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Effect of Soil Redox Potential andpH on Nutrient Uptake by Rice With Special Reference to Arsenic Forms and Uptake.

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EFFECT OF SOIL REDOX POTENTIAL AND PH ON NUTRIENT UPTAKE BY RICE WITH SPECIAL REFERENCE TO ARSENIC FORMS AND UPTAKE

A Dissertation

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To Elsa, Maria Fernanda, Pablo and Virginia

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ABSTRACT

Studies dealing with the availability and speciation of arsenic (As) as affected by soil redox potential and pH were initiated because of the lack of information on As chemistry in flooded soils. The chemistry of native and applied As was studied in a Crowley silt loam soil (Typic Albaqualf). Arsenic uptake and its toxic effect on two rice cultivars as affected by As chemical form and concentration were also studied.

Soil redox potential and pH were shown to affect speciation and solubility of both native and applied As. Upon soil reduction, indigenous-As solubility increased, and arsenite [As(III)] comprised most of the soluble As. At the lowest redox potential (-200 mV) 7.3, 2.2 and 1.4% of soil As became soluble at pH's 5.5, 6.5 and 7.5 respectively. Under oxidized conditions, As solubility was lower, and arsenate [As(V)] constituted most of the soluble As. When 4 mg kg^{-1} monomethylarsenic acid (MMAA) was added, a similar trend occurred. Solubility of applied MMAA increased when the soil was reduced. In the two experiments, the greater availability of As under reduced conditions led to a greater As uptake by rice plants.

Studies with plants grown in nutrient solution showed As chemical form to be the most important factor determining As availability and toxicity. Arsenic phytoavailability followed the trend As(III) > MMAA > As(V) > DMAA (dimethylarsenic acid), while As toxicity followed the trend MMAA > As(III) > As(V) ~ DMAA. Arsenic taken up as MMAA, As(III) or As(V) was stored in the

root, but As taken up as DMAA was readily translocated to the shoot. Phosphorus uptake decreased with increasing As application. Zinc tissue concentration and uptake was decreased by all chemical forms of As. In both the soil and the nutrient solution experiments, the uptake of As as MMAA interfered with the translocation of Zn and, to a lesser degree, Cu.

Root applied DMAA at a concentration of 1.6 mg As L^{-1} inhibited photosynthetic activity, photosynthetic capacity and plant growth, leading eventually to death. Photosynthetic activity was inversely related to tissue As concentration.

INTRODUCTION

Sources of Arsenic

Arsenic (As) is ubiquitous in our environment and has both natural and anthropogenic sources. Natural As comes from the weathering of rocks and soils, and the geologic history of a particular soil determines its As content (Greaves, 1913). Volcanic activity introduces As into the atmosphere as high-temperature volatile gases. Arsenic then returns to earth as dust or in precipitation (Woolson, 1983; Peterson, 1985). Contribution of vulcanism to concentration of As is small at the present time; however, it added much of the sedimentary As over geological times (Reuter, 1975). The abundance of As in the continental crust of the earth is generally estimated as 1.5-2 ppm (National Academy of Sciences, 1977; Woolson, 1983). Recently, Chilvers and Peterson (1987) estimated the emission of natural As to the atmosphere as 45,480 T yr^{-1}. The major sources are volcanic activity and low temperature volatilization.

Man has introduced a large amount of As into the environment through unintended contamination from industrial activities or through intentional use, as a pesticide, medicine, or feed additive. Some of the As is easily recycled in nature (that from pesticides, medicines, etc), but other As (such as that used as additives in metal and glass) is not easily recycled, thereby increasing As accumulation (National Academy of Sciences, 1977). An important portion of As contamination is originated in the burning of fossil fuels (Ferguson and Gavis, 1972), coal and petroleum by-products (Campbell et al., 1978; Piver,1983), or as a by-product of the smelting of ores

(Schroeder and Balassa, 1966). Chilvers and Peterson (1987) estimated the emission of As into the atmosphere from anthropogenic sources as 28,060 T yr^{-1}.

Attention has focussed on As in the environment for a long time, because the intensive use of arsenical compounds as pesticides has led to the pollution of some agricultural soils. The amount of available As in virgin soils is small and averages about one-tenth of the total As present in most cultivated soils (Grimmett, 1939; Woolson et al., 1971; Colbourn et al., 1975). The accumulation of As in soils reduces their productivity (Liebig, 1966). Concern was also expressed due to the possibility of As entering the food chain. Plants growing in As-contaminated soils generally have higher residues than plants grown in uncontaminated soils. However, there appears to be little chance that animals would be poisoned by consuming plants that contain As residues from contaminated soils, because plant injury occurs before toxic concentrations could appear (National Academy of Sciences, 1977).

Toxicity of Arsenic

Elemental As is not considered poisonous (Liebig, 1966; Schroeder and Balassa, 1966), but many of its compounds are extremely toxic and have been used as pesticides for many years. Arsenical compounds have been also used because of their medicinal properties. Schroeder and Balassa (1966) documented mountaineers eating As for endurance at high altitude. The pharmacology of arsenical compounds depends on the dose given; no action, useful or toxic (Peoples, 1975). Arsenic also shows differing toxicities and effectiveness in different oxidation states and when combined with different organic ligands (Ferguson and Gavis, 1972). It has recently been proven that

As is essential for animal metabolism, although it has not been shown to be an essential plant nutrient. However, stimulation of plant growth by As additions has been reported by several workers (Peterson et al., 1981).

Different mechanisms have been proposed to explain the toxicity of inorganic As. It is generally agreed that trivalent As is considerably more toxic than pentavalent As (National Academy of Sciences, 1977). Trivalent As has great affinity for thiol groups, and combination or chelation of As with thiols effectively inhibits key enzymes containing active thiols (Schroeder and Balassa, 1966). Unlike trivalent As, pentavalent As species do not react directly with the active sites of enzymes. The similarities between arsenic acid and phosphoric acid, make possible the competition between arsenate and phosphate in many enzymatic reactions (Long and Ray, 1973) or essential molecules, such as ATP (National Academy of Sciences, 1977).

The relatively good understanding that researchers have concerning the toxic mechanism of inorganic arsenicals contrasts considerably with the confusion about the effect of organic arsenicals. While the biological function of As methylation is not clearly known, it has been suggested to be a detoxification process in nature (Ferguson and Gavis, 1972; Chau and Wong, 1978). Peters (1955) proposed that organic arsenicals exert their toxicity after being reduced in vivo to As(III). The organic forms of As have been recognized as less toxic to animals (Peoples, 1975) and to plants when applied in soil (Peterson et al., 1981). However, Shroeder and Balassa (1966) cite inorganic arsenite as less inhibitory than organic As. Work with monosodium methanearsonate (MSMA) has suggested that organic arsenicals may act on

photosynthesis (Spilsbury, 1972) and respiration (Pillai et al., 1973). However, experimental data have not been reported to support this hypothesis.

Importance of Arsenic Speciation

Recognition that different As compounds vary in their toxicities has directed attention towards the specific compounds or chemical forms of the element present in the environment. Over the years researchers realized that the toxicity of As is not only dependent on its chemical-form, but also on its solubility and mobility in soils. Thus, studies concerning the speciation and species transformation of As are essential to understanding the behavior of As in the environment and its toxic effect on plants and animals. The chemical form determines the availability to plants and animals, and plant uptake. Recent development and improvement of techniques to determine As species (Masscheleyn et al., 1991b) have allowed the studies of As speciation and its mutual transformations under different soil conditions.

The biogeochemistry of As has been reviewed by several researchers (Ferguson and Gavis, 1972; Braman, 1975), who agree that As biogeochemistry is complex and not completely understood. Ferguson and Gavis (1972) summarized the stability of inorganic As species in a redox-pH diagram; however, they did not include organic arsenicals. In natural waters, soils and sediments, the As species of interest are the arsenate oxyanions, As(V); the arsenite oxyanions, As(III); monomethylarsonic acid, As(III); and dimethylarsinic acid, As(I). Arsenic chemistry is governed by many factors. The solubility of their salts, the complexing ability of solid and soluble ligands,

biological reactions, pH and redox potential, and the presence of other ions are all reported to control As concentration and speciation.

Soils can experience redox potential ranging from -300 to +700 mV. In oxidized (aerated) soils, redox potential (Eh) is reported to range from about +400 to +700 mV (Patrick and Mikkelsen, 1971). When soils are flooded, such as occurs in flooded rice fields, oxygen demand by microorganisms and plant roots rapidly depletes soil oxygen, and reduced conditions usually result. Upon flooding, various chemical and biological transformations take place, resulting in a decrease in Eh. Soils with Eh of about -300 to -100 mV are considered highly reduced, while soils with Eh between +100 and +400 are considered moderately reduced. The pH of both acid and alkaline soils tends to converge toward neutrality when these soils are inundated (Patrick and Mikkelsen, 1971; Ponnamperuma, 1965; 1972). Usually, a thin layer of oxidized soil develops in the water-flooded soil interface. This thin oxidized layer is very important in the chemical transformation and nutrient cycling that occurs in flooded soils (Patrick and Mikkelsen, 1971). Changes in the physicochemical properties of soils due to flooding or draining often influence the chemical behavior and the bioavailability of nutrients (Patrick et al., 1985) and toxic heavy metals (Gambrell et al., 1976).

Several researchers (Epps and Sturgis, 1939; Deuel and Swoboda, 1972; Brannon and Patrick, 1987; Masscheleyn et al., 1991a) have reported that upon flooding a soil, the solubility of As increased. Masscheleyn et al. (1991a) showed that under oxidized soil conditions, As solubility was low and most of the As in solution was As(V). Upon reduction, As(III) became the major As specie, and As solubility

increased considerably. Information about the behavior of organic arsenicals under reduced condition is completely lacking. Methanearsonates have been reported to be broken down by soil microorganisms with the residual As retained in the soil in its inorganic form under aerobic conditions (Von Endt et al., 1968; Johnson and Hiltbold, 1969). Methanearsonates are reduced to an alkylarsine form under anaerobic conditions (Kearney and Woolson, 1971). The reverse of these processes (the methylation of As from arsenate and arsenite) can also occur in flooded soils (McBride and Wolfe, 1971; Onken et al., 1987). At extremely low Eh values, organic arsenical compounds are stable (Ferguson and Gavis, 1972).

Effect of Arsenic on Rice Plants

Rice is a morphologically non-aquatic plant that thrives better in flooded soil (Senewiratne and Mikkelsen, 1961; Chaudry and McLean, 1963). When soils are flooded, several physico-chemical and biological changes occur, such as a decrease in Eh, changes in pH, an increase in conductance, reduction of Fe, Mn, NO_3^- and SO_4^-, an increase in the availability of P, Si and Mo, and generation of organic products of anaerobic metabolism (De Datta, 1981). Several of these changes are beneficial to rice plants. One of the few disadvantages that flooding soils has for rice plants is straighthead disease. Straighthead is a physiological disease of flooded rice, which results in blank florets, distorted palea and lemma and, in extreme cases, failure of panicles to form (Tisdale and Jenkins, 1921, 1940; Atkins, 1958, 1974, 1975). The affected panicles are erect rather than deflexed and have few filled florets. Several field researchers have associated straighthead with As toxicity (Wells and Gilmour, 1977;

Gilmour and Wells, 1980; Marin, 1989). The fact that straighthead is found only in flooded rice suggests its close relationship with flooded conditions and with the geochemistry of As in flooded soils. Marin (1989) determined that under reduced soil conditions rice plants took up more As than when fields were drained in mid-season. Higher rates of applied As resulted in increased straighthead incidence (Wells and Gilmour, 1977; Marin, 1989).

Arsenic has shown antagonistic interaction with P, either in nutrient or soil solution (Schweizer, 1967; Woolson et al., 1973) or within the plant (Wallace et al., 1980; Marin, 1989), and Zn (Batjer and Benson, 1958; Oh and Sedberry, 1974; Marin, 1989). Except for those antagonisms, little information is found in the literature about As interaction with other plant nutrients.

Plant species and varieties are known to vary in their susceptibility to toxic elements or to interactions of toxic elements with plant nutrients (Joshi et al., 1975). Detailed studies on the effect of As on different rice varieties have not been done. Such studies offer the possibility that plants could be selected to fit an arsenic-contaminated soil or the soil environment could be changed to provide a medium for better rice growth.

Research Objectives

The importance of a better understanding of the chemistry of As under different soil conditions and its absorption and toxicity to plants led to studies of the effect of redox potential and pH on As speciation and solubility. The effect of As on plant toxicity and reduced photosynthesis in rice was also studied.

The objectives of this research were to:

1) Study the speciation and transformation of As in soils as affected by redox potential and pH.

2) Learn more about the toxicity and uptake of different As chemical forms by rice plants and its influence on other plant nutrients.

3) Determine the effect of root-applied organic arsenicals on photosynthesis and plant growth.

The first objective was accomplished with a laboratory experiment controlling redox potential and pH. The results and conclusions are presented in chapter 1. The toxicity and uptake of different As chemical forms on rice plants and their influence on other plant nutrients were studied through an experiment with plants grown in nutrient solution. These results are reported in chapters 2 and 3. For the third objective, a second nutrient solution experiment was established. The outcome is discussed in chapter 4.

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CHAPTER 1

REDOX STABILITY OF SOIL ARSENIC CHEMICAL FORMS AND ITS INFLUENCE ON ARSENIC UPTAKE BY RICE.

Introduction

Arsenic (As) is not considered essential for plants and appears not to be involved in specific metabolic reactions when supplied at low concentrations (Liebig, 1966). At higher concentrations, however, As has been reported to interfere with metabolic processes and to inhibit plant growth, sometimes leading to death (Reed and Sturgis, 1936; Schweizer, 1967; Baker et al., 1976; Marin et al., 1992).

Accumulation of As by plants depends on plant species (Liebig, 1966; Walsh and Keeney, 1975), the concentration of As present (National Academy of Sciences, 1977), and the presence of other ions (Woolson et al., 1973; Khattak et al., 1991). Interactions between arsenate and phosphate have been frequently reported in the literature (Barrow, 1974; Wauchope, 1975). In general, increasing the amount of P reduces As accumulation by plants, and vice versa. In a recent paper (Marin et al., 1992), we reported As phytoavailability and phytotoxicity to rice was also affected by the chemical form of As present. While application of arsenate [As(V)] and dimethyl arsenic acid [DMAA] did not affect plant growth, both arsenite [As(III)] and monomethyl arsenic acid [MMAA] were phytotoxic to rice. Availability of As to rice followed the trend: DMAA < As(V) < MMAA < As(III). However, these

observations were made with plants grown in nutrient solutions amended with different As chemical forms.

The present study was undertaken with the objective to investigate the effect of soil redox-pH condition on As speciation and plant As uptake. This was achieved by studying As uptake by rice plants growing in soil suspensions equilibrated under controlled redox-pH conditions. The effect of changing soil redox-pH conditions on the solubility and stability of As chemical forms in solution was determined and related to uptake and translocation of As in the plants. Elevated As in soils from rice-producing areas are most commonly associated with monosodium or disodium salts of MMAA, a herbicide widely used as a direct spray for postemergence weed control in cotton (Frans et al., 1985). Monomethyl arsenic acid residues can cause severe damage to succeeding rotational crops, such as rice (Gilmour and Wells, 1980; Frans et al., 1985; Marin, 1989). In rice-producing areas of the southern United States, the monosodium salt of MMAA is considered the probable cause for straighthead, a physiological disease of flooded rice (Gilmour and Wells, 1980) that results in blank florets, and distorted palea and lemma (Johnston et al., 1959). Therefore, we also investigated the redox stability of monomethyl arsenic acid (MMAA) in soil suspensions and its bioavailability and phytotoxicity to rice.

Materials and Methods

Soil

Crowley silt loam soil (Typic Albaqualf) was collected from a rice farm, with a history of straighthead disease, in Cameron Parish, LA (USA). The oxidized soil had

a pH=5.2, 1.2% organic matter, and a total As content of 3.2 mg kg^{-1} dry soil. The soil was air-dried, screened through a 6 mm hardware cloth, and well mixed.

Redox control system - plant microcosms

Five hundred g soil (amended with 0.2% (w/w) ground dried rice straw) was added to 2 L deionized water and equilibrated in laboratory microcosms under controlled redox-pH conditions. The apparatus used (Fig. 1.1) is a modification of the redox-pH control system developed by Patrick et al. (1973). It consists of a desiccator, a plexiglass plate designed to support the rice seedlings, pH and platinum electrodes, a calomel half cell, and a gas inlet and outlet. The soil is kept in suspension by a magnetic stirrer. The pH and platinum electrodes, the calomel half cell, and the gas inlet and outlet are fitted into holes on the plexiglass plate. The plate covers the desiccator and is sealed with silicone rubber sealant. The outer surfaces of both the desiccator and plexiglass plate are painted with silver paint to prevent exposure of the soil suspension to light.

In a first set of treatments, 12 equilibrations were performed and the following redox-pH combinations were used: redox -200, 0, +200, and +400 mV; pH 5.5, 6.5, and 7.5. In the redox control systems the soil redox potential is maintained at a preset value automatically (Patrick et al., 1973). Sodium hydroxide (2N) or HCl (2N) were added to the plant microcosms with a syringe as required to adjust pH. Soil suspensions were equilibrated under the controlled redox-pH conditions for 3 days prior to plant introduction.

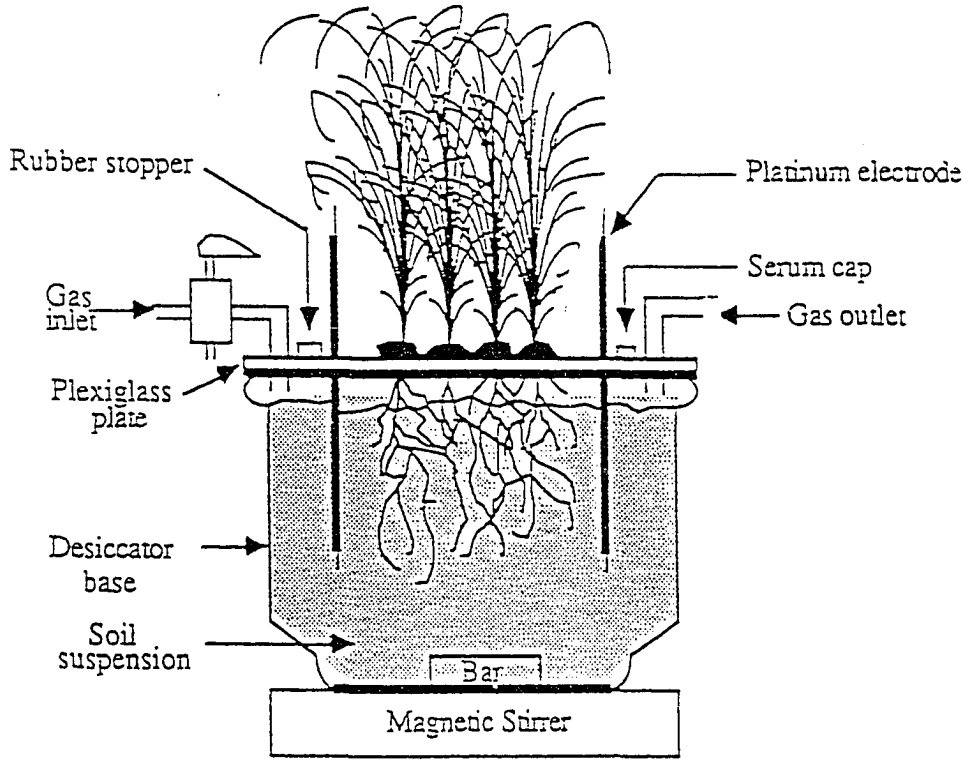

Figure 1.1: Experimental setup used in the plant growing experiment under controlled redox and pH conditions.

In the second set of treatments, the soil was amended with 4 mg As kg^{-1} dry soil as the monosodium salt of MMAA prior to equilibration under specific soil redox-pH conditions. The same soil redox-pH conditions were used as in the first group of treatments. Plant As uptake was examined in relation to the redox-pH stability and solubility of amended MMAA.

Plant material

Rice (*Oryza sativa*, L.) seeds from the cultivars Lemont and Mercury were germinated in sterilized sand. Lemont, an early maturing, semidwarf, long grain cultivar, and Mercury, an early maturing, semidwarf, medium-grain cultivar are considered to be moderately tolerant (Bollich et al., 1985) and very susceptible to As toxicity (McKenzie et al., 1988), respectively. Eight days after germination, uniform seedlings were selected and grown in a nutrient solution (Yoshida et al., 1976) for two weeks.

Plant growth experiment

Seedlings were transplanted in soil suspensions equilibrated under controlled redox-pH conditions. A single microcosm, representing one specific soil redox-pH condition, contained 6 seedlings from each cultivar (Fig. 1.1). Plants were placed through holes in the plexiglass plate. Some cotton wool was placed around the seedlings and held the seedlings in place. Every other day, deionized water was added to the microcosms to replace the water lost through evapotranspiration. Plants were grown in the redox control-plant microcosms until they were 56 days old. Continuous illumination was provided by a set of fluorescent tubes and 100 Watt flood lamps.

The experiment was conducted with both the native soil and with the MMAA amended soil. No symptoms of vegetative injury could be observed during the experiment.

Sampling procedures

During the growing period under controlled redox-pH conditions, the concentration and chemical forms of As present in the soil suspensions were determined weekly. Thirty mL soil suspensions were withdrawn from the microcosm, centrifuged, and filtered through a 0.45 μm micropore filter under an inert N_2 stream for the reduced treatments (Patrick and Henderson, 1981). Concentrations of As(III), As(V), MMAA, and DMAA in the supernatants were determined using the As speciation technique described by Masscheleyn et al. (1991a).

At the end of the growing period, plants were harvested. Eight seedlings (4 of each variety) were randomly selected for tissue analysis. Roots were carefully washed with tap water, rinsed with 0.1 N HCl solution followed by 3 rinses with deionized water. Roots and shoots were separated and dry matter yields determined after drying at 65° C for 48 hr. Dried samples were ground in a stainless-steel Wiley mill to pass a 20 mesh sieve. Plant tissue samples (1 and 0.5 g for shoot and root samples, respectively) were digested with 5 mL conc. HNO_3 (AR select, Mallinkrodt Inc.) for 4 hr at 130° C. Digested samples were filtered (Whatman # 42) and diluted with deionized water to 50 mL. Arsenic, Fe, Zn and Cu in the extracts were determined with a Jarrel Ash (Atom Comp 800 series) ICP. The detection limit of the ICP for As is 15 $\mu g\ L^{-1}$.

Statistical analyses were performed using the PROC CORR and PROC GLM procedures available in SAS (Statistical Analysis System, 1987).

Results and Discussion

Effect of soil redox-pH condition on arsenic speciation and solubility.

In the treatments with the unamended soil, redox potential and pH greatly affected the speciation and solubility of indigenous As. Figure 1.2 shows the amount of water-soluble As in two chemical forms at four redox levels (-200, 0, +200, and +400 mV) in combination with three pH levels (5.5, 6.5, and 7.5). Results represent the average concentration of soluble As chemical forms, calculated from the weekly analyses of the soil suspensions, and represent the As concentration and chemical forms available for uptake by the rice plants during the growing period.

Water-soluble As concentrations were inversely related to redox and pH. At the lowest redox potential studied (-200 mV) 7.3, 2.2, and 1.4 % of the total As in the soil (3.2 mg kg^{-1} dry soil) became water-soluble at a pH 5.5, 6.5, and 7.5, respectively. Irrespective of soil redox potential, most water-soluble As was found at pH 5.5. The effect of pH on As solubility was most pronounced at a soil redox condition of -200 mV. At redox potentials of +400 and +200 mV, As(V) was the major dissolved As species constituting from 51 to 90% of the total dissolved As. Except at pH 7.5, As(III) became the dominant As chemical form in solution upon reduction (0 and -200 mV) of the soil suspensions. Although thermodynamically unstable, a considerable amount of As(V) remained in solution under reduced conditions. It is interesting to note that both

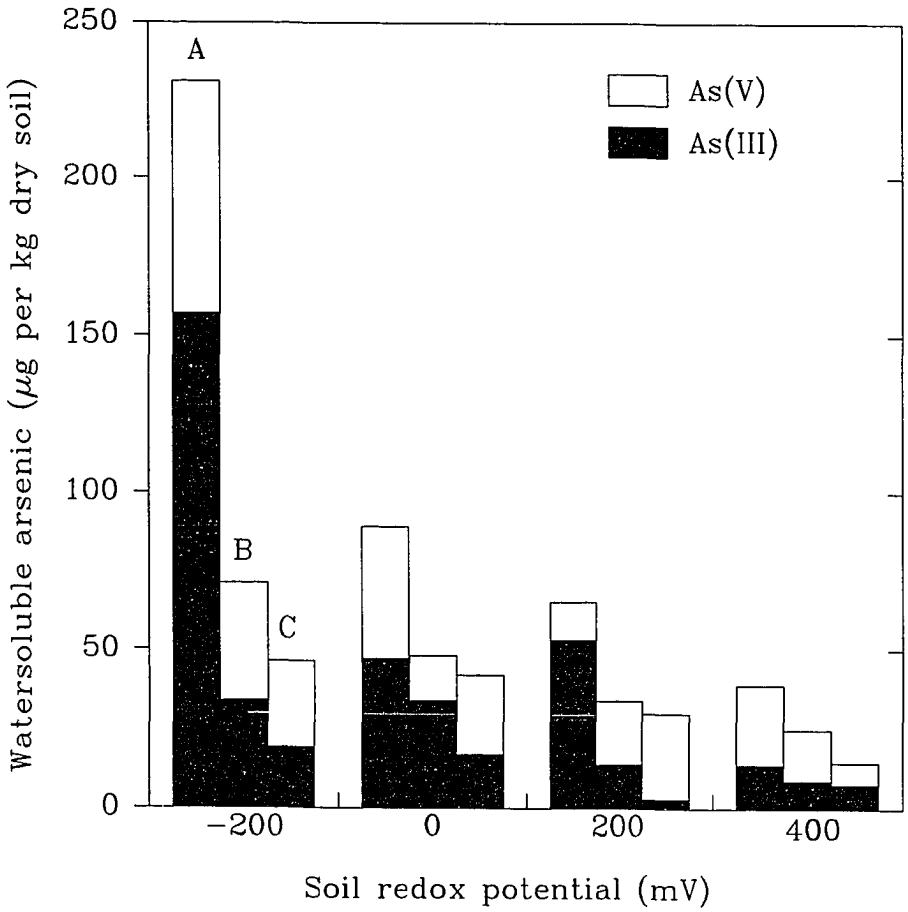

Figure 1.2: Speciation and solubility of indigenous As as affected by redox condition for soil equilibrated at pH's 5.5 (A), 6.5 (B) and 7.5 (C).

the amount of water-soluble As(III) and As(V) increased with decreasing redox. For the treatment at pH 7.5, the As(III)/As(V) concentration ratio increased with decreasing redox, but As(V) remained the most important As species in solution at all times. No water-soluble organic arsenicals could be detected. Our data illustrate that a decrease in pH and/or a decrease in soil redox level will result in increased As availability to plants.

According to Livesey and Huang (1981), soluble As concentrations were controlled by sorption/desorption reactions rather than through precipitation/dissolution reactions. Convincing evidence for a species specific sorption behavior of As on soils and mineral phases has been presented (Pierce and Moore, 1982). Under the redox-pH conditions encountered in this study, As(V) will be negatively charged (as $H_2AsO_4^-$ or $HAsO_4^{2-}$), while As(III) will be predominantly present as the uncharged H_3AsO_3 chemical form (Masscheleyn et al. 1991b). As the soil pH increases, hydroxyl ions will replace As on the soil sorption sites and As will be released into solution. Furthermore, the increasing negative soil surface charge with increasing pH will facilitate desorption of As anions. Deuel and Swoboda (1972) reported an increase of total soluble As under reduced soil conditions and attributed this increase to the reduction of ferric arsenate compounds. More recently, Masscheleyn et al. (1991b) found the influence of redox on As solubility in soils to be governed by 1) reduction of As(V) to As(III) followed by desorption, and 2) the dissolution of Fe-oxyhydroxides and concurrent release of coprecipitated As(V). Results of our study are in accordance with the latter findings. Water-soluble Fe concentrations (Fig. 1.3) were highly correlated [$P<0.001$] with

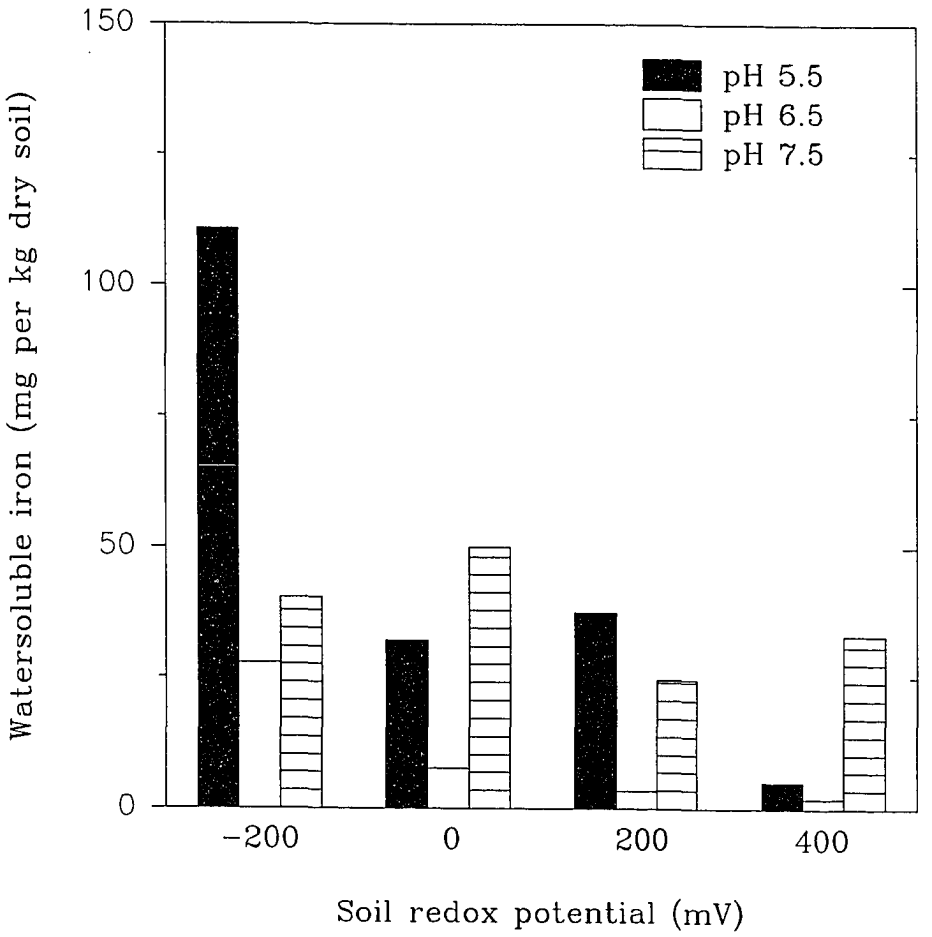

Figure 1.3: Solubility of iron as affected by redox condition for the MMAA-unamended soil equilibrated at pH's 5.5, 6.5 and 7.5.

dissolved total As (r=0.86), As(III) (r=0.83), and As(V) (r=0.62) concentrations suggesting the relation between the reduction of Fe-compounds and the solubility of As. Furthermore, an increase in dissolved total As was generally associated with an increase in the As(III)/As(V) concentration ratio (Fig. 1.2). For example, in the equilibrations at pH 7.5 the observed increase in dissolved As concentration was due to the reduction of As as the amount of reduced (water-soluble) Fe remained approximately the same for all redox levels studied.

When the soil was amended with MMAA (at a rate of 4 mg As kg^{-1}) soil physicochemical condition, as indicated by redox potential and pH, affected the speciation and solubility of both inorganic As and MMAA, the organic arsenical added (Fig. 1.4). Considering the addition of MMAA as the main effect, the amount of water-soluble As(III+V) increased significantly [$P < 0.05$] in the MMAA amended soil (Table 1.1) as compared to the unamended soil. This was associated with a significant increase in dissolved As(V). At pH 6.5 and 7.5, As(V) remained the dominant inorganic As species in solution even under strongly reducing (0, and -200 mV) conditions. In the MMAA amended soil, water-soluble Fe concentrations were not significantly correlated with dissolved As(III+V), As(V), or MMAA. However, dissolved Fe concentrations were correlated [$P < 0.001$] with soluble As(III) [r=0.61]. Although other soil biogeochemical processes may be involved, the observed increase in As(V) concentration may be due to demethylation of amended MMAA. The rupture of C-As bonds and production of As(V) from MMAA has previously been observed by Dickens and Hiltbold (1967), Von Endt et al. (1968), and Odanka et al. (1985a, b). Methylation

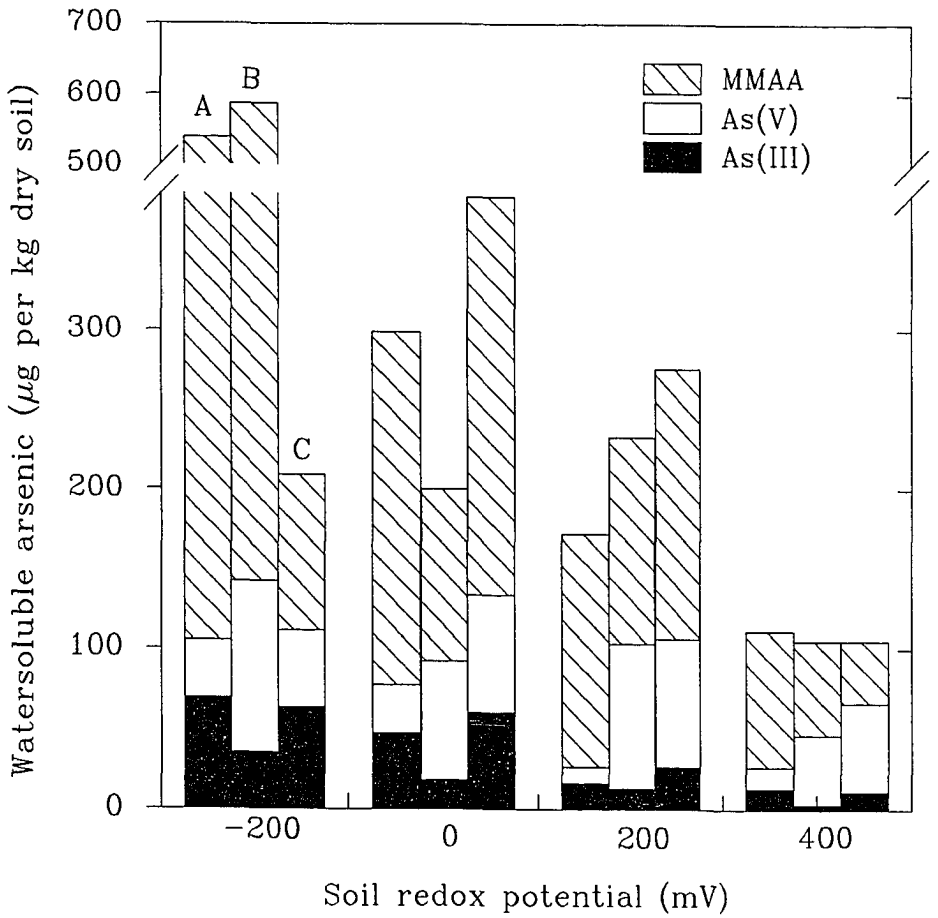

Figure 1.4: Arsenic speciation and solubility in the MMAA amended soil suspension as affected by redox condition for soil equilibrated at pH's 5.5 (A), 6.5 (B) and 7.5 (C).

Table 1.1: Effect of MMAA addition on soil arsenite, arsenate, Zn and Cu content[a].

MMAA added	Inorganic As As(III+V)	Arsenite As(III)	Arsenate As(V)	Zn	Cu
	—— $\mu g\ kg^{-1}$ ——			—— $mg\ kg^{-1}$ ——	
4 mg As kg^{-1}	83.4 a[b]	30.3 a	53.1 a	0.18 a	0.03 b
0 mg As kg^{-1}	55.3 b	31.4 a	23.9 b	0.16 a	0.06 a

[a] Data represent mean values of n=12.
[b] Means followed by the same letter in a column do not differ at $P<0.05$, compared by Duncan's Multiple Range Test.

of the amended MMAA and dimethyl arsenic acid (DMAA) formation was not observed in our experiment. Higher soil redox levels led to lower dissolved MMAA concentrations. The effect of pH on water-soluble MMAA concentrations was less clear. As for the inorganic As chemical forms (Livesey and Huang, 1981; Pierce and Moore, 1982) Fe-oxides have been implicated in the sorption of MMAA by soils (Holm et al., 1980). The observed responses of MMAA solubility to redox and pH are likely due to reduced sorption capacity of the soil caused by decreasing redox or increasing pH.

Some questions remain concerning the persistence of As(V) under reducing soil conditions (Fig. 1.2 and 1.4). Although the ratio As(III)/As(V) generally increased with decreasing redox the observed As(III)/As(V) ratios do not agree with equilibrium

thermodynamic considerations. Under the redox-pH conditions encountered in our experiments $H_2AsO_4^-$ and H_3AsO_3 are the thermodynamically stable As(V) and As(III) chemical forms, respectively (Masscheleyn et al., 1991b). The As(V) to As(III) reduction can thus be written as $H_2AsO_4^- + 3H^+ + 2e^- = H_3AsO_3 + H_2O$. If we assume thermodynamic equilibrium and an equilibrium constant = 10^{-11} (Masscheleyn et al., 1991b), the As(III)/As(V) concentration ratio should follow the equation: log [As(III)/As(V)] = 22-2[pe+3/2pH]. Clearly, the observed As(III)/As(V) concentration ratios do not conform to this equation, suggesting that chemical kinetics could play an important role in the conversion of As(V) to As(III). The presence of the rice plants in the soil suspensions could have been another important factor altering the As(III)/As(V) concentration ratio. Recently, Marin et al. (1992) illustrated the importance of the chemical form of As in the uptake of the element from nutrient solutions by plants. It was shown that, in nutrient solutions, As(III) is the As chemical form most readily taken up by rice plants. Assuming the same is true in soil suspensions, the rice plants could selectively remove As(III), thereby altering the As(III)/As(V) concentration ratio's in the soil suspensions.

Arsenic uptake by rice as affected by soil redox-pH condition and MMAA application

Since soil redox-pH conditions affected the speciation and solubility of As in the soil, one could expect soil redox-pH to also determine As phytoavailability and phytotoxicity. We found soil physicochemical (redox-pH) condition and the application of MMAA to affect plant growth and tissue As concentration. There were no significant

differences in dry matter production and tissue As concentration due to cultivar effect. Therefore, cultivar could be used as replications in the statistical analysis.

In the treatments with the native (unamended) soil, tissue As concentration increased with decreasing redox and pH (Table 1.2). Arsenic absorbed by the plants was accumulated in the roots. Shoot As was only detected at the lower redox levels. When the pH was 6.5 and 7.5 in the unamended soil (Table 1.2), plants did not take up any As at the highest soil redox level (+400 mV) studied. Under this soil redox-pH condition As solubility was lowest and the major part of soil As was present as As(V) (Fig. 1.2). Arsenic tissue concentrations were highest at pH 5.5. This in agreement with the higher As solubility in the soil at pH 5.5 (Fig. 1.2). Root As concentrations were significantly [$P<0.001$] correlated with As(III) ($r=0.76$), and As(V) ($r=0.76$) concentrations in solution.

Total plant As uptake, calculated by multiplying tissue As concentrations by the dry weight of the corresponding plant part and total stem + root values, was also affected by soil redox-pH condition (Fig. 1.5A). Due to the low dry matter production at the -200mV-5.5 and 0mV-5.5 soil redox-pH conditions the total plant As uptake was low under these physicochemical soil conditions. Plant As uptake was not correlated with soluble As(III), nor with soluble As(V) concentrations. Plant As uptake from soil suspensions agreed with what we observed in a previous reported hydroponic study (Marin et al., 1992). When plants were grown in nutrient solutions amended with As(III) the element was readily taken up and stored in the root. In the soil suspensions under reduced conditions, As(III) was the dominant As chemical form present. A

considerable amount of As was taken up by the plants, and the major part remained in the root (Table 1.2).

Table 1.2: Tissue arsenic concentration as affected by soil redox-pH condition[a].

MMAA added	Redox	Plant part	pH 5.5	pH 6.5	pH 7.5
			mg kg^{-1} dry wt.		
0 mg As kg^{-1}	-200 mV	Shoot	1.0	2.0	1.0
		Root	107.0	78.5	74.5
	0 mV	Shoot	1.5	ND[b]	0.5
		Root	59.5	37.5	17.5
	+200 mV	Shoot	1.0	ND	ND
		Root	49.0	8.5	6.5
	+400 mV	Shoot	ND	ND	ND
		Root	35.5	ND	ND
4 mg As kg^{-1}	-200 mV	Shoot	28.5	11.5	6.0
		Root	277.5	176.5	168.0
	0 mV	Shoot	20.5	7.0	7.0
		Root	228.0	117.0	80.0
	+200 mV	Shoot	5.0	0.5	1.0
		Root	128.0	54.0	27.5
	+400 mV	Shoot	ND	ND	ND
		Root	102.5	18.0	16.5

[a] Data represent mean values of n=2.
[b] Not detected.

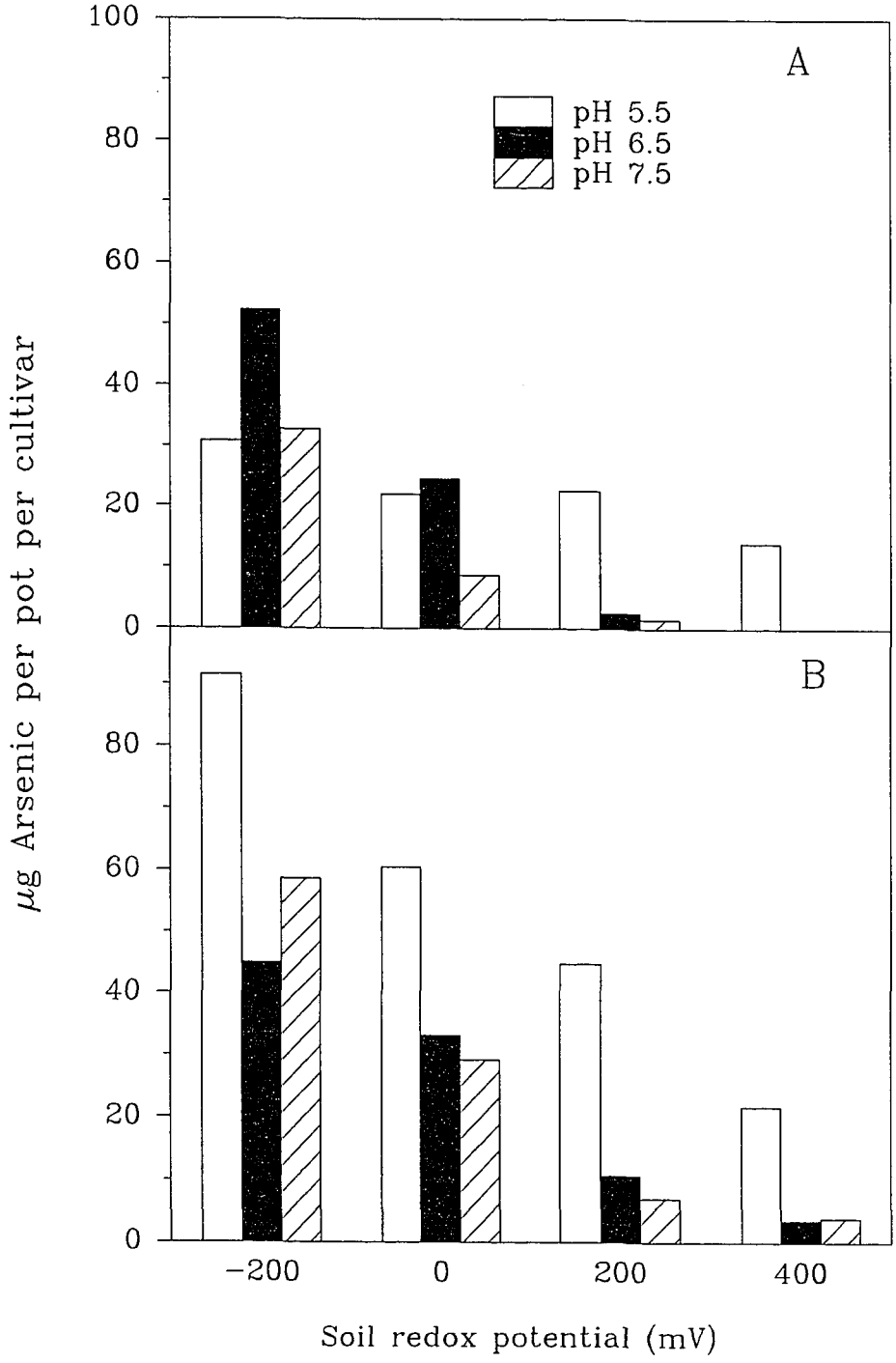

Figure 1.5: Total plant As uptake as affected by soil redox-pH condition in A) the native soil, and B) the MMAA amended soil.

When As was added as MMAA at a rate of 4 mg As kg^{-1} dry soil, a significant [$P<0.05$] decrease in total dry matter production was observed reflecting the negative impact of MMAA on plant growth. Although application of MMAA resulted in lower dry matter production the total As uptake by the plants significantly increased compared to unamended soils (Table 1.3). On the average, the addition of MMAA resulted in approximately a two fold increase in total As uptake. When plants were grown in the MMAA amended soil suspensions the tissue As concentration ratio (shoot As concentration / root As concentration) and As uptake ratio (shoot As uptake / root As uptake) significantly increased. This suggests that when more As is taken up by the plant in the MMAA form, more As is translocated to the above ground plant parts.

Table 1.3: Effect of MMAA addition on plant dry weight and tissue arsenic concentration and uptake[a].

MMAA added	Total dry wt.	As uptake			As conc. ratio	As upt. ratio
		Shoot	Root	Total		
	g	——— μg per pot ———				
4 mg As kg^{-1}	1.02 b[b]	5.04 a	29.1 a	34.1 a	0.04 a	0.12 a
0 mg As kg^{-1}	1.60 a	0.64 b	16.9 b	17.6 b	0.01 b	0.03 b

[a] Data represent mean values of n=24.
[b] Means followed by the same letter in a column do not differ at $P<0.05$, compared by Duncan's Multiple Range Test.

However, most As still remains stored in the root leading to concentration and uptake ratios lower than one. This is in agreement with the findings from our previous study (Marin et al., 1992) where plants were grown in MMAA-amended nutrient solutions. Monomethyl arsenic acid was shown to be the most phytotoxic As chemical form, and the concentration ratio increased with an increase in the rate of application.

Addition of MMAA also affected Zn and Cu phytoavailability. In our experiment, application of MMAA caused a significant decrease in Zn tissue concentration, uptake, and translocation to the rice shoot (Table 1.4). Due to the addition of MMAA, the Zn concentration and uptake ratios decreased from 1.32 and 3.88 to 0.71 and 2.35, respectively (Table 1.4). Solubility of Zn in the soil suspensions

Table 1.4: Effect of MMAA addition on tissue Zn concentration, uptake and mobility[a].

MMAA added	Tissue Zn		Zn conc. ratio	Zn uptake		Zn upt. ratio
	Shoot	Root		Shoot	Root	
	—— mg kg^{-1} ——			—— μg per pot ——		
4 mg As kg^{-1}	49.1 b[b]	77.9 a	0.71 b	38.5 b	18.2 b	2.35 b
0 mg As kg^{-1}	105.5 a	83.3 a	1.32 a	125.4 a	35.0 a	3.88 a

[a] Data represent mean values of n=24.
[b] Means followed by the same letter in a column do not differ at $P<0.05$, compared by Duncan's Multiple Range Test.

was not influenced by MMAA application (Table 1.1), nor by the soil redox-pH condition. The observed antagonistic effect of As on Zn absorption may have been another factor related to the consistent negative effect of solution MMAA on plant growth. Similar antagonistic MMAA-Zn interactions have been observed previously in rice (Oh and Sedberry, 1974; Marin, 1989) and in peaches (Thompson and Batjer, 1950; Batjer and Benson, 1958). Addition of MMAA also influenced Cu solubility in the soil. Soil Cu concentrations significantly decreased due to MMAA (Table 1.1). The reason for the decreased Cu solubility is still unclear. As a consequence of the effect of MMAA on Cu solubility, several Cu plant uptake parameters were negatively correlated with As uptake and soil As solubility. For example, plant Cu and As uptake [$r=-0.67$; $P<0.001$], plant Cu uptake and soluble inorganic As content [$r=-0.55$, $P<0.01$], and root Cu and root As uptake [$r=-0.66$, $P<0.001$] were all negatively correlated. As soil redox status did not affect soil Cu solubility, the observed decrease in Cu uptake with decreasing soil redox level may be related to the increased As absorption by the plants.

The effect of soil redox-pH condition on tissue As concentration and plant As uptake in the treatments with the MMAA amended soil suspensions are given in Table 1.2 and Figure 1.5B, respectively. Both plant As root and shoot concentrations were significantly [$P<0.001$] correlated with the amount of As(III) ($r=0.71$ and 0.83) and MMAA ($r=0.59$ and 0.61) in solution. In contrast to the unamended soil, plant As concentrations were negatively correlated with the amount of As in solution present as As(V). Seedlings subjected to MMAA accumulated As in their tissues at all soil

redox-pH conditions studied (Fig. 1.5B). The lower the pH and redox the higher the amount of As accumulated by the rice plants. A decrease in soil redox level from +400 to -200 mV caused approximately a 4, 13, and 15 fold increase in plant As uptake for soils equilibrated at pH 5.5, 6.5, and 7.5, respectively. Application of MMAA affected the As shoot/root uptake ratio, as mentioned above. Except for the +400 mV treatments, part of the As taken up by the plants was translocated to the shoot.

In summary, soil redox potential and pH were shown to affect As speciation and solubility, thereby determining As phytoavailability and phytotoxicity to rice. The lower the soil redox potential and pH, the higher the amount of water-soluble As found. Although As(III) became the major As species in solution under reduced conditions, some As remained as As(V). Plant As tissue concentrations and uptake were highest under reduced soil conditions. Flooding a rice soil will lead to higher dissolved As concentrations and the presence of As(III) will enhance As uptake by the plants. In plants grown under controlled redox-pH conditions, most of the As that was taken up by the rice plants remained in the roots. However, when rice is grown on acreage previously treated with MMAA herbicides, greater concentrations of MMAA are likely to be found in the soil, and more As would be expected to be taken up by the plants and translocated to the shoots. In our experiment, the addition of 4 mg MMAA-As kg^{-1} dry soil decreased plant growth but resulted in significantly higher plant As concentrations and uptake. Low soil redox conditions increased the solubility and phytoavailability of MMAA. Monomethyl arsenic acid also affected the absorption of Zn and Cu.

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CHAPTER 2

THE INFLUENCE OF CHEMICAL FORM AND CONCENTRATION OF ARSENIC ON RICE GROWTH AND TISSUE ARSENIC CONCENTRATION.[1]

Introduction

The toxicity of arsenic (As) to biological systems has made it a useful constituent of insecticides, herbicides, fungicides, desiccants and wood preservatives (Johnson and Hiltbold, 1969; US Department of Agriculture, 1980). However, the use of these arsenicals has led to elevated concentrations of plant-available As in many soils. Arsenic accumulation in soils reduces soil productivity (Liebig, 1966) and is toxic to plants (Baker et al., 1976; Deuel and Swoboda, 1972; Schweizer, 1967). In the southern United States, for example, As toxicity has been associated with straighthead, a physiological disease of flooded rice (Gilmour and Wells, 1980; Marin, 1989; Wells and Gilmour, 1977). Straighthead results in blank florets, distorted palea and lemma and, in extreme cases, failure of panicles to form (Johnston et al., 1959). The affected panicles are erect rather than deflexed and have few filled florets.

Arsenic in soil-water environments can be present in at least four different chemical forms: arsenate (As(V)), arsenite (As(III)), monomethyl arsenic acid (MMAA), and dimethyl arsenic acid (DMAA). Arsenic is subject to chemically and/or microbiologically mediated oxidation-reduction (Brannon, 1983; Masscheleyn et al.,

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1991a), and methylation (Brannon, 1983) reactions in soils. Both As solubility (Masscheleyn et al., 1991a) and toxicity to animals and humans (Clemens and Munson, 1947; Ferguson and Gavis, 1972; Tsusumi and Takahashi, 1974) depend on its chemical form.

Absorption of arsenic by plants is influenced by many factors including plant species (Liebig, 1966; Walsh and Keeney, 1975), the concentration of As in the soil (National Academy of Sciences, 1977), soil properties such as pH and clay content (Dickens and Hiltbold, 1967; Johnson and Hiltbold, 1969; Von Endt et al., 1968), and the presence of other ions (Khattak et al., 1991; Rumberg et al., 1960; Woolson et al., 1973). Phytotoxicity studies with As have been conducted with particular As compounds (usually As(V) and As(III)); however, interpretations were always based on analyses of total soil As (Peoples, 1975) or on specific soil-As fractions (Woolson et al.,1971b). Woolson et al. (1971a,b) related plant-available As to the amount of soil-As, using several selective extractants and found that the amount of water-soluble As and the content of reactive Ca, Fe, and Al in a soil determined As phytoavailability.

No information is available on As phytoavailability as influenced by its chemical form in solution. Furthermore, the relation of As phytotoxicity to the chemical form of As present in solution has not been investigated. Even though different degrees of susceptibility to As toxicity (straighthead) exist among rice cultivars, no explanation has been proposed for the mechanism involved in this tolerance. We designed a laboratory experiment, using two rice cultivars, that allowed the study of As absorption and phytotoxicity in relation to its chemical form. The effect of different chemical forms

of As on rice growth and the distribution of the absorbed As chemical forms between shoot and root is reported here.

Materials and Methods

Rice (*Oryza sativa,* L.) was grown in a nutrient solution containing different chemical forms and concentrations of As. The factorial treatments (4 x 3 x 2) were applied using four replicates of a complete randomized design. The treatments consisted of four chemical forms of As (As(V), As(III), MMAA, and DMAA) with three As concentrations (0.05, 0.20, and 0.80 mg As L^{-1}), and two rice cultivars (Lemont, and Mercury). Two controls, one for each cultivar, with no added As were also included. The chemical forms of As were added as their sodium salts. Lemont, an early maturing, semidwarf, long-grain cultivar, and Mercury, an early maturing, semidwarf, medium-grain cultivar are considered to be moderately tolerant (Bollich et al., 1985) and very susceptible to As toxicity (McKenzie et al., 1988), respectively.

Seeds were germinated in sterilized sand. Eight days after germination, uniform seedlings from each variety were selected. The sand was washed from the root system with distilled-deionized water, and seedlings transferred to 2.5 L plastic pots containing 2 L nutrient solution. A single pot, representing a specific As form - As concentration treatment, contained 4 seedlings from both cultivars. The basal nutrient solution (Yoshida et al., 1976) contained: 40 mg L^{-1} of N, K, Ca and Mg; 10 mg P L^{-1}; 2 mg Fe L^{-1}; 0.5 mg Mn L^{-1}; 0.05 mg Mo L^{-1}; 0.2 mg B L^{-1}; and 0.01 mg L^{-1} of Zn and Cu. The pH of the nutrient solution was adjusted to 4.0 $\pm$ 0.2 in order to avoid iron

deficiency (Judsujinda, 1976). Seedlings were passed through holes in a styrofoam plate (12-cm diameter and 4-mm thick) floating on the nutrient solution. The plants were grown in the laboratory (26±2°C) and received continuous illumination from a set of fluorescent tubes and 100 Watt flood lamps situated 50 cm above the plants.

After four days of acclimatization plants were subjected to the different As treatments. The As in solution was analyzed regularly using a hydride generation atomic absorption technique (Masscheleyn et al., 1991b) to verify that the chemical form of the added As did not change over time. Arsenic forms were found to be stable with respect to oxidation/reduction and methylation/demethylation reactions for a period of 2 days. Thus, the nutrient solutions containing specific As forms were replaced every other day in order to maintain the desired treatments.

Plants were grown for 4 weeks, and then harvested. Roots were washed with tap water, rinsed with a 0.1 *M* HCl solution followed by 3 rinses with distilled-deionized water. Roots and shoots were separated and dry matter yields determined after drying at 65°C for 72 hr. Samples were ground in a stainless-steel Wiley mill to pass a 20-mesh sieve. Due to the small sample size obtained for several treatment combinations, samples of two replications were combined for tissue analysis prior to digestion. Plant tissue samples (0.5 g) were digested with 5 mL conc. HNO_3 (AR Select, Mallinckrodt Inc.) for 4 h at 130°C. Digested samples were filtered (Whatman #42) and diluted with distilled deionized water to 50 mL. Arsenic in the extracts was determined with a Jarrel Ash (Atom Comp 800 series) ICP. Acid blanks were analyzed

in order to assess possible As contamination. The As content of the HNO_3 used was below the detection limit of the ICP (15 ugL^{-1}).

Statistical analyses were performed using the PROC GLM procedure available in SAS (SAS Institute Inc., Cary, NC).

Results and Discussion

Rice growth: total, root, and shoot dry weight

Rice growth, as represented by shoot, root, and total (shoot+root) dry weight was significantly affected by As treatment. Both the concentration and chemical form of As present in the nutrient solution influenced plant growth (Table 2.1). Our results demonstrate that the As form is more important than the As level in solution in determining the phytotoxic effect of As to rice. In Figure 2.1 control plants and plants subjected to 0.8ppm of DMAA, As(V), As(III), and MMAA, respectively, are depicted.

There were no significant differences in total dry matter production due to cultivar effect. Rice dry matter production was influenced by As chemical form as well as by the concentration of As in solution (Fig. 2.2). When As was applied as DMAA at levels of 0.05, or 0.2 mg As L^{-1} an increase in total dry matter was observed as compared to the control. At the highest level of DMAA addition (0.8 mg As L^{-1}), total dry weight was the same as for the control. Seedlings subjected to the DMAA treatments were thicker, developed more tillers, and shorter thicker roots as compared to the other treatments (Fig. 2.1). The reason for the observed positive growth response

Table 2.1: Effect of arsenic concentration and chemical form on dry matter production of rice.

Factor	Total dry wt.	Shoot dry wt.	Root dry wt.	Dry wt. Ratio
	g per pot			
Variety				
Lemont	0.605 a[a]	0.438 a	0.166 a	2.52 b
Mercury	0.591 a	0.449 a	0.142 b	3.11 a
Arsenic Form				
As(III)	0.586 b	0.427 c	0.159 a	2.64 c
As(V)	0.683 a	0.510 b	0.173 a	3.00 b
mmAs	0.361 c	0.246 d	0.115 b	2.09 d
dmAs	0.762 a	0.591 a	0.170 a	3.53 a
Arsenic Rate				
0.05	0.691 a	0.516 a	0.174 a	2.99 a
0.2	0.617 a	0.461 a	0.156 b	2.88 a
0.8	0.486 b	0.353 b	0.133 c	2.57 b
As form X As Rate				
Control[b]	0.664	0.508	0.155	3.28
AsIII 0.05	0.692	0.518	0.174	3.01
AsIII 0.2	0.660	0.492	0.167	2.94
AsIII 0.8	0.406	0.271	0.135	1.98
AsV 0.05	0.684	0.520	0.165	3.19
AsV 0.2	0.725	0.547	0.178	3.12
AsV 0.8	0.640	0.464	0.176	2.67
mmAs 0.05	0.546	0.382	0.164	2.36
mmAs 0.2	0.312	0.206	0.106	1.91
mmAs 0.8	0.225	0.149	0.076	1.98
dmAs 0.05	0.842	0.647	0.195	3.39
dmAs 0.2	0.772	0.599	0.173	3.53
dmAs 0.8	0.672	0.528	0.143	3.66
F test for interactions				
Var x As form	NS[c]	NS	*	NS
Var x As rate	NS	NS	NS	NS
As form x As rate	NS	NS	**	**
Var x AsForm x AsRate	NS	NS	NS	NS

a Values followed by the same letter are not significantly different ($p<0.05$), Duncan Multiple Range Test.

b Control values were not included in the Analysis of Variance.

c NS=non significant F ratio ($p<0.05$), * significant at $p<0.05$, ** significant at $p<0.01$.

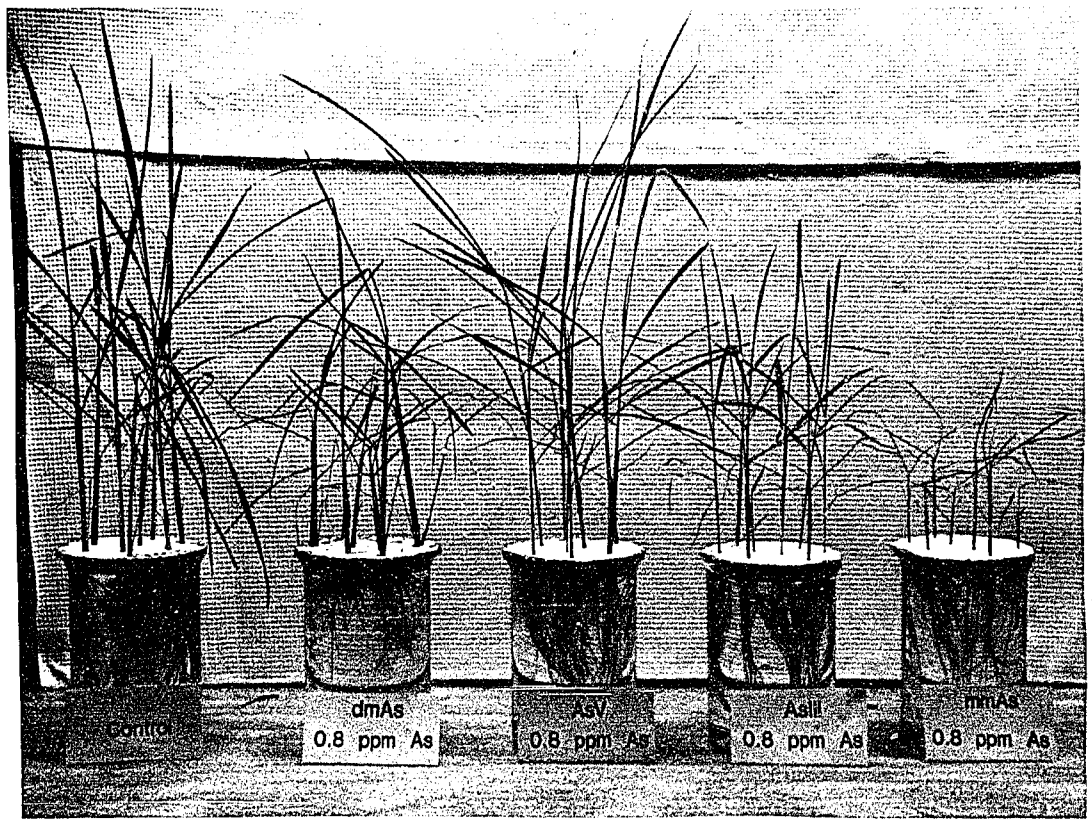

Figure 2.1: Effect of arsenic chemical form (0.8 mg As L^{-1} nutrient solution) on rice growth.

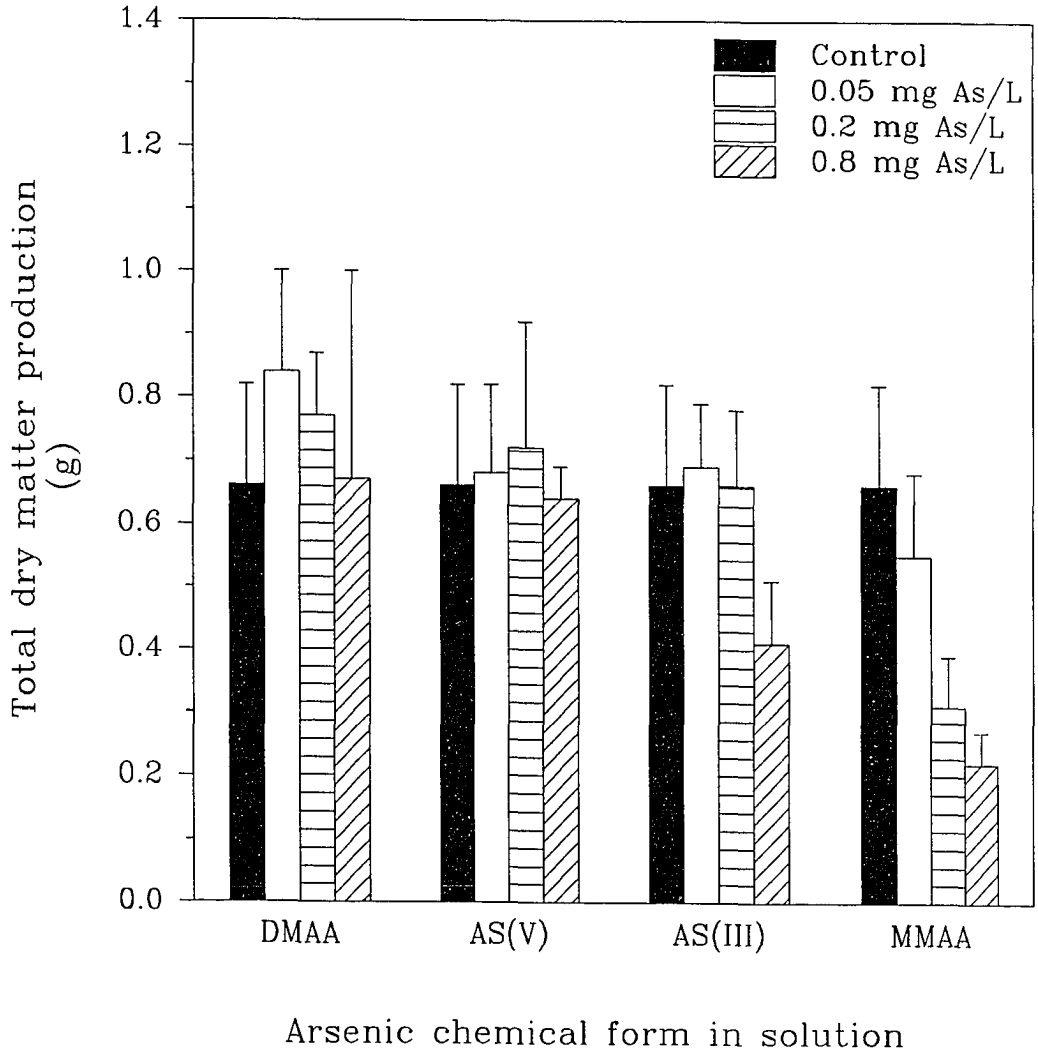

Figure 2.2: Rice dry matter production as affected by As concentration and chemical form.

is unclear. Although As is reported not to be an essential nutrient for plants (Liebig, 1966), low concentrations of As have been reported to increase growth of maize (Woolson et al., 1971a), and potatoes (Jacobs et al., 1970). It has been suggested (Deuel and Swoboda, 1972; Jacobs et al., 1970) that a displacement of soil phosphate by arsenate results in an increased plant-P availability, thereby affecting growth. However, this speculation cannot be responsible for the increased rice dry matter production observed in our experiments since we worked in a soil-free system.

When plants were grown in a nutrient solution containing As(V) at levels ranging from 0.05 to 0.8 mg As L^{-1}, the total dry matter production was not affected (Table 2.1, Fig. 2.2).

Both As(III) and MMAA were phytotoxic to rice (Table 2.1, Figs. 2.1 and 2.2). Arsenic (III) caused a significant decrease in growth when applied at the maximum rate (0.8 mg As L^{-1}). Monomethyl arsenic acid was the most toxic As form with respect to total dry matter production. A steady decrease in growth was observed with increased MMAA concentrations in the nutrient solution. Total dry matter production was significantly reduced at any of the applied MMAA rates. Arsenic toxicity in plants was described by Machlis (1941) as consisting of root plasmolysis and leaf wilting followed by root discoloration and necrosis of leaf tips and margins. Plants grown in the nutrient solution containing MMAA at a rate of 0.8 mg As L^{-1} were stunted, with necrosis in leaf tips and margins. These symptoms indicated a limitation in the movement of water into the plant resulting in death. Their total dry weight was only 34 % as compared to the control, and it was evident that these plants were going to die.

Root dry weight was different for the two cultivars, with Lemont showing a significantly higher root production than Mercury (Table 2.1). Root production in both cultivars responded in a similar way to the methylated (MMAA, and DMAA) As forms. A significant increase in root dry weight was obtained when As was applied as DMAA at a rate of 0.05 mg As L^{-1}. The application of MMAA at levels of 0.2, and 0.8 mg As L^{-1} significantly decreased root dry weight in both cultivars. The application of the inorganic As species led to a differential response in root production between the two cultivars. At the 0.2, and 0.8 mg As(V) L^{-1} levels, root dry matter weight in Mercury was significantly increased. This was not the case for Lemont. However, a significant decrease in Lemont root dry weight was observed when As was applied at 0.8 mg As(III) L^{-1}. Root dry weight of Mercury was not affected by the latter treatment.

The As treatments affected shoot dry weights in a similar manner as they did total dry weights. This is not surprising since dry weight of shoots contributed the major portion (66 to 79 %) of total dry weight.

As a consequence of the significant difference in root production between the two cultivars, the shoot/root dry weight ratio was also significantly different (Table 2.1). Mercury had a significantly higher shoot/root dry weight ratio than Lemont. Again, both As concentration and chemical form affected these ratios in a similar way for both varieties. A consistent increase in shoot/root dry weight ratio was observed with increasing DMAA concentration (Table 2.1). This, and the above described symptoms, suggest that a metabolite re-allocation process rather than a simple translocation of As to the shoot may be responsible for the positive growth response of rice to DMAA. In

contrast to the DMAA treatment, the shoot/root dry weight ratio was not significantly affected by an increase in As(V) application. However, the highest level (0.8 mg As L^{-1}) of As(V) reduced the shoot/root dry weight ratio as compared to the control. When As(III) was absorbed, the reduction in shoot/root dry weight ratio was significant at concentrations of 0.2, and 0.8 mg L^{-1}. Monomethyl arsenic acid application caused a decrease in the shoot/root dry weight ratio at any rate of applied As.

Tissue arsenic concentration

The amount of As taken up by the rice plants followed the trend: DMAA < As(V) < MMAA < As(III), regardless of the rate of addition (Table 2.2). Once the As compounds were present in the root, a differential preference was exhibited for translocation to the shoot. On a dry weight basis, the root contained the highest mean As concentration when As was applied as As(III), As(V), or MMAA. The data on root As concentration were hard to interpret since all two- and three-way interactions were significant.

Shoot As concentration was not influenced by cultivar (Table 2.2). Arsenic concentrations in the shoot increased significantly with increasing As levels in the nutrient solution. At equal As concentrations in solution, the amount of accumulated As in shoot tissue was highly dependent on the chemical form of As present (Fig. 2.3). The As concentrations in shoots from control plants were below our detection limit. The As concentration ratio (shoot As concentration/root As concentration) was not different between cultivars, but was significantly influenced by both As chemical form and As concentration in the nutrient solution.

Table 2.2: Effect of arsenic concentration and chemical form on tissue arsenic concentration of rice.

Factor	Tissue arsenic		As conc. Ratio
	Shoot	Root	
	mg kg^{-1}		
Variety			
Lemont	13.38 a[a]	78.17 b	0.61 a
Mercury	14.71 a	105.42 a	0.53 a
Arsenic Form			
As(III)	21.50 a	192.42 a	0.18 b
As(V)	11.58 c	102.17 b	0.20 b
mmAs	16.58 b	70.67 c	0.18 b
dmAs	6.50 d	1.92 d	3.33 a
Arsenic Rate			
0.05	1.75 c	10.50 c	0.36 ab
0.2	8.12 b	52.19 b	0.16 b
0.8	32.25 a	212.69 a	1.05 a
As form X As Rate			
Control[b]	0.00	0.00	-
AsIII 0.05	3.00	11.25	0.29
AsIII 0.2	13.50	99.50	0.14
AsIII 0.8	48.00	466.50	0.11
AsV 0.05	2.00	6.50	0.32
AsV 0.2	7.50	51.75	0.16
AsV 0.8	25.25	248.25	0.11
mmAs 0.05	1.50	24.00	0.06
mmAs 0.2	9.00	57.50	0.16
mmAs 0.8	39.25	130.50	0.31
dmAs 0.05	0.50	0.25	2.00
dmAs 0.2	2.50	0.00	-
dmAs 0.8	16.50	5.50	3.67
F test for interactions			
Var x As form	NS[c]	**	NS
Var x As rate	NS	**	NS
As form x As rate	**	**	NS
Var x AsForm x AsRate	NS	*	NS

a Values followed by the same letter are not significantly different ($p<0.05$), Duncan Multiple Range Test.

b Control values were not included in the Analysis of Variance.

c NS=non significant F ratio ($p<0.05$), * significant at $p<0.05$, ** significant at $p<0.01$.

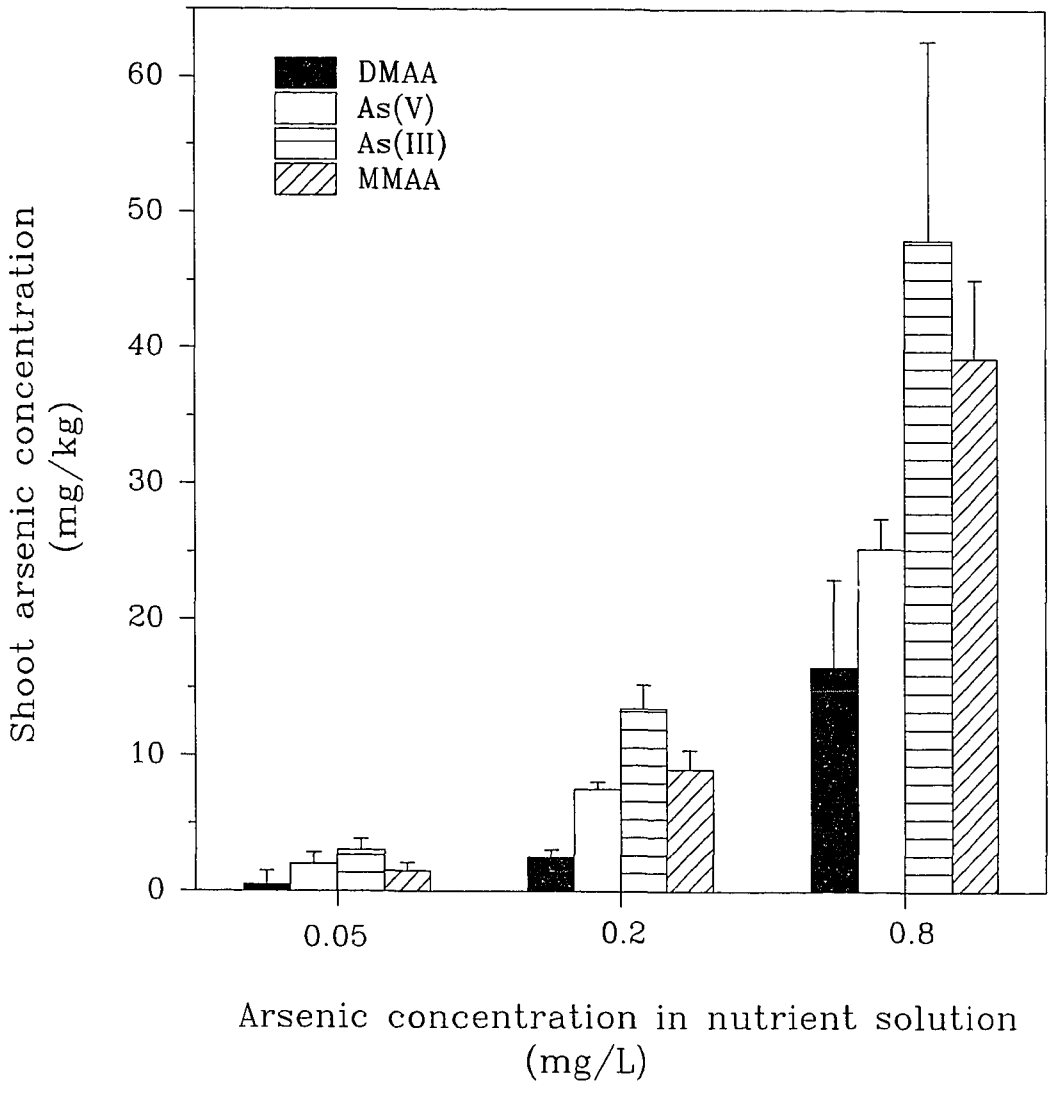

Figure 2.3: Shoot arsenic concentration as affected by As concentration and chemical form.

The data on tissue As concentration and tissue As concentration ratio indicate that the chemical form under which As is present not only determines the phytoavailability of As to rice but also determines the mobility or translocation of As in the plant. When DMAA was absorbed, most As was translocated to the shoot (Table 2.2). Seedlings grown in a nutrient solution containing DMAA at a level of 0.8 mg As L^{-1} concentrated up to 3.7 times more As in the shoot than in the root. For the other chemical As forms, plants accumulated As in the roots and only minor amounts of As were translocated to the shoot. As the As(III) and As(V) concentrations in the nutrient solution increased, the tissue As concentration ratio decreased, this due to As accumulation in the roots. In the MMAA treatments, however, As was translocated to the shoot upon increased As application (Table 2.2). For the 0.8 mg As(MMAA) L^{-1} treatment, the tissue As concentration ratio was 0.35 and 0.27 for the Lemont and Mercury cultivar, respectively. Although less As was absorbed than with As(III), MMAA was more phytotoxic to rice (Table 2.2). The higher degree of MMAA translocation in the plant probably contributed to the observed larger intrinsic toxicity of MMAA.

The results presented demonstrate that the concentration and chemical form of As applied had significant effects on the dry matter production and on As uptake and translocation by rice plants. Residual arsenicals have shown to damage rice with symptoms similar to straighthead (Gilmour and Wells, 1980; Marin, 1989; Wells and Gilmour, 1977). However, As levels causing straighthead disease appear to be much lower than those causing a decrease in vegetative growth. Because vegetative growth is not affected by straighthead disease (Wells and Gilmour, 1977), it is impossible to

identify any straighthead symptoms before emergence of the panicles (Atkins, 1974). The differential absorption and translocation of As chemical forms observed in this experiment, could be important to As levels leading to straighthead development.

A major portion of the rice acreage in the southern United States was previously cropped to cotton where the use of MMAA (as monosodium methane arsenate or disodium methane-arsenate) as herbicides was a common practice. We clearly demonstrated that MMAA is the most phytotoxic arsenical to rice. Monomethyl arsenic acid was readily translocated to the shoot, thereby increasing its possibility of affecting rice yield. Furthermore, straighthead is reported to be only a problem in flooded rice cultivation (Atkins, 1974, 1975; Reed and Sturgis, 1936). One reason for the high availability of As to flooded rice is thought to be related to the increased solubility of As upon flooding (reduction) of the soil. In a recent field study, Marin (1989) illustrated that application of As (as monosodium methanearsenate) under flooded conditions increased straighthead severity. However, when the soil was drained mid-season there was no straighthead development regardless of As addition. These and similar (Wells and Gilmour, 1977) findings suggest that chemical changes in the soil, due to flooding and drainage, greatly affect As chemistry and thereby its availability for plant uptake. Recent studies in our laboratory (Masscheleyn et al., 1991a) have demonstrated that the reduction of As(V) to As(III), upon flooding of a soil, leads to an increased solubility of As. The data reported here show that As(III) is the form most readily accumulated by rice. Draining and drying the soil midseason, a common straighthead control practice, will result in all inorganic As being oxidized to As(V),

a non-phytotoxic As form. Although no information is currently available on the redox stability of methylated arsenicals, it would not be surprising that MMAA and DMAA, in which As is trivalent and monovalent, respectively, are also being oxidized to As(V). In view of these considerations, it is very likely that changes in the chemical form of soil-As are directly responsible for the straighthead disorder observed in rice and attributed to As.

Arsenic treatments were applied to two rice varieties, Lemont and Mercury, a cultivar moderately tolerant and very susceptible to straighthead, respectively. Root dry weight production in Lemont was higher than in Mercury (Table 2.1). The higher root production possibly increased the root-holding capacity for As, thereby limiting As translocation to the aboveground plant parts. Mercury accumulated more As than Lemont in both roots and shoots (Table 2.2), regardless of As form or rate applied. However, differences in As shoot concentrations between the two cultivars were not statistically significant. Significant two- and three-way interactions between treatments made it impossible to interpret the observed differences in As root concentrations. It is interesting to note that, with increasing MMAA application, the shoot/root As concentration ratio was higher for Mercury than for Lemont. Differences in both root dry weight production and As uptake and translocation could contribute to the difference in As tolerance.

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CHAPTER 3

THE INFLUENCE OF CHEMICAL FORM AND CONCENTRATION OF ARSENIC ON THE ABSORPTION OF SELECTED PLANT NUTRIENTS BY RICE PLANTS.

Introduction

Arsenic (As) is an ubiquitous element in nature, and it is present in almost all living organisms. However, it is not known to be an essential mineral nutrient for plants (Liebig, 1966; Walsh and Keeney, 1975). Instead, As has been recognized as a phytotoxic element, and rice has been mentioned among the most sensitive to As toxicity (Reed and Sturgis, 1936; Schweizer, 1967). The effect of any element on a plant depends not only on its chemical properties, but also on its concentration and the presence and concentration of other elements (Mengel and Kirkby, 1987).

Arsenic in soil-water environments can be present at least in four different chemical forms: arsenate [As(V)], arsenite [As(III)], monomethyl arsenic acid (MMAA), and dimethyl arsenic acid (DMAA). In soils, As is subject to chemically and/or microbially mediated oxidation-reduction (Brannon and Patrick, 1987; Masscheleyn et al., 1991a), methylation (Brannon and Patrick, 1987; Onken et al., 1987) and demethylation (Dickens and Hiltbold, 1967) reactions. Both As solubility (Masscheleyn et al., 1991a) and toxicity to animals and humans (Ferguson and Gavis, 1972) depend on its chemical form. For plants, As(III) has been shown to be more phytotoxic than As(V) (Clements and Munson, 1947; Tsusumi and Takahashi, 1974).

Plant growth depends on many interacting factors such as nutrient supply, rate of nutrient absorption, distribution of the nutrient to functional sites, and nutrient mobility within the plant (Olsen, 1972). Interactions may occur between micronutrients, macronutrients and toxic elements, modifying the nutrient supply to the plant. Such interactions may take place in the soil or within the plant. The chemical similarity between the As and P molecule, and the replacement of P by As in essential molecules has been mentioned as a possible mechanism for As phytotoxicity (National Academy of Sciences, 1977). Most of previous work on the As-P interaction has been done with inorganic arsenical forms only: arsenate, or arsenate and arsenite. As a result, several researchers have reported As toxicity to be a function of P concentration in the culture solution (Hurd-Karrer,1939; Woolson et al., 1973). Data on the interaction of P with organoarsenicals is lacking. The mechanism of phytotoxicity of organoarsenicals is not known (Hiltbold, 1975), and the phytotoxicity of organoarsenicals has not been tested experimentally by application to roots. Methylation of As has been shown to decrease its toxicity (Peoples, 1975). The antagonistic action of As on Zn absorption was also mentioned in regard to peach (Thompson and Batjer, 1950; Batjer and Benson, 1958) and rice (Oh and Sedberry, 1974; Marin, 1989) plants.

When the rate of plant growth exceeds the rate of uptake of a particular nutrient due to changes in environmental conditions, the concentration of that nutrient in the tissue decreases or is 'diluted' in the plant tissue (Olsen, 1972). When the inverse situation occurs, a 'concentration effect' takes place (Aldrich, 1973; Martin and Matocha, 1973). Toxic materials are among the environmental conditions capable of

altering plant growth to cause 'concentration' or 'dilution' effects. In situations where concentration or dilution effects are suspected, Jarrel and Beverly (1981) recommended analyzing the results considering concentration and total uptake.

In a previous report (Marin et al., 1992) we have shown that for rice plants, the toxicity, absorption and mobility within the plant of the As molecule is dependent mainly on its chemical form. However, very little is known about the influence of As, especially different As chemical forms, on the absorption and uptake of other plant nutrients. The objectives of this paper are to determine the influence of chemical form and rate of As on tissue concentration, uptake and distribution of other nutrients, such as P, K, Ca, Mg, Na, S, Zn and Cu in rice plants.

Materials and Methods

Rice (*Oryza sativa* L.) was grown in a nutrient solution containing different chemical forms and concentrations of As. The factorial treatments (4 x 3 x 2) were applied in four replicates of a complete randomized design. The treatments consisted of four chemical forms of As [As(V), As(III), MMAA, and DMAA] with three As concentrations (0.05, 0.20, and 0.80 mg As L^{-1}), and two rice cultivars (Lemont, and Mercury). Two controls, one for each cultivar, with no added As were also included. The chemical forms of As were added as their sodium salts. Lemont, an early maturing, semidwarf, long-grain cultivar, and Mercury, an early maturing, semidwarf, medium-grain cultivar are considered to be moderately tolerant (Bollich et al., 1985) and very susceptible to As toxicity (McKenzie et al., 1988), respectively.

Seeds were germinated in sterilized sand. Eight days after germination, uniform seedlings from each variety were selected. The sand was washed from the root system with distilled-deionized water, and seedlings transferred to 2.5-L plastic pots containing 2 L of nutrient solution. A single pot, representing a specific As form - As concentration treatment, contained 4 seedlings from both cultivars, and constituted one replication. The basal nutrient solution (Yoshida et al., 1976) contained: 40 mg L^{-1} of N, K, Ca and Mg; 10 mg P L^{-1}; 2 mg Fe L^{-1}; 0.5 mg Mn L^{-1}; 0.05 mg Mo L^{-1}; 0.2 mg B L^{-1}; and 0.01 mg L^{-1} of Zn and Cu. The pH of the nutrient solution was adjusted to 4.0 $\pm$ 0.2 in order to avoid iron deficiency (Judsujinda, 1976). Seedlings were passed through holes in a styrofoam plate (12-cm diameter and 4-mm thick) floating on the nutrient solution. The plants were grown in the laboratory (26$\pm$2°C) and received continuous illumination from a set of fluorescent tubes and 100 Watt flood lamps situated 50 cm above the plants.

After 4 days of acclimatization, plants were subjected to the different As treatments. The As in solution was analyzed regularly using a hydride generation atomic absorption technique (Masscheleyn et al., 1991b) to verify that the chemical form of the added As did not change over time. Arsenic forms were found to be stable with respect to oxidation/reduction and methylation/demethylation reactions for a period of 2 days. Thus, the nutrient solutions containing specific As forms were replaced every other day in order to maintain the desired treatments.

Plants were grown for 4 weeks, and then harvested. Roots were washed with tap water, rinsed with a 0.1 *M* HCl solution followed by 3 rinses with distilled

deionized water. Roots and shoots were separated and dried at 65°C for 72 hr. Samples were ground in a stainless-steel Wiley mill and passed through a 20-mesh sieve. Due to the small sample size obtained for several treatment combinations, samples of two replications were combined for tissue analysis prior to digestion. Plant tissue samples (0.5 g) were digested with 5 mL conc. HNO_3 (AR Select, Mallinckrodt Inc.) for 4 h at 130°C. Digested samples were filtered (Whatman #42) and diluted with distilled deionized water to 50 mL. Phosphorous, K, Ca, Mg, Na, S, Zn, Cu, Fe, Mn and B in the extracts were determined with a Jarrel Ash (Atom Comp 800 series) ICP. Acid blanks were analyzed in order to assess possible As contamination.

Statistical analyses were performed using the PROC GLM procedure available in SAS (SAS Institute Inc., Cary, NC. 1987).

Results and Discussion

Chemical forms and rates of As did not influence the tissue concentration of B, Fe and Mn, either in shoot or in root. However, they did significantly influence the concentrations (in shoot and root) of P, K, Ca, Mg, Na, S, Zn and Cu. In the case of Fe, we suspect that sample contamination with rust during the milling process may have hidden some kind of response. Data on elemental uptake (concentration times dry matter) are not presented here, but are discussed when it is convenient. Interested readers can calculate them using the dry weight data in the previous paper (Marin et al., 1992).

Effect on phosphorus concentration and uptake

Shoot P concentration was affected by cultivar and by chemical form of applied As. Lemont contained significantly higher concentrations of P in the shoot than did Mercury. Phosphorus concentrations in shoot and root did not differ significantly ($p<0.05$) considering the rate of applied As as a main effect (Table 3.1). However, uptake data shows a decrease in P uptake as the rate of applied As increased for all chemical forms of As (Fig. 3.1).

When As(V) was applied, the concentration of P in shoot decreased as the rate of applied As increased (Table 3.1). The application of As(III) did not show a clear trend on shoot tissue P. Due to their chemical similarity As and P molecules compete in nutrient or soil solutions (Woolson et al., 1973; National Academy of Sciences, 1977). Wallace et al. (1980) reported a decrease in P uptake in leaves, stems and roots of beans plants following an application of arsenate (As (V)). Benson et al. (1981) found that arsenate and phosphate are absorbed by identical carrier mechanisms in most cells. Assuming the As-P interaction works in a similar way when the concentration of either one is changed in the nutrient solution, we can compare our results with previous work. Increased P level in solution reduced the absorption of pentavalent As by wheat (Hurd-Karrer, 1939), bean, sudan-grass, tomato (Clements and Munson, 1947), oats (Rumburg et al., 1960), and alfalfa plants (Khattak et al., 1991). However, P had little, if any, effect on the absorption of trivalent As (Clements and Munson, 1947).

The As chemical form that produced the highest concentration of P in shoot and root was MMAA, yet shoot P concentration increased as rate of applied As increased

Table 3.1: Effect of arsenic concentration and chemical form on concentration of P and K in shoots and roots of rice plants.

Factor	Tissue P Shoot	Tissue P Root	P Conc Ratio	Tissue K Shoot	Tissue K Root	K Conc Ratio
	—— mg kg^{-1} ——			—— mg kg^{-1} ——		
Variety						
Lemont	13710 a[a]	5891 a	2.4 a	26110 a	6654 a	4.3 a
Mercury	10650 b	5849 a	1.9 b	24546 b	6752 a	4.0 a
Arsenic Form						
As(III)	11944 b	5380 b	2.3 a	26803 a	6115 bc	5.0 a
As(V)	10547 b	5294 b	2.0 b	26707 a	6934 b	4.0 b
mmAs	15300 a	6555 a	2.4 a	20503 b	5294 c	4.4 ab
dmAs	10930 b	6251 ab	1.8 b	27302 a	8470 a	3.3 c
Arsenic Rate						
0.05	12100 a	6317 a	1.9 b	27416 a	8471 a	3.3 c
0.2	12181 a	5820 a	2.1 ab	25754 b	6381 b	4.2 b
0.8	12260 a	5472 a	2.3 a	22815 c	5257 c	5.0 a
As form X As Rate						
Control[b]	11324	6356	1.8	28744	7756	3.7
AsIII 0.05	11963	6669	1.8	28918	8807	3.4
AsIII 0.2	11028	5345	2.1	27276	5934	4.6
AsIII 0.8	12841	4126	3.1	24213	3603	7.0
AsV 0.05	11535	5669	2.0	28204	8376	3.4
AsV 0.2	10719	5090	2.2	27206	6866	4.1
AsV 0.8	9387	5121	1.8	24710	5562	4.6
mmAs 0.05	13193	6888	1.9	24452	8339	3.0
mmAs 0.2	16302	6590	2.5	20731	4610	4.5
mmAs 0.8	16407	6188	2.7	16324	2934	5.6
dmAs 0.05	11709	6044	2.0	28090	8364	3.4
dmAs 0.2	10676	6256	1.8	27804	8116	3.5
dmAs 0.8	10404	6454	1.6	26013	8930	2.9
F test for interactions						
Var x As form	NS[c]	NS	NS	*	NS	NS
Var x As rate	NS	NS	NS	NS	NS	NS
As form x As rate	*	NS	**	NS	**	**
Var x AsForm x AsRate	NS	NS	NS	NS	NS	NS

a Values followed by the same letter in a column are not significantly different ($p<0.05$), Duncan Multiple Range Test.

b Control values were not included in the Analysis of Variance.

c NS=non significant F ratio ($p<0.05$), * significant at $p<0.05$. ** significant at $p<0.01$.

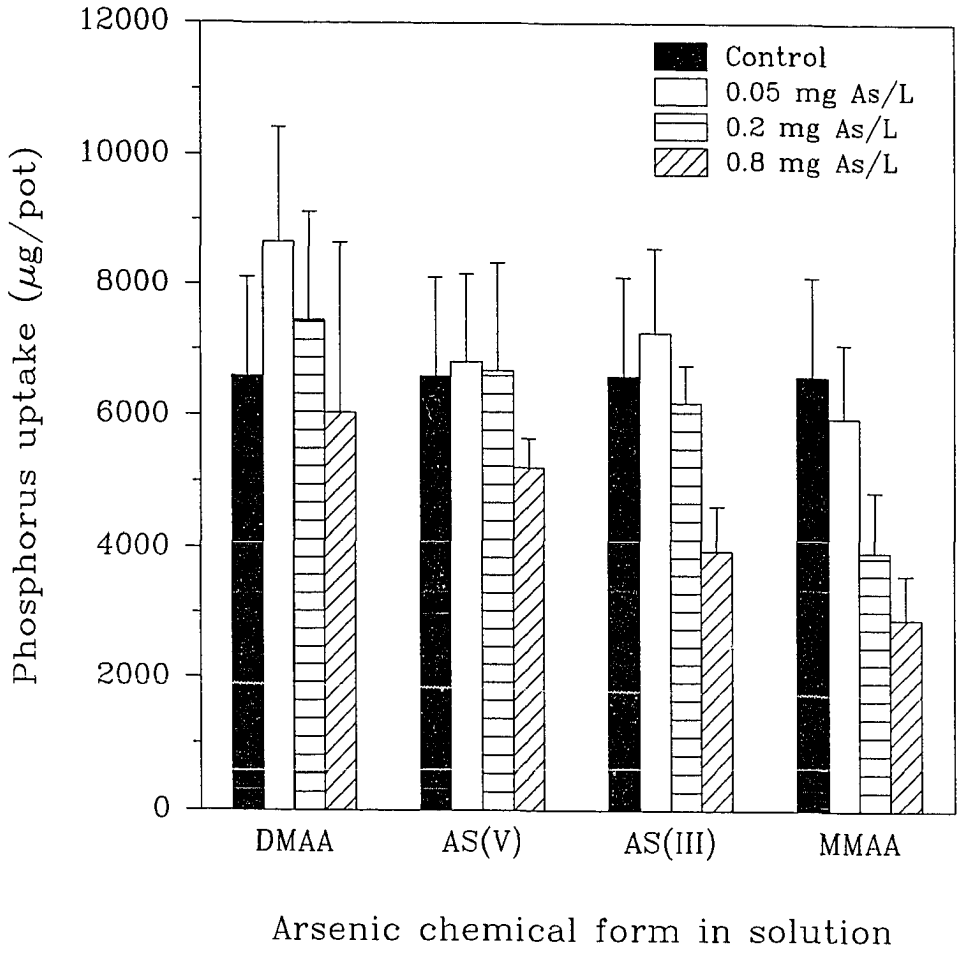

Figure 3.1: Total P uptake as affected by As concentration and chemical form in rice plants.

(Table 3.1). However, since MMAA was the As chemical form most phytotoxic to rice (Marin et al., 1992), the increase in concentration of P through application of MMAA was a 'concentration effect' due to depressed growth. When As was applied as MMAA, P uptake was significantly lower, and decreased as the rate of As increased (Fig. 3.1).

When rates of applied As as DMAA increased, shoot P concentration decreased slightly, while root P concentration increased (Table 3.1). Uptake of P increased at the lower As rate in relation to the control (Fig. 3.1), due to an increase in dry matter production (Marin et al., 1992).

The P concentration ratio (shoot P concentration/root P concentration) was significantly higher for Lemont than for Mercury (Table 3.1), indicating a relatively greater concentration of P in the shoot by Lemont than by Mercury. The trend of P concentration ratio was dependent on the As form applied. For As(III) and MMAA the P concentration ratio increased as the rate of As increased, while for DMAA the trend was the reverse.

Several researchers have reported As toxicity to be a function of P concentration in the culture solution. Hurd-Karrer (1939) mentioned a P:As ratio greater than 4:1 to reduce As toxicity. Other investigators have demonstrated similar results with bean, sudan grass, tomato (Clements and Munson, 1947), and oat plants (Rumburg et al., 1960). Woolson et al. (1973) observed a reduction of As toxicity in soil at P:As ratios of 0.7:1 to 42.5:1. However, our results suggest chemical form of As to be the most important factor. For example, when As was applied as MMAA it was toxic at any rate of As. For the intermediate rate (0.2 mg As L^{-1}) the P:As ratio was 200:1 (40 mg

P L^{-1}:0.2 mg As L^{-1}). In contrast, when As was applied as DMAA at the maximum rate, the P:As ratio was 50:1 (40 mg P L^{-1}:0.8 mg As L^{-1}), and there was no toxic effect. Probably, the control of As toxicity by a certain P:As ratio in solution is valid when considering arsenate, but not for other As chemical forms.

Although, As influences and interferes with the absorption and mobility of P in the plant, its toxic effect cannot be explained by a P deficiency. In all treatments considered the tissue P content was above the critical level of P for rice plants (Yoshida, 1981; Sedberry et al., 1987).

Effect on potassium

Concentrations of K in the shoot were significantly higher for Lemont than for Mercury (Table 3.1). Potassium concentrations in shoots were also significantly influenced by chemical form of applied As. Monomethyl arsenic acid caused the lowest, while DMAA resulted in the highest concentration of K in both shoot and root. As the rate of applied As increased, concentration of K in shoot and root was significantly reduced regardless of the chemical form (Table 3.1). Data for K uptake confirmed all those results, indicating a true decrease in K content as rate of applied As increased. The decrease was most notable when MMAA was applied. Wallace et al. (1980) working with culture solution, reported depression of K in roots due to increase in arsenate (As V) rate in bush beans plants. However, the decrease in K absorption could be the result of competition with Na in the solution, since the arsenicals were added as its Na salts.

The K concentration ratio changed depending upon the As rate and the As chemical form applied (Table 3.1). For As(III), As(V) and MMAA, the K concentration ratio increased consistently as the rate of applied As increased. When DMAA was used, the ratio was not only lower than the control but also decreased for the higher rate of As, indicating an accumulation of K in the root.

Effect on calcium

The effect of As rate on shoot tissue Ca differed depending on the chemical form applied (Table 3.2). Arsenic applied as MMAA produced a significant increase in Ca concentration in shoots, however it was a 'concentration effect', since shoot uptake of Ca steadily decreased. Marin (1989) found MMAA application to soil decreased the concentration of Ca in the Y-leaf of rice, with no 'concentration effect', since reduction in growth was not apparent.

Wallace et al. (1980) reported a decrease in Ca in all parts of bean plants due to the application of arsenate (As (V)). Our data does not show consistent changes in shoot and root tissue Ca when As(III), As(V) or DMAA were applied. However, it is worth noting that the shoot Ca concentration was higher than the control for all treatments except for DMAA at the highest As rate.

Data for Ca uptake in shoot and root show a significant decrease in Ca uptake as the rate of applied As increased. The Ca concentration ratio was significantly higher for Lemont than for Mercury, indicating a greater accumulation of Ca in the shoot in Lemont cultivar.

Table 3.2: Effect of arsenic concentration and chemical form on concentration of Ca and Mg in shoots and roots of rice plants.

Factor	Tissue Ca Shoot	Tissue Ca Root	Ca Conc Ratio	Tissue Mg Shoot	Tissue Mg Root	Mg Conc Ratio
	— mg kg^{-1} —			— mg kg^{-1} —		
Variety						
Lemont	1942 a[a]	366 a	5.4 a	5097 a	1110 a	5.0 a
Mercury	1828 a	441 a	4.4 b	4062 b	1005 b	4.7 a
Arsenic Form						
As(III)	1731 b	350 b	5.0 a	4176 b	1007 b	4.5 b
As(V)	1701 b	355 b	5.0 a	4166 b	1171 a	3.6 c
mmAs	2496 a	507 a	5.6 a	5568 a	830 c	7.6 a
dmAs	1611 b	403 ab	4.1 b	4408 b	1220 a	3.6 c
Arsenic Rate						
0.05	1803 a	396 a	4.9 a	4628 a	1255 a	3.7 c
0.2	1918 a	387 a	5.1 a	4620 a	1074 b	4.6 b
0.8	1934 a	428 a	4.8 a	4491 a	843 c	6.2 a
As form X As Rate						
Control[b]	1592	348	4.9	4308	1249	3.5
AsIII 0.05	1745	329	5.3	4453	1327	3.4
AsIII 0.2	1616	339	4.8	4103	1073	3.8
AsIII 0.8	1831	381	4.8	3972	622	6.4
AsV 0.05	1642	361	4.7	4346	1374	3.2
AsV 0.2	1747	317	5.7	4214	1187	3.6
AsV 0.8	1716	388	4.4	3938	952	4.1
mmAs 0.05	2077	466	5.3	5108	1108	4.6
mmAs 0.2	2756	458	5.5	5840	841	7.2
mmAs 0.8	2656	596	6.1	5755	542	11.0
dmAs 0.05	1748	429	4.1	4603	1211	3.8
dmAs 0.2	1552	434	3.7	4325	1195	3.6
dmAs 0.8	1533	347	4.5	4298	1255	3.4
F test for interactions						
Var x As form	NS[c]	NS	*	NS	NS	NS
Var x As rate	NS	NS	NS	*	NS	NS
As form x As rate	**	NS	NS	NS	**	**
Var x AsForm x AsRate	NS	NS	*	NS	NS	NS

a Values followed by the same letter in a column are not significantly different ($p<0.05$), Duncan Multiple Range Test.

b Control values were not included in the Analysis of Variance.

c NS=non significant F ratio ($p<0.05$), * significant at $p<0.05$. ** significant at $p<0.01$.

Effect on magnesium

Lemont cultivar had significantly higher tissue concentrations and total uptake of Mg in both shoot and root than did Mercury (Table 3.2). The application of As as MMAA resulted in higher Mg concentrations in shoot due to a 'concentration effect'. For the other As chemical forms, the increase in As rate produced a steady, but not statistically significant decrease in shoot tissue Mg. In roots, the decrease in Mg concentration and uptake was consistent for all chemical forms of applied As, except DMAA, which did not show any change. Wallace et al. (1980) reported similar results in bean plants due to application of arsenate.

The Mg concentration ratio showed a consistent increase as rate of applied As increased for As(III), As(V) and MMAA, indicating greater accumulation of Mg in shoot as the rate of applied As increases. For DMAA the trend was reversed with very small changes.

Effect on sodium

Concentration of Na in shoot was not affected by cultivar. In the root, tissue Na was significantly higher in Mercury than in Lemont (Table 3.3). The concentration of Na in shoot was significantly increased as rate of applied As increased, when As(III), MMAA and DMAA were applied. That increase was not surprising since As compounds were applied as their Na salts. The trend was exactly the opposite in the root, with higher rates of applied As resulting in lower Na concentration. Those results for tissue Na in root, were confirmed by uptake data, which followed similar trend.

Table 3.3: Effect of arsenic concentration and chemical form on concentration of Na and S in shoots and roots of rice plants.

Factor		Tissue Na Shoot	Tissue Na Root	Na Conc Ratio	Tissue S Shoot	Tissue S Root	S Conc Ratio
Variety		—— mg kg^{-1} ——			—— mg kg^{-1} ——		
Lemont		3483 a[a]	1680 b	2.4 a	7643 a	3189 b	2.4 a
Mercury		3649 a	2450 a	1.7 b	6273 b	3767 a	1.7 b
Arsenic Form							
As(III)		3220 b	1988 bc	1.9 b	7125 b	3711 a	1.9 b
As(V)		2704 b	2194 ab	1.3 c	5900 c	3670 a	1.6 c
mmAs		5560 a	1701 c	3.8 a	8870 a	3105 c	2.9 a
dmAs		2780 b	2379 a	1.3 c	5938 c	3427 b	1.7 bc
Arsenic Rate							
0.05		2599 c	2290 a	1.2 c	6165 b	3562 a	1.8 c
0.2		3408 b	2074 ab	1.9 b	6766 b	3426 a	2.0 b
0.8		4691 a	1832 b	3.1 a	7944 a	3447 a	2.4 a
As form X As Rate							
Control[b]		3335	2591	1.3	5910	3533	1.7
AsIII	0.05	1975	2239	0.9	5944	3666	1.6
AsIII	0.2	2692	2157	1.3	6115	3560	1.8
AsIII	0.8	4994	1568	3.5	9316	3907	2.4
AsV	0.05	2587	2367	1.1	5920	3673	1.6
AsV	0.2	2434	2021	1.2	5884	3565	1.7
AsV	0.8	3091	2194	1.4	5896	3774	1.6
mmAs	0.05	3575	2114	1.8	6891	3470	2.0
mmAs	0.2	5859	1579	4.0	9623	3160	3.0
mmAs	0.8	7246	1410	5.5	10096	2684	3.8
dmAs	0.05	2260	2442	0.9	5905	3437	1.7
dmAs	0.2	2647	2537	1.1	5443	3421	1.6
dmAs	0.8	3433	2158	1.8	6467	3424	1.9
F test for interactions							
Var x As form		NS[c]	NS	NS	NS	*	NS
Var x As rate		NS	NS	NS	NS	NS	NS
As form x As rate		**	NS	**	**	*	**
Var x AsForm x AsRate		NS	NS	NS	NS	NS	NS

a Values followed by the same letter in a column are not significantly different ($p<0.05$), Duncan Multiple Range Test.

b Control values were not included in the Analysis of Variance.

c NS=non significant F ratio ($p<0.05$), * significant at $p<0.05$. ** significant at $p<0.01$.

When As(V) was applied Na concentration in shoot and root did not follow any particular pattern.

The greater concentration of Na in root by Mercury cultivar resulted in a lower Na concentration ratio for Mercury than for Lemont. The Na concentration ratio also increased as the rate of applied As increased for all the As forms applied, which indicate that increased As rates resulted in more Na stored in the shoot than in the root (Table 3.3).

Effect on sulfur

Lemont cultivar showed significantly higher content of S in shoot than Mercury. The opposite was the case in root, where Mercury showed higher concentration of S (Table 3.3). The chemical form of applied As influenced shoot tissue S in different ways. When As(III) or MMAA were applied, S concentration in shoot increased as rate of applied As increased, due to a 'concentration effect', since a decrease in S uptake was noted due to As application. In roots, the trend was the opposite for MMAA, and was not consistent for As(III). Applications of As(V) and DMAA did not show consistent influence on shoot and root concentration of S. Epps and Sturgis (1939), found that additions of S to soils decreased the amount of soluble As in soil and decrease the intake of As by the rice plants.

The S concentration ratio was significantly higher for Lemont than for Mercury, and it increased consistently as rate of applied As increased when As(III) and MMAA were used. When As(V) or DMAA were applied the S concentration ratio did not change.

Effect on zinc

Shoot tissue Zn was significantly affected by chemical form of applied As, with the two most toxic forms of As to rice plants (MMAA and As(III)), causing significantly lower concentration of Zn (Table 3.4). In roots, the application of MMAA resulted in significantly higher concentration of Zn, but there was no change for As(III). Concentration of Zn in shoot and root did not show major changes when As(V) or DMAA were applied.

Data in Table 3.4 shows that for all rates and chemical forms of As, the concentration of Zn in shoots was far below the concentration in the control (48.2 mg kg^{-1}). This appears to confirm the antagonism between As and Zn mentioned by other researchers (Thompson and Batjer, 1950; Batjer and Benson, 1958; Oh and Sedberry, 1974; Marin, 1989). Uptake data (Fig. 3.2) corroborate that the values for concentration represent true decreases in Zn intake due to As application. Oh and Sedberry (1974) reported soil application of As (as arsenite) to reduce Zn uptake by rice plants. Marin (1989) also found As to decrease Zn concentration in Y-leaves of rice after application of monosodium methanearsonate (MSMA), a MMAA derivative, in two field experiments. In roots, there was not much difference except when MMAA was applied.

The Zn concentration ratio was significantly lower for MMAA treatments than for the other As chemical forms, evidencing that a very small proportion of the Zn was translocated to the shoot (Table 3.4). That means, As (when applied as MMAA), inhibited the movement of Zn to the shoot. The high concentration of Zn in the root

Table 3.4: Effect of arsenic concentration and chemical form on concentration of Zn and Cu in shoots and roots of rice plants.

Factor	Tissue Zn Shoot	Tissue Zn Root	Zn Conc Ratio	Tissue Cu Shoot	Tissue Cu Root	Cu Conc Ratio
	—— mg kg^{-1} ——			—— mg kg^{-1} ——		
Variety						
Lemont	21.21 a[a]	17.33 a	1.56 a	20.83 a	66.29 a	0.32 b
Mercury	23.58 a	18.62 a	1.63 a	19.38 b	54.62 b	0.37 a
Arsenic Form						
As(III)	23.17 b	12.17 b	1.92 a	18.83 b	51.25 b	0.38ab
As(V)	29.17 a	15.00 b	2.03 a	20.33 b	59.75 b	0.34 b
mmAs	11.67 c	31.75 a	0.40 b	17.92 c	72.00 a	0.26 c
dmAs	25.58 ab	13.00 b	2.03 a	23.33 a	58.83 b	0.41 a
Arsenic Rate						
0.05	23.75 a	18.06 a	1.77 a	22.31 a	65.00 a	0.35 a
0.2	23.56 a	17.00 a	1.75 a	20.62 b	62.75 a	0.34 a
0.8	19.88 b	18.88 a	1.26 b	17.38 c	53.62 b	0.35 a
As form X As Rate						
Control[b]	48.20	12.50	3.95	23.80	65.00	0.37
AsIII 0.05	28.50	12.50	2.28	22.50	65.50	0.34
AsIII 0.2	24.75	12.00	2.07	19.75	54.25	0.37
AsIII 0.8	16.25	12.00	1.39	14.25	34.00	0.43
AsV 0.05	28.50	12.25	2.35	22.75	66.00	0.35
AsV 0.2	29.75	15.00	2.03	20.75	59.50	0.35
AsV 0.8	29.25	17.75	1.70	17.50	53.75	0.33
mmAs 0.05	14.75	36.25	0.43	20.25	68.50	0.30
mmAs 0.2	10.50	29.00	0.37	18.00	71.75	0.26
mmAs 0.8	9.75	30.00	0.39	15.50	75.75	0.22
dmAs 0.05	23.25	11.25	2.01	23.75	60.00	0.40
dmAs 0.2	29.25	12.00	2.52	24.00	65.50	0.37
dmAs 0.8	24.25	15.75	1.56	22.25	51.00	0.44
F test for interactions						
Var x As form	NS[c]	NS	NS	NS	NS	NS
Var x As rate	**	NS	NS	NS	NS	NS
As form x As rate	NS	*	NS	NS	NS	NS
Var x AsForm x AsRate	NS	NS	NS	NS	NS	NS

a Values followed by the same letter in a column are not significantly different ($p<0.05$), Duncan Multiple Range Test.

b Control values were not included in the Analysis of Variance.

c NS=non significant F ratio ($p<0.05$), * significant at $p<0.05$. ** significant at $p<0.01$.

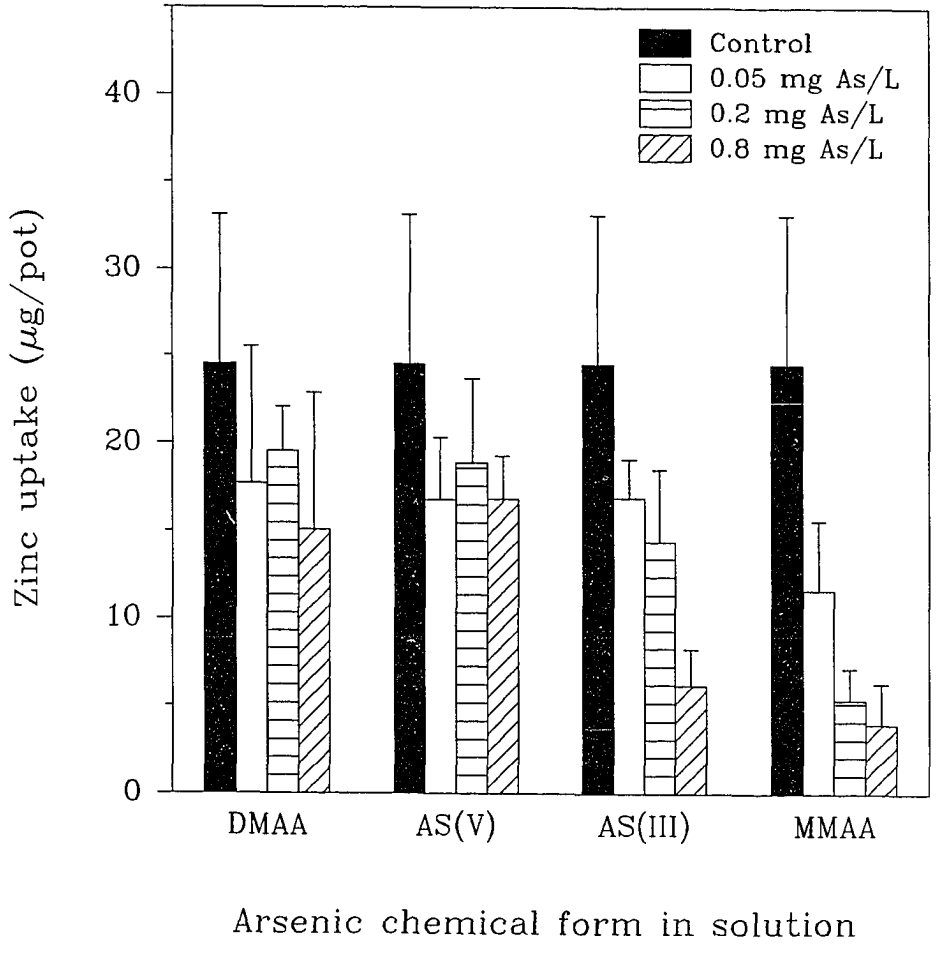

Figure 3.2: Total Zn uptake as affected by As concentration and chemical form in rice plants.

indicate that the lower level of Zn in the aboveground part of the plant was an inhibition in the transport from root to shoot, not a lack of absorption. Calculations of Zn uptake for root and shoot separately (data not shown) confirm this. In spite of the reduction in both shoot and root dry matter production due to MMAA effect, the amount of Zn in the root was the highest, while the amount of Zn in the shoot was the lowest of the experiment for the MMAA treatments. With small differences, the scenario was similar for the As(III) treatment, which was the next most toxic As chemical form.

This differential effect of As chemical form on the movement of Zn into the plant could explain the controversy about the effect of Zn applications in decreasing As toxicity. Thompson and Batjer (1950) reported reduction of As toxicity in peach trees with application of $ZnSO_4$. Batjer and Benson (1958) reported reduction of As toxicity with foliar application of Zn-EDTA. Oh and Sedberry (1974) reported soil application of $ZnSO_4$ to reduce As toxicity in rice. However, Stansel et al. (1978) and Marin (1989) did not get response to Zn application on soils treated with As in rice. In none of these studies was the As chemical form available to (and presumably absorbed by) the plant determined.

The impairing of Zn movement by MMAA, and in a lesser degree by As(III), appears to be just part of a more general physiological process, since the concentration of Zn in the shoot does not appear to be low enough to justify by itself the toxic effect of As. The results suggest that future studies looking for ways to control the toxicity

of As in plants should be done through foliar applications of Zn. However, studies looking for the antagonism As-Zn should be done through soil applications.

Effect on copper

Lemont cultivar showed significantly higher tissue Cu in both shoot and root than Mercury. An increase in the rate of applied As resulted in significantly decreased tissue Cu in both shoots and roots for all chemical forms applied (Table 3.4). Copper concentrations in shoots and roots were dependent on the As chemical form applied. The scenario was quite similar to the one observed for effect of MMAA on Zn absorption and movement within the plant. When MMAA was applied, the concentration of Cu in shoot was lower, in root higher, and the concentration ratio lower than for the other As chemical forms, indicating an impairing in Cu transport to the shoot. However, the differences were no as big as in the case of Zn. Data on Cu uptake (Fig. 3.3) confirmed all the results for concentration.

Applications of As as As(V) or DMAA did not influence tissue Cu concentration. Chisholm (1972) found that lead arsenate (As(V)) did not affect Cu absorption by several crops in two different sandy loam soils.

In summary, although tissue P concentration did not show a very clear trend due to a 'concentration effect', P uptake was decreased by As application regardless of the chemical form applied. The chemical form of As present in solution appears to be more important than the P:As ratio in determining As toxicity. Arsenic was highly toxic at P:As ratio as high as 200:1 when applied as MMAA. However, there was no toxic effect when As was applied as DMAA at a P:As ratio as low as 50:1. Increasing As

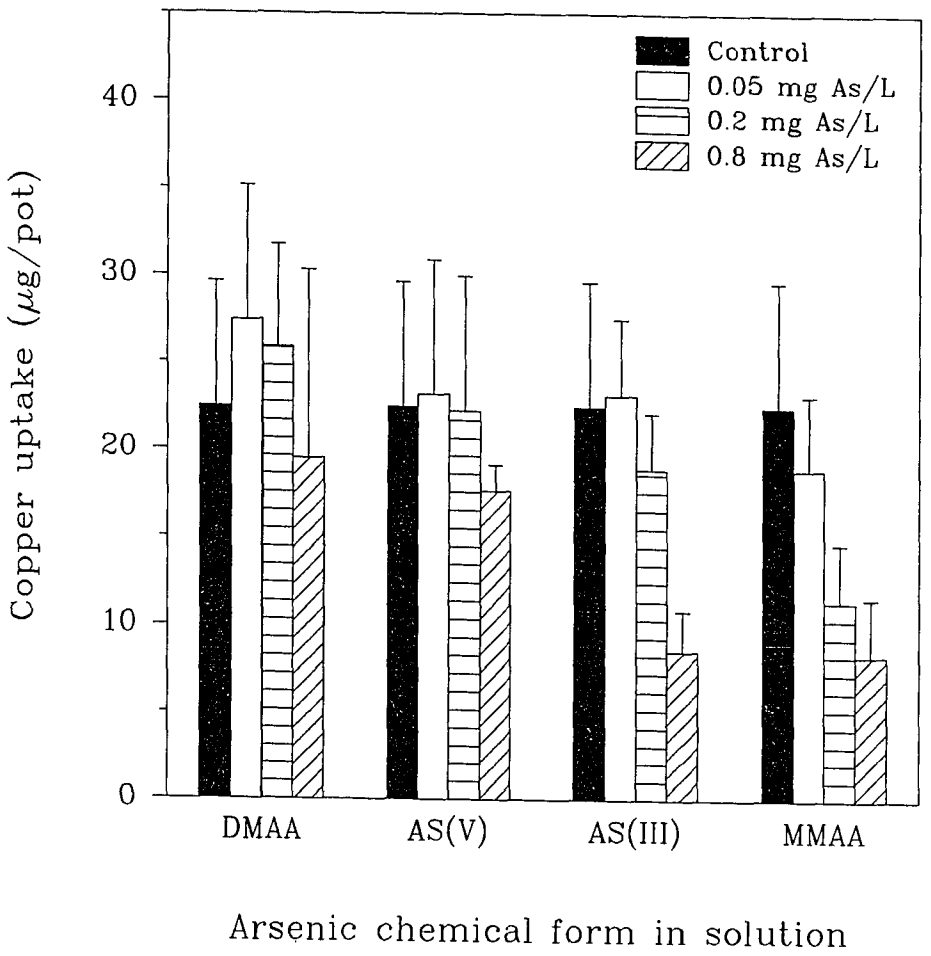

Figure 3.3: Total Cu uptake as affected by As concentration and chemical form in rice plants.

concentration in solution resulted in significant decline in both shoot and root tissue concentration and uptake of K for all As chemical forms applied. Data showed the antagonistic effect of As on Zn uptake regardless of the As chemical form applied. When As was taken up as MMAA it interfered with the translocation of Zn to the aboveground plant part. The same occurred when As(III) was absorbed, although to a smaller degree. Copper movement into the plant was also affected by MMAA in a similar pattern as Zn.

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CHAPTER 4

EFFECT OF DIMETHYLARSENIC ACID (DMAA) ON GROWTH, TISSUE ARSENIC AND PHOTOSYNTHESIS OF RICE PLANTS.

Introduction

Dimethylarsenic acid (herein after DMAA or its synonymous cacodylic acid = CA) is a nonselective, postemergent, foliar contact herbicide (Woolson and Kearney, 1973). It is used to defoliate or desiccate a wide variety of plant species (Ashton and Crafts, 1981).

In the past, indiscriminate use of inorganic arsenicals as pesticides, desiccants and wood preservatives led to pollution of many agricultural soils that reduced their productivity. Thus, research on arsenic (As) toxicity has emphasized its inorganic forms. As a result, the toxic mechanism of arsenite (As(III)) and arsenate (As(V)) is quite well understood. However, in the last 30 years there was a shift away from the inorganic arsenical pesticides to organic herbicides (methanearsonic acid and its salts, and cacodylic acid and its salts) (Woolson, 1983), which are applied at lower rates and have lower toxicity than inorganic arsenicals to animals and humans. Their lower rate of application and lower toxicity compared to inorganic arsenicals have caused organic arsenicals to receive less attention by researchers than their inorganic counterparts.

Very little is known about the toxic effects of organic arsenicals on plant species other than the ones receiving pesticidal application. The mechanism of toxicity

for the organic As species is unknown. The possibility of inhibitory effects of organic arsenicals on photosynthesis was suggested, but never received experimental support (Hance and Holly, 1990). Marin et al. (1992) hypothesized that DMAA may influence the allocation of carbohydrates. Sckerl and Frans (1969), suggested that organic arsenical metabolite may block protein synthesis or some other biosynthetic pathway.

The studies on cacodylic acid (CA) effects on plants have emphasized its herbicidal activity when applied to the foliage, neglecting the possibility of root absorption. However, Braman (1975) pointed out that DMAA may be an ubiquitous As compound found in all soils and may predominate in many. Woolson et al. (1982) found CA to be present in field soils one year after treatment with either arsenite, monosodium methanearsonate (MSMA) or CA. Soil As can undergo a variety of reactions: e. g., oxidation-reduction (Brannon and Patrick, 1987; Masscheleyn et al., 1991a) and methylation-demethylation (Dickens and Hiltbold, 1967; Braman and Foreback, 1973; Brannon and Patrick, 1987; Onken et al., 1987). Thus, the chemical form dominant in the soil will depend upon soil conditions. Under reduced conditions, such as developed in flooded rice fields, organic arsenical compounds may be more stable (Ferguson and Gavis, 1972).

Arsenic uptake by plants is influenced by many factors including plant species (Liebig, 1966; Baker et al., 1976), As concentration in the soil (National Academy of Sciences, 1977), soil properties such as pH and clay content (Dickens and Hiltbold, 1967; Von Endt et al., 1968; Johnson and Hiltbold, 1969), presence of other ions (Rumberg et al. 1960; Woolson et al., 1973; Khattak et al., 1991), and the chemical

form of those As ions (Marin et al., 1992). Recent research has shown that the toxicity of arsenical compounds to rice plants, and the mobility of As ions once taken up by the plant, depend primarily on its chemical form (Marin et al., 1992). Studies comparing the toxicity of different arsenical compounds when applied to the root at relatively low rate have shown DMAA to be the least toxic (Sachs and Michael, 1971; Marin et al., 1992). However, the reaction of plants when exposed to relatively high rate of root applied DMAA has not been investigated.

The objectives of this research were to determine the effect of dimethylarsonic acid (DMAA) applied to the root on photosynthesis and growth of rice plants, and to determine if DMAA affects the allocation of nutrients in rice plants.

Materials and Methods

Rice (*Oryza sativa* L.) plants were grown in nutrient solution containing 0, 0.2, 0.8 and 1.6 mg As L^{-1}, as dimethylarsenic acid (DMAA). The DMAA was added as its sodium salt. The treatments were applied using four replicates in a complete randomized design. Mercury, an early maturing, semidwarf, medium-grain cultivar considered to be very susceptible to As toxicity (McKenzie et al., 1988) was used.

Seeds were germinated in sterilized sand. Eight days after germination, uniform seedlings were selected. The sand was washed from the root system with distilled-deionized water, and seedlings (8 seedlings pot^{-1}) were transferred to 2.5-L plastic pots containing 2 L nutrient solution. The nutrient solution (Yoshida et al., 1976) contained: 40 mg L^{-1} of N, K, Ca and Mg; 10 mg P L^{-1}; 2 mg Fe L^{-1}; 0.5 mg

Mn L^{-1}; 0.05 mg Mo L^{-1}; 0.2 mg B L^{-1}; and 0.01 mg L^{-1} of Zn and Cu. The pH of the nutrient solution was adjusted to 4.0 $\pm$ 0.2 in order to avoid iron deficiency (Judsujinda, 1976). Seedlings were passed through holes in a styrofoam plate (12 cm diameter and 4 mm thick) floating on the nutrient solution. The plants were grown in the laboratory (26 $\pm$ 2 °C), and they received continuous illumination from a set of fluorescent tubes and 100 Watt flood lamps situated 50 cm above the plants.

After four days of acclimatization, the plants were subjected to the different As treatments. The concentration of As in solution was analyzed regularly using a hydride generation technique (Masscheleyn et al., 1991b) to verify that the chemical form of the added As did not change over time. DMAA was found to be stable with respect to oxidation/reduction and methylation/demethylation reactions for a period of 4 days. Thus, the nutrient solutions containing the DMAA treatment were replaced every four days in order to maintain the desired treatments.

Net photosynthesis was measured on intact attached leaves of plants using a portable photosynthetic system (ADC, model A120, Analytical Development Co., North Andover, MA). Measurement were done on days 1, 3, 5, 7, 8 and 25 after DMAA treatment started. Leaf area was determined at harvest using an SI 700 Leaf Area Analysis System (SKYE Instrument, Buckingham, PA).

Plants were grown for 5 weeks, and then harvested. Roots were washed with tap water, rinsed with a 0.1 N HCl solution followed by 3 rinses with distilled-deionized water. Roots were separated from the shoots and dry weights were determined after drying at 65 °C for 48 hours. Samples were ground in a stainless-steel

Wiley mill to pass a 20-mesh sieve. Due to the small sample size obtained for several treatment combinations, samples of two replications were combined for tissue analysis prior to digestion in order to obtain large enough tissue samples for accurate analysis. Plant tissue samples were digested by wet ashing technique, digesting 0.5 g sample aliquots in 5 ml concentrated nitric acid. Temperature during the digestion was controlled to a maximum of 140 °C to avoid As volatilization. Arsenic and nutrient concentrations were determined with a Jarrel Ash (Atom Comp 800 series) ICP.

In addition to the determinations above, other parameters calculated were:

Total dry wt. = Shoot dry wt. + Root dry wt.

Dry weight Ratio = Shoot dry wt./Root dry wt.

As concentration Ratio=Shoot As conc./Root As conc.

As uptake = Tissue As conc. x Dry wt.

Nutrient conc. Ratio=Shoot nutrient conc./Root nutrient conc.

Nutrient uptake Ratio=Shoot nutrient upt./Root nutrient upt.

Photosynthetic capacity = Net Photosynthesis x Leaf area

Statistical analyses were performed using the PROC ANOVA and PROC GLM procedure available in SAS (SAS Institute Inc., Cary, NC).

Results and Discussion

Arsenic uptake and tissue arsenic concentration

Arsenic uptake (dry weight times concentration) and tissue As concentration in both shoot and root increased significantly in rice plants as the concentration of

DMAA in solution increased (Fig. 4.1). Figure 4.1 also shows the dramatic increase in As uptake as the level of DMAA increased in spite of the reduction in dry weight observed in Figure 4.2. Absorption of DMAA from solution culture was also reported for beans (Sachs and Michael, 1971), and rice (Marin et al., 1992). However, Rumburg et al. (1960) pointed out that absorption of arsenicals from a soil medium may differ substantially from nutrient solution absorption.

Both concentration and uptake data in Figure 4.1 shows that As taken up was readily translocated and stored in the shoot. Similar results were obtained with beans (Sachs and Michael, 1971) and rice plants (Marin et al., 1992). The easy and quick translocation to the shoot appears to be specific for DMAA chemical form. When As is absorbed in its other chemical forms it is stored in the root (Liebig, 1966; Frans et al., 1988; Marin et al., 1992). Its facile translocation appears to confirm that DMAA is primarily or exclusively translocated in the apoplast (National Academy of Sciences, 1977; Ashton and Crafts, 1981). A compound moved apoplastically will be transported primarily to the expanded leaves, an ideal pattern of distribution for any compound whose mode of action is inhibition of photosynthesis (Caseley and Walker, 1990).

Effect on other nutrients

The data on Table 4.1 shows shoot and root mineral concentration at harvest. The increase in concentration of some nutrients at the highest rate of DMAA application can be explained by a concentration effect due to reduction in dry matter production at that treatment. Shoot tissue K concentration decreased with an increase in DMAA

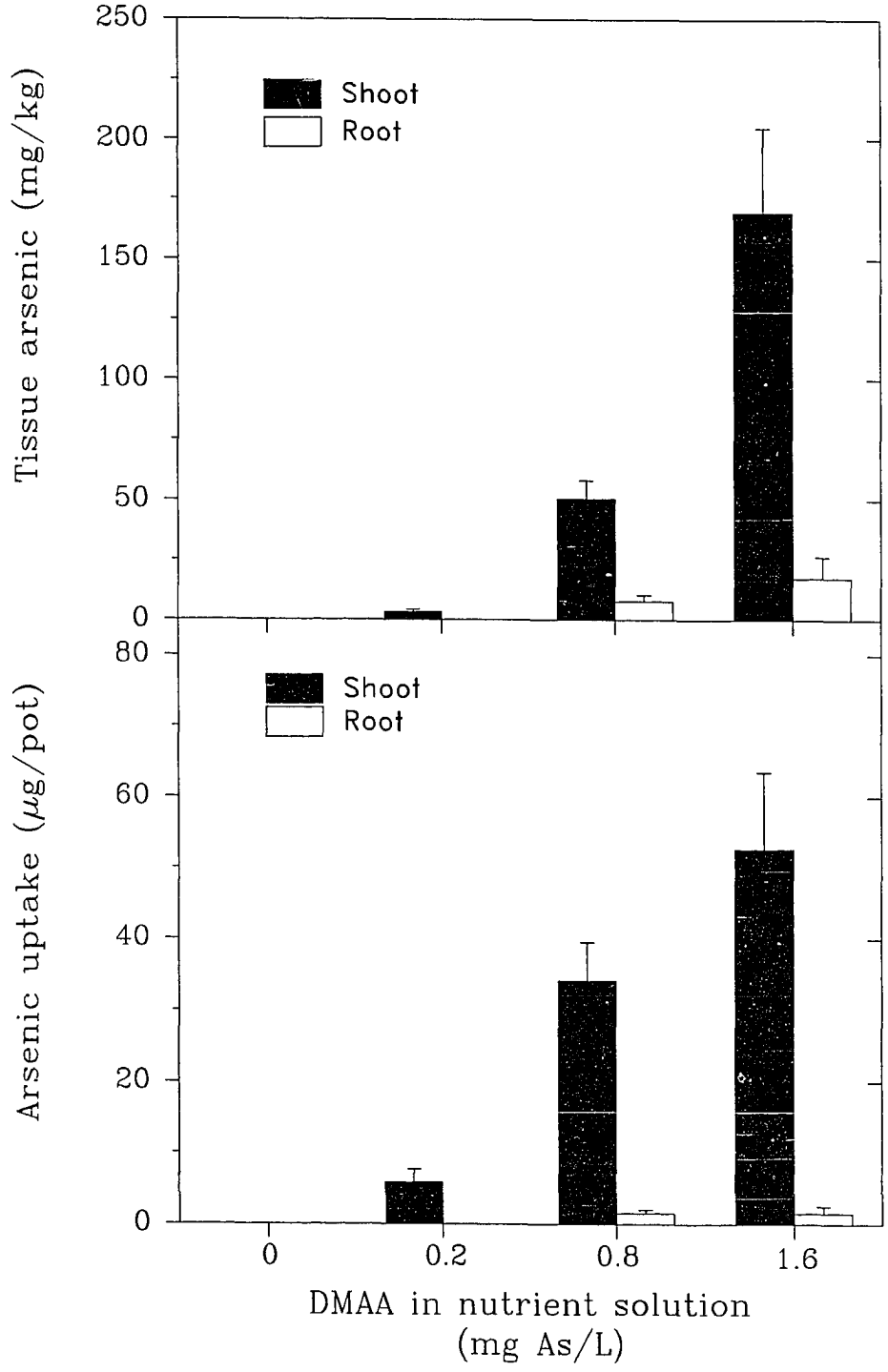

Figure 4.1: Effect of DMAA on arsenic uptake and tissue arsenic concentration in rice plants.

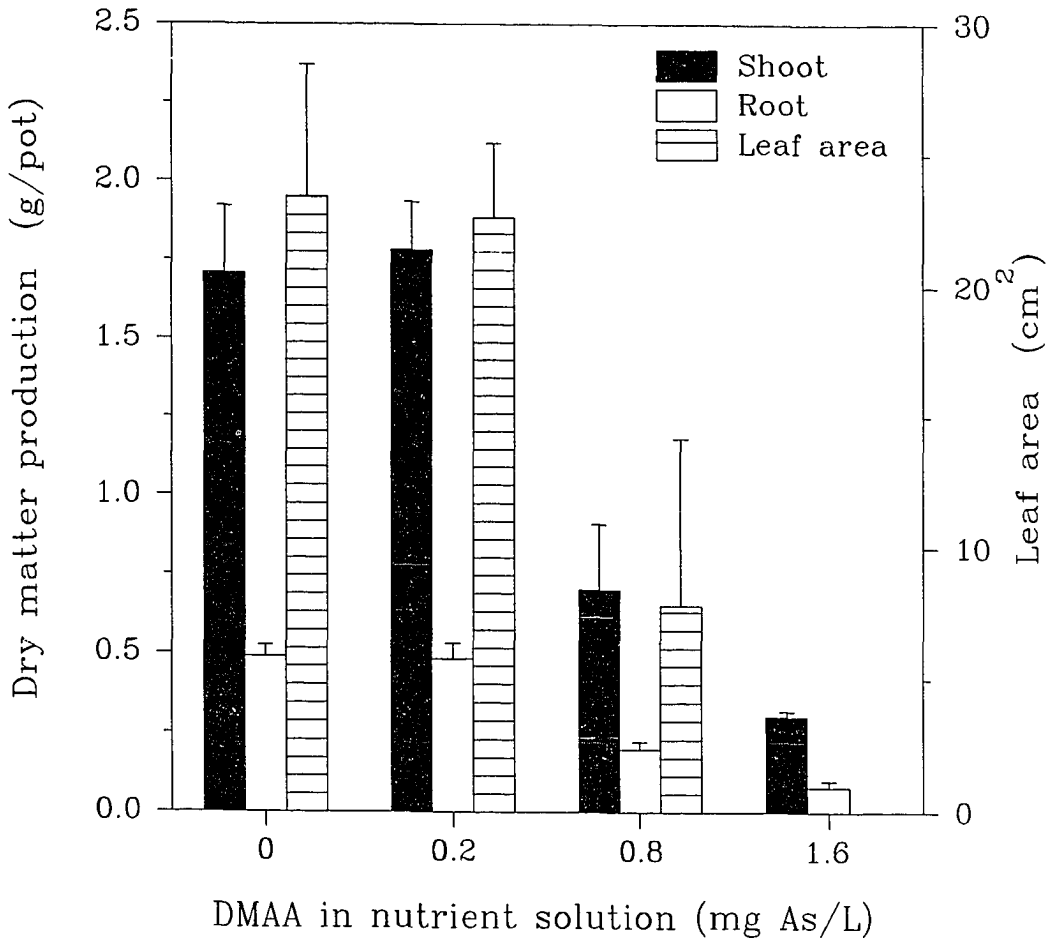

Figure 4.2: Rice leaf area and dry matter production as affected by DMAA application in nutrient solution.

TABLE 4.1: Effect of dimethylarsenic acid (DMAA) on mineral concentration in shoot and root of 'Mercury' rice plants[a].

Mineral content		DMAA applied (mg As L^{-1})			
(mg kg^{-1})		0	0.2	0.8	1.6
P	Shoot	8319 a[b]	8302 a	8974 a	9432 a
	Root	4694 a	4935 a	5939 a	5717 a
K	Shoot	30794 a	31206 a	18505 b	13634 c
	Root	8213 a	8077 a	7626 a	6856 a
Ca	Shoot	1509 b	1525 b	1899 b	2390 a
	Root	477 c	627 bc	885 b	1438 a
Mg	Shoot	3286 b	3377 b	3696 b	4676 a
	Root	812 a	835 a	816 a	792 a
Mn	Shoot	309 a	277 a	262 a	301 a
	Root	25.0 a	33.0 a	22.7 a	20.3 a
Fe	Shoot	282 a	238 a	337 a	279 a
	Root	3204 b	3560 ab	4661 ab	5314 a
Cu	Shoot	24.7 a	21.6 b	21.0 b	26.1 a
	Root	47.4 a	55.0 a	53.6 a	97.5 a
Zn	Shoot	50.3 b	45.4 b	48.5 b	81.4 a
	Root	28.6 b	110.3 a	51.7 ab	70.6 ab
Mo	Shoot	6.62 b	6.66 b	7.81 ab	8.30 a
	Root	28.5 c	30.7 bc	35.4 ab	41.1 a
Na	Shoot	967 c	771 c	2405 b	3852 a
	Root	856 a	848 a	855 a	678 a

a Data represent mean values of n=4.

b Values within a row followed by the same letter are not significantly different ($p<0.05$), Duncan Multiple Range Test.

concentration. This was probably due to competition with Na in the nutrient solution. The sharp increase in tissue Na concentration upon increased DMAA rate is not surprising since the DMAA was applied as its sodium salt. Even though the difference in concentration of some nutrients is due to DMAA effect, the nutritional status of the plants appears to be adequate. All nutrients in all treatments were above the sufficient level for rice plants as defined by Sedberry et al., (1987). Thus, the toxic effect of DMAA on rice plants cannot be explained as a nutritional disorder.

Uptake data (data not shown) show a general decrease in mineral uptake as level of DMAA in nutrient solution increased, which was expected due to the decrease in dry matter production in response to DMAA effect. Both the concentration ratio and uptake ratio for nutrients in plant tissue in general did not show major difference among treatments, with the exception of Mg and Mn that increased its uptake ratio at the highest rate of DMAA applied. The dry matter ratio also did not show difference among treatments (Fig. 4.3). Hence, the present data does not support the hypothesis that DMAA caused changes in carbohydrate allocation.

Effect on photosynthesis

Net photosynthesis (Pn) and photosynthetic capacity decreased significantly at the two higher rates of applied DMAA (Fig. 4.4). In fact, the average value of Pn for the highest rate of DMAA shows a negative value (prevailing respiration) at that treatment. At the minimum rate of applied DMAA, both Pn and photosynthetic capacity were similar to the control, which is further confirmed by the observed pattern of growth responses to the treatments (Fig. 4.2).

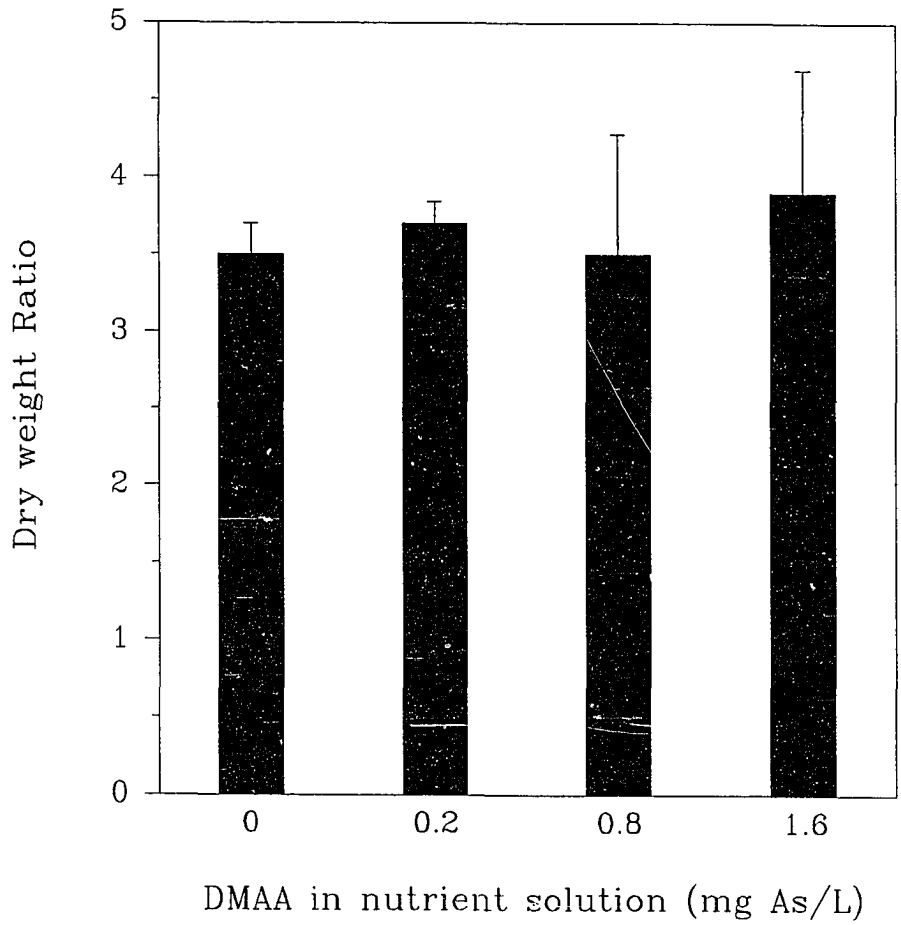

Figure 4.3: Effect of DMAA on dry weight ratio of rice plants.

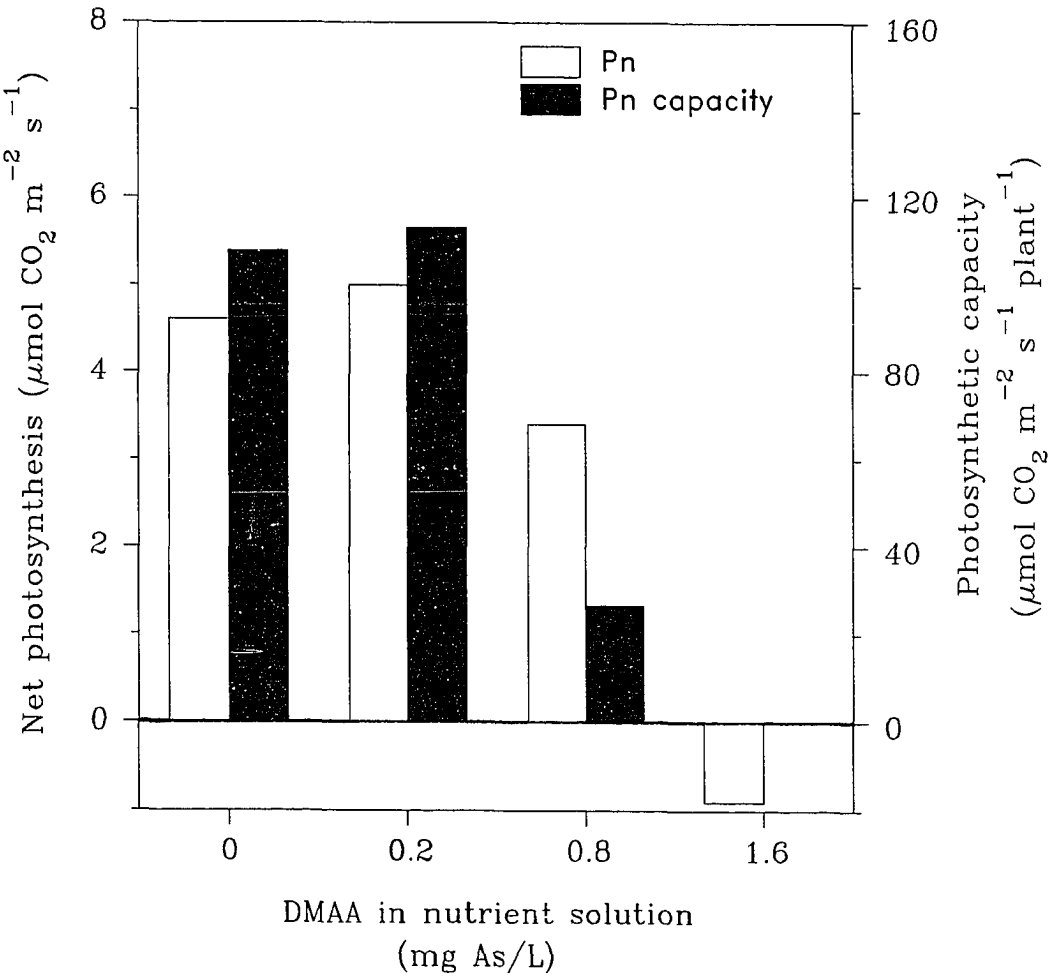

Figure 4.4: Net photosynthesis and photosynthetic capacity in rice plants as affected by DMAA concentration in nutrient solution.

The time-course of Pn response to application of DMAA is shown in Figure 4.5. At the highest level of DMAA addition (1.6 mg As L^{-1}), an early and sharp decline in Pn was noted. At day 3, no positive Pn activity was detected. The dramatic Pn response in this treatment was followed by the death of plants at harvest. On the other hand, the lower concentrations of DMAA treatments did not show significant differences in Pn activity compared to the control plants up to day 8. At the last Pn measurement (day 25) treatment 3 and 4 showed significant decreases as compared to the control. At day 25, plants under 0.2 mg As L^{-1} treatment also showed a slightly (but not significant) lower Pn activity than the control. Whether or not the Pn will continue to decrease or this was just a random effect is not known. Regression calculations of Pn activity at day 25 versus concentration of As in the shoot showed a significant linear decline (Fig. 4.6). The regression of Pn at day 25 versus DMAA concentration in solution also showed a significant negative relationship, with a significant decrease in Pn as the concentration of DMAA in solution increased, rendering the equation $Y = 3.66-6.767 X$, ($R^2=0.58$, $P<0.001$).

Workers with monosodium methanearsonate (MSMA) have suggested that organic arsenicals may act on Pn (Spilsbury, 1972) and respiration as an inhibitory uncoupler (Pillai et al., 1973). No information was found in the literature about the mechanism of action of DMAA on photosynthetic processes. The results presented clearly demonstrate that DMAA inhibited Pn, however identification of the mechanism/s responsible for this was beyond the scope of this study.

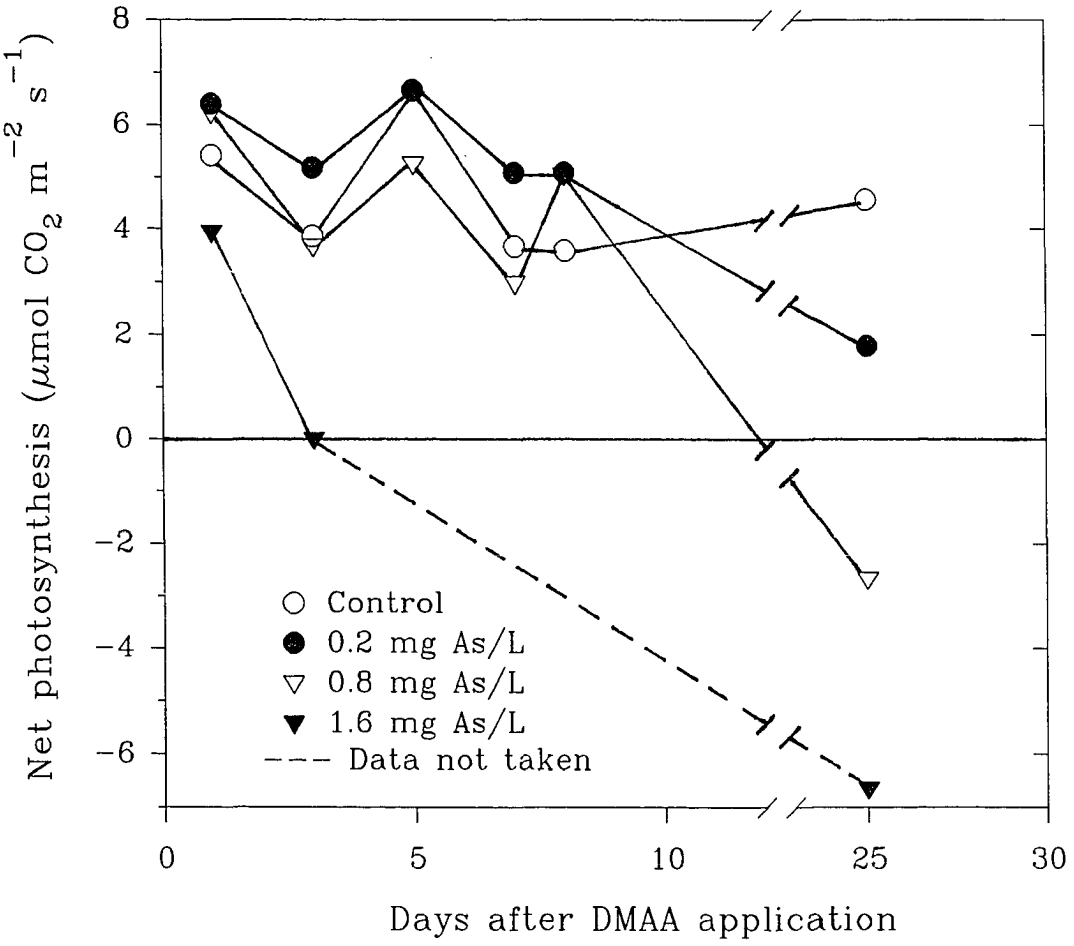

Figure 4.5: Time-course response of net photosynthesis to DMAA application in rice plants.

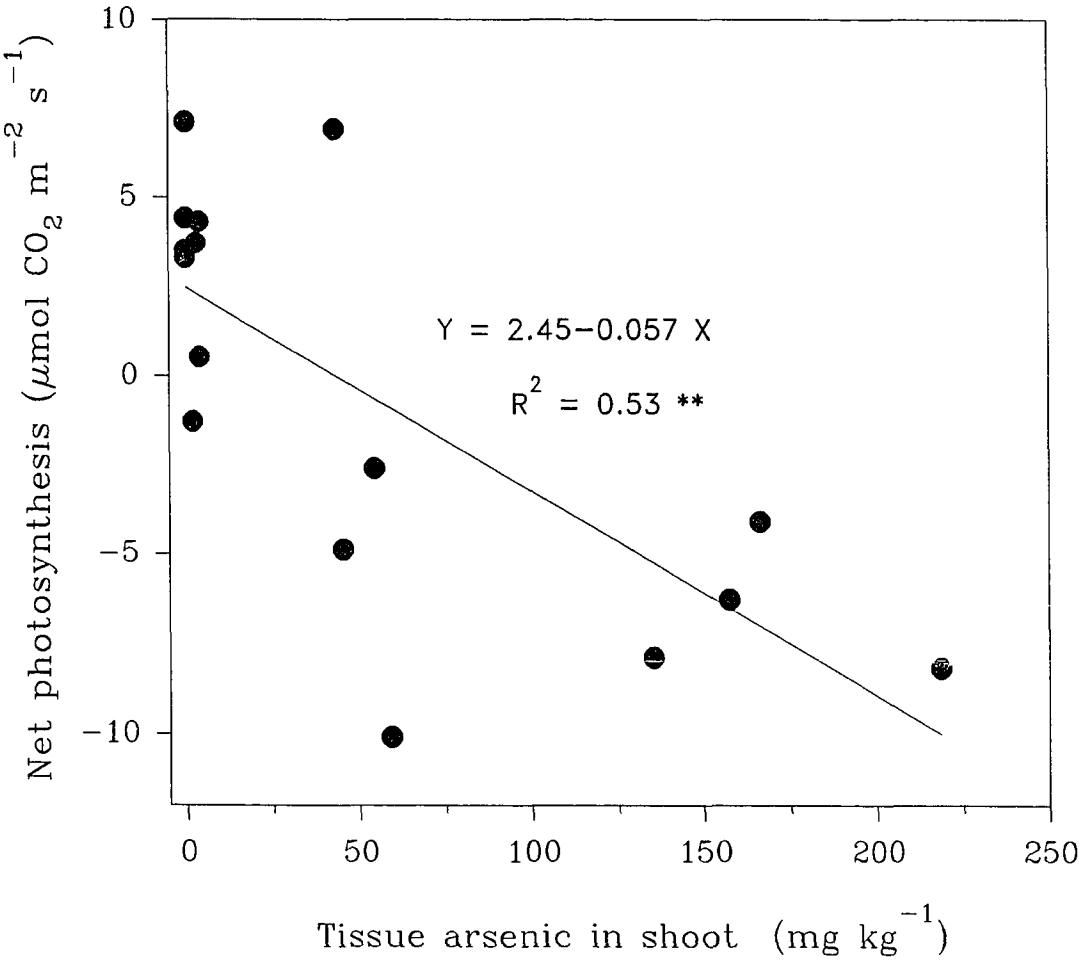

Figure 4.6: Relationship between net photosynthesis and shoot arsenic concentration in rice plants.

Effect on growth parameters

Rice growth, as represented by shoot, root and total (shoot + root) dry weight, and leaf area was significantly reduced at the two higher (0.8 and 1.6 mg As L^{-1}) rates of DMAA treatments (Fig. 4.2), and plants showed visible chlorosis symptoms. At the highest rate of applied As, most of the plants were dead at harvest time, consequently leaf area was not determined for that treatment. However, when DMAA was applied at the rate of 0.2 mg As L^{-1}, there was no difference between the treatment and control in any of the measured parameters. Marin et al. (1992) did not find any changes in dry matter production of rice plants due to DMAA application up to a rate of 0.8 mg As L^{-1}. This was probably due to slight differences in the experimental procedure. In the present experiment, the growing solution was changed every 4 days, while in the former experiment, the solution was changed more frequently. The more frequent changes in solution may have precluded As absorption for a time leading to lower As concentration in tissue, and therefore, no damage at the 0.8 mg As L^{-1} rate.

The shoot/root dry weight ratio was not affected by DMAA treatment, and ranged between 3.5 and 3.9 (Fig. 4.3). Other researchers have observed toxicity symptoms in beans (Sachs and Michael, 1971) and soybeans and radish (Woolson and Isensee, 1981) plants due to root application of DMAA (or CA) at high rates (27 to 37 mg As L^{-1}).

Research comparing the toxicity of different As chemical forms based on similar As concentration has shown DMAA to be the least toxic when applied to the root (Sckerl et al., 1966; Sach and Michael, 1971; Marin et al., 1992). Therefore, our

results demonstrate that DMAA applied at relatively high doses may be toxic to rice plants, although its toxicity is low compared to the other arsenical chemical forms.

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SUMMARY AND CONCLUSIONS

Over the years it has become more evident that it is important to know the chemical form of the element present in the soil solution in order to assess the effect and toxicity of arsenic (As) on plants. Currently, little data are available on the distribution and stability of As in soils and its availability to plants. The lack of experimental data and the importance of a better understanding of As chemistry in soils, and especially in flooded soils, warranted a study dealing with the speciation, species transformation, solubility, plant uptake and effect of As on plant growth.

First, the speciation and redox chemistry of native and added As and its influence on two rice cultivars was investigated. Secondly, the effect of As-chemical forms and As-concentration on the growth of rice plants, and their influence on the uptake of other plant nutrients was studied. Finally, the effect of a relatively high dose of an organic arsenical compound (DMAA) on plant growth and photosynthesis was examined.

The influence of redox potential and pH on As availability, speciation, and uptake by rice plants was studied using a Crowley silt loam soil (Typic Albaqualf). A set of treatments was performed without As addition, and another set of treatments was carried out after the addition of 4 mg As kg^{-1} as monosodium metanearsonate (MSMA). Soil suspensions were equilibrated at four redox potentials (-200, 0, +200 and +400 mV) and three pH's (5.5, 6.5 and 7.5). The As concentration and species distribution were determined. Major cations and metals were also determined.

Soil redox potential and pH were shown to affect As speciation and solubility, thereby determining As phytoavailability and phytotoxicity to rice. In the soil suspension without As addition, water-soluble As concentration increased upon soil reduction, and As(III) constituted most of the total soluble As present in the soil solution. Water-soluble As also increased as soil pH decreased. At the lowest redox potential studied (-200 mV) 7.3, 2.2, and 1.4% of the total As in the soil (3.2 mg kg^{-1}) became water-soluble at a pH 5.5, 6.5, and 7.5 respectively. Under oxidized condition, As solubility was lower and As(V) constituted most of the soluble As present.

When the soil was amended with MMAA, soil redox potential and pH also affected speciation and solubility of both inorganic As and MMAA. The amount of water-soluble inorganic As [As(III)+As(V)] increased significantly in the amended soil as compared to the unamended one, due to a large increase in the arsenate fraction. Lower redox potential led to higher dissolved MMAA. The effect of pH was less clear than in the unamended soil. Although thermodynamically unstable, a considerable amount of As(V) remained present under reduced conditions in both the unamended and As amended soils.

As soil redox-pH conditions affected the speciation and solubility of As in the soil, one could expect soil redox-pH to also determine As phytoavailability and phytotoxicity. Arsenic uptake increased considerably in plants grown under reduced conditions, for the two set of treatments, with and without MSMA addition. Overall, the addition of MMAA to the soil resulted in a significant increase in As intake and a significant decrease in dry matter production. Plants stored a greater percentage of As

in the roots for all treatment combinations. In the unamended soil, shoot As was only detected at the lower redox levels (-200 and 0 mV). No significant difference in As uptake between Lemont and Mercury rice cultivars was observed.

The addition of MMAA also affected Zn and Cu phytoavailability. Due to the addition of MMAA, the Zn concentration ratio and uptake ratio decreased from 1.32 and 3.88 to 0.71 and 2.35, respectively. Besides affecting Zn availability, addition of MMAA also decreased Cu solubility in soil. Higher uptake of As resulted in lower Cu uptake.

In a second experiment, with plants grown in nutrient solution, As was applied at 4 levels (0, 0.05, 0.2 and 0.8 mg As L^{-1}), and 4 chemical forms [monomethyl arsenic acid (MMAA), arsenite As(III), arsenate As(V) and dimethyl arsenic acid (DMAA)]. Chemical form of As was shown to be the most important factor responsible for As toxicity to rice plants. Monomethyl arsenic acid was the most toxic As form at relatively low concentrations and As(III) was the next most toxic As form. Arsenic(V) and DMAA did not shown toxic effects on rice plants up to the concentration of (0.8 mg As L^{-1}) used in solution. At the lowest rate used (0.05 mg As L^{-1}), DMAA shown a beneficial effect on plant growth. The amount of As taken up by the rice plants followed the trend DMAA < As(V) < MMAA < As(III). The mobility of As in the plant was also dependent on chemical form. While As(III), As(V) and MMAA accumulated in the roots, DMAA was readily translocated to the shoot. No significant differences in the effect of As chemical form or concentration were found between Lemont and Mercury cultivars.

The study of plant nutrient uptake as affected by As concentration and chemical form applied in solution showed that shoot tissue P uptake decreased with increasing As application. Increasing As concentration in solution caused significant decline in both shoot and root tissue K for all As chemical forms applied. Shoot Zn concentration and uptake was lowered by As for all chemical forms applied. Zinc translocation to the shoot was impaired by As when applied as MMAA, and to a lesser degree by As(III). The scenario was similar for Cu, although not so noticeable as in the case of Zn.

The data obtained from the experiment with DMAA applied to the root at relatively high concentrations showed that DMAA may be toxic. Dimethylarsenic acid has been shown to be less toxic than the other As chemical forms when applied at an equimolar base. When applied at rate of 1.6 mg As L^{-1}, DMAA inhibited plant growth, reduced photosynthesis activity and photosynthetic capacity, and eventually caused plant death. A time-course of photosynthesis response to applied DMAA showed an early and sharp decrease in photosynthesis activity, as early as one day after DMAA application started. A linear regression equation was calculated for photosynthesis activity at day 25 vs. shoot tissue As concentration. The equation determined was $Y = 245\text{-}0.057\ X$ ($R^2 = 0.53^{**}$).

Results generated in this study indicate that both redox potential and pH affect speciation and solubility of As in soils. Therefore, changes in redox-pH conditions can significantly influence As bioavailability and determine toxicity to plants.

APPENDIX

Letter of permission to reprint:

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Date April 28, 1992

Mr A.R. Marin
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Dear Mr Marin,

Further to your letter of April 9, 1992, regarding your article: "The influence of chemical form and concentration of arsenic on rice growth and tissue arsenic concentration" Plant and Soil 139: 175-183, 1992.

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Your contribution to Plant and Soil is much appreciated.

I wish you every success in defending your Thesis.

Yours sincerely,

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VITA

Alfredo Rubén Marín was born October 28, 1954, at Itati, Corrientes, Argentina, son of Alberto Marín and Maria Luisa Babín. He graduated from the Universidad Nacional del Nordeste in Corrientes, in August 1979, with a Bachelor of Science in Agronomy (as an Ingeniero Agrónomo).

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Major Field: Agronomy

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