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Competitive Research Grant

Sub-Project Completion Report

on

Inhibition of arsenic accumulation in rice with phytofortification of microelements for nutritional safety

Project Duration

May 2017 to September 2018

Department of Agricultural Chemistry
Patuakhali Science and Technology University
Dumki, Patuakhali-8602

Submitted to
Project Implementation Unit-BARC, NATP 2
Bangladesh Agricultural Research Council
Farmgate, Dhaka-1215

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Acronyms

As- Arsenic

ANOVA-Analyses of variances

BRRI-Bangladesh Rice Research Institute

CRD-Completely Randomized Design

Fe-Iron

Kg-Kilogram

mg-Milligram

Mn-Manganese

P-Phosphorus

SD- Standard Deviation

Se-Selenium

Si-Silicon

TSW-Thousand Seed Weight

TGW-Thousand grain weight

Wt. –Weight

@-at the rate of

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Executive Summary

Arsenic (As) is a carcinogenic metalloid causing global environmental problem and adversely affects the health of millions of people. Rice represents a major route of As exposure in human after water. Inhibition of excessive As accumulation in rice is one of the major concerns in As affected areas. We hypothesized that phytofortification of micronutrients like iron (Fe), manganese (Mn), silicon (Si) and selenium (Se)in rice growing medium can reduce As bioaccumulation in rice and produce micronutrients enriched rice. So the objective of the current investigation was the reduction of As bioaccumulation in rice by phytofortification of these micro elements in rice growing medium.

The research was conducted in both artificially and naturally As contaminated soils. The naturally As contaminated soil was collected from *Faridpur* district, and soil from Dumki, Patuakhali was considered as uncontaminated of As. These soils was used for *Aus, Aman* and *Boro* rice cultivation in a pot experiment with different treatments of Fe, Mn, Si and Se, added from their respective salts. *Aus* rice (BRRI dhan 55) was cultivated in Patuakhali soil with artificially treatment of As,Fe, Mn, Si and Se. Then *Aman* (BRRI dhan 39) and *Boro* (BRRI dhan 47) rice was cultivated in naturally contaminated Faridpur soil along with different treatment of Fe, Mn, Si and Se. Different agronomic and elemental parameters of root, stem, leaf, husk and grain of rice were determined for As, Fe, Si, Mn and Se treatments.

Data revealed that, lower dose of Si (25 mg kg^{-1}) and Se (2.5 mg kg^{-1}) reduced the toxic effects of As at lower concentration (25 mg As kg^{-1}) in *Aus* rice and increased the photosynthetic pigments hence increased the yield in case of artificially As contamination of soil. The yield of *Aman* and *Boro* rice increased due to Si and Fe treatments in naturally As contaminated soil. Arsenic accumulation in rice grain is always lower than shoot for the Si and Fe treatment and the order is root > shoot > grain. Arsenic accumulation significantly ($p<0.01$ and 0.05) reduced in dehusked *Aman* and *Boro* grain which is below the recommended level (0.2 mg kg^{-1}) compare to control at previously Si and Fe treated soil. Mn and Se have discrete effect on the As accumulation in different rice varieties.

It is concluded that Si and Fe have the potentiality against As accumulation in above ground plant parts for all of experimental rice varieties. These trace elements reduced the As accumulation in rice shoot, husk and grain both in artificially and naturally As contaminated soil in addition to reduce the phytotoxicity of As at different levels hence increases the yield. The better effect was found in naturally As contaminated soil (grain As < 0.2 mg kg^{-1}, the recommended As level in rice) than artificially As incubated soil due to addition of different microelements. The increased concentrations of Si, Fe, Mn and Se in grain in As treated and naturally As contaminated soil indicated the enrichment of these elements into the rice grain. The findings of this research can be applied for the less As content and micro nutrients enriched rice production in arsenic prone area.

CRG Sub-Project Completion Report (PCR)

A. Sub-project Description

1. Title of the CRG sub-project: **Inhibition of arsenic accumulation in rice with phytofortification of microelements for nutritional safety**

2. Implementing organization: Patuakhali Science and Technology University

3. Name and full address with phone, cell and E-mail of PI/Co-PI (s):

Principal Investigator (PI): Dr. Md. Shariful Islam
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4. Sub-project budget (Tk):

   4.1 Total: 24,98,020.00 (Twenty Four Lac Ninety Eight Thousand and Twenty Taka Only)

   4.2 Revised (if any): 24,13,876.00 (Twenty Four Lac Thirteen Thousand Eight Hundred Seventy Six Taka Only)

5. Duration of the sub-project: From May, 2017 to September, 2018

   5.1 Start date (based on LoA signed): 8th May, 2017

   5.2 End date: 30 September 2018

6. Justification of undertaking the sub-project:

Arsenic is one of carcinogenic elements causing global health problem. Rice represents a major route of arsenic exposure in populations and about half of the total arsenic intake occurs through rice consumption grown in the arsenic affected areas of Bangladesh, India, China etc. So inhibition of excessive arsenic accumulation in rice is one of the major concerns in arsenic affected areas. It is very difficult to treat huge amount of arsenic contaminated soil/water for arsenic free rice production but phytofortification (part of biofortification) of micro/trace elements can inhibit arsenic uptake in rice in addition to enrich micronutrients. Micronutrients are not only essential for plant growth and development but also integral to human and animal health. More than two billion people are micronutrient deficient. Micronutrient deficiencies or "hidden hunger" affect about 38% of pregnant women and 43% of preschool children worldwide and are most prevalent in developing countries. The micronutrients like iron (Fe), manganese (Mn), silicon (Si), selenium (Se) etc. are of particular interest, given that all are essential micronutrients for higher organism. For example, Fe serves as an important cofactor for various enzymes performing basic functions in humans. About 15% of the total population suffering from anemia due to Fe decencies. Si is not only causes the strengthening of connective tissues and bones, but also useful in taking care of nails, hair and skin. Si also plays a vital role in the prevention of arthrosclerosis, insomnia, skin disorders and tuberculosis. The application of 1 m M Si significantly reduces the As (III) content in tolerant rice variety by 31% and 24% when plant is exposure to 10 and 25µM As (III) concentration (Tripathi *et al*., 2013). But in case of sensitive variety the As content reduction were 24% and 18% with the same concentration of arsenite exposure (Tripathi*et al*., 2013). Mn decencies cause a serious problem and can lead to asthma and severe birth defects. Se is also an essential micronutrient for humans and animals to form seleno proteins which play critical roles in reproduction, thyroid hormone metabolism, DNA synthesis, and protection from oxidative damage and infection. There are approximately one billion people facing with Se malnutrition in the world (Carey *et al*., 2012). Se deficiency and low Se daily dietary intake can cause endemic diseases or other significant environmental health problems, such as *Keshan* disease (a degenerative heart disease observed in *Keshan*, China). The combined effects of these micronutrient decencies pose significant threat to human health. Plant-derived food products contain different amounts of micronutrients because concentrations of micronutrients in soil vary substantially in the natural environment. We hypothesized that phytofortification of micro/trace elements in rice growing medium can inhibit/reduce arsenic bioaccumulation in rice and produce micronutrients enriched rice. There are some scattered researches about arsenic uptake within crop plants affected by micronutrients/trace elements. But there are no such findings about phytofortication of micronutrients and inhibition of arsenic uptake simultaneously in rice. The findings of this research will be beneficial for millions of arsenic affected peoples because they can apply micronutrients as fertilizer for inhibition of arsenic accumulation in foods and concurrently get rid of suffer from micronutrients malnutrition.

7. **Sub-project goal**: To inhibit arsenic bioaccumulation in rice in arsenic affected areas through micro/trace element(s) phytofortification

8. **Sub-project objective**(s):

- To examine the potentiality of different micro/trace elements like Fe, Mn, Si and Se for inhibition of arsenic bioaccumulation in rice.
- To find out the suitable dose of micro/trace elements for application in different arsenic contaminated soils to avoid deficiency and/or toxicity, and
- To analyze the bioaccumulation/enrichment of micro/trace elements and arsenic in rice and soil.

9. **Implementing location (s):** Patuakhali Science and Technology University

10. Methodology(in brief):

10.1 Soil sampling, rice cultivation and agronomic data collection

Arsenic uncontaminated and naturally As contaminated surface soils were collected from Patuakhali and Faridpur district, respectively. Uncontaminated surface soil was incubated with As and other microelements like Fe, Mn, Si and Se at different doses. The rates of As were 0, 25 and 50 mg As Kg^{-1} from sodium arsenite ($NaAsO_2$); and the rates of micronutrients were 0, 50 and 100 mg Fe Kg^{-1}; 0, 50 and 100 mg Mn Kg^{-1}; 0, 25 and 50 mg Si Kg^{-1}; 0, 2.5 and 5.0 mg Se Kg^{-1} soil from the ferrous sulphate ($FeSO_4.7H_2O$), manganese sulphate ($MnSO_4.H_2O$), sodium metasilicate ($Na_2SiO_3.9H_2O$) and sodium selenite (Na_2SeO_3), respectively. The combination of As and micronutrient was As-Fe, As-Mn, As-Si and As-Se. These four combinations were added separately and replicated for thrice with control. In case ofAs-Fe experiments, the numbers of treatments were 9 (3 x 3) and including three replication the total number of pots were 27 (9x 3). Others treatments were done in the same way. Ten kg of air dried debris free meshed soil were taken in each pot and treatments were added and mixed thoroughly. The N, P, K, S and Zn were supplied as recommended dose for each variety. *Aus* rice (BRRI dhan 55) seedling (2 to 3) was transplanted in each pot for incubated soils. Irrigation was done from As free ground water and plastic shading were arranged for the protection of rain.

Naturally arsenic contaminated soil samples were collected from five different spot of Faridpur district and analysed for As, Fe and Mn by Atomic absorption spectrophotometer and the total As concentration was 33, 25, 11, 10 and 8 ppm. Then 33 and 11 ppm naturally As contaminated soils were selected and collected for Aman rice cultivation. Aman rice (BRRI dhan 39) seedlings (2 to 3) were transplanted in the treated soil and replicated for thrice. After harvesting Aman rice, Boro (BRRI dhan 47) rice (2seedlings/pot) was transplanted in the same soil. Agronomic data were collected at vegetative and reproductive stage. Chlorophyll a, b, total chlorophyll and carotenoid were determined at vegetative stage. Chlorophyll and carotenoid concentration in leaves were extracted using 80% chilled acetone, contents of these were estimated using the equation given by Lichtenthaler and Wellburn (1983). Anthocyanin content was estimated in leaves as described by Sims and

Gamon (2002). The rice was harvested and root, stem, husk, grain were separated and processed for further analysis.

10.2 Soil analysis

A sub-sample weighing of 0.5-1 g was transferred into a dry clean digestion flask. 10 mL of conc. HNO_3 was then added to it. After leaving for overnight, the flask was heated with a digestion chamber (RAYPA Compact Digestion system, MBCM-12, Spain) at a temperature slowly raised to 130^{o}C. The contents of the flask were heated until they became clean and colorless. The digests were cooled and 2 mL H_2O_2 was added into it and again heated for 4 hrs. The digest were cooled and diluted with distilled water and filtered through Whatman No. 42 filter paper. The volume was made up to 100 mL with distilled water and kept into dry plastic bottle and stored in freeze for further analysis of As, Fe, Mn, Si and Se. Arsenic, Fe,Ca, Mg and Mn were determined with the help of Atomic Absorption Spectrophotometer and Si by UV-VIS spectrophotometer. The soil samples was also extracted with different reagent for determination of exchangeable K, available P and S. K was determined by flame emission spectrophotometric method, P and S by UV-VIS spectrophotometry. Nitrogen and organic carbon was determined by Kjeldahl method and wet oxidation method, respectively.

10.3 Plant analysis

Exactly 0.2-0.5 g root, stem, husk and grain sample was extracted separately with the help of HNO_3 and H_2O_2 as described in soil analysis. Then total As, Fe, Mn, Si and Se were determined followed by the instrumental methods of analysis as above.

10.4 Statistical analysis

Results were expressed as the means ± SE of three replicates. Significance degree is calculated using a *t*-test. The data obtained from different agronomic parameters were analyzed using Minitab 17 statistical software (Minitab Inc, State College, PS, USA) to find out the significance of variation resulting from the experimental treatments. ANOVA for different parameters were performed by general linear model (GLS) and the means were separated with Tukey at 1% and 5% level of probability.

11. Results and discussion

11.1. Exp-1: Aus rice (BRRI dhan 55) cultivation in artificially As contaminated and microelements incubated soil

Agronomic data like plant height, number of tillers, thousand seed weight, filled and unfilled grains, fresh weight, yield per pot and photosynthetic pigments data like chlorophyll a, chlorophyll b, total chlorophyll, anthocyanin and carotenoids are presented in the Table 1-4.The effect of different micronutrients on As accumulation in shoot husk and grain of *Aus* rice (BRRI dhan 55) were given in Fig. 1-4.

11.1.1 Effects of Si and As application on agronomic parameters and photosynthetic pigments of *Aus* rice

Maximum number of grain (114.50 grains $panicle^{-1}$), filled grains (103.50 $panicle^{-1}$), thousand grain weight (24.82 g) and yield (24.40 g pot^{-1}) were recorded in *Aus* rice at $As_{25}Si_{25}$ (25 mg As and 25 mg Si Kg^{-1} soil) treatment (Table 1). Plant height was reduced due to increasing rate of only Si application (50 mg Si Kg^{-1} soil, 92.38 cm) compared to control (107.5 cm)**.** But the combined application of As with Si (25 mg As and 25 mg Si Kg^{-1} soil) increased the plant height, though the increment was less than that of control (Table 1). It indicated that at a certain levels of concentration Si and As has synergistic effects on plant height.

Photosynthetic pigments play an important role in accumulation of carbohydrate in rice grain that finally leads to the yield of rice. The results indicated that the concentrations of chlorophyll-a, chlorophyll-b, carotenoid and anthocyanin were increased with the single application of Si @ of 25 mg Kg^{-1} soil. But the concentrations of these biochemical substances drastically reduced with the increasing rate of Si application (50 mg Si Kg^{-1} soil). On the other hand, higher levels of As application (50 mg As Kg^{-1} soil) lead to accumulate these biochemical substance in the green leaves of rice. In case of combined application of Si and As (25 mg Si and 25 mg As Kg^{-1} soil), higher contents of chlorophyll-a, chlorophyll-b, carotenoid and anthocyanin were observed (Table 1). For this reason yield might be increased at this treatment. Although higher levels of combined application of Si and As reduced the concentration of these biomolecules. So at certain levels of Si treatment (i.e., 25 mg kg^{-1}) reduced the toxic effect of As in BRRI dhan 55 and increased the yield.

11.1.2 Effects of Fe and As application on agronomic parameters and photosynthetic pigments of *Aus* rice

Compared to control (107.5 cm), the increasing rate of Fe application (50 and 100 mg Fe Kg^{-1} soil) reduced the plant height of *Aus* rice**.** The single (25 and 50 mg As Kg^{-1} soil) and combined application of As with Fe (25 mg As and 50 mg Fe Kg^{-1} soil) increased the plant height compared to single application of Fe, but the increment was less than that of control (Table 2). It indicated that at a certain levels of concentration Fe and As has synergistic effects on plant height. Except thousand seed weight, the number of effective tiller, total number of grain, filled grain and yield of *Aus* rice also decreased with the treatment of Fe and As both singly and combined, compared to control (Table 2).

Table 1. Agronomic and photosynthetic pigments parameters of *Aus* rice treated by As and Si

Treatment / Parameter	Plant Height (cm)	No. of Effective Tillers	Total Grains	Filled Grains	Fresh wt (g)	TGW (g)	Yield (g/pot)	Chl. a (mg/g)	Chl. b (mg/g)	Caroten. (mg/g)	Anthocyanin (mg/g)
As_0Si_0	107.50^{a}	10.33^{a}	107.33^{ab}	96.67^{ab}	21.99^{bc}	24.40^{a}	24.37^{ab}	0.55^{ab}	0.78^{b}	0.31^{a}	1.61^{b}
As_0Si_{25}	88.05^{d}	9.00^{a}	62.0^{f}	95.50^{ab}	23.83^{b}	23.26^{a}	19.99^{bc}	0.63^{a}	1.13^{a}	0.42^{a}	2.17^{a}
As_0Si_{50}	$92,38^{cd}$	11.00^{a}	70.50^{ef}	95.50^{ab}	35.85^{a}	23.80^{a}	25.00^{a}	0.01^{c}	0.01^{b}	0.03^{b}	0.21^{e}
$As_{25}Si_0$	97.60^{bc}	9.00^{a}	95.00^{bcd}	79.50^{de}	24.93^{b}	23.64^{a}	16.91^{cd}	0.54^{ab}	1.04^{a}	0.38^{a}	1.01^{cd}
$As_{50}Si_0$	100.13^{b}	7.00^{a}	87.00^{cd}	81.50^{cd}	17.73^{d}	24.86^{a}	14.18^{d}	0.78^{a}	1.20^{a}	0.40^{a}	2.52^{a}
$As_{25}Si_{25}$	102.45^{ab}	9.50^{a}	114.50^{a}	103.50^{a}	24.53^{b}	24.82^{a}	24.33^{ab}	0.73^{a}	1.21^{a}	0.37^{a}	2.65^{a}
$As_{50}Si_{25}$	97.03^{bc}	8.00^{a}	99.50^{bc}	90.50^{bc}	22.77^{bc}	23.72^{a}	17.17^{cd}	0.33^{b}	0.45^{c}	0.20^{ab}	2.20^{a}
$As_{25}Si_{50}$	99.98^{b}	9.50^{a}	90.5^{cd}	69.50^{e}	22.11^{bc}	27.72^{a}	18.30^{cd}	0.56^{ab}	0.62^{bc}	0.37^{a}	0.53^{de}
$As_{50}Si_{50}$	86.80^{d}	7.00^{a}	81.5^{d}	70.00^{e}	19.09^{cd}	24.14^{a}	11.83^{e}	0.55^{ab}	0.72^{b}	0.39^{a}	1.21^{bc}
Level of sig.	**	NS	**	**	**	NS	**	**	**	**	**
CV%	7.03	19.86	18.59	13.97	21.55	7.36	24.70	45.12	49.18	45.42	54.85

[In a column, values having different letter(s) differ significantly, but with common letter(s) do not differ significantly at 5% and 1% level of probability analyzed by Tukey, denoted by * and **, respectively. NS= Not significant, CV = Coefficient of variation]

Table 2. Agronomic and photosynthetic pigments parameters of *Aus* rice treated by As and Fe

Treatment/ Parameter	Plant Height (cm)	No. of Effective Tillers	Total Grains	Filled Grains	Fresh wt (g)	TGW (g)	Yield (g/pot)	Chl. a (mg/g)	Chl. b (mg/g)	Caroten. (mg/g)	Anthocyanin (mg/g)
As_0Fe_0	107.50^{a}	10.33^{a}	107.33^{a}	96.67^{a}	21.99^{abc}	24.40^{a}	24.37^{a}	0.55^{a}	0.78^{b}	0.31^{a}	1.6^{a}
As_0Fe_{50}	87.65^{c}	11.00^{a}	89.00^{c}	73.50^{c}	24.27^{ab}	24.96^{a}	20.18^{abc}	0.02^{d}	0.01^{d}	0.11^{b}	0.52^{a}
As_0Fe_{100}	85.83^{c}	11.50^{a}	87.50^{c}	73.50^{c}	21.03^{abc}	23.86^{a}	20.17^{abc}	0.29^{bc}	0.29^{c}	0.35^{a}	0.40^{a}
$As_{25}Fe_0$	97.60^{abc}	9.00^{a}	95^{abc}	79.50^{b}	24.93^{a}	23.64^{a}	16.91^{bcd}	0.54^{ab}	1.04^{a}	0.38^{a}	1.01^{a}
$As_{50}Fe_0$	100.13^{ab}	7.00^{a}	87^{c}	81.5^{b}	17.73^{cd}	24.86^{a}	14.18^{d}	0.78^{a}	1.20^{a}	0.40^{a}	2.52^{a}
$As_{25}Fe_{50}$	90.80^{bc}	9.50^{a}	95.50^{abc}	79^{b}	18 81^{bcd}	24.68^{a}	18.52^{bcd}	0.01^{d}	0.01^{d}	0.11^{a}	0.31^{a}
$As_{50}Fe_{50}$	97.57^{abc}	9.00^{a}	89^{c}	67.50^{d}	21.10^{abc}	25.12^{a}	15.26^{cd}	0.29^{bc}	0.29^{c}	0.31^{a}	0.81^{a}
$As_{25}Fe_{100}$	87.38^{c}	9.50^{a}	106.50^{ab}	93^{a}	13.98^{d}	24.84^{a}	21.95^{ab}	0.11^{cd}	0.11^{cd}	0.29^{a}	0.30^{a}
$As_{50}Fe_{100}$	90.47^{bc}	11.00^{a}	93.00^{bc}	73^{c}	22.59^{abc}	24.94^{a}	20.01^{abc}	0.13^{cd}	0.13^{cd}	0.23^{a}	0.28^{a}
Level of sig.	*	NS	**	**	**	NS	**	**	**	NS	NS
CV%	8.21	21.83	8.93	11.75	17.64	7.07	18.44	88.84	103.63	46.71	96.47

[In a column, values having different letter(s) differ significantly, but with common letter(s) do not differ significantly at 5% and 1% level of probability analyzed by Tukey, denoted by * and **, respectively. NS= Not significant, CV = Coefficient of variation]

The concentrations of chlorophyll-a, chlorophyll-b, carotenoid and anthocyanin were drastically decreased at lower dose of Fe (50 mg Fe Kg^{-1} soil) and combined lower dose of Fe and As (50 mg Fe and 25 mg As Kg^{-1} soil), but at higher dose of only Fe application (100 Fe Kg^{-1} soil), the concentration of these biomolecules were slightly increased though the values were less than that of control. On the other hand, lower dose Fe and higher dose of As (50 mg Fe and 50 mg As Kg^{-1} soil) also increased the concentration of these photosynthetic pigments. Although higher levels of combined application of Fe and As (100 mg Fe and 50 mg As Kg^{-1} soil) reduced the concentration of these biomolecules.

Table 3. Agronomic and photosynthetic pigments parameters of Aus rice treated by As and Mn

Treatment / Parameter	Plant Height (cm)	Effective Tillers	Total Grains	Filled Grains	Fresh wt (g)	TGW (g)	Yield (g/pot)	Chl. a (mg/g)	Chl. b (mg/g)	Caroten (mg/g)	Anthocyanin (mg/g)
As0Mn0	107.50[a]	10.33[a]	107.33[a]	96.67[a]	21.99[b]	24.40[a]	24.37[a]	0.55[b]	0.78[bc]	0.31[cd]	1.61[b]
As0Mn50	86.70[cde]	11.00[a]	88.00[bcd]	73.50[b]	21.20[bc]	24.96[a]	19.90[a]	0.32[c]	1.11[a]	0.27[de]	0.42[g]
As0Mn100	82.30[e]	11.50[a]	89.00[bc]	73.50[b]	21.04[bc]	23.86[a]	20.37[a]	0.28[c]	1.11[a]	0.31[de]	0.44[fg]
As25Mn0	97.60[abc]	9.00[a]	95.00[b]	79.50[b]	24.93[a]	23.64[a]	16.91[cd]	0.54[b]	1.04[a]	0.38[ab]	1.01[c]
As50Mn0	100.13[ab]	7.00[a]	87.00[bcd]	81.50[b]	17.73[e]	24.86[a]	14.18[de]	0.78[a]	1.20[a]	0.40[a]	2.52[a]
As25Mn50	91.77[bcde]	10.00[a]	74.50[e]	49.50[c]	18.16[de]	24.68[a]	12.22[ef]	0.55[b]	0.44[de]	0.22[e]	0.47[f]
As50Mn50	93.95[bcd]	8.00[a]	80.50[cde]	73.50[b]	17.15[e]	24.38[a]	14.34[de]	0.15[c]	0.21[e]	0.33[bc]	0.72[d]
As25Mn100	84.08[de]	7.50[a]	76.5[de]	58.00[c]	18.47[cde]	24.54[a]	10.67[f]	0.62[ab]	0.57[cd]	0.22[e]	0.54[e]
As50Mn100	87.2[cde]	8.00[a]	94.00[b]	76.50[b]	20.61[bcd]	23.98[a]	14.68[de]	0.64[ab]	0.55[cd]	0.23[e]	1.00[c]
Level of sig.	*	NS	**	**	**	NS	**	**	**	**	**
CV%	9.28	19.08	11.80	18.07	12.62	3.86	26.27	40.79	43.87	22.34	69.13

[In a column, values having different letter(s) differ significantly, but with common letter(s) do not differ significantly at 5% and 1% level of probability analyzed by Tukey, denoted by * and **, respectively. NS= Not significant, CV = Coefficient of variation]

11.1.3 Effects of Mn and As application on agronomic parameters and photosynthetic pigments of *Aus* rice

The increasing rate of Mn application (50 and 100 mg Mn Kg^{-1}) in soil decreased the plant height of *Aus* rice compared to control (107.5 cm)**.** The single (25 and 50 mg As Kg^{-1} soil) and combined application of As with Mn (25 mg As and 50 mg Mn Kg^{-1} and 50 mg As and 100 mg Mn Kg^{-1} soil) increased the plant height compared to single application of Mn, but the increment was less than that of control (Table 3). It indicated that at a certain levels of concentration Mn and As has synergistic effects on plant height. Except thousand seed weight, the number of effective tiller, total number of grain, filled grain and yield of *Aus* rice also decreased with the single and combined treatment of Mn and As (Table 3).The higher dose of As and Mn (50 mg As and 100 mg Mn Kg^{-1}

soil) tends to increase the concentration of chlorophyll-a, chlorophyll-b, carotenoid and anthocyanin than lower dose As and Mn (25 mg As and 50 mg Mn Kg^{-1} soil), though the concentrations of all the biomolecules were less than that of control (Table 3). The values obtained from the chemical analyses indicated the negative effects of As and Mn in accumulation of carbohydrate accumulating biomolecules in the leaves of *Aus* rice plants.

Table 4. Agronomic and photosynthetic pigments parameters of Aus rice treated by As and Se

Treatment/ Parameter	Plant Height (cm)	No. of Effective Tillers	Total Grains	Filled Grains	Fresh wt (g)	TGW (g)	Yield (g/pot)	Chl. a (mg/g)	Chl. b (mg/g)	Caroten. (mg/g)	Anthocyanin (mg/g)
$As_0Se_{0.0}$	107.50^{a}	10.33^{a}	107.33^{a}	96.67^{a}	21.99^{bc}	24.40^{bc}	24.37^{a}	0.55^{b}	0.78^{b}	0.31^{ab}	1.61^{d}
$As_0Se_{2.5}$	85.32^{de}	8.50^{a}	70.5^{de}	56.50^{de}	27.03^{a}	26.06^{ab}	12.52^{e}	0.25^{cd}	0.58^{bc}	0.18^{b}	2.08^{c}
$As_0Se_{5.0}$	94.13^{bc}	9.50^{a}	58.00^{f}	54.50^{de}	26.95^{a}	24.84^{abc}	12.86^{de}	0.14^{d}	0.24^{de}	0.15^{a}	1.32^{e}
$As_{25}Se_{0.0}$	97.60^{bc}	9.00^{a}	95.00^{b}	79.50^{bc}	24.93^{ab}	23.64^{c}	16.91^{b}	0.5^{b}	1.04^{a}	0.38^{ab}	1.01^{fg}
$As_{50}Se_{0.0}$	100.13^{ab}	7.00^{a}	87.00^{c}	81.50^{bc}	17.73^{d}	24.86^{abc}	14.18^{cd}	0.78^{a}	1.20^{a}	0.40^{ab}	2.52^{b}
$As_{25}Se_{2.5}$	89.83^{cde}	10.00^{a}	98.50^{b}	90.00^{ab}	22.88^{bc}	26.75^{a}	24.47^{a}	0.41^{bc}	0.44^{cd}	0.40^{ab}	1.15^{ef}
$As_{50}Se_{2.5}$	81.92^{e}	6.50^{a}	73.50^{d}	63.00^{d}	12.65^{e}	24.66^{bc}	10.10^{f}	0.52^{b}	0.61^{bc}	0.27^{ab}	1.70^{d}
$As_{25}Se_{5.0}$	91.12^{cd}	10.50^{a}	97.00^{b}	75.50^{c}	23.18^{bc}	19.34^{d}	15.33^{c}	0.12^{d}	0.14 e	0.12^{b}	0.82^{g}
$As_{50}Se_{5.0}$	89.78^{cde}	7.50^{a}	63.00^{ef}	49.50^{e}	20.3^{cd}	24.98^{abc}	9.27^{f}	0.54^{b}	0.60^{bc}	0.37^{ab}	2.95^{a}
Level of sig.	*	NS	**	**	**	**	**	**	**	**	**
CV%	8.53	18.31	20.41	22.79	20.49	8.58	34.29	50.85	53.81	69.79	40.92

[In a column, values having different letter(s) differ significantly, but with common letter(s) do not differ significantly at 5% and 1% level of probability analyzed by Tukey, denoted by * and **, respectively. NS= Not significant, CV = Coefficient of variation]

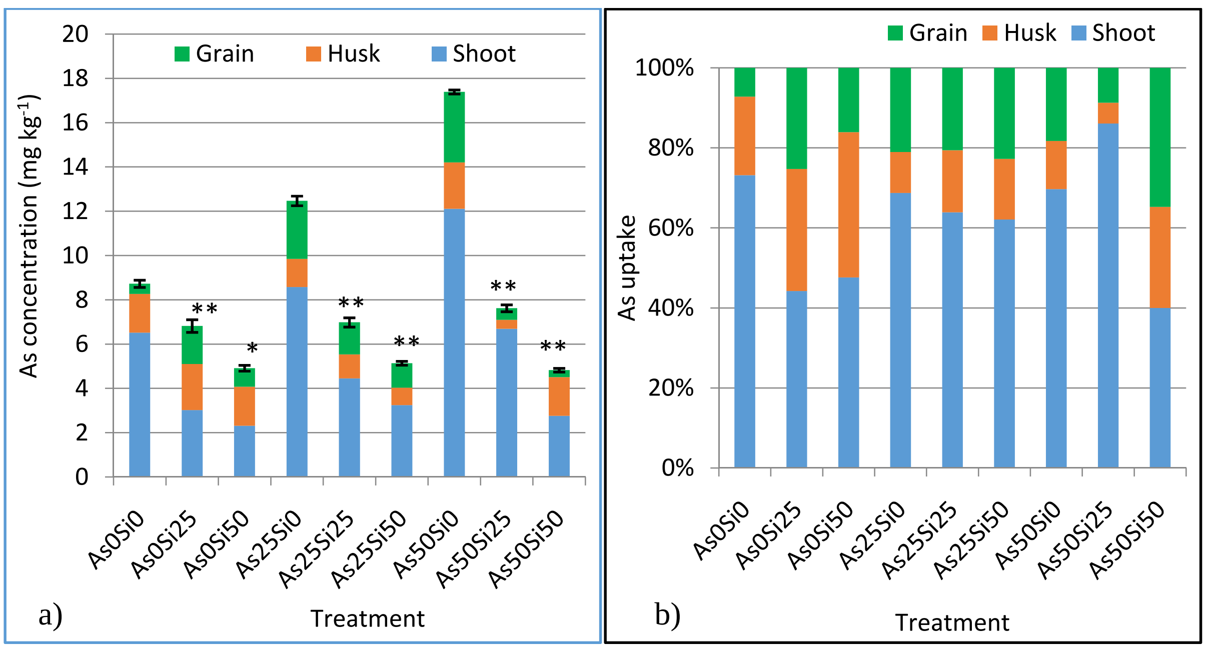
11.1.4 Effects of Se and As application on agronomic parameters and photosynthetic pigments of *Aus* rice

Except thousand grain weight and yield the values of almost all agronomic parameters were decreased with the single and combined application of Se and As (Table 4).Compared to control thousand seed weight and yield were slightly increased with lower combined dose of Se and As (2.5 mg Se and 25 mg As Kg^{-1} soil).At control the thousand seed weight was 24.40 g and it was 26.75 g when soil was treated with 2.5 mg Se Kg^{-1} in combination with 25 mg $AsKg^{-1}$(Table 4). It is indicated that at lower concentrations of Se might be reduced the toxic effects of As on *Aus* rice yield.

Data from Table 4 explained that except anthocyanin, the values of other photosynthetic pigments were decreased with the single and combined increasing rates of Se and Se with As application to the soil. But anthocyanin content was remarkably increased with higher levels of Se in combination with higher levels of As.

11.1.5 Effects of Si, Fe, Mn and Se on As contents in shoot, husk and grain of *Aus* rice

The effects of Si, Fe, Mn and Se on As uptake characteristics in shoot, husk and grain of *Aus* rice are depicted in Fig. 1-4.



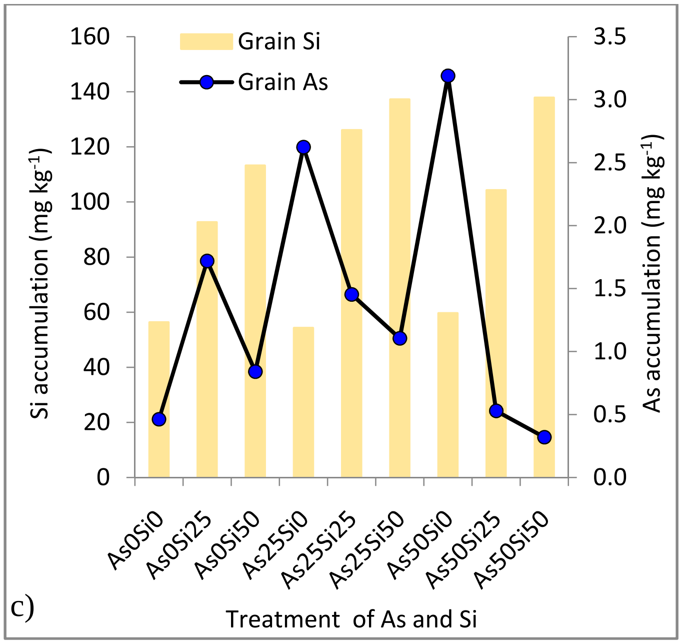


Fig. 1. a) As concentration (mg kg^{-1}), b) As uptake rate (%) in shoot, husk and grain, and c) As and Si accumulation in grain after treated with Si in Aus rice [Error bar indicates grain Mean± SD. ** and * indicates significantly difference against respective Si control at p < 0.01 and 0.05, respectively]

11.1.6 Effects of Si on As accumulation in *Aus* rice

Arsenic content in shoot was increased with the increasing application of As (Fig.1a). Maximum As accumulation in shoot (12.112 mg As kg^{-1}) was determined when maximum amount of As (50 mg As kg^{-1}) was applied. It indicates that the rice plant has ability to uptake As from the soil. But when soil was treated with Si singly and in combination with As, the concentration of As in shoot was reduced. Fig.1a also reflected that shoot As accumulation is minimum when soil was treated with 50 mg Si kg^{-1} and 50 mg As kg^{-1}. Compared to control higher dose of As application also accumulate higher amount of As in husk. It is found that husk As accumulation is minimum at 25 mg Si kg^{-1} and 50 mg As kg^{-1} treated soil (Fig.1a).

Grain As content was also increased with the increasing rate of applied As. Application of Si in soil @25 and 50 mg kg^{-1} reduced the As content in rice grain even when the soil was treated with 50 mg As kg^{-1}(Fig. 1c). Arsenic uptake percentile indicated that the lowest As was accumulated in grain when soil was treated with 25 mg Si kg^{-1} and 50 mg As kg^{-1} but in that case shoot As concentration was highest (Fig.1b).

11.1.7 Effects of Fe on As accumulation in *Aus* rice

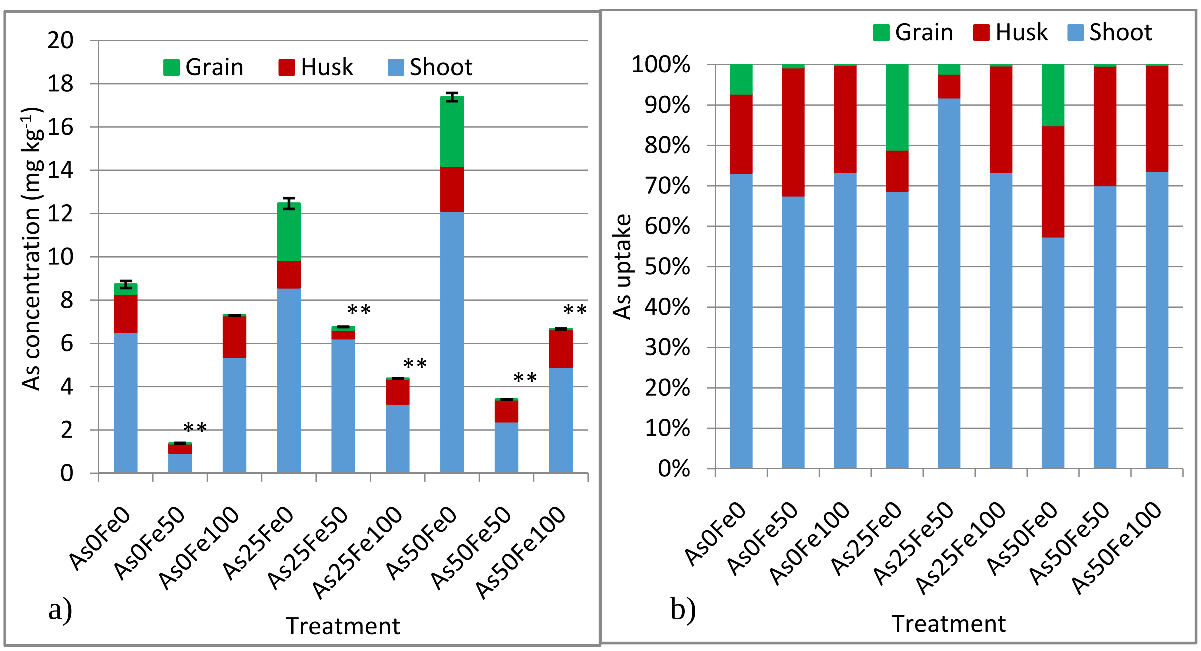
Grain As contents were decreased with the increasing rate of single and combined application of Fe in soil. Fig. 2a reflected that in all cases both single and combined application of Fe with As, the concentrations of As in grain was very minimum or below the detection limit (0.01 mg As kg^{-1}). So, Fe application in soil significantly inhibits the uptake and accumulation of As in rice grain (Fig. 2a,b,c).

Arsenic contents in the shoots of Fe treated soils were reduced compared to control and As treated soils. The lowest As content in shoot (2.39 mg kg^{-1}) was determined with the application of 50 mg As and 50 mg Fe kg^{-1} soil. The increase of Fe application from 50 mg kg^{-1} to 100 mg kg^{-1} in soil contaminated with 25 mg As kg^{-1} then the concentration of As in shoot was reduced almost 50% (Fig. 2b). Husk As concentrations were decreased with the increasing rate of Fe application in soil. The lowest As accumulation in husk was obtained at 50 mg kg^{-1} Fe and 25mg kg^{-1} As contaminated soil compared to soil with other treatments (Fig. 2a). Grain As concentration was below 0.5 mg kg^{-1} for both of Fe treatments (Fig. 2c).

11.1.8 Effects of Mn on As accumulation in *Aus* rice

Grain As accumulation was higher than husk for almost all of the Mn and As treated soil (Fig. 3a,b). Application of Mn prevents the As contents in the shoot of rice. The shoot As concentration was minimum (5.928 mg As kg^{-1}) when soil was treated with 50 mg Mn kg^{-1} and contaminated with 25 mg As kg^{-1} soil.

Husk As concentrations were decreased with increasing the rate of Mn application. Fig. 3a indicated that the lowest As concentration (0.436 mg As kg^{-1}) in husk was determined when 100 mg kg^{-1}Mn was applied in soil contaminated with 50 mg kg^{-1} As.



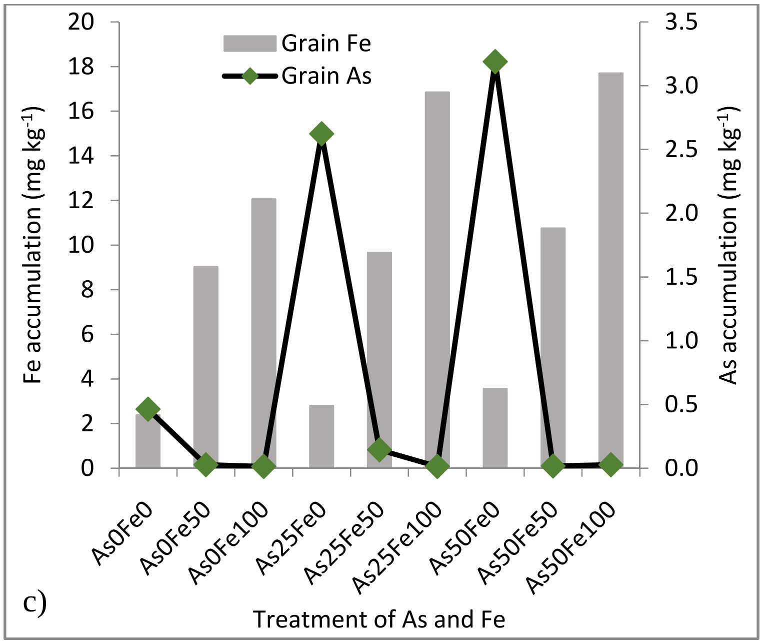
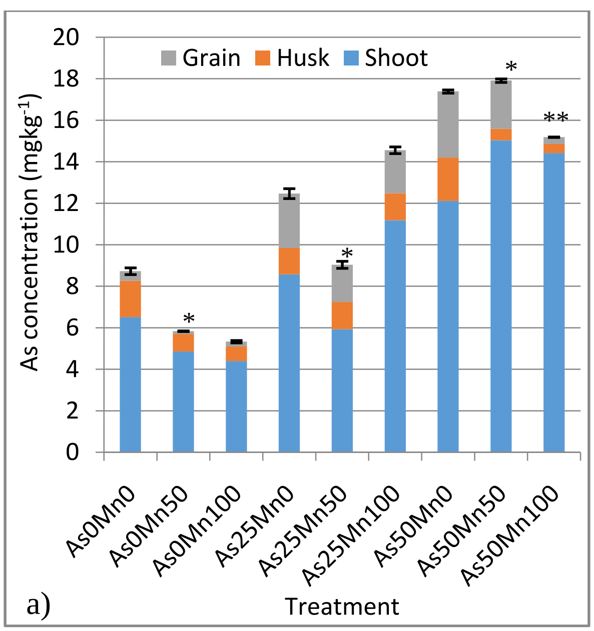
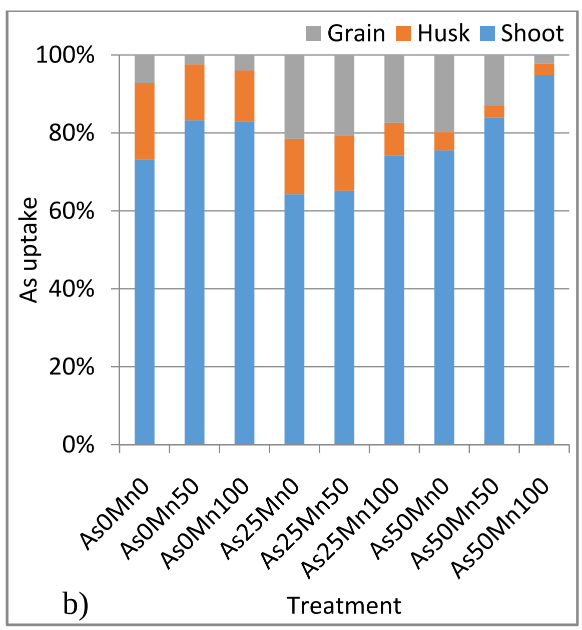


Fig. 2. a) As concentration (mg kg^{-1}), b) As uptake rate (%) in shoot, husk and grain, and c) As and Fe accumulation in grain after treated with Fe in Aus rice [Error bar indicates grain Mean± SD. ** and * indicates significantly difference against respective Fe control at $p < 0.01$ and 0.05, respectively].

Grain As contents were decreased with the increasing rate of single and combined application of Mn in soil. Fig. 3a reflected that in all cases both single and combined application of Mn with arsenic, the concentration of As in grain was comparatively minimum (0.343 mg kg^{-1}) at 100 mg $Mnkg^{-1}$ and 50 mg As kg^{-1} application to the soil. Therefore, Mn application in soil inhibits the uptake of As in rice grain (Fig. 3c).





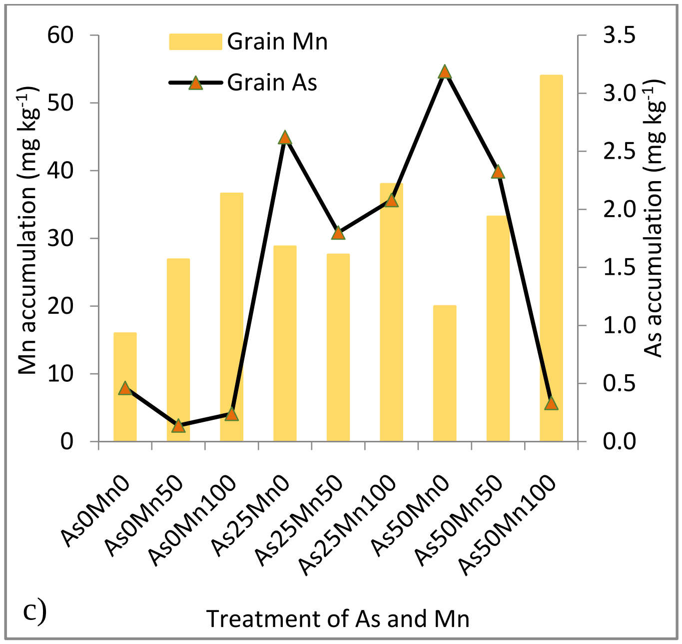
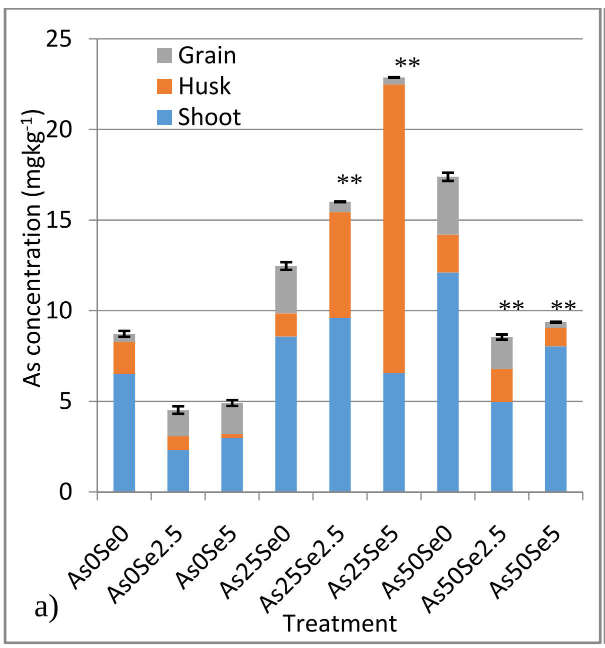
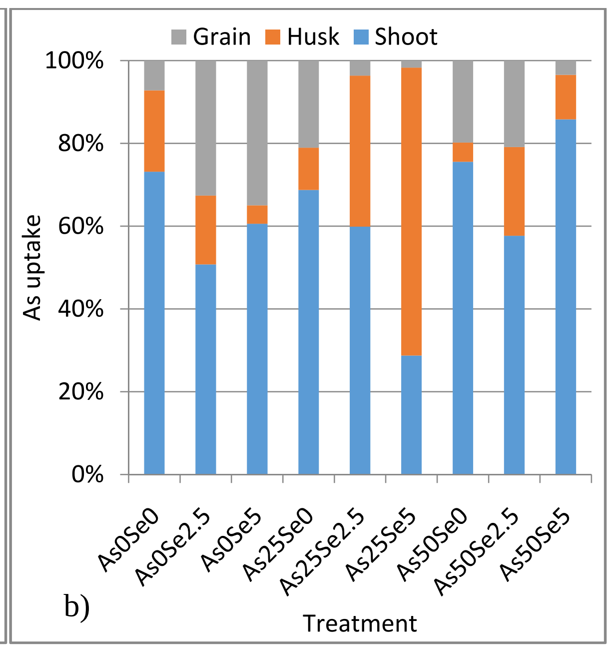


Fig. 3. a) As concentration (mg kg^{-1}), b) As uptake rate (%) in shoot, husk and grain, and c) As and Mn accumulation in grain after treated with Mn in Aus rice [Error bar indicates grain Mean± SD. ** and * indicates significantly difference against respective Mn control at p < 0.01 and 0.05, respectively].





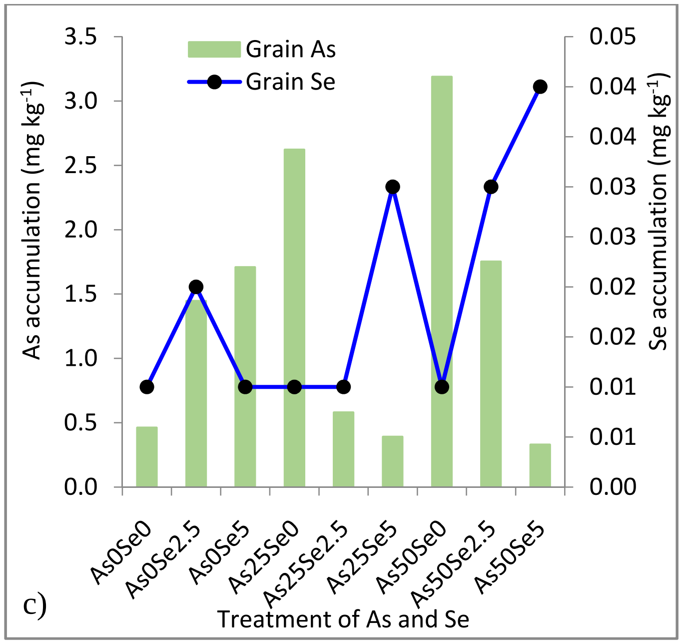


Fig. 4. a) As concentration (mg kg^{-1}), b) As uptake rate (%) in shoot, husk and grain, and c) As and Se accumulation in grain after treated with Mn in Aus rice [Error bar indicates grain Mean± SD. ** and * indicates significantly difference against respective Mn control at $p < 0.01$ and 0.05, respectively].

11.1.9 Effects of Se on As accumulation in *Aus* rice

Grain As concentration was decreasing with increasing of Se application in soil (Fig. 4a,b). Minimum concentration of As in husk (1.006 mg As kg^{-1}) and grain (0.343mg As kg^{-1}) were determined with the increasing rate of Se application in soil. But shoot As content (4.954 mg kg^{-1}) was comparatively lower at low dose of Se with high dose of As application in soil ($As_{50}Si_{2.5}$).

11.1.10 Conclusion from 1st Exp

It is concluded that, lower dose of Si (25 mg kg^{-1}) and Se (2.5 mg kg^{-1}) reduced the toxic effects of As at lower concentration (25 mg As kg^{-1}) in *Aus* rice and increased the yield whereas lower dose of Si in combination with lower dose of As increased the photosynthetic pigments in *Aus* rice leaves. So Si and Se have the potentiality to reduce the toxic effect of As (up to 25 mg kg^{-1}) in *Aus* rice production. Arsenic accumulation was significantly reduced in shoot, husk and grain with the application of different levels of added Si and Fe at different As treated soils. Accumulation of As was reduced in husk and grain at different levels of Mn application. In case of Se treatment, only grain As accumulation was reduced compared to Se control.

11.2. Exp. 2. Aman rice (BRRI dhan 39) cultivation in naturally As contaminated and microelements incubated soil

To evaluate the effect of micro element on As uptake and micro element phytofortification by *Aman* rice var. BRRI dhan 39 in naturally As contaminated soil, we carried out 2nd experiment in two naturally As contaminated soil with control (uncontaminated), the soil As concentration was 11mg As Kg^{-1}, 33mg As Kg^{-1} and 4.61 mg As Kg^{-1} (control). The control and these two soils were incubated with different doses of Si, Fe, Mn and Se. The doses of incubations were 0, 25, 50 mg Kg^{-1} for Si; 0, 50,100 mg Kg^{-1} for Fe; 0, 50, 100 mg Kg^{-1} for Mn and 0, 2.5, 5.0 mg Kg^{-1} for Se. After incubation with micro elements, *Aman* rice var. BRRI dhan 39 was cultivated in this soils following CRD with three replications. However, the effects of mentioned micro elements on agronomic, photosynthetic pigments, As uptake and treated micronutrient phytofortification are discussed here.

11.2.1 Effects of Si on agronomic parameters and photosynthetic pigments of *Aman* rice

Results shown in Table 5, reflected that compared to control in low As contaminated soil, number of effective tiller and grain yield of *Aman* rice was increased in 25mg Si Kg^{-1} incubated soil.

Table 5. Agronomic and photosynthetic pigments parameters of *Aman* rice grown in naturally As contaminated soil and incubated with Si

Treatment \ Parameter	Plant Height (cm)	No. of Effective Tillers	Filled Grains	TGW(g)	Yield (g/pot Oven DW)	Chl. a (mg/g)	Chl. b (mg/g)	Caroten. (mg/g)	Anthocyanin (mg/g)
As_0Si_0	66.3^{bc}	11^{a}	82.3^{a}	23.96^{a}	21.61^{a}	0.10^{b}	0.24^{e}	0.34^{a}	0.25^{e}
As_0Si_{25}	68.2^{abc}	12^{a}	72^{a}	24.48^{a}	21.15^{a}	0.30^{ab}	0.27^{de}	0.34^{a}	0.20^{e}
As_0Si_{50}	68.3^{abc}	10^{a}	89.3^{a}	24.48^{a}	21.79^{a}	0.12^{b}	0.30^{d}	0.27^{c}	0.37^{b}
$As_{11}Si_0$	62.2^{c}	12^{a}	75^{a}	22.12^{a}	19.91^{a}	0.39^{a}	0.46^{b}	0.30^{b}	0.28^{de}
$As_{11}Si_{25}$	75.6^{abc}	14^{a}	72^{a}	23.56^{a}	23.75^{a}	0.23^{ab}	0.29^{d}	0.36^{a}	0.71^{ab}
$As_{11}Si_{50}$	81.5^{a}	13^{a}	78^{a}	22.48^{a}	22.80^{a}	0.35^{ab}	0.18^{f}	0.28^{bc}	0.80^{a}
$As_{33}Si_0$	81.2^{a}	11^{a}	73^{a}	19.98^{a}	19.85^{a}	0.18^{ab}	0.57^{a}	0.12^{e}	0.57^{c}
$As_{33}Si_{25}$	82.1^{a}	13^{a}	68^{a}	24.08^{a}	21.29^{a}	0.35^{ab}	0.56^{a}	0.15^{d}	0.65^{bc}
$As_{33}Si_{50}$	80.5^{ab}	13^{a}	72^{a}	24.48^{a}	22.91^{a}	0.36^{ab}	0.36^{c}	0.16^{d}	0.29^{de}
Level of sig.	*	NS	NS	NS	NS	*	**	**	**
CV%	11.55	17.03	9.40	9.49	9.40	49.81	37.66	34.15	47.83

[In a column, values having different letter(s) differ significantly, but with common letter(s) do not differ significantly at 5% and 1% level of probability analyzed by Tukey, denoted by * and **, respectively. NS= Not significant, CV = Coefficient of variation]

The higher dose (50mg Si Kg^{-1} soil) of Si application also increased the plant height, total number of grain, number of filled grain, thousand seed weight and yield of *Aman* rice in As uncontaminated and naturally As contaminated soil**.** It is indicated that at a certain levels of concentration Si has synergistic effects on yield of rice. Compared to control the increasing rate of Si application increased the concentration of photosynthetic pigments of rice leaves. In low As contaminated soil photosynthetic pigments of rice leaves were decreased with increasing rate of Si. But in higher As contaminated soil, the increasing rate of Si application increased the concentration of photosynthetic pigments of rice leaves.

11.2.2 Effects of Si application on As uptake in shoot, husk and grain of *Aman* rice

To determine the As accumulation inhibition potentiality of Si, *Aman* rice was grown on naturally As contaminated soils treated with different levels of Si. From Fig. 5, it is very much clear that As uptake in root, shoot, husk and grain was remarkably decreased when Si was applied @ 25 and 50mg kg^{-1} both in 11 and 33 mg kg^{-1} naturally As contaminated. But root and shoot As contents were comparatively higher than husk and grain. It indicated that the application of Si in naturally As contaminated soil inhibits the As accumulation in the different above ground parts of rice plant. As accumulation in rice grain was reduced by 8.40% and 30.25% when 33 mg kg^{-1} naturally As contaminated was treated with 25 and 50mg Si kg^{-1} soil, respectively (Fig. 6). On the other hand it was 96.96% when soil As concentration was 11 mg kg^{-1}. In case of husk due to the Si treatment As accumulation reduction were 96.87% and 97.56%.

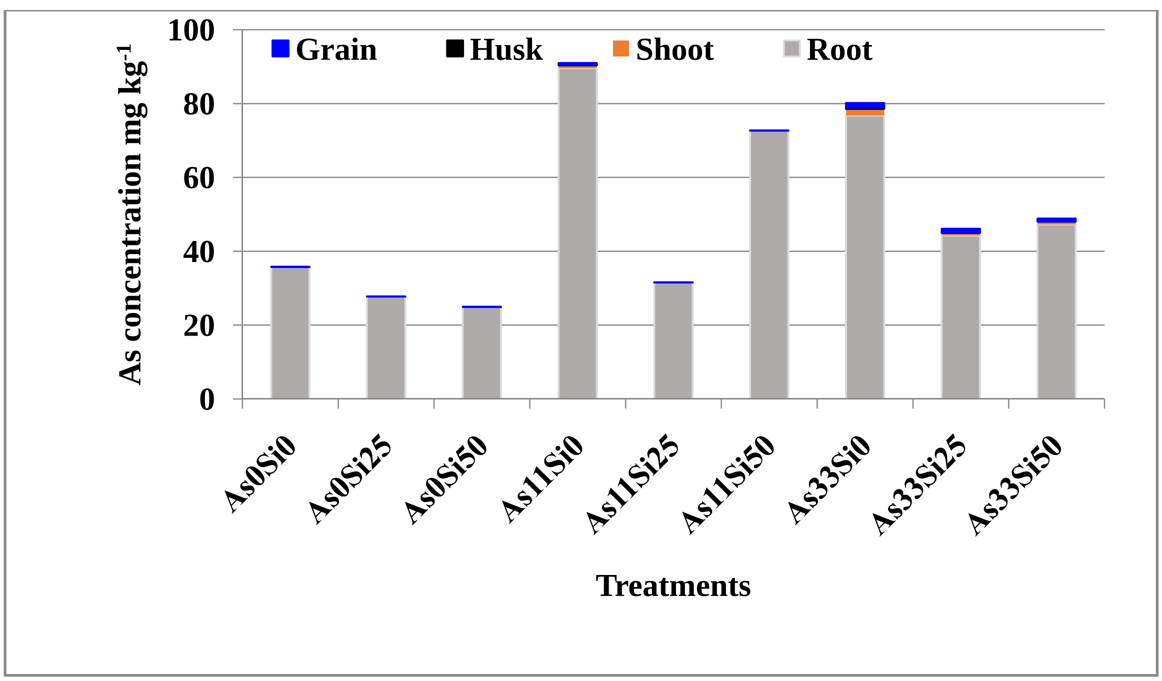


Fig. 5As concentration in Aman shoot, husk and grain after treated with Si

11.2.3 Effects of Fe on agronomic parameters and photosynthetic pigments of *Aman* rice

Table 6 indicated that compared to control the application of Fe at higher levels in As control soil, comparatively lower and higher As contaminated soil increased the yield and other agronomic parameters of rice. The highest yield (24.97g pot^{-1}) was obtained when control soil was treated with 100 mg Fe Kg^{-1} soil and 2^{nd} highest yield (24.01gpot^{-1}) also obtained when 11mg Kg^{-1} As contaminated soil was treated with the same concentration of Fe.

In control and low As contaminated soil, the increasing rate of Fe application increased the concentration of photosynthetic pigments like chlorophyll-a, chlorophyll-b in rice leaves. But in higher As contaminated soil the increasing rate of Fe application decreased the concentrations of carotenoid and anthocyanin. Compared to control at higher As contaminated soil, the increasing rate of Fe application decreased the concentration of photosynthetic pigments of rice leaves.

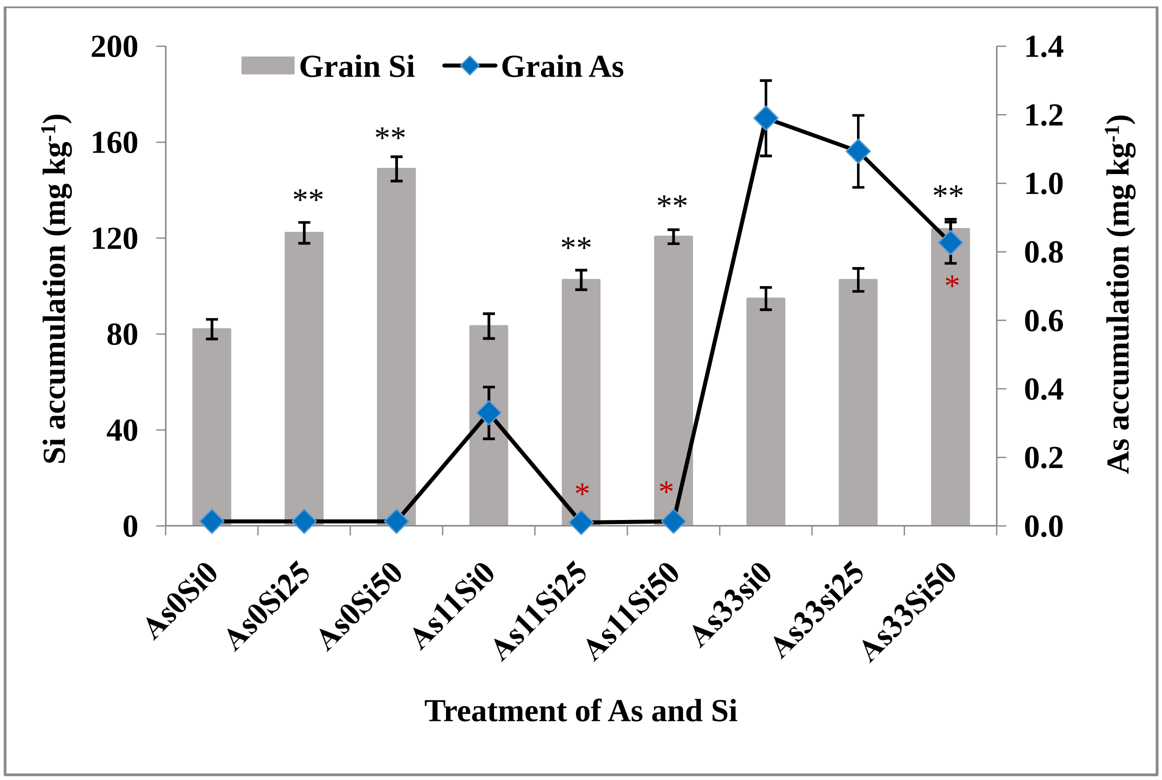


Fig. 6 Accumulation of As and Si in dehusked Aman grain after treated with Si [Error bar indicates grain Mean± SD. ** and * indicates significantly difference against respective Si control at p< 0.01 and 0.05, respectively]

Table 6. Agronomic and photosynthetic pigments parameters of *Aman* rice grown in naturally As contaminated soil and incubated with Fe

Treatment \ Parameter	Plant Height (cm)	No. of Effective Tillers	Filled Grains	TGW(g)	Yield (g/pot Oven DW)	Chl. a (mg/g)	Chl. b (mg/g)	Caroten. (mg/g)	Anthocyanin (mg/g)
As_0Fe_0	56.33^{cd}	11^{a}	82^{a}	23.96^{a}	21.61^{a}	0.10^{g}	0.24^{f}	0.34^{c}	0.25^{e}
As_0Fe_{50}	74.80^{abc}	11^{a}	90^{a}	23.56^{a}	23.32^{a}	0.20^{e}	0.19^{g}	0.35^{a}	0.57^{c}
As_0Fe_{100}	78.20^{ab}	12^{a}	85^{a}	24.48^{a}	24.97^{a}	0.56^{a}	0.50^{d}	0.30^{g}	0.24^{e}
$As_{11}Fe_0$	$62\ 20^{d}$	12^{a}	75^{a}	22.12^{a}	19.91^{a}	0.39^{d}	0.46^{e}	0.30^{f}	0.28^{e}
$As_{11}Fe_{50}$	82.00^{a}	12^{a}	78^{a}	25.28^{a}	23.66^{a}	0.48^{c}	0.69b	0.32^{d}	0.73^{b}
$As_{11}Fe_{100}$	78.30^{ab}	12^{a}	82^{a}	24.40^{a}	24.01^{a}	0.51^{b}	0.87^{a}	0.34^{b}	0.42^{d}
$As_{33}Fe_0$	81.20^{ab}	11^{a}	73^{a}	19.98^{a}	19.85^{c}	0.18^{f}	0.57^{c}	0.12^{h}	0.57^{c}
$As_{33}Fe_{50}$	71.90^{bc}	13^{a}	85^{a}	23.64^{a}	23.91^{a}	0.07^{i}	0.07^{i}	0.31^{e}	0.65^{bc}
$As_{33}Fe_{100}$	73.10^{abc}	13^{a}	78^{a}	23.52^{a}	23.85^{a}	0.07^{h}	0.10^{h}	0.08^{i}	0.89^{a}
Level of sig.	**	NS	NS	NS	NS	*	*	*	**
CV%	9.38	12.42	7.24	8.13	9.43	67.58	64.98	35.30	43.22

[In a column, values having different letter(s) differ significantly, but with common letter(s) do not differ significantly at 5% and 1% level of probability analyzed by Tukey, denoted by * and **, respectively. NS= Not significant, CV = Coefficient of variation]

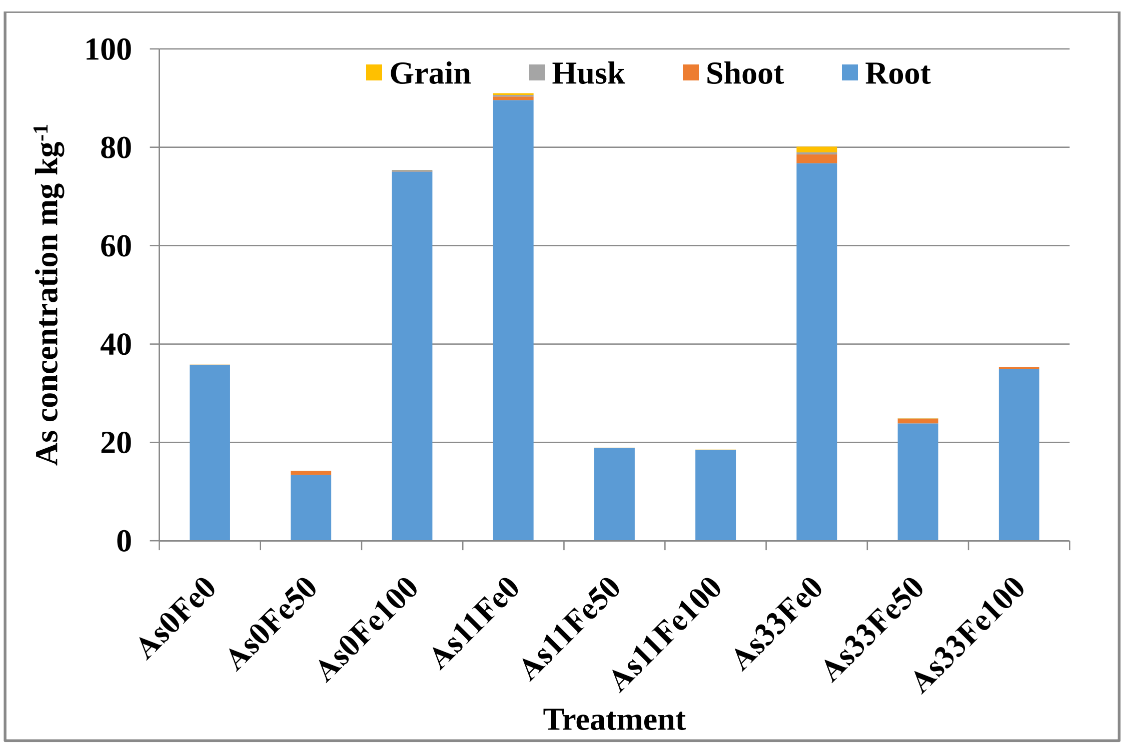


Fig. 7As concentration in Aman shoot, husk and grain after treated with Fe

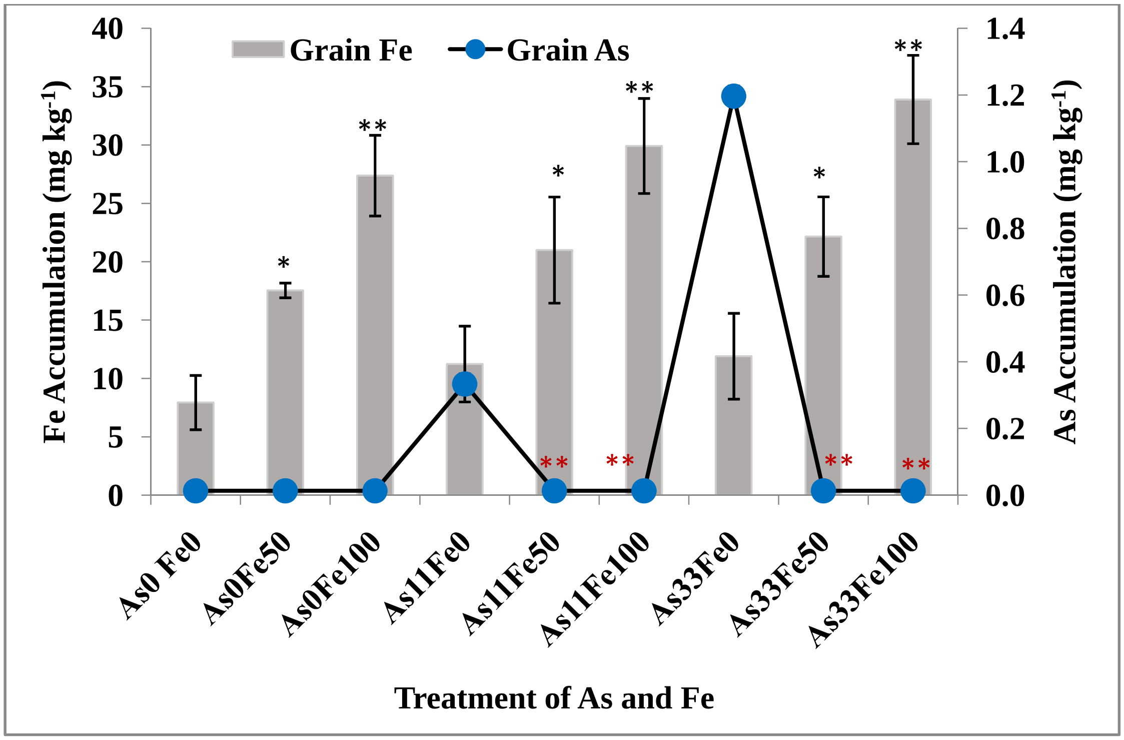


Fig. 8 Accumulation of As and Fe in dehusked Aman grain after treated with As and Fe[Error bar indicates grain Mean± SD. ** and * indicates significantly difference against respective Fe control at $p < 0.01$ and 0.05, respectively]

11.2.4 Effects of Mn on agronomic parameters and photosynthetic pigments of *Aman* rice

Results showed that in low As contaminated soil (11 mg AsKg^{-1} Soil) the application of Mn @ 50 mg Kg^{-1} increased the number of effective tiller and yield of *Aman* rice (Table 7). But compared to control in higher As contaminated soil (33 mg AsKg^{-1} Soil) the increasing rate of Mn application increased the number of effective tiller and yield. In case of low As contaminated soil (11 mg AsKg^{-1} Soil) 50 mg Mn Kg^{-1} Soil application contributed the highest yield but in case of higher As contaminated soil (33 mg AsKg^{-1} Soil) 100 mg Mn Kg^{-1} Soil application gave the highest yield.

Like Fe in As free (4.61 mg kg^{-1} or control), comparatively lower (11 mg kg^{-1}) and higher As contaminated soil (33 mg kg^{-1}), the increasing rate of Mn application, increased the concentration of photosynthetic pigments like chlorophyll-a and chlorophyll-b in rice leaves.

Table 7. Agronomic and photosynthetic pigments parameters of *Aman* rice grown in naturally As contaminated soil and incubated with Mn

Treatment \ Parameter	Plant Height (cm)	No. of Effective Tillers	Filled Grains	TGW (g)	Yield (g/pot Oven DW)	Chl. a (mg/g)	Chl. b (mg/g)	Caroten. (mg/g)	Anthocyanin (mg/g)
As_0Mn_0	66.33^{cde}	11^{abc}	82^{a}	23.96^{a}	21.61^{bc}	0.10^{f}	0.24^{f}	0.34^{a}	0.25^{f}
As_0Mn_{50}	58.60^{e}	14^{a}	71^{bcd}	24.84^{a}	24.69^{a}	0.28^{d}	0.13^{g}	0.27^{c}	0.53^{bc}
As_0Mn_{100}	63.50^{cde}	8^{c}	62d	23.20^{a}	11.50^{e}	0.37^{c}	0.38^{e}	0.34^{a}	0.46^{cd}
$As_{11}Mn_0$	62.20^{de}	12^{ab}	75^{abc}	22.12^{ab}	19.91^{cd}	0.39^{bc}	0.46^{d}	0.30^{b}	0.28^{ef}
$As_{11}Mn_{50}$	68.20^{cd}	14^{a}	74^{abc}	23.92^{a}	24.78^{a}	0.42^{b}	0.38^{e}	0.31^{b}	1.93^{a}
$As_{11}Mn_{100}$	86.10^{a}	10^{bc}	76^{ab}	24.88^{a}	18.90^{cd}	0.54^{a}	0.49^{c}	0.32^{ab}	0.25^{f}
$As_{33}Mn_0$	81.20^{ab}	11^{abc}	73^{abc}	19.98^{b}	19.85^{cd}	0.18^{e}	0.57^{b}	0.12^{d}	0.57^{b}
$As_{33}Mn_{50}$	72.00^{bc}	12^{ab}	66^{cd}	23.16^{a}	18.34^{d}	0.04^{g}	0.04^{h}	0.27^{c}	0.44^{cd}
$As_{33}Mn_{100}$	70.00^{cd}	14^{a}	70^{bcd}	24.24^{a}	23.76^{ab}	0.06^{fg}	0.75^{a}	0.32^{ab}	0.38^{de}
Level of sig.	**	**	**	*	**	**	**	**	**
CV%	12.90	18.59	8.57	7.27	19.91	64.84	55.48	22.81	89.30

[In a column, values having different letter(s) differ significantly, but with common letter(s) do not differ significantly at 5% and 1% level of probability analyzed by Tukey, denoted by * and **, respectively. CV = Coefficient of variation]

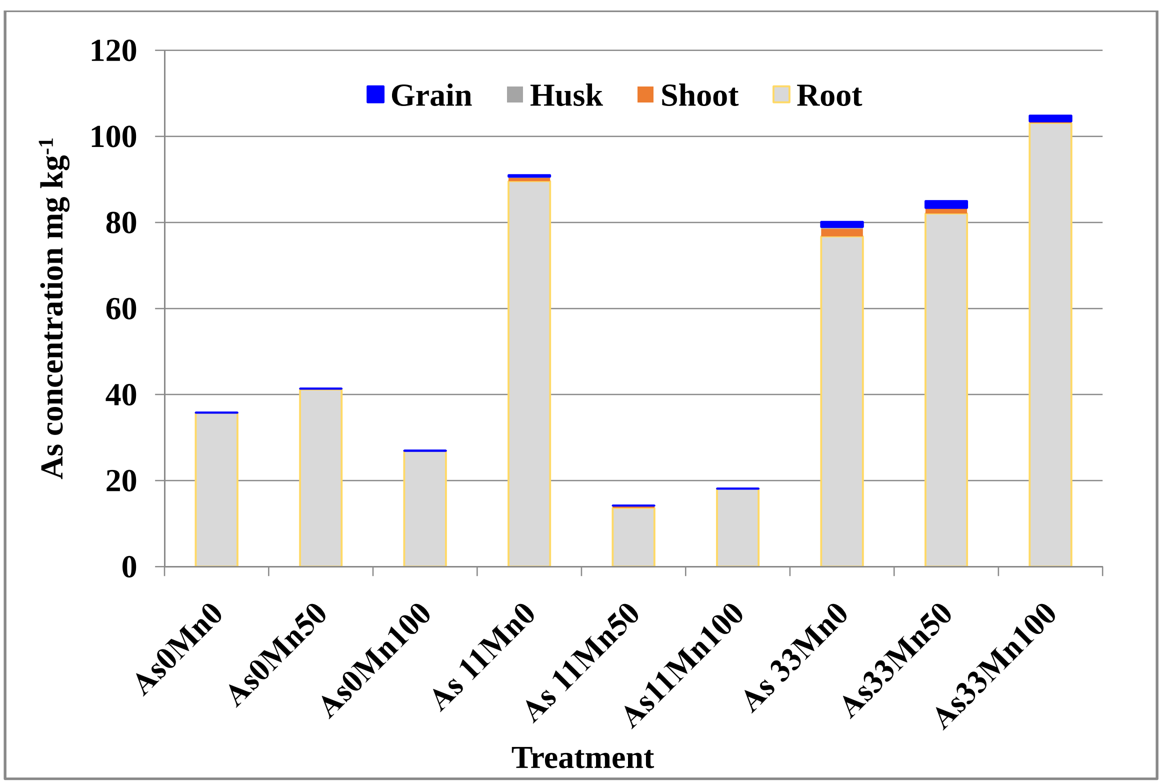


Fig. 9 As concentration in Aman shoot, husk and grain after treated with Mn

11.2.5 Effects of Fe and Mn application on As uptake in shoot, husk and grain of *Aman* rice

In the same naturally As contaminated soil, the addition of Fe and Mn @ 50 and 100mg kg^{-1} soil also showed As accumulation reduction trend in root, shoot, husk and grain of rice (Fig. 7). In case of Fe the grain and husk As accumulation reduction rate were almost similar to the rate of Si. But in case of Mn, 50 and 100mg kg^{-1} Mn application in low As contaminated soil (11 mg Askg^{-1}) reduced grain As accumulation by 93.93% and for husk it was 96.87%. While in higher As contaminated soil (33mg As kg^{-1}) Mn had no inhibitory effect of As accumulation in the grain of *Aman* rice (Fig. 9).

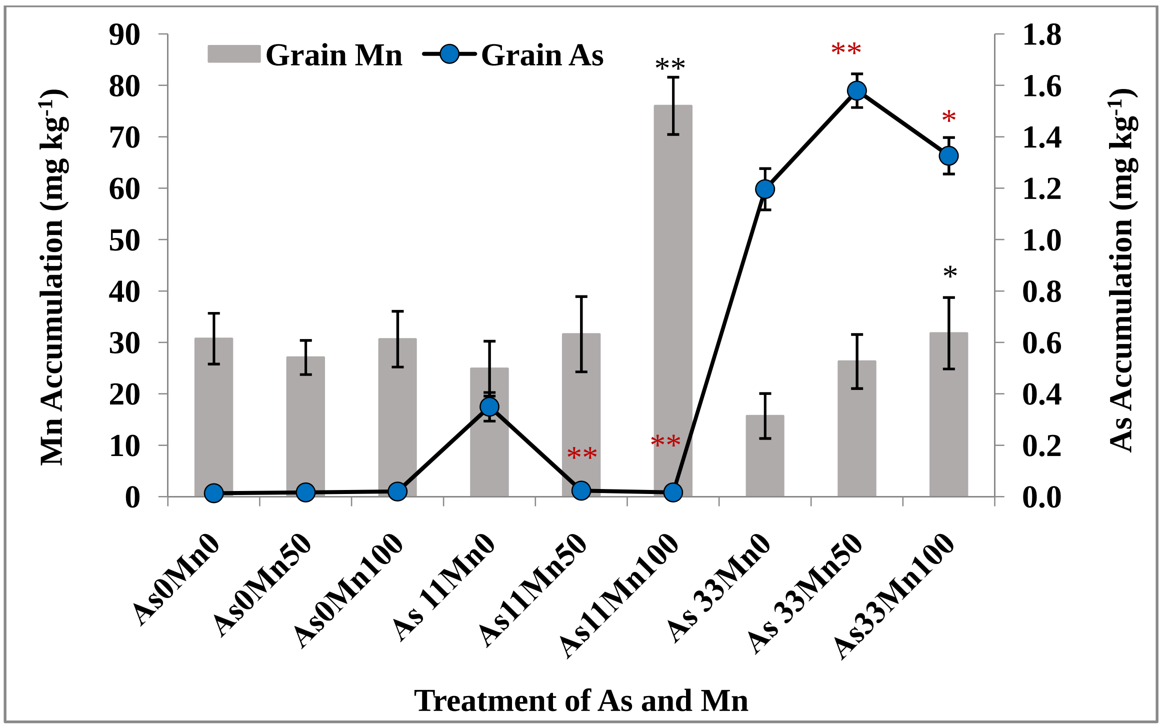


Fig. 10Accumulation of As and Mn in dehusked Aman grain after treated with As and Mn[Error bar indicates grain Mean± SD. ** and * indicates significantly difference against respective Mn control at $p < 0.01$ and 0.05, respectively]

11.2.6 Effects of Se on agronomic parameters and photosynthetic pigments of *Aman* rice

In As control soil except thousand seed weight almost all agronomic parameters were decreased with the application of Se (Table 8). While in naturally As contaminated soil, Se had positive impact on the number of effective tillers and yield of *Aman* rice. Compared to control thousand seed weight and yield were slightly increased in lower As contaminated soil (11.0 mg As Kg^{-1} soil) at higher dose of Se (5.0 mg Se Kg^{-1} soil).Except higher As contaminated soil (33.0 mg As Kg^{-1} soil) with the application of Se the values of photosynthetic pigments were decreased in As free and lower As contaminated soil (Table 8).

Table 8. Agronomic and photosynthetic pigments parameters of *Aman* rice grown in naturally As contaminated soil and incubated with Se

Treatment \ Parameter	Plant Height (cm)	No. of Effective Tillers	Filled Grains	TGW(g)	Yield (g/pot Oven DW)	Chl. a (mg/g)	Chl. b (mg/g)	Caroten. (mg/g)	Anthocyanin (mg/g)
$As_0Se_{0.0}$	66.33^{bc}	11^{a}	82.00^{a}	23.96^{a}	21.61^{a}	0.10^{g}	0.24^{g}	0.34^{ab}	0.24^{f}
$As_0Se_{2.5}$	66.00^{bc}	10^{a}	49.00^{d}	23.56^{a}	11.54^{c}	0.03^{h}	0.18^{h}	0.26^{d}	0.55^{bc}
$As_0Se_{5.0}$	64.00^{c}	11^{a}	48.00^{d}	24.32^{a}	14.00^{bc}	0.03^{i}	0.01^{i}	0.19^{e}	0.45^{cd}
$As_{11}Se_{0.0}$	62.20^{c}	12^{a}	75.00^{ab}	22.12^{ab}	19.91^{a}	0.39^{d}	0.46^{f}	0.30^{c}	0.28^{ef}
$As_{11}Se_{2.5}$	$73\ 10^{b}$	14^{a}	63.00^{c}	24.84^{a}	21.9^{a}	0.56^{b}	0.56^{c}	0.36^{a}	1.05^{a}
$As_{11}Se_{5.0}$	66.70^{bc}	12^{a}	67.00^{bc}	25.28^{a}	22.01^{a}	0.56^{a}	0.55^{d}	0.32^{bc}	0.34^{ef}
$As_{33}Se_{0.0}$	81.20^{a}	11^{a}	$73\ 00^{b}$	19.98^{b}	19.85^{a}	0.18^{f}	0.57^{b}	0.12^{f}	0.57^{b}
$As_{33}Se_{2.5}$	68.80^{bc}	13^{a}	67.00^{bc}	24.08^{a}	20.97^{a}	0.30^{e}	0.51^{e}	0.33^{b}	0.37^{de}
$As_{33}Se_{5.0}$	69.10^{bc}	15^{a}	42.33^{d}	24.92^{a}	15.69^{b}	0.55^{c}	0.76^{a}	0.30^{c}	0.45^{cd}
Level of sig.	**	NS	**	*	**	*	*	**	**
CV%	8.56	16.57	21.21	7.89	20.55	72.00	52.93	27.02	49.08

[In a column, values having different letter(s) differ significantly, but with common letter(s) do not differ significantly at 5% and 1% level of probability analyzed by Tukey, denoted by * and **, respectively. NS= Not significant, CV = Coefficient of variation]

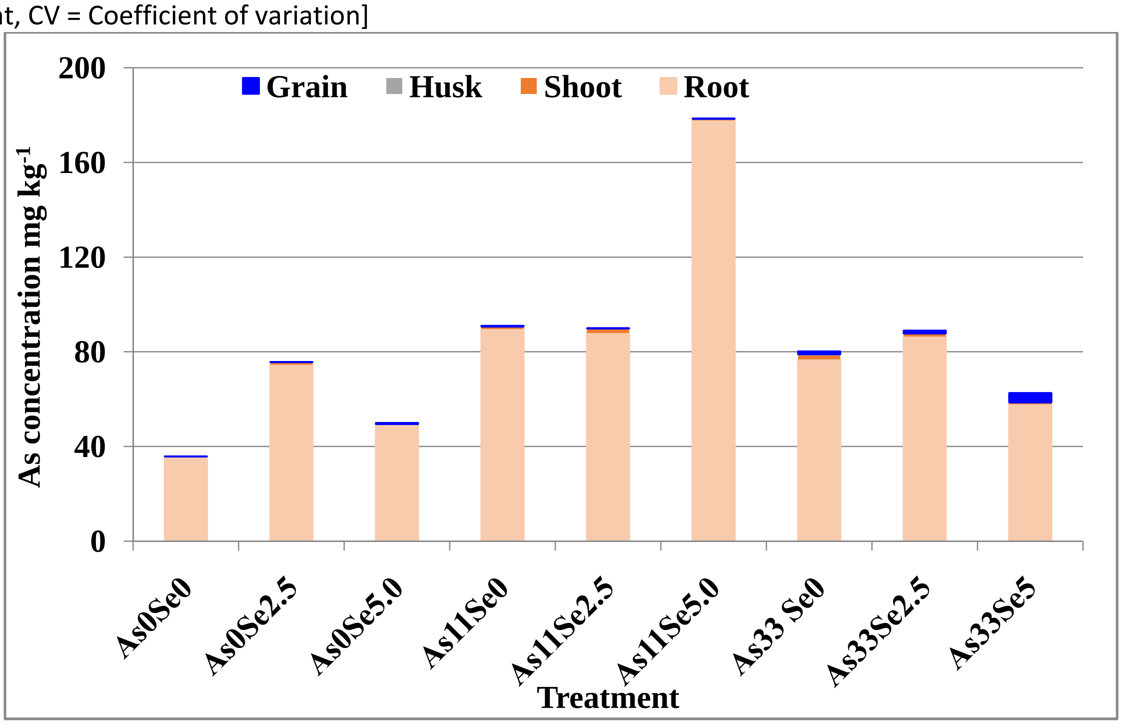


Fig. 11As concentration in Aman shoot, husk and grain after treated with Se

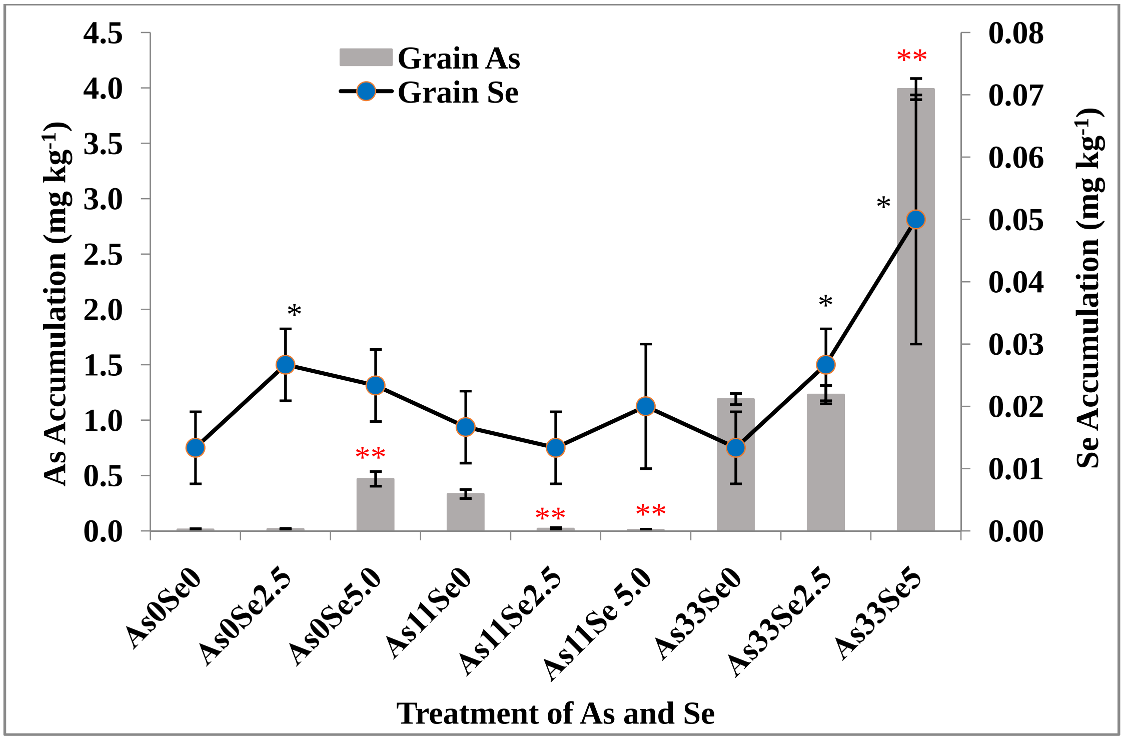


Fig. 12 Accumulation of As and Se in dehusked Aman grain after treated with As and Se [Error bar indicates grain Mean± SD. ** and * indicates significantly difference against respective Se control at $p < 0.01$ and 0.05, respectively]

11.2.7 Effects of Se application on As uptake in shoot, husk and grain of *Aman* rice

Compared to Se control soil, application of Se @ 2.5 and 5.0mg kg^{-1} in naturally As contaminated soil reflected the antagonistic effect on accumulating As in rice grain. It reduced As accumulation in husk and grain of rice. Both the doses were effective for the prevention of As accumulation in husk and grain when applied at lower As contamination of soil (Fig. 11 and 12).

11.2.8 Nutrient phytofortification in rice grain by treating soil with trace elements

Fe, Mn, Si and Se are important mineral elements for human and animals. Since the main function of food material is to supply the nutrients for all sorts of animals including human being. Food material must be phytofortified with various mineral elements. The addition of Fe, Mn, Si and Se in naturally As contaminated soils of different degrees helps to inhibit As accumulation in the different above ground parts of rice plant like shoot, husk and grain. Similarly, the application of these elements in the As contaminated soil also helps to increase the accumulation of these elements in the rice grain and that is the nutrient phytofortification.

Highest amount (148.89mg Kg^{-1}) of Si content in grain was found when control soil was treated with50mg Kg^{-1} Si. But in case of naturally As contaminated soil highest amount (123.89mg Kg^{-1}) of Si content in grain was determined when 50mg Kg^{-1} Si was applied in 33mg Kg^{-1} As containing soil (Fig. 6).

Fig. 8 indicated that compared to control highest amount (33.90mg Kg^{-1}) of Fe content in grain was determined when 100mg $FeKg^{-1}$ was applied in 33 $mgKg^{-1}$ naturally As contaminated soil.
Similarly, maximum amount (76.04mg Kg^{-1}) of grain Mn was found when 100mg Kg^{-1}Mn was applied in 11mg Kg^{-1} naturally As contaminated soil. In the same dose, grain Mn accumulation was 31.82mg Kg^{-1} for 33 $mgKg^{-1}$ naturally As contaminated soil (Fig. 10).
Compared to control (0.01mg Se Kg^{-1}) comparatively higher amount of Se (0.05mg Se Kg^{-1}) was determined from the rice grain when 33mg Kg^{-1} naturally As contaminated soil was treated with 5.0 mg Se Kg^{-1}(Fig. 12).

11.2.9 Conclusion from 2nd exp.

From the experiment 2, it is found that yield of *Aman* rice increased due to Si, Fe and Mn treatment in naturally As contaminated soil. Arsenic accumulation in grain is lower than shoot for the Si and Fe treatment. Arsenic accumulation is significantly ($p<0.01$ and 0.05) reduced in dehusked grain compare to control due to treatment of microelements in soil which is below the recommended level (0.2 mg kg^{-1}) for Si, Fe, Mn and Se treatment at lower level of As contamination (11 mg kg^{-1}) but only Fe treatment can reduce the uptake of As in grain at both of the As contaminated soil (11 and 33 mg $kg\text{-}1^{-1}$). There are significant ($p<0.01$ and 0.05) accumulation or fortification of microelements in grain is happened due to different treatment of these elements.

11.3 Exp. 3: Boro rice (BRRI dhan 47) cultivation in As contaminated and microelements incubated soil followed by Aman rice

11.3.1 Effect of Si and Fe on agronomic and photosynthetic pigments parameters of *Boro* rice

Yield of boro rice was significantly increases with the increasing of micronutrients treatment like Fe and Si in soil compare of control (Table 9 and 10). The maximum yield was found in Si fortified soil (28.90 g pot^{-1}at $As_{11}Si_{50}$ treatment) then Fe treated soil (28.66 g pot^{-1} at $As_{11}Fe_{100}$ treatment). The increasing of yield is highly correlated with the other agronomic parameters like effective tiller, filled graintiller^{-1}, thousand grain weight (TGW) and photosynthetic pigments contents in these treatments(Table 9 and 10).In case of Mn and Se incubated soil, the yield was not increased rather decreased compare to Mn and Se control treatment. This is might be due to the less number of effective tillers, filled grain per tiller and TGW (Table 11 and 12).

11.3.2 Effects of Si and Fe application on As uptake in grain of *Boro* rice

Due to the reduction of yield by Mn and Se treatment (Table 11 and 12), analyses were done only for inhibition of As accumulation in grain by Si and Fe application and data were depicted in Fig. 13 and 14. In both cases, As accumulation were significantly ($p < 0.01$ and 0.05) decreased in grain compare to their respective control (0 mg kg^{-1}) Si and Fe treatment. The decreasing rate of As accumulation was higher in Fe treated soil than Si. Arsenic accumulation was recorded as 0.110-0.350 and 0.013-0.104 mg As kg^{-1}in grain grown in Si and Fe treated soil, respectively (Fig. 13 and 14).

Table 9. Agronomic and photosynthetic pigments parameters of *Boro* rice grown in naturally As contaminated soil and incubated with Si followed by *Aman* rice

Treatment/ Parameter	No. of Effective Tillers	Filled Grains	TGW (g)	Yield (g/pot Oven DW)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)
As_0Si_0	8^a	113.33^{ab}	25.40^a	22.96^{cd}	3.98^{abc}	0.26^b
As_0Si_{25}	8^a	118^{ab}	26.04^a	24.58^{bc}	4.10^{ab}	0.26^b
As_0Si_{50}	9^a	122^a	24.52^a	26.92^{ab}	4.18^a	0.29^b
$As_{11}Si_0$	7^a	109^b	26.68^a	20.36^d	3.32^{abc}	0.29^b
$As_{11}Si_{25}$	9^a	109^b	26.32^a	25.82^{bc}	1.70^c	0.11^c
$As_{11}Si_{50}$	9^a	122^a	26.32^a	28.90^a	4.81^a	0.43^a
$As_{33}Si_0$	8^a	107^b	24.84^a	21.26^d	1.77^{bc}	0.12^c
$As_{33}Si_{25}$	9^a	111^b	23.20^a	23.18^{cd}	2.83^{abc}	0.09^c
$As_{33}Si_{50}$	9^a	112^b	26.36^a	26.57^{ab}	3.43^{abc}	0.13^c
Level of sig.	NS	*	NS	**	*	**
CV%	12.86	5.72	5.72	11.51	36.99	50.13

[In a column, values having different letter(s) differ significantly, but with common letter(s) do not differ significantly at 5% and 1% level of probability analyzed by Tukey, denoted by * and **, respectively. NS= Not significant, CV = Coefficient of variation]

Table 10. Agronomic and photosynthetic pigments parameters of *Boro* rice grown in naturally As contaminated soil and incubated with Fe followed by *Aman* rice

Treatment/ Parameter	No. of Effective Tillers	Filled Grains	TGW (g)	Yield (g/pot Oven DW)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)
As_0Fe_0	8^a	113.33^{ab}	25.4^a	22.96^b	3.98^{ab}	0.26^{bc}
As_0Fe50	9^a	108.33^{ab}	23.44^a	22.78^b	3.39^{ab}	0.24^{bcd}
As_0Fe100	8^a	110^{ab}	26.16^a	23.02^b	3.10^{ab}	0.20^{de}
As_{11}Fe0	7^a	109^{ab}	26.68^a	20.36^b	3.32^{ab}	0.29^b
As_{11}Fe50	9^a	115^{ab}	26.76^a	27.70^a	4.65^a	0.39^a
As_{11}Fe100	9^a	119^a	26.76^a	28.66^a	4.70^a	0.22^{cde}
As_{33}Fe0	8^a	107^b	24.56^a	21.26^b	1.77^b	0.12^f
As_{33}Fe50	9^a	118^{ab}	24.84^a	26.38^a	2.71^{ab}	0.17^{ef}
As_{33}Fe100	9^a	119^a	25.24^a	27.03^a	3.19^{ab}	0.21^{cde}
Level of sig.	NS	**	NS	**	*	**
CV%	12.86	5.03	5.54	12.31	34.65	32.29

[In a column, values having different letter(s) differ significantly, but with common letter(s) do not differ significantly at 5% and 1% level of probability analyzed by Tukey, denoted by * and **, respectively. NS= Not significant, CV = Coefficient of variation]

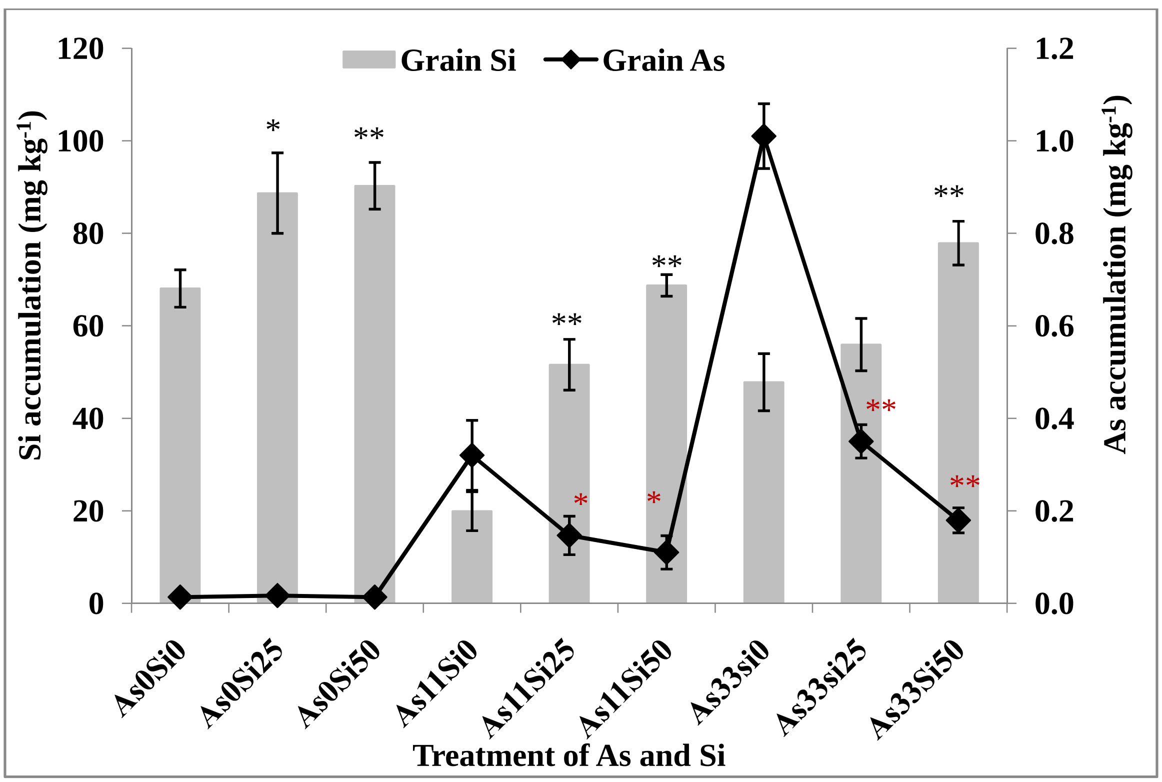


Fig. 13 Accumulation of As and Si in dehusked Boro grain after treated with Si [Error bar indicates grain Mean± SD. ** and * indicates significantly difference against respective Si control at $p < 0.01$ and 0.05, respectively]

11.3.3 Phytofortification of Si and Fe in grain of *Boro* rice

Analyses were done for Si and Fe accumulation in the grain of *Boro* rice and data were represented in the Fig. 13 and 14. Grain Si and Fe accumulation were significantly increased in Si and Fe treated soil compare to their control indicated the phytofortification of these elements. Grain Si accumulation ranges from 51.96-90.22 mg kg^{-1} for different level of Si treated soil whereas 19.74-68.07mg kg^{-1} for no Si treatment (Fig 13). Fe accumulation in grain was ranges from 17.47-36.08 and 6.95-11.90 mg kg^{-1} for Fe treated soil and control, respectively (Fig. 14).

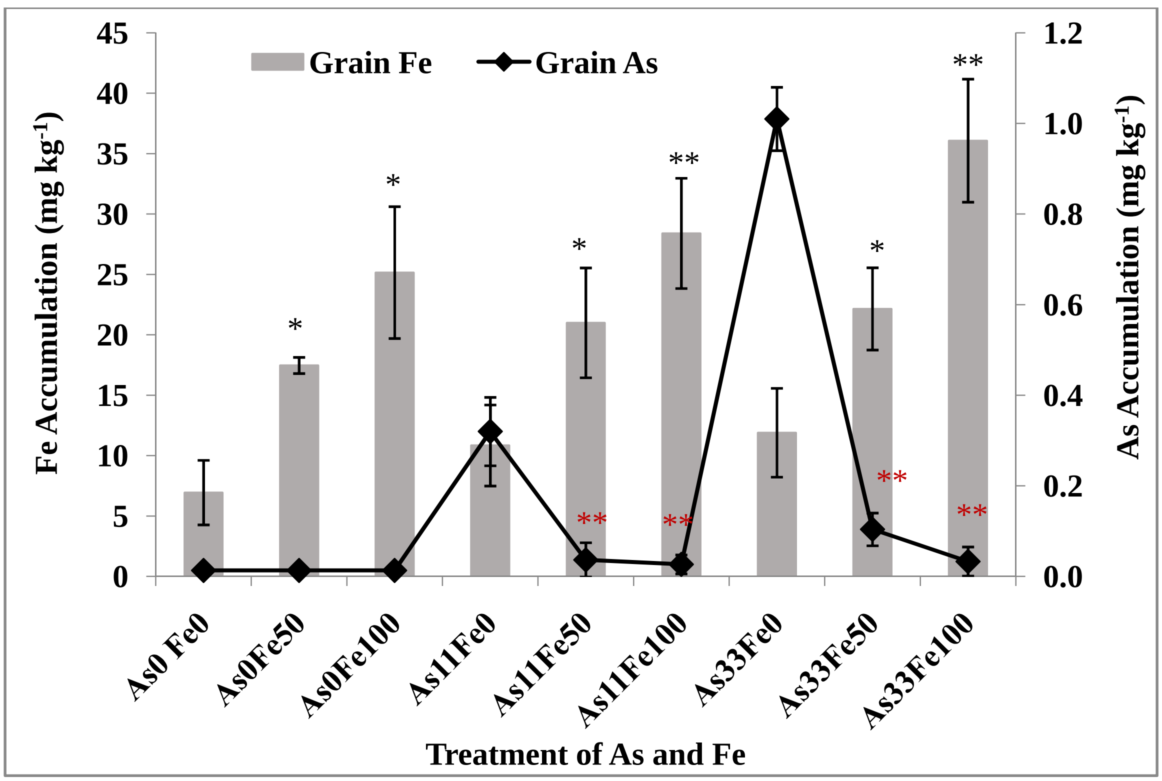


Fig. 14 Accumulation of As and Fe in dehusked Boro grain after treated with Fe [Error bar indicates grain Mean ± SD. ** and * indicates significantly difference against respective Fe control at $p < 0.01$ and 0.05, respectively]

Table 11. Agronomic and photosynthetic pigments parameters of *Boro* rice grown in naturally As contaminated soil and incubated with Mn followed by *Aman* rice

Treatment/ Parameter	No. of Effective Tillers	Filled Grains	TGW (g)	Yield (g/pot Oven DW)	Chlorophyl a (mg/g)	Chlorophyl b (mg/g)
As_0Mn0	8^{ab}	113.33^{a}	25.40^{a}	22.96^{a}	3.98^{abc}	0.26^{cd}
As_0Mn50	7^{b}	87^{b}	21.68^{a}	13.20^{e}	4.43^{abc}	0.22^{de}
As_0Mn100	7^{b}	68^{c}	21.44^{a}	10.21^{f}	2.28^{c}	0.10^{g}
$As_{11}Mn0$	7^{b}	109^{a}	26.68^{a}	20.36^{abc}	3.32^{bc}	0.29^{c}
$As_{11}Mn_{50}$	8^{ab}	88^{b}	24.84^{a}	17.49d	6.16^{a}	0.53^{a}
$As_{11}Mn100$	7^{b}	95^{b}	26.52^{a}	17.64^{cd}	5.17^{ab}	0.45^{b}
$As_{33}Mn0$	8^{ab}	107^{a}	24.56^{a}	21.26^{ab}	1.77^{c}	0.12^{fg}
$As_{33}Mn50$	7^{b}	105^{a}	25.32^{a}	18.61^{bcd}	4.07^{abc}	0.27^{cd}
$As_{33}Mn100$	10^{a}	94^{b}	24.72^{a}	22.24^{a}	3.11^{bc}	0.17^{ef}
Level of sig.	**	**	*	**	**	**
CV%	16.58	14.42	405.35	22.75	40.29	51.74

[In a column, values having different letter(s) differ significantly, but with common letter(s) do not differ significantly at 5% and 1% level of probability analyzed by Tukey, denoted by * and **, respectively.CV = Coefficient of variation]

Table 12. Agronomic and photosynthetic pigments parameters of *Boro* rice grown in naturally As contaminated soil and incubated with Se followed by *Aman* rice

Treatment/ Parameter	No. of Effective Tillers	Filled Grains	TGW (g)	Yield (g/pot Oven DW)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)
As_0Se0	8^{ab}	113.33^{a}	25.40^{a}	22.96^{a}	3.98^{ab}	0.26^{cd}
$As_0Se_{2.5}$	6^{b}	90^{bc}	22.36^{a}	10.07^{e}	4.66^{a}	0.27^{cd}
$As_0Se5.0$	7^{ab}	95^{b}	23.56^{a}	15.68^{c}	4.53^{a}	0.28^{cd}
$As_{11}Se0$	7^{ab}	109^{a}	26.68^{a}	20.36^{ab}	3.32^{ab}	0.29^{c}
$As_{11}Se2._{5}$	8^{ab}	114.33^{a}	24.40^{a}	22.25^{a}	5.77^{a}	0.54^{a}
$As_{11}Se5.0$	9^{a}	84^{cd}	23.56^{a}	17.81^{bc}	5.01^{a}	0.34^{b}
$As_{33}Se0$	8^{ab}	107^{a}	24.56^{a}	21.26^{a}	1.77^{bc}	0.12^{e}
$As_{33}Se2._{5}^{ab}$	9^{a}	77^{d}	21.44^{a}	14.86cd	0.51^{c}	0.02^{f}
$As_{33}Se5.0$	7	79^{d}	21.72^{a}	12.01^{de}	3.38^{ab}	0.24^{d}
Level of sig.	**	**	NS	**	**	**
CV%	16.58	15.0	9.30	25.97	47.78	52.71

[In a column, values having different letter(s) differ significantly, but with common letter(s) do not differ significantly at 5% and 1% level of probability analyzed by Tukey, denoted by * and **, respectively. NS= Not significant, CV = Coefficient of variation]

11.3.4 Conclusions from 3rd Exp.

Yield of Boro rice (28.90 and 28.66 g/pot) increased due to increasing Si and Fe treatment in naturally As contaminated soil. Arsenic accumulation significantly ($p<0.01$ and 0.05) reduced in dehusked Boro grain compare to control at previously Si and Fe treated soil but no significant effects for Mn and Se. Accumulation of As in dehusked grain of Boro rice is below the recommended level (0.2 mg kg^{-1}) for previously Si and Fe treated naturally As contaminated soil (11 and 33 mg kg^{-1}) but no significant effect for Se and Mn treatment. There are significant ($p<0.01$ and 0.05) increase of Si and Fe in grain with decrease of As accumulation.

11.4 General Conclusions

Research findings reflected that Si and Fe were highly effective against As accumulation in above ground plant parts for all of experimented rice varieties. These trace elements inhibit As accumulation in rice shoot, husk and grain both in artificially and naturally As contaminated soil in addition to reduce the phytotoxicity of As at different levels hence increases the yield. Arsenic accumulation pattern is always- root > shoot > grain. The better effect was found in naturally As contaminated soil (grain As < 0.2 mg kg^{-1}, the recommended As level in rice) than artificially As incubated soil due to addition of different microelements. The increased concentrations of Si, Fe, Mn and Se in grain in As treated and naturally As contaminated soil indicated their phytofortification ability.

11.5 Future Research

- Further research should be conducted at field level of different As contaminated areas using Si and Fe at different level with other rice varieties.
- Advance research should be conducted to find out the molecular mechanism for inhibition of As accumulation by adding these elements in soil

12. Research highlight/findings

- Si (25 and 50 mg kg^{-1}) and Fe (50 and 100 mg kg^{-1}) were shown to be highly potential against As accumulation in above ground plant parts for all of experimented rice varieties
- Arsenic accumulation pattern was always- root > shoot > grain.
- Arsenic accumulation was significantly ($p<0.01$ and 0.05) lower in dehusked grain compare to control due to treatment of microelements in soil
- The better effect was found in naturally As contaminated soil (grain As < 0.2 mg kg^{-1}, the recommended As level in rice) than artificially As incubated soil due to addition of Si and Fe.
- Si and Fe are help to reduce the phytotoxicity of As in rice at different levels hence increases the yield.
- The increased concentrations of Si, Fe, Mn and Se in rice grain in As treated and naturally As contaminated soil indicated their phytofortification ability.

13. References:

Carey, AM., Lombi, E., Donner, E. 2012. A review of recent developments in the speciation and location of arsenic and selenium in rice grain. Anal Bioanal Chem (2012) 402: 3275.

Lichtenthaler, H.K., Wellburn, A.R. 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochem. Soc. Trans. 11, 1-592.

Sims, D.A., Gamon, J.A. 2002. Relationships between leaf pigment content and spectral reflectance across a wide range o species, leaf structures and developmental stages. Remote Sens. Environ. 81, 337-354.

Tripathi, P., Tripathi, R.D., Singh, R.P., Dwivedi, S., Goutam, D., Shri, M., Trivedi, P.K., Chakrabarty, D. 2013. Silicon mediates arsenic tolerance in rice (Oryza sativa L.) through lowering of arsenic uptake and improved antioxidant defense system. Ecol. Eng. 2013, 52, 96–103.

B. Implementation Position

1. Procurement:

Description of equipment and capital items	PP Target		Achievement		Remarks
	Phy (#)	Fin (Tk)	Phy (#)	Fin (Tk)	
(a) Office equipment	Desktop computer, Laser printer, UPS, Scanner, Digital camera, executive table, Executive chair, steel almirah, visitor chair, computer chair	187500	Desktop computer, Laser printer, UPS, Scanner, Digital camera, executive table, Executive chair, steel almirah, visitor chair, computer chair	185000	
(b) Lab &field equipment	Soil and plant digestion chamber, Analytical balance, Auger	489000	Soil and plant digestion chamber, Analytical balance, Auger	488000	
(c) Other capital items	-	-	-	-	-

2. Establishment/renovation facilities: Not applicable

Description of facilities	Newly established		Upgraded/refurbished		Remarks
	PP Target	Achievement	PP Target	Achievement	

3. Training/study tour/ seminar/workshop/conference organized: Not applicable

Description	Number of participant			Duration (Days/weeks/ months)	Remarks
	Male	Female	Total		
(a) Training					
(b) Workshop					

C. Financial and physical progress

Fig in Tk

Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
A. Contractual staff salary	416466	416466	416466	0	100	-
B. Field research/lab expenses and supplies	993827	993827	993827	0	100	-
C. Operating expenses	202727	202727	202727	0	100	-
D. Vehicle hire and fuel, oil & maintenance	57500	57500	57500	0	100	-
E. Training/workshop/seminar etc.	0			0		-
F. Publications and Printing	121500	38356	15000	23356	10	Back to NATP
G. Miscellaneous	33000	32000	32000	0	99	-
H. Capital expenses	673000	673000	673000	0	100	-

D. Achievement of Sub-project by objectives: (Tangible form)

Specific objectives of the sub-project	**Major technical activities performed in respect of the set objectives**	**Output(i.e. product obtained, visible, measurable)**	**Outcome(short term effect of the research)**
To examine the potentiality of different micro/trace elements like Fe, Mn, Si, Se etc. for inhibition of arsenic bioaccumulation in rice.	Arsenic contaminated and uncontaminated soil sampling, micronutrient incubation at different rates, pot preparation, Aus, aman and boro rice cultivation, agronomical parameters collection, photosynthetic pigments and chemical analyses, data analysis etc.	As free rice (grain As < 0.2 mg kg^{-1}) is produced	Si and Fe is highly potential to inhibit the accumulation of As in rice grain
To find out the suitable dose of trace elements for practical application in different arsenic contaminated soils to avoid deficiency and/or toxicity	Arsenic and treated micronutrients data analysis with yield and yield contributing characters of different rice varieties grown in aus, aman and boro season	Suitable dose is found for As inhibition in rice	Si @ 25 and 50 mg kg^{-1} and Fe @ 50 and 100 mg kg^{-1} is suitable for As inhibition in rice grown in naturally As contaminated soil collected from Faridpur
To analyze the bioaccumulation/enrichment of micro/trace elements and arsenic in rice and soil	Chemical and biochemical analyses of rice root, shoot, husk, and grain for different treated and untreated soil	Micronutrient enriched rice produced	Si, Fe, Mn and Se contents in rice grain increased for all of the micronutrient incubated soil

E. Materials Development/Publication made under the Sub-project:

Publication	Number of publication		Remarks (e.g. paper title, name of journal, conference name, etc.)
	Under preparation	Completed and published	
Technology bulletin/ booklet/leaflet/flyer etc.			
Journal publication	02		
Information development			
Other publications, if any (MS Thesis)		**02**	

F. Technology/Knowledge generation/Policy Support (as applied):

i. Generation of technology (Commodity & Non-commodity)

Arsenic free rice production technology through Si and Fe phytofortificaiton

ii. **Generation of new knowledge that help in developing more technology in future**

Agronomic phytofortification can be applied for enrichment of specific elements in grain

iii. Technology transferred that help increased agricultural productivity and farmers' income

Si and Fe inhibit the As accumulation in rice which can be applied in the field condition for As free rice production

iv. Policy Support

After field experimentation in several places, this technology can be incorporated in national policy for execution in the As contaminated areas

G. Information regarding Desk and Field Monitoring

i) Desk Monitoring [description & output of consultation meeting, monitoring workshops/seminars etc.):

ii) **Field Monitoring (time& No. of visit, Team visit and output):** Three times by internal (RTC, PSTU) and external (BARC) teams and gave some suggestions.

H. Lesson Learned (if any)

i) Arsenic accumulation can be inhibited in grain using Fe and Si in soil without disturbing the soil environment.

I. Challenges (if any)

i) Time was too short to complete the field and lab activities using limited budget

Signature of the Principal Investigator
Date ……………………….
Seal

Counter signature of the Head of the organization/authorized representative
Date ………………………..
Seal