H_2S toxicity is a multifaceted nutritional disorder. Further examination of soil chemistry, soil physical characteristics, biotic and abiotic influences are necessary to understand the causes of H_2S toxicity.

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Table 2.1 Selected soil chemical and physical properties from locations used in greenhouse experiments

Location ID	H_2S Occurrence	County	Soil Series	Soil Classification	Soil Texture†			pH‡	LOI§	TN¶	TC¶	P#	K#	S#
					Sand	Silt	Clay							
					-------g/kg--------				----------g/kg----------			----mg kg^{-1}----		
HR-W	Always	Cross	Henry Silt Loam	Coarse-silty, mixed, active, thermic Typic Fragiaqualfs	6	79	15	8.1	2.69	0.1261	1.3694	24	71	14
HR-E	Sometimes	Cross	Henry Silt Loam	Coarse-silty, mixed, active, thermic Typic Fragiaqualfs	12	74	14	8.1	2.03	0.0661	0.9426	19	46	10
H	Always	Woodruff	Hillemann Silt Loam	Fine-silty, mixed, active, thermic Albic Glossic Natraqualfs	14	73	13	7.9	2.01	0.0677	0.9425	16	53	16
PTRS	Never	St. Francis	Calloway Silt Loam	Fine-silty, mixed, active, thermic Aquic Fraglossudalfs	35	48	17	7.6	2.08	0.0806	0.9128	70	114	9

† Soil texture determined by hydrometer method (Gavlak et al., 2003).
‡ pH determined by 1:2 soil/water ratio (Thomas, 1996).
§ LOI determined by muffle furnace 360°C (Combs et al., 1998).
¶ TN and TC determined by combustion (Bremner, 1996; Nelson and Sommers, 1996).
# P, K, and S determined by Mehlich 3 extractable (1:10 ratio) analysis by Spectro Arcos ICP (Helmke and Sparks, 1996).

Table 2.2. Mean concentrations of soluble SO_4^{2-}, soluble Fe, and Eh on day 21 for each soil location.

Location	H_2S Occurrence	SO_4^{2-}†	Fe	Eh
		------mg L^{-1}------		mV
H	Always	21.11 a	6.8	-131
PTRS	Never	19.4 a	44.77	60.7
HR-W	Always	16.24 ab	8.69	-286
HR-E	Sometimes	13.31 b	4.71	-59

†-Locations not followed by the same letter are significantly different.

Table 2.3. Mean soluble Fe concentrations over time for each treatment.

Treatment	Time in days							
	1	7	14	21	28	35	42	49
H Sterilized	0.05	1.34	4.81	7.30	8.2	11.55	13.61	12.39
H Unsterilized	0.03	1.86	6.16	7.57	8.53	10.71	11.57	11.01
HR-W Sterilized	0.05	2.12	6.17	9.33	10.14	12.61	14.14	13.98
HR-W Unsterilized	0.03	1.48	4.63	6.69	7.88	10.77	12.21	13.43
HR-E Sterilized	0.04	0.38	2.87	4.95	6.89	6.49	9.63	10.74
HR-E Unsterilized	0.03	0.54	3.46	4.82	5.72	7.85	8.70	9.83
PTRS Sterilized	0.12	7.65	31.92	50.72	56.36	69.24	68.49	56.90
PTRS Unsterilized	0.02	0.68	3.31	3.91	3.53	4.51	4.98	4.99

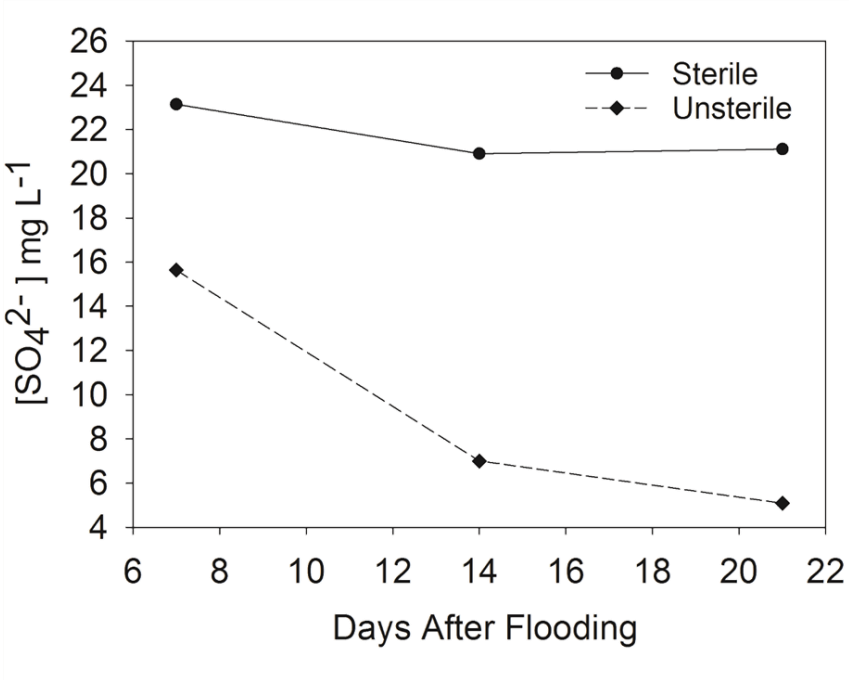

Figure 2.1. Sulfate concentration over time for sterilized and non-sterilized soil treatments.

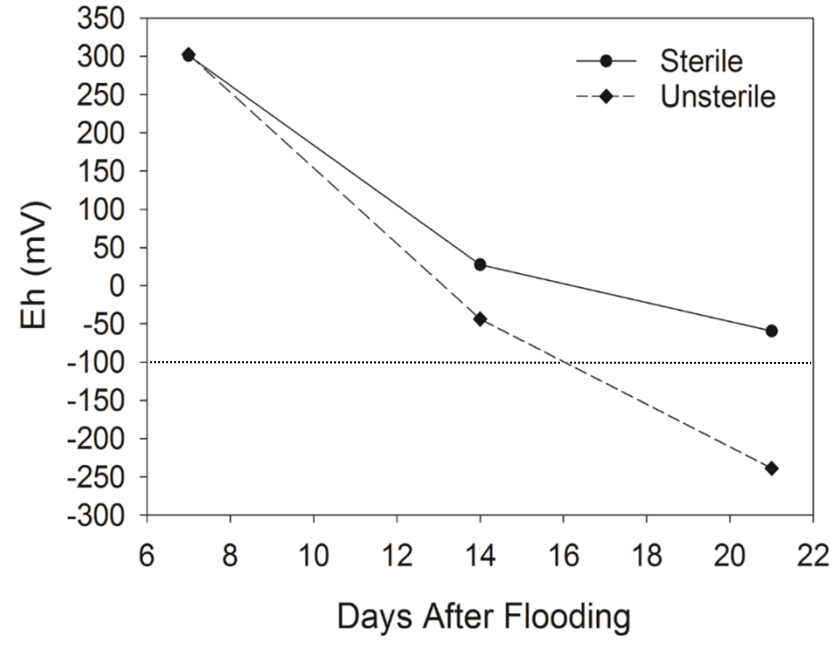

Figure 2.2. Redox potential over time for sterilized and non-sterilized soil treatments.

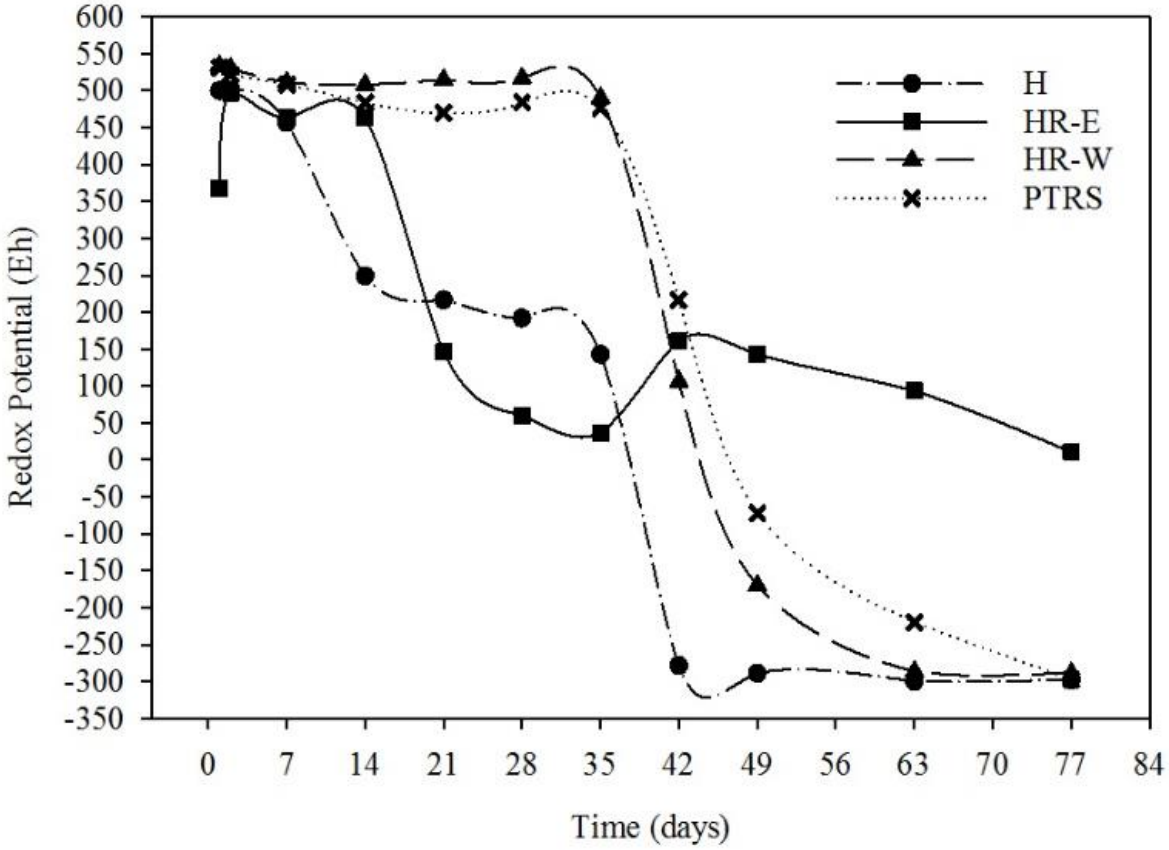

Figure 2.3. Mean redox potential of each soil location over time after flooding during rice growth.

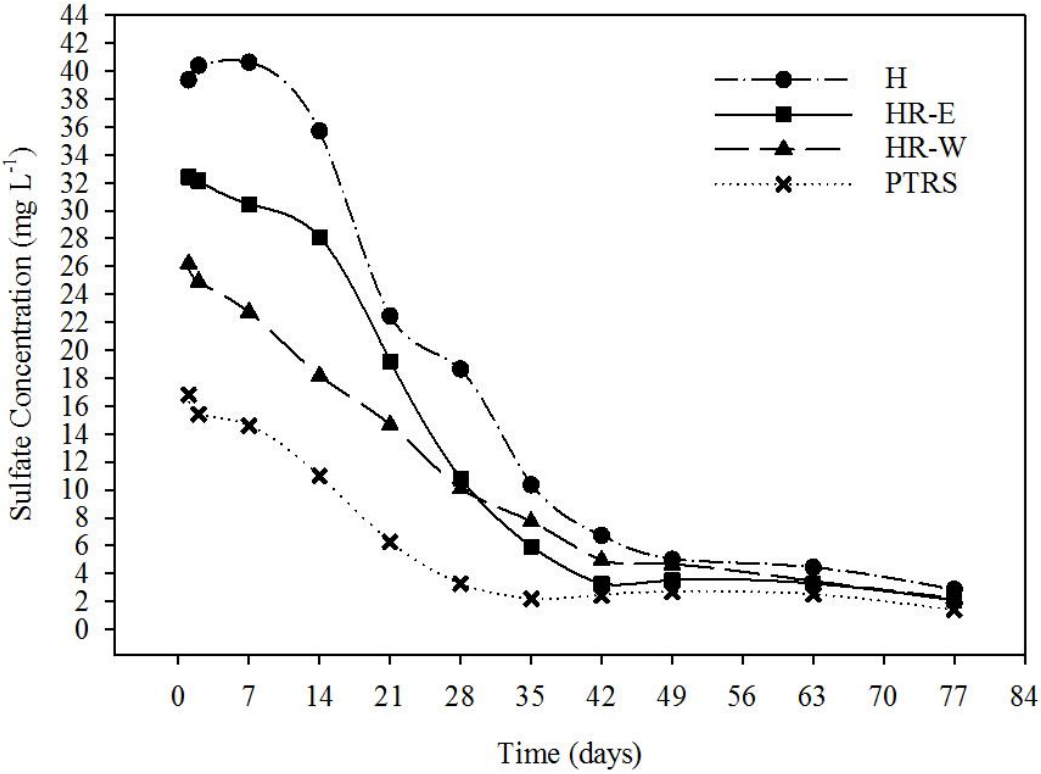

Figure 2.4. Mean sulfate concentration for each soil location over time after flooding during rice growth.

CHAPTER 3

Effect of Ammonium Sulfate Fertilizer Additions on Hydrogen Sulfide Toxicity

ABSTRACT

Hydrogen sulfide (H_2S) toxicity is an increasingly problematic physiological disorder reported in certain Arkansas rice (*Oryza sativa* L.) fields. Though the exact causes of this disorder are unknown, one contributing factor may be the use of ammonium sulfate $(NH_4)_2SO_4$ fertilizer. A greenhouse study was designed to investigate the physical and chemical differences in four soils in Arkansas where this disorder regularly occurs (H and HR-Y) and never has been reported (HR-N and PTRS) and to investigate the effects of different rates of $(NH_4)_2SO_4$ fertilizer on those soils. Mehlich 3 extractable nutrients were similar between soils prone to H_2S toxicity and those that are not. With Zn deficiencies appearing in PTRS and HR-N, several factors likely influenced this deficiency including P fertilization and flooding effects. Significant differences in soluble SO_4^{2-} concentration between soil locations occurred for the first 21 days after flooding. HR-N contained significantly more SO_4^{2-} and levels did not change over time. However, the other locations contained less SO_4^{2-} and concentrations increased, particularly during the third week. Differences between fertilizer treatments were also significant from days 2-21 after flooding. The highest treatment of $(NH_4)_2SO_4$ contained the highest concentration of SO_4^{2-} in solution, followed by the low treatment of $(NH_4)_2SO_4$, with the 0 kg control having the lowest concentration. Concentrations of SO_4^{2-} increased in each soil over time. The increase in SO_4^{2-} was likely caused by decomposition of soil organic matter (SOM). Lack of evidence of H_2S toxicity in the root examination and in the above ground biomass nutrient content, along with the soil solution results, indicate that H_2S toxicity is influenced by more than the SO_4^{2-} content in the soil. A combination of other factors such as Eh, microorganisms, SOM content, and environmental conditions are likely major contributors to the occurrence of H_2S toxicity.

INTRODUCTION

Approximately half of the rice produced in the United States is grown in Arkansas (Hardke, 2015). As the leader of rice production in the country, rice significantly contributes to Arkansas's economy, with sales reaching nearly one billion dollars in 2016 (USDA-NASS, 2017) and providing over 25,000 jobs (Richardson and Outlaw, 2010). With an average yield of 8,221 kg ha^{-1} in 2015, Arkansas producers achieve the second highest yield in the United States (Hardke, 2016). As a vital part of Arkansas's economy, researchers must stay proactive in developing solutions for current and potential production problems.

One challenge that is becoming more problematic in Arkansas is hydrogen sulfide (H_2S) toxicity. Also referred to as autumn decline or akiochi (Japanese for 'autumn decline'), H_2S toxicity is thought to be caused by excessive reduction of SO_4^{2-} to H_2S in the soil. Under anaerobic conditions, SO_4^{2-} reduces to H_2S, a toxic gas, but then typically reacts with reducible Fe in the soil and precipitates out as insoluble FeS, preventing the buildup and toxicity of H_2S (Yoshida, 1981). This phenomena is difficult to classify and has been termed a "physiological disease" (Baba et al., 1964) and was later classified as a "nutritional disorder" which is a more accurate term (Yoshida, 1981). Though often classified as a disease, H_2S toxicity is not caused by a pathogen and therefore is not truly a disease. However, H_2S toxicity does weaken the plant by interfering with water and nutrient uptake, making plants more susceptible to invasion of opportunistic diseases such as brown spot (historically referred to as *Helminthosporium* leaf spot), rice blast, caused by *Pyricularia oryzae*, and crown rot, caused by the fungus *Gaeumannomyces graminis* var. *graminis* (Tanaka and Yoshida, 1966; Wamishe, 2013; Wamishe et al., 2013).

Once symptoms of H_2S toxicity are identified in the field, only one rescue technique is currently available: draining and drying the field. By removing the flood from the field, the surface soil is able to re-oxygenate which stops the reduction reactions occurring in the anaerobic soils (Hardke and Wamishe, 2015). Once oxygen reenters the root zone, new roots form and then the field can be flooded again (Wamishe, 2015). If this rescue technique is performed early enough in the growth cycle of the rice, the majority of the rice is likely to recover, though yield loss may still occur (Hardke, 2015). However, rice is not a drought tolerant plant, and aerobic conditions increase the potential for other disease to take over, such as blast, and may influence yield (Wamishe et al., 2013). In Arkansas, fields should be drained at the time recommended to drain for straighthead using the DD-50 program (Wamishe et al., 2013).

Though the exact causes of H_2S toxicity are unknown, there are two major management practices that likely impact the occurrence and severity of H_2S toxicity: irrigation water source and fertilizer choice. In Arkansas, 76.4% of the rice acreage is irrigated using ground water (Hardke, 2016). With SO_4^{2-} concentrations ranging from 2 to over 100 mg L^{-1} in the ground water, this can be a major source of SO_4^{2-} regularly added to the field throughout the season (Norman et al., 2013). In soils already high in SO_4^{2-} or SOM, additional SO_4^{2-} from irrigation water could likely increase the chance of H_2S toxicity occurring. Irrigation water is a suspected contributor to the occurrence of H_2S toxicity in Arkansas (Hardke and Wamishe, 2015).

In addition to irrigation water potentially influencing this disorder, fertilizer choice can also increase the chance of H_2S toxicity occurring, particularly $(NH_4)_2SO_4$ fertilizer. Though $(NH_4)_2SO_4$ is a more expensive fertilizer, this fertilizer is recommended as a pre-flood N source particularly on sandy soils that tend to be deficient in SO_4^{2-} (Norman et al., 2013). Based on data collected by the Soil Testing and Research Laboratory in Marianna, AR, 31%, 35%, and 52% of

all soils tested in Cross County, Woodruff County and St. Francis County respectively were below optimal in SO_4^{2-} in 2014 (DeLong et al., 2016). Ammonium sulfate is a practical choice for fertilization to increase both S and N, however, application to soils sufficient in SO_4^{2-} may increase the likelihood of H_2S toxicity occurring.

In Arkansas, N, P, and K are typically required to maximize rice yield potential (Hardke et al., 2017). Recommended fertilizer application rates depend upon soil test report information. Proper N fertilization and management are vital for excellent rice grain yield (Norman et al., 2013). The two typical N sources for rice used in Arkansas are urea and $(NH_4)_2SO_4$ (Hardke et al., 2017). Due to the higher cost of $(NH_4)_2SO_4$, this fertilizer is only recommended when there is potential for S deficiencies (Norman et al., 2013). Since sandy soils are typically low in SOM and SO_4^{2-} is easily lost from the soil through leaching, S fertilizer is usually only required on sandy soils (Norman et al., 2013).

The objective of this study is to evaluate the effects of $(NH_4)_2SO_4$ fertilizer on H_2S toxicity. Three rates of $(NH_4)_2SO_4$ will be utilized and soluble SO_4^{2-} and Eh monitored throughout the experiment. The goal for the highest $(NH_4)_2SO_4$ rate is to induce H_2S toxicity, particularly in the soils prone to H_2S toxicity.

MATERIALS AND METHODS

Soil was collected from three locations in Arkansas during 2016. Surface soil was collected from the Pine Tree Research Station (PTRS) in Pine Tree, AR where H_2S toxicity has never been reported, from a producer field in Hunter, AR (H) where H_2S toxicity occurs every time when planted to rice, and two samples were taken from two locations in a producer's field in Hickory Ridge, AR where H_2S toxicity always occurs in one location when planted to rice (HR-Y) and another location within the same field where H_2S has not occurred when planted to

rice (HR-N). Surface soil was collected from each location and brought back to Fayetteville, AR for the greenhouse experiment. A soil test was conducted to assess pH (1:2 v:v soil:water ratio) (Thomas, 1996), soil texture (Gavlak et al., 2003), total nitrogen (TN) (Bremner, 1996), total carbon (TC) (Nelson and Sommers, 1996), soil OM via weight loss on ignition (LOI) (Schulte and Hopkins, 1996), and Mehlich 3 extractable nutrients, P, K, and S (Helmke and Sparks, 1996). Detailed soil and agronomic information is listed in Table 3.1.

Soil was passed through a one cm sieve to remove clods and large pieces of crop residue. A volume of 3.79 L of each soil was placed into a 7.57 L bucket. Each location had nine buckets with 3.79 L of soil per bucket. Three $(NH_4)_2SO_4$ fertilizer treatments were applied for this experiment: 0 kg ha^{-1}, 115 kg ha^{-1}, and 230 kg ha^{-1}. Buckets were arranged randomly on two benches in the greenhouse with 18 buckets on each bench. Fertilizer was incorporated in the top few centimeters of the soil at rates equivalent to 89.74 kg P_2O_5 ha^{-1} and 134.62 kg K_2O ha^{-1}. The P and K fertilizer rates were doubled for this experiment to avoid deficiency symptoms that occurred in prior trials with these soils. Deionized water was used in this experiment to eliminate excess SO_4^{2-} being added through the water source. Cultivar 'CL151' seeds were germinated in a damp paper towel to ensure viable seeds were used. Soil was wetted with deionized water and 24h later, 10 rice seedlings were transplanted into each bucket. Deionized water was misted heavily in each bucket then the buckets were covered with plastic wrap to trap humidity and promote plant establishment and growth. Deionized water was misted in each bucket daily until seedlings were established. Buckets were hand weeded as needed. Six days later, each bucket was thinned to 5 uniform plants per bucket.

At the 1-2 leaf stage, $(NH_4)_2SO_4$ fertilizer treatments were surface applied to their respective buckets and the soil was allowed to dry before the next watering. Once plants reached

the 5 leaf stage, supplemental urea fertilizer was added to the soil at rates equivalent to 352 kg urea ha^{-1} for the 115 kg ammonium sulfate ha^{-1} treatment, 300 kg urea per ha^{-1} for the 230 kg ammonium sulfate ha^{-1}, and 692 kg urea ha^{-1} for the 0 kg ammonium sulfate ha^{-1} treatment to give each treatment the same total N application rate of 319 kg N ha^{-1}. Urea rates were double what is recommended for rice growth in Arkansas to account for the closed system in the greenhouse. One porous ceramic cup sampler (IRROMETER® Soil Solution Access Tube – Model SSAT, Riverside, CA) was placed in each bucket approximately 8 cm deep, and one platinum electrode redox sensor (Sensorex® electrochemical ORP sensor) was placed approximately 8 cm deep throughout one replication of the experiment for a total of 18 redox sensors (Patrick et al., 1993). Twenty four hours after the addition of urea, all buckets were flooded with deionized water to approximately 10 cm above the soil surface. This flood level was maintained for the duration of the experiment. Redox was continuously monitored by the electrodes and logged into a data logger, and soil solution was sampled 1, 2, 7, 14, 21, 28, 35, 42, 49, 63, 77, and 91 days after flooding, following the protocol of Gao et al. (2002).

Before extracting soil solution, a 60 cc syringe was used to extract and discard all fluid in the porous ceramic cup sampler. To extract soil solution samples, pressure was drawn to 60 centibars to create a vacuum in each porous ceramic cup sampler using a hand vacuum pump. The vacuum was maintained for 3h before the solution was collected. A clean 60 cc syringe was double rinsed with deionized water, and used to extract the solution which was then placed in a scintillation vial. All scintillation vials contained two drops of concentrated HCl (37%) to acidify the solution to prevent precipitation of nutrients and to reduce microbial activity. The syringe was double rinsed with deionized water between each sample. Samples were stored at room temperature until analyzed by inductively coupled argon plasma (ICAP) spectrophotometry. Soil

solution samples were analyzed for P, K, Ca, Mg, Na, S, Fe, Mn, Zn, Cu, and B. After the final sampling on day 91, root of all plants were washed and examined for any buildup of FeS. Above ground biomass was removed, dried, and analyzed for nutrient contents by ICAP spectrophotometry following a nitric acid digest.

On the first day of flooding, one sixth of a mosquito dunk was added to each bucket as a control for fungus gnats. This method was ineffective, so Fury® Insecticide was applied to all plants two days after flooding. Four days after the application of Fury®, all plants grown in the PTRS and HR-N soils were notably pale green to yellow. Plants were monitored but new leaves remained yellow and leaf tips became necrotic and growth was stunted. Thirteen days after flooding, a foliar treatment of zinc sulfate ($ZnSO_4$) was applied to all plants. After a few days, leaf tissue began to darken again. One more application of zinc sulfate was applied as a foliar treatment to only PTRS and HR-N plants one week later. The application of $ZnSO_4$ corrected nutrient deficiencies in the plants, but unfortunately a few plants were lost, giving a smaller plant stand in a few of the buckets.

Statistical Analysis

This experiment contained four soil locations and three treatments: 0, 115 and 230 kg $(NH_4)_2SO_4$ ha^{-1}. Each treatment was replicated three times totaling 36 individual buckets. Buckets were arranged in a randomized complete block design with three blocks. This experiment was designed so that one replication appeared within each block. Redox sensors were placed at random within one replication. Soil solution samples were collected from each individual bucket on the designated sampling days and redox potential was monitored continuously. An analysis of variance (ANOVA) was conducted for each day of soil solution data using JMP® Pro 12 (SAS Institute, Inc., Cary, NC). Comparisons were made at the $p \leq 0.05$

significance level to evaluate if the different rates of $(NH_4)_2SO_4$ impacted SO_4^{2-} reduction and if SO_4^{2-} reduction differed between soils from different locations. Student's T Test was used to separate significant means.

Our statistical hypotheses were that SO_4^{2-} levels would be elevated in treatments with added ammonium sulfate and that SO_4^{2-} levels would decrease more rapidly in H and HR-Y soils than the HR-N and PTRS soils. Soil solution data were analyzed by an ANOVA, and redox data were used to support the findings of the soil solution ANOVA.

RESULTS AND DISCUSSION

Physical and Chemical Soil Characteristics

After soils were collected for this experiment, subsamples from each location were analyzed for physical and chemical properties. Differences between locations prone to H_2S toxicity and those not prone to the disorder were not major. Oddly, H and HR-N were similar to each other overall. However, a few differences may be important to note.

Mehlich 3 extractable S and Fe both varied between locations. Soluble S in HR-N exceeded all other locations by 70-80 mg SO_4^{2-} kg^{-1} soil, and Fe concentrations were highest in H and HR-N. Again, H and HR-N were similar in TC, TN, and LOI. Though soil test report information can be useful, this data does not appear to point to any major differences between locations that could be the cause of H_2S toxicity.

One major nutrient that proved problematic during this experiment was Zn. Deficiency symptoms occurred eight days after flooding though not severe. Thirteen days after flooding, Zn deficiency symptoms had worsened with pale green and yellow leaves, necrotic leaf tips, very little internode elongation, minimal tillering, and even death of a few plants. Zinc deficiencies are fairly common in lowland rice soils after flooding though sometimes plants can recover on

their own after 6-8 weeks (Yoshida, 1981). In Arkansas, Zn fertilizer is only recommended on silt and sandy silt loam textures (Slaton et al., 2002). All soils used in this experiment fall into that category (Table 3.1). Deficiency may have occurred for several reasons. Though PTRS contained the highest amount of Zn (14.82 mg SO_4^{2-} kg^{-1} soil) symptoms occurred most severely in PTRS and HR-N soils regardless of $(NH_4)_2SO_4$ treatment. PTRS contained Zn levels well above the critical level, however HR-N was below the critical level of 3.5 mg Zn kg^{-1} soil (Slaton et al., 2002). Keeping in mind that the Mehlich 3 data shows the nutrient status of the soil before flooding, once flooded, chemical changes take place (Slaton et al., 2006). After flooding, pH rises in acidic soils and decreases in alkaline soils to settle in the range of 6.8-7.2 (Ponnamperuma, 1972). Solubility of Zn decreases by two orders of magnitude for every one unit of increase in pH (Dobermann and Fairhurst, 2000). With the soil pH ranging from 7.26-8.27, pH likely decreased up to one order of magnitude. This change in pH may have influenced availability of Zn, though it is not likely. However, flooding increases availability of Fe, Ca, Mg, Cu, Mn and P and therefore suppresses Zn uptake (Dobermann and Fairhurst, 2000). Both HR-N and PTRS contained higher concentrations of Ca, Mg, and Mn. Decline in Eh is another chemical factor that changes after flooding. Under low Eh conditions, sulfides of Zn can form and cause Zn deficiencies (Dobermann and Fairhurst, 2000). Additionally, after high rates of P fertilizer are applied to the soil, Zn-phosphate commonly forms and reduces Zn availability (Dobermann and Fairhurst, 2000). Since the same P rate was applied to each of the soil locations, all locations showed P levels above the critical level, and P becomes more plant available after flooding (Slaton et al., 2006), Zn likely reacted with P in all soil location to form Zn-phosphate to some extent. With the greatest pH change likely occurring in HR-N and PTRS, higher

concentration so nutrients that can suppress Zn uptake, and the addition of high levels of P fertilizer added to the soils, the severe Zn deficiencies in these two locations can be rationalized.

Along with differences in nutrient concentrations, texture differed between locations. H contained the highest percentage of sand with all other soil locations containing less than 10% sand. Since surface soil was collected for this experiment, accuracy of the subsample tested for nutrients, SOM, and texture is likely not high since soils vary greatly within fields (Norman et al., 2013). In order to get a true index of nutrients and texture of a field, soil cores should be taken across the field in a zigzag pattern at a depth of 10 cm (Norman et al., 2013). However, since surface soil was collected and utilized for this experiment, the soil collection method was appropriate, but proper sampling could have been used to support findings.

Flooding Effect on Sulfate Concentration and Redox Potential

Although new redox sensors were used for this experiment, data from eight sensors were lost due to a malfunction with the data logger. One sensor from the working data logger appeared to malfunction as Eh increased over 1000 mV over time. Data from that sensor was discarded, but data from the other seven sensors will be used to support soil solution data.

Days 1-21

Despite the different fertilizer treatments, significant differences between soil locations were present for the first three weeks after flooding (Table 3.2). With higher concentrations of SO_4^{2-} in solution, approximately 51-57 mg SO_4^{2-} L^{-1}, HR-N had significantly higher concentrations of SO_4^{2-} compared to all other soil locations, likely due to the high concentration of SO_4^{2-} already present in the soil (Table 3.1). For the duration of the experiment, no statistical difference between PTRS and HR-Y were found. These results were unexpected since, in the field, H_2S toxicity has never been a reported problem on HR-N soil but has been reported on HR-

Y soils. Based on field reports, we would expect SO_4^{2-} concentrations to be similar between HR-N and PTRS, not HR-Y and PTRS. Though our soil tests are representative of the samples used in this study, reports from whole field sampling would be interesting to compare to the samples used. If the samples used in this experiment are indeed representative of the whole field, results from this study indicate that H_2S toxicity is not driven by SO_4^{2-} concentrations alone. Other factors may contribute to the occurrence of H_2S toxicity such as microbial populations and concentrations of substrates in the soil. Microorganisms are vital in the chemical changes that take place in soil. The reduction of compounds, such as SO_4^{2-}, and changes in Eh would occur at a much slower rate without microbes (Ponnamperuma, 1972; Strawn et al., 2015). The different species of microorganisms also affect what reactions take place in the soil. With certain species of microbes responsible for the majority of the SO_4^{2-} reduction, *Desulfovibrio* and *Desulfotomacuclum* (Ponnamperuma, 1984; Reddy et al., 1986), these species are likely present in soils prone to H_2S toxicity. Of course, concentrations of substrates in the soil greatly affect chemical changes as well. With high concentrations of SO_4^{2-} in the soil and low concentrations of Fe^{2+} to react with H_2S as the SO_4^{2-} reduces, H_2S toxicity could be an expected result.

Another possible contributing factor is additional SO_4^{2-} being added throughout the growing season by well water (Hardke and Wamishe, 2015). Though this factor was controlled in this greenhouse experiment by using deionized water, well water may contribute to the problem in the field, causing different changes in soil solution in the field compared to the controlled greenhouse environment.

Unlike the first greenhouse study and results found in the literature, SO_4^{2-} did not rapidly decrease after flooding (Ogata and Bower, 1965; Connell and Patrick, 1969; Ponnamperuma, 1981; Gao et al., 2004). Approximately two weeks passed after flooding with SO_4^{2-} levels

remaining fairly constant in all soil locations. Though Eh began to drop immediately after flooding in all recovered data, all soils were above -100 mV except HR-N with 0 kg $(NH_4)_2SO_4$ applied. Since Eh had not reached the SO_4^{2-} reducing potential in the majority of the soils by two weeks after flooding, this could explain why SO_4^{2-} concentrations remained steady. However, after two weeks of flooding, SO_4^{2-} concentrations increased in all locations except HR-N rather than decreasing (Figure 3.1). Though SO_4^{2-} may have been added to the system in low quantities due to the foliar application of $ZnSO_4^{2-}$ to correct the Zn deficiency, this increase in soluble SO_4^{2-} was likely due to SOM mineralization rather than an outside source. Since mineralization occurs most rapidly in a soil pH of 6.5-7, this increase in SO_4^{2-} was likely due to microbial activity (Ponnamperuma, 1984).

Days 2-21

Regardless of soil location, soluble SO_4^{2-} concentrations differed significantly between fertilizer treatments for the first three weeks after flooding (Table 3.2). As expected, the control treatment with 0 kg $(NH_4)_2SO_4$ ha^{-1} had the least SO_4^{2-} in solution, the medium treatment of 115 kg $(NH_4)_2SO_4$ ha^{-1} contained more, and the highest treatment of 230 kg $(NH_4)_2SO_4$ ha^{-1} contained the highest concentration of soluble SO_4^{2-} (Figure 3.2). With the addition of sulfate to the soil via $(NH_4)_2SO_4$, soluble SO_4^{2-} in the soil solution is expected to be elevated according to the addition of $(NH_4)_2SO_4$. Though there was a numerical increase in soluble SO_4^{2-} when 115 kg $(NH_4)_2SO_4$ ha^{-1} was added to the soil, this was not enough to raise the soluble SO_4^{2-} concentrations in solution significantly compared to the 0 kg $(NH_4)_2SO_4$ ha^{-1} rate.

For the first three weeks after flooding, SO_4^{2-} concentrations in the 0 kg $(NH_4)_2SO_4$ ha^{-1} and 115 kg $(NH_4)_2SO_4$ ha^{-1} treatment remained fairly steady, beginning to increase around the third week after flooding. However, SO_4^{2-} concentrations in the 230 kg $(NH_4)_2SO_4$ ha^{-1} treatment

gradually increased during that time. Mineralization of organic matter is promoted when the pH of the soil solution is between 6.5 and 7 (Ponnamperuma, 1984). Microbial activity increasing due to the ideal pH when flooded and from the warm environment are likely causing an increase in mineralization of organic S, causing the increase in soluble SO_4^{2-}. Based on the recovered Eh data, soils with 230 kg $(NH_4)_2SO_4$ ha^{-1} did not go below -100 mV for the duration of the experiment, but the 0 kg $(NH_4)_2SO_4$ ha^{-1} and 115 kg $(NH_4)_2SO_4$ ha^{-1} treatments achieved -100 mV around 19 days after flooding.

After the termination of the experiment, plants were removed from buckets to wash and examine the roots. As expected due to the above ground appearance, root blackening signature of H_2S toxicity did not appear in any of the rice roots. A few black streaks were noted in the 230 kg $(NH_4)_2SO_4$ L^{-1} treatment in the H soils. According to the suggested protocol to identify if the blackening is due to H_2S toxicity, these roots were left in the sun for an hour, then examined again for signs of blackening (Hardke and Wamishe, 2015).

Though there was sufficient $(NH_4)_2SO_4$ applied in the treated buckets to intensify SO_4^{2-} reduction and induce H_2S toxicity symptoms, there are several reasons that may be responsible for the lack of symptomology. First, rice roots are known to oxidize the rhizosphere which prevents toxic substances produced by the reduced conditions from affecting the roots (Ando et al., 1983). The large volume of roots confined in a relatively small bucket likely produced enough O_2 to effectively oxidize any H_2S that was produced, therefore avoiding the buildup and toxicity of H_2S. Even though a straighthead susceptible cultivar was used to improve chances of H_2S toxicity occurring (Joshi et al., 1975), the sheer volume of roots in each bucket appeared to produce ample O_2 to prevent H_2S toxicity from occurring. Another possible explanation for the lack of symptoms in the roots is the high temperature in the greenhouse during this experiment

which was near 40°C during the day. According to Vamos (1964), H_2S toxicity occurred in rice fields under cool, cloudy conditions with low atmospheric pressure, but H_2S toxicity did not occur under hot conditions with higher atmospheric pressure. In a study to investigate if temperature influenced the occurrence of H_2S toxicity, Vamos (1964) found that H_2S toxicity occurred when temperatures were dropped from 30 to 6°C, but no symptoms appeared when 30°C temperatures were maintained. Severe symptoms of H_2S toxicity near cold well water inlets in Arkansas have also made cold temperature a suspect in causing this disorder (Wamishe, 2015). However, field reports in Arkansas show that H_2S toxicity has been most severe in hot, dry years compared to more mild weather years (Wamishe, 2015).

Even though symptoms did not appear above or below ground, all above ground biomass was analyzed for nutrient content (Table 3.3). With Zn concentrations in the leaf tissue all above the critical content, we can conclude that our foliar application of $ZnSO_4$ was effective in correcting the deficiency (Tanaka and Yoshida, 1966). The major effect of H_2S toxicity is the root damage that prevents nutrient and water uptake (Tanaka and Yoshida, 1966). With this in mind, nutrient content would be expected to be below optimal in plants affected by H_2S toxicity. However, P was above optimal in all plants as was K except in HR-N soils with all fertilizer treatments and PTRS with the 0 kg $(NH_4)_2SO_4$ ha^{-1} treatment where K was below optimal. For S content, above ground biomass for all treatments was above the critical deficiency level (Dobermann and Fairhurst, 2000). Interestingly, Fe content in all plants except those with the 0 kg $(NH_4)_2SO_4$ ha^{-1} and 115 $(NH_4)_2SO_4$ ha^{-1} treatment grown in HR-Y soils had concentrations of Fe above the toxicity level (Dobermann and Fairhurst, 2000). Typically in plants affected by H_2S toxicity, Fe concentrations in the leaf tissue are extremely high (Tanaka et al., 1968). Despite the high Fe contents, symptoms of H_2S toxicity were not present. Based on the leaf tissue report with

the majority of nutrients above the critical content, we can conclude that the roots were not damaged by H_2S and were able to take up sufficient nutrients from the soil.

SUMMARY

The objective of this study was to evaluate the effect of $(NH_4)_2SO_4$ fertilizer additions on soils prone to H_2S toxicity compared to soils where this disorder has never been reported. Our hypothesis was that symptoms of H_2S toxicity would occur in the treatments with the highest rate of $(NH_4)_2SO_4$ fertilizer, 230 kg ha^{-1}, in the soils most prone to H_2S toxicity, H and HR-Y. A straighthead susceptible cultivar of rice (CL 151) was used in this experiment since these cultivars also tend to be susceptible to H_2S toxicity. In order to evaluate the effects of $(NH_4)_2SO_4$ and the different soil locations, soluble SO_4^{2-} and Eh were monitored. At the end of the experiment, all roots were washed and evaluated for the blackening symptom. Tissue analysis was digested in concentrated HNO_3 and 30% for total S (Jones and Case, 1990).

An initial soil test was performed on the soils collected and used for this experiment. Unexpectedly, the two soils prone to H_2S toxicity, H and HR-N, were not similar to each other. Instead, H and HR-Y were very similar in their physical and chemical attributes as were PTRS and HR-N.

After these soils were flooded, one major problem occurred; severe Zn deficiencies appeared in the rice grown primarily in PTRS and HR-N soils. Since flooding increases the availability of certain nutrients, particularly P, increased uptake of P likely suppressed the uptake of any available Zn causing plant deficiency (Dobermann and Fairhurst, 2000). This is most likely the major cause due to the over application of P in this experiment to avoid P deficiencies.

Results from the soil solution collected throughout the experiment showed a significant difference between soil locations for the first three weeks after flooding (*p* values ranged from

0.0183 to < 0.0001). H and HR-N were not statistically different from each other but were different from HR-Y and PTRS. Since the results from HR-N and HR-Y were opposite of what was expected, this likely means that H_2S toxicity is driven by a multitude of other factors and conditions such as microbial populations, Eh, OM content, as well as SO_4^{2-} content.

Rather than SO_4^{2-} concentrations immediately declining after flooding, as seen in the literature (Ogata and Bower, 1965; Connell and Patrick, 1969; Ponnamperuma, 1981; Gao et al., 2004), SO_4^{2-} concentrations remained fairly constant in all soil locations for the first two weeks after flooding, then began to increase. While Eh did decline immediately in all locations, only HR-N dropped below the SO_4^{2-} reducing potential by the end of three weeks.

Along with the differences between locations, fertilizer treatments were significantly different for the first three weeks after flooding (*p* values range from 0.0275 to < 0.0001). As expected, SO_4^{2-} concentrations were highest in soils with the highest rate of $(NH_4)_2SO_4$ applied, and the lowest SO_4^{2-} concentration was in the soils with no $(NH_4)_2SO_4$ added, while soils with the middle rate of $(NH_4)_2SO_4$ had concentrations in between. For the 0 kg $(NH_4)_2SO_4$ ha^{-1} and 115 kg $(NH_4)_2SO_4$ ha^{-1} rates, concentrations of SO_4^{2-} in solution remained steady for the first two weeks after flooding then began to increase. In the 230 kg $(NH_4)_2SO_4$ ha^{-1}, SO_4^{2-} concentrations slowly increased for the first three weeks after flooding. The increase in soluble SO_4^{2-} concentrations after two weeks was, again, likely due to mineralization of SOM (Ponnamperuma, 1984), but is unclear.

After terminating the experiment, washing all rice roots revealed minimal root blackening, which is the most predominant symptom of H_2S toxicity. Due to the small area that the roots were able to inhabit, the oxidation via the aerenchyma likely prevented H_2S toxicity (Ando et al., 1983).

Nutrient content of the above ground biomass collected at the end of the experiment also indicated that H_2S toxicity did not occur. With the majority of nutrients at or above the optimal levels, roots did not appear to be damaged by H_2S (Tanaka and Yoshida, 1966).

Though symptoms of H_2S toxicity were not present in this experiment, we can conclude from the different soils used and $(NH_4)_2SO_4$ treatments used that H_2S toxicity is driven by more than soil SO_4^{2-} content. This disorder is multifaceted, and further study should be aimed at understanding how SO_4^{2-} content, Eh, microbial populations, OM content, and water temperature influence the occurrence of H2S toxicity.

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Table 3.1 Selected soil chemical and physical properties from locations used in greenhouse experiment.

Location ID	H_2S Toxicity Frequency	County	Soil Series	Soil Classification	Soil Texture†			pH‡	LOI§	TN¶	TC¶	P#	K#	S#	Zn#
					Sand	Silt	Clay								
					-------g kg^{-1}-------				----------g kg^{-1}----------			-------mg kg^{-1}-------			
HR-Y	Always	Cross	Henry Silt Loam	Coarse-silty, mixed, active, thermic Typic Fragiaqualfs	7	84	9	7.9	1.54	0.0547	0.6703	42	91	8	7
HR-N	Never	Cross	Henry Silt Loam	Coarse-silty, mixed, active, thermic Typic Fragiaqualfs	10	76	14	7.9	2.09	0.0805	1.0254	54	116	92	3
H	Always	Woodruff	Hillemann Silt Loam	Albic Glossic Natraqualfs	33	55	12	7.3	2.50	0.0819	1.1730	30	147	21	5
PTRS	Never	St. Francis	Calloway Silt Loam	Aquic Fraglossudalfs	6	78	16	8.3	1.85	0.0455	0.6794	28	93	9	15

† Soil texture determined by hydrometer method (Gavlak et al., 2003).
‡ pH determined by 1:2 soil/water ratio (Thomas, 1996).
§ LOI determined by muffle furnace 360°C (Combs et al., 1998).
¶ TN and TC determined by combustion (Bremner, 1996; Nelson and Sommers, 1996).
# P, K, S, and Zn determined by Mehlich 3 extractable (1:10 ratio) analysis by Spectro Arcos ICP (Helmke and Sparks, 1996).

Table 3.2. Analysis of variance with significant level α=0.05

Effects	DF	1	2	7	14	21	28	35	42	49	63	77	91
		----------Days----------											
Location	3	**0.0051**	**<0.0001**	**<0.0001**	**<0.0001**	**0.0183**	0.4468	0.4012	0.2513	0.2513	0.6250	0.8862	0.9994
Fertilizer Treatment	2	0.5488	**0.0275**	**0.0140**	**<0.0001**	**<0.0001**	0.1461	0.1166	0.9923	0.9923	0.9997	0.9999	0.9977
Location x Fertilizer Treatment	6	0.3641	0.6209	0.4796	0.7415	0.6645	0.5661	0.2321	0.6386	0.6386	**0.0016**	**0.0197**	0.2322

Table 3.3. Mean plant tissue content.

Location	Fertilizer Rate	P	K	Ca	Mg	S	Na	Fe	Mn	Zn	Cu	B
	kg ha^{-1}	----------%----------					----------mg kg^{-1}----------					
H	0	0.20	0.98	0.47	0.23	0.09	461	1200	903	32.6	1.15	3.87
H	115	0.19	1.06	0.49	0.20	0.09	293	878	952	35.8	1.03	4.20
H	230	0.20	1.03	0.49	0.23	0.10	441	556	996	37.5	1.14	4.25
HR-Y	0	0.19	1.00	0.37	0.22	0.08	146	265	751	36.5	0.82	4.75
HR-Y	115	0.19	0.96	0.43	0.23	0.09	144	210	747	39.0	0.95	5.22
HR-Y	230	0.18	1.06	0.44	0.22	0.40	193	465	766	34.8	122	5.08
HR-N	0	0.22	1.51	0.32	0.25	0.09	1050	285	619	21.6	0.83	6.09
HR-N	115	0.23	1.52	0.26	0.23	0.09	946	367	574	23.2	0.83	5.76
HR-N	230	0.21	1.58	0.32	0.25	0.11	730	1010	645	24.0	1.50	5.65
PTRS	0	0.22	1.62	0.31	0.34	0.12	677	469	1136	48.0	1.95	6.89
PTRS	115	0.22	1.42	0.30	0.32	0.13	663	241	905	53.9	2.34	4.86
PTRS	230	0.19	1.46	0.35	0.29	0.12	448	1753	1068	48.7	3.10	4.90

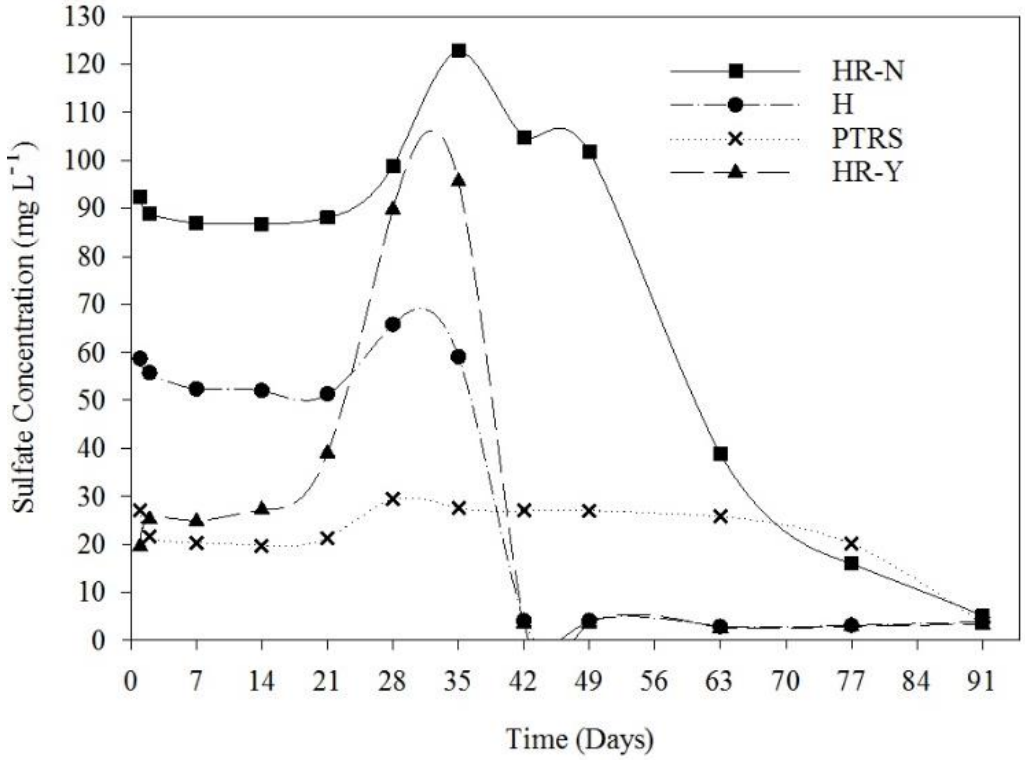

Figure 3.1. Sulfate concentration for each soil location over time.

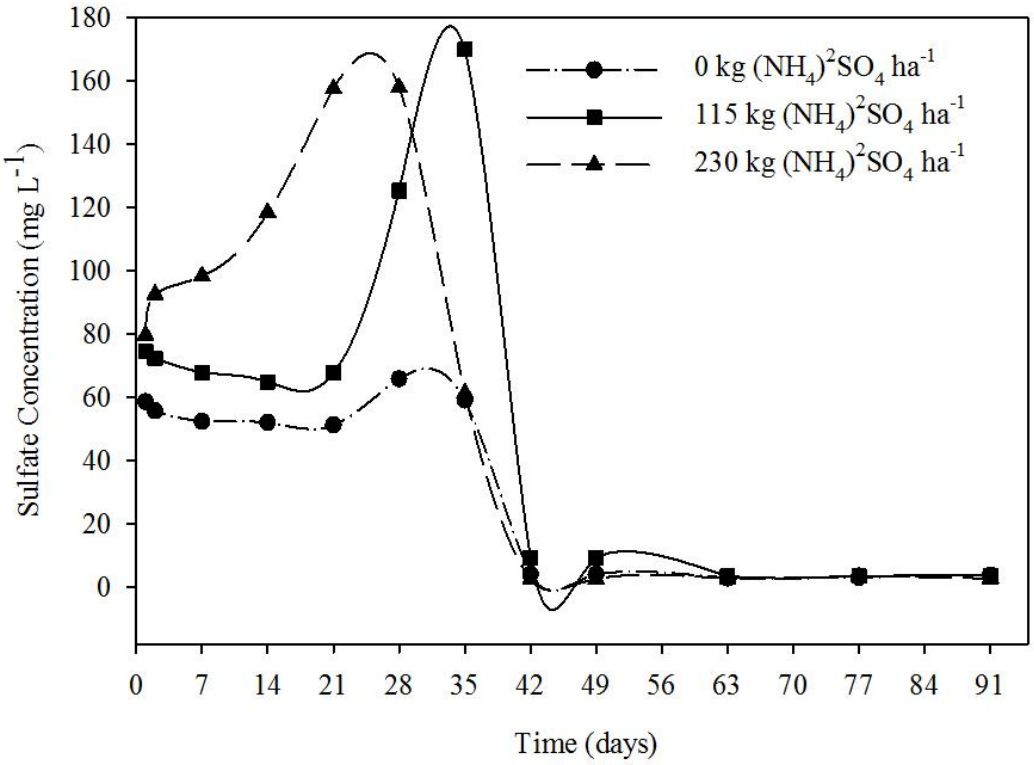

Figure 3.2. Sulfate concentration for each ammonium sulfate fertilizer treatment over time.

CHAPTER 4

Conclusions

Correctly identifying and understanding different physiological disorders is important for successful crop production. Hydrogen sulfide (H_2S) toxicity in rice (*Oryza sativa* L.) fields has become a growing concern in Arkansas and is poorly understood. With reduced yield and potential for crop loss, understanding the causes of H_2S toxicity and how to address or avoid this disorder are pertinent. The overall research goal of this investigation was to understand the chemical and physical properties of soils prone to H_2S toxicity compared to soils that rarely or have never had the disorder reported. Specific objectives to accomplish the goal were to: i) understand the S cycle and Eh in the soil, ii) determine any physical and/or chemical soil characteristics that may influence the occurrence of H_2S toxicity both with and without rice growing, iii) determine the degree of microbial influence in this disorder, and iv) determine if $(NH_4)_2SO_4$ fertilizer influences the occurrence and severity of H_2S toxicity.

The sterilization treatment revealed that microorganisms are highly influential in the chemistry behind H_2S toxicity. Rather than SO_4^{2-} concentrations declining rapidly after flooding, SO_4^{2-} in the sterilized soils declined at a slower rate and had significantly more SO_4^{2-} in solution than the non-sterilized soils (*p* values ranged from 0.0231 to <0.0001). The decline in Eh to below -100 mV was also delayed in the sterilized soils by 14 to 28 days, indicating that microbes are a major player in overall soil reduction and ultimately the occurrence of H_2S toxicity. Without the normal population of microbes, chemical changes are slowed in the soil, and at this rate would delay or even prevent the production of H_2S during the early stages of rice growth when they are most susceptible to damage.

When comparing soils from different locations with varying degrees of H_2S toxicity, differences in soils were observed between locations (*p* values ranged from 0.0105 to <0.0001), however soluble SO_4^{2-} concentrations in the most prone soil and the least prone soil were not

statistically different, indicating that soluble SO_4^{2-} concentration is not the best indicator of where H_2S toxicity will occur. Concentrations of other substrates, such as Fe and soil organic matter (SOM), and microbial population and density likely controlled the rate of reduction and the subsequent toxicity of H_2S.

By investigating the soluble SO_4^{2-} concentrations and Eh under flooded conditions in the soils alone then in the same soils with rice growing, differences in chemistry were identified. Differences between soil locations differed in the rice experiment, with soluble SO_4^{2-} in the most prone and least prone soils significantly different from each other (p values ranged from 0.0405 to 0.0095). Rather than SO_4^{2-} concentrations declining immediately after flooding, when rice was grown SO_4^{2-} concentrations remained steady for approximately one week before SO_4^{2-} began to reduce. This difference in the behavior of the chemistry indicates that the diffusion of O_2 into the rhizosphere via the roots delayed the immediate reduction of SO_4^{2-}. Though the cause of H_2S toxicity is still unclear, this disorder appears to be multifaceted with many factors contributing to the occurrence.

Addition of $(NH_4)_2SO_4$ fertilizer treatments revealed that soluble SO_4^{2-} concentration in solution increases as fertilizer rate increases, regardless of soil location, with significant differences in SO_4^{2-} concentrations between treatments (*p* values ranged from 0.0183 to < 0.0001). Though this would indicate that $(NH_4)_2SO_4$ would increase the likelihood of H_2S toxicity occurring, symptoms did not appear in this study. Despite the fertilizer rate, there was no difference in SO_4^{2-} concentration between a prone soil and a soil not prone to H_2S toxicity. Since both soils had similar concentrations and rates of SO_4^{2-} reduction, this indicates that amount of SO_4^{2-} in the soil is not the driving force behind H_2S toxicity. Though adding $(NH_4)_2SO_4$ does increase the SO_4^{2-} concentration which creates more H_2S as the SO_4^{2-} is reduced, results from

this study again indicate that there are multiple factors that interact to create H_2S toxicity in the field.

In summary, all three experiments indicated that the chance of H_2S occurring does not depend on the amount of soluble SO_4^{2-} in the soil solution alone but, rather, is caused by several factors. Though the exact cause has still not been determined, microorganisms, particularly the sulfate reducing species, play a vital role in this disorder. Other factors such as OM content, Fe content, Eh, and temperature likely influence the occurrence of this complex disorder.