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Trace element accumulation in rice :effects of soil arsenic, irrigation management, phosphate application and iron oxide amendment

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TRACE ELEMENT ACCUMULATION IN RICE: EFFECTS OF SOIL ARSENIC, IRRIGATION MANAGEMENT, CULTIVAR, PHOSPHATE APPLICATION AND IRON OXIDE AMENDMENT

by

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ABSTRACT

A concern is growing over the accumulation of trace contaminants in rice, a plant which has been estimated to supply 50% of the caloric intake to the world's population. Contamination with arsenic (As) and other trace elements have been shown in many areas including the U.S. and rice intake poses a potential risk to human health. This research investigated the accumulation of selected trace elements including arsenic (As), selenium (Se), molybdenum (Mo) and cadmium (Cd) in as many as six rice cultivars and two control soils common to the south central U.S. A field study was completed to evaluate the impact of As content in soil, irrigation management and cultivar on total rice grain element accumulation. Results indicated soil amended with monosodium methanearsonate (MSMA) increased the accumulation of As and Se, but decreased Mo accumulation in rice under all irrigation treatments. Grain-Cd increased for most cultivars in MSMA-amended soil. In addition, intermittent flooding significantly decreased total grain-As, Se and Mo, but increased Cd. Greenhouse studies were also completed to evaluate the impact of phosphate application and iron oxide amendments on total grain concentrations of As, Se, Mo and Cd. Correlations between soil-As and grain elements were also studied. Results indicated that grain-As concentration is directly proportional to the soil-As concentration, but phosphate application has no substantial impact on grain element concentration. Iron oxide amendments significantly reduced grain-As accumulation in rice. Results also indicated increasing the soil-As concentration increased grain-As, increased grain-Se and Cd, and decreased grain-Mo accumulation.

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TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

LIST OF TABLES

Page

NOMENCLATURE

Symbol	Description
As	Arsenic
Cd	Cadmium
Mo	Molybdenum
MSMA	Monosodium methanearsonate (CH_4AsNaO_3)
Se	Selenium

1. BACKGROUND

The primary caloric intake of 50% of the world's population is sustained by rice and prompts/assumes a risk to human health caused by the accumulation of arsenic (As) and other trace elements including cadmium (Cd) [1]. Multiple market surveys have identified elevated As concentrations in rice grain (grain-As) and rice food product that exist as both inorganic and organic As [2-4]. Inorganic As has been determined a human carcinogen and the methylation of inorganic As to organic metabolites has been portrayed as a toxification pathway [5, 6]. Among all food categories, consumption of rice makes up the largest contribution to the dietary intake of As [7].

The source of As contamination in rice crops varies globally. Some rice crops are grown in areas contaminated by As polluted irrigation water. Others are grown with elevated arsenic concentrations in the soil (soil-As) due to high amounts of As byproducts around large mining areas [8, 9]. Rice crops in the south central U.S. are grown in areas formerly known as the "cotton belt" plagued by elevated soil-As attributed to the historic application of inorganic and organic As herbicides, pesticides and desiccants for a century between the mid-nineteenth century and the 1950s [10, 11]. During this time period an estimated 3 million pounds of such herbicides including monosodium methanearsonate (MSMA) were applied annually based on U.S. EPA's Screening Level Use Analysis data [10]. Due to the soil characteristics of this region, favorable conditions stabilize As in the soil and cause the decades of As application to have a cumulative effect on soil-As concentrations.

As a result of arsenical herbicide application, the average grain As concentrations of rice grown in the south central U.S. is 0.27 μg g^{-1}. This concentration is substantially higher than global rice grain concentrations in Bangladesh (0.12 μg g^{-1}), China (0.11 μg g^{-1}) and India (0.05 μg g^{-1}) [12-15]. For the United States, rice consumption continues to be a substantial part of diets. Including rice, annual grain consumption was 45% higher in 2000 than in 1970 [16]. Global paddy rice crops account for an estimated 75% of global rice production, growing at an annual rate of 3% [17, 18]. The overall average consumption of rice in the U.S. is 25 g per day, where individuals considered as ‘rice consumers’ (intake > 3.5 g rice per day) consume 61.2 g [2]. Asian-American rice-subsistent diets consume up to 400 g $cap^{-1}d^{-1}$ [19], estimating a per capita rice consumption in the U.S. of up to 108 μg As d^{-1} compared to 45.1 μg As d^{-1} in China [2, 15, 20, 21]. Therefore, current estimates suggest the greatest risk is posed against those with Asian-American diet consuming rice from the south central U.S.

Further exacerbating the risk associated with high grain-As is the lack of national regulation to control As food standards in the U.S. Global As standards present today include the Bangladesh National Drinking Water As Standard (50 μg inorganic As g^{-1}), the Chinese Food Safety Standard (0.15 μg inorganic As g^{-1}), the UK Food Statutory General Limit (1.0 mg kg^{-1} fresh weight in food) and the U.S. EPA and World Health Organization (WHO) drinking water limit of 10 μg L^{-1} [9, 22, 23]. According to Williams, et al. [4], only 21% of rice produced in the south central U.S. would meet Chinese regulations. Based on average grain-As in the south central U.S., the average Asian-American diet would easily exceed the equivalent of U.S. EPA and WHO maximum tolerable daily intake (MTDI) [21, 24, 25].

Previous research has studied the impact of particular environmental conditions including soil-As, irrigation management and genotypic variables that may decrease the risk of arsenic toxicity to human health. It is well known that elevated soil-As corresponds to higher levels of As in the rice grain (grain-As) [24, 26, 27]. Grain-As is exacerbated further, irrespective of soil-As levels, when flooding conditions create a reducing environment that can lead to an increase in As uptake [24, 28]. Plant As uptake also varies based on cultivar [13, 29, 30] and the potential to significantly reduce grain-As has been shown through cultivar selection [31]. Straighthead disease is common to rice crops grown with elevated soil-As conditions, where panicle sterility and significant yield loss result from As toxicity. Cultivar selection may also be used to assist in minimizing the effects of straighthead disease [20, 32]. Minimizing grain-As uptake through the combination of irrigation management and straighthead resistance has been demonstrated in previous research [33, 34]. The presence of hazardous trace elements in rice thus merits national safety standards in the U.S. and continued research to link environmental conditions to food As levels and ultimately protect human health.

2. GOALS AND OBJECTIVES

The goal of this research was to link soil concentrations to total grain concentrations in rice under varying environmental and experimental conditions for select trace elements of concern, including arsenic, selenium (Se), molybdenum (Mo) and cadmium (Cd). To provide a comprehensive understanding of this goal, specific objectives and corresponding hypotheses were developed.

Objective 1: Determine the impact of soil-As on total grain concentrations of elements of concern. The hypothesis stated increased soil-As would significantly impact plant accumulation of As and potentially Se, Mo and Cd.

Objective 2: Determine the impact of irrigation management techniques on total grain concentrations of elements of concern. The hypothesis stated the practice of intermittent flooding would significantly impact plant accumulation of As and potentially Se, Mo and Cd.

Objective 3: Evaluate correlations between concentrations of total grain arsenic and other trace elements of concern. The hypothesis stated significant correlations exist between total grain As and grain Se, Mo and Cd.

Objective 4: Determine the impact of phosphate application on total grain concentrations of elements of concern. The hypothesis stated phosphate application would significantly impact accumulation of grain As, Se, Mo and Cd.

Objective 5: Determine the impact of iron oxide amendments on total grain concentrations of elements of concern. The hypothesis stated iron oxide amendments would significantly impact accumulation of grain As, Se, Mo and Cd.

3. LITERATURE REVIEW

Literature on As and selected trace elements including Se, Mo and Cd was reviewed on mobility in soil, subsequent rice plant uptake and potential mitigation techniques for rice crops. The impact of these factors on speciation was also evaluated. The review is presented with emphasis on the most prominent environments in which rice is grown in the south central U.S., mainly on soil of a silt-loam type, including continuous flooding and intermittent irrigation management practices.

3.1. TRACE ELEMENT MOBILITY IN SOIL

Trace element mobility in a soil environment is directly related to the solubility and bioavailability to plant uptake. The main factors impacting element mobility are soil redox, pH and the presence of other minerals and nutrient groups. Additional factors include temperature, biologic activity and water flux through the sol column. The soil chemistry at this level is not fully dependent on one factor, but is instead a function of the interaction between factors. Figure 3.1 illustrates factors that influence trace element mobility and uptake.

Soil redox potential is directly associated with the irrigation management of rice crops and has impact on element speciation and subsequent mobility. Continuous flooding is the most widely accepted irrigation management practice for rice crops grown today with a soil redox potential estimated at 500-200 mV (pH 5-8) [28]. In a continuously flooded field, flooding creates a physical barrier of oxygen diffusion and a reducing (anaerobic) soil environment [31]. Anaerobic condition may cause As-retaining in the soil to partially dissolve, releasing As and increasing bioavailability [28].

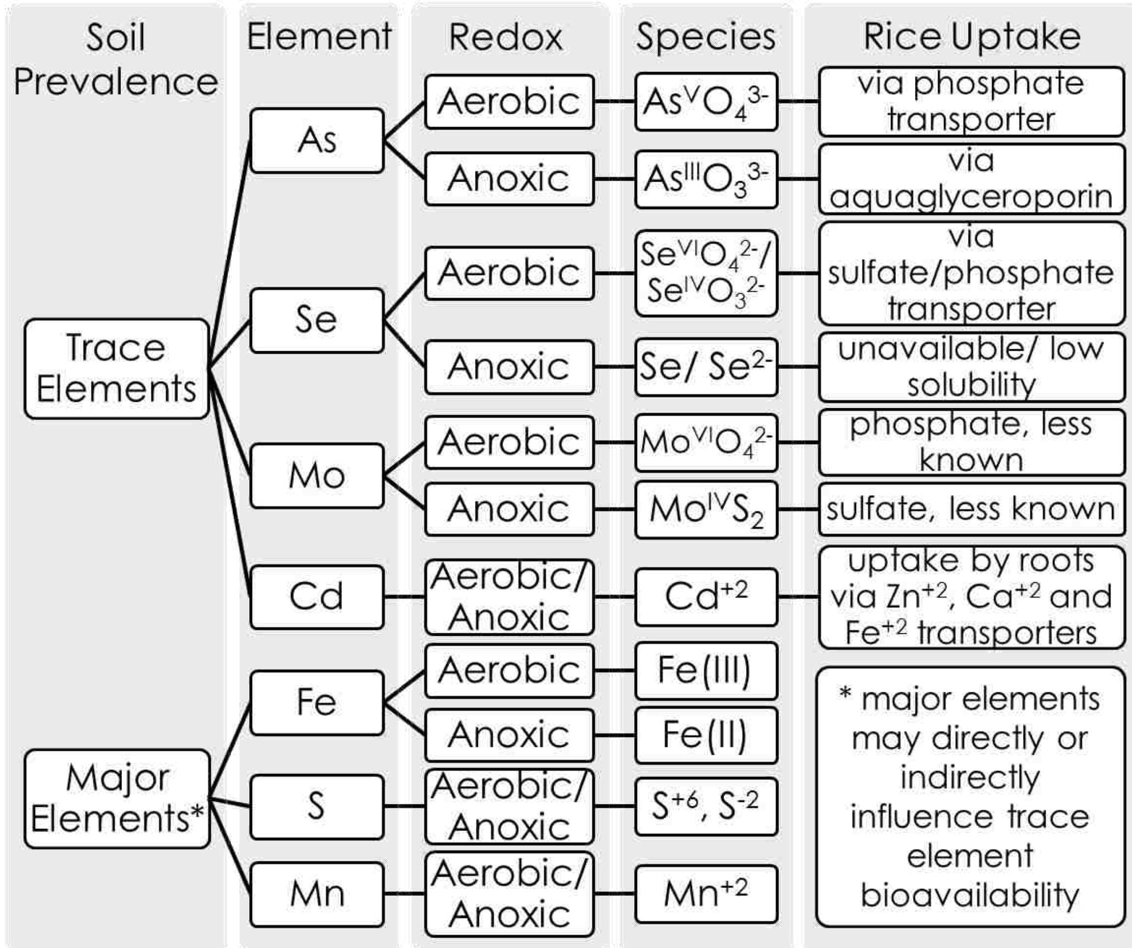

Figure 3.1. Flow diagram identifying trace element bioavailability and uptake into rice plants as influence by particular major elements. Diagram shows the predominant ionic or molecular species and uptake pathways into rice under generalized conditions.

Intermittent flooding, for example, flooding is alternated intermittently between flooding conditions with approximately 10 cm water and then allowed to reach less than field capacity [31]. This intermittent flooding creates a partially oxidized (aerobic) soil environment in between flooding periods. In this environment, the reduction of inorganic

As species is well known to occur with decreasing soil redox potential (0 to -200 mV), transforming As from primarily (> 95%) arsenate (As^{V}) to the generally more mobile and majorly dissociated arsenite (As^{III}). This may increase solubility by as much as 13 times [28]. The increased As solubility is directly associated with the release of As^{V} bound under aerobic conditions to Fe-(hydr)oxide complexes [35].

Iron is known as an essential soil element and offers high adsorption capacity in the oxidized state (Fe^{III}) for sorbates such as As^{V}. In an anoxic environment, these As^{V}-Fe^{III} complexes generally reduce to the more soluble forms of As^{III} and Fe^{II} in a reduced environment and subsequently may increase As bioavailability to plant uptake [35, 36].

In an aerobic environment, Se is present primarily in the more soluble form selenite (Se^{IV}) and in its highest oxidation state of selenate (Se^{VI}) [37]. Mo is present predominantly as Mo^{VI} in aerobic conditions with greatest affinity for oxides and sulfur-containing groups [38]. In an anaerobic environment, Se is present in the stable forms elemental Se (Se^{0}) and selenide (Se^{2-}), however, Se^{2-} tends to form metal complexes with decreased solubility. Se reduction can also occur from microorganisms [39].

Mo is primarily in the molybdate (Mo^{IV}), or MoO_4^{-2} form in a reducing environment and competing for sorption sites similarly as the sulfate anion (SO_4^{-2}) [38]. The mobility of Mo is strongly influenced by redox conditions, existing predominantly as Mo^{IV} in the anoxic environment and Mo^{VI} in the oxic environment. In an anoxic environment, Mo^{IV} is predominantly present as the insoluble sulfide mineral molybdenite (MoS_2) [40]. Pyrite (FeS_2) is an important sediment sink for Mo [41]. Phosphate and molybdate compete with Se^{IV} uptake as a competitive adsorption onto Fe oxides [42]. Further, increased PO_4^{3-} may also assist in the formation of phosphomolybdate complexes in soils that are readily adsorbed by plants [43]. The effect of sulfur on Mo adsorption into plants may exist as direct competition between SO_4^{-2} and MoO_4^{-2} [44].

Plant roots facing stress in Fe-deficient soils have been shown to exude phytosiderophores (PS) that can chelate Cd and form soluble Cd-PS complexes by mobilizing formerly insoluble CdS and enhance plant Cd uptake [45]. In addition, plant Cd has been shown to express a significant positive correlation with plant Fe [46]. With regards to other soil minerals such as zinc (Zn), rice grain-Cd concentrations increase with higher Zn:Cd soil ratios [47].

Arsenic incorporation onto solid Fe-(hydr)oxide and other minerals such as those containing manganese (Mn) at higher soil redox levels (500-200 mV) may also decrease As solubility and corresponding plant uptake [28]. Belefant-Miller [48] has shown soil from rice exhibiting straighthead contains lower soil-Mn and lower pH than rice showing no straighthead; suggesting higher soil-As may contain less soil-Mn for straighthead susceptible cultivars. Therefore, MSMA-amended soil with higher redox levels may support the formation of As-Mn complexes and decrease As bioavailability.

Iron plaque can form on the root surface in an aerobic rhizosphere environment and significantly impact As uptake due to the oxidation of Fe^{II} to Fe^{III} stimulated by oxygen root diffusion or irrigation management such as intermittent flooding [49, 50]. Further, the As^{V} sorption on root plaque has even been shown to account for 80-84% of the total As adsorbed on plaque [34, 42, 51]. Root-plaque has been shown to exceed root concentrations [49, 50], and Somenahally, et al. [34] discovered root-plaque concentrations of up to 10 times greater than the adjacent rhizosphere soil. Continuous flooding conditions have been found to increase the As:Fe molar ratio as compared to intermittent flooding. Maximum concentrations of As on root plaque have been shown to occur in untreated (native) continuously flooded soil, even when compared to As concentrations on root plaque in MSMA-amended intermittent flooded soil [34].

3.2. ESTIMATING UPTAKE MECHANISMS INTO PLANTS

Under aerobic conditions, soluble As has been found to be as much as three times higher in lower pH due to a change in adsorption characteristics, illustrating the adsorption of As^{V} onto (iron) oxide surfaces to be a function of equilibrium pH [52, 53]. The variation of removal of As^{V} by iron (hydr)oxide adsorption in the pH range of 4-10 has been shown to be as great at 92% with maximum adsorption occurring in pH range of 4.5-6 [53, 54]. Cullen and Reimer [5] have shown pH primarily impacts As^{V} mobility.

Previous studies provide conclusive evidence that inorganic and organic As uptake by rice plants utilize separate uptake mechanisms, and further still, the uptake of inorganic As^{III} and As^{V} follow two different uptake mechanisms [56]. This difference was shown by Abedin, et al. [57] through competitive inhibition as the presence of phosphates strongly suppressed As^{V}, whereas As^{III} was not affected. As^{V} is characterized by ion size, symmetry and acid dissociation steps as a phosphate (P^{V}) analogue [58]. The uptake of As^{V} occurs dominantly in aerobic conditions and is readily available for plant uptake as a phosphate analogue via the phosphate transporter pathway [57, 59]. Abedin, et al. [57] has shown As^{III} uptake at high rates following Michaelis-Menten kinetics at the root symplast. The influx of As^{III} is primarily mediated as an active process and the majority of plant As uptake, is transported as a silicic acid analogue by aquaglyceroporins [60, 61].

Selenium in the form of selenate (Se^{VI}) follows the sulfate pathway via sulfate transporters. The uptake of selenite (Se^{IV}) is less understood, although it has been shown that Se^{IV} bonds to iron oxides and hydroxides more strongly than Se^{VI} [62]. Se^{IV} is predominantly mediated into rice plants via phosphate transporters and readily converted to other less soluble forms and accumulates in roots [63]. Antagonisms to sulfate- Se^{VI} are shown to be stronger than phosphate- Se^{IV} [63].

Mo is required by some plants for nitrogen fixation and to support proper plant growth [64]. Plant uptake mechanisms are much less known than, for example, As, Se and Cd. Three Cd transport mechanisms have been identified in rice, including uptake by roots, xylem loading into shoots and translocation up the plant via phloem [65]. Uptake of Cd in roots is a key process, whereby molecular approaches indicate essential element transporters such as Zn^{+2}; Fe^{+2} and Ca^{+2} mediate Cd^{+2} into roots [65, 66]. Cd is most well-known to be transported from rice roots to rice shoots by the xylem following absorption and to accumulate after remobilization from unknown transporters [65, 67].

3.3. IDENTIFYING MITIGATION STRATEGIES

There is a narrow window between nutrition and toxicity for trace elements, therefore well-deserved attention has been given towards mitigation techniques to minimize grain-As and grain-Cd concentrations. Previous research has proven mitigation potential in areas of irrigation management, cultivar selection and soil amendment.

3.3.1. Irrigation Management. Continuous flooding increased As bioavailability to plants, regardless of the initial soil-As concentration [24, 28]. According to Somenahally, et al. [34], intermittent flooding as compared to continuous flooding decreased grain-As concentrations by 30% in MSMA-amended soil and 45% in native soil. Spanu, et al. [68] used a sprinkler type irrigation to further reduced grain-As and achieved 98% reduction in grain-As for 37 cultivars.

Intermittent flooding has been shown to increase the uptake of the more soluble $Se^{IV/VI}$ [37]. Further, intermittent flooding may offer the combined benefit of increasing grain-Se and decreasing grain-As. Intermittent flooding can significantly increase crop yield by decreasing grain-As [31]. In addition, increased grain yield has been linked to a decrease in grain-Mo and suggests intermittent flooding could decrease grain-Mo [69]. Continuous flooding practices in paddy rice fields combine Cd in the soil system with sulfur (S) to form minimally soluble CdS and MoS_2, however the oxidative potential of intermittent flooding converts CdS to the more soluble $CdSO_4$ [70]. Using continuous flooding, [71] reported an 84% reduction in grain-Cd as compared to intermittent flooding.

3.3.2. Soil Amendment. The effect of phosphate application on As mobility and uptake is primarily influenced by competitive adsorption between As^V and to a lesser extent, As^{III} with P^V for available adsorption sites at Fe-oxide surfaces [72]. Research has shown an increase in As mobility to results from the addition of phosphate [73, 74]. Phosphate displaces the sorbed As^V from exchange sites, thus increasing solubility, phyto-availability, and movement down the soil profile [57]. Further, a direct correlation in soil between sodium dithionite-sodium citrate-sodium bicarbonate (DCB)-extractable PO_4^{3-} concentrations and soil-As has been demonstrated by Hua, et al. [33]. However, at least some research has shown phosphate decreases the uptake of As species by rice plants due to a competitive uptake mechanism [57]. In addition, Liu, et al. [46] showed As concentration in iron plaque to be significantly lower in plants treated with PO_4^{3-}

solution (1.3 mM KH_2PO_4). When phosphorus (P) was present, four times adsorbent (Fe_2O_3) was required to adsorb the same amount of As without P (3.7x) [54]. Evidence suggests increased PO_4^{3-} application to As-susceptible cultivars may even suppress As^V uptake in plants and uptake PO_4^{3-} more efficiently [75]. Wang, et al. [78] has shown PO_4^{3-}phosphorus (P) in the form of phosphates (PO_4^{3-}), as phosphates have a greater affinity for soil sorption sites [38, 64]. Further, Abedin, et al. [57] showed application of PO_4^{3-} was more neutral and does not significantly impact plant yield or plant As concentrations, except for rice husk. Therefore, the impact of PO_4^{3-} application on As uptake remains unknown.

The effect of PO_4^{3-} application on Se mobility and uptake is primarily influenced by the competitive inhibition between increasing PO_4^{3-} and Se^{IV} [63]. Se transport mechanisms corresponding to PO_4^{3-} applications deserve further study to improve rice Se concentrations and minimize Se biofortification practices [76]. Increased Mo mobility may increase further with increased PO_4, as added phosphate also assist in the formation of phosphomolybdate complexes in soils that can then be readily adsorbed by plants [43].

The effect of phosphate application on Cd mobility and uptake is primarily enhanced by phosphate application. Waterlot, et al. [55] shows P amendment generally increased Cd uptake in rye grass shoots by 17.9-79%. Wang, et al. [77] has shown phosphate fertilizer addition to significantly decrease soluble Cd (1.5-30.7%) and uptake in cabbage by 16.5-66.9%. The effect of phosphate amendments on Cd mobility and uptake is also a function of the soil physiochemical properties, reducing Cd mobility by as much as 40% with a 3:5 amendment ratio of phosphate to Cd [46].

A mixture of in situ Fe-oxide and Al-oxide soil amendments have shown As adsorption potential in the order of greatest to least: ferrihydrite > boehmite > goethite and adsorption was determined primarily a function of porosity [54, 78]. One remediation strategy found a combination of steel shot and beringite (aluminosilicate) (1% and 5%, respectively) to largely immobilize soil As by adsorption on the aluminum (Al) and Fe oxides present in beringite [79-81]. Another performed by Manning, et al. [82] took the approach of applying zerovalent iron (Fe^0) and found Fe^0 immediately begins to corrode to Fe^{II} using water as the primary oxidant of the Fe^0 surface and then is reacts with OH- and oxidizes a step further to a mixed Fe^{II}/Fe^{III}. In aerobic conditions Manning, et al. [82] found an Fe^{II}/Fe^{III} mixture assisted with oxidation of As^{III} to As^V. Further, the study confirmed Fe as goethite as a substantial absorbent, but found hematite and magnetite to only form weak bonds with As^{III} [82]. Zhang, et al. [54] has shown As^V follows a Langmuir adsorption isotherm onto majorly hematite and goethite iron ore deposits with a capacity of 0.4 mg As^V g^{-1} adsorbent.

Perhaps the largest reduction provided by in situ soil amendments was achieved by Moore and Patrick Jr [83] using ferrous sulfate to reduce As^V concentrations in soil by 99.5%. The potential offered by the application of commercial grade $FeSO_4$ at 1.89% (w/w) has also been shown by Warren and Alloway [84] as a method for reducing As uptake in plants by up 84%. However, using ferrous sulfates to bind soil-As requires supplemental agricultural lime to adjust pH [83, 84].

Other elements such as Mo in the form of molybdate Mo^{VI} also have structural similarities, but a lower adsorption affinity towards Fe^{III} comparable to As^V [38, 83].

Although the release mechanism(s) primarily occur as reductive dissolution for As and Mo, the impact of iron oxide on the uptake of Se and Cd is largely unknown.

3.3.3. Other Strategies. Microbial activity has been shown to cause significant impact on As bioavailability [54, 85]. In the soil environment, oxygen depletion occurs with microbial activity and organic matter decomposition during flooded conditions [31]. The concentration of organic As species, dimethylarsinic acid (DMA^{V}), in soil pore-water has been positively correlated to the microbial methylation of As^{III} to DMA^{V} in anoxic conditions [85]. Further, the presence of sorbents strongly reduces As^{V} reduction rate by bacteria [78].

4. ACCUMULATION OF SELECTED TRACE ELEMENTS IN RICE: EFFECTS OF SOIL ARSENIC, IRRIGATION MANAGEMENT AND CULTIVAR

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4.1. ABSTRACT

Accumulation of trace elements of concern in rice has been reported in many regions of the world, including the United States. This research investigated the accumulation of selected trace elements including arsenic (As), selenium (Se), molybdenum (Mo) and cadmium (Cd) in six rice cultivars commonly grown in the south central U.S. The effects of As content in soil, irrigation management and cultivar on total grain element accumulation were evaluated. Results indicated MSMA-amended soil increased the accumulation of As and Se, but decreased Mo under all irrigation treatments. Grain-Cd increased for most cultivars. In native soil, intermittent flooding significantly decreased total grain Mo with negligible on As, Se and Cd. However, under MSMA-amended soil conditions, intermittent flooding significantly decreased total grain-As, but significantly increased Se and Cd and only slightly increased grain-Mo. A negative grain As-Se correlation, but a positive grain As-Mo correlation was identified based on all samples in this study. However, the grain As-Cd correlation was negligible. Statistical analysis revealed significant interactions between factors for As, Se and Mo accumulation, with negligible impact on Cd and concluded As, Se, Mo and Cd uptake to be a complex function rather than a function of a single factor.

4.2. INTRODUCTION

Inorganic arsenic (As) has been determined a human carcinogen by the U.S. EPA [10]. Rice contamination by As , though primarily composed of less toxic organic As, has also been recognized as a carcinogenic human health risk [86, 87]. Arsenic contamination in food crops could be caused by elevated As levels in soils of traditional cotton growing regions of the south central U.S., which were treated with the application of arsenical herbicides such as monosodium methanearsonate (MSMA) [11]. The average total grain-As concentration of rice grown in the south central U.S. is 0.27 µg As g^{-1} with approximately 0.11 µg As g^{-1} as inorganic As [2, 4]. However, As contamination is primarily in the inorganic form in subsequent rice food products such as toddler formula, cereal bars, rice syrup and energy shots [3, 21, 25]. Therefore, the daily consumption of these majorly unregulated rice and rice food products has exceeded the equivalent of U.S. EPA and WHO drinking water standard of 10 µg L^{-1} [24, 25]; making it imperative that the agricultural practices directly responsible for total As in foods be studied further.

Environmental conditions that relate to As uptake by rice plants have included As content in soil (soil-As), irrigation management and genotype variables such as cultivar and heading date in previous studies. Elevated soil-As corresponds to higher As content in grain (grain-As) [24, 26]. Straighthead disease is common to rice crops grown with elevated soil-As conditions, where panicle sterility and significant yield loss are realized [32]. Grain-As is exacerbated further irrespective of soil-As levels, where flooded conditions create a reducing environment that can lead to an increase in As uptake [24, 28, 34]. Plant As uptake also varies based on cultivar [13, 29], therefore potential to significantly reduce grain-As exists through cultivar selection [31].

In addition to As, the accumulation of other trace elements such as selenium (Se), molybdenum (Mo) and cadmium (Cd) in rice grain could also be of concern. Selenium is an essential trace element with known deficiencies that cause negative health effects such as cardiovascular disease and cancer [19]. However, Se is also regulated by the U.S. EPA in drinking water with a maximum contaminant level (MCL) of 50 $\mu g\ L^{-1}$ as intake exceeding this level may also negatively impact human health [88]. By understanding Se transport mechanisms, rice Se concentrations may be regulated as necessary and minimize Se biofortification practices [89, 90]. Contrary to As uptake in continuous flooding, Mikkelsen, et al. [37] has shown Se uptake to decrease in rice as compared to non-flooded upland crops. Premarathna, et al. [90] has shown irrigation management impact on total grain Se to follow the order: field capacity > submerged > submerged/drained.

Molybdenum (Mo) is an essential trace element to human, though listed on the U.S. EPA drinking water Contaminant Candidate List 3 (CCL3) due to its potential negative effect under high concentrations [88]. Mo has been found to regulate calcium (Ca), magnesium (Mg) and copper (Cu) metabolism in humans, where the greatest risk of Mo toxicity has been shown to occur in humans with Cu deficiencies [91]. Dunn and Dunn [69] discovered an increase in grain yield (or a decrease in total grain-As) resulted in a decrease in grain-Mo, suggesting a positive As-Mo correlation. Molybdenum toxicity may be a factor for crops grown in poorly drained soils; where tolerance varies with cultivar [92].

In recent years the accumulation of Cd in rice has been reported in many areas, especially China [70]. Unlike As, Se and Mo, which occur mostly as aqueous anionic complexes, Cd is soluble generally as the Cd^{2+} ion in rice soils. The bioaccumulation of non-essential elements such as Cd in rice may be greater than some essential elements [46]. Negative health impacts are known for Cd, including kidney dysfunction [93]. Further, Cheng, et al. [94] showed a positive As-Cd correlation in rice roots and straw; however, these studies did not evaluate total grain-Cd.

In previous research, most studies focused on the uptake of individual elements by rice under various environmental conditions. However, the uptake of multiple elements of concern may be related due to interaction between elements. In this research, the grain accumulation of multiple elements of concern, including As, Se, Mo and Cd were studied. The objectives of this research were to provide a comprehensive understanding and the significance of (a) the impact of soil-As on total grain concentrations of elements of concern; (b) the impact of irrigation management techniques on total grain concentrations of elements; and (c) correlations between elements of concern.

4.3. EXPERIMENTAL

4.3.1. Design. The field study was conducted in Stuttgart, Arkansas at the USDA ARS Dale Bumpers National Rice Research Center in 2009. The experiment was arranged in a split-split plot design with soil main plots and irrigation management sub-plots, where the main plot acted as the blocking factor. Each main plot was divided into four completely randomized sub-plots, for a total of eight treatment combinations. Each sub-plot included six cultivars completely randomized in 1.8 m by 1.5 m plots prepared according to Pillai, et al. [31].

The native soil is a Dewitt silt-loam (fine, smectitic, thermic Typic Albaqualf). The field of MSMA-amended soil was treated with MSMA at a rate of 6.7 kg ha^{-1} in alternate years since the 1980s for straighthead resistance evaluation [32]. Fields of non-MSMA soil (e.g. native soil) was located next to the field receiving MSMA treatment. Field soil samples were collected for analysis from both sites at the surface horizon (0-20 cm) before rice planting according to Hua, et al. [33].

Six cultivars commonly grown in the south central U.S. (4484-1693, Cocodrie, GP-2, Spalcik, Wells and Zhe 733) were included in this study. Cultivars included early, mid and late maturity periods with varying As induced straighthead susceptibility (resistant, moderate, and susceptible) [95, 96]. Arsenic tolerant cultivars that are given commercial precedence included Zhe 733. Therefore, the current research will concur with a practical solution to minimize grain-As in field production and focus on the impact of treatment factors on Zhe 733. The approach used for Zhe 733 analysis was used to analyze trends for the five additional cultivars.

The four irrigation management practices used in this study included one intermittently flooded condition and three continuously flooded conditions. Continuous flooding varied based on field draining 10, 20 and 35 days after rice plant heading date (50% panicle emergence from flag leaf). Intermittent flooding conditions were maintained in keeping soil moisture at or above field capacity, irrigating up to 10 cm from a nearby rainfall reservoir (pH ~7.0, < 2 µg As L^{-1}) when soil surface moisture decreased to less than capacity. Cultivars were triplicated within each treatment combination. At maturity, rice grain from each plot was harvested and stored at room temperature. Prior to digestion, samples were de-hulled using ceramic mortar and pestle and oven dried at 65°C for 48 h or until constant weight and stored in a desiccator.

4.3.2. Sample Digestion Procedure. A modified EPA Method 3052 was used for the total digestion of the rice grain, using a pressure/temperature (p/T) sensor for control. Pre-acid cleaned Rotor 48 (MF50) digestion vessels were used and 0.5 g rice grain was measured into the vessels with 8.0 mL optima grade nitric acid (HNO_3) and 0.3 mL 30% hydrogen peroxide (H_2O_2) (Fisher Scientific Co., Boston, U.S.). The digestion program consists of a 10 min ramp to 100°C with a 10 min hold, followed by a 10 min ramp to 140°C with a 15 min hold, then a 10 min ramp to 170°C with a 10 min hold before cool down.

A modified EPA Method 3051A was used to digest the soil and determine the acid extractable elements. Pre-acid cleaned Rotor 8 (XF100) digestion vessels were used and 0.5 g soil was measured into the vessels with 9.0 mL trace metal grade HNO_3 and 3.0 mL trace metal grade hydrochloric acid (HCl). The digestion program consists of a 10 min ramp to 1100W with a 20 min hold before cool down.

Digestion vessels were sealed in the rotor assemblies and samples were digested using a Multiwave 3000 microwave digester (Anton Paar, Austria). All digested samples were allowed to cool down to < 50°C before venting. A blank cleaning digestion using HNO_3 was performed for the MF50 and XF100 rotors between sample digestions.

Standard reference materials (NIST, Gaithersburg, MD) for rice grain (SRM1568a) and soil (SRM2711a Montana II) were included in respective digestions for quality assurance and quality control (QA/QC). Additional digestion QA/QC included one sample blank, one sample duplicate and one sample spike per 10 samples for rice and soil digestion. Spike concentrations were analyzed at an estimated two times average sample concentrations. Digested samples were rinsed from digestion vessels to acid cleaned 50 mL conical tubes and diluted to 20 mL using Millipore water (18.2 MΩ-cm).

4.3.3. Chemical Analysis. Total As, Se, Mo and Cd concentrations in digested grain samples and extractable Se, Mo and Cd from soil samples were determined using inductively coupled mass spectrometry (ICP-MS) (PerkinElmer Elan DRC-e, MDS Sciex, Concord, Ontario, Canada). Extractable As from soil was determined using graphite furnace atomic adsorption (GFAA) (PerkinElmer AAnalyst™ 600, Shelton, CT, USA). Digested grain and soil samples were diluted to HNO_3 concentrations of 10% and 5% prior to ICP-MS analysis, respectively. Sample method detection limits (MDLs) for all tested elements were determined for the GFAA and ICP-MS according to EPA Method 200.9 and 200.8, respectively. Sample concentrations greater than 2-3 times MDL were required. Estimated soil MDLs for As, Se, Mo and Cd were 0.10, 0.08, 0.02 and 0.02 mg kg^{-1}, respectively. Estimated rice grain MDLs for As, Se, Mo and Cd were 0.004, 0.031, 0.003 and 0.003 mg kg^{-1}, respectively. QA/QC was validated if the following criteria were met: (a) relative difference of a duplicate sample less than 20%; (b) spike recovery between 85-115%; and (c) the accuracy of a calibration standard remained within 10% per 10 samples analyzed. Concentrations related to soil and grain accumulation in this study are reported as total concentrations unless stated otherwise.

4.3.4. Statistical Analysis. The analysis of variance (ANOVA) procedure appropriate for the split-split-plot design utilized in this research was accrued out using SAS 9.2 to estimate the impact of experimental variables on element uptake. The experimental variables (factors) are soil-As (Soil), irrigation management (Water) and Cultivar, with Soil treated as the main-plot factor, Water treated as the sub-plot factor and Cultivar acting as the sub-sub-plot factor. ANOVA allows interaction terms representing the combined impact of different factors on element uptake to be tested, whereby a significant interaction term signifies that the impact of the changing levels of one factor is influenced by the level of the other factor(s).

Experiment design was limited by the absence of replication at the main-plot level. Within each main-plot, Soil × Water treatment combinations were replicated three times, allowing Cultivar × Soil × Water, Cultivar × Water, Cultivar × Soil and Cultivar to be tested. Least square means with Tukey's adjustment were used to contrast means of specific Soil-Water combinations. Tukey's procedure allowed experiment-wise error to be kept at the selected rate, $\alpha = 0.05$ [97].

4.4. RESULTS AND DISCUSSION

4.4.1. Soil Characterization. Characterization and major physiochemical properties of two soils used in this study, native soil and MSMA-amended soil are presented in Yan, et al. [95] and Hua, et al. [33]. The average soil-As in native and MSMA-amended soil was determined to be 5.9 ± 0.25 and 19.5 ± 0.36 mg kg^{-1}, respectively, confirming Hua, et al. [33], which were 5.0 and 18.3 for the native and MSMA-amended soil, respectively. Further, native soil contained 0.23 ± 0.004 mg kg^{-1} Se, 0.38 ± 0.041 mg kg^{-1} Mo and 0.06 ± 0.003 mg kg^{-1} Cd and MSMA-amended soil contained 0.53 ± 0.086 mg kg^{-1} Se, 0.63 ± 0.239 mg kg^{-1} Mo and 0.06 ± 0.006 mg kg^{-1} Cd.

4.4.2. Effect of Soil As. Grain concentrations were evaluated based on soil-As present in native and MSMA-amended plots. The importance of evaluating native soil was not only to provide a control for MSMA-amended soil, but also to determine the accumulation behavior of the trace elements of concern for low-As soil, and the effect of element interaction on their accumulation. It was hypothesized that multiple elements interfere with each other during the accumulation process. It has been reported that elevated grain-As concentrations can occur in low-As soils under continuous and intermittently flooded conditions, and the presence of trace quantities of MSMA can strongly affect grain-As [98]. Yan, et al. [32] showed increased grain-As and yield reduction due to straighthead susceptibility results from MSMA-amendments under continuous flooding and similar environmental conditions. Therefore, both soil As and irrigation management impact grain-As and other elements in grain.

The effect of soil-As on average grain concentrations of As, Se, Mo and Cd under continuous and intermittent flooding conditions for the As-tolerant Zhe 733 is illustrated in Figure 4.1. Zhe 733 was selected based on its straighthead resistance and commercial use.

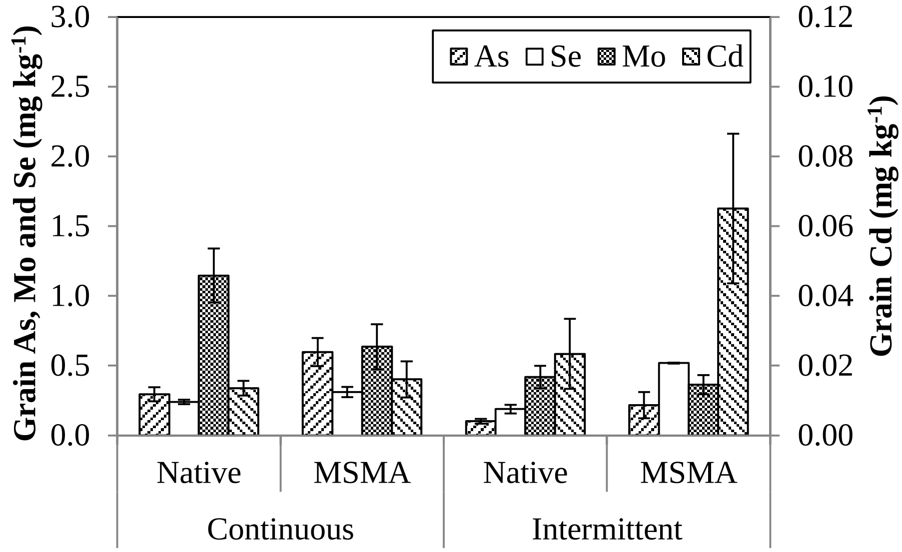

Figure 4.1. Average total grain element concentrations for Soil × Water combinations of Zhe 733 comparing soil-As (main plot) and irrigation management (sub-plot) effects on total grain concentration of As, Se, Mo and Cd; ± bars = std. dev.

Under continuous flooding conditions, MSMA-amendments increased As from 0.30 to 0.60 mg kg^{-1}, increased Se from by 0.24 to 0.31 mg kg^{-1}, decreased Mo from 1.15 to 0.64 mg kg^{-1} and increased Cd from 0.01 to 0.02 mg kg^{-1}. Under intermittent flooding conditions, MSMA-amendments increased As from 0.10 to 0.22 mg kg^{-1}, increased Se from 0.19 to 0.52 mg kg^{-1}, decreased Mo from 0.42 to 0.36 mg kg^{-1}, and increased Cd from 0.02 to 0.07 mg kg^{-1}. Therefore, increased soil-As not only increased grain-As, but also significantly impacted the uptake of Se, Mo and Cd, especially under intermittent flooding conditions.

A general trend of the effect of soil-As on average grain concentrations of As, Se, Mo and Cd for five other cultivars under continuous and intermittent flooding conditions is illustrated in Figure 4.2. For the five cultivars under continuous flooding conditions, MSMA amendments increased As by 250%, increased Se by 29%, decreased Mo by 51% and increased Cd by 35%. Under intermittent flooding conditions, MSMA amendments increased grain-As by 46%, increased Se by 71%, decreased Mo by 25% and decreased Cd by 12%. Overall, increasing soil-As positively impacted grain-As, grain-Se and grain-Cd, but negatively impacted grain-Mo.

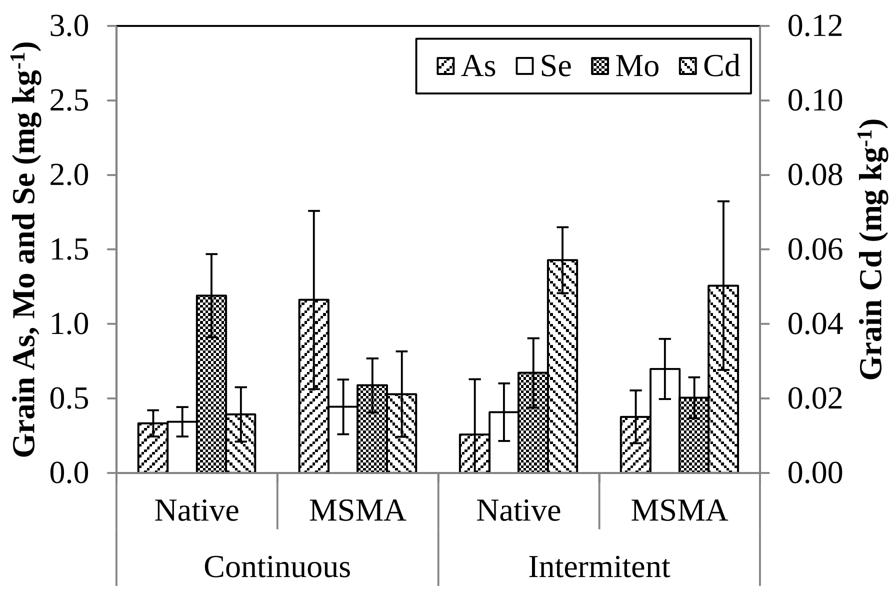

Figure 4.2. Average total grain element concentrations for Soil × Water combinations of five cultivars comparing soil-As (main plot) and irrigation management (sub-plot) effects on total grain concentration of As, Se, Mo and Cd

4.4.3. Irrigation Management Effect – Native Soil. Also shown in Figure 4.1, for Zhe 733 in native soil and compared to continuous flooding, intermittent flooding significantly decreased grain-As from 0.30 to 0.10 mg kg^{-1} ($p = 0.003$), significantly decrease grain-Se from 0.24 to 0.19 mg kg^{-1} ($p = 0.04$), significantly decreased grain-Mo from 1.14 to 0.42 mg kg^{-1} ($p = 0.001$, $F = 14.78$) and slightly increased grain-Cd from 0.01 to 0.02 mg kg^{-1} ($p = 0.111$).

In the case of the five other cultivars under native soil and compared to continuous flooding, intermittent flooding slightly decreased grain-As from 0.33 to 0.26 mg kg^{-1}, slightly increased grain-Se from 0.34 to 0.41 mg kg^{-1}, significantly decreased grain-Mo from 1.19 to 0.67 mg kg^{-1} ($p < 0.001$, $F = 16.89$) and slightly increased grain-Cd from 0.02 to 0.06 mg kg^{-1} (Figure 4.2).

Redox potential largely impacted the fate and transport of As, due to the presence of insoluble Fe(III) (hydr)oxides and the formation of As(V)-Fe(III) complexes [36, 99]. Therefore, results from the current research support As incorporation onto Fe and other minerals such as manganese (Mn) at higher soil redox levels (500-200 mV) occurred in the intermittent flooding conditions, which may decrease As solubility and corresponding plant uptake [28]. In addition, under high redox levels, As is also in the less mobile As(V) form [5]. Therefore, intermittent flooding decreases As mobility and plant uptake.

For Zhe 733, intermittent flooding also significantly decreased grain-Se. However, the overall trend of the other cultivars indicated a slight increase in grain-Se under intermittent flooding, confirming Mikkelsen, et al. [37]. This further suggests the Se uptake mechanism is a function of cultivar. Therefore, cultivar selection based on minimizing grain-As may also have implications on other grain concentrations. Similar to grain-As, overall grain-Mo significantly decreased as a result of intermittent flooding. The primary uptake mechanism for Mo and As under aerobic conditions exists via the phosphate pathway [42]. Therefore, it is evident the more oxidized environment regulates and effectively reduces Mo bioavailability by the formation of immobile iron (hydr)oxide complexes, resulting in lower mobility and thus plant uptake [83]. Overall grain-Cd concentrations significantly increased under the intermittent flooding condition. Due to the more oxidized conditions associated with intermittent flooding, insoluble CdS may convert to the more soluble $CdSO_4$, resulting in a greater availability of Cd [51].

The average As, Se, Mo and Cd grain concentrations in native soil for five individual cultivars are given in Figure A.1. Tukey's adjustment was used to identify the cultivar that has the most significant increase or decrease of a given element as compared to others. This provides a process to minimize or maximize one element uptake in a given Soil × Water combination by cultivar selection while evaluating this impact on other elements. The significant impact of cultivars for the Soil × Water combinations of the six cultivars in native and MSMA-amended soil is given in Table 4.1. Among the six cultivars in native soil under continuous and intermittent flooding conditions, cultivar selection has at least one significant impact on the uptake of each element. Therefore, this research provides evidence that cultivar selection alone can significantly increase or decrease grain element concentration and varies with irrigation management.

Table 4.1. Effect of cultivar for Soil × Water combinations of six cultivars on total grain concentration of As, Se, Mo and Cd

	Native		MSMA-amended	
	Continuous	Intermittent	Continuous	Intermittent
As	Spalcik ↑	Zhe 733 ↓ Spalcik ↑	-	-
Se	Zhe 733 ↓ GP-2 ↑	-	-	-
Mo	4484-1693 ↓ Wells ↑	Zhe 733 ↓ Spalcik ↑	Spalcik ↓	-
Cd	GP-2 ↑	-	GP-2 ↑	-

4.4.4. Irrigation Management Effect – MSMA-Amended Soil. Figure 4.1 also shows the effect of irrigation management on average grain concentrations of As, Se, Mo and Cd for Zhe 733 under MSMA-amended soil conditions. In MSMA-amended soil and compared to continuous flooding, intermittent flooding significantly decreased grain-As from 0.60 to 0.22 mg kg^{-1} ($p = < 0.001$, $F = 27.09$), significantly increased grain-Se from 0.31 to 0.52 mg kg^{-1} ($p < 0.001$, $F = 25.76$), significantly decreased grain-Mo from 0.64 to 0.36 mg kg^{-1} ($p = 0.023$) and significantly increased grain-Cd from 0.02 to 0.07 mg kg^{-}

^{1} ($p = 0.001$, $F = 15.53$). Therefore, irrigation management resulted in similar effect on As, Mo and Cd uptake in native soil and MSMA-amended soil; however, the effect on grain-Se was opposite.

In the case of the other five cultivars in MSMA-amended soil and compared to continuous flooding, intermittent flooding significantly decreased grain-As from 1.16 to 0.38 mg kg^{-1} ($p < 0.001$, $F = 12.01$), significantly increased grain-Se from 0.44 to 0.70 mg kg^{-1} ($p < 0.001$, $F = 8.86$), slightly decreased grain-Mo from 0.59 to 0.50 mg kg^{-1} and significantly increased grain-Cd from 0.02 to 0.05 mg kg^{-1} ($p < 0.001$, $F = 20.96$) (Figure 4.2). Therefore, as compared to native soil, intermittent flooding under MSMA-amended soil conditions has a greater impact on the grain-As decrease. The grain-As concentrations in MSMA-amended soil determined from this study were slightly different from those reported by other researchers [4, 31], most likely due to the cultivar section in different studies.

The increase in grain-Se found as a result of intermittent flooding in MSMA-amended soil in the current research confirms Mikkelsen, et al. [37] and corresponds to native soil results. It is known that Se is most prevalent in the mobile selenate form under aerobic conditions, therefore causing the increase in grain-Se in intermittent flooding [37]. The increase in grain-Cd as a result of intermittent flooding discovered in MSMA-amended soil in the current research corresponds with Arao, et al. [70]. This increase in Cd concentrations of rice grain due to aerobic treatments makes it difficult to simultaneously decrease human exposure to both As and Cd elements based on irrigation management.

Figure A.1 also shows the average grain concentrations in MSMA-amended soil for the other five cultivars, and Tukey's adjustment was used to identify the cultivar that has the most significant increase or decrease for a given element as compared to others.

Table 4.1 also shows that among the six cultivars in MSMA-amended soil under continuous and intermittent flooding conditions, cultivar selection has a significant impact on grain-Mo and grain-Cd. Therefore, this research validates evidence that cultivar selection alone can significantly increase or decrease grain element concentration due to differences in uptake mechanisms and plant transport. Though the cultivar impact on grain- As, Se, Mo and Cd in MSMA-amended intermittent flooding conditions was also determined negligible, the cultivar Zhe 733 minimized grain-As in MSMA-amended soil, corresponding with Hua, et al. [33]. Combined with results from native soil plots, this result agrees with previous research and indicates the importance of cultivar selection as a control of element uptake [13, 29, 100].

A field experiment using 25 diverse rice cultivars including the study of the heading date impact on grain-As and plant yield was conducted by Pillai, et al. [31]. A positive correlation between grain-As and heading date was found, which was attributed to extended vegetative growth and exposure to soil-As due to delayed heading [31].

4.4.5. Element Correlations in Rice Grain. The six cultivars in this experiment were evaluated ($p < 0.05$, $R^2 > 0.60$) individually based on the correlations between grain-As concentrations and grain- Se, Mo and Cd concentrations in both native and MSMA-amended soil. Figure 4.3 shows the impact of grain-As on grain Se, Mo and Cd concentrations and the corresponding correlations.

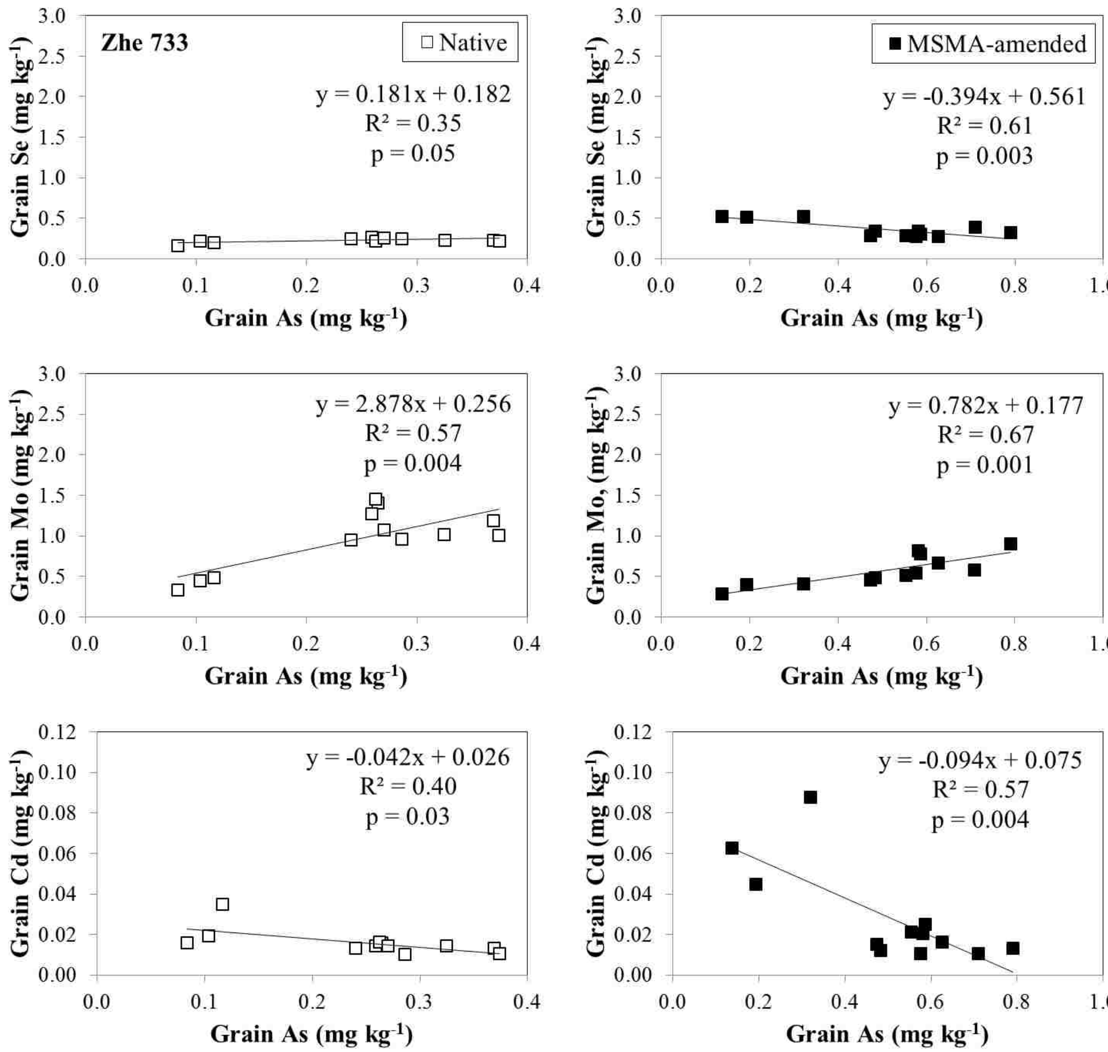

Figure 4.3. Element correlations for the Zhe 733 cultivar in native (left) and MSMA-amended soil (right), including As-Se, As-Mo and As-Cd (top to bottom); evaluated based on coefficient of determination, $R^2 = 0.60$.

In native soil, grain-As had a positive impact on grain-Se in native soil ($R^2 = 0.35$, $p = 0.05$), but had a negative impacted in MSMA-amended soil ($R^2 = 0.61$, $p = 0.003$) (Figure 4.3). Grain-As had a positive impact on grain-Mo in both native ($R^2 = 0.57$, p =

0.004) and MSMA-amended (R^2 = 0.67, p = 0.001) soil. However, grain-As had a negative impact on grain-Cd in native (R^2 = 0.40, p = 0.03) and MSMA-amended (R^2 = 0.57, p = 0.004) soil. These results contradict findings of a positive As-Cd correlation (Cd = 0.4162×As) in rice root and straw discovered by Cheng, et al. [94].

Figure A.3 shows correlations between grain- As, Se, Mo and Cd are shown for five additional cultivars in native and MSMA-amended soil. An overall negative trend between grain-As and grain-Se was observed, though no strong correlations were found. Strong positive grain As-Mo correlations were discovered for 4484-1693 (native), Cocodrie (native) and Wells (native) similar to Zhe 733 presented above. Dunn and Dunn [69] discovered plant Mo is negatively correlated with straighthead score; suggesting total grain Mo decreases as straighthead score and total grain-As increase, contradicting the overall positive grain As-Mo correlation found in the current research. A negative grain As-Mo correlation was also shown in Norton, et al. [101]. Strong neutral As-Cd correlations were discovered for Cocodrie (MSMA-amended), GP-2 (native), Spalcik (MSMA-amended), and Zhe 733 (MSMA-amended).

4.4.6. Analysis of Variance. ANOVA was performed on Zhe 733 using experiment-wise mean squared error to determine significance of main effects and the corresponding interaction specific to Zhe 733. The significance of the Soil × Water interaction term was tested first and succeeded by main effects, Soil and Water. Table 4.2 also shows significance levels of interaction and main effects. Testing interaction terms for Zhe 733, the Soil × Water interaction is significant for Mo only (p = 0.04), indicating the impact of soil-As on grain-Mo is influenced by irrigation management. Testing main effects for Zhe 733, soil-As is significant for As and Se (p = 0.01 and 0.01, respectively), confirming the increase in grain-As and grain-Se in MSMA-amended soil as compared to native soil that was discovered in the current research.

Table 4.2. Total grain concentration analysis of variance for six cultivars as impacted by Soil, Water and Cultivar in split-split plot, including results for Zhe 733 (as impacted by Soil and Water) and six cultivars (as impacted by Soil, Water and Cultivar).

			Element (Pr > F) †			
	DF	F_C †	As	Se	Mo	Cd
			Zhe 733			
Soil	1	4.49	0.01**	0.01**	< 0.001***	0.59
Water	3	3.25	0.09	0.59	< 0.001***	0.73
Soil × Water	3	3.25	0.69	0.19	0.04*	0.9
			4484-1693, Cocodrie, GP-2, Spalcik, Wells and Zhe 733			
Cultivar	5	2.31	< 0.001***	< 0.001***	< 0.001***	0.3557
Cultivar × Soil	5	2.31	< 0.001***	0.0114*	0.0002***	0.5190
Cultivar × Water	15	1.77	0.0498*	0.7769	0.0041**	0.1933
Cultivar×Soil×Water	15	1.49	< 0.001***	0.8419	0.1503	0.7902

$MS_{\text{experiment-wise}}$ = 0.051, 0.014, 0.023, and 0.003 for As, Se, Mo and Cd, respectively

*, **, *** indicate significance when $p < 0.05$, 0.01, and 0.001

† $\alpha = 0.05$

Table 4.2 also shows the ANOVA performed on the split-split plot experiment for all six cultivars to test significance of treatment main effects and interactions for elements As, Se, Mo and Cd. Testing interactions for all six cultivars, Cultivar × Soil × Water is significant for As ($p < 0.001$); Cultivar × Water is significant for Mo ($p = 0.004$); and Cultivar × Soil is significant for Se ($p = 0.011$). Further analysis using Tukey's test was utilized to contrast Cultivar means within Soil × Water combinations, allowing irrigation management, soil effects and existing correlations to be evaluated. The occurrence of multiple significant interactions for the six cultivars indicate As, Se, Mo and Cd uptake to be a complex function rather than a function of a single element.

4.5. ASSOCIATED CONTENT

4.5.1. Supporting Information. Details on method detection limits, average total grain concentration based on cultivar, heading date and total grain-As analysis, and element correlations based on cultivar.

4.5.2. Acknowledgements. This research was funded by USDA (Award No. 2008-38814-04727) to Missouri University of Science & Technology through Lincoln University. The authors would like to thank Ms. Tiffany Sookaserm for sample collection and preparation, Ms. Brandi Clark for assistance in method development, and Prof. V. A. Samaranayake for assistance of statistical analysis.

5. ACCUMULATION OF SELECTED TRACE ELEMENTS IN RICE: EFFECTS OF SOIL ARSENIC, PHOSPHATE, AND FERRIC OXIDE – A GREENHOUSE STUDY

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5.1. ABSTRACT

Approximately half of the world's population is majorly reliant upon rice for its caloric intake and is at risk of contamination by arsenic (As) and other trace elements. Though contamination varies globally, recent research has directly the use of specific environmental factors to reduce As uptake and subsequent human health. However, previous research has largely produced mixed results. This research was completed as a greenhouse study to replicate contaminated rice grown south central U.S. on native and high-As soils contaminated by arsenical herbicides applied to the former cotton belt. Studies included the impact of soil-As, phosphate application and iron oxide amendments on total grain concentrations of As, Se, Mo and Cd. Correlations between elements in rice grain were also evaluated. It was found that when compared to native soil, MSMA-amended soil increased grain-As, increased grain-Se, decreased grain-Mo and increased grain-Cd. Phosphate application was found to have a substantial impact on the uptake and accumulation of Se. It was also found that iron oxide amendments had a significant impact on grain-As.

5.2. INTRODUCTION

Rice is a staple food crop for which 50% of the world's population depends for its primary caloric intake and is at risk of contamination by arsenic (As) and other trace elements including cadmium (Cd) [102]. Arsenic contamination of rice varies globally with crops grown in some areas contaminated by As polluted irrigation water, others grown on soil with high amounts of As byproducts due to large mining areas, and still others are extensively cultivated on As impacted soil from the historic application of arsenical pesticides and defoliants [11, 103]. Global paddy rice crops account for an estimated 75% of global rice production, growing at an annual rate of 3% and further exacerbating the risk to human health [17, 18].

Elevated soil-As levels in the south central U.S. are the result of monosodium methanearsonate (MSMA) application as an herbicide in this former cotton growing region that currently produces the majority of paddy rice supplied to the U.S. [20, 30]. It is well known that plant As accumulated in rice grain increases as soil-As levels increase [24]. As a result, the typical rice subsistent Asian-American diet (400 g d^{-1} cap^{-1}) may consume up to six times more than the average rice consumer in the U.S. [2, 104] and easily exceed the drinking water equivalent standard set by the U.S. EPA and World Health Organization (WHO) as a maximum tolerable daily intake (MTDI) of 10 µg L^{-1} [21, 24, 25]. Evidence suggests the human health risk associated with As may persist and or impact other trace elements [105].

The mobility, uptake and accumulation of As and elements such as selenium (Se), molybdenum (Mo) and cadmium (Cd) is heavily influenced by complex soil properties, minerals and is further impacted by the application of phosphate containing crop fertilizers [77, 106]. Arsenic is thermodynamically stable in most soils and residual soil-As persists decades after crop applications of MSMA ceased in the U.S [10]. Among the factors that influence As mobility in soil are speciation, soil redox (Eh) and the presence of mineral surfaces such as iron (Fe). Studies have shown roots can uptake organic and inorganic As species using separate uptake mechanisms [56]. Inorganic arsenate (As^{V}) is dominant in aerobic conditions and is readily available for plant uptake as a phosphate analogue via phosphate transporter pathway [57, 59]. However, inorganic arsenite (As^{III})

accounts for the majority of uptake by plants using a different uptake mechanism, transported as a silicic acid analogue by aquaglyceroporins [60, 61, 107, 108].

Rice plant accumulation and toxicity from As, Se, Mo and Cd has been studied with varying phosphate applications and identified a narrow window between nutrient deficiency and toxicity for Se and Mo [63, 109].

The effect of phosphate on As uptake by rice is still unclear. Arsenic in the form of arsenate is a phosphate analogue relating to physiochemical soil characteristics. Some research suggests that the addition of phosphate to soils containing As should increase the mobility of As through competitive sorption towards available particle surfaces [73, 74] and increase the uptake of As by rice. Hua, et al. [33] has shown a direct correlation in soil between DCB-extractable phosphate concentrations and soil-As, however, Abedin, et al. [110] has shown phosphate decreases the uptake of some As species by rice plants due to a competitive uptake mechanism. Phosphate and arsenate are also analogues with respect to root plasma membrane transporters, where arsenate enters at rice root via phosphate transporters [110]. Abedin, et al. [110] shows application of phosphate does not affect plant As concentrations, except for rice husk. Effects of phosphate on the mobility and uptake of other elements (Se, Mo and Cd) have also been reported [38, 55, 63, 109].

Iron is an essential element and a prevalent sorbent mineral in soil with high adsorption capacities for sorbates such as As^{V} in aerobic conditions with high Eh. In an anaerobic environment, Eh decreases causing As(V) bound on Fe-oxide or Fe-hydroxide Fe^{III} complexes dissolute and release Fe^{II} and As^{III} into solution, increasing As bioavailability [76]. Other factors can control As mobility, including pH, which primary impacts As^V [111], and the formation of iron plaque on root surfaces, where As^V accounts for 80-84% of total As adsorbed on plaque [34, 49, 81]. In addition, recent attempts of in situ iron oxide amendments have been shown to increase soil adsorption capacity for As [79]. The impact of iron oxide on the uptake of Se, Mo and Cd is less known.

The objectives of the current research were to provide a comprehensive understanding and the significance of: (a) the impact of increasing soil-As conditions; (b) the impact of phosphate application; and (c) the impact of soil iron oxide amendments on element uptake.

5.3. EXPERIMENTAL

5.3.1. Design. Multiple greenhouse studies were conducted in Rolla, Missouri at the Missouri S&T Chester Baker Greenhouse between February 2010 and January 2012. Included are a total of three studies, including: soil As impact, phosphate impact and iron oxide impact on total rice grain accumulation (Figure 5.1). The soil used in this study is a Dewitt silt-loam (fine, smectitic, thermic Typic Albaqualf) obtained from the USDA ARS Dale Bumpers National Rice Research Center (Stuttgart, Arkansas) in winter 2009. The two control soils in the soil-As experiment were native soil and MSMA-amended soil obtained from the field, where MSMA-amended soil was treated with MSMA at a rate of 6.7 kg MSMA ha^{-1} in alternate years since the 1980s for straighthead resistance evaluation [95]. Native soil was used in the phosphate and iron oxide impact. Native soil was also amended (As-amended) to achieve As concentrations equal to that in MSMA-amended soil for these two studies. Soil was homogenized using an industrial concrete mixer (Lancaster Concrete Mixer, Type SW, Year 1942, No. 153, Lancaster, PA).

Continuous flooding conditions were maintained throughout the greenhouse experiments. Supplement fertilizer applications were given to each pot using general 20:20:20 fertilizer solution by mixing 31.7 g fertilizer L^{-1} tap water.

The soil-As impact study, illustrated in Figure 5.1a, included the two control soils and four additional soil As levels achieved by dosing native soil with a sodium hydrogen arsenate heptahydrate ($Na_2HAsO_4*7H_2O$). A 1L solution was added during soil homogenization prior to planting to achieve soil-As concentrations of 8.9, 11.9, 20.9 and 25.9 mg kg^{-1}. Three cultivars commonly grown in the south central U.S. were included in this study with origin in China (Zhe 733) and the U.S. (Cocodrie and Rondo). Cultivars included early, mid, and late maturity periods and varying As induced straighthead susceptibility (resistant, moderate, and susceptible) [69, 91].

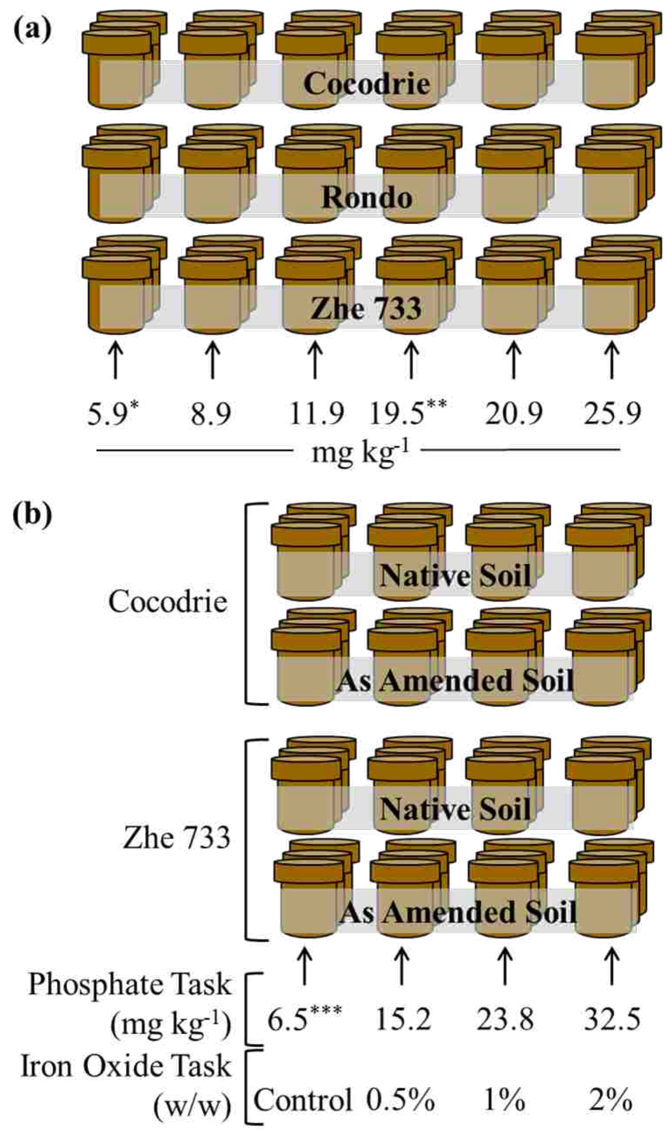

Figure 5.1. Experimental design of soil-As, phosphate and iron oxide studies, *, **, *** indicate control soil, As-amended soil and background phosphate, respectively

The phosphate impact study, illustrated in Figure 5.1b, included two soil As conditions, native soil and an As-amended soil dosed with Na_2HAsO_4*$7H_2O$ to replicate background soil-As found in MSMA-amended soil [33]. Native and As-amended soil was treated with phosphate applications using calcium dihydrogen phosphate $Ca(H_2PO_4)_2$ solution to minimize the addition of other nutrients such as potassium and ammonia. After plants achieved five to six leaves, phosphate applications of 15.2, 23.8 and 32.5 mg P L^{-1} were applied as solution in 50 mL increments once per two week period to achieve phosphate concentrations (w/w) of up to five times background levels [74]. Two cultivars were used in this study, including Cocodrie and Zhe 733.

The iron oxide impact study, also illustrated in Figure 5.1b, included the same soil treatments as the phosphate impact study. The iron oxide used in this experiment was obtained as an affordable milling byproduct from an iron ore production facility in the U.S. The byproduct, also known as fines (-500 mesh or 0.025 mm), was composed of 80% magnetite and 20% hematite. Iron oxide was amended into each pot and homogenized at 0.5, 1.0 and 2.0% (w/w) with 3.0 kg of soil [84, 112]. Treatments were completed in triplicates including a control unless stated otherwise. Two cultivars were used in this study, including Cocodrie and Zhe 733.

5.3.2. Sample Preparation. Grain samples were harvested from rice plants at maturity, stored in sterile plastic sample bag and allowed to dry in laminar flow vent hood (NUAIRE Model NU-425-600) for 21 days. Grain samples were dehulled, milled and ground according to Pillai, et al. [31]. Prior to digestion, samples were oven dried at 65°C for 48h or until constant weight and stored in a desiccator.

5.3.3. Sample Digestion Procedure. A modified EPA Method 3052 was used for the total digestion of the rice grain, using a pressure/temperature (p/T) sensor for control. Acid cleaned Rotor 48 (MF50) digestion vessels were used and 0.5 g rice grain was measured into the vessels with 8.0 mL optima grade nitric acid (HNO_3) and 0.3 mL 30% hydrogen peroxide (H_2O_2) (Fisher Scientific Co., Boston, U.S.). The digestion program consists of a 10 min ramp to 100°C with a 10 min hold, followed by a 10 min ramp to 140°C with a 15 min hold, then a 10 min ramp to 170°C with a 10 min hold before cool down. All digested samples were allowed to cool < 50°C before venting.

Standard reference material (NIST, Gaithersburg, MD) for rice grain (SRM1568a) was included in respective digestions for quality assurance and quality control (QA/QC). Additional digestion QA/QC included one sample blank, one sample duplicate and one sample spike per 10 samples for rice and soil digestion. Digested samples were rinsed from digestion vessels to acid cleaned 50 mL conical tubes and diluted to 20 mL using Millipore water (18.2 MΩ-cm).

5.3.4. Chemical Analysis. Total grain-As, Mo, Se and Cd concentrations were determined using inductively coupled mass spectrometry (ICP-MS) (PerkinElmer Elan DRC-e, MDS Sciex, Concord, Ontario, Canada). Grain samples were diluted to acid concentrations of 10% prior to ICP-MS analysis. Sample method detection limits (MDLs) for elements were determined for the ICP-MS according to EPA Method 200.8. MDLs were estimated for rice grain As, Se, Mo and Cd as 0.004, 0.031, 0.003 and 0.003 mg kg^{-1}, respectively. Sample concentrations greater than 2-3 times MDL were required QA/QC was validated if the following criteria were met: (a) relative difference of a duplicate sample less than 20%; (b) spike recovery between 85-115%; and (c) the accuracy of a calibration standard within 10% per 10 samples analyzed. Concentrations related to soil and grain accumulation in this study are reported as total concentrations unless stated otherwise.

5.3.5. Statistical Analysis. The analysis of variance (ANOVA) procedure appropriate for the completely randomized design utilized in this experiment was accrued out using SAS 9.2 to estimate the impact of treatment variables (soil-As, phosphate, iron oxide and cultivar) on the measured responses. Total grain concentrations of As, Se, Mo and Cd were the measured responses for all experiments in this study.

In the soil-As impact study, experimental design includes the treatment factors of soil-As (Soil) and Cultivar. The phosphate impact study includes treatment factors of Soil, Cultivar and phosphate application (Phosphate). The iron oxide impact study includes treatment factors of Soil, Cultivar and iron oxide amendment (Iron oxide). ANOVA allowed interaction terms representing the combined impact of factors on element uptake to be tested, whereby a significant interaction term signifies that the impact of the changing levels of one factor is influenced by the level of the other factor(s). Least square means with Tukey's adjustment was used to contrast means within treatment factors when interactions were determined significant. Tukey's procedure allowed experiment-wise error to be kept at the selected rate, $\alpha = 0.05$ [97].

5.4. RESULTS AND DISCUSSION

5.4.1. Soil Characterization. Select soil characterization and physiochemical properties of two soils used in the As impact study, native and MSMA-amended soil were presented in Farrow, et al. [105]. Native soil contained 5.9 mg kg^{-1} As, 0.23 mg kg^{-1} Se, 0.38 mg kg^{-1} Mo and 0.06 mg kg^{-1} Cd and MSMA-amended soil contained 19.5 mg kg^{-1} As, 0.53 mg kg^{-1} Se, 0.63 mg kg^{-1} Mo and 0.06 mg kg^{-1} Cd. Both soils had background phosphorus levels of 6.5 mg P kg^{-1} soil [33]. Background iron concentrations were measured as 7.0-10.0 mg g^{-1} in native soil and 12.0-15.0 mg g^{-1} in As-amended soil according to Hua, et al. [33].

5.4.2. Effect of Soil-As. The soil-As impact on grain- As, Se, Mo and Cd concentrations for the three cultivars were compared between the six soil-As conditions. Figure 5.2 shows the correlations between soil-As and grain concentrations for Cocodrie, Rondo and Zhe 733. For the Zhe 733 cultivar and compared to native soil, MSMA-amended soil increased grain-As by 254%, increased grain-Se by 35%, decreased grain-Mo by 23% and increased grain-Cd by 15%. Therefore, increased soil-As impacts not only significantly increased grain-As, but also substantially impacted the uptake of Se, Mo and Cd and confirmed Farrow, et al. [105].

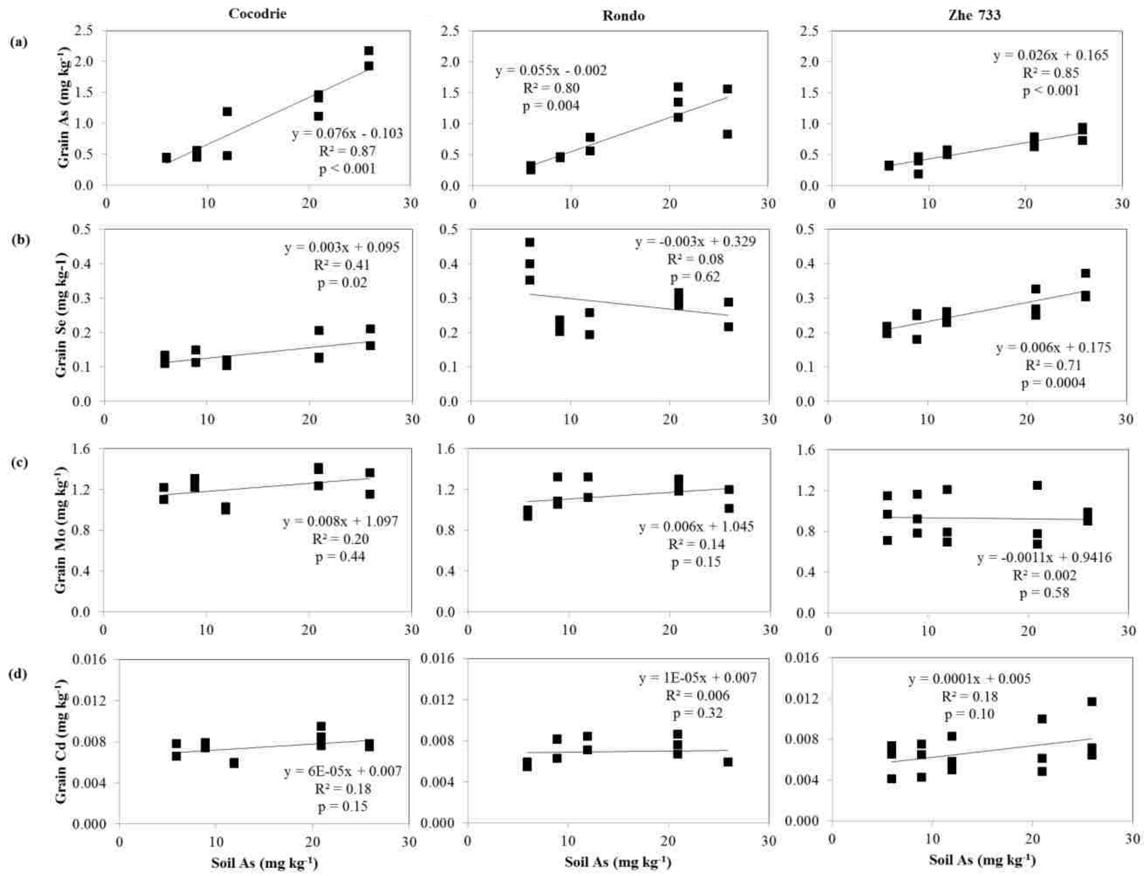

Figure 5.2. Correlations between Soil-As and concentrations of: (a) grain-As, (b) grain-Se, (c) grain-Mo, and (d) grain-Cd for Cocodrie, Rondo and Zhe 733 (left to right); evaluated based on coefficient of determination, $R^2 = 0.60$

The ratio of grain-As to soil-As for rice plants grown in native soil ranged from 0.02 to 0.08. Grain-As was significantly increased for all cultivars grown in MSMA-amended soil (19.5 mg As kg^{-1}) and soil-As equal to 25.9 mg kg^{-1}, where the mean ratios of grain-As to soil-As were 0.08-0.13 and 0.03-0.09, respectively, indicating As uptake is not transport limited. The As-susceptible Rondo cultivar had the maximum grain-As to soil-As ratio estimated at 0.13, and thus, the most efficient uptake of soil-As. Figure 5.2a indicated strong positive correlations between soil-As and grain-As for the three cultivars, and validated an increase in grain-As corresponds to an increase in soil-As [24]. As compared to grain-As, grain-Se was less significantly impacted by soil-As for all cultivars.

Figure 5.2b also shows soil-As significantly increased grain-Se for Cocodrie ($p = 0.02$) significantly increased grain-Se for Zhe 733 ($p = 0.0004$). When compared to native soil, grain-Se concentrations for Zhe 733 increased by 30% in As-amended soil. This confirms findings by Farrow, et al. [105] that showed MSMA-amended soil increased grain-Se by 68% for Zhe 733 grown in similar soil under continuous flooding conditions. This provides further evidence that a correlation may exist between soil-As and grain-Se. The result also indicated that the grain-Se increase is associated with As uptake rather than changing irrigation condition. The correlation between soil-As and grain-Se for Rondo was negligible, as were correlations between soil-As and grain- Mo and Cd for all cultivars. Figure 5.2c-d shows correlations between soil-As and grain-Mo and Cd.

The cultivar impact on grain- As, Se, Mo and Cd concentrations were compared between the three cultivars for all soil-As conditions. As shown in Table 5.1, cultivar significantly impacts grain-Mo accumulation ($p < 0.001$, $F = 13.48$) where Zhe 733 significantly decreased grain-Mo. Combined with findings in Farrow, et al. [105], the significance of cultivar selection can either increase or decrease the uptake of Mo. In addition, cultivar had a substantial impact on the uptake and accumulation of As ($p < 0.001$, $F = 26.99$) and Se ($p < 0.001$, $F = 45.40$), where Zhe 733 substantially decreased grain-As and Cocodrie significantly decreased grain-Se. Negligible cultivar impact was realized on grain-Cd in the soil-As impact study.

Table 5.1. Analysis of variance for soil-As, phosphate application and iron oxide studies in completely randomized experimental design

	Element (Pr > F)†			
	As	Se	Mo	Cd
	Soil-As Study			
Cultivar	< 0.001***	< 0.001***	< 0.001***	0.226
Soil-As	< 0.001***	< 0.001***	0.638	0.055*
Cultivar*Soil-As	< 0.001***	< 0.001***	0.308	0.268
	Phosphate Study			
Cultivar	< 0.001***	0.029**	0.047**	< 0.001***
Soil-As	< 0.001***	0.124	0.880	0.516
Phosphate	0.401	0.015**	0.660	0.290
Cultivar × Soil-As	0.890	0.544	0.279	0.114
Cultivar × Phosphate	0.521	0.108	0.756	0.123
Soil-As × Phosphate	0.857	0.094*	0.321	0.707
Cultivar × Soil-As × Phosphate	0.917	< 0.001***	0.618	0.183
	Iron Oxide Study			
Cultivar	< 0.001***	0.001***	< 0.001***	< 0.001***
Soil-As	< 0.001***	0.021**	0.227	0.027**
Iron oxide	0.027**	0.946	0.351	0.464
Cultivar × Soil-As	< 0.001***	0.247	< 0.001***	0.606
Cultivar × Iron oxide	0.084*	0.398	0.047**	0.129
Soil-As × Iron oxide	0.057*	0.475	0.335	0.412
Cultivar × Soil-As × Iron oxide	0.172	0.971	0.080*	0.692

*, **, *** indicate significance when $p < 0.05$, 0.01, and 0.001

† $\alpha = 0.05$

In addition, the soil-As impact on grain- As, Se, Mo and Cd concentrations were compared for each of the three cultivars. Grain-As was significantly increased in As-amended soil for Rondo, with negligible soil-As impact on grain-As for other cultivars. Grain- Se, Mo and Cd were negligibly impacted by soil-As for all cultivars.

Correlations between the grain elements were evaluated. Figure 5.3 shows a strong positive grain As-Se correlation for Cocodrie (R^2 = 0.61, p = 0.002). This positive grain As-Se trend is also supported by Rondo (Figure 5.3b, R^2 = 0.05, p = 0.42) and Zhe 733 (Figure 5.3c, R^2 = 0.20, p = 0.07). This slight positive grain-As to grain-Se trend contradicts the slight negative trend found in Farrow, et al. [105]. The range of grain-As concentrations for Zhe 733 (0.18 to 1.28 mg kg^{-1}) extended beyond those found in Farrow, et al. [105] (0.14 to 0.79 mg kg^{-1}) and indicate a nonlinear uptake of As for Zhe 733. This results aligns with uptake kinetics and adsorption modeled by Abedin, et al. [57] and Zhang, et al. [54], respectively. Correlations between grain-As and other elements were negligible (Figure B.1).

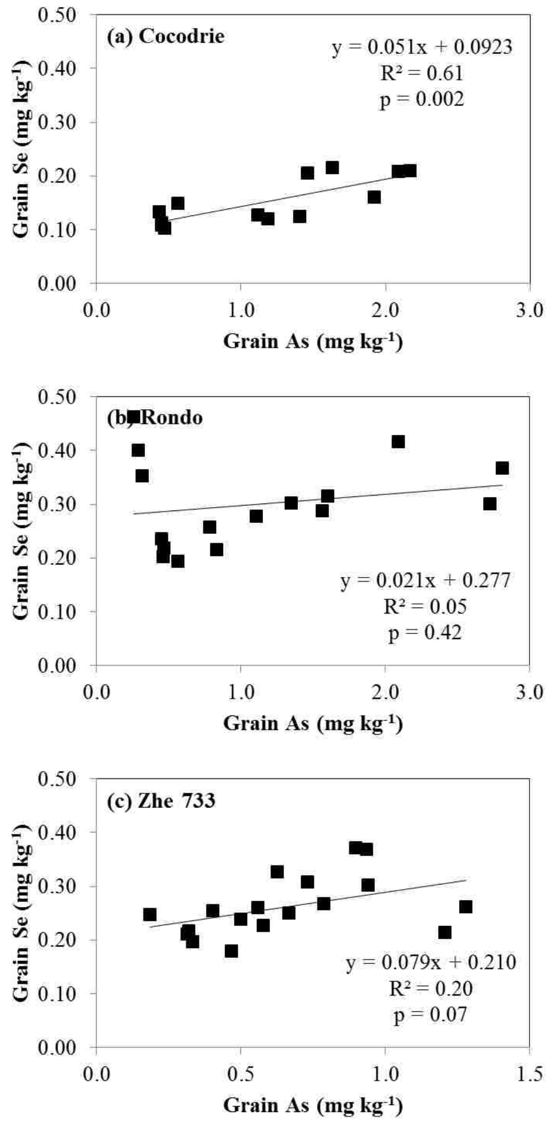

Figure 5.3. Correlations between grain arsenic and total grain selenium concentrations for cultivars Cocodrie, Rondo and Zhe 733; evaluated based on coefficient of determination, $R^2 = 0.60$.

Table 5.1 shows the significance levels of the interaction (Cultivar*Soil) and treatment factors (Cultivar, Soil) for the soil-As impact study, where Cultivar*Soil is significant for As ($p < 0.001$, $F = 6.57$) and Se ($p < 0.001$, $F = 4.33$). The presence of a Cultivar*Soil interaction indicates a change in cultivar is impacted by the level of soil-As, and further suggests a cultivar may accumulate As and Se differently in native and MSMA-amended soils. Therefore, Tukey's adjustment was used to further evaluate the impact of treatment factors on grain- As, Se, Mo and Cd. Table 5.1 also indicates soil-As has a substantial impact on grain-As ($p < 0.001$, $F = 55.73$) and grain-Se ($p < 0.001$, $F = 6.01$), though the interaction term Cultivar × Soil was significant for both elements.

5.4.3. Effect of Phosphate Application. The impact of phosphate application on grain- As, Se, Mo and Cd concentrations were compared between the two soil-As conditions for the two cultivars. As shown in Table 5.1, phosphate application had a substantial impact on the uptake and accumulation of Se ($p = 0.02$), though the interaction term Cultivar × Soil-As × Phosphate was significant. Hopper and Parker [63] found plant uptake of the more phytotoxic selenite (SeO_3) to occur following the phosphate transporter protein pathway, therefore, results suggest the decrease in grain-Se may be due to the phosphate-selenite antagonism. As the availability of phosphate increase with concentrations in rhizosphere soil, phosphate transporter proteins substantially decrease uptake of the analogous selenite.

Phosphate impact per cultivar was also evaluated and found increased phosphate application had negligible impact on grain-As, but grain-Se slightly decreased for Zhe 733 in both native and As-amended soil (Figure B.2). For Zhe 733 plants, phosphate application equal to 11.2 mM PO_4, or double background levels, reduced grain-Se by 9%.

Increasing phosphate application to 16.8 mM PO_4 reduced grain-Se by 57%. Further increasing phosphate application to five times background levels resulted in negligible change in grain-Se, illustrating phosphate has maximum effect on Se uptake when in the range of three and four times background levels. Plant Se uptake in the presence of phosphate is less known. Previous research has shown competitive inhibition of selenite uptake to occur with increasing soluble phosphate [63, 113, 114]. By understanding Se transport mechanisms, further research can sufficiently improve rice Se concentrations and minimize Se biofortification practices [76]. The phosphate impact on grain-As for Cocodrie was negligible (Figure B.3).

Plant Mo uptake can be enhanced by phosphates; whereby phosphates have a greater affinity for soil sorption sites [115, 116] and mobilize soil bound molybdenum [109, 117]. Further, increased PO_4 may also assist in the formation of phosphomolybdate complexes in soils that can then be readily adsorbed by plants [43]. The impact of phosphate application on grain-Mo in this study was shown negligible (Figure B.2-3).

Plant Cd uptake can be enhanced by phosphate application. Waterlot, et al. [55] shows P amendment generally increased Cd uptake in rye grass shoots by 17.9-79%. Wang, et al. [77] has shown phosphate fertilizer addition to significantly decrease soluble Cd (1.5-30.7%) and uptake in cabbage by 16.5-66.9%. The impact of phosphate application on grain-Cd in this study was also shown negligible (Figure B.2-3).

The soil-As impact on grain- As, Se, Mo and Cd concentrations for the two cultivars were compared between the two soil-As conditions. Table 5.1 shows significance levels of the treatment factors (Cultivar, Soil, Phosphate) and respective interactions for the phosphate impact study. Cultivar*Soil*Phosphate was found to be significant for Se ($p < 0.001$, $F = 9.62$). As shown in Table 5.1, Soil-As is a significant factor for grain-As accumulation ($p < 0.001$). Further, grain-As was significantly increased for Cocodrie and Zhe 733 cultivars in As-amended soil, suggesting the same positive soil-As to grain-As correlation found in the soil-As impact study. The soil-As impact on grain- Se, Mo and Cd was negligible.

The cultivar impact on grain- As, Se, Mo and Cd concentrations were compared between the two cultivars. Also shown in Table 5.1, cultivar was a significant factor for the uptake of As ($p < 0.001$, $F = 14.27$), Mo ($p = 0.05$) and Cd ($p < 0.001$, $F = 73.55$). Further, cultivar had a substantial impact on the uptake and accumulation and Se ($p = 0.03$), though the interaction term Cutlivar × Soil-As × Phosphate was significant ($p < 0.001$). For the Zhe 733 cultivar, as compared to Cocodrie, grain- As, Se, Mo and Cd were all significantly decreased. Therefore, this data confirms previous research and indicates the importance of cultivar selection as an uptake control mechanism of multiple elements [13, 29, 100].

5.4.4. Effect of Iron Oxide Amendment. The impact of iron oxide amendments on grain- As, Se, Mo and Cd concentrations were compared between the two soil-As conditions and the two cultivars. As shown in Table 5.1, iron oxide amendments had a significant impact on grain-As ($p = 0.03$). Figure 5.4 shows the impact of iron oxide amendments to the Cocodrie (As-susceptible) cultivar in native soil. A substantial reduction ($R^2 = 0.29$, $p = 0.07$) in grain-As (Figure 5.4) concentrations was achieved with 2% (w/w) iron oxide amendments for a 67% decrease from 0.161 mg kg^{-1} to 0.080 mg kg^{-1}. Figure 5.5 shows grain-As was also decreased ($R^2 = 0.43$, $p = 0.04$) by 2% iron oxide amendments in As-amended soil with a decrease of 55% from 1.407 mg kg^{-1} to

0.796 mg kg^{-1} (Figure 5.5a). In addition, iron oxide amendments also reduced grain-Mo ($p = 0.05$) (Figure 5.5b) and increased grain-Cd ($p = 0.05$) (Figure 5.5c). This suggests increased soil adsorption sites from Fe could sorb anions with a greater affinity to iron oxide and release Cd into solution. This also confirms iron oxide adsorption potential shown in previous studies [79, 84].

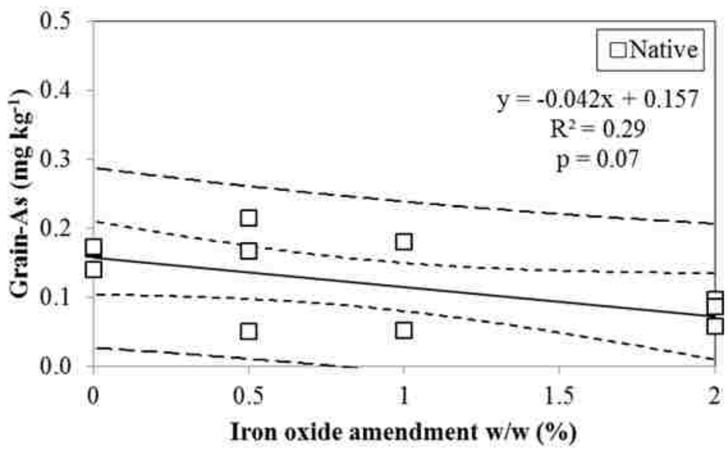

Figure 5.4. Iron oxide impact on Cocodrie concentrations of grain-As in native soil; including 95% prediction and confidence intervals

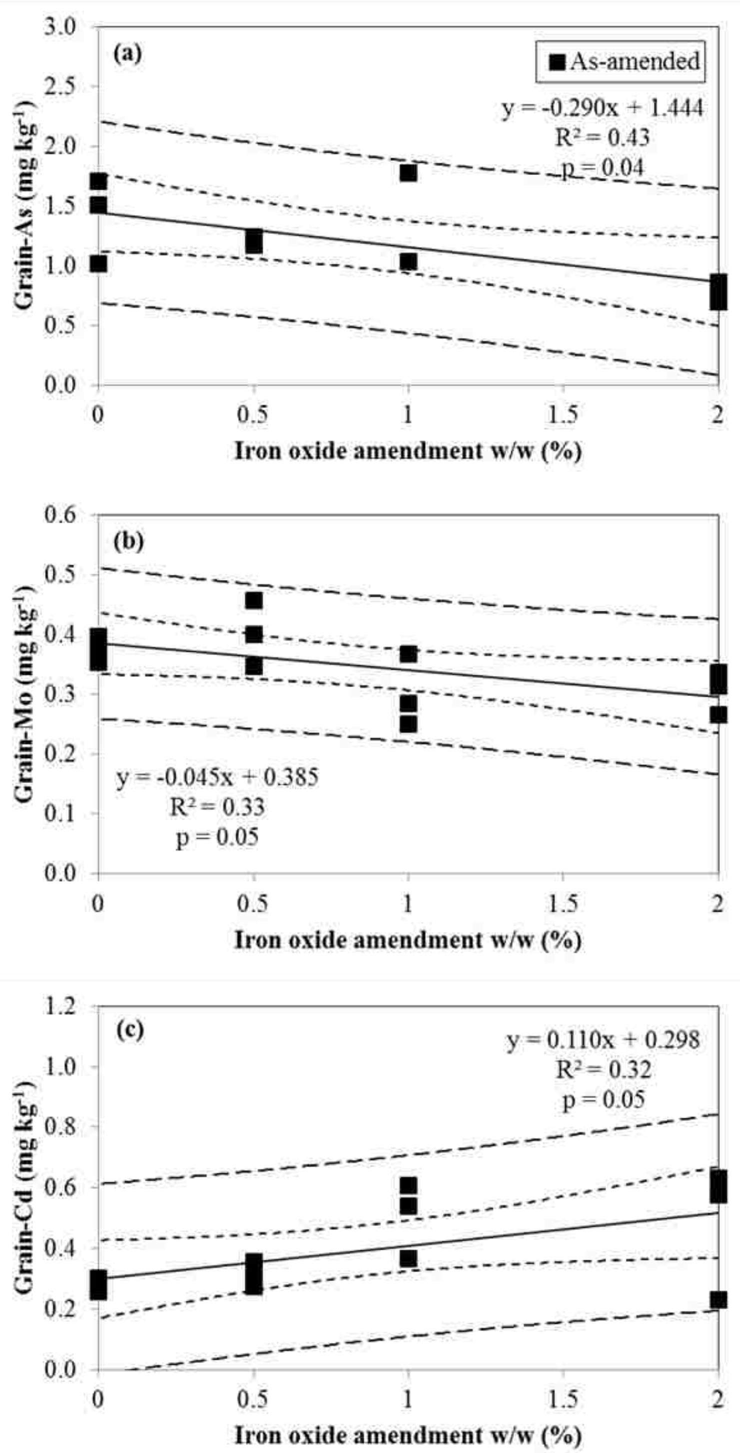

Figure 5.5. Iron oxide impact on Cocodrie concentrations of (a) grain-As, (b) grain-Mo and (c) grain-Cd in As-amended soil; including 95% prediction and confidence intervals

Arsenic sorption onto magnetite in solution, although slow and weak, has been shown to occur as zerovalent iron oxidized to Fe^{2+}/Fe^{3+} mixed phase [82]. Research by Su, et al. [118] shows soil-As in a natural environment approaching equilibrium over time increases with soil-Fe concentrations. Additional correlations were found for Cocodrie (Figure B.4) and Zhe 733 (Figure B.5).

The As adsorption capacity of the magnetite and hematite iron oxide mixture used in this study is less than granular ferric hydroxide and other forms of iron oxide, annual soil amendments may provide a cumulative increase in the soil adsorption capacity under flooded conditions [119]. As compared to Cocodrie, grain-As concentrations were significantly less in Zhe 733 and the impact of iron oxide expressed only a weak negative trend in both native and As-amended soil.

The soil-As impact on grain- As, Se, Mo and Cd concentrations for the two cultivars were compared between the two soil-As conditions. Table 5.1 shows significance levels of the interactions (Cultivar × Soil) and treatment factors (Cultivar, Soil, Iron oxide) for the iron oxide impact study, where Cultivar × Iron oxide is significant for Mo ($p = 0.047$) and Cultivar × Soil-As is significant for As ($p < 0.001$, $F = 57.86$) and Mo ($p < 0.001$, $F = 13.67$). Table 5.1 indicates Soil-As was a significant factor for the uptake of grain-Se ($p = 0.02$) and grain-Cd ($p = 0.03$), with a substantial impact on grain-As ($p < 0.001$), though the Cultivar × Soil-As interaction was significant for grain-As.

Further, As-amended soil yielded higher grain-Se than native soil, a common trend in the above soil-As, phosphate and iron oxide impact studies. Elevated grain-Se in As-amended soil suggests a relationship between soil-As and Se uptake, one area of research with little information available. However, grain-Cd in native soil was greater than grain-Cd in As-amended soil (inconclusive in soil-As and phosphate studies).

In addition, grain-As was significantly increased in As-amended soil, suggesting the positive soil-As to grain-As correlation is confirmed. Next, the soil-As impact on grain- As, Se, Mo and Cd were compared between the two soil-As conditions for each of the two cultivars, individually. For Zhe 733, MSMSA-amended soil significantly increased grain-As and grain-Se, significantly decreased grain-Mo and increased grain-Cd (insignificant). For Cocodrie, MSMA-amended soil significantly increased grain-As, decreased grain-Se and grain-Mo (insignificant) and increased grain-Cd (insignificant). The interaction term Cultivar × Soil-As was significant for As and Mo.

The cultivar impact on grain- As, Se, Mo and Cd concentrations were compared between the two cultivars. As shown in Table 5.1, cultivar was a significant factor for the uptake of grain-Se ($p = 0.001$) and grain-Cd ($p < 0.001$, $F = 36.13$). Further, cultivar had a substantial impact on grain-As ($p < 0.001$, $F = 61.06$) and grain-Mo ($p < 0.001$, $F = 48.50$), though the Cultivar × Soil-As interaction was significant for grain- As and Mo. Zhe 733 as compared to Cocodrie, significantly increased grain-As, significantly decreased grain-Se, significantly increased grain-Mo and significantly increased grain-Cd. Therefore, this data also confirms previous research and indicates the importance of cultivar selection as a control of element uptake [13, 29, 100].

5.5. ASSOCIATED CONTENT

5.5.1. Supporting Information. Details on average total grain concentrations (soil-As impact study), effect of soil-As on grain-As (soil-As impact study), element correlations (soil-As impact study), impact of phosphate application (phosphate impact study), and impact of iron oxide amendments (iron oxide impact study).

5.5.2. Acknowledgements. This research was funded by USDA (Award No. 2008-38814-04727) to Missouri University of Science & Technology through Lincoln University. The authors would like to thank Ms. Tiffany Sookaserm for sample collection and preparation and Prof. V. A. Samaranayake for assistance of statistical analysis.

APPENDIX A:

SUPPORTING INFORMATION FOR:

ACCUMULATION OF SELECTED TRACE ELEMENTS IN RICE: EFFECTS OF SOIL ARSENIC, IRRIGATION MANAGEMENT AND CULTIVAR

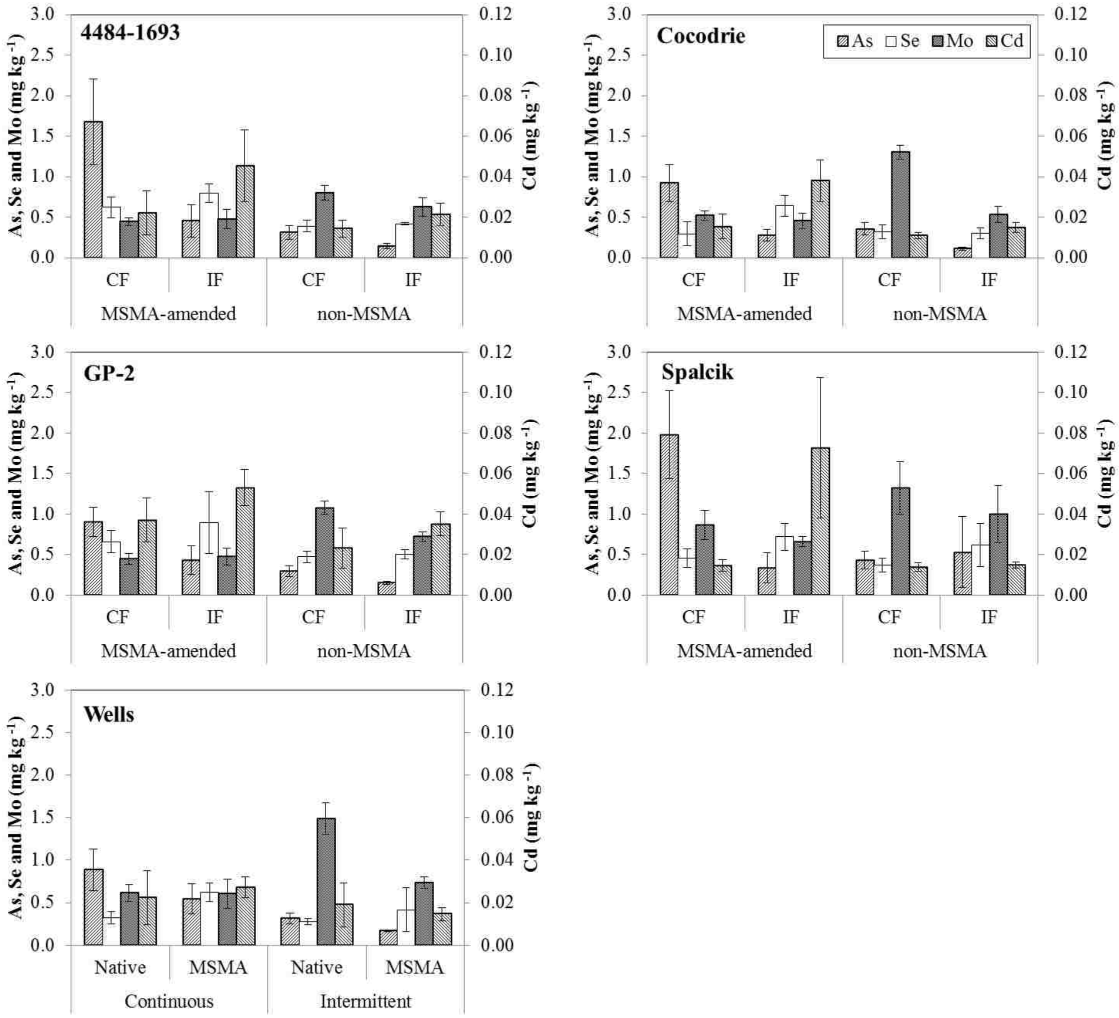

Figure A.1. Average values for Soil × Water combinations of six cultivar comparing main plot and sub-plot effects on total grain concentrations. Outliers removed according to Grubbs test.

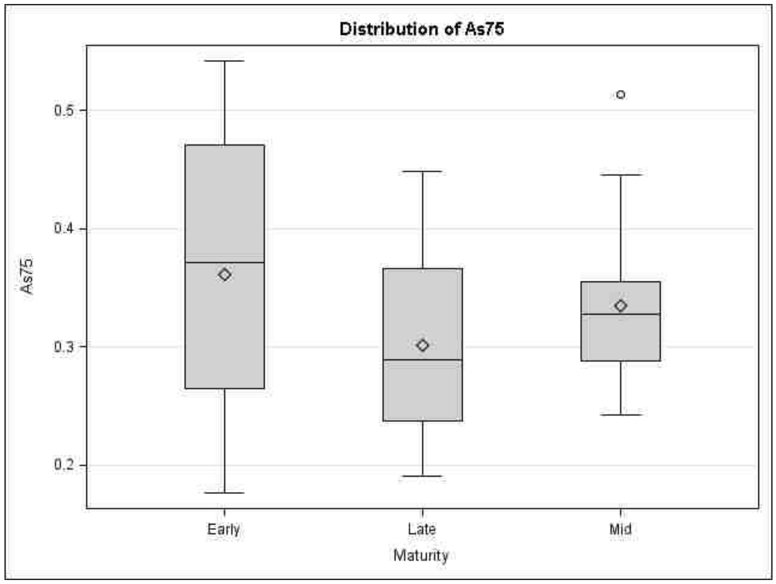

Figure A.2. Distribution boxplot between total grain-As and heading date in continuous flooding non-MSMA soil for six cultivars

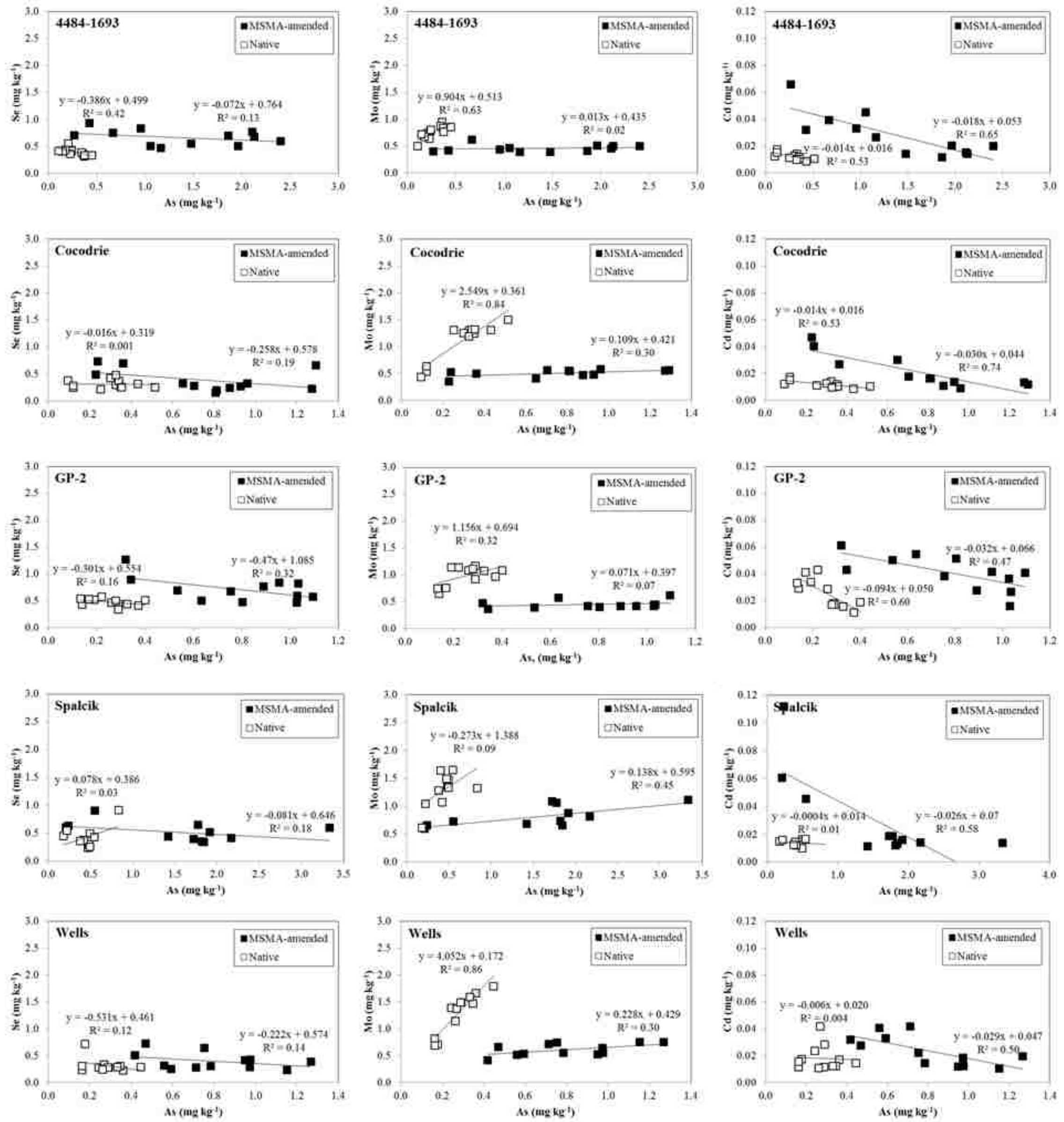

Figure A.3. Element correlations between As-Se, As-Mo and As-Cd (columns, left to right) for MSMA-amended (solid) and Native (open) soil of five cultivars, including 4484-1693, Cocodrie, GP-2, Spalcik and Wells (rows, top to bottom)

APPENDIX B:

SUPPORTING INFORMATION FOR:

ACCUMULATION OF SELECTED TRACE ELEMENTS IN RICE: EFFECTS OF SOIL ARSENIC, PHOSPHATE, AND FERRIC OXIDE – A GREENHOUSE STUDY

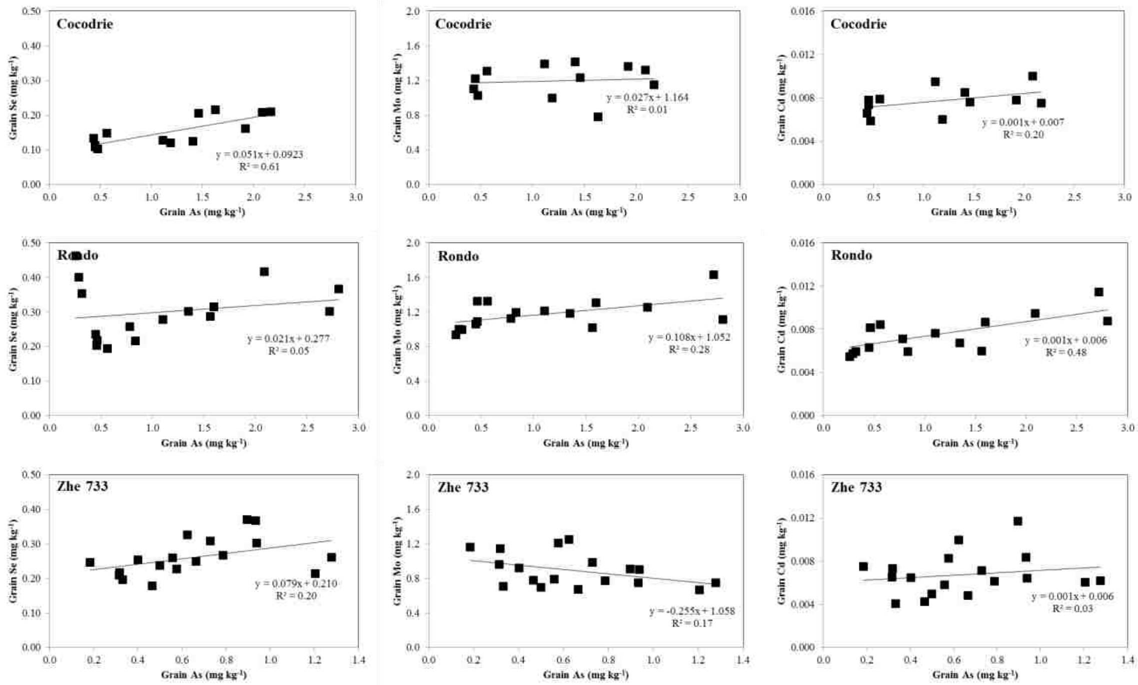

Figure B.1. Element correlations between As-Se, As-Mo and As-Cd (columns, left to right) for three cultivars, including Cocodrie, Rondo and Zhe 733 (rows, top to bottom); evaluated based on coefficient of determination, $R^2 = 0.60$.

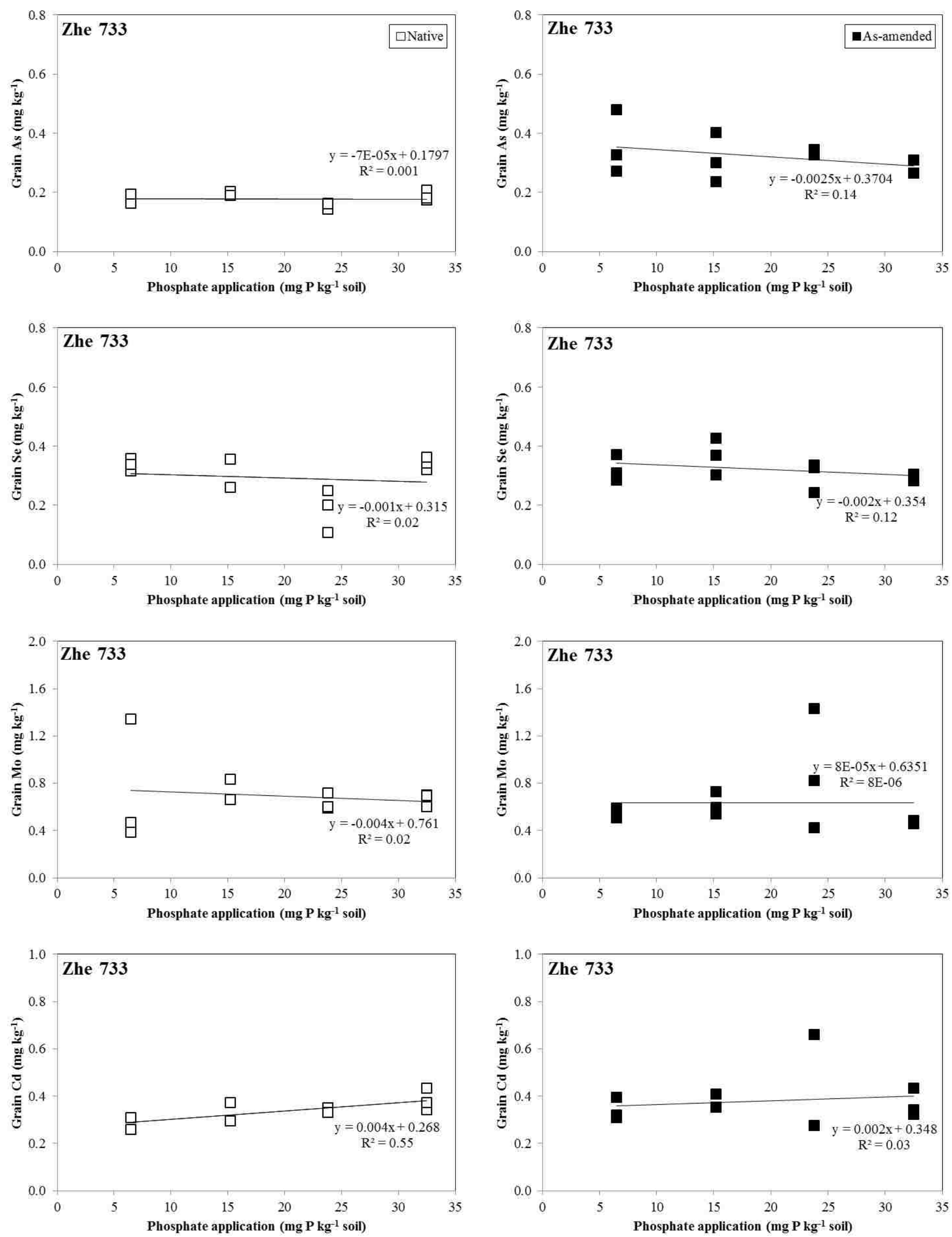

Figure B.2. Impact of phosphate application on grain- As, Se, Mo and Cd uptake in Zhe 733 rice plants under native and As-amended soil conditions; evaluated based on coefficient of determination, R^2 = 0.60.

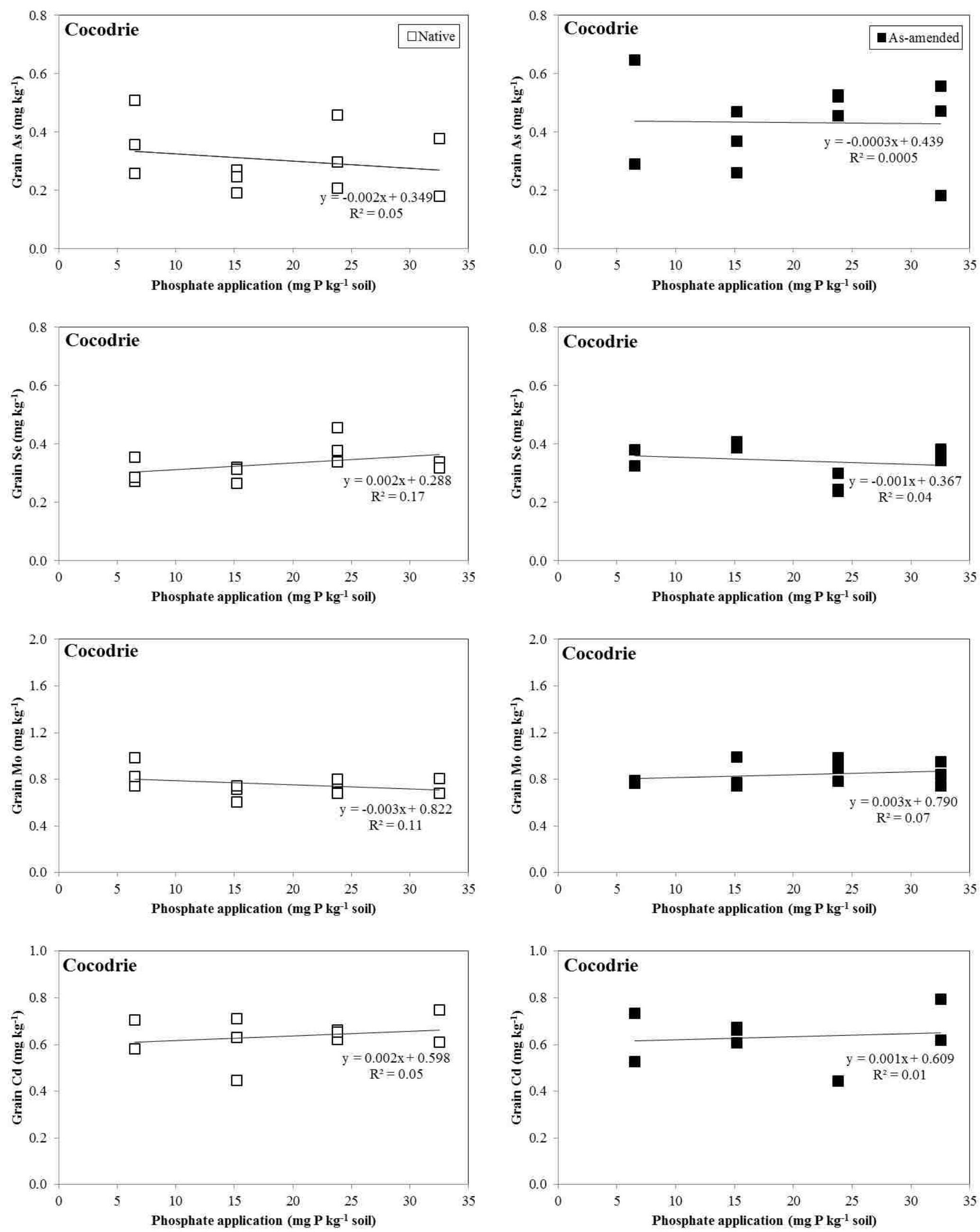

Figure B.3. Impact of phosphate application on grain- As, Se, Mo and Cd uptake in Cocodrie rice plants under native and As-amended soil conditions; evaluated based on coefficient of determination, $R^2 = 0.60$.

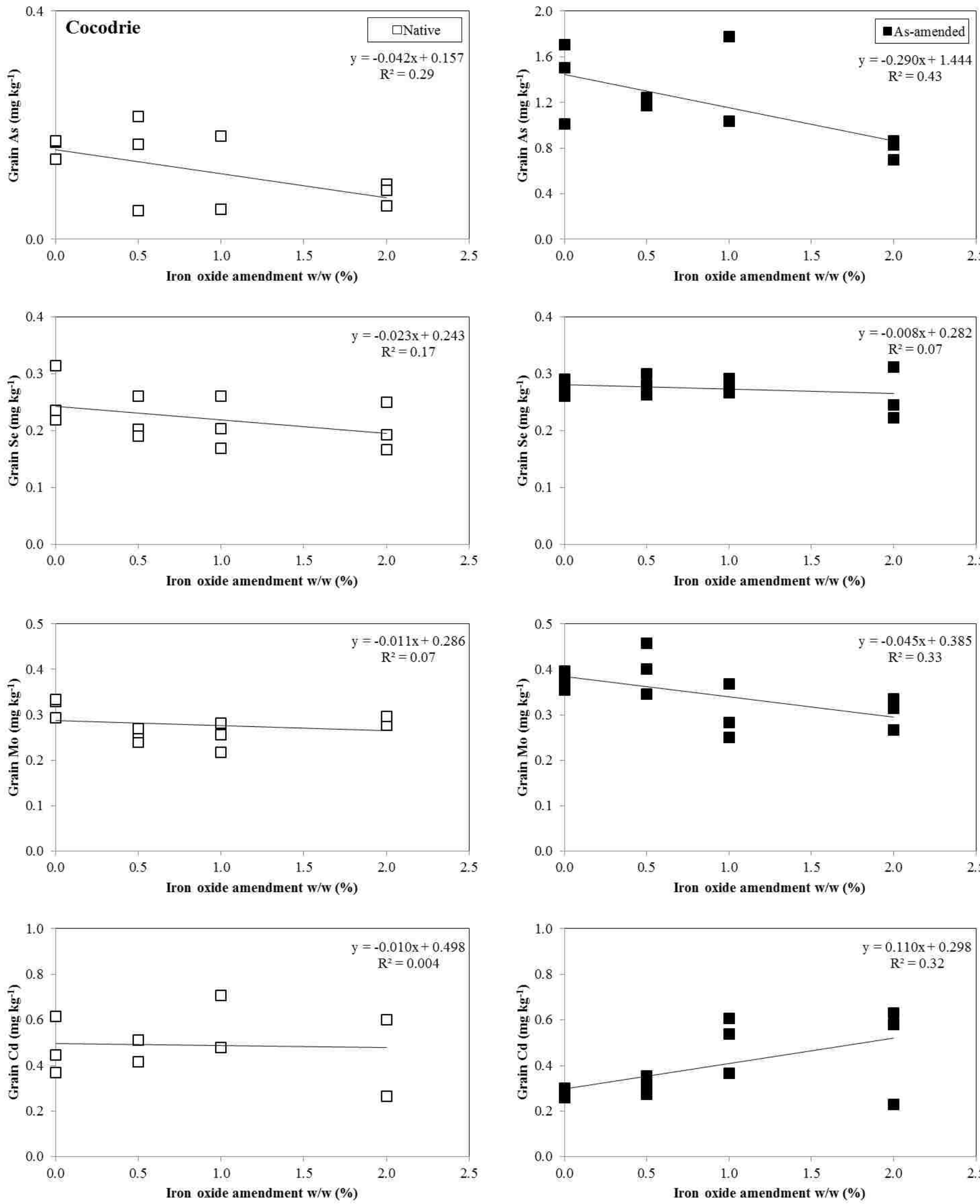

Figure B.4. Impact of iron oxide soil amendments on grain- As, Se, Mo and Cd uptake in Cocodrie rice plants under native and As-amended soil conditions; evaluated based on coefficient of determination, R^2 = 0.60.

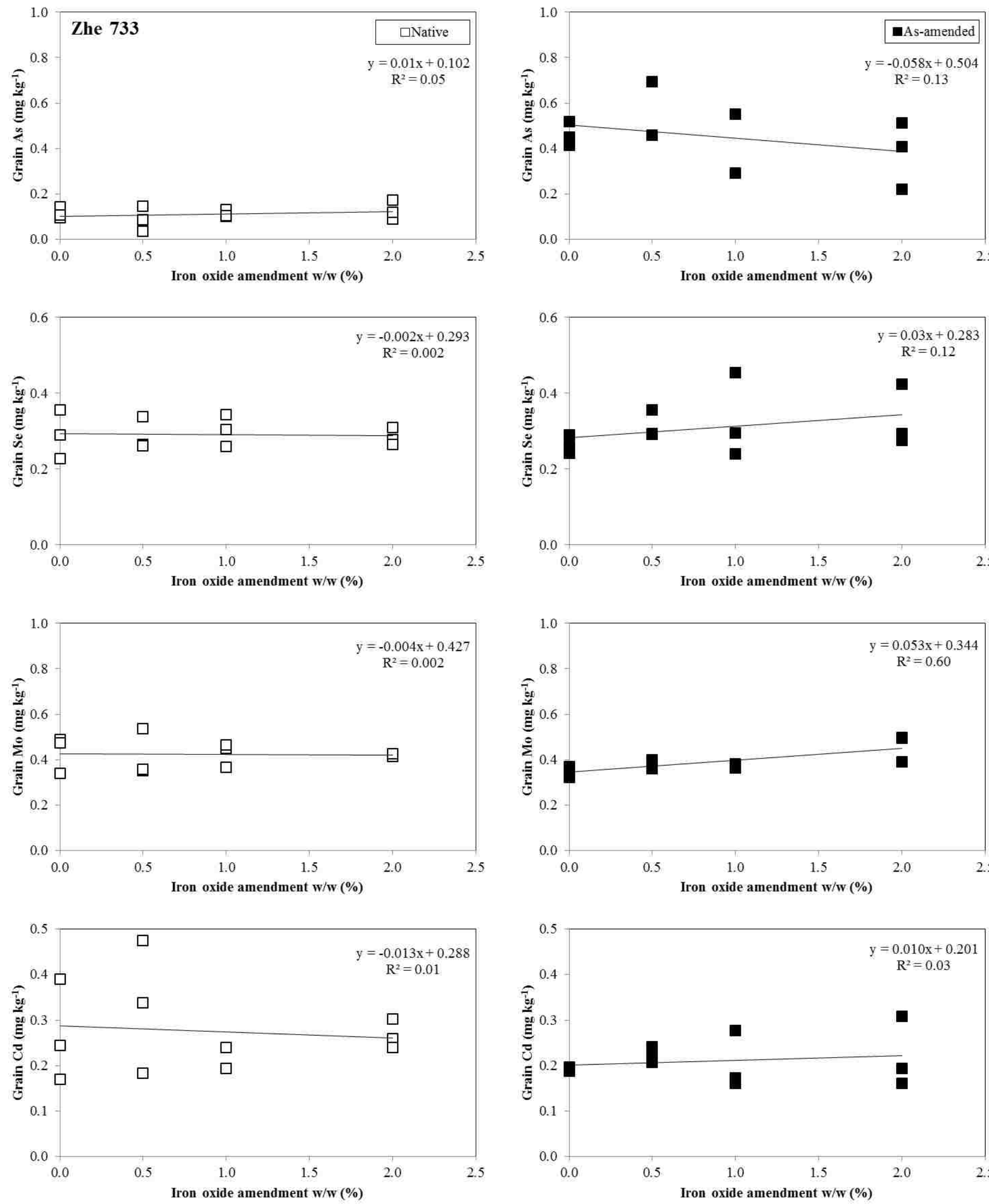

Figure B.5. Impact of iron oxide soil amendments on grain- As, Se, Mo and Cd uptake in Zhe 733 rice plants under native and As-amended soil conditions; evaluated based on coefficient of determination, $R^2 = 0.60$.

APPENDIX C:

EXPERIMENTAL PHOTOS

Homogenizing soil for pot experiment using concrete mixer

Pot experiment soil preparation

Rice seedlings shortly after transplant

Mature rice plants in greenhouse

Rice grain sample taken at harvest

Pots following harvest after rice straw has been removed for soil to dry

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