



Effects of phosphorous application on arsenic toxicity to and uptake by rice seedlings in As-contaminated paddy soils



Chia-Hsing Lee¹, Chung-Hung Wu¹, Chien-Hui Syu, Pei-Yu Jiang, Chia-Chen Huang, Dar-Yuan Lee^{*}

Department of Agricultural Chemistry, National Taiwan University, No. 1, Sec. 4, Roosevelt Rd., Taipei 10617, Taiwan

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ABSTRACT

Arsenate (As^{V}) has been proven to share the same absorption pathway from soil to rice roots as phosphate (P_i). Therefore, soil application of phosphorous was assumed able to reduce arsenate uptake by rice. However, solution cultivations, pot tests and field experiments have shown differing results regarding the efficacy of phosphorous applications. Moreover, the effects of P_i on Arsenic (As) uptake by rice in As-enriched soils may depend on multiple factors, such as soil properties, As levels in soils or the application rate of P_i . In this study, we aimed to determine the effects of a wide range of application rates of P_i on As release and phytotoxicity in soils. Two geogenically As-enriched soils, two background soils, and two As-spiked soils were tested. The rice seedlings were cultivated in the soils treated with $\text{Ca}(\text{H}_2\text{PO}_4)_2$ for 40 days. The results show that P_i application into soils did not inhibit As uptake and accumulation in rice seedlings for all soils. For the soils with high retention capacity of P_i , the P_i treatments did not result in any obvious change in As concentration of the soil solution while the P_i concentration in the soil solution increased but did not reach a level that can compete with As for rice uptake. For the soils with low retention capacity of P_i , the application of P_i largely increased both the As and P_i concentrations in the soil solutions, which could be attributed to competitive adsorption by solids. This resulted in a small increase in the P_i/As molar ratio of the soil solution and an absence of competitive uptake by rice. Conversely, the As toxicity was aggravated when As concentration in soil solution was increased with high P_i application. We conclude that for paddy soils with high levels of As content, P_i application at a much higher level than plant requirements may be needed, which is not appropriate due to environmental concerns. We suggest future testing be aimed at determining whether foliar applications of P would induce enough competitive uptake between P and As to sufficiently reduce As uptake by rice seedlings grown in As-contaminated soils.

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1. Introduction

Arsenic (As) is widely distributed in soil, water, food and even the particulate matter in the air despite its concentration in the environment being generally low (Mandal and Suzuki, 2002). Arsenic is a carcinogen and can pose significant human health risks as a contaminant of drinking ground water, which has been found in many countries, including Taiwan (e.g. Chen et al., 1995; Huang et al., 1992). In anaerobic soil environments, arsenite (As^{III}) is the major species and its proportion among all the As species in soil solutions of paddy fields is high (Bolan et al., 2013a, 2013b; Roberts et al., 2011). Paddy rice is a staple foodstuff in Asia and unfortunately, it accumulates As more efficiently in comparison with other cereals (Su et al., 2010). In recent years, there has been speculation that rice consumption may be a primary route for human exposure to As on lands with As-enriched soils or groundwater, and excessive As accumulation in the rice grain can pose a significant health risk to consumers (Meharg, 2004; Meharg et al., 2009; Mondal and

Polya, 2008; Williams et al., 2007). Moreover, the phytotoxicity of As increases with high levels of As content in soils and consequently, the rice growth or yields in paddy fields decrease (Panaullah et al., 2009; Talukder et al., 2014; Tripathi et al., 2013). It is important, therefore, to understand As mobility and its affecting factors in the soil–rice system.

Arsenate (As^{V}) is a chemical analog of phosphate (P_i) and shares the uptake pathway of rice with the same transporters (Abedin et al., 2002; Shin et al., 2004). Therefore, P_i supply to rice has been assumed able to reduce As^{V} toxicity through competitive uptake by rice plants. Previous works have lent credible support to this assumption through hydroponic experiments. For example, in solution cultures with high As^{V} concentrations up to 3.75 mg L^{-1} , addition of P_i into nutrient solutions to increase the P/As ratio did in fact reduce As uptake and increase the biomass of rice (Geng et al., 2005; Guo et al., 2007; Huang et al., 2010; Wang and Duan, 2009). Choudhury et al. (2011) also provided evidence of better metabolism in rice plants in the presence of P_i for a solution culture with high As concentrations. Similar effects have also been found for other plants (Grifoni et al., 2015; Lou et al., 2010). Wu et al. (2011) reported the evidence that showed sharing of transport pathways between P_i and As^{V} in a hydroponic experiment. However,

^{*} Corresponding author.

E-mail address: dylee@ntu.edu.tw (D.-Y. Lee).

¹ Dr. Chia-Hsing Lee and Mr. Chung-Hung Wu are equal contributors to this paper.

they further showed that the pathway contributed little to As uptake and transport to grain in rice plants grown in flooded soils, presumably a result of particular As species distributions in the soils (i.e. when As^{III} dominates in the flooding soil system).

Since the adsorption of As by soil solids is similar to that of P_i , competitive adsorption between P_i and As (both As^{III} and As^{V}) may occur when P_i is applied to an As-enriched soil, and this may result in an increase of As release into soil solutions. The extent of this effect depends on the soil properties related to adsorption capacity (Signes-Pastor et al., 2007; Zeng et al., 2012). Bolan et al. (2013a and 2013b) have clearly described the aspects that the effect of P_i on As mobility in soil and its uptake by plant depends on three factors: (i) the competition of P_i for As adsorption by soil particles, (ii) the competition of phosphate for As uptake by plant roots, and (iii) the effect of phosphate on As translocation from root to shoot. Therefore, both the effect of competitive adsorption on soil solids (i.e. enhancing As release into soil solutions) and the effect of competitive uptake between As and P by rice plants (i.e. inhibition of As uptake) may occur when P_i is applied to As-enriched soils, and the subsequent uptake of As by plant roots depends on the relative concentrations of these two solutes in the soil solution.

However, an investigation of the overall effect of P_i soil applications on the As phytoavailability to paddy rice would be very valuable because several soil experiments have revealed that As accumulation is not always affected or even increased by P application for rice (Hossain et al., 2009) or other plants (Brackhage et al., 2014; Lewińska and Karczewska, 2013). Probable factors controlling the effects of As–P interactions on As mobility and phytoavailability include soil binding capacity for P and As, soil pH, soil redox potential, As species distribution in soil solutions, amounts of P application and rice genotypes (Lou-Hing et al., 2011; Signes-Pastor et al., 2007; Zeng et al., 2012). Furthermore, co-application of Ca and P into As enriched soils can form Ca–As–P minerals and thus cause a decrease in As mobility (Neupane and Donahoe, 2013). Accordingly, Ca–P combined fertilizers such as single superphosphate and triple superphosphate fertilizers might be better for decreasing As toxicity while formation of Ca–P–As minerals could offset P_i induced As release and applied P_i could compete for rice uptake against As. P_i is an essential element for plants, and therefore could affect As uptake by supporting plant growth as well. Lessl and Ma (2013) denoted that in a soil system with low available P, better development of root systems and more As uptake by *Pteris vittata* can be observed compared to that with high available P.

Iron plaque on roots could be an effective restraint on the uptake of As by rice plants (Lee et al., 2013; Syu et al., 2014). Moreover, iron oxidation and iron mineral formation are known to be affected by the chemistry of the background solution. Therefore, the presence of P_i in soil solutions could lead to changes in the amount and mineral composition of iron plaque on rice roots and subsequent As uptake and accumulation by the rice. Hu et al. (2005) revealed decreasing amounts of iron plaque and increasing transportation of As from roots to shoots with increasing application of P fertilizer, which resulted in only subtle differences in As accumulation in shoots between P treatments.

The related soil factors that control the effects of P_i application on As mobility and phytoavailability remain unclear. Therefore, we conducted a pot experiment to test As mobilization and competitive uptake against P through application of a wide range of P_i rates into soils with distinct properties which allowed varied retention capacities of As and P.

2. Materials and methods

2.1. Soil sampling and As treatments of soils

Guandu (Gd) soils were collected from the paddy fields of the Guandu plain, northern Taiwan, which has been identified as an As-contaminated area caused by irrigation with As-contaminated spring water (Chiang et al., 2010). Two Gd soils were collected, one considered

un-contaminated soil from Guandu (designated GdL) with a low level of As (9 mg kg^{-1}) and the other considered contaminated soil from Guandu (designated GdH) with a much higher level of As (87 mg kg^{-1}), both of which are still cultivated for rice production to date. Two uncontaminated paddy soils were also collected from the topsoils of Pinchen (Pc) and Chenchung (Cf) soil series, which are widely distributed in northern and southern Taiwan, respectively, and are substantially different in their soil pH values and Fe/Al oxide content. The soils were air-dried, sieved through a 10-mesh sieve, well mixed and then stored in plastic containers (labeled un-spiked PCL and CFL soils, respectively). A portion of Pc and Cf soils were spiked by spraying disodium hydrogen arsenate solution to reach a total As content of 90 mg As kg^{-1} soil. The As-spiked soils (PcH and CfH) were then subjected to three cycling processes of wetting ($v:w = 1:1$) and air-drying at room temperature for a total of approximately 60 days, followed by further sieving and mixing. Subsamples were taken and the total As content of each soil was analyzed. Accordingly, three uncontaminated CFL, PCL, and GdL soils and three As-contaminated/spiked GdH, PcH, and CfH soils were tested. Soil texture and pertinent chemical properties of the six soils are given in Table 1.

2.2. Batch experiments for P adsorption isotherm

The tested soils were added into series of KH_2PO_4 solutions at the ratio of 1:10 (g:mL). The ion strength of the systems was adjusted by adding CaCl_2 to reach 0.01 M. Two drops of toluene were added to inhibit microbial activity. The suspensions were then shaken at 25°C for 7 days followed by centrifugation and filtration ($0.45 \mu\text{m}$). The P_i concentrations of the filtrates were determined and the Langmuir equation was used to obtain the maximum capacity of P_i retention (MPR) of the tested soils (Fox and Kamprath, 1970). The MPR values are given in Table 1.

2.3. P treatments of soils

The available P (Bray- P_i) values of the prepared soils were determined as 65, 48, and 23 mg kg^{-1} for Cf, Pc, and Gd soils (Table 1), respectively. According to the classification of P fertility and recommended amount of P fertilizer (RAP) for rice cultivation in Taiwan soils, the corresponding RAPs for Cf, Pc, and Gd soils were 30, 30, and $40 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$, respectively. Supposing that topsoil weight of paddy field is $2200 \text{ tons ha}^{-1}$, the RAPs approximately equal to 6, 6, and 8 mg P kg^{-1} , respectively. We conducted a series of P treatments by adding $\text{Ca}(\text{H}_2\text{PO}_4)_2$ at the rates of 0, 1, 2, 5, and 10 folds of RAP (denoted by P0, P1, P2, P5, and P10, respectively). Soils were then applied with urea and potassium chloride at the rates of 118 and 142.5 mg kg^{-1} as base fertilizers (i.e. 220 kg N ha^{-1} and $180 \text{ kg K}_2\text{O ha}^{-1}$). As mentioned above, previous studies have suggested that P_i applications into soils may be ineffective regarding As uptake inhibition because they probably provide insufficient amounts of P_i to adequately outcompete As for the absorption pathway. Therefore, in this study P fertilizer was applied up to a high level of 10 RAP to investigate whether the inhibition effect of P_i application on As uptake by rice plants could be achieved with high P fertilization.

2.4. Pot experiments of rice seedlings

The cultivar of paddy rice (*Oryza sativa* L.), Tai-ken 9 (TK 9; Japonica), was used in this study, which is widely cultivated in Taiwan. The details of seed germination and seedling raising procedures were the same as those described in our previous research (Lee et al., 2013). Five hundred grams of each soil was added into a pot and treated with fertilizer containing equal amounts of N and K but different levels of P, and then water is added to reach a soil/water ratio of 1:1. The pots were then moved into a phytotron glasshouse and kept under controlled conditions

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