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Silicon Fertilization in Rice: Establishment of Critical Silicon Level and Its Impact on Availability of Nutrients in Soils of Louisiana

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SILICON FERTILIZATION IN RICE: ESTABLISHMENT OF CRITICAL
SILICON LEVEL AND ITS IMPACT ON AVAILABILITY OF NUTRIENTS IN
SOILS OF LOUISIANA

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
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in

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by
Wooiklee S. Paye
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Abstract

While silicon (Si) fertilization is widely practiced in paddy rice production, the establishment of critical soil Si levels has remained understudied. This study was undertaken to: 1) determine the critical soil Si level for rice production in Louisiana using different extraction procedures, and 2) document the relationship between plant-available Si and select essential plant nutrients in soil and their uptake by rice. Field trials were established at 12 sites across Louisiana from 2013 to 2015. Before planting, Si was applied as silicate slag (SiO_3 , 12% Si) at the rates of 0, 1, 2, 4, 6, and 8 Mg ha^{-1} . Agricultural lime was also applied at (2 and 4 Mg ha^{-1}) to evaluate the liming effect of SiO_3 slag in the main treatments. Treatments were arranged in a randomized complete block design with four replications. Soil samples collected at harvest were analyzed for pH, soil Si, heavy metals and plant-essential nutrients, while plant samples were analyzed for elemental composition. Plant-available soil Si was extracted using different solutions: 0.5 M acetic acid, 0.01 M calcium chloride, 1 M sodium acetate, 0.5 M ammonium acetate, 0.1 M citric acid and deionized water. Silicon content in sample extracts was determined using Molybdenum Blue Colorimetric (MBC). Silicon in both straw and panicle was determined using Oven Induced Digestion procedure followed by MBC. Soil pH was measured using a 1:1 (weight/volume) soil to water solution. Heavy metals and plant-essential nutrients in soil were determined using Mehlich-3 extraction procedure, followed by Inductively Coupled Plasma-Optical Emission Spectroscopy procedure (ICP-OES). The heavy metal and nutrient contents of rice panicle was determined by HNO_3 - H_2O_2 digestion followed by ICP-OES. Analysis of variance and correlation analysis were performed for all measured variables using SAS 9.4. Slag application significantly increased the soil pH by as much as 1.4 units ($p < 0.05$) in several sites. The Si contents of soils having high initial Si and pH was not significantly increased by slag application. However, Si contents in soils with low pH and low initial Si was significantly increased as a result of slag

application. Rice grain yield was significantly ($p < 0.01$) increased in several sites by slag application, with the highest average grain yield obtained at application rates ranging between 1-4 Mg ha⁻¹. The critical plant-available Si levels in soils estimated using soil Si extracted by different solutions and plant Si uptake ranged from 11.8 mg kg⁻¹ (0.01 M CaCl₂) to 771 mg kg⁻¹ (0.1 M citric acid). The Si content in rice straw was negatively correlated with the panicle P ($r = -0.25$) and S ($r = -0.38$). Silicon content of panicle was also negatively correlated with As ($r = -0.33$) and Cd ($r = -0.39$), but positively correlated with Mn ($r = 0.35$). In general, soils with high initial Si and pH gave minimal responses to Si fertilization, while the Si content of those soils with low initial Si was significantly increased. Soil Si did not interfere with the uptake of most plant-essential nutrients, but the decrease in As and Cd contents of panicle shows that Si fertilization could be essential for improving grain quality of rice.

Chapter 1. Introduction

Silicon (Si) is the second most abundant element in the earth's crust after oxygen and accounts for about 28% of the soil weight, but it is not considered essential for growth of higher plants (Epstein, 1999). Despite its abundance in the soil, Si exists mostly as silica (SiO_2), a form that is not available for plant uptake. To be taken up by plants, Si must be in the form of monosilicic acid (H_4SiO_4) but the natural release of H_4SiO_4 from SiO_2 is a very slow process (Raven, 1983). Today, Si is a highly controversial element in plant nutrition as both crop and soil scientists cannot come to a conclusion on whether the element is "essential" or "required" for plant growth. One reason for such disagreement is because most plants can complete their life cycle without external addition of Si to the growing medium (Marschner, 1995). Another reason Si is often not regarded as essential for plant growth could be due to its ubiquity in nature. It is extremely difficult to completely exclude it from nutrient culture solution thus, making some scientists to neglect the presence of Si in culture solutions (Epstein, 1994). While its essentiality is still debated, there are many reports of plant response to the addition of Si to their growing medium. For instance, Yoshida et al. (1959) reported that rice (*Oryza sativa*) plants growing in nutrient solution significantly deficient in Si were physically inferior, prone to insects and disease attacks and produced less biomass than those growing in solution supplied with Si. Narayanaswamy and Prakash (2009) also observed yield increases in rice growing in soils testing low for plant available Si when amended with Si fertilizer.

The most notable contribution of Si to plant growth and development may be attributed to the ability of the element to induce plant resistance to biotic stresses from pest and disease pressures and abiotic stresses arising from the soil system and the environment (Datnoff et al., 2001). Following its absorption by plants roots, H_4SiO_4 is transported through the apoplastic

and symplasmic pathways and deposited either as amorphous silica ($\text{SiO}_2 \cdot n\text{H}_2\text{O}$) or opal phytolith in the cell lumens of the stem and leaves (Raven, 1983). While in the plant system, H_4SiO_4 loses water through the process of transpiration and become more concentrated to form SiO_2 gels; and with plant age, these can develop into SiO_2 bodies (Parry and Smithson, 1964). A buildup of these silica bodies in older leaves acts as a physical barrier and forms a basis for the formation of harder leaf surfaces which cannot be easily penetrated by insects and plant pathogens. The more rigid leaf surfaces also serve as a deterrent for herbivores (Herrera, 1985). Miyake and Takahashi (1983) observed that increasing the Si concentration of a solution in which a cucumber (*Cucumis sativa*) plant was grown led to an increase in shoot Si content and a subsequent reduction in the incidence of powdery mildew disease relative to plants growing in solution low in Si. Similar observation was made by Menzies et al. (1991), who reported reduced infection efficiency, colony size and germination of conidia when cucumbers were grown in nutrient solution containing high concentrations of Si. High nitrogen (N) fertilization can lead to the development of succulent plant tissues which encourages pest and disease attacks. Ma (2004) reported decreased occurrence of blast in rice fields under high N fertilization with the addition of Si. Datnoff et al. (1997) also reported decreased incidence of blast and sheath blight in rice under Si fertilization. Wheat (*Triticum aestivum*) plants treated with Si produced phytoalexins and inhibited powdery mildew infection (Remus-Borel et al., 2005)

Several studies have also been done to evaluate the role of Si in inducing plant resistance to abiotic stresses from a variety of sources; the soil and plant system, the earth's gravitational pull, as well as wind pressure on plants. For example, high N and phosphorus (P) fertilization programs can lead to increased production of above ground biomass causing plants to easily lodge under windy conditions hence, leading to reduction in yield and profitability (Berry et al., 2000). Excessive N uptake by plants can also lead to production of heavy and overgrown leaves

especially in grasses such as rice, wheat and sugarcane (*Saccharum officinarum*). This can make the leaves become droopy, leading to shading of the lower leaves and overall reduction in the rate of photosynthesis in lower leaves with subsequent reduction in yield (Sivasankar et al., 1993). It has been shown that Si fertilization can alleviate this problem by inducing erectness of the leaves and reduce shading of the lower leaves; thereby enhancing even distribution of light within the canopy which can positively influence the photosynthetic capacity of plants (Epstein, 1994). In an experiment involving high N rates and Si fertilization, the number of blank spikelets per panicle was reduced and weight of 1000 seeds obtained in rice increased with Si application under high N fertilization (Mauad et al., 2003).

It is been suggested that Si also alleviates the toxic effects of heavy metals and other micronutrients which is now becoming prevalent in many crop production systems (Corrales et al., 1997). Although the mechanism by which Si ameliorates the toxic effects of heavy metals is still under study, Cocker et al. (1998) proposed that Si inhibits aluminum (Al) toxicity either by forming insoluble aluminosilicates or hydroxyaluminosilicates which reduces the concentration of free Al^{3+} in soil solution, or by blocking the apoplastic pathway which is the main transport route for metal cations within plant cells. From a pot experiment where corn (*Zea mays*) was planted in soil contaminated with high levels of cadmium (Cd) and zinc (Zn), Cunha et al. (2008) discovered that addition of Si up to 200 mg kg^{-1} drastically reduced the bioavailability of both heavy metals and led to an increased corn biomass production. Fleck et al. (2013) reported that application of Si to paddy rice resulted in reduction of arsenic (As) concentration in straw, shoot, flag leaves and husk up to 50% and also reduced the As^{+3} content of brown polished rice up to 22%. Under flooded conditions, Si fertilization facilitates the transport of oxygen to the root system of rice plants and increases the oxidative capacity of the roots thus causing oxidation

and subsequent deposition of toxic levels of Mn^{+2} and Fe^{+2} on roots surfaces; and reducing their uptake and translocation to the shoot (Yuan and Chang, 1978).

Drought is another environmental factor that impedes agricultural productivity. Under drought stress, the rate at which plants lose water through transpiration can be severely increased, causing disruption in physiological processes such as photosynthesis, thus decreasing the activity of photochemical and enzymes in the Calvin cycle (Monakhova and Chernyad'ev, 2002). The deposition of Si in the outer walls of epidermal cells on both surfaces of leaves in rice (Agarie et al., 1998) and sugarcane (Savant et al., 1999) is reported to have reduced water loss through transpiration and maintained normal growth under drought stress. In a separate study, Gong et al. (2003) observed that wheat plants treated with Si had thicker leaves and a better water use efficiency when compared with those without Si. Therefore, they postulated that Si was involved with inducing drought tolerance in wheat by reducing water loss through transpiration.

While its essentiality as a plant nutrient is yet not established, some researchers have resolved to use terms such as “beneficial” or “quasi-essential” to describe the role of Si in certain plants growing under different stressful conditions (Epstein and Bloom, 2005). Recognizing the benefits of Si in crop production as well as the positive response of many agronomic and horticultural crops to Si fertilization both in culture solution and soil, Si has become an important component of integrated nutrient management and sustainable agriculture across Asia, South America and the U.S. (Vasanthi et al., 2012). Perhaps rice and sugarcane are the two crops showing the most notable beneficial effects of Si under field conditions. In Japan, rice grown in degraded paddy fields have shown the physiological disorder called “Akiochi” (a disease caused by the restriction of nutrient uptake by hydrogen sulfide coupled with increased fungi attack and

poor plant growth, usually associated with Si deficiency), which can be corrected by Si fertilization (Yoshida et al., 1959). Ma et al. (1989) reported 20 and 50% loss in straw and grain yields respectively when rice plants were denied Si during the reproductive stage, contrary to 24 and 30% increases in straw and grain yields obtained by those supplied with Si.

The abundance of Si in soil does not negate the occurrence of its deficiency as a nutrient element. While it is the second most abundant element in the earth's crust, it has been shown that soils with certain physical and chemical properties can often have low levels of plant-available Si. Soils classified as Oxisols and Histosols have been reported to have limited amount of plant-available Si (Foy, 1992). Snyder et al. (1986) documented that soil having greater than 80% organic matter can usually be deficient in other plant essential nutrients and Si. Soils that are highly weathered and acidic with high rate of water infiltration have been shown to have limited amount of Si (Datnoff et al., 1997). Continuously planting a field to crops such as rice and sugarcane both of which are classified as high Si accumulators, can also lead to the depletion of soil Si and cause reduction in crop yield if not supplemented with appropriate Si fertilization (Elaward and Green, 1979).

Rice is the most important food crop worldwide, serving as a major source of calories for more than half of the world's population (Greenland, 1997). Since its domestication about 8,000 to 10,000 years ago, the crop has sustained more lives for a longer period of time than any other domesticated plant (Evan, 1998). Global per capital consumption of rice has been estimated at over 100 Kg with more than three billion people; mostly in Asia, Africa and Latin America relying on the crop as their staple food (Nguyen and Ferrero, 2006). It is reported that rice provides about 27% of dietary calories as well as 20% of dietary protein; and its cultivation provides employment and serves as a major source of income for nearly 100 million households

in more than 114 developing countries worldwide (Nguyen and Ferrero, 2006; FAO, 2004). Most of the world's rice is produced and consumed in Asia where population densities are very high, with China and India ranking as the top producers and consumers (FAO, 2014). Outside of Asia, Brazil and the U.S. are the leading producers of rice but most of the rice produced in the U.S. is not consumed locally, because the U.S. does not have a large rice consuming population (USITC, 2015). With limited local consumption, most of the U.S. rice is exported to other regions of the world where demand for the crop is high, making the U.S. to rank the fifth highest rice exporting country in the world (FAO, 2014). Rice production in the U.S. is mostly concentrated across six states including Arkansas, California, Louisiana, Mississippi, Missouri and Texas, with Louisiana ranking third highest (USDA NASS, 2014). In Louisiana, rice is the second most economically important crop after sugarcane; with total production covering an area of 188, 584 hectares and a total value of \$454 million (USDA NASS, 2014). In spite of these well noted statistics, Louisiana's rice producers are faced with many challenges arising from insect pests and diseases pressures, nutrient deficiencies, as well as environment factors that can lead to reduction in yield and profitability. Rice is one of the plants classified as a high Si accumulator, meaning that the plant can accumulate more than 5% Si in its straw (Datnoff et al., 2001). With more research proving that Si fertilization can alleviate both biotic and abiotic stresses and improve rice yield, there is a need for the establishment of Si fertilization guidelines for rice production in Louisiana.

Unlike the state of Florida where Si fertilization is widely practiced in rice and sugarcane production; there is limited research on Si fertilization in the rice and sugarcane industries of Louisiana. The initial step for the implementation of any fertilization program would be the establishment of the critical level of the nutrient in question in the soil and an appropriate laboratory determination procedure for its critical level. While the interaction of Si with heavy

metals and other cations is widely reported, there is limited information as to how Si affects the uptake of other plant essential nutrients in rice. The objectives of this study therefore were to: a) determine the critical soil Si level for rice production in Louisiana using different extraction procedures, and 2) document the relationship between plant-available Si and select essential plant nutrients in soil and their uptake by rice.

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Chapter 2. Determination of Critical Soil Silicon Levels for Rice Production in Louisiana using Different Extraction Procedures

2.1 Introduction

Silicon (Si) is the second most abundant element in the earth's crust after oxygen and accounts for about 28% of the soil weight, but it is not considered essential for growth of higher plants (Epstein, 1999). Despite its abundance in the soil, Si exists mostly as silica (SiO_2), a form that is not available for plant uptake. To be taken up by plants, Si must be in the form of monosilicic acid (H_4SiO_4) but the natural release of H_4SiO_4 from SiO_2 is a very slow process (Raven, 1983). Today, Si is a highly controversial element in plant nutrition as both crop and soil scientists cannot come to a conclusion on whether the element is "essential" or "required" for plant growth. One reason for such disagreement is because most plants can complete their life cycle without external addition of Si to the growing medium (Marschner, 1995). Another reason Si is often not regarded as essential for plant growth could be due to its ubiquity in nature. It is extremely difficult to be completely excluded from nutrient culture solution thus, making some scientists to neglect the presence of Si in culture solutions (Epstein, 1994). While its essentiality is still debated, there are many reports of plant response to the addition of Si to their growing medium. For instance, Yoshida et al. (1959) reported that rice (*Oryza sativa*) plants growing in nutrient solution significantly deficient in Si were physically inferior, prone to insects and disease attacks and produced less biomass than those growing in solution supplied with Si. Narayanaswamy and Prakash (2009) have also documented yield increases in rice plant growing in soils testing low for plant available Si when amended with Si fertilizer.

Although not considered a plant essential nutrient, numerous studies suggest that when under certain kind of stress, higher plants especially those from the poaceae and gramineae family can benefit greatly when adequately supplied with plant available Si (Jones and

Handreck, 1967). The most notable contribution of Si to plant growth and development may be attributed to the ability of the element to induce plant resistance to biotic stresses from pest and disease pressures and abiotic stresses arising from the soil system and the environment (Datnoff et al., 2001). Following its absorption by plants roots, H_4SiO_4 is transported through the apoplasmic and symplasmic pathways and is deposited either as amorphous silica ($SiO_2 \cdot nH_2O$) or opal phytolith in the cell lumens of the stem and leaves (Raven, 1983). While in the plant system, H_4SiO_4 lose water through the process of transpiration and become more concentrated to form SiO_2 gels; and with plant age, these can develop into SiO_2 bodies (Parry and Smithson, 1964). A buildup of these SiO_2 bodies in older leaves acts as a physical barrier and forms a basis for the formation of harder leaves surfaces which cannot be easily penetrated by insects and plant pathogens. The more rigid leaf surfaces also serve as a deterrent for herbivores (Herrera, 1985). Miyake and Takahashi (1983) observed that increasing the Si concentration of a solution in which a cucumber (*Cucumis sativus*), plant was grown led to an increase in shoot Si content and a subsequent reduction in the incidence of powdery mildew disease relative to plants growing in solution low in Si. Similar observation was made by Menzies et al. (1991), who reported reduced infection efficiency, colony size and germination of conidia when cucumbers were grown in nutrient solution containing high concentrations of Si. High N fertilization can lead to the development of succulent plant tissues which encourages pest and disease attacks. Ma (2004) reported decreased occurrence of blast in rice fields under high N fertilization with the addition of Si. Datnoff et al. (1997) also reported decrease incidence of blast and sheath blight in rice under Si fertilization. Wheat plants treated with Si produced phytoalexins, a compound which inhibits powdery mildew infection (Remus-Borel et al., 2005).

The impact of Si on inducing plants resistance to abiotic stress is also well-documented. For example, high N and phosphorus (P) fertilization can lead to increased production of above

ground biomass causing plants to easily lodge under windy conditions hence, leading to reduction in yield and profitability (Berry et al., 2000). Excessive N uptake by plants can also lead to production of heavy and overgrown leaves especially in grasses such as rice, wheat (*Triticum aestivum*) and sugarcane (*Saccharum officinarum*), which can become droopy and causes shading of the lower leaves; thereby leading to an overall reduction in the rate of photosynthesis in lower leaves with subsequent reduction in yield (Sivasankar et al., 1993). It has been shown that Si fertilization can alleviate this problem by improving rigidity of the cell wall, inducing erectness of the leaves and reduce shading of the lower leaves; thereby enhancing even distribution of light within the canopy which can positively influence the photosynthetic capacity of plants (Epstein, 1994). The number of blank spikelets per panicle was reduced and weight of 1000 seeds obtained in rice increased with Si application under high N fertilization (Mauad et al., 2003).

It is been suggested that Si also alleviates the toxic effects of heavy metals and other micronutrients which is now becoming prevalent in many crop production systems, especially for crops grown under anaerobic conditions (Corrales et al., 1997). Although the mechanism which by Si ameliorates the toxic effects of heavy metals is still under study, Cocker et al. (1998) proposed that Si inhibits aluminum (Al) toxicity either by forming insoluble aluminosilicates or hydroxyaluminosilicates which reduces the concentration of free Al^{3+} in soil solution, or by blocking the apoplastic pathway which is the main transport route for metal cations within plant cells. From a pot experiment where corn (*Zea mays*) was planted in soil contaminated with high levels of cadmium (Cd) and zinc (Zn), Cunha et al. (2008) discovered that addition of Si up to 200 mg kg^{-1} drastically reduced the bioavailability of both heavy metals and led to increase in corn biomass production. Fleck et al. (2013) reported that application of Si to paddy rice resulted in a reduction of arsenic (As) concentration in straw, shoot, flag leaves and husk up to 50% and

also reduced the As^{+3} content of brown polished rice up to 22%. Under flooded condition, Si facilitates the transport of oxygen to the root system of rice plants and increases the oxidative capacity of the roots; which can lead to oxidation and subsequent deposition of toxic levels of Mn^{+2} and Fe^{+2} on roots surfaces; thus, reducing their uptake and translocation to the shoot (Yuan and Chang, 1978).

Recognizing the benefits of Si in crop production as well as the positive response of many agronomic and horticultural crops to Si fertilization both in culture solution and soil, Si has become an important component of integrated nutrient management and sustainable agriculture across Asia, South America and the U.S. (Vasanthi et al., 2012). Perhaps rice and sugarcane are the two crops showing the most notable beneficial effects of Si under field conditions. In Japan, rice grown in degraded paddy fields have shown the physiological disorder called “Akiuchi” (a disease caused by the restriction of nutrient uptake by hydrogen sulfide coupled with increased fungi attack and poor plant growth usually associated with Si deficiency), which can be corrected by Si fertilization (Yoshida et al., 1959). Ma et al. (1989) reported 20 and 50% loss in straw and grain yields, respectively, when rice plants were denied Si during the reproductive stage, contrary to 24 and 30% increase in straw and grain yields obtained by those supplied with Si.

The abundance of Si in soil does not negate the occurrence of its deficiency as a nutrient element. While it is the second most abundant element in the earth’s crust, it has been shown that soils with certain physical and chemical properties can often have low levels of plant-available Si. Most soils classified as Oxisols, Entisols and Histosols have been reported to have limited amount of plant- available Si (Foy, 1992). Snyder et al. (1986) documented that soil having greater than 80% organic matter can usually be deficient in other plant essential nutrients and Si. Soils that are highly weathered and acidic with high rate of water infiltration have been shown to

have limited amount of Si (Datnoff et al., 1997). Continuously planting a field to crops such as rice and sugarcane, both of which are classified as high Si accumulators can also lead to the depletion of soil Si and causes reduction in crop yield if not supplemented with appropriate Si fertilization (Elaward and Green, 1979).

Rice is the most important food crop worldwide, serving as a major source of calories for more than half of the world's population (Greenland, 1997). Since its domestication about 8,000 to 10,000 years ago, the crop has sustained more lives for a longer period of time than any other domesticated plant (Evan, 1998). Global per capital consumption of rice has been estimated at over 100 Kg with more than three billion people; mostly in Asia, Africa and Latin America relying on the crop as their staple food (Nguyen and Ferrero, 2006). It is reported that rice provides about 27% of dietary calories as well as 20% of dietary protein; and its cultivation provides employment and serves as a major source of income for nearly 100 million households in more than 114 developing countries worldwide (FAO, 2004; Nguyen and Ferrero, 2006). Most of the world's rice is produced and consumed in Asia where population densities are very high; with China and India ranking as the top producers and consumers (FAO, 2014). Outside of Asia, Brazil and the U.S. are the leading producers of rice but most of the rice produced in the U.S. is not consumed locally, because the U.S. does not have a large rice consuming population (USITC, 2015). With limited local consumption, most of the U.S. rice is exported to other regions of the world where demand for the crop is high, making the U.S. to rank the fifth highest rice exporting country in the world (FAO 2014). Rice production in the U.S. is mostly concentrated across six states including Arkansas, California, Louisiana, Mississippi, Missouri and Texas, with Louisiana ranking third highest (USDA, NASS, 2014). In Louisiana, rice is the second most economically important crop after sugarcane; with total production covering an area of 188,584 hectares; and a total value of \$454 million (USDA, NASS, 2014). In spite of these

well noted statistics, Louisiana's rice producers are faced with many challenges arising from insect pests and diseases pressures, nutrient deficiencies, as well as environmental factors that can lead to reduction in yield and profitability. Rice is one of the plants classified as a high Si accumulator, meaning that the plant can accumulate more than 5% Si in its straw on a dry weight basis (Datnoff et al., 2001). With more research proving that Si fertilization can alleviate both biotic and abiotic stresses and improve rice yield, there is a need for the establishment of Si fertilization guidelines in Louisiana.

In Louisiana, rice is the second most important economic crop with several hundred thousands of hectares cultivated each year. Rice is a Si accumulator and its cultivation can lead to depletion of soil Si if not amended. Unlike the state of Florida where Si fertilization is widely practice in rice production on Histosols, Si fertilization is not practiced in rice cultivation of Louisiana. Although the Si status of Louisiana's soils is not well documented, Breitenbeck et al. (2006) observed a limited deterioration of growth and yield in rice fields of southwestern Louisiana which they attributed to Si deficiency. However, for a fertilizer recommendation to be made, it is first important to determine, the critical limits in the soil for a particular nutrient in question for plant growth and development. Such information will guide producers with respect to the degree of nutrient deficiency and the appropriate amount of fertilizer needed to correct such deficiency (Korndorfer et al., 2001). In order to develop recommendations for field applications of Si fertilizer to rice in Louisiana, an understanding of the Si status of soil and an appropriate laboratory determination procedure is required. Although critical limits of most plant essential nutrients are already established for Louisiana, there is limited knowledge on the Si status of Louisiana soils. The aim of this study therefore was to determine the critical soil Si level for rice production in Louisiana using different extraction procedures.

2.2 Materials and Methods

2.2.1 Locations, Trial Establishment, and Experimental Design

The study was conducted in Southwest and Northeast Louisiana from 2013 to 2015 with a total of 12 site-years. In 2013 and 2014, field trials were established in five parishes including Acadia (Crowley) on a Crowley silt loam soil (Fine, smectitic, thermic Typic Albaqualfs), Evangeline (Mamou) on a Mowata silt loam (Fine, smectitic, thermic Typic Glossaqualfs), Vermilion (Lake Arthur) on a Kaplan silt loam and St. Landry (Palmetto) on a Tensas Sharkey complex all of which are in the Southwest, and Franklin parish (Gilbert) on a Sharkey clay soil (Very-fine, smectitic, thermic Chromic Epiaquerts) in the Northeast of Louisiana. In 2015, trials were established at two sites, Crowley and Lake Arthur. Table 2.1 provides details of each site, the soil type and initial soil Si content determined by different extraction procedures. Soil classes such as Alfisols, Entisols, Inceptosols and Vertisols generally predominate in these areas. These soils have aquic moisture regime, mostly comprise of clay with mixed sand and silt mineralogy, poorly drained and thermic. They are very deep, nearly flat to gently undulating and generally fit in one of two soil textural classes: loamy or clayey (Weindorf, 2008). They differ in pH, organic matter and mineralogical composition. The initial soil pH, organic matter content, and mineralogical composition of the soils are summarized in Table 2.2. These soils are part of the alluvial plain found along the Mississippi river valley and have been subjected to continual annual rice production for decades if not centuries. All trials were established in fields that are under current rice cultivation.

Before sowing, the amount of fertilizer required per plot was determined and weighed out in large zip lock plastic bags. These were then broadcast by hand and incorporated into soil to a depth of 7 cm. The source of Si used for this study was silicate slag which is a by-product of the steel and iron industry. This material contains about 14% Si and may also contain other elements

such as Ca, Mg, Fe, and S. The elemental composition of slag material used for this study is presented in Table 2.3. The treatment structure consisted of five slag rates applied at 1, 2, 4, 6, and 8 Mg ha⁻¹ which are equivalent to 140, 280, 560, 840 and 1120 Kg Si ha⁻¹, respectively, along with two lime treatments of 2 and 4 Mg ha⁻¹. All treatments were arranged in a randomized complete block with four replications. Treatment structure and fertilizer rates are summarized in Table 2.4.

After slag application, rice seeds were drilled at the rate of 300 seeds m² with 20 cm row spacing in plots measuring 1.5 m x 4.9 m, with a total of 7 rows per plot. Three rice varieties including CL111, Jupiter, and CLXL 729, were used for this study. These varieties were selected based on the predominant variety cultivated in each site or location. Phosphorus and potassium fertilizers were applied where needed, to ensure that their deficiency did not limit growth and production; and at four leaves growth stage, a urea based N fertilizer was applied according to the LSUAgCenter recommendation and thereafter, permanent flooding was maintained in the field until physiological maturity. Table 2.5 gives details of field layout and all major field activities carried out during the course of the experiment. All other pests and weed management practices were carried out in line with those recommended by the LSUAgCenter.

2.2.2 Harvest and Grain Yield

At physiological maturity, whole plant samples were taken from a 1-m section of the third row from each opposite side of the plots by cutting the entire above grown biomass and then combining them into one composite sample for each plot for yield component determination. The samples were oven dried at 55°C until they reached a constant weight; and separated into straw and panicle and their weights were determined separately. The straw and panicle samples were used for elemental composition analysis. The entire plot was then

harvested using a plot combine harvester equipped with a computerized weighing system to determine the grain weight from individual plot. The moisture content of the grains was adjusted to 12% and plot grain yield was converted to kilograms per hectare.

2.2.3 Soil Sampling and Processing

Close to or immediately after harvest in August or September of each year, soil samples were collected for elemental composition analysis. Sampling method involved taking twelve core samples at a depth of 15 cm, with six cores taken from the third row on the opposite sides of each plot from where plant biomass samples were taken. These were thoroughly mixed and homogenized as a composite sample for individual plot. The samples were placed in paper bags and oven dried for about a week using a Despatch oven (LBB series; model number LBB2-18-1) set at 60°C. After drying, the samples were ground using a Humboldt soil grinder to pass through a 2 mm sieve and then stored in 200 mL screw cap plastic cups with proper labeling for laboratory analysis.

2.2.4 Soil Analysis

2.2.4. a. Soil pH Determination

The soil pH was measured using a 1:1(weight/volume) soil to water ratio. Ten (10) grams of dried soil was weighed and placed in a 50 mL screw cap plastic centrifuge tube; and 10 mL of deionized water was added to each tube and then screwed tightly. The tubes were placed on a reciprocal shaker set at high speed for one hour and allowed to sit undisturbed for another hour to permit soil to settle. After that, the soil pH was determined using a SevenCompact™ pH/ Ion S220 digital pH meter.

Table 2.1. Initial soil silicon content as determined by different extraction procedures.

Site	Soil series	Deionized water	0.01 M CaCl ₂	1 M NaOAc	0.5 M NH ₄ OAc	0.5 M [†] OAc-1	0.5 M [‡] OAc-2	0.1 M Citric acid	Mean
-----mg kg ⁻¹ -----									
Crowley	Crowley silt loam	24	20	84	78	103	134	814	180
Gilbert	Sharkey clay	28	17	145	115	156	161	827	207
Mamou	Mowata silt loam	14	9	25	52	16	58	280	65
Lake Arthur	Kaplan silt loam	9	7	12	36	11	36	306	60
St. Landry	Tensas Sharkey complex	11	6	39	18	33	36	297	63

[†] Samples were extracted after shaking for one hour. [‡] Samples were rested in solution for 24 hours and then shaken for one hour before extraction.

Table 2.2. Initial pH, organic matter content, and elemental composition of soils used in this study.

Site	Soil Series	³ Organic matter	pH 1:1 water	†Si	Ca	Cu	P	K	Na	S	Mg	Zn
Crowley	Crowley silt loam	1.1	7.6	103	1463	1.9	25	73	83	9.0	256	6.7
Gilbert	Sharkey clay	1.9	6.8	156	4971	5.5	78	408	71	10.6	1013	4.9
Mamou	Mowata silt loam	1.8	5.7	16	1325	1.6	14	129	64	18.4	1413	2.1
Lake Arthur	Kaplan silt loam	1.1	4.9	11	997	0.9	35	63	40	11.7	110	4.1
Palmetto	Tensas Sharkey complex	2.6	7.1	33	3770	2.8	78	228	92	6.3	681	2.8

†Initial soil Si status was determined by 0.5 M acetic acid and 1 hour shaking and then followed by Molybdenum Blue Colorimetric procedure. Organic matter was determined colorimetrically using the Walkley and Black method (Walkley and Black, 1936). All essential plant nutrients were determined using Mehlich-3 extraction procedure followed by ICP analysis.

Table 2.3. Elemental composition of slag material used for this study.

Element	Percent
Aluminum	7.0
Calcium	23.0
Iron	14.0
Magnesium	7.0
Manganese	1.6
Silicon	14.0
Sulfur	0.5

Table 2.4. Treatment structures and fertilizer rates.

Treatment	Material	Rate (Mg ha ⁻¹)	Equivalent Si application kg ha ⁻¹
1 *	Check	0	0
2	slag	1	140
3	slag	2	280
4	slag	4	560
5	slag	6	840
6	slag	8	1120
7	lime	2	0
8	lime	4	0

*check plots did not receive lime or Si fertilizer

Table 2.5. Field activities carried out during the cropping season in 2013, 2014, and 2015.

Location	Year	Treatment Application and Planting	Variety	Flooding	Harvest
Gilbert	2013	20-May-13	CL111	21-Jun-13	17-Sep-13
	2014	6-May-14	CL111	11-Jun-14	16-Sep-14
Mamou	2013	18-Mar-13	Jupiter	7-May-13	9-Aug-13
	2014	21-Apr-14	Jupiter	20-May-14	16-Aug-13
Palmetto	2013	19-Mar-13	CL111	9-May-13	19-Aug-13
	2014	24-Mar-14	CL111	12-May-14	23-Aug-14
Crowley	2013	14-Mar-13	CL111	12-May-13	5-Aug-13
	2014	13-Mar-14	CL111	16-May-13	6-Aug-14
	2015	20-Mar-15	CL111	15-May-15	10-Aug-15
Lake Arthur	2013	19-Mar-13	CLXL729	8-May-13	27-Aug-13
	2014	13-Mar-14	CLXL729	9-May-14	10-Aug-14
	2015	23-Mar-15	CLXL729	12-May-14	12-Aug-14

2.2.4. b. Extraction and Determination of Plant-Available Soil Silicon

Plant-available Si was extracted from the soil using seven different extraction procedures. Details of each extraction procedure and the referenced researchers are provided in Table 2.6. After extraction, the plant-available Si in each aliquot was quantified by Molybdenum Blue Colorimetric (MBC) procedure as described by Korndorfer et al. (2001). Based on each extraction procedure used, a predetermined volume of aliquot was transferred to a 50 mL plastic centrifuge tube, added with 10 mL of deionized water and a 0.5 mL of 1:1 hydrochloric acid (HCl), plus 1 mL of 10% ammonium molybdate $[(\text{NH}_4)_6\text{Mo}_7\text{O}_2]$. The solution was allowed to rest for about 5 minutes and thereafter, 1 mL of 20% tartaric acid was added to the solution, shaken/swirled for about 10 seconds and allowed to sit again for 2 minutes. After 2 minutes, 1 mL of ANSA (prepared by adding 0.5 g of amino naphthol n-sulphonic acid + 1 g of sodium sulfite + 30 g of sodium bisulfite and dissolved in 250 mL of deionized) was added as a reducing agent. After 5 minutes, the absorbance reading of the final solution was measured using an UV visible spectrophotometer (Hach DR 5000) set at 630 nm.

Standards series containing 0.2, 0.4, 0.6, 0.8, 1.2, and 1.6 mg L⁻¹ Si were also obtained by pipetting 0.5, 1, 2, 3, 4, and 5 ml of 10 mg L⁻¹ Si and were all treated in the same manner like soil extracts as described above.

Table 2.6 Different procedures used for extracting plant-available silicon from soil collected after harvest.

Extraction Procedure	Solution	Soil: solution ratio g ml ⁻¹	Shaking time	References
OAc-1 †	0.1M CH ₃ COOH †	1:10	1 hr	Korndorfer et al., 1999
CaCl ₂	0.01M CaCl ₂	1:10	1 hr	Korndorfer et al., 1999
NaOAc	1M NaCH ₃ COOH	1:10	1 hr	Fox et al., 1967
Water	Deionized water	1:10	1 hr	Korndorfer et al., 1999
NH ₄ OAc	0.5M NH ₄ CH ₃ COOH	1:10	1 hr	Korndorfer et al., 1999
Citric acid	0.1M C ₆ H ₈ O ₇	0.5:25	2 hrs, 24 hrs rest, 1 hr	Acquaye and Tinsley, 1965
OAc-2 ‡	0.1M C ₂ H ₄ O ₂ ‡	1:10	24 hrs rest, 2 hrs	Snyder, 2001

† samples were immediately shaken for 1 hr after addition of acetic acid
‡ samples were allowed to rest in acetic acid for 24 hrs and then shaken for 2 hrs

2.2.5 Plant Analysis

Prior to harvesting of the entire plot, whole plant samples were taken for Si and elemental composition analysis. Plant samples were oven dried at 55°C and separated into straw and panicle; and these were ground, digested, and analyzed separately before determining their Si content. Plant sample digestion was carried out following the Oven-Induced Digestion procedure as described by Kraska and Breitenbeck (2010). One hundred (100) mg of finely ground samples were weighed out into 50 mL polyethylene screw-cap centrifuge tubes. The tubes were then capped loosely and placed in a conventional oven set at 60°C for 15 minutes to get rid of any atmospheric moisture that may have come in contact with the samples during storage. The samples were then removed and 5 drops of octanol (octyl-alcohol) were added in order to prevent excessive foaming upon addition of hydrogen peroxide (H₂O₂) and sodium hydroxide (NaOH). Two mL of 30% H₂O₂ were added to the

samples while ensuring that no particles were left on the wall of the tubes and then all tube were loosely capped and placed back into the oven set at 95°C for 30 minutes. After that, 4 mL of 50% NaOH was added and the tubes were placed back into the oven for four hours of continuous digestion; mixing the samples every 15 minutes with a vortex mixer. The samples were removed after four hours and 1 mL of 5 mM ammonium fluoride (NH₄F) was added in order to facilitate the formation of H₄SiO₄ and then diluted with deionized water to a final volume of 50 mL.

Molybdenum Blue Colorimetric procedure (Hallmark et al., 1982) was followed to determine the Si concentration in the digested plant samples. Two mL of the digests were transferred to 50 mL polyethylene screw-cap centrifuge tubes, added with 10 mL of 20% acetic acid and swirled for about 10 seconds. Four mL of 0.26 M ammonium molybdate was added and the samples were allowed to stand for 5 minutes. Two mL of ANSA was then added as a reducing agent and 20% acetic was added to make the final volume to 30 mL. The samples were then tightly screwed and allowed to sit for 30 minutes after which they were vigorously shaken before taking absorbance reading using an UV spectrophotometer (Hach DR 5000) set at 630 nm. Standard series of 0.4, 0.8, 1.6, 3.2, 4.8 and 6.4 mg L⁻¹ were also prepared by pipetting 0.5, 1, 2, 4, 6, and 8 mL of 24 mg L⁻¹ Si and treated in like manner as the digested plant samples.

2.2.6 Data Analysis

2.2.6. a. Correlation between Soil Silicon and Plant Silicon

The relationship between soil Si extracted by different procedures and various plant response variables was determined using regression analysis in the PROC REG procedure in SAS 9.4 (SAS Institute, 2012). The relationship between extractable soil Si based on different procedures and Si rates was also evaluated using regression analysis with the PROC REG procedure. The coefficient of

determination (r^2) and P -value were used as standards to determine the significance of their relationship.

2.2.6. b. Determination of Critical Soil Silicon Levels

The critical soil Si level was determined by plotting the soil Si concentration extracted by different extractants on the X-axis against either the grain yield or the total Si uptake on the Y-axis. Two different statistical methods were used for estimating the critical soil Si level; including the quadratic regression and linear-plateau model. The critical soil Si level was defined as the soil Si concentration above which there was no significant increase either in grain yield or Si uptake by plant; and below which there were significant increases in grain yield or plant Si uptake (Waugh et al. 1973). The quadratic regression model was used to estimate the optimum soil Si concentration. This model estimate factors that were separately derived from individual site and soil type across the 12 site years; and this was achieved by plotting the soil extractable Si on the x-axis against the total Si uptake on the y-axis in the PROC REG procedure using SAS 9.4 (SAS institute, 2012). The critical soil Si concentration on the x-axis was considered as the value corresponding to the plant Si uptake on the y-axis at the peak of the regression line.

According to Boquet et al. (2009), the linear-plateau model establishes the yield corresponding to the ideal soil nutrient concentration and it is the point at which the linear and plateau are joint. Plant response to additional fertilization remains constant after this point and there is no statistically significant difference between all response data points that fall along the plateau. After this point, it is highly unlikely that crops will benefit from increasing soil nutrient concentration and yield may start to decrease if the soil nutrient is continuously increased above this point. Conversely, plants response to additional fertilization is highly expected at any point below

the joint of the plateau and crops plants are expected to benefit from addition of fertilizer. The linear-plateau model was performed using the NLIN procedure in SAS 9.4.

2.3 Results and Discussion

2.3.1 Estimates of Plant-Available Silicon Based on Different Extraction Procedures

The soil Si content differs greatly with soil type and extractants used. Out of the five different soil series that involve this study, the Sharkey clay soil at Gilbert site had the highest initial soil Si content across the seven extractants with an average initial Si of 207 mg kg⁻¹ followed by Crowley silt loam soil with an average initial Si of 180 mg kg⁻¹ (Table 2.1). The lowest average initial soil Si of 60 mg kg⁻¹ across all seven extraction procedures was observed on Kaplan silt loam soil at the Lake Arthur site. St. Landry (Tensas Sharkey complex soil) and Mamou (Mowata silt loam soil) sites presented initial average Si of 63 and 65 mg kg⁻¹, respectively (Table 2.1). The plant-available Si extracted from soil using different extractants which included 0.5 M OAc-1, 0.5 M OAc-2, 0.1 M citric acid, deionized water, 0.01 M CaCl₂, 0.5 M NH₄OAc and 1 M NaOAc ranged from 8.8-297, 10.4-183, 91-1761, 3.1-100, 15.4-231, 2.2-50.5, and 5.9-255 ug g⁻¹, respectively. The plant-available soil Si extracted by individual extractant regardless of the soil type were in the order of highest to lowest 0.1 M citric acid > 0.5 M OAc-2 > 0.5 M OAc-1 > 0.5 M NH₄OAc > 1 M NaOAc > deionized water > 0.01 M CaCl₂.

From these results, it can be seen that 0.1 M citric acid, 0.5 M OAc-2, 0.5 M NH₄OAc, and 1 M NaOAc extracted the greatest amount of soil Si while deionized water and 0.01 M CaCl₂ extracted the least. Narayanaswamy and Prakash (2009) observed similar trends from their experiment involving six different rice producing soils in India and suggested that the amount of Si extracted from soil may vary depending on several factors such as the ability of the extractant to

extract both exchangeable and adsorbed Si, pH of the soil, as well as the extracting solution and shaking period. In a separate experiment, Fox et al. (1967) also reported that acetic acid, sulfuric acid and calcium dihydrogen phosphate extracted more Si from soil than deionized water and CaCl₂ and therefore concluded that CaCl₂ and deionized water extract only the easily soluble or exchangeable forms of Si while solutions with lower pH extract other forms of Si that may not be readily available for plant uptake.

2.3.2 Effect of Slag Application on Soil pH and Soil Silicon Content

The results of the analysis of variance on the effect of slag application on pH and soil extractable Si based on different extractant are summarized in Table 2.7. Slag application significantly increased the soil pH in five out of twelve sites. The increases in pH were observed in Lake Arthur in 2014 and 2015 but not in 2013. In Mamou, St. Landry and Crowley, pH was increased only in 2014. There was no significant change in pH observed in Gilbert site on a Sharkey clay soil. Except for St. Landry which has a Tensas Sharkey complex soil, the other three sites where pH increases were observed consists of light textured or silt loam soils having low initial pH and Si. The differences observed in pH across site years can be due to many factors. Although slag is widely used for correcting low soil pH, its liming potential depends on the particle size, management practices, contact period with soil, and the climate of the region (Alcarde and Rodella 2003). In most of the sites where pH was affected, slag was more effective than lime in increasing the soil pH. These results are in line with those found by Ramos et al. (2006), who studied the effects of slag and lime on pH at different depths under a greenhouse condition and discovered that slag had a greater potential to increase the soil pH than lime at depths of 25 and 30 cm respectively, when applied at the rates of 500 and 1000 kg ha⁻¹. The increase in pH as affected by slag ranged from 0.14 -1.42 units over the control plots. Nolla et al. (2006) reported a similar increase of 0.5 and 0.6 units in pH

with slag application at the rate of 6000 kg ha⁻¹ on an Oxisol. However, in a separate study conducted to determine the liming potential of slag and lime, Louzada (1987) concluded that when limestone and slag having the same particle size are applied, slag can be less effective in increasing the soil pH than limestone.

The effect of slag application on soil Si also varied with site and extractant used. Based on 0.5 M OAc-1 extraction, there was a significant increase in plant-available Si with increasing slag rates for all sites in 2014 excluding Lake Arthur (Table 2.7). However, soil Si only increased with Si application up to 300 kg ha⁻¹ Si in the Gilbert site, after which a decline in soil Si with increasing application rate became evident. This is because the application of slag material causes a fast release of H₄SiO₄ into the soil, causing the soil Si concentration to increase (Kato, 1996). Notwithstanding, the high clay as well as high initial soil Si content of the soil in Gilbert may have caused adsorption of plant-available Si onto clay minerals, or led to polymerization and the formation of polysilicic acid, thereby reducing the availability of monosilicic acid with increasing Si application (Kato 1996). Soil pH could be a factor inhibiting increases in Si in in the Sharkey clay soil in Gilbert. This soil had high initial pH. When pH increases, the exchangeable Si content can be significantly increased; but Si can be adsorbed to the surfaces of variably charged soil colloids such as Fe and Al hydroxides. Such adsorption is highly pH-dependent because at high pH higher proportion of total Si in solution can be present as H₃SiO₄⁻ instead of H₄SiO₄. Because Si is favorably adsorbed in the anionic H₃SiO₄⁻ form, greater Si adsorption occurs at higher soil pH (Haynes 2014). With 0.5 M acetic acid-2, slight increases were observed in soil Si with increasing application, except for the Crowley site in 2015 where an initial decline in soil Si followed by a gradual increase as the rate increases was observed (Figure 2.2). Excluding St. Landry which has a heavy texture soil; all the other sites have light textured soils and it was expected that Si application would increase the soil Si.

Similar trend observed previously in the Gilbert site on Sharkey clay may have been the cause of the trend observed in St. Landry. However, deionized water extractable Si showed an initial increase with Si rate up to 300 kg ha⁻¹ above which soil Si started to decline with application rate up to 900 kg ha⁻¹, and then started increasing again in Gilbert but continued to decline in St. Landry (Figure 2.3). This could be due to the high initial soil Si observed in Gilbert. The NH₄OAc-extractable Si had good relationship with slag rate for all sites in 2014 except for Gilbert, while NaOAc-extractable Si show a positive relationship with slag rate for Lake Arthur in both 2013 and 2014, and for all other sites in 2014 (Figures 2.4 and 2.5). There are several factors that can affect the amount of plant-available Si in soil. For example, the type and amount of clay mineral present in a soil have been shown to have an effect on the availability of soil Si. Takahashi and Sato (2000) reported that the available Si in paddy soils in Japan increased with the amount of clay when the clay content was <290 g kg⁻¹ soil. Similarly, Schwanders et al. (2001) reported that the amount of available Si in Ultisols in the state of Florida increased with clay content. Such report suggest that the high initial Si content of the Sharkey clay soil at Gilbert site could have been due to the high clay content, and that increasing the Si application rate may not necessarily increase the Si availability in this site. Another factor that can influence the soil Si availability is pH. According to Szulc et al. (2015), soils having pH between six and seven are usually sufficient in available Si, while those with pH below six may be deficient in Si depending on texture, and at the pH of 9.8, maximum adsorption of Si can occur. Although we did not attain a pH of 9.8 in our study, the limited effect of slag application on the soil Si content in Gilbert for both 2013 and 2014 as well as in Crowley in 2013 and 2015 might be due to the high pH of the soils in these sites, which may have caused some level of Si adsorption. On the other hand, soil having coarse texture and low pH as in Lake Arthur, Mamou, and St. Landry sites showed some increases in soil Si with increasing rate when extracted with NH₄OAc and NaOAc. All

these sites showed lower initial pH and soil Si than the Gilbert and Crowley sites. As indicated earlier, the Si availability in soil is highly pH dependent. Since low pH can facilitate reaction of Si with Al^{3+} and Fe; and with increasing levels of exchangeable Al and Fe been reported at lower pH, it is likely that the changes in pH brought about by slag application might have caused an increase in Si availability in these sites. These results are similar to those reported by Narayanaswamy and Prakash (2010) who also observed a good relationship between pH, applied Si, plant-available soil Si and various plant response variables using NaOAc as extractant.

2.3.3 Effect of Silicon Application on Grain Yield and Silicon Content of Straw and Grain

In general, Si application positively affected the grain yield in most sites. The most evident increases in grain yield as affected by Si application was observed in Gilbert 2014 ($p < 0.1$) and in Crowley 2014 ($p < 0.1$) (Figures 2.6 and 2.7, respectively). Slag application rates ranging from 1- 4 Mg ha^{-1} seem to be the optimum rates for rice production in Louisiana, since the highest responses in term of grain yield were mostly observed at these rates (Figures 2.6-2.10). Although higher yields were observed at higher rates of 6 and 8 Mg ha^{-1} in some sites, these yield differences are not high enough to warrant such high application rates. Moreover, while it is true that slag is a by-product and may be relatively inexpensive, it should also be taken into consideration that this material is very cumbersome. Thus, transportation expenses can vary with location of the producer; and this may affect the cost of application depending on the distance of the producer from source of fertilizer. Since slag and limestone are not significantly different in their abilities to neutralize soil acidity, Korndorfer et al. (2003) has recommended that slags should be applied at the same rates as limestone. Increases in flooded rice yield arising from Si fertilization were also noticed in Sri-Lanka (Takjima et al., 1970) and India (Singh et al., 2006; Prakash et al., 2007). Snyder et al. (1986) also

Table 2.7 Analysis of variance for treatment effect on soil pH and extractable silicon based on different extraction procedures.

		Crowley 2013							
Slag rate Mg ha ⁻¹	Si rate kg ha ⁻¹	pH	OAc-1†	OAc-2‡	Citric acid	Deionized water	NH ₄ OAc	CaCl ₂	NaOAc
0	0	8.0ab	103abc	134ab	814ab	25a	79abc	20b	84ab
1	140	8.0ab	102bc	130abc	783b	25a	78abc	23a	77abc
2	280	8.0ab	114ab	138ab	867a	24a	84ab	20b	86a
4	560	7.9b	113ab	135ab	775b	24a	77abc	20ab	79abc
6	840	8.0ab	116a	134ab	842ab	26a	84ab	21ab	87a
8	1120	8.1a	113ab	145a	808ab	25a	85a	21ab	86a
2 lime	0	8.0ab	103bc	109c	835ab	25a	76bc	21ab	77bc
4 lime	0	8.0ab	96c	123bc	756b	26a	71c	21ab	70c
<i>P</i> -value		NS	NS	NS	NS	NS	NS	NS	NS

		Crowley 2014							
Slag rate Mg ha ⁻¹	Si rate kg ha ⁻¹	pH	OAc-1†	OAc-2‡	Citric acid	Deionized water	NH ₄ OAc	CaCl ₂	NaOAc
0	0	7.0c	35e	90.cd	467b	19a	29e	14ab	48d
1	140	7.5b	53cd	101b	496b	17ab	32de	15a	50cd
2	280	7.5b	55bcd	100b	538ab	19a	35c	15a	65b
4	560	8.0a	59c	121a	562ab	17ab	41b	13ab	70b
6	840	8.0a	76b	117a	651a	17ab	45a	13ab	91a
8	1120	8.0a	90a	111a	496b	18ab	47a	12b	90a
2 lime	0	7.6b	43de	97bc	546ab	17ab	34cd	13ab	55cd
4 lime	0	7.65b	37e	81d	467b	16b	32cd	13b	55c
<i>P</i> -value		<0.001	<0.0001	<0.01	NS	NS	<0.001	NS	<0.001

Table 2.7 Continued

Slag rate Mg ha ⁻¹	Si rate kg ha ⁻¹	pH	Crowley 2015						
			OAc-1†	OAc-2‡	Citric acid	Deionized water	NH ₄ OAc	CaCl ₂	NaOAc
			-----Soil Si, mg kg ⁻¹ -----						
0	0	8.0a	78abc	137a	856abc	75a	82bc	24a	176a
1	140	8.0a	97abc	116c	860abc	62abc	89abc	20a	165a
2	280	7.9b	122a	124bc	954a	73ab	88abc	18a	178a
4	560	8.0a	112ab	119c	737bcd	64abc	91ab	18a	178a
6	840	8.0a	119a	123bc	896ab	62abc	97a	28a	167a
8	1120	8.0a	71bc	132ab	636d	49c	86abc	29a	160a
2 lime	0	8.0a	58c	115c	730bcd	56bc	77c	20a	167a
4 lime	0	8.0a	65c	122c	694cd	49c	84abc	18a	152a
<i>P</i> -value		NS	NS	NS	NS	NS	NS	NS	NS

Slag rate Mg ha ⁻¹	Si rate kg ha ⁻¹	pH	Gilbert 2013						
			OAc-1†	OAc-2‡	Citric acid	Deionized water	NH ₄ OAc	CaCl ₂	NaOAc
			-----Soil Si, mg kg ⁻¹ -----						
0	0	7.3a	165a	163ab	1302a	21bc	120a	17ab	160a
1	140	7.4a	178a	164ab	1342a	24abc	124a	16b	171a
2	280	7.2a	170a	150bc	1241a	28ab	120a	18a	155a
4	560	7.4a	178a	169a	1374a	20bc	129a	18b	174a
6	840	7.3a	196a	155abc	1298a	17c	121a	16b	184a
8	1120	7.3a	170a	163ab	1336a	37a	125a	17ab	176a
2 lime	0	7.3a	158a	146c	1385	27ab	130a	16b	169a
4 lime	0	7.2a	169a	156abc	1389	26abc	121a	16ab	166a
<i>P</i> -value		NS	NS	NS	NS	0.01	NS	NS	NS

Table 2.7 Continued

Slag rate Mg ha ⁻¹	Si rate kg ha ⁻¹	pH	Gilbert 2014						
			OAc-1†	OAc-2‡	Citric acid	Deionized water	NH ₄ OAc	CaCl ₂	NaOAc
			-----Soil Si, mg kg ⁻¹ -----						
0	0	7.0b	168a	158a	1174ab	40bc	70d	19b	139ab
1	140	7.1b	160a	155a	1135ab	48ab	77abc	19b	124b
2	280	7.3a	212a	161a	1082b	34c	80ab	18b	155a
4	560	7.0b	181a	165a	1312a	34c	83a	21ab	154a
6	840	7.1b	167a	157a	1188ab	33c	75bcd	19b	142ab
8	1120	7.1b	172a	162a	1219ab	46ab	76bcd	120ab	150a
2 lime	0	7.1ab	179a	157a	1055b	49a	74bcd	21ab	146a
4 lime	0	7.0b	162a	155a	1225ab	42ab	72c	23a	139ab
<i>P</i> -value		NS	NS	NS	NS	<0.01	0.08	NS	NS

Slag rate Mg ha ⁻¹	Si rate kg ha ⁻¹	pH	Lake Arthur 2013						
			OAc-1†	OAc-2‡	Citric acid	Deionized water	NH ₄ OAc	CaCl ₂	NaOAc
			-----Soil Si, mg kg ⁻¹ -----						
0	0	5.3a	15bc	46a	330a	10a	37a	7a	9c
1	140	5.4a	21b	43a	305ab	10a	39a	7a	14bc
2	280	5.3a	20b	40a	307ab	10a	41a	6a	17ab
4	560	5.2a	24ab	48a	340a	9a	41a	7a	14abc
6	840	5.4a	20bc	48a	363a	10a	39a	7a	20a
8	1120	5.3a	27a	40a	316ab	9a	39a	7a	17ab
2 lime	0	5.3a	27a	30a	315ab	10a	41a	7a	16ab
4 lime	0	5.3a	15c	41a	247b	9a	37a	7a	22a
<i>P</i> -value		NS	NS	NS	NS	NS	NS	NS	NS

Table 2.7 Continued

Slag rate Mg ha ⁻¹	Si rate kg ha ⁻¹	pH	Lake Arthur 2014						
			OAc-1†	OAc-2‡	Citric acid	Deionized water	NH ₄ OAc	CaCl ₂	NaOAc
			-----Soil Si, mg kg ⁻¹ -----						
0	0	4.8e	17a	25bc	211b	7a	6b	5abc	7c
1	140	4.8e	19a	22c	191b	7a	6b	4c	12bc
2	280	4.9de	18b	24bc	198b	7a	7ab	5ab	15ab
4	560	4.9cd	24a	25bc	177b	7a	6b	5bc	17ab
6	840	5.0bc	30a	28ab	207b	7a	8a	6a	17b
8	1120	5.1ab	24a	31a	273a	7a	8a	5abc	18a
2 lime	0	4.9c	20a	23c	132c	7a	6b	4c	5c
4 lime	0	5.2a	19a	23c	199b	6a	7ab	5ab	5c
<i>P</i> -value		<0.0001	0.016	NS	0.03	NS	NS	NS	0.06

Slag rate Mg ha ⁻¹	Si rate kg ha ⁻¹	pH	Lake Arthur 2015						
			OAc-1†	OAc-2‡	Citric acid	Deionized water	NH ₄ OAc	CaCl ₂	NaOAc
			-----Soil Si, mg kg ⁻¹ -----						
0	0	4.9e	68ab	18a	130ab	18abc	27a	11a	42a
1	140	4.9e	74ab	26a	163ab	26a	26	10a	46a
2	280	5.2a	80a	35a	232a	14bc	23a	8a	45a
4	560	5.2a	71ab	30a	84b	14bc	25a	10a	44a
6	840	4.9bc	67ab	25a	209ab	23ab	29a	9a	36a
8	1120	5.0ab	64ab	28a	219ab	17abc	31a	9a	49a
2 lime*	0	4.9c	74ab	32a	156ab	10c	27a	11a	50a
4 lime**	0	5.1ab	72ab	23a	114ab	14bc	24a	10a	51a
<i>P</i> -value		0.09	NS	NS	NS	NS	NS	NS	NS

Table 2.7 Continued

Slag rate Mg ha ⁻¹	Si rate kg ha ⁻¹	pH	Mamou 2013						
			OAc-1†	OAc-2‡	Citric acid	Deionized water	NH ₄ OAc	CaCl ₂	NaOAc
			-----Soil Si, mg kg ⁻¹ -----						
0	0	5.8ab	19ab	70ab	402	15b	59abc	12b	30ab
1	140	6.0ab	19b	64ab	428b	15ab	46bc	13ab	26ab
2	280	6.0ab	19b	73ab	468ab	19a	59abc	13ab	33a
4	560	6.0ab	13ab	76ab	431ab	16ab	54abc	13ab	30ab
6	840	6.26a	19a	79ab	542a	18ab	48bc	14a	29ab
8	1120	6.0ab	21a	90a	479ab	17ab	70a	13ab	23b
2 lime*	0	5.8ab	18ab	55b	476ab	15b	44c	12b	22a
4 lime**	0	6.0ab	17ab	68ab	408b	17ab	65ab	12ab	26a
<i>P</i> -value		NS	Ns	NS	NS	NS	NS	NS	NS

Slag rate Mg ha ⁻¹	Si rate kg ha ⁻¹	pH	Mamou 2014						
			OAc-1†	OAc-2‡	Citric acid	Deionized water	NH ₄ OAc	CaCl ₂	NaOAc
			-----Soil Si, mg kg ⁻¹ -----						
0	0	5.7d	23abc	28bc	214cd	5c	10bc	7a	14c
1	140	5.9cd	5de	39b	223cd	9ab	9bc	5ab	14c
2	280	6.0dc	21bca	31bc	215cd	11a	10bc	6ab	14c
4	560	6.4b	18bca	38bc	271a	11a	14b	6a	24b
6	840	6.7b	31ab	56a	269ab	10ab	21a	7a	27ab
8	1120	7.1a	34a	65a	221bcd	11a	25a	6ab	36a
2 lime*	0	6.1c	4e	24c	179d	8abc	8c	4b	13c
4 lime**	0	6.4b	9cde	24c	224bc	7bc	9bc	5b	12c
<i>P</i> -value		<0.0001	0.009	<0.001	0.04	0.06	<0.0001	NS	0.002

Table 2.7 Continued

			St. Landry 2013						
Slag rate Mg ha ⁻¹	Si rate kg ha ⁻¹	pH	OAc-1†	OAc-2‡	Citric acid	Deionized water	NH ₄ OAc	CaCl ₂	NaOAc
			-----Soil Si, mg kg ⁻¹ -----						
0	0	5.4a	55b	44a	416bc	12ab	39a	7a	16ab
1	140	5.4a	55b	45a	201bc	12ab	42a	7a	16ab
2	280	5.3a	49bc	43a	382c	11ab	41a	6b	16ab
4	560	5.4a	72a	41a	435bc	10b	39a	2ab	17ab
6	840	5.5a	74a	46a	462ab	10b	41a	6ab	19ab
8	1120	5.3a	76a	47a	413bc	11b	42a	7a	22a
2 lime*	0	5.4a	37c	40a	519a	13a	39a	7a	14b
4 lime**	0	5.3a	60b	41a	519a	10b	44a	6a	21a
<i>P</i> -value		NS	NS	NS	0.003	NS	NS	NS	NS

			St. Landry 2014						
Slag rate Mg ha ⁻¹	Si rate kg ha ⁻¹	pH	OAc-1†	OAc-2‡	Citric acid	Deionized water	NH ₄ OAc	CaCl ₂	NaOAc
			-----Soil Si, mg kg ⁻¹ -----						
0	0	5.7d	176cd	153c	1289ab	19b	83cd	18abc	142d
1	140	5.9cd	191c	164ab	1519a	19b	83cd	18abc	154cd
2	280	6.0dc	193bc	155c	1315ab	24a	86bcd	18abc	162bcd
4	560	6.4b	196bc	169a	1468ab	20b	90ab	19ab	174bc
6	840	6.8b	220ab	160bc	1368ab	19b	88bc	14d	183ab
8	1120	7.1a	227a	168a	1234b	16c	96a	15cd	202a
2 lime*	0	6.0c	172cd	141d	1366ab	21ab	81d	20a	142d
4 lime**	0	6.4b	151d	131e	1454ab	19b	79d	19ab	144d
<i>P</i> -value		<0.0001	0.001	<0.0001	NS	0.01	0.004	0.009	0.001

† - one-hour shaking using acetic acid solution; ‡ - 24 rest + two -hour shaking using acetic acid solution

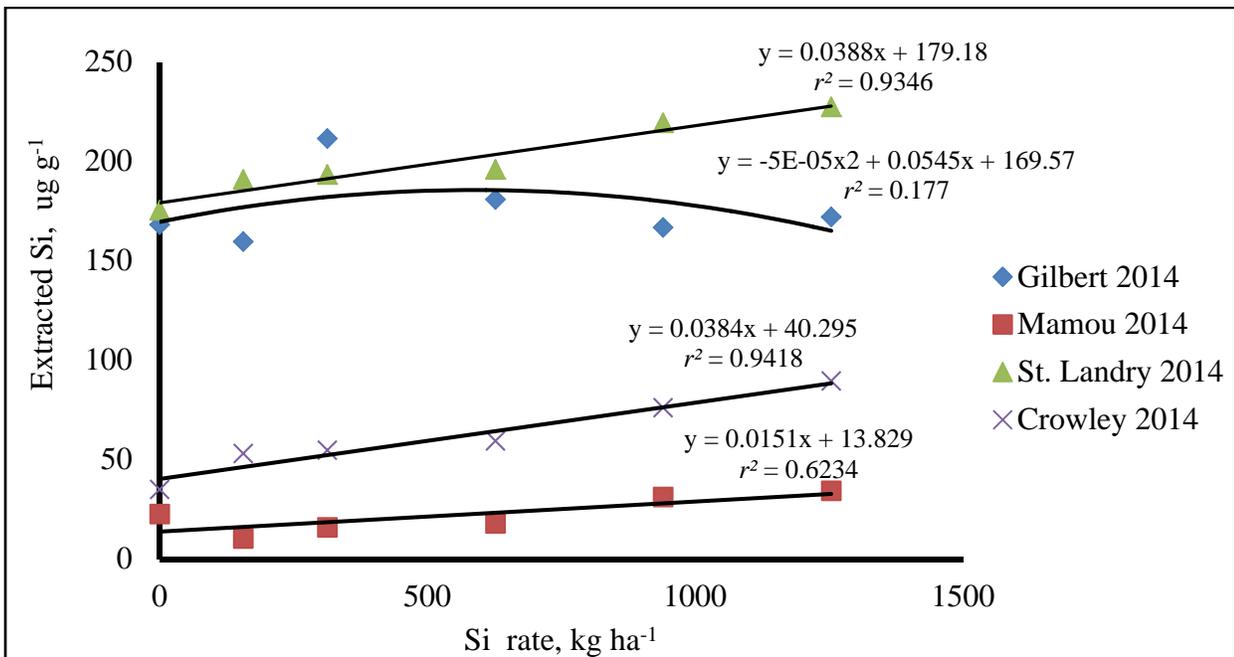


Figure 2.1 Effect of silicon rates on soil silicon as determined by 0.5 M acetic acid-1.

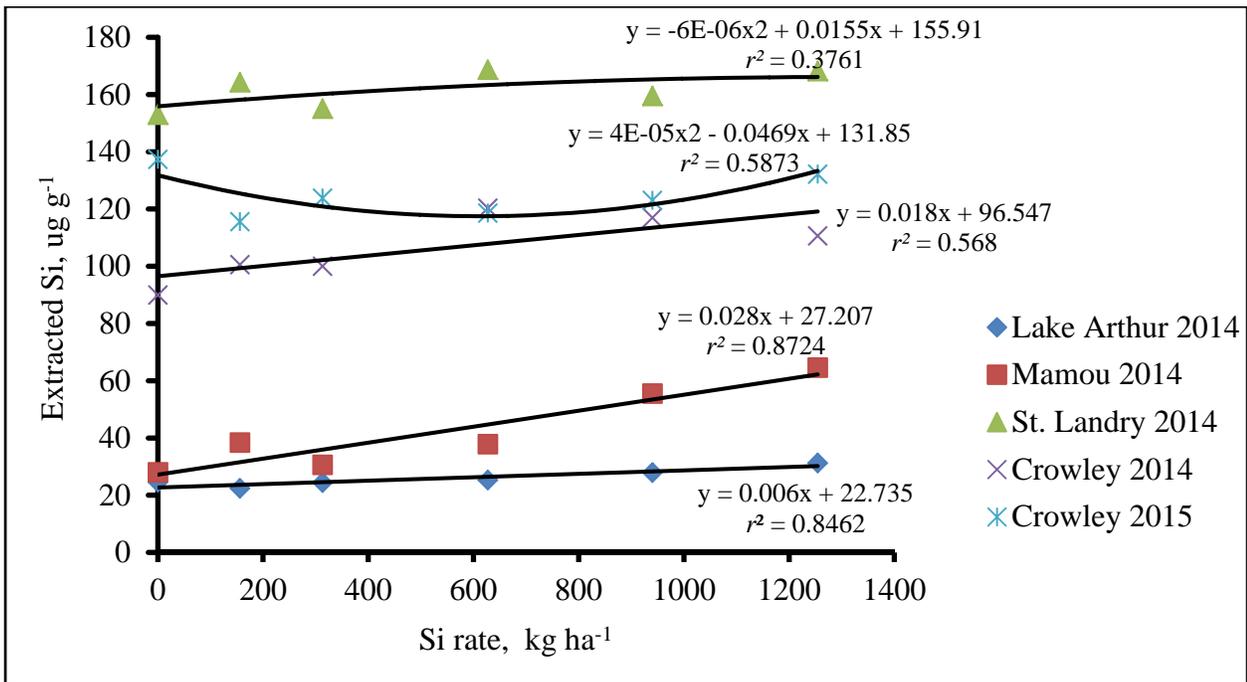


Figure 2.2 Effect of silicon rates on soil silicon as determined by 0.5 M acetic acid-2.

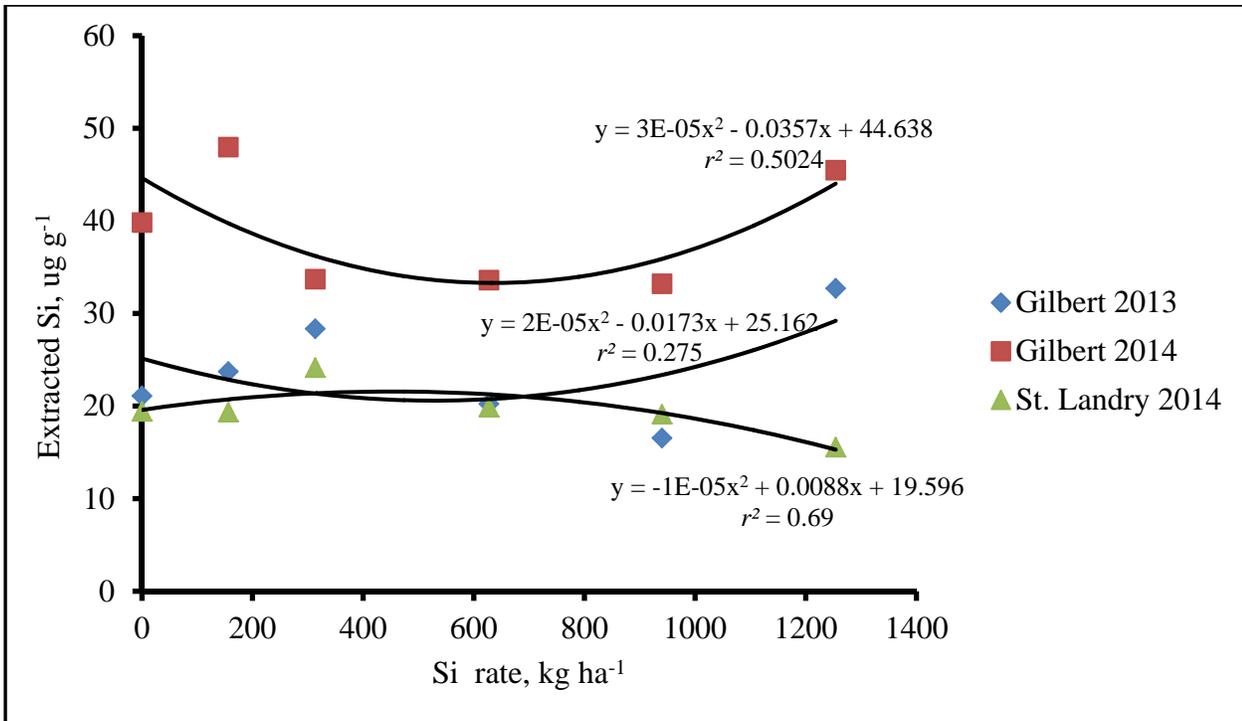


Figure 2.3 Effect of silicon rates on soil silicon as determined by deionized water.

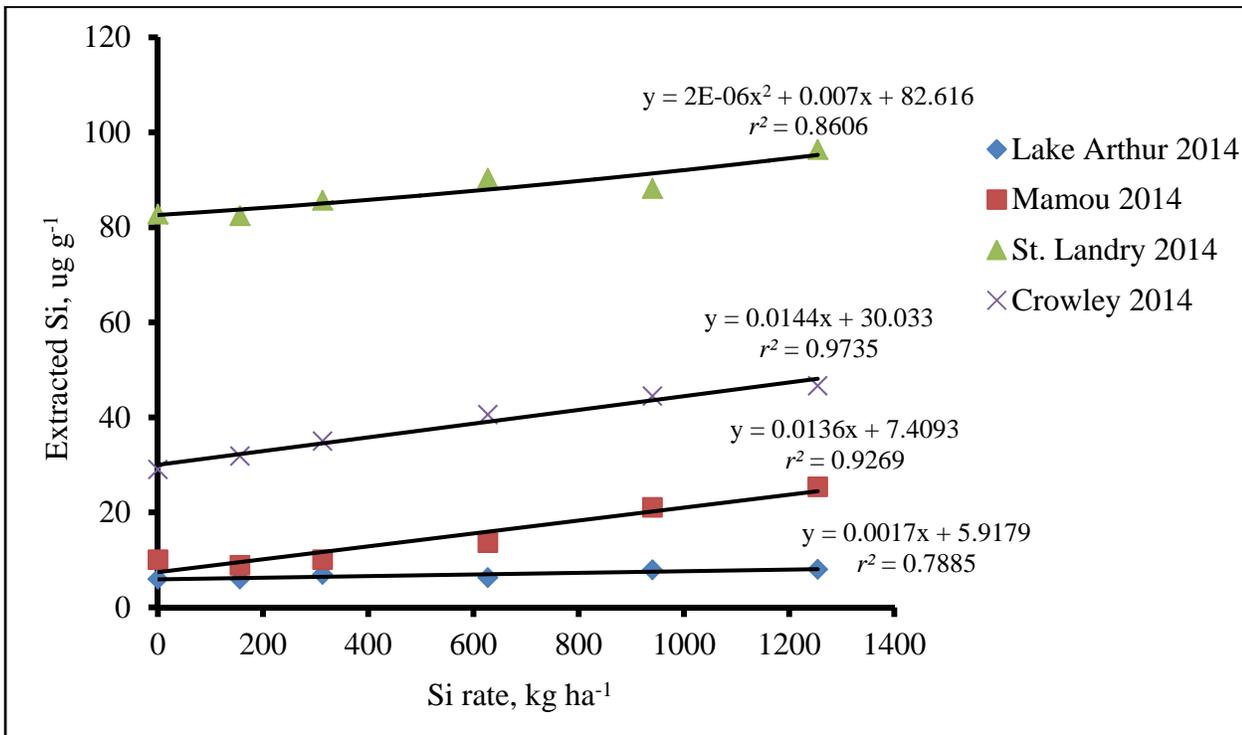


Figure 2.4 Effect of silicon rates on soil silicon as determined by 0.5 M ammonium acetate.

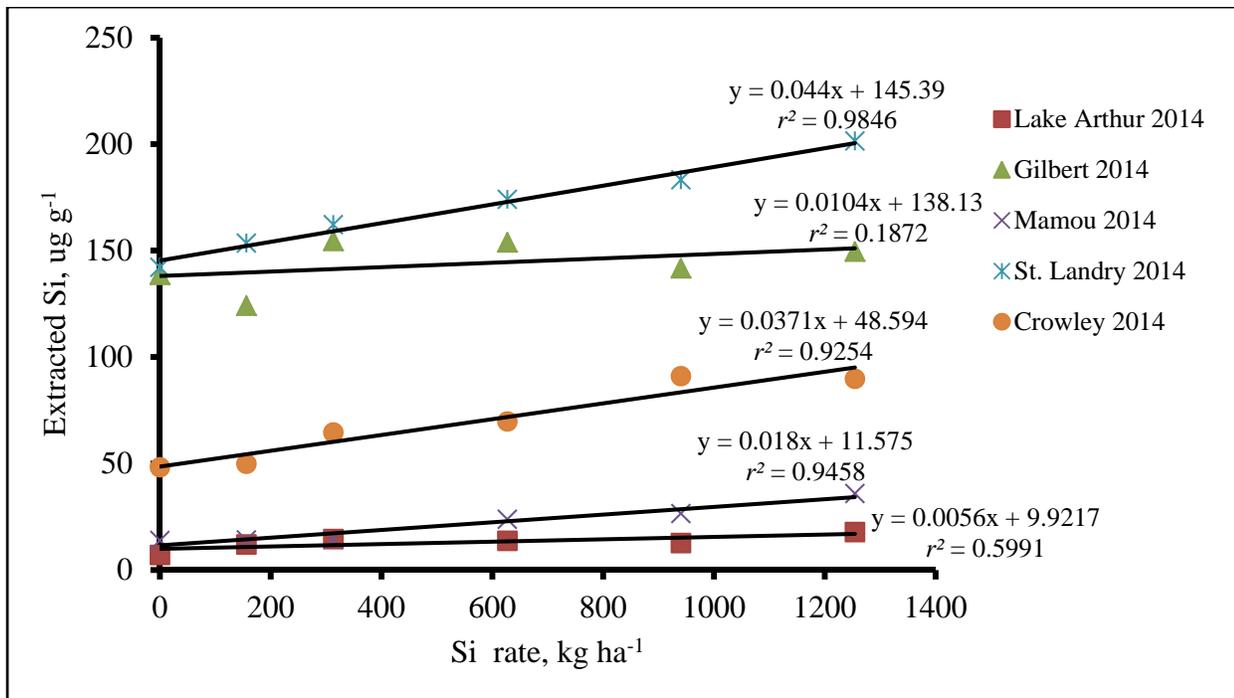


Figure 2.5 Effect of silicon rates on soil silicon as determined by 1 M sodium acetate.

showed that the application of calcium silicate increased rice yields in Histosols mainly because of the supply of Si and not those of other nutrients. The effect of Si on decreasing incidence of diseases may have contributed to increased yield, but Si fertilization can also increase crop yield in the absence of diseases (Datnoff et al., 1992). The increase in grain yield could be attributed to more utilization of solar radiation, moisture and nutrients initiated by adequate Si nutrition, since enhanced Si uptake helps provide rigidity in the plant cell and enable the rice plant to be more erect during the various growth stages for efficient utilization of solar radiation (Rani et al., 1997). Agarie et al. (1992) reported that the maintenance of photosynthetic activity due to Si fertilization could be one of the reasons for increased dry matter production. Korndorfer et al. (2001) reported yield increases in 19 out of 28 sites in Florida where soils were amended with Si application. The increase in grain yield as affected by Si application may be due to the increase in plant-available Si resulting from Si application. Silicon application did not affect the grain Si content but had an effect on the

straw Si content especially in Lake Arthur in 2014 and Mamou in 2014 (Table 2.8). Generally regarding Si and rice grain yield, Si fertilization may increase the Si availability when soil with low inherent Si is amended with adequate Si fertilization, thus leading to increase in Si uptake by rice with a subsequent increase in straw Si content and grain yield.

Table 2.8 Effect of silicon application on rice grain yield, grain and straw silicon content and plant silicon uptake across all site years.

Location	Year	Grain Yield <i>P</i> -values	Si Content		Plant Si uptake <i>P</i> -values
			Grain	Straw	
Lake Arthur	2013	0.1563	0.9201	0.7101	0.6324
	2014	0.5682	0.9002	0.0971	0.9234
	2015	0.7270	0.8811	0.9292	0.8255
Gilbert	2013	0.1146	0.8806	0.5409	0.6564
	2014	0.0129	0.4224	0.6778	0.1393
Mamou	2013	0.9328	0.8113	0.4068	0.7864
	2014	0.1348	0.1279	0.0773	0.1008
St. Landry	2013	0.8399	0.2077	0.9125	0.9291
	2014	0.3467	0.2979	0.5566	0.4532
Crowley	2013	0.6106	0.3519	0.5491	0.2897
	2014	0.0227	0.3898	0.3534	0.5708
	2015	0.3328	0.1687	0.6157	0.9224

2.3.4 Relationship between Soil Si Extracted by Different Solutions and Plant Response Variables.

The relationship between the plant-available Si in soil and various growth parameters of rice was determined using the PROC CORR procedure in SAS. All seven extractants used in this study showed a significant positive relationship of Si concentration in soil and plant response (Table 2.9). However, the highest levels of significant correlation were observed among Si extracted by OAc-1, OAc- 2, citric acid, NH₄OAc, and NaOAc with panicle Si content, straw Si content and total Si content. Similar results were obtained by Narayanaswamy and Prakash (2010) for both NaOAc and OAc-2, and suggested that these two extractants could be the best for estimating plant-available Si in soils.

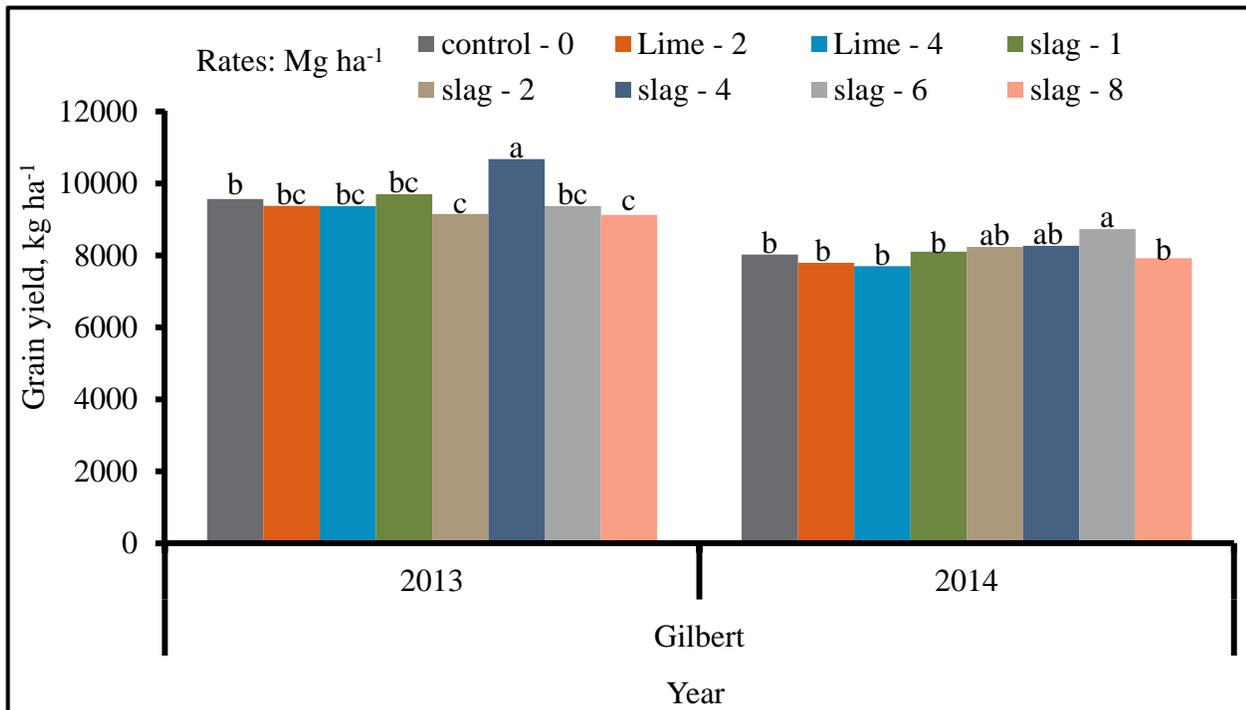


Figure 2.6 Grain yield of rice supplied with varying rates of silicate slag and lime in Gilbert in 2013 and 2014. Bars labeled with the same letter within years are not significantly different based on Fisher's LSD at ($p < 0.1$).

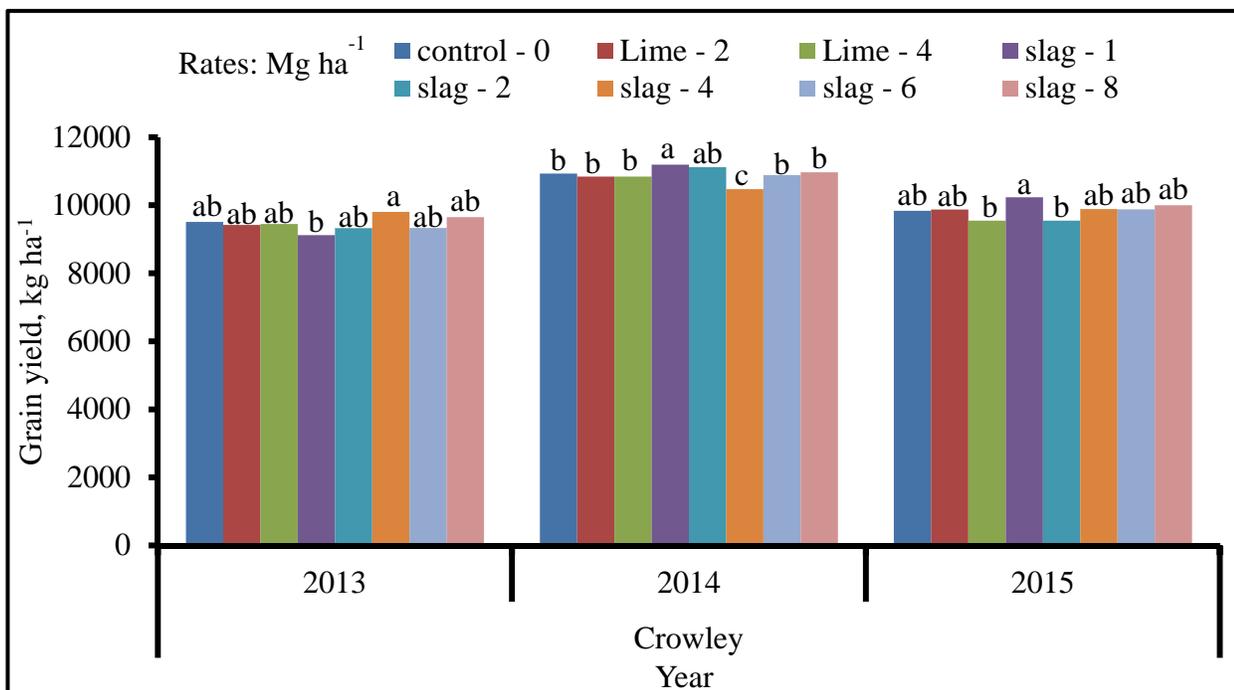


Figure 2.7 Grain yield of rice supplied with varying rates of silicate slag and lime in Crowley in 2013, 2014 and 2015. Bars labeled with the same letter within years are not significantly different based on Fisher's LSD at ($p < 0.1$).

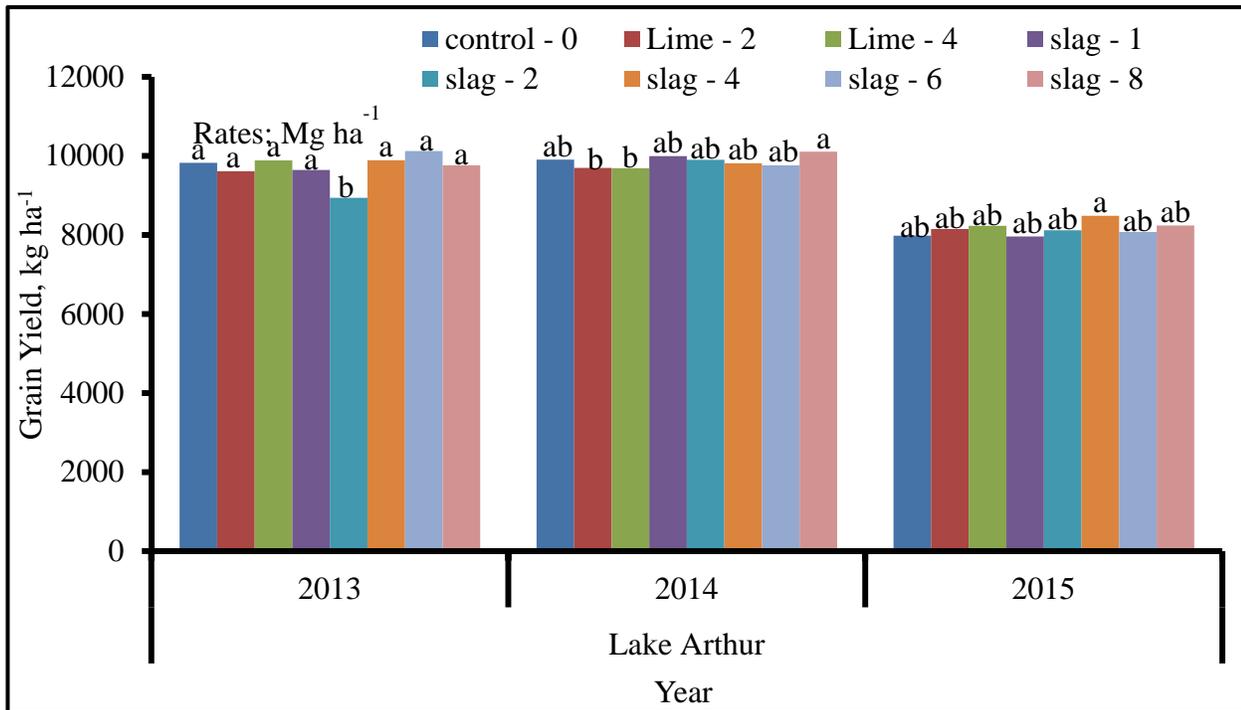


Figure 2.8 Grain yield of rice supplied with varying rates of silicate slag and lime in Lake Arthur in 2013, 2014 and 2015. Bars labeled with the same letter within years are not significantly different based on Fisher's LSD at ($p < 0.1$).

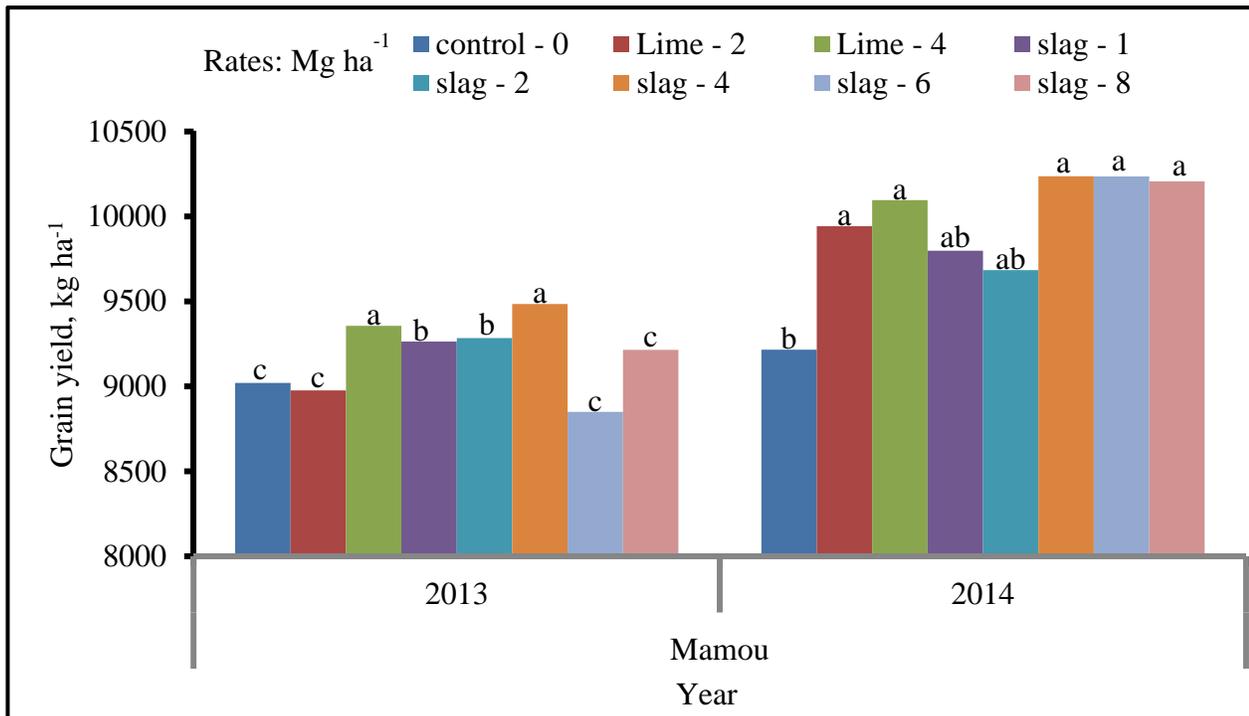


Figure 2.9 Grain yield of rice supplied with varying rates of silicate slag and lime in Mamou in 2013 and 2014. Bars labeled with the same letter within years are not significantly different based on Fisher's LSD at ($p < 0.1$).

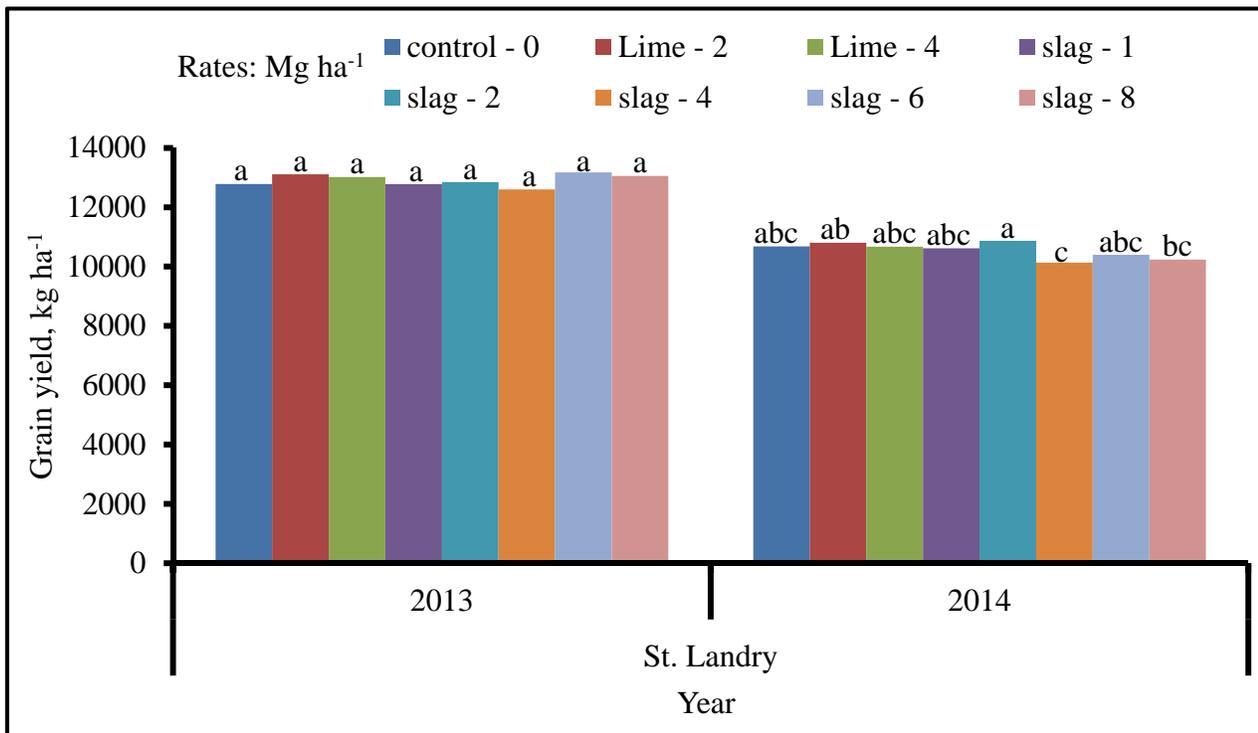


Figure 2.10 Grain yield of rice supplied with varying rates of silicate slag and lime in St. Landry in 2013 and 2014. Bars labeled with the same letter within years are not significantly different based on Fisher's LSD at ($p < 0.1$).

Although NH_4OAc - and citric acid-extractable Si showed good relationships with plant response variable, they can also extract adsorbed forms of Si due to their low pH and high extraction powers, especially when used in soil with high clay content and high adsorbed Si content. Hence, their usage could overestimate the amount of plant-available Si in soils. Calcium chloride and deionized water have lower extraction powers and they mostly extract the exchangeable Si in soil solution. Hence, when used on soils mostly containing adsorbed Si, these two extractant could underestimate the plant-available Si.

2.3.5. Critical Soil Silicon level for Rice in Louisiana

The critical soil Si level (level below which an increase in yield or plant response to Si fertilization can be expected, and above which to significant plant response is expected) varied

Table 2.9 Correlation between soil Si extracted by different solutions and plant response variables.

Plant response variables	Extractors						
	0.5 M OAc-1†	0.5 M OAc-2‡	0.1 M Citric Acid	Deionized Water	0.5 M NH ₄ OAc	0.01 M CaCl ₂	1 M NaOAc
	----- Coefficients of correlation -----						
Panicle Si content	0.53***	0.55***	0.55***	0.26***	0.42***	0.40***	0.48***
Straw Si content	0.42***	0.64***	0.54***	0.10*	0.56***	0.33***	0.35***
Total Si content	0.53***	0.68***	0.61***	0.20**	0.57***	0.40***	0.45***
Panicle Si uptake	0.35***	0.35***	0.35***	0.51***	0.28***	0.44***	0.45***
Straw Si uptake	0.18**	0.18**	0.22***	0.24***	0.30***	0.29***	0.34***
Total Si uptake	0.27***	0.28***	0.30***	0.38***	0.34***	0.29***	0.34***

*** = $p < 0.001$; ** = $p < 0.01$; * = $p < 0.05$

widely across sites and extractants. Several researchers have used the relative biomass yield to report critical soil Si levels (Narayanaswamy and Prakash, 2009; Kondorfer et al., 2001). For this study, the actual grain yield and plant Si uptake were used as response variables for estimation of the critical Si level. The linear-plateau gave an estimate of critical level at 37 ug g^{-1} based on 0.5 M OAc-1 extraction procedure using grain yield as response variable for five sites (Figure 2.11). This was the only extractant that was able to show a critical level between soil Si and grain yield. There was no clear pattern between grain yield and soil Si levels using the rest of the extraction procedures evaluated in this study. Narayanaswamy and Prakash (2009) reported a critical Si level of 54 ug g^{-1} Si using 0.5 M OAc as the extractant but using relative biomass yield as response variable. The linear-plateau model gave similar critical level of 54 ug g^{-1} for 0.5 M OAc-1 when plant Si uptake was used as response variable (Figure 2.12). The critical soil Si levels determined using 0.5 M OAc-2, 0.1 M citric acid, deionized water, 0.5 M NH_4OAc , 1 M NaOAc, and 0.01 M CaCl_2 versus plant Si uptake were 116, 771, 47, 87, 198, and 11.8 ug g^{-1} , respectively (Figures 2.13- 2.18). The critical Si levels based on deionized water and CaCl_2 extraction procedure are lower than the than those already established in previous studies. The critical levels determined by OAc-2, citric acid, NH_4OAc , and NaOAc are much higher than those already established by other researchers. These high critical levels were somehow expected for these extractants because acidic solutions have higher extracting power and can easily dissolve slag material, bringing more Si into solution. Haynes et al. (2013) cautioned that acidic extractants can extract more Si from soil treated with slag because this material is easily soluble in acids, and that extracting slag-treated soil with acidic extractant should be done with caution because some Si extracted by these solutions may come from undissolved slag material that may still be present in the soil. Comparable observations have been made for the NaOAc solution

buffered at (pH 4.0) method by Imaizumi and Yoshida (1958), and also by both Sumida (2002) and Wang et al. (2001). It is therefore advisable to consider the use of these extractants with caution in order to avoid overestimation of plant-available Si.

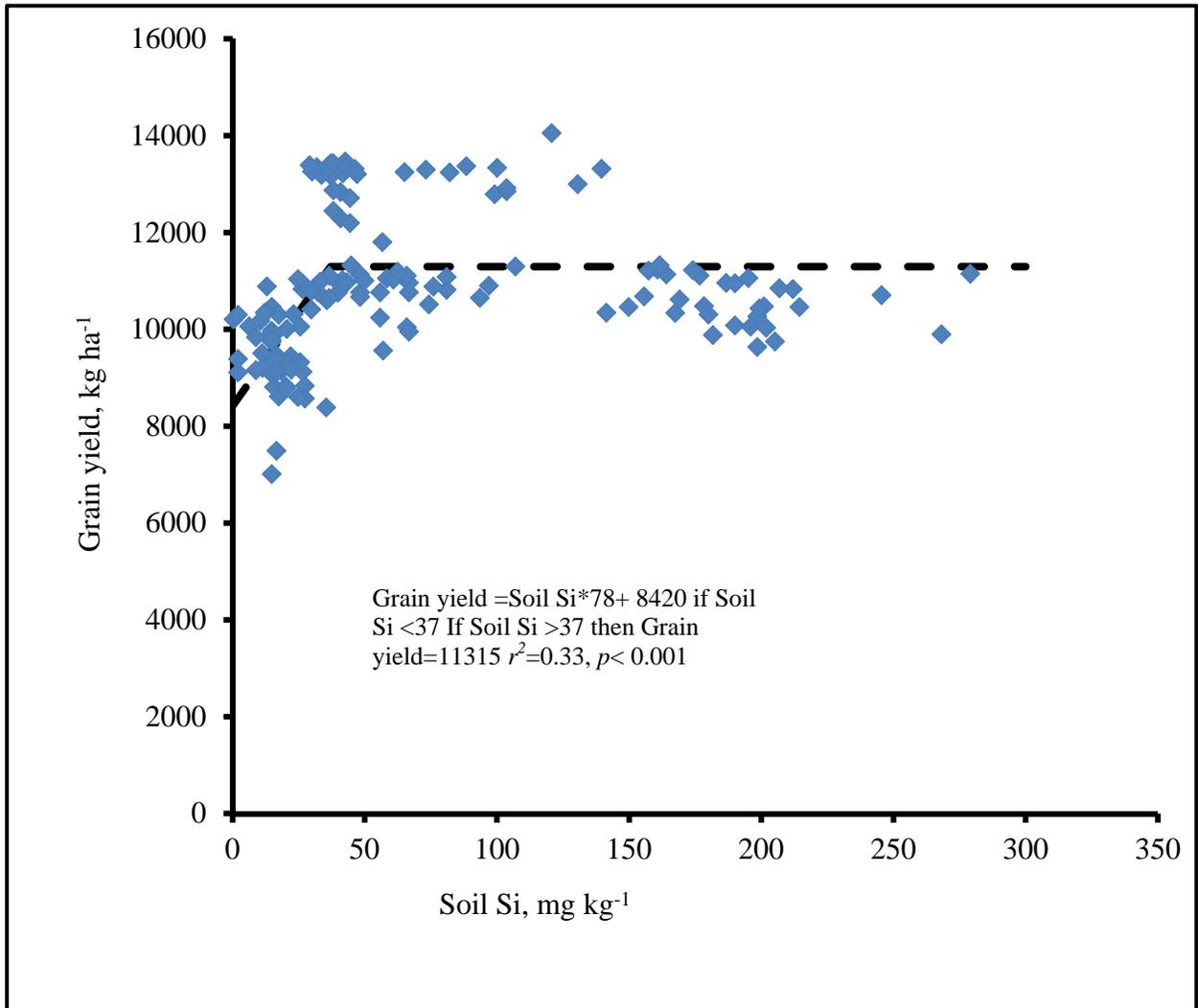


Figure 2.11 Critical silicon level for five sites estimated by linear-plateau model using 0.5 M acetic acid-1 extraction procedure.

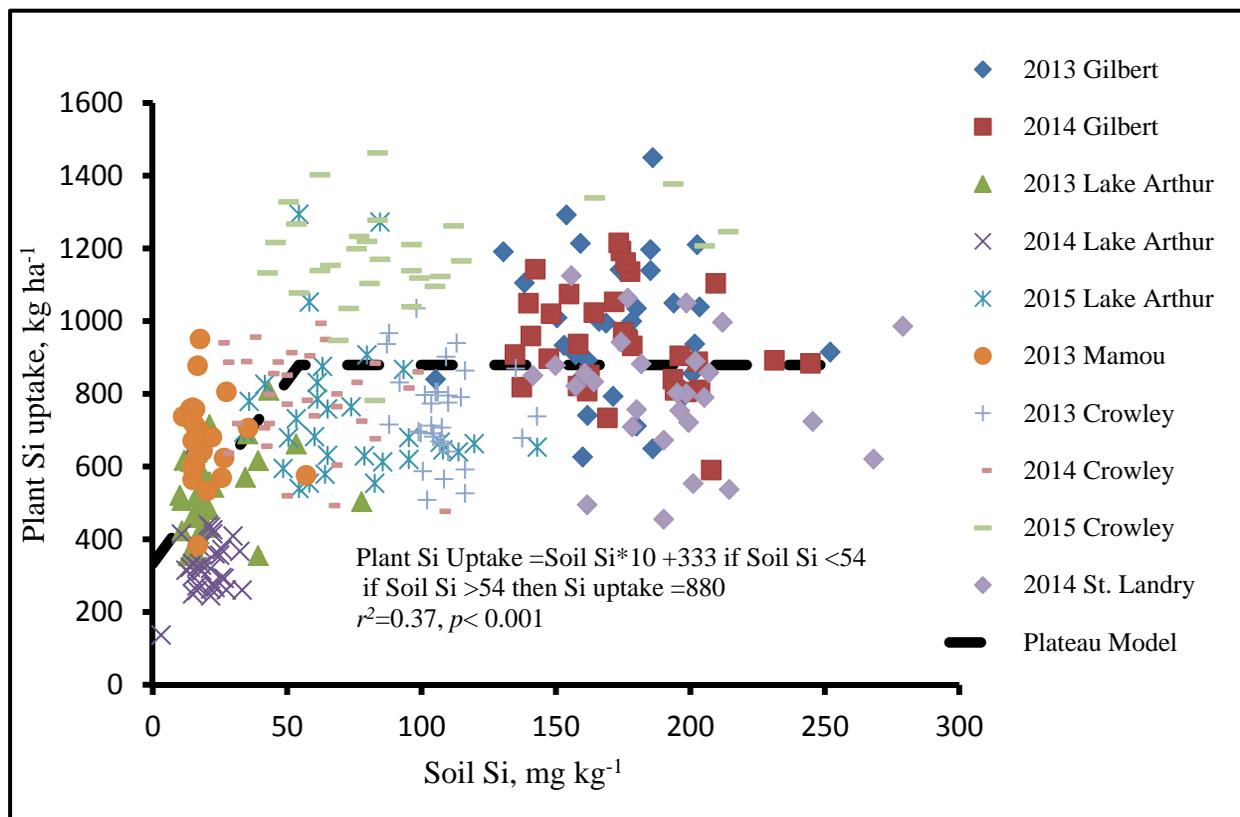


Figure 2.12 Critical silicon levels in soil estimated by linear-plateau model for 0.5 acetic acid-1 extraction procedure.

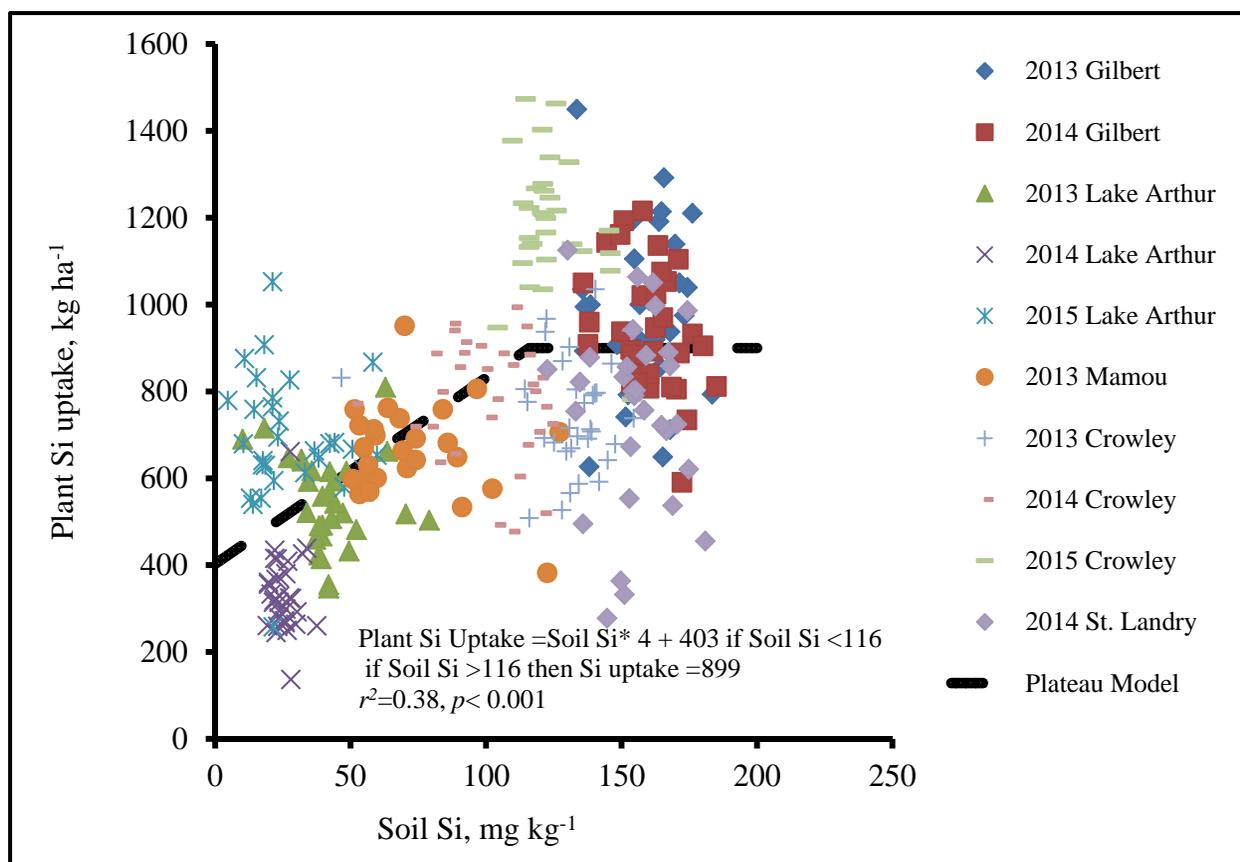


Figure 2.13 Critical silicon level 1 in soil estimated by linear-plateau model for 0.5 acetic acid-2 extraction procedure.

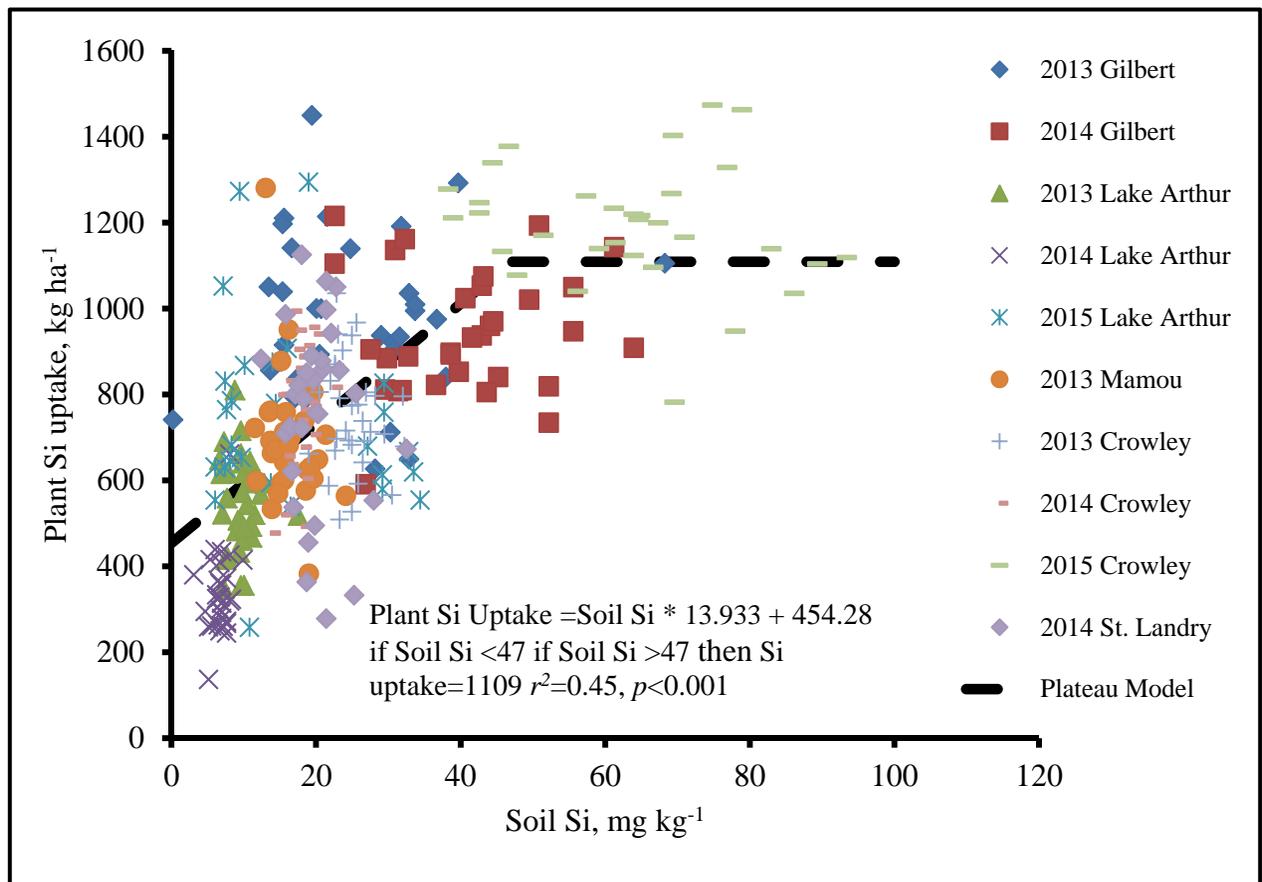


Figure 2.14 Critical silicon level in soil estimated by linear-plateau model using 0.1M citric acid extraction procedure.

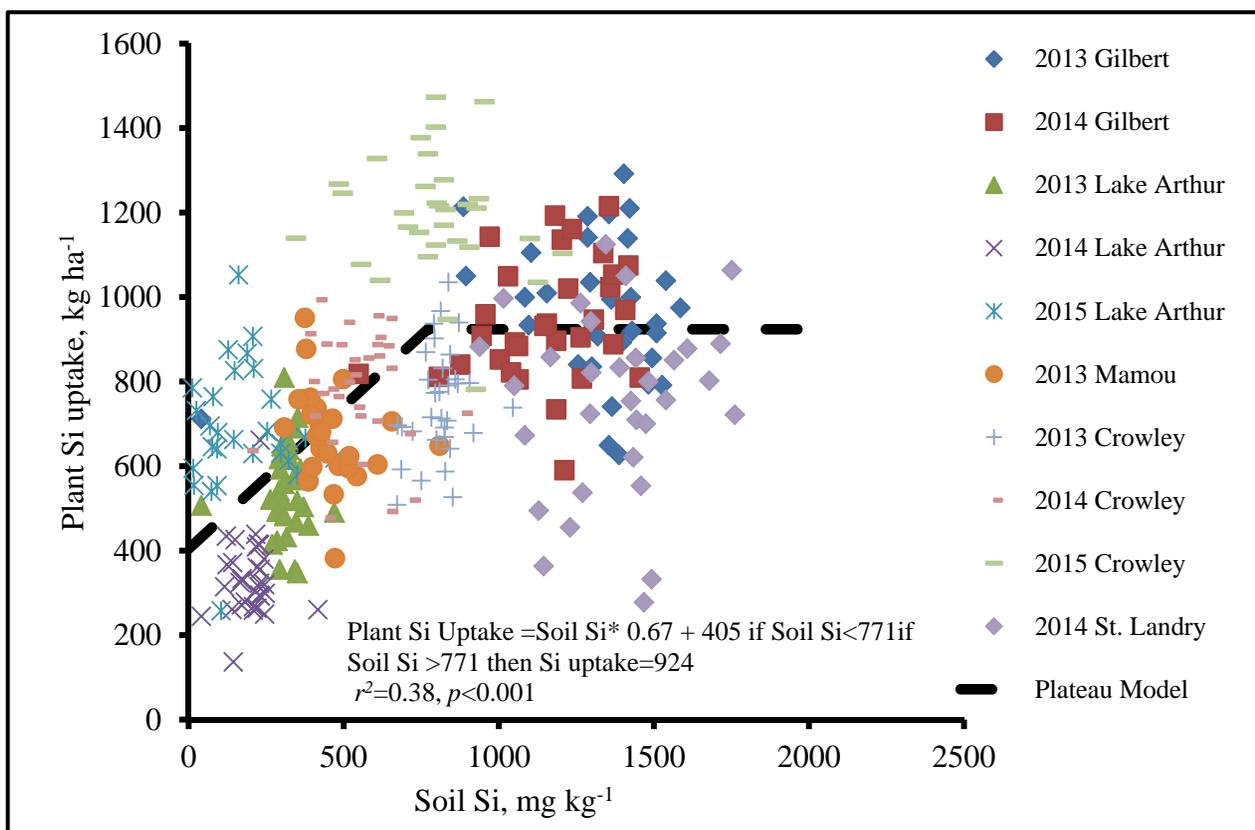


Figure 2.15 Critical silicon level in soil estimated by linear-plateau model using deionized water extraction procedure.

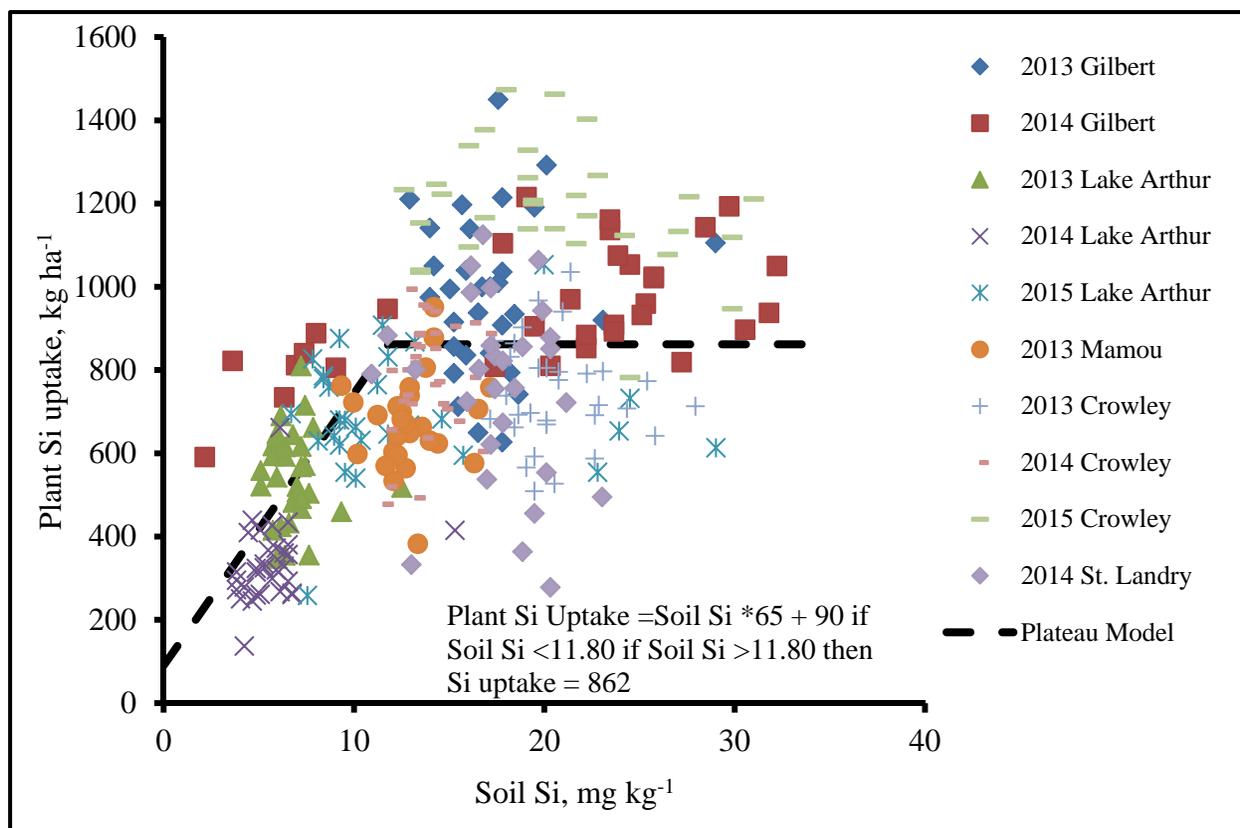


Figure 2.16 Critical silicon levels in soil estimated by linear-plateau model using 0.01 M calcium chloride extraction procedure.

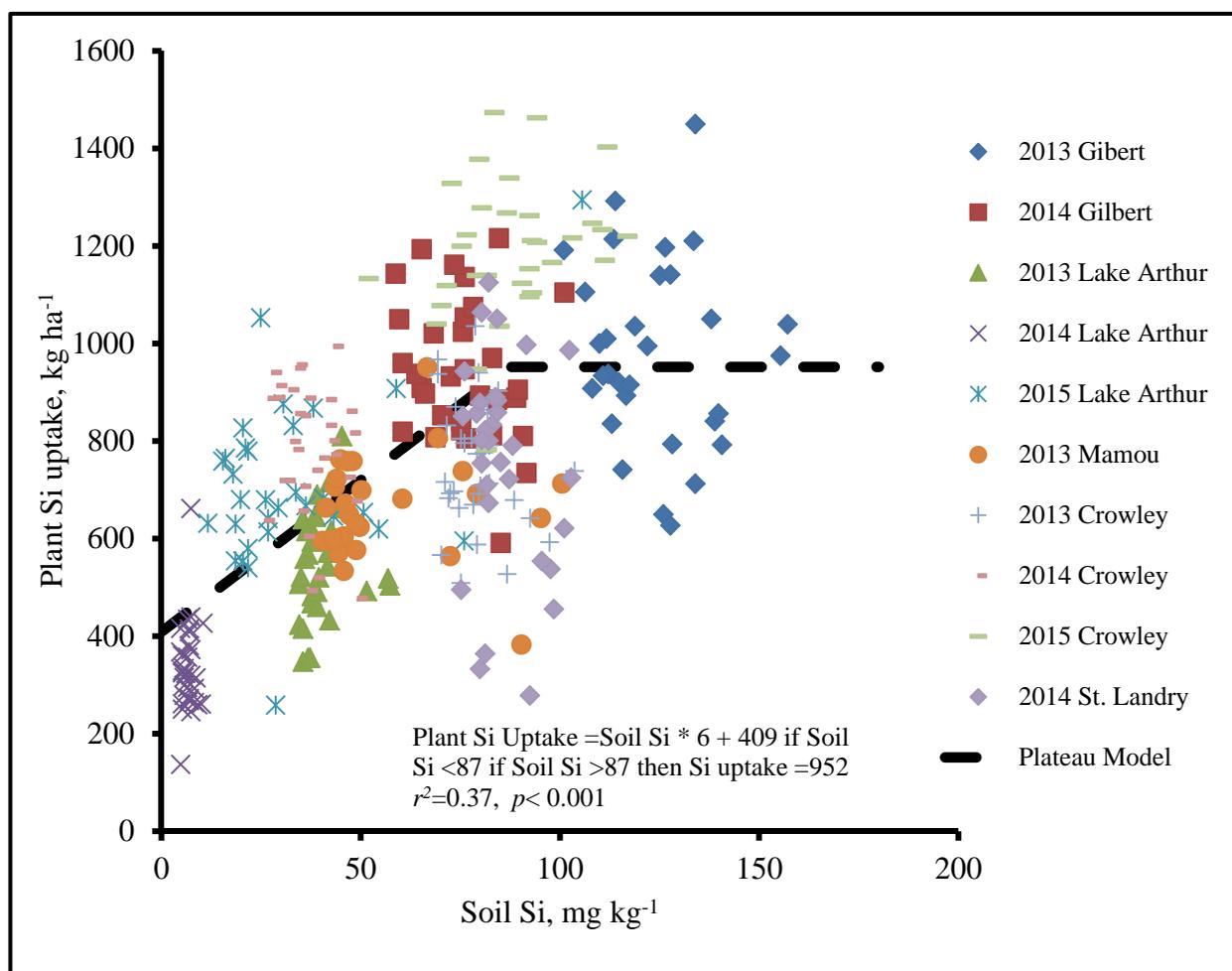


Figure 2.17 Critical silicon levels in soil estimated by linear-plateau model using 0.5M ammonium acetate extraction procedure.

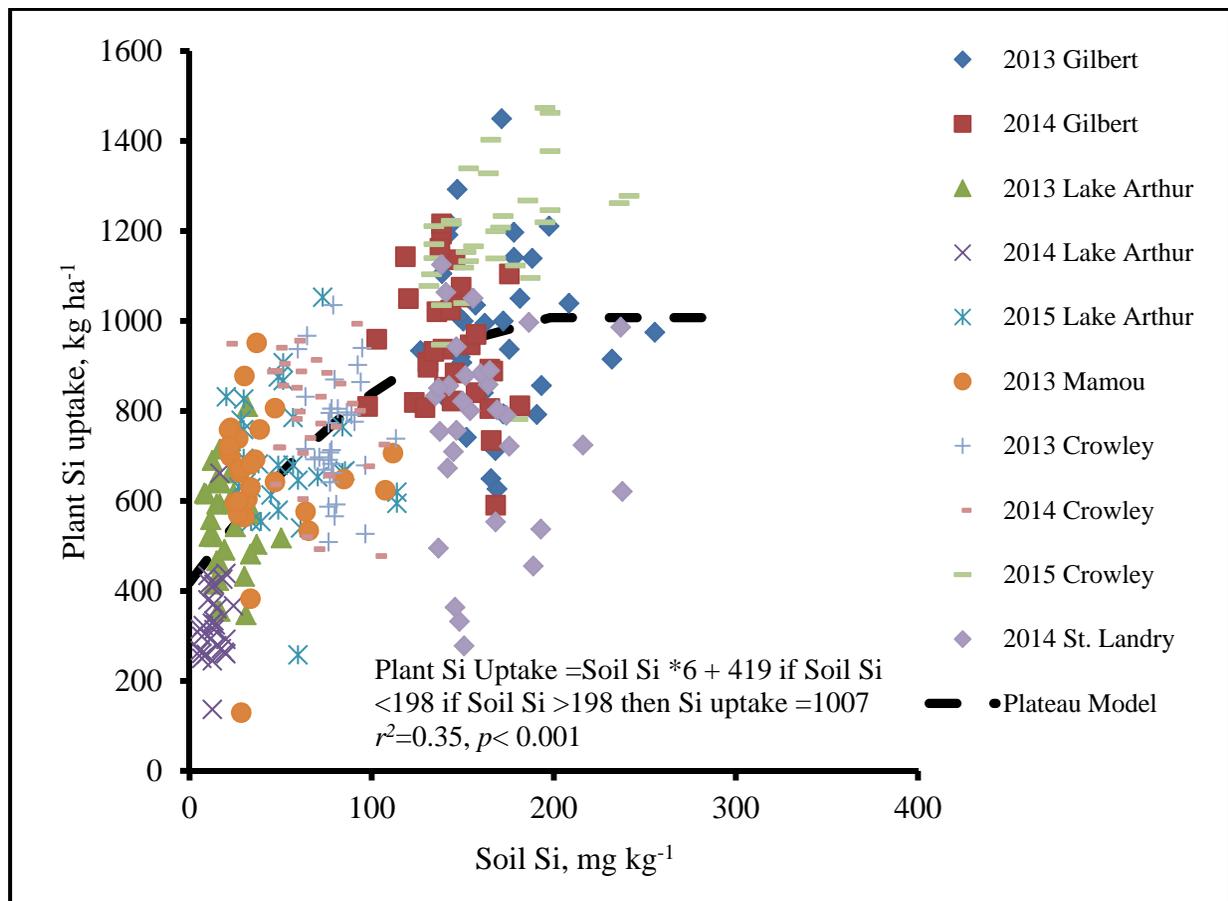


Figure 2.18 Critical silicon level in soil estimated by linear quadratic model using 1M sodium acetate extraction procedure.

2.4 Conclusions

Slag application significantly increased soil pH in five out of twelve site-years. Except for the St. Landry site which has a Tensas Sharkey complex (a medium texture soil), all other sites where pH increased have light textured soil. While there were increases in available soil Si with slag application, the increases were not always linear for all sites, suggesting that slag application may not always lead to increasing dissolve Si (H_4SiO_4) concentration in solution. Several factors including polymerization, the amount and type of clay present in soil, and management practice can influence the Si availability in soil. Critical Si levels determined by different extraction procedures were highly variable. The critical level of 37 $\mu g\ g^{-1}$ and 54 $\mu g\ g^{-1}$ determined by 0.5 M OAc-1 using grain yield and plant Si uptake, respectively was in agreement with previous critical levels already established, while deionized water and 0.01 M $CaCl_2$ resulted in a critical level below those already established. Depending on the soil texture and Si availability, the use of these two extractants may underestimate Si availability in soil. Solution such as 0.5 M OAc-2, citric acid, NH_4OAc and $NaOAc$ should be used with caution, as these have the potential to extract both available and adsorbed forms of Si and may have the potential to overestimate the availability of Si in soils. While soil Si determination procedures are still been evaluated, all factors that affect Si availability in soil must be considered when deciding the choice of extractant.

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Chapter 3. Evaluating the Impact of Silicon Fertilization on the Availability of Nutrients in Soil and their Uptake by Rice

3.1 Introduction

Silicon (Si) is not an essential nutrient for higher plants but it is often taken up in large amounts, and its concentration in plant tissues can be higher than some essential macronutrients (Epstein, 1999). Although not considered essential, but several beneficial effects of Si nutrition have been well-documented especially in crops growing under certain stressful conditions usually imposed by biotic and abiotic factors (Epstein, 1994; Datnoff et al., 2001). Recognizing the benefits that Si nutrition renders to sustainable crop production, Si fertilization is gaining wide acceptance especially in rice (*Oryza sativa*) and sugarcane (*Saccharum officinarum*) production, but little attention has been paid to the interaction between Si and other plant nutrients in soils. In a field experiment where wheat (*Triticum aestivum*) plants were supplied with different sources and rates of nitrogen (N) fertilizer along with foliar applied Si, Hellal et al. (2012) discovered that foliar application of 4% Si resulted in increased grain and straw yield and nutrient content of plants supplied with farmyard manure and ammonium sulfate, respectively as N sources. On the contrary, Wallace (1989) reported that Si concentration in plant tissues was decreased with increasing tissue N content. Similarly, a decline in N and phosphorous (P) contents of rice was also reported with enhance Si uptake in straw (Deren, 1997).

Perhaps, studies involving the interaction of Si with other elements in soil and plant have focused mostly on heavy metals that become toxic to plants when available in high concentration. For example, iron (Fe) and aluminum (Al) toxicity in low pH soils can reduce crop yield up to 40% (Nolla et al., 2013). However, Si application to soil in the form of slags is reported to have reduced aluminum toxicity in a wide range of crops including corn (*Zea mays*), cotton (*Gossypium hirsutum*), rice and sorghum (*Sorghum bicolor*) (Cocker et al., 1998; Korndorfer et al., 2003). It is been debated that amelioration of Al phytotoxicity by Si is due to a pH effect of the slag material (Korndorfer et al., 2003). However, Hodson and Evans (1995) have attributed it to the formation of insoluble hydroxyl aluminum silicates resulting from increasing the soil Si concentration. In a separate experiment where corn was planted in soil containing a toxic level of Al, it was discovered that the addition of Si in the form of silicic acid significantly reduced the root growth inhibiting effect of toxic levels of Al (Ma and Matsumoto, 1997). The interaction between Si and Al seems not only to occur in the soil solution but also within the plant system. Accumulation of Si in the nutrient transport pathways within plant

system is reported to have reduced the translocation of Al from root to shoot (Cocker et al., 1998). It is reported that cadmium (Cd) and zinc (Zn) bioavailability was decreased by 24 and 41%, respectively when corn plants were treated with 200 mg kg⁻¹ of Si (Cunha et al., 2008). Treder and Cieslinski (2005) reported that soil application of Si prior to planting strawberry (*Fragaria ananassa*) plants was more effective in reducing Cd content of stem, leaves and fruit when compared to those supplied with foliar Si. Fleck et al. (2013) reported that Si fertilization in rice resulted in a 50% decrease in arsenic (As) contents of the shoot, flag leaf and husk, and a 24% decrease in As⁺³ content of the polished grains in brown rice. In another study involving Si and cation macronutrients, it was reported that tissues potassium (K) content increased with increased application of Si in rice (Gerami and Rameeh, 2012).

The ever growing world's population places high demands for food on the world's arable land, causing farmers to engage in crop intensification practices with little regards to adequate nutrient management. This has led to a decline in crop yield and quality, often associated with inadequate N fertilizer management in some places (Meena et al., 2014). Nevertheless, numerous research works by crop and soil scientists have shown that depletion of the Si level in soils subjected to continuous cropping is also a possible cause for declining yield, especially in rice and sugarcane (Ma et al., 1989; Datnoff et al., 2001). Much of the research involving Si nutrition in plants has focused mostly on the role of Si in inducing plant resistance to biotic and abiotic stresses. Although it has been reported that Si concentration in plant tissues can be higher than most essential macronutrients, it is also necessary to understand the interaction between Si and plant essential nutrients in soil, and how they correlate within the plants. The objective of this study was to document the relationship between plant- availability Si and select essential plant nutrients in soil and their uptake by rice.

3.2. Materials and Methods

3.2.1. Locations, Trial Establishment, and Experimental Design

The study was conducted in Southwest and Northeast Louisiana from 2013 to 2015 with a total of 12 site years. In 2013 and 2014, field trials were established in five parishes including Acadia (Crowley) on a Crowley silt loam soil (Fine, smectitic, thermic Typic Albaqualfs), Evangeline (Mamou) on a mowata silt loam (Fine, smectitic, thermic Typic Glossaqualfs), Vermilion (Lake Arthur) on a Kaplan silt loam and St. Landry (Palmetto) on a Tensas Sharkey complex all of which are in the Southwest, and Franklin parish (Gilbert) on a sharkey clay soil

(Very-fine, smectitic, thermic Chromic Epiaquerts) in the Northeast of Louisiana. In 2015, trials were established in two sites, Crowley and Lake Arthur. Table 3.1 provides details of each site, the soil type and initial pH, organic matter content and extractable nutrients. Soil classes such as Alfisols, Entisols, Inceptosols and Vertisols generally predominate in these areas. These soils have an aquic moisture regime, mostly comprised of clay with mixed sand and silt mineralogy, poorly drained and thermic. They are very deep, nearly flat to gently undulating and generally fit in one of two soil textural classes: loamy or clayey. These soils are part of the alluvial plain found along the Mississippi river valley and have been subjected to continual annual rice production for decades if not centuries. All trials were established in fields that are under current rice cultivation.

Before sowing, the amount of fertilizer required per plot was calculated and weighed out in large zip lock plastic bags. These were then broadcasted by hand and incorporated into soil to a depth of 7 cm. The source of Si was silicate slag which is a by-product of the steel and iron industry. This material contains about 14% Si and may also contain other elements such as Ca, Mg, Fe and S. It can also be used for correcting soil pH in acidic soils. The elemental composition of slag material used for this study is provided in Table 3.2. Agricultural lime was also included in the treatment structure in order to separate the liming effect of slag on soil pH. The treatment structure consisted of five slag rates applied at 1, 2, 4, 6 and 8 Mg ha⁻¹ which are equivalent to 140, 280, 560, 840 and 1120 Kg ha⁻¹ Si, respectively along with two lime treatments of 2 and 4 Mg ha⁻¹. All treatments were arranged in a randomized complete block with four replications.

After slag application, rice seeds were drilled at the rate of 300 seeds m² with 20 cm row spacing in plots measuring 1.5 m x 4.9 m, with a total of 7 rows per plot. Three rice varieties (CL111, Jupiter and CLXL 729) were used for this study. These varieties were selected based on the predominant variety cultivated in each site or location. Phosphorus (P) and K fertilizers were applied where needed, to ensure that their deficiency did not limit growth and production; and at four leaf growth stage, a urea based N fertilizer was applied in accordance with LSUAgcenter recommendation and thereafter, permanent flooding was maintained in the field until physiological maturity. Table 3.3 gives details of field layout and all major field activities carried out during the course of the experiment.

Table 3.1 Initial soil pH, organic matter content, and extractable nutrient contents of different soils.

Site	Soil Series	Organic matter (%)	pH 1:1 water	*Si	Ca	Cu	P	K	Na	S	Mg	Zn
							mg kg ⁻¹				
Crowley	Crowley silt loam	1.44	7.4	103	1595	1.4	6.7	55	59	9.0	256	4.6
Gilbert	Sharkey clay	1.87	6.8	156	4971	5.47	78	408	71	10.6	1013	4.9
Mamou	Mowata silt loam	1.83	5.6	16	1325	1.57	14.4	129	64	18.4	413	2.1
Lake Arthur	Kaplan silt loam	1.47	4.8	11	792	1.1	4.5	101	31	12.8	156	4.9
St. Landry	Tensas Sharkey complex	2.59	7.1	33	3770	2.8	78	228	92	6.3	681	2.8

*Initial soil Si status was determined by 0.5 M acetic acid and 1 hour shaking and then followed by molybdenum blue colorimetric procedure. Organic matter was determined colorimetrically by the Walkley and Black method (Walkley and Black 1936). All essential plant nutrients were extracted using Mehlich-3 procedure followed by ICP analysis.

Table 3.2 Elemental composition of silicate slag.

Element	Percent
Aluminum	7
Calcium	23
Iron	14
Magnesium	7
Manganese	1.6
Silicon	14
Sulfur	0.5

Table 3.3 Location and field activities carried out from 2013 to 2015.

Location	year	Treatment Application and Planting	Variety	Flooding	Harvest
Gilbert	2013	20-May-13	CL111	21-Jun-13	17-Sep-13
	2014	6-May-14	CL111	11-Jun-14	16-Sep-14
Mamou	2013	18-Mar-13	Jupiter	7-May-13	9-Aug-13
	2014	21-Apr-14	Jupiter	20-May-14	16-Aug-13
Palmetto	2013	19-Mar-13	CL111	9-May-13	19-Aug-13
	2014	24-Mar-14	CL111	12-May-14	23-Aug-14
Crowley	2013	14-Mar-13	CL111	12-May-13	5-Aug-13
	2014	13-Mar-14	CL111	16-May-13	6-Aug-14
	2015	20-Mar-15	CL111	15-May-15	10-Aug-15
Lake Arthur	2013	19-Mar-13	CLXL729	8-May-13	27-Aug-13
	2014	13-Mar-14	CLXL729	9-May-14	10-Aug-14
	2015	23-Mar-15	CLXL729	12-May-14	12-Aug-14

3.2.2. Plant Biomass Sampling and Processing

At physiological maturity, plant biomass samples were taken for Si and elemental composition analysis. Whole plant samples were taken from a 1-m section of the third row from each opposite side of the plots by cutting the entire above ground biomass and then combining them into one composite sample for each plot. The samples were then oven dried at 55°C until a constant weight was obtained for each sample and then separated into straw and panicle; and these were ground, digested and analyzed separately before determining their nutrient and Si content.

3.2.3. a. Sample Digestion for Si Analysis

In order to determine their Si content, plant samples digestion was carried out following the Oven-Induced Digestion (OID) procedure as described by Kraska and Breitenbeck (2010). One hundred milligram (100 mg) of finely grind samples were weighed out in 50 mL polyethylene screw-cap centrifuge tubes. The tubes were then capped loosely and placed in a conventional oven set at 60°C for 15 minutes to remove any atmospheric moisture that may have come in contact with the samples during storage. The samples were then removed and 5 drops of octanol (octyl-alcohol) were added in order to prevent excessive foaming upon addition of hydrogen peroxide (H_2O_2) and sodium hydroxide (NaOH). Two mL of 30% H_2O_2 were added to the samples while ensuring that no particles were left on the wall of the tubes and then all tube were loosely capped and placed back into the oven set at 95°C for 30 minutes. After that, 4 mL of 50% NaOH were added and the tubes were placed back into the oven for four hours of continuous digestion; mixing the samples every 15 minutes with a vortex mixer. The samples were removed after four hours and 1 mL of 5 mM ammonium fluoride (NH_4F) was added in order to facilitate the formation of H_4SiO_4 and then diluted with deionized water to a final volume of 50 mL.

3.2.3. b. Plant Si Analysis

Molybdenum Blue Colorimetric (MBC) procedure (Hallmark et al., 1982) was followed to determine the Si concentration in the digested plant samples. Two mL of the digests were transferred to 50 mL polyethylene screw-cap centrifuge tubes, added with 10 mL of 20% acetic acid and swirled for about 10 seconds. Two mL of 0.26 M ammonium molybdate was added and the samples were allowed to stand for 5 minutes. Two mL of ANSA was then added as a

reducing agent and 20% acetic acid was added to make the final volume to 30 mL. The samples were then tightly capped and allowed to sit for 30 minutes after which they were vigorously shaken before taking absorbance reading using an UV spectrophotometer (Hach DR 5000) set at 630 nm. Standard series of 0.4, 0.8, 1.6, 3.2, 4.8, and 6.4 mg L⁻¹ were also prepared by pipetting 0.5, 1, 2, 4, 6, and 8 mL of 24 mg L⁻¹ Si and treated in like manner as the digested plant samples.

3.2.3. c. Plant Tissue Analysis for Elemental Composition

Plant tissues sample digestion for elemental composition was carried out following the nitric acid- hydrogen peroxide (HNO₃-H₂O₂) digestion procedure as outlined by (Jones et al., 1991). A 0.5 g ground plant tissue sample was weighed out and placed into a (5 x 5 cm) kimwipe paper; the ends of the paper were twisted to enclose the sample and then placed into a 100 mL glass digestion tube. Five mL of concentrated (67-70%) HNO₃ acid was added to each tube ensuring that the acid washed down every plant residue on the wall of the tube. The samples were allowed to sit for 50 minutes; during which time the digestion block was turned on to reach a temperature of 153-155°C (temperature maintained during the entire digestion period). The samples were then mixed with a vortex mixer for 5 seconds and placed in the digestion block for 5 minutes to initiate vigorous boiling. After 5 minutes, the tubes were withdrawn and allowed to cool for 10 minutes, and then 3 mL of 30% H₂O₂ was added to each tube and capped with a small glass funnel to prevent the samples from overflowing during digestion. The samples were placed back into the digestion block for two hours and forty-five minutes (2 hrs, 45 minutes). At the end of the digestion period, the tubes were removed and allowed to cool, the digests were vortexed and poured into 15 mL centrifuge tubes and deionized water was added to make the final volume to 12.5 mL. The tubes were tightly capped and kept in the refrigerator awaiting elemental composition analysis which was carried out by Inductively Coupled Plasma (ICP) - Optical Emission Spectroscopy (OES). Immediately before ICP analysis, samples were filtered using Whatman No. 1 filter papers to ensure that there were no precipitated materials in them. Results from ICP analysis (ug mL⁻¹) were multiplied by a dilution factor of 25 (12.5 mL / 0.5 g) to express concentration in ug g⁻¹ or mg kg⁻¹. Elemental composition (mg kg⁻¹) was then divided by 1,000 and multiplied by yield (Mg ha⁻¹) so that uptake could be expressed in kg ha⁻¹.

3.2.3. d. Soil pH, Si and Elemental Composition Analysis

Soil samples collected after harvest were also analyzed for pH, plant-available Si, and select nutrient elements. The soil pH was determined using a 1:1(weight/volume) soil to water

ratio. Ten (10) grams of dried soil was weighed and placed in a 50 mL screw cap plastic centrifuge tube; and 10 mL of deionized water was added to each tube and then capped tightly. The tubes were placed on a reciprocal shaker set at high speed for one hour and allowed to sit undisturbed for another hour to permit larger soil particles to settle. After that, the soil pH was measured using a SevenCompact™ pH/ Ion S220 digital pH meter. Plant-available Si was extracted using seven extraction procedures as outlined by different researchers. The details of each extraction procedure are summarized in Table 3.4. After extraction, a predetermined volume of sample extract based on each extraction procedure was obtained and analyzed for plant-available Si following the MBC procedure as outlined by Korndorfer et al. (2001). A desired volume of aliquot from each sample was pipetted into 50 mL polyethylene centrifuge tubes and 10 mL of deionized water was added. This was followed by the addition of 0.5 mL of 1:1 hydrochloric acid (HCl). One mL of 10% ammonium molybdate $[(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}]$ was added and the samples were allowed to sit for 5 minutes. After 5 minutes, 1 mL of 20% tartaric acid was added followed by 1 mL of ANSA (prepared by adding 0.5 g of amino naphthol n-sulphonic acid + 1 g of sodium sulfite + 30 g of sodium bisulfite and dissolved in 250 mL of deionized water) as a reducing agent. After 5 minutes, Si in each sample was measured by taking absorbance readings through an UV spectrophotometer (Hach DR 5000) set at 630 nm. Standards series containing 0.2, 0.4, 0.6, 0.8, 1.2, and 1.6 mg L⁻¹ Si were also prepared by pipetting 0.5, 1, 2, 3, 4, and 5 mL of 10 mg L⁻¹ Si and were all treated in the same manner like soil extracts as described above. Essential plant nutrients in soil samples were extracted using the Mehlich-3 solution (Mehlich 1984). Two grams of dried soil sample was weighed into 100 mL plastic bottles and 20 mL of Mehlich-3 solution (diluted acid-fluoride-EDTA solution adjusted to pH 2.5) was added. The samples were placed in a reciprocal shaker set at high speed and shaken for 5 minutes and then filtered using Whatman No. 42 filter paper. The extract was then analyzed using ICP-OES for select plant essential nutrients.

3.2.4. Data Analysis

Analysis of variance (ANOVA) procedure was performed to determine the treatment effect of varying rates of silicate slag on soil pH and Si availability in soil using SAS 9.4 (SAS Institute 2012). The relationship between applied Si in the form slag and soil Si content, pH and nutrient concentration was evaluated using the PROC CORR procedure in SAS 9.4 (SAS Institute 2012).

Table 3.4 Different procedures used for extracting plant-available silicon from collected soil samples after harvest.

Extraction Procedure	Solution	Soil: solution ratio g ml ⁻¹	Shaking time	References
OAc-1 †	0.1M CH ₃ COOH †	1:10	1 hr	Korndorfer et al., 1999
CaCl ₂	0.01M CaCl ₂	1:10	1 hr	Korndorfer et al., 1999
NaOAc	1M NaCH ₃ COOH	1:10	1 hr	Fox et al., 1967
Water	Deionized water	1:10	1 hr	Korndorfer et al., 1999
NH ₄ OAc	0.5M NH ₄ CH ₃ COOH	1:10	1 hr	Korndorfer et al., 1999
Citric acid	0.1M C ₆ H ₈ O ₇	0.5:25	2 hrs, 24 hrs rest, 1 hr	Acquaye and Tinsley, 1965
OAc-2 ‡	0.1M C ₂ H ₄ O ₂ ‡	1:10	24 hrs rest, 2 hrs	Snyder, 2001

† samples were immediately shaken for 1hr after addition of acetic acid

‡ samples were allowed to rest in acetic acid for 24 hrs and then shaken for 2 hrs

Correlation analysis was also done to evaluate the relationship between enhanced Si uptake in rice straw and panicle on the content of selected elements in rice panicle.

3.3 Results and Discussion

3.3.1 Effect of Slag Application on Soil pH

The application of silicate slag significantly ($p < 0.1$) increased soil pH in five out of twelve sites. The increase in pH as affected by slag application was observed in St. Landry, Mamou, Lake Arthur, and Crowley in 2014, and also in Lake Arthur in 2015 (Table 3.5). Although there were some increases obtained for other sites, they were not always linear with slag or lime application. The most significant ($p < 0.05$) linear increase in pH which was as much as 1.4 units was obtained in Mamou 2014, Lake Arthur 2014, Crowley 2014, and St. Landry 2013 (Figure 3. 1). This was however expected because slag is often used as a liming material in places where soil acidity is a problem for crop production. Increases in pH of 0.5 and 0.6 units have been reported by Nolla et al. (2006) when slag was applied at 6000 kg ha^{-1} . It has been reported that silicate slag has equal potential for correcting soil acidity and in some cases may be more effective in correcting soil pH than limestone (Nolla et al., 2013). It was also reported that slag application have a superior agronomic effect than lime when applied to soil because unlike lime which is primarily used to increase soil pH, slag can also be used as fertilizer and for remediation of soils contaminated with heavy metals (Negim et al., 2010). Containing about 12-17% Si as well as a host of several plant essential nutrients including Ca, Fe, Mg, Mn and S, the application of silicate slag may also replenish the depletion of these nutrients arising from continuous and consecutive cropping (Korndorfer et al., 2003; Nolla et al., 2006). While verifying the corrective effect on pH of slag and limestone applied to Ultisols, Pereira (1978) concluded that there was no difference found between the liming potential of the two materials. Hence, Korndorfer et al. (2003) recommended that the use of slag as a soil amendment should be in line with liming requirement of the soil to be treated.

3.3.2 Effect of Slag Application on Soil Silicon

The initial soil Si content determined by 0.5 M OAc-1 was highly variable across sites. Among the five soil series involved in this study, the Sharkey clay soil at Gilbert Site had the highest initial Si of 164 mg kg^{-1} followed by the silt loam soil in Crowley which had an initial Si of 103 mg kg^{-1} . The other three sites had soils lower in initial Si and pH. For instance, the Mowata silt

loam in Mamou site had initial soil Si of 16 mg kg⁻¹; Tensas Sharkey complex in St. Landry had initial Si of 33 mg kg⁻¹ while Kaplan silt loam in Lake Arthur had initial Si of 11 mg kg⁻¹ (Table 3.1). High initial Si availability in Crowley and Gilbert may have been influenced by the high pH of the soils in these sites. The amount of H₄SiO₄ availability in soil is highly dependent on pH and Al and Fe content of the soil (Szulc et al., 2015). With higher soil pH, the amount of H₄SiO₄ increases in soil but continuous increase in H₄SiO₄ concentration can promote adsorption especially in soils having high clay content. The maximum availability of H₄SiO₄ in soil occurs at pH of 6.5 -8.5; maximum adsorption occurs at pH 9.8, while soil having pH of 5-6 or below, are often deficient in plant-available Si due to the leaching of H₄SiO₄ from soil (Frings et al., 2014; Hynes, 2014). The addition of slag significantly ($p<0.01$) increased the soil pH and Si availability especially in soil with pH of six and below as determined by 0.5 M OAc-1, 0.5 M OAc-2, deionized water, 1 M NaOAc, and 0.5 M NH₄OAc (Table 3.5). However, increases in soil Si were not always linear with slag application rate. Among the seven used extractants for this study, 0.5 M OAc-1 extracted Si showed significant ($p<0.1$) positive effects of silicate slag application on soil Si content (Table 3.6) and also correlated well with plant Si uptake. Most of the increases in soil Si derived from slag application were observed on light textured (silt loam) soils and soils having a pH lower than six. For the Gilbert site where the soil pH is above 7, slag application did not significantly alter the soil Si. Similar trends were observed in Crowley in 2013 and 2015 where the soil pH ranged from 7-8. In 2014 however, there was a significant increase in soil Si with slag application ($p<0.01$) for most sites (Table 3.5) Although the maximum adsorption of Si has been reported at pH of 9.8 (Hynes, 2014), slag application may have resulted into the already high H₄SiO₄ levels in Crowley and Gilbert to be increased thus, promoting the conversion of H₄SiO₄ which is plant-available, to polysilicic acid which is unavailable to plants. Liming can also increase the availability of Si in the soil. Based on the findings of Miles et al. (2014) who studied different soil types in a given region, soil Si extractability can be positively correlated with pH. Hence, in soils with high amount of adsorbed Si as in Gilbert and Crowley, liming alone may bring enough Si into solution for plant uptake but this may not work for sites such as Lake Arthur, Mamou and St. Landry where pH and soil Si are very low.

Table 3.5 *P*-values of analysis of variance for treatment effects on soil pH and extractable silicon based on different extraction procedures.

Location	Year	pH (1:1 water)Extractable soil silicon.....						
			OAc-1 [†]	OAc-2 [‡]	Citric	Water	NH ₄ OAc	CaCl ₂	NaOAc
Lake Arthur	2013	0.8782	0.827	0.9134	0.3477	0.8272	0.8462	0.9006	0.0747
	2014	0.0003	0.3129	0.0203	0.1506	0.7891	0.0899	0.2013	0.0654
	2015	0.0947	0.9016	0.7631	0.5393	0.2989	0.8987	0.7601	0.9163
Gilbert	2013	0.7696	0.5873	0.3855	0.9709	0.0872	0.7585	0.253	0.7098
	2014	0.2668	0.0086	0.6198	0.4157	0.0069	0.1297	0.854	0.0993
Mamou	2013	0.7879	0.671	0.7117	0.4429	0.3129	0.2911	0.7032	0.4156
	2014	<0.0001	0.0997	0.009	0.1821	0.1119	0.0009	0.704	0.0127
St. Landry	2013	0.9108	0.8272	0.9364	0.4088	0.7618	0.7558	0.3534	0.6582
	2014	<0.0001	0.0862	0.0139	0.5284	0.0279	0.0335	0.0242	0.0136
Crowley	2013	0.4506	0.2871	0.2495	0.3288	0.9256	0.4836	0.4305	0.3758
	2014	0.0006	<0.0001	0.001	0.5407	0.6909	<0.0001	0.1487	<0.0001
	2015	0.1723	0.4011	0.0278	0.1676	0.2009	0.4459	0.5046	0.9718

[†] - one-hour shaking using acetic acid solution; [‡] - 24 rest + two -hour shaking using acetic acid solution

Table 3.6 Treatment effect of lime and silicate slag on soil silicon content determined by 0.5 M OAc-1.

		2013					2014					2015		
	0.5 M OAc-1 extractable soil Si, mg kg ⁻¹												
Slag rate Mg ha ⁻¹	Si rate kg ha ⁻¹	Crowley	Gilbert	Lake Arthur	Mamou	St. Landry	Crowley	Gilbert	Lake Arthur	Mamou	St. Landry	Crowley	Lake Arthur	
0	0	103abc	165a	15bc	19ab	23abc	35e	168a	17a	55b	176cd	78abc	68ab	
1	140	102bc	178a	21b	19b	5.0de	53cd	160a	19a	55b	191c	97abc	74ab	
2	280	114ab	170a	20b	19b	21bca	55bcd	212a	18b	49bc	193bc	122a	80a	
4	560	113ab	178a	24ab	13ab	18bca	59c	181a	24a	72a	196bc	112ab	71ab	
6	840	116a	196a	20bc	19a	31ab	76b	167a	30a	74a	220ab	119a	67ab	
8	1120	113ab	170a	27a	21a	34a	90a	172a	24a	76a	227a	71bc	64ab	
2*	0	103bc	158a	27a	18ab	4.0e	43de	179a	20a	37c	172cd	58c	74ab	
4**	0	96c	169a	15c	17ab	9.0cde	37e	162a	19a	60b	151d	65c	72ab	
<i>P-values</i>		NS	NS	NS	Ns	0.009	<0.0001	NS	0.016	NS	0.001	NS	NS	

Means with same letter within columns are statistically similar as determined by Fisher's LSD at $p < 0.1$.

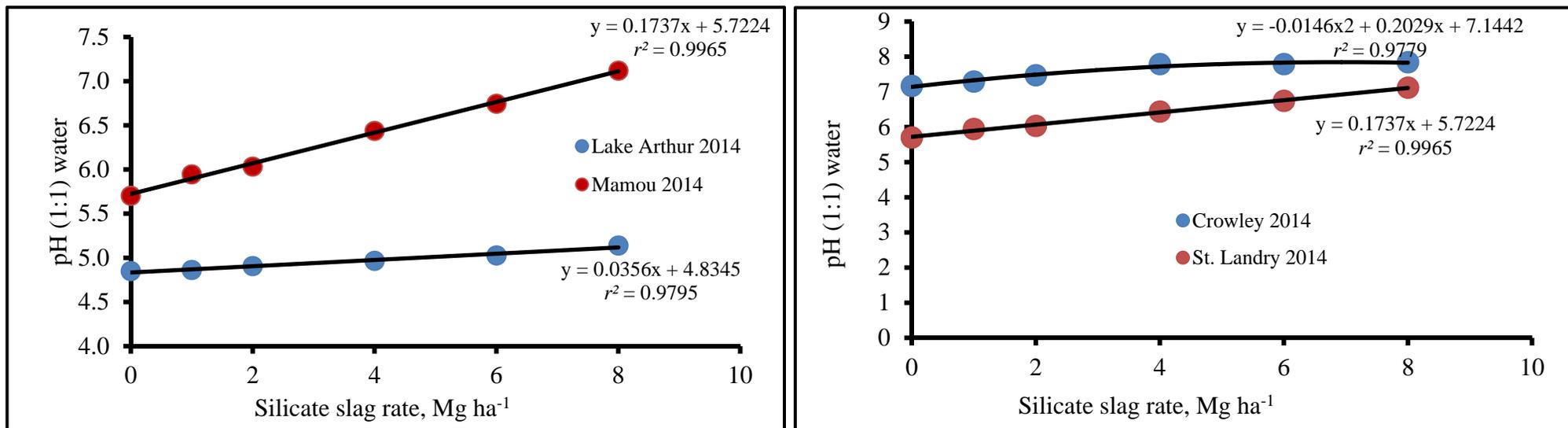


Figure 3.1 Effect of silicate slag applications on soil pH in four different sites in Louisiana

3.3.3 Relationship of Soil Silicon with Plant Silicon Content

Seven extraction procedures were used to determine Si availability in soil after slag application. As mentioned earlier, there were good correlations observed between Si extracted by different extractants and various plant response parameters. However, 0.5 M OAc-1 extractable Si show a more consistent relationship between Si availability in soil as well as its uptake by rice as observed in rice straw and panicle content; thus, it was selected among the rest of the extractants for our correlation study. The application of slag significantly increased the availability of soil Si in several sites which also had a significant linear relationship with both straw and grain Si contents of rice based on 0.5 M OAc-1 extracted Si (Figures 3.2 and 3.3). Increasing the soil Si was also positively correlated with the whole plant Si content (Figure 3.4). These results are in agreement with those found by Osuna-Canizalez et al. (1991) who reported significant increases in Si content of the leaf blades in rice when soil Si content was increased through fertilization. Adequate Si availability in soil or culture solution has always been associated with enhanced level of Si uptake by plants (Yoshida et al., 1959). However, it should be pointed out that once taken up by plants, Si is deposited either as amorphous silica or opal phytoliths and cannot be redistributed within the plant system (Raven, 1983; Marschner, 1995). Hence, for even distribution of Si to occur in both straw and panicle of rice, the soil must contain a steady supply of available Si. Moreover, Si is the only element in soil whose phytotoxicity has not been reported. With limited interaction occurring between Si and most plant essential nutrients, high tissue Si levels may not negatively affect the physiological processes of plants. Many researchers have reported that rice shoots can contain higher concentration of Si than most plant essential nutrients and in some cases may even be higher than the trio of nutrients; NPK (Agarie et al., 1998; Epstein, 1999; Ma and Takahashi, 2002). Rice exhibits the highest uptake of

H₄SiO₄ among grasses and in the absence of sufficient supply of Si; growth can be considerably reduced, thus leading to marked decrease in yield (Ma, 2003). A vast amount of research has confirmed that increase in tolerance of rice to biotic and abiotic stresses is largely attributed to enhanced levels of Si in their shoots, which is also dependent on the amount of Si present in the soils in which they are grown (Agarie et al., 1993; Matsuo et al., 1995; Datnoff et al., 2001).

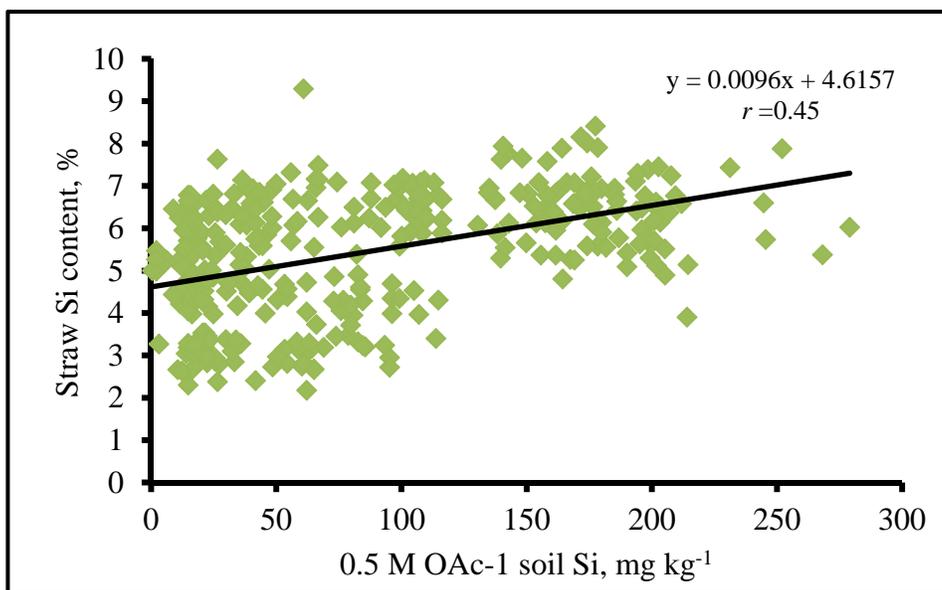


Figure 3.2 Correlation of soil silicon with straw silicon content.

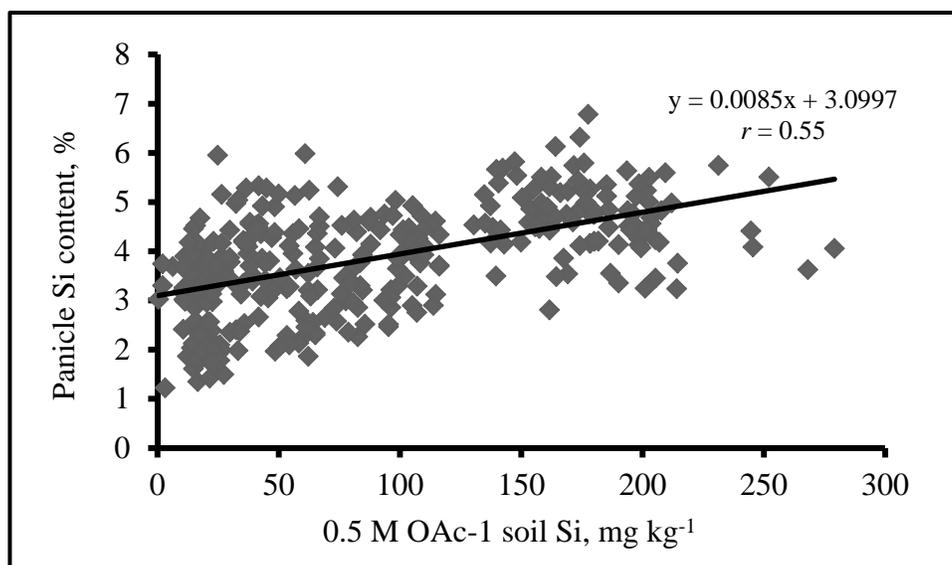


Figure 3.3 Correlation of soil silicon with panicle silicon content.

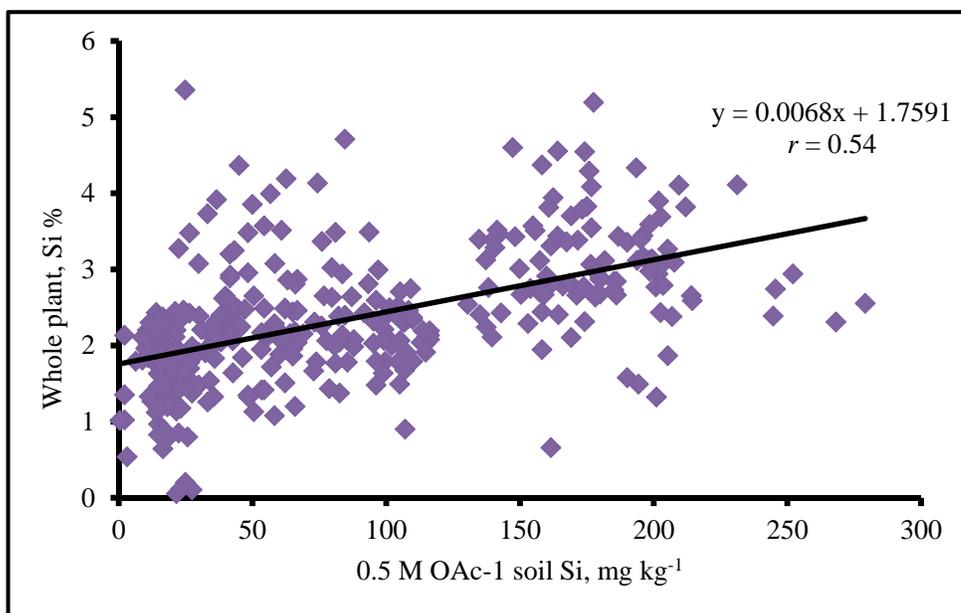


Figure 3.4 Relationship of soil silicon with whole plant silicon content.

3.3.4 Relationship of Silicon with Other Nutrients in Rice

A correlation analysis was performed in order to determine the relationship of soil and plant Si contents with selected elements in rice straw and panicle. Results indicate that straw Si content was negatively correlated ($r = -0.25$) with P content of rice panicle (Figure 3.5). Slag is a by-product of steel or elemental P production and may contain some amounts of P. However, the P in slag may not be plant available; and slag fertilization may not necessarily increase P availability and uptake by plants (Anderson et al., 1992). Notwithstanding, Silva (1971) reported increased P uptake following the application of Si fertilizer on an Oxisoil in Hawaii. Deren (1997) also reported that P concentration was increased in both straw and grain in rice with Si fertilization. Lin and Hung (1980) found the concentration of P in rice increased when Si was added to the nutrient solution. Our results are however similar to results obtained by Anderson (1991), who reported that Si application was either poorly or negatively correlated with P uptake

in sugarcane in the Everglades Histosols of Florida. Snyder et al. (1986) and Epstein (1994) also observed P concentration in plants were either not affected, or decreased with Si uptake.

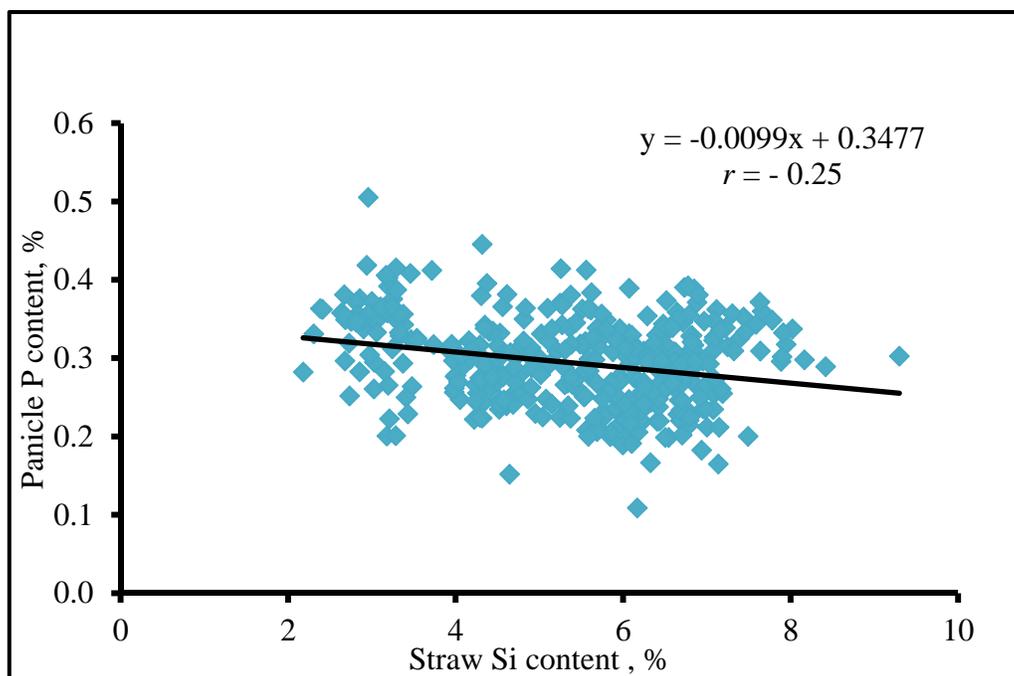


Figure 3.5 Correlation of silicon content in rice straw with phosphorus content of panicle.

Increases in soil Si was also negatively correlated with the uptake of sulfur (S) in rice panicle (Figure 3.6). Similarly, both straw and panicle S contents decreased with their Si content (Figures 2.7 and 2.8). The means by which Si inhibits S uptake by plants has not been documented. However, one possible explanation could be due to the fact that at high concentration, Si exists most as a negatively charged H_3SiO_4^- rather than uncharged H_4SiO_4 (Haynes, 2014). Since Si is preferentially adsorbed as H_3SiO_4^- to soil colloids at high concentration and with increasing pH, there could be a potential competition between H_3SiO_4^- and SO_4^- for exchange sites; and this could lead to the release of SO_4^- from the soil exchange sites, making them easily leachable from the soil with the infiltration of water within soil. Interaction between Si, S, and heavy metals in plants may also explain the rationale behind the

inhibitory effects of Si on S uptake by plants. In an experiment where rapeseed (*Brassica napus* L.) was treated with varying rates of arsenic (As) and S, Zhong et al. (2001) found that the As content in various plant components decreased with S content. But since Si also has the ability to decrease the availability of heavy metals in soils and their uptake by plants, it is likely that the mechanism of transport could be the same for Si, S, and heavy metals. Therefore, a competition between these ions could be limiting the uptake of S.

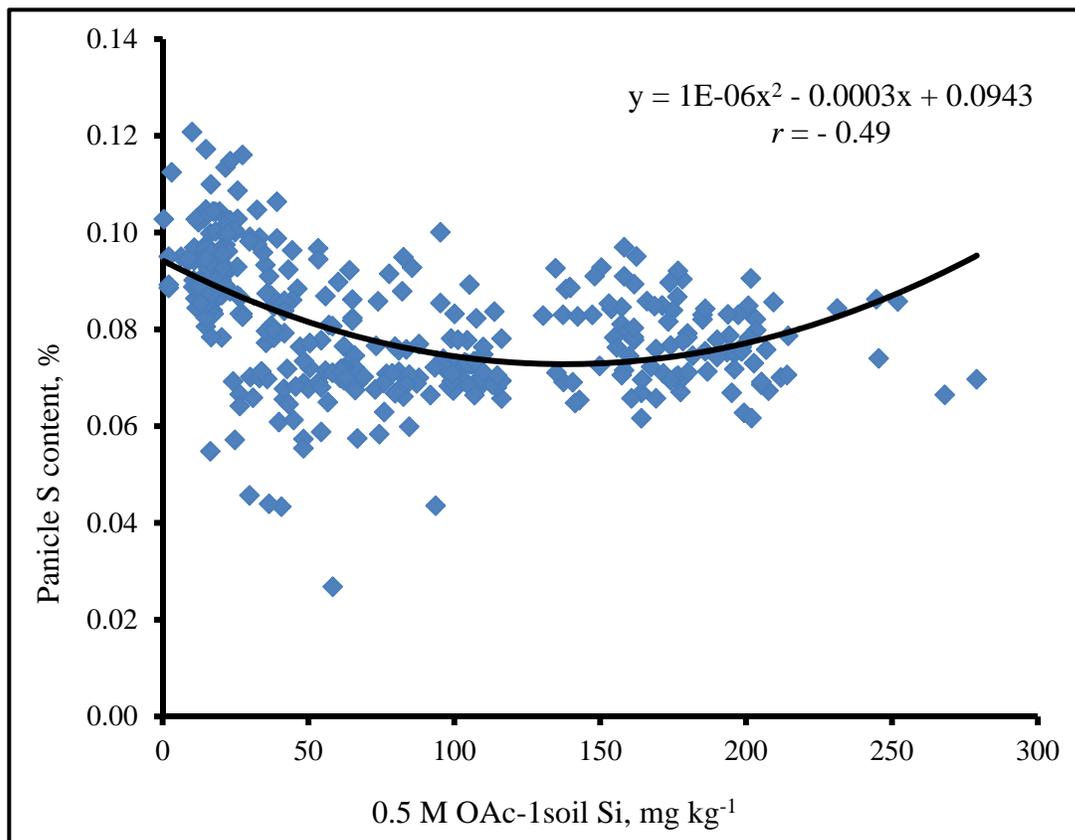


Figure 3.6 Relationship of soil silicon and sulfur in rice panicle.

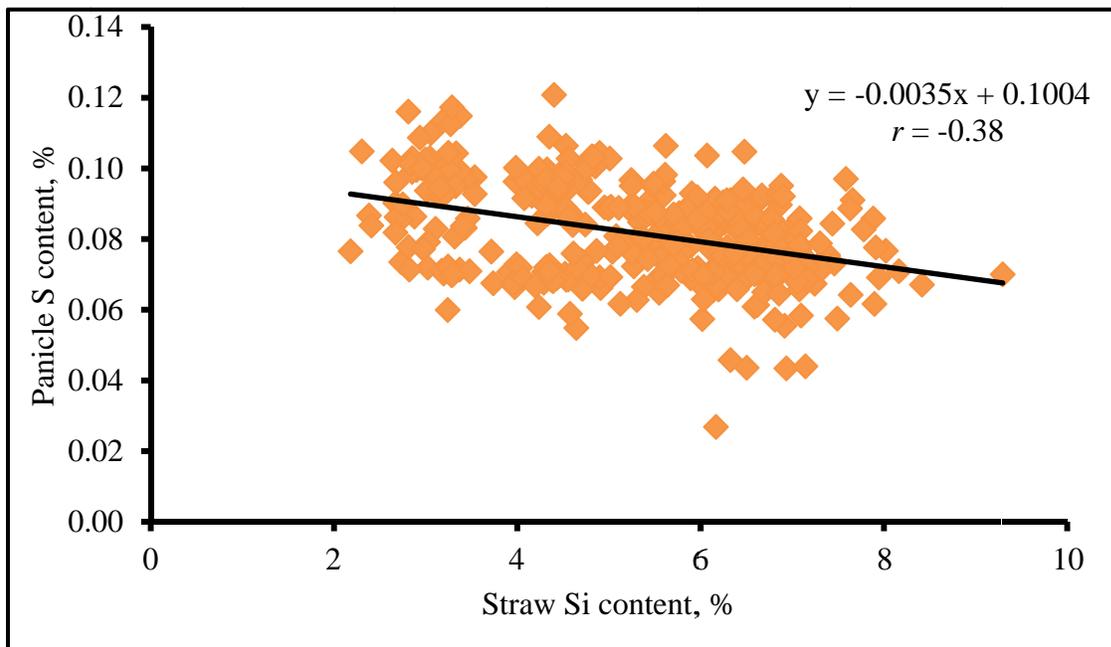


Figure 3.7 Relationship of silicon in rice straw with panicle sulfur content.

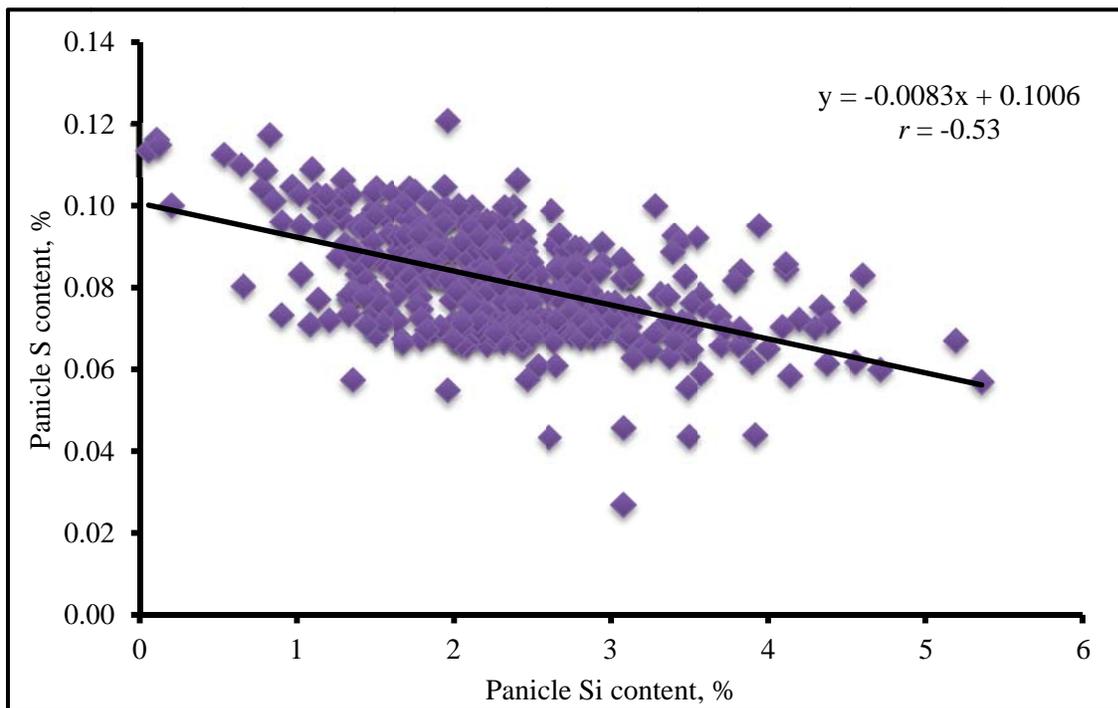


Figure 3.8 Relationship of silicon in rice panicle with panicle sulfur content.

3.3.5. Relationship of Silicon with Heavy Metals in Rice

The impact of enhanced Si uptake in rice straw and panicle was also evaluated on the uptake of heavy metals in rice. Several studies showed that increased uptake of Si can be negatively correlated with heavy metals uptake by plants. The current study however, did not find a definitive relationship between heavy metals such Al and Fe contents in rice with increasing Si content. Nevertheless, alleviation of Al and Fe toxicities have been widely reported in plants treated with Si (Hudson and Evans, 1995; Cocker et al., 1998; Korndorfer et al., 2003).

It was however interesting to notice that increased Si content in rice panicle was positively correlated with its manganese (Mn) content (Figure 3.9). This result differs from what has been reported in published literature that Si reduces the uptake of Mn. An alleviating effect of Si on Mn toxicity has been observed in rice grown in hydroponics by Okuda and Takahashi (1962), barley (*Hordeum vulgare*- Horiguchi and Morita, 1987), bean (*Phaseolus vulgaris* - Horst and Marschner, 1978), and pumpkin (*Cucurbita*- Iwasaki and Matsumura, 1999). However, Williams and Vlamis (1957) indicated that the addition of Si to culture solution did not restrict Mn uptake, but rather led to an even distribution of Mn in the leaves and shoot and therefore, they concluded that alleviation of Mn toxicity is not only due to inhibition of Mn uptake by Si, but also due to Si facilitating the homogeneous distribution of Mn within plant tissues.

Regarding other heavy metals such as As and cadmium (Cd), there is a growing body of research documenting increase availability of these heavy in soils which is often associated with improper handling or disposal of industrial by-products, use of sewage sludge as fertilizer and other agrochemicals that may contain these heavy metals. High As and Cd levels in food can

pose health risks to both humans and animals (Nascimento and Xing, 2006). From their study on the concentration of As present in rice from various regions in Brazil, Batista et al. (2011) reported that the total levels of As in Brazilian rice samples collected from the various rice markets ranged from 108 to 428 ng g⁻¹ with the highest As content reported in brown rice. This might sound worrisome but for As to be lethal to human, it must be consumed in a single dose of at least 70- 80 mg of As trioxide (Vallee et al., 1960).

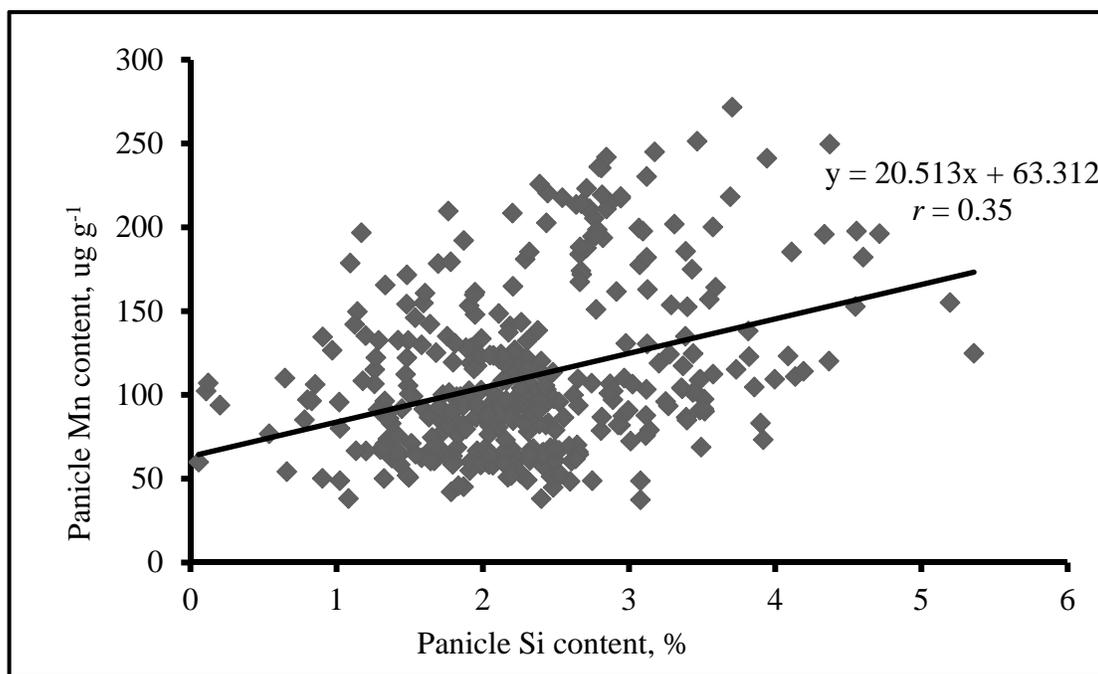


Figure 3.9 Relationship of silicon content in rice Panicle with Manganese.

The result from our study found that increases in soil Si brought about by slag application lead to an increased uptake of Si which was negatively correlated with the rice straw and panicle contents of both Cd and As contents (Figure 3.10). These results are in agreement with results reported by several other researchers. Cunha et al. (2008) reported decreased uptake of Cd and Zn by corn plants growing in soil contaminated with high levels of these heavy metals when amended with Si. In a separate experiment, Guo et al. (2005) studied the interactive effect of Si,

As and P in rice in nutrient culture solution. Their results indicated that plants treated with Si had significantly low As concentration as compared to those without Si. The reduction in heavy metals availability in soil has been associated with the increases in soil pH resulting from lime or slag application to soil; and may not be a direct effect of Si in soil solution, since slag and lime have equal potentials to cause increases in soil pH (Korndorfer et al. 2003). In our study however, there was no significant negative effect of soil pH found on the availability and uptake of As and Cd by rice. Reduction in heavy metal toxicity may not only be due to inhibiting effect of Si on the uptake of these heavy metals, but could also be derived from other benefits associated with enhanced levels of Si in plants. Cunha et al. (2008) found corn biomass production was highly positively correlated with its Si content but Cd concentration in the shoot was negatively correlated with biomass production and therefore suggested that increased biomass production resulting from Si fertilization could lead to dilution of high concentration of Cd in plants thereby reducing the deleterious effects of the metal in plants.

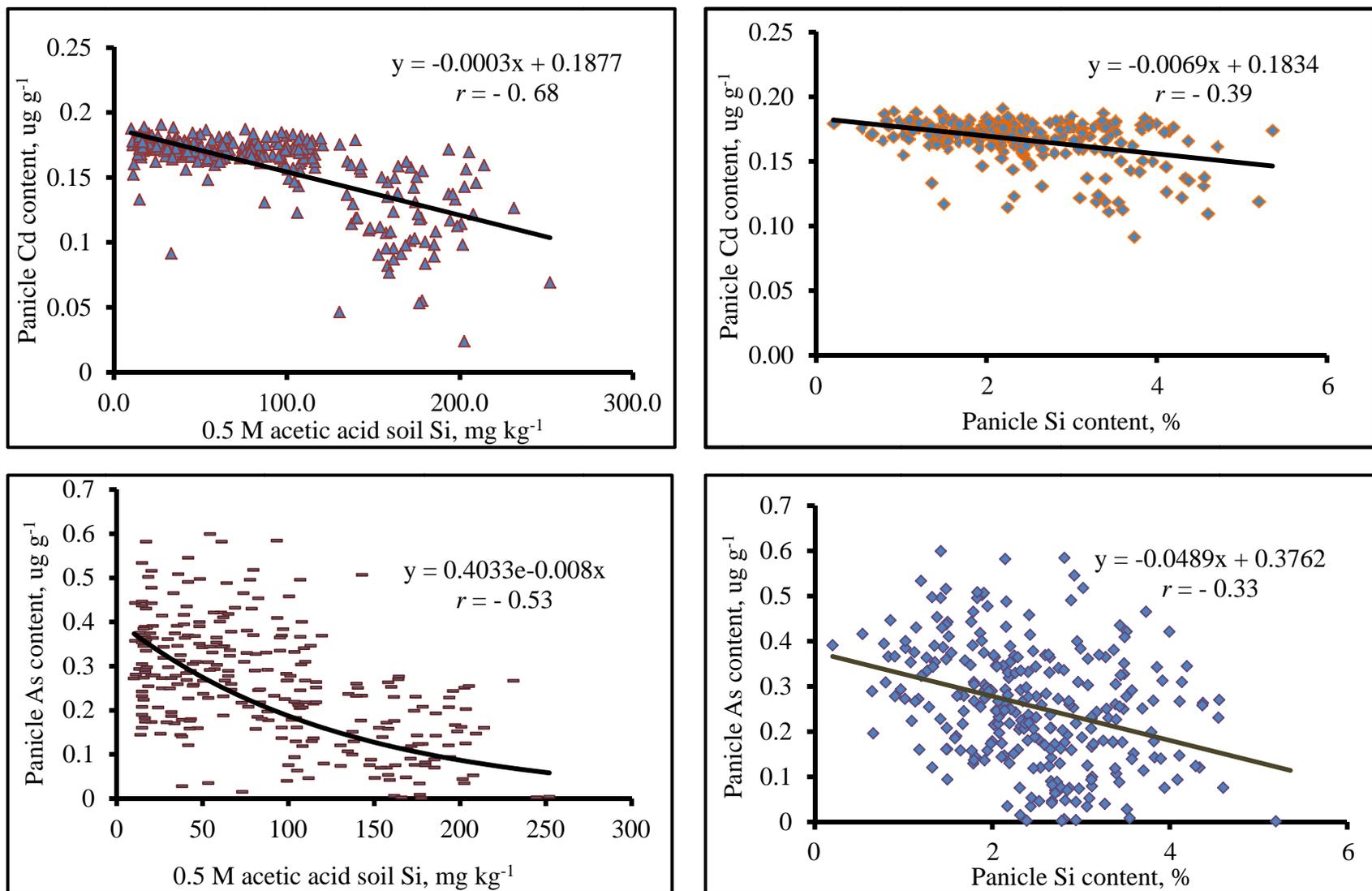


Figure 3.10. Relationship of soil silicon and As and Cd content of rice panicle.

3.4 Conclusions

In general slag application was effective in increasing soil pH but mostly in soils with low pH (below 6). Most of the increases in pH were observed in light textured soils and soils having low pH and low initial Si contents. In most sites where pH increases were observed, slag was as much effective as lime in increasing soil pH and in some cases as observed in Crowley, Lake Arthur, Mamou and St. Landry in 2014 and in Lake Arthur in 2015, slag application resulted in higher pH increases than lime application. These results confirm previous findings that slag is an effective liming material. In soils with low initial Si (Mowata silt loam, Kaplan silt loam and Tensas Sharkey complex), slag application was effective in increasing the plant-available Si; but this was not observed in soils where pH, clay and initial Si contents were high (Crowley silt loam and Sharkey Clay). Hence, addition of slag may not necessarily increase the availability of Si in soil especially when soil pH is high or when there is high native Si and clay content present in soil. Adding Si to soils with these characteristics can either promote Si adsorption to soil colloids or lead to conversion of H_4SiO_4 to other forms of Si that may be unavailable for plant uptake. However, low pH and light textured soils are inherently low in plant-available Si; and the addition of slag material can promote increases in pH and Si availability in these soils. Hence, soil pH and native Si content are key factors to be considered in a Si fertilization program especially when slag is the source of fertilizer. Results from this study suggest that Si fertilization could be more beneficial to be practiced in Lake Arthur, Mamou, and St. Landry where soils are inherently low in plant-available Si. Among the seven extraction procedure used in this study, 0.5 M OAc-1 extractable soil Si showed a more consistent correlation with grain yield, plant Si uptake and uptake of other elements by rice. There was no evident correlation between soil Si extracted by the other procedures and elemental composition of rice panicles. Therefore, 0.5 M OAc-

1extraction procedure was more reliable in estimating plant-available soil Si which also correlated well with plant Si uptake and uptake of other elements by rice. Increased Si uptake by rice was negatively correlated with the panicle P and S contents, suggesting that there could be an antagonistic relationship between H_4SiO_4 and phosphate and sulfate ions either in the soil solution or within plants. Although slag material contains some amounts of P and S, but its application as an agronomic practice may have an adverse effect on the uptake of these two elements by rice. There was no clear-cut relationship found between Si content in rice straw and panicle and those of other nutrients. Silicon fertilization was effective in reducing the availability and uptake of heavy metals such as As and Cd by rice. These results suggests that Si fertilization may not only be effective in protecting plant against mechanical or physiological damage, but can also lead to yield increases and improve the grain quality of rice.

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Chapter 4. Conclusions

In general, slag application resulted in increases in soil pH and soil Si content mostly in areas with low pH and low native Si concentration. It was noticed that increasing soil Si through slag application can be influenced by soil property. Among the soil studies, Kaplan silt loam in Lake Arthur, Mowata Silt loam in Mamou and Tensas Sharkey clay in St. Landry had low pH and low initial Si. On the other hand, the Crowley silt loam soil in Crowley and Sharkey clay in Gilbert had high pH and high initial Si regardless of the extractant used. However, the addition of Si through slag application had minimal effects on the Si availability in Crowley and Gilbert, but had a significant increase in soil Si content in Lake Arthur, Mamou and St. Landry. Rice grain yield was highly influenced by silicate slag application with the most significant influence on yield obtained at application rates ranging from 1- 4 Mg ha⁻¹. Although application rates of 6 and 8 Mg ha⁻¹ resulted in the highest grain yield obtained in some sites, it may not be economically practical to apply slag at these rates. Among the seven extractants evaluated in this study, 0.5 M OAc-1 showed the most consistent relationship between soil Si, plant Si uptake and grain yield of rice. Hence, the native soil Si determined by 0.5 M OAc-1 and pH of the soil will be essential in determining the need for slag fertilization for Louisiana soils.

The critical soil Si level was highly variable with the extractant used. Using grain yield as response variable, 0.5 M OAc-1 gave a critical level of 37 mg kg⁻¹. When total plant Si uptake was used as response variable, a critical level of 54 mg kg⁻¹ was also determined using 0.5 M OAc-1. A critical level could not be determined using grain yield as response variable for the other six extractants. However, when total plant Si uptake was used as the response variable, critical levels of 116, 771, 198, 87, 47, 11.8 mg kg⁻¹ were determined by 0.5 M OAc-2, 0.1 M citric acid, 1 M NaOAc, 0.5 M NH₄OAc, deionized water, and 0.01 M CaCl₂, respectively. Based

on these results, 0.5 M OAc-1 thus far is considered a suitable extractant for estimating Si availability in Louisiana soils.

Correlation study showed that increases in soil Si derived from Si fertilization and uptake by rice can affect the uptake of P and S suggesting that there could be an antagonistic interaction among Si, P, and S within plants. Based on 0.5 M OAc-1 extractable Si, there was also a negative correlation between Si availability in soil and As and Cd content of soil. Silicon contents of rice panicle was also negatively correlated with its As and Cd contents. The reduction in As and Cd contents of rice panicle with increasing Si level suggest that enhance Si nutrition in rice may improve the grain quality for rice grown in soils containing high levels of these heavy metals. The outcomes of this study suggest that there is a potential for Si fertilization to improve performance and productivity of rice especially when grown in soils testing low for plant-available Si in Louisiana. With rice being a high Si accumulator, ignoring Si fertilization in low Si soils and soils with low pH will gradually lead to Si deficiency in many rice producing areas of Louisiana which may eventually compromise yield and grain quality.

Vita

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