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Alleviation of arsenic accumulation in rice by applying silicon-rich rice husk residues in Bangladesh soil

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Abstract

While the accumulation of arsenic in rice (*Oryza sativa* L.) has been highlighted as a major concern in Bangladesh, sustainable measures are critically needed to reduce the uptake of arsenic by rice plants. In the present study, a pot-experiment was conducted using a Boro rice variety (BRRI dhan-29) in two geomorphologically different soils from Holocene floodplains and Pleistocene terraces, in which silicon-rich fresh rice husk (FRH) and rice husk ash (RHA) were applied, as silicon fertilisers, in the soils at the rate of 1% (w/w) of rice residue:soil.In the Holocene floodplain soils, the application of FRH was found to decrease arsenic in grain, husk and straw by 42, 56 and 51%, respectively, whereas the soil incorporation of RHA decreased arsenic in grain, husk and straw by 26, 37.5 and 36%, respectively. In the Pleistocene terrace soils, the application of FRH reduced the grain, husk and straw arsenic by 38, 38 and 44%, respectively, whereas the RHA decreased the grain, husk and straw arsenic by 26, 30 and 29%, respectively. Fresh rice husk was found to be more effective in alleviating arsenic accumulation in rice than RHA. In both the Holocene floodplain and Pleistocene terrace soils, the grain concentrations of calcium, phosphorus, silicon, and zinc were found to be increased with the decrease of arsenic in the grain due to the use of FRH and RHA. The present study suggests that silicon-rich rice husk residue scan be used as silicon fertilisers to reduce arsenic accumulation in rice in Bangladesh.

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Introduction

Arsenic contamination of paddy cultivated soils irrigated with arsenic laden groundwater in Bangladesh is well documented (Meharg and Rahman, 2003; Huq and Naidu, 2003; Chowdhury *et al.*, 2017). Rice crops grown in the arsenic enriched soils can accumulate elevated concentrations of arsenic into the plant parts, particularly into the grains (Meharg *et al*., 2001; Abedin *et al*., 2002; Williams *et al.,* 2006; Huq 2008). Rice accumulates an order of magnitude higher arsenic concentration in its grain compared to other cereals (Williams *et al.*, 2007). The high concentration of arsenic in rice grain is due to a number of factors. Arsenic in flooded paddy soils is more available compared to non-flooded soils, as it is present predominantly as arsenite, which is more mobile and toxic compared to arsenate (Xu *et al.*, 2008; Takahashi *et al.*, 2004). Additionally, rice is a high accumulator of silicon (Ma *et al.*, 2001a; Ma *et al.*, 2001b), which is a non-toxic and beneficial element for rice, comprising up to ten percent of the dry matter in rice husk and straw being much higher than other mineral nutrients (Epstein, 1994; 1999; 2009). This is important as it has been shown that arsenic is taken up by the same transporter genes (*Lsi1* and *Lsi2*), which is responsible for the accumulation of silicon from the soil (Ma *et al.*, 2006; Ma *et al.*, 2008). Rice is the staple food of one hundred and sixty million people in Bangladesh, and seventy percent of total calorific intake by the population is from rice (GRiSP, 2013).

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It indicates that rice dependent diets, such as those of Bangladeshi people, pose a serious issue for human arsenic intake (Schoof *et al.*, 1999). Therefore, developing a sustainable strategy to alleviate arsenic accumulation in rice, thereby improving the quality of food and human health across the world is indispensable.

Use of silicon is an emerging method to reduce arsenic uptake by rice (Ma *et al.*, 2008; Seyfferth *et al.*, 2016; Limmer *et al.*, 2018). Dissolved and plant-available silicon in soil can improve the yield and quality of rice by reducing arsenic uptake/toxicity (Bogdan and Schenk, 2008; Li *et al.*, 2009; Seyfferth and Fendorf, 2012) and the severity of fungal diseases (Datnoff *et al.*, 2001; Seebold *et al.*, 2001). Increasing plant-available silicon in the porewater of well-weathered, silicon-depleted rice soils (Savant *et al.*,1997) can decrease grain arsenic as a result of competitive interactions between silicon (silicic acid) and arsenic (arsenite) for plant uptake and adsorption onto soil solids (Luxton *et al.*, 2006; Li *et al.*, 2009; Bogdan and Schenk, 2008; Seyfferth and Fendorf, 2012). However, the source of silicon matters, as various silicon sources affect rice arsenic uptake differently. Silicon-rich materials that increase porewater silicon in high concentrations, such as silica gel, decrease grain arsenic (Li *et al.*, 2009; Seyfferth and Fendorf, 2012), whereas silicon-rich materials with a low silicon solubility, such as diatomaceous earth, may increase grain arsenic (Seyfferth and Fendorf, 2012). The application of silica fertilisers such as calcium silicate ($CaSiO_3$) in paddies may reduce arsenic concentration in rice (Guo *et al.*, 2005; Guo *et al.*, 2007), but these may contain toxic trace metals which can negatively affect rice yield and quality (Gu *et al.*, 2011). While rice farmers in developing countries, like in Bangladesh, may have limited access to synthetic silicon fertilisers, agronomic practices like incorporation of silicon-rich rice residues such as rice straw and rice husk can be a holistic approach to reduce arsenic in rice grain (Ma *et al.*, 2014; Seyfferth *et al.*, 2016; Penido *et al.*, 2016; Limmer *et al.*, 2018). Incorporation of silicon-rich rice straw has been reported to increase arsenic uptake by rice in grain (Ma *et al.*, 2014), whereas incorporation of silicon-rich rice husk and husk ash decreased arsenic accumulation in rice grain (Penido *et al.*, 2016; Seyfferth *et al.*, 2016). Rice husk contains less arsenic and labile carbon and provides more silicon to soil porewater compared to rice straw which releases more arsenic and less silicon in soil porewater than fresh husk or huskash (Penido *et al.*, 2016). In addition, incorporation of rice husk generates less methane emissions, which is a potential greenhouse gas, from rice paddies than incorporation of straw (Penido *et al.*, 2016; Gutekunst *et al.*, 2017). Therefore, rice husk is advantageous over rice straw as a sustainable solution to mitigate arsenic contamination in rice worldwide. However, the performance of the silicon-rich amendments relative to different rice varieties as well as different soil types in different geographic regionsneeds to be better understood.

Bangladesh has three major geomorphological units (Brammer, 1996; Huq and Shoaib, 2013). These are hill, terrace, and floodplain areas. The uplifted terrace areas are of Pleistocene age, and the floodplains are of Holocene age. These geomorphological units are related to the parent geological formations, and they are also characterized by land topography and age of the soil formation through sediment deposition over time (Brammer, 1996). Chowdhury *et al.* (2017) demonstrated that the Pleistocene terrace paddy soils were generally low in the concentrations of a range of elements, including arsenic and essential macro-and micro-nutrient elements, compared to the Holocene floodplain paddy soils. Provided that the mobilityand biogeochemistry of arsenic in the geomorphologically different paddy soils of Bangladesh are highly and inherently variable (Chowdhury *et al.*, 2017; Chowdhury *et al.*, 2018), it is indeed important to investigate the potential of silicon fertilisation in reducing arsenic accumulation in rice grown in the different soil types of Bangladesh.

In the present study, we evaluated the effects of silicon-rich rice husk residues, both fresh husk and husk ash, amendments on the accumulation of arsenic, calcium, phosphorus,silicon, and zinc in rice (BRRI dhan 29 variety) grown in two geomorphologically and biogeochemically different soils of Bangladesh, the Holocene floodplain and Pleistocene terrace soils, which were also naturally variable in arsenic concentrations.

Materials and methods

Collection and processing of soil samples

Bulk soil samples were collected from 2 geomorphologically different regions of Bangladesh: (i) the Holocene floodplain; the soils belonged to the physiographic region of Meghna Estuarine floodplain located at Baidder Bazar union, Sonargaon upazila, Narayanganj district, 23° 39' N and 90° 37' E, and (ii) the Pleistocene terrace; the soils belonged to the physiographic region of Madhupur Tract, located at Bhawal Rajabari union, Sreepur upazila, Gazipur district, 24° 06' 35.96" N and 90° 29' 56.89" E. The collected soil samples were processed and prepared for pot experiment and background analysis following the procedures described in Chowdhury *et al.* (2010).

Pot culture experiment

A pot culture experiment was conducted using a lowland crop, rice (*Oryza sativa* L., BRRI dhan 29 variety) and the soils collected from the geomorphologically different areas of Holocene floodplain and Pleistocene terrace. In order to determine the impact of silicon-rich ricehusk incorporation to soil on arsenic uptake by rice, powered fresh rice husk (FRH, obtained from a rice millin Savar, Dhaka) and rice husk ash (RHA, obtained by combustion of the same rice husk) were utilised as silicon amendment treatment at a rate of 1% (w/w) of rice residue:soil, as recommended by Penido *et al* .(2016), although this level of amendment could be higher than feasible for application on a large scale considering the production of husk per crop cycle (Seyfferth *et al.* 2016). Rice grown in the soils amended with the silicon-rich rice residues were compared to non-amended controls. All the treatments and non-amended control were conducted in triplicates. Therefore, a total of 18 pots (earthen pots of 8 kg in size, having no hole at the bottom, with 5 kg of air-dried and 5 mm sieved soil samples) were used in the pot experiment. The FRH and RHA (50 g) were gently mixed by hand into the soil in the pots 7 days prior to the plantation of rice seedlings collected from a farmer's field, and the pots were flooded to 4 cm above the soil surface using distilled water. All the pots were placed in a net-house in a randomized arrangement. Transplantation of the rice seedlings and culture of the plants during the rice growing period were done according to the protocols described in Chowdhury *et al.* (2010).

Collection and processing of plants and residual soils

Rice plants were harvested after 110 days of seedling transplantation. At harvest, the rice plantsamples (separated into 3 parts: roots, straw and grains) and the residual soil samples were collected and processed manually by following the procedures described in Chowdhury *et al.* (2010).

Chemical analysis

The initial and residual soils, and the FRH and RHA were analyzed for pH, organic carbon, arsenic, calcium, nitrogen, phosphorus, potassium, silicon, sulfur, and zinc, and the rice grain, husk, and straw were analyzed for total arsenic, calcium, phosphorus, silicon, and zinc following the analytical procedures described in Huq and Alam (2005). For the analysis of the elements, the soils and plant samples were digested using aqua regia (a mixture of nitric acid and hydrochloric acid in the ratio of 1:3). The concentrations of phosphorus in the soils and plant samples were determined colorimetrically using a spectrophotometer. The concentrations of arsenic, calcium, silicon, and zincin the soils and plant samples were determined using atomic absorption spectrometer (Shimadzu AA-7000 was used for analysing arsenic and silicon, and Varian AA-240 was used for analysing calcium and zinc). The quality control/quality assurance of the analysis was maintained following the standard procedure.

Statistical analysis

Statistical analyses were performed using the statistical software Minitab v.19 (State College PA) and SigmaPlot v.14 (Systat Software Inc., CA). The data were checked for normality and were transformed prior to statistical analysis where appropriate.

Results and discussion

Background properties of the soil and silicon-rich rice residues

The concentrations of arsenic, calcium, nitrogen, organic carbon, phosphorus, and zinc werefound to be higher in the Holocene floodplain soil compared to the Pleistocene terrace soil, which had higher concentrations of potassium, silicon and sulfur (Table I). The paddy soils of the Holocene floodplains have been found to be generally higher in a range of geochemical elements including arsenic compared to the Pleistocene terracesoils across Bangladesh (Chowdhury *et al.* 2017). Higher concentrations and mobilization of arsenic in the Holocene floodplain soils due to enhanced influence of the pedoenvironmental properties in the soils have also been reported by Martin *et al.* (2014; 2015). Silicon is the most abundant element in soil (Kabata-Pendias, 2011); however, silicon in the paddy soil svaries with the amount of crop residues remaining in the field that adds large amounts of silicon-rich phytoliths in the soils (Wilding, 1967; Parr and Sullivan, 2005; Wickramasinghe and Rowell, 2006).

The pH and the concentrations of all the elements analysed, except sulfur, were observed to be higher in the RHA compared to in the FRH (Table I). This was perhaps due to the fact that when the rice husks were burnt, the strongly bound fractions of the elements were released and came into the solution when extracted. Igwebike-Ossi (2017) also found lower concentrations of 13 different elements in fresh rice husks than in rice husk ash, which could be attributed to the presence of greater amounts of water and lignocellulosic components in fresh rice husk giving it a larger weight and volume of materials than in the husk ash (Mansaray and Ghaly, 1998; Stroevena *et al.*, 1999). When these components were removed during combustion, the reduction in weight and volume of the rice husk ash residue gave rise to higher concentrations of the elements in the husk ash.

Table I. Background properties of the initial soil and silicon-rich rice residues

Soil properties	Value			
	Holocene floodplain soil	Pleistocene terrace soil	Fresh rice husk	Rice husk ash
pH	6.86	5.61	7.10	7.10
Organic carbon (%)	1.25	0.84	6.00	-
Organic matter (%)	2.18	1.47	10.32	-
Arsenic (mg kg^{-1})	8.32	5.55	1.55	1.73
Calcium (mg kg^{-1})	18110	8310	2273	3706
Nitrogen (%)	0.16	0.09	1.04	1.15
Phosphorus (%)	0.06	0.03	0.004	0.63
Potassium (%)	0.27	0.34	0.90	1.71
Silicon (mg kg^{-1})	189	268	467	1800
Sulfur (%)	0.05	0.06	0.12	0.10
Zinc (mg kg^{-1})	77.75	45.45	72	91

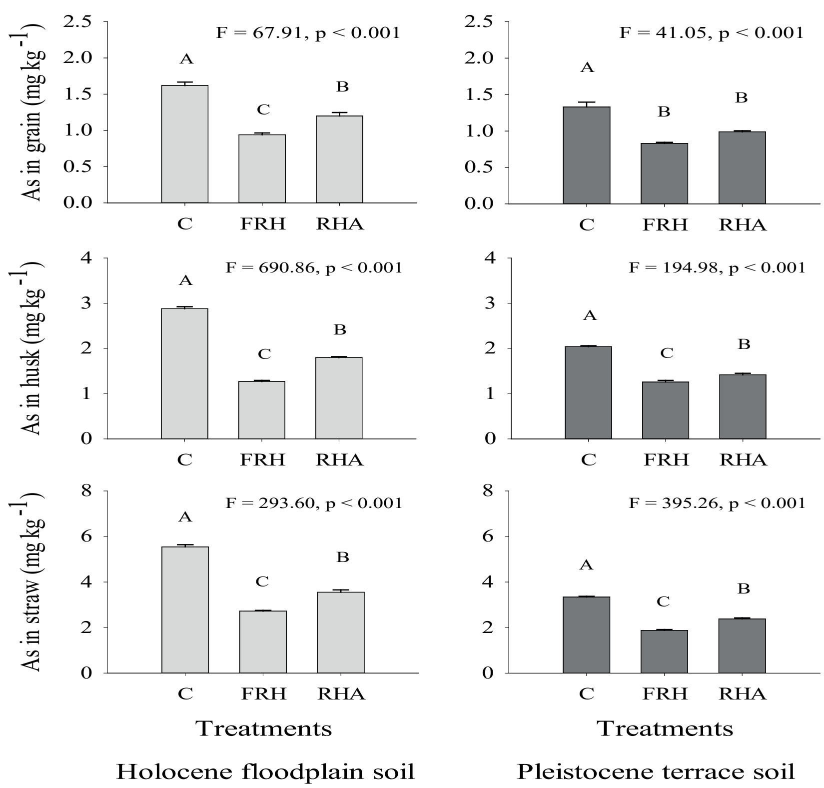

Fig. 1. The concentrations of total arsenic (As) in rice grain, husk, and straw grown in non-amended control (C) and silicon-rich fresh rice husk (FRH) and rice husk ash (RHA) amended Holocene floodplain and Pleistocene terrace soils. One-way analysis of variance was used to compare pair-wise the means of arsenic concentrations in grain, husk and straw individually at each of the treatments (C, FRH and RHA). Treatments that share the same letter (A – C) are not significantly different. The letters indicate Tukey groupings for the treatments with respect to their mean arsenic concentrations in rice grain, husk and straw

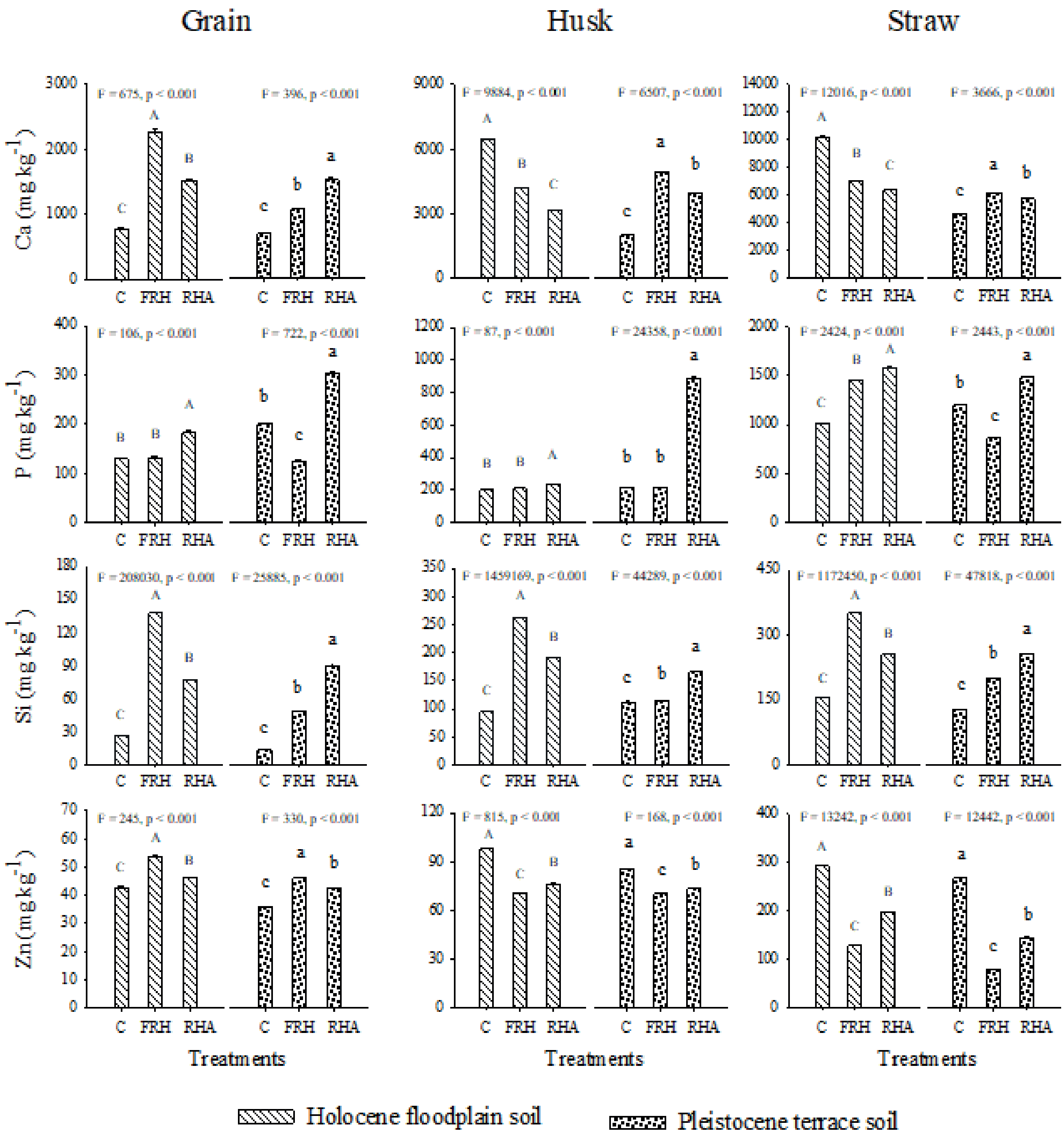

Fig. 2. The concentrations of total calcium (Ca), phosphorus (P), silicon (Si), and zinc (Zn) in rice grain, husk, and straw grown in non-amended control (C) and silicon-rich fresh rice husk (FRH) and rice husk ash (RHA) amended Holocene floodplain and Pleistocene terrace soils. One-way analysis of variance was used to compare pair-wise the means of the concentrations of calcium, phosphorus, silicon and zinc in grain, husk and straw individually at each of the treatments (C, FRH and RHA). Treatments that share the same letter (A – C) are not significantly different. The letters indicate Tukey groupings for the treatments with respect to their mean concentrations of calcium, phosphorus, silicon and zinc in rice grain, husk and straw

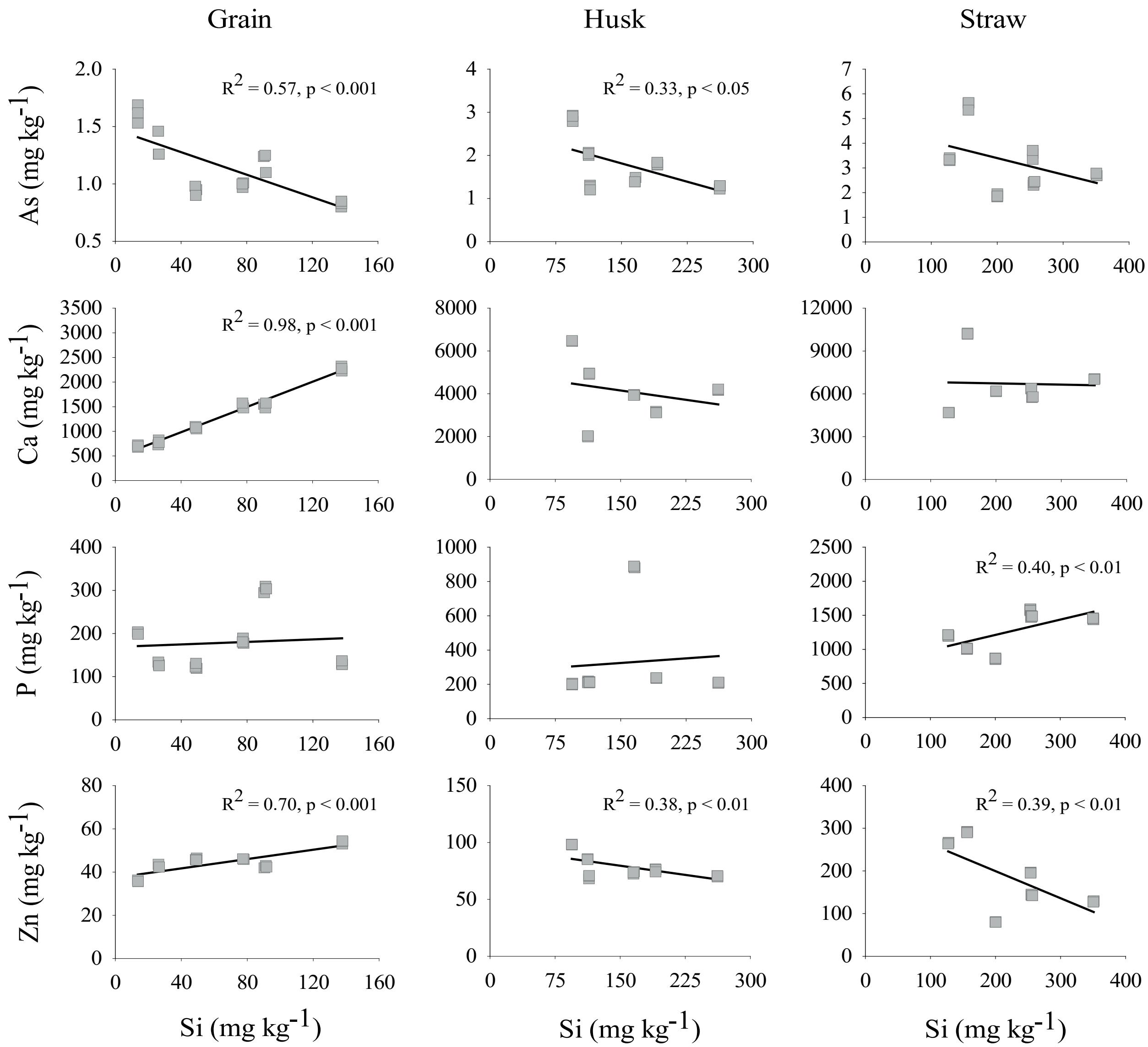

Fig. 3. Relationships between silicon (Si) and arsenic (As), calcium (Ca), phosphorus (P), and zinc (Zn) concentrations in rice grain, husk, and straw. The data are irrespective of the soil types (Holocene floodplain and Pleistocene terrace soils) and silicon-rich rice husk residues (fresh rice husk and rice husk ash)

Impacts of silicon fertilisation on rice

The incorporation of silicon-rich rice husk residues into soil was found to decrease total arsenic in rice grain, husk and straw (Fig. 1). In the Holocene floodplain soils, FRH reduced arsenic in grain, husk and straw by 39 – 45%, 55 – 58% and 50 – 51%, respectively, whereas RHA decreased arsenic in grain, husk and straw by 23 – 32%, 36 – 38% and 33 – 39%, respectively, compared to arsenic concentrations in grain, husk and straw in the non-amended control. In the Pleistocene terrace soils, FRH reduced arsenic in grain, husk, and straw by 36 – 40%, 36 – 41% and 42 – 45%, respectively, while RHA decreased arsenic in grain, husk and straw by 23 – 27%, 27 – 32% and 27 – 31%, respectively, compared to arsenic concentrations in grains, husk and straw in the non-amended control. These indicated that the silicon-rich rice husk residues had potentials to reduce arsenic accumulations in rice plants, the FRH being more efficient in reducing rice arsenic concentrations compared to the RHA. Significant variations in the concentrations of arsenic in grain

($^{ANOVA}F = 67.91$ and $^{ANOVA}F = 41.05$; $p < 0.001$, respectively, for Holocene floodplain and Pleistocene terrace soils), husk ($^{ANOVA}F = 690.86$ and $^{ANOVA}F = 194.98$; $p < 0.001$, respectively, for Holocene floodplain and Pleistocene terrace soils) and straw ($^{ANOVA}F = 293.60$ and $^{ANOVA}F = 395.26$; $p < 0.001$, respectively, for Holocene floodplain and Pleistocene terrace soils) were observed within the silicon amended and non-amended soils (Fig. 1). The mean concentration of arsenic in rice grain in the FRH amended Holocene floodplain soils was found to be significantly lower than the mean grain arsenic concentration in RHA treated soils, while in the FRH and RHA amended Pleistocene terrace soils, the reduction in grain arsenic was not observed to be statistically significant which could be related to the higher inherent silicon concentration in the Pleistocene terrace soil (Table I). Significant differences in arsenic concentrations in rice grain, husk and straw in response to different silicon amendments (fresh husk, husk ash and calcium silicate) were also observed by Teasley *et al.* (2017). Seyfferth *et al.* (2016) also reported reductions in toxic inorganic arsenic in rice grain and straw by 25 – 50% and at least 50%, respectively, for 3 different rice cultivars (*Oryza sativa* L., cv. M206, IR66 and Nipponbare) due to soil incorporation of silicon-rich fresh rice husk residues. This study also found FRH more promising than RHA in reducing the accumulation of inorganic or total arsenic in rice plants, particularly in rice grain. However, charred rice husk was found to be more effective than fresh rice husk in reducing arsenic accumulation in rice plant, which was perhaps due to the presence of more amorphous silica in the char as prepared under lower temperature than ash (Mansaray and Ghaly, 1998; Hanafi and Abo-El-Enein, 1980) that rendered its silicon to be more soluble in the media (Limmer *et al.*, 2018). Teasley *et al.* (2017) observed about 40% decrease in grain arsenic concentration due to fresh rice husk amendment, whereas neither calcium silicate nor rice husk ash amendments significantly affected total grain arsenic. Leksungnoen *et al.* (2019) also reported decreased inorganic grain arsenic by 20-24% as a result of the incorporation of RHA in soil. Alleviation of arsenic accumulation in rice by using other sources of silicon amendments, such as silica-gel (Li *et al.*, 2009; Seyfferth and Fendorf, 2012; Fleck *et al.*, 2013, Liu *et al.*, 2014) and different silicate minerals as silica fertilisers (Guo *et al.*, 2005; Guo *et al.*, 2007; Tripathia *et al.*, 2013; Wang *et al.*, 2016; Limmer *et al.*, 2018) have also been reported.

The decrease in the concentrations of arsenic,due to silicon-rich FRH and RHA amendments,were found to related to the increase in silicon concentrations in the grain, husk and straw. In the Holocene floodplain soils, FRH significantly increased ($p < 0.001$) silicon in grain, husk and straw by 423%, 178% and 124%, respectively, whereas RHA significantly increased ($p < 0.001$) silicon in grain, husk and straw by 195%, 102% and 62%, respectively, compared to silicon concentrations in grain, husk and straw in the non-amended control (Fig. 2). In the Pleistocene terrace soils, FRH significantly increased ($p < 0.001$) silicon in grain, husk, and straw by 258%, 1.42% and 57%, respectively, while RHA significantly increased ($p < 0.001$) silicon in grain, husk and straw by 565%, 47% and 100%, respectively, compared to silicon concentrations in grains, husk and straw in the non-amended control (Fig. 2). Silicon enrichment in the rice plantsdue to silicon fertilization (Seyfferth *et al.*, 2016; Teasley *et al.*, 2017; Cuong *et al.*, 2017) had potentials to decrease arsenic in the rice (Seyfferth *et al.*, 2016; Teasley *et al.*, 2017). The decrease in arsenic in the rice plants due to FRH and RHA amendments in the soils was perhaps regulated bythecompetitive interactions between silicon and arsenic for plant uptake and sorption. The addition of silicon-rich rice husk residues has been reported to increase plant-available silicon in soil porewater, which limitsthe uptake of arsenite by suppressing the expression of the Low silicon 1 (Lsi1) and 2 (Lsi2) genesin roots (Ma *et al.*, 2006; Ma and Yamaji, 2015), and enhance the competition of silicon with arsenite for uptake (Bogdan and Schenk, 2008; Seyfferth and Fendorf, 2012). Thereduced uptake of arsenic by roots, along with the reducedadsorption of arsenic onto soil solids due to enhanced competition with the released silicon from FRH and RHA in soil porewater (Luxton *et al.*, 2006), increases the concentration of arsenic available in soil porewater that may undergo redistribution and remobilisation through irrigation/ monsoon flooding (Roberts *et al.*, 2010) depending on the land topographical condition (Chowdhury *et al.*, 2021). However, the mobilization and retention of arsenic and silicon in paddy soils are also affected by a a number of other factors related to the properties and composition of the soil, such as temperature, pH, redox potential, clay and organic matter content, ionic constituents as well as the microbially mediated biogeochemical interactions that control the biogeochemical cycling of the elements in the soil (Bissen and Frimmel, 2003; Mahimairaja *et al.*, 2005; Sommer *et al.*, 2006; Moreno-Jiménez *et al.*, 2012; Pati *et al.*, 2016).

The FRH and RHA amendments significantly ($p < 0.001$) affected the concentrations of calcium, phosphorus and zinc in rice grain, husk and straw in the Holocene

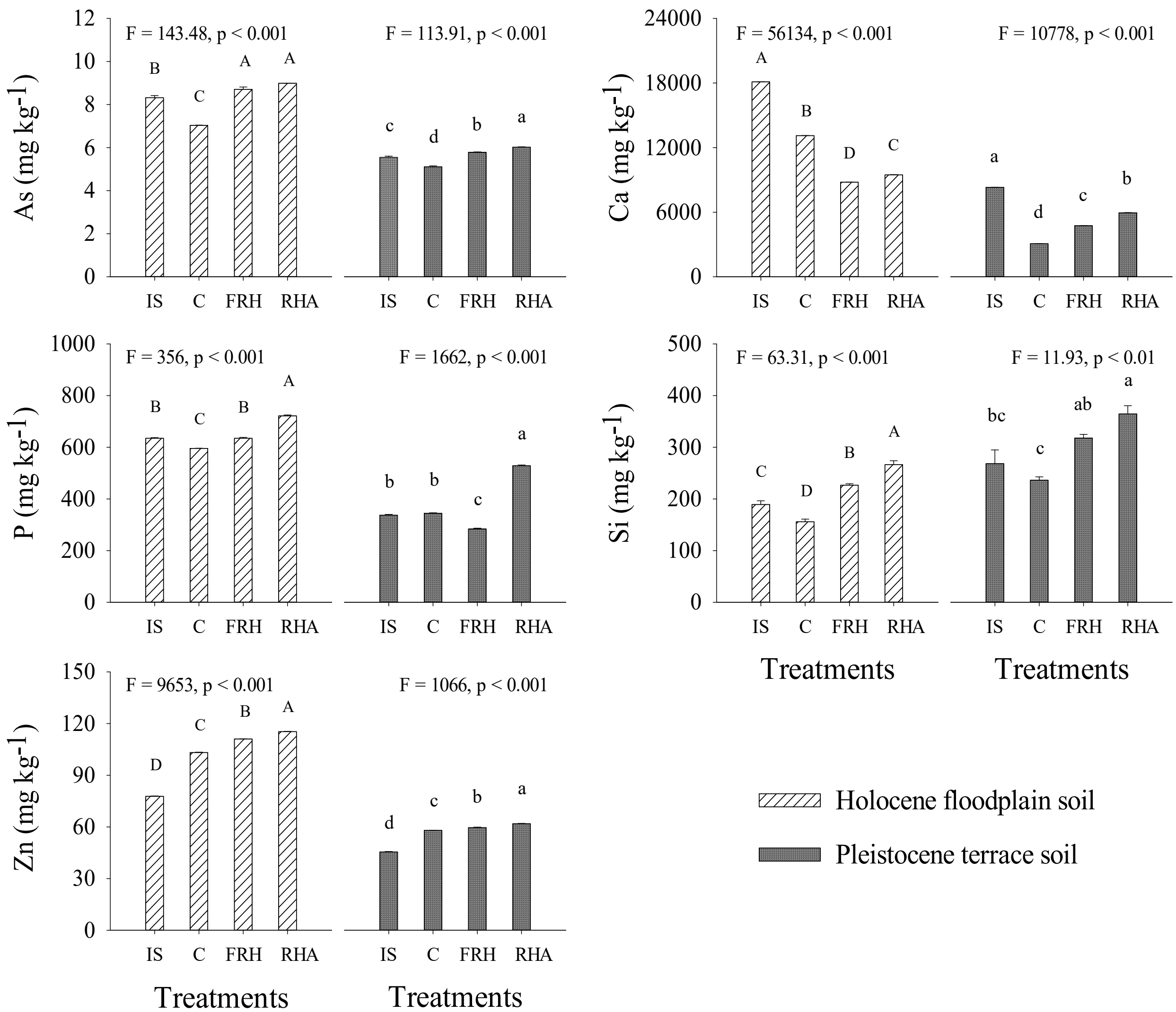

Fig. 4. Concentrations of arsenic (As), calcium (Ca), phosphorus (P), silicon (Si), and zinc (Zn), in the initial soil (IS) and the post-harvest soils of non-amended control (C) and fresh rice husk (FRH) and rice husk ash (RHA) amended soils of the Holocene floodplain and Pleistocene terrace. One-way analysis of variance was used to compare pare-wise the means of elemental concentrations in the initial and post-harvest soils at each of the treatments (C, FRH, and RHA). Treatments that share the same Tukey letter (A-D, for Holocene floodplain soils; a-d for Pleistocene terrace soils) are not significantly different. The bars are mean ± standard error of the mean

floodplain and Pleistocene terrace soils (Fig. 2). The mean concentrations of calcium, phosphorus (except for FRH in Pleistocene terrace soil), silicon and zinc in grain were found to be increased in both the FRH and RHA amended soils (Fig. 2). The accumulations of the nutrient elements were enhanced perhaps due to the less arsenic accumulation within the rice grain, as higharsenic concentrations within rice grains impacts negatively on other grain nutrient elements (Williams *et al.*, 2009; Norton *et al.*, 2010). While the increased silicon concentration in grain had negative impact on grain arsenic, it showed positive relationships with calcium ($^{linear\ regression}R^2 = 0.98$, $p < 0.001$) and zinc ($^{linear\ regression}R^2 = 0.70$, $p < 0.001$) concentrations within the grain (Fig. 3). Increased silicon concentration in soil solution perhaps mobilized the essential nutrients that enhanced the concentrations of the nutrients in rice grain (Cuong *et al.*, 2017; Swain and Rout, 2018).

Residual concentrations of the elements in soil

The incorporation of the silicon-rich FRH and RHA was found to increase, in general, the concentrations of all the elements, but calcium, in the post-harvest soils, compared to the concentrations in the initial soil (Fig. 4). The concentrations of arsenic were found to be significantly decreased in the post-harvest soils of the non-amended controls (by 16 and 8% in the Holocene floodplain and Pleistocene terrace soils, respectively), whereas the concentrations of arsenic were observed to be significantly increased by 4-8% in the FRH and RHA amended Holocene floodplain and Pleistocene terrace soils. Silicon was also found to be increased by 19-20% in the FRH amended post-harvest soils, and by 36-41% in the RHA amended post-harvest soils of the Holocene floodplain and Pleistocene terrace.

Conclusion

Silicon-rich rice husk residues (fresh huskand husk ash) have potentials to alleviate arsenic accumulation in rice grown in the geomorphologically different soils (Holocene floodplain and Pleistocene terrace soils) of Bangladesh. Soil amendments with rice husk residues could be an effective measure to mitigate arsenic contamination of the food chain in the arsenic affected areas of Bangladesh.

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