Silica sources for arsenic mitigation in rice: Machine learning based predictive modeling and risk assessment

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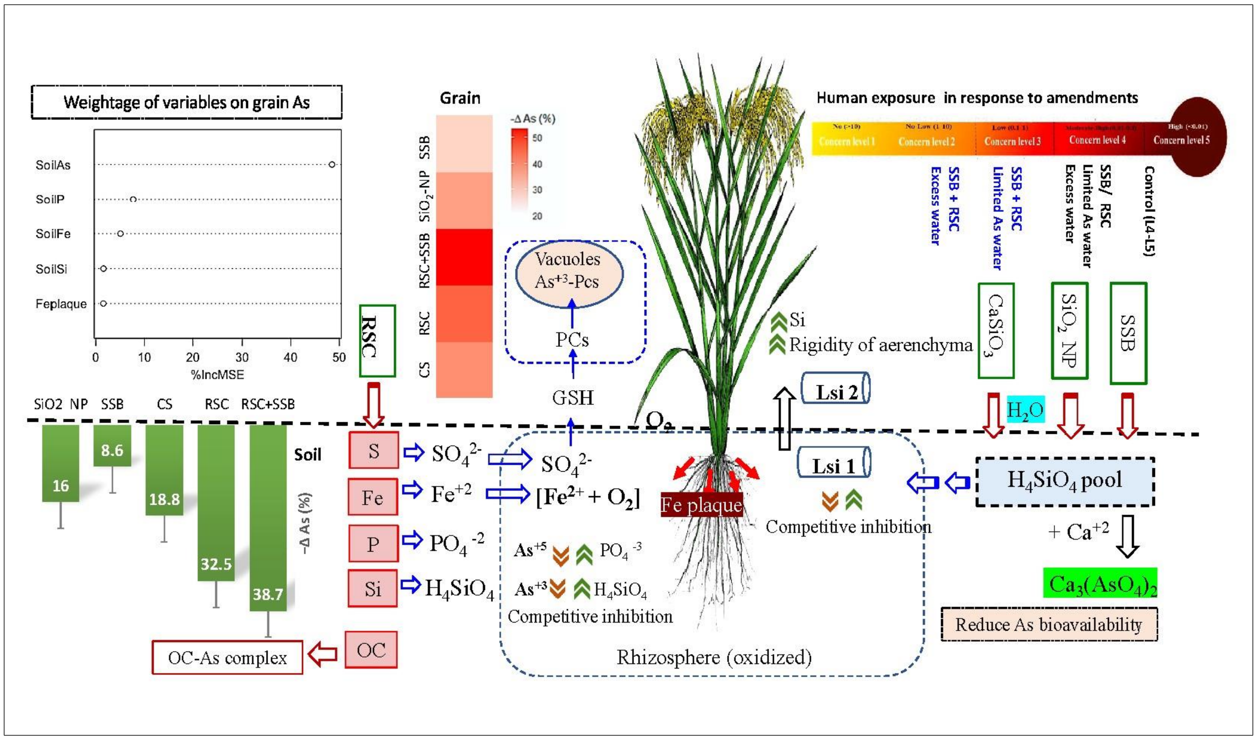
Abstract

Arsenic (As) is a well-known human carcinogen and the consumption of rice is the main pathway for the South Asian people. The study evaluated the impact of the amendments involving $CaSiO_3$, SiO_2 nanoparticles, silica solubilizing bacteria (SSB), and rice straw compost (RSC) on mitigation of As toxicity in rice. The translocation of As from soil to cooked rice was tracked and the results showed that RSC and its combination with SSB were the most effective in reducing As loading in rice grain by 53.2%. To determine the risk of dietary exposure to As, the average daily intake (ADI), hazard quotient (HQ), and incremental lifetime cancer risk (ILCR) were computed. The study observed that the ADI was reduced to one-third (0.24 μg kg^{-1} BW) under RSC+SSB treatments compared to the control. An effective prediction model was established using random forest model and described the accumulation of As by rice grains depend on bioavailable As, P and Fe which explained 48.5, 5.07 % and 2.6% of the variation in the grain As, respectively. The model anticipates that to produce As benign rice grain, soil should have P and Fe concentration more than 30 mg kg^{-1} and 12 mg kg^{-1}, respectively if soil As surpasses 2.5 mg kg^{-1}.

Keywords

Arsenic; Rice; Silicon; Machine learning; Random forest model; Human exposure

Graphical Abstract



1 Introduction

Arsenic (As) poisoning is reported to affect 200 million individuals globally, either through the consumption of As-contaminated groundwater or As-laced food crops, especially rice (Oryza sativa L.) (Khanam et al. 2023). As a class I carcinogen, As has been associated with a wide range of human illnesses, including fatigue, chronic respiratory disease, liver fibrosis, cardiovascular disorders and cancer (Guha Mazumder and Dasgupta, 2011). The As accumulation in rice is influenced by a number of environmental, biological, and geochemical factors. These factors regulate the solubility of As, its bioavailability in soil and its translocation to plant. (Islam et al. 2016; Mawia et al. 2021). Many studies aim to limit As accumulation in rice grains, employing diverse management strategies such as agronomic, biotechnological, nanotechnological interventions, and microbial supplementation. However, a majority of the studies that aimed to decrease As levels in the paddy-rice system were conducted in isolation, focusing on one or two amendments at a time. The primary goal was to assess their influence on As loading in grains, with limited understanding of the contributing factors and mechanisms involved in As transportation from soil to cooked rice and its detrimental effects. Reduction in bioavailable As and subsequently in grain As dose not only regulated by one or two elements supplied from any particular amendments. It involves the changes occurred in soil –plant system viz., change in pH, Eh, bioavailable P, Si, S, Fe and organic carbon and change in morpho-physiological characters (such as plant height, root biomass, reductive oxygen loss, Fe plaque deposition, phytochelatine production, number of nodes and internodes); and the expression of transporters in the plant on imposing of the amendments (Pan et al. 2020; Khanam et al. 2022).

In soil, Si addition either through ($CaSiO_3$ or SiO_2 nanoparticles), exhibits a dual action in influencing As mobility from soil to plant. Firstly, Si may improve As bioavailability by replacing

As from soil adsorption sites (Kumarathilaka et al. 2019; Khanam et al. 2023); In addition, Si has the ability to hinder the uptake and movement of As (III) within plants by competitively inhibiting transporters (*OsLsi1* and *OsLsi2*) (Pan et al. 2020). Interestingly, the accompanied Ca of $CaSiO_3$ may precipitate As as sparingly soluble $Ca_3(AsO_4)_2$, causing a net drop in As bioavailability outweighing the influence of SiO_2 nanoparticles. Like inorganic Si sources (such as $CaSiO_3$ and SiO_2), bio-organic amendments (such as rice straw compost and silicon solubilizing bacteria) also exerting multiple pathways to influence As mobility from soil to plant. Rice straw compost (RSC) is a rich source of not only Si (7%), but also C (28%), P (0.30%), Fe (835 mg kg^{-1}), Zn (35 mg kg^{-1}), cellulose (15%) and lignin (7%). The Fe released from amendments and reduced iron oxy-hydroxides can form Fe plaques in the rice rhizosphere. These plaques might work like a filter around rice roots, limiting the uptake of As by the roots. Further, the supply of P may reduce As translocation inside the plants by imposing competition for the same transporters (*OsPht1*; *OsPT8*). The addition of cellulose, organic C and other nutrients (such as S, Zn, Mn, Cu) enhance the phytochelatine (PCs) production, root biomass, plant height and number of nodes leading to further restriction of As to accumulate in grain.

To impose any management practices for reduction of grain As, prediction of the most important factors which directly or indirectly regulate grain As concentration is a priority. Therefore, in this investigation, an attempt has been made to identify the most important soil variables for predicting grain As concentration through machine learning technique (*Random forest model*). Such prediction of grain As concentration from changes in the important soil factors may give some scope to get As benign grain through maneuvering the identified soil parameters.

With all of the aforementioned factors considered, the current research was carried out to assess the (i) effect of the four amendments and their combinations on bioavailable Si, P, Fe and S and their influence on regulation of As mobility from soil to grain (ii) effect of soil amendments on the As distribution in grain fractions (iii) influence of the amendments on As loading in cooked rice and to human, and lastly (iv) to identify most influencing soil parameter in predicting grain As concentration through machine learning technique (*Random forest model*).

To date, the current investigation is the first of its kind to compare expansively the impact of most important Si sources (Viz., $CaSiO_3$, SiO_2 NPs, silica solubilizing bacteria, rice straw compost and their combinations and doses) on whole journey of As from soil to human, interplays of the factors, and their influence on As enrichment/depletion during processing of the grains and cooking of polished rice. Lastly, identifying the most important soil factors to predict grain As through machine learning techniques.

2 Materials and methods

2.1 Location of the study

The experiment was conducted using pot with a soil capacity of 10.0 kg at the ICAR-National Rice Research Institute, Cuttack, India. The experimental site is a hot and humid climate with an annual average rainfall of about 1668 mm, and maximum and minimum monthly temperatures of 35 ± 2.0°C and 25 ± 2.0°C, respectively. The soil used in this experiment was collected from topsoil (0− 20 cm) of a farmer's field at Nadia (N 23°01.901' and E 88°34.722'), West Bengal, which is reported as one of the worst arsenic hotspots and typical rice-growing area of South East Asia (Rahman et al. 2011; Khanam et al. 2021). The detailed physico- chemical properties of experimental soil is presented in Supplementary Table S1. The As level in irrigation water used for the experiment was maintained at 500 µg l^{-1} and 1000 µg l^{-1} during nursery preparation and throughout the growth period, respectively.

2.2 Crop cultivation and treatments details

IR 64, a high yielding rice variety predominantly grown in rice-growing region of eastern India (recognized as As hotspot), was used in the experiment. Seedlings of IR 64 were raised in small pots containing two kilograms of soil during the Rabi season of 2019-20 and 2020-21. The seedlings were irrigated on an as-needed basis with water treated with 500 µg l^{-1} of As. Twenty-one days old seedlings were transplanted in a hill of two plants per pot with three replications. Fertilizers were applied to each pot as per the recommended practices (N, P_2O_5 and K_2O at 80, 40 and 40 kg ha^{-1}). When necessary, irrigation was administered, keeping the water level in the pots at 7.0 cm from the time of transplanting to grain filling stage. Manual weeding was performed to control the weeds.

A total of seven different treatments were applied using four different sources of both organic and inorganic silicon (Si). The sources included silica solubilizing bacteria (SSB), rice straw compost (RSC), calcium silicate (CS), and SiO_2 nanoparticles (Si Np), which were used individually or in various combinations and doses. The details of the treatments including the doses and associated cost are given in supplementary table S2. The used SiO_2 nanoparticles were procured from Merck, Sigma Aldrich, America with particle size < 20 nm. The selected level of Si Nps (i.,e SiO_2 Np at 80 mg kg soil^{-1}, and SiO_2 Np at 40 mg kg soil$^{-1)}$ was based on the previous studies **(**Alvarez et al. 2018**).** The SSB culture was procured from ICAR- National Rice Research Institute and the precise amount of SSB culture is mixed completely with 2 kilograms of soil, resulting in a ratio of 2 milligrams of SSB culture to 2 kilograms of soil. Then, 10 grams of the soil mixture is moved into the selected pots intended for SSB treatment. The calculated quantity of RSC (at 5 t ha^{-1}) was added to respective pots for the respective treatment receiving RSC before transplanting of rice seedlings. The elemental composition of RSC used in this experiment is furnished in supplementary table S3

2.3 Sample collection and preparations

Samples were collected from all 7 treatments, with four replications each, at three distinct growth stages, namely tillering, panicle initiation (PI), and maturity, according to the standard protocol (Khanam et al. 2021a). The samples included root, shoot, and grain (upon maturity). The collected samples thus were subsequently rinsed with tap water free of As, as well as separately with deionized water and distilled deionized water. The samples were dried at 65 °C in an oven for 48 hours and ground using a stainless-steel mini-grinder. The grinder was opened and carefully cleaned with a nylon brush in between samples to prevent cross-contamination.

2.4 Processing of whole grain

Using a compact mill (THU-35C, Satake, Japan), the dried rice grain was hulled and milled. All the husk obtained after hulling was retained for future analysis. The resulting brown rice was further milled to produce polished rice and bran. The polished rice and bran were gathered and kept for subsequent analysis.

2.5 Sample digestion and instrumental measurement

Concentrated HNO_3 was used to digest the rice and soil samples for As analysis, following the method by Rahman et al. 2009. The As content was then measured using hydride generation atomic absorption spectrometry (HS-AAS), with the aid of analytical standard sodium borohydride (3%; Merck), sodium hydroxide (2.5%; Merck), and hydrochloric acid (6 M; Merck) for hydride generation. The detected As concentrations in all samples exceeded the instrumental detection limits (0.2 ppb), as reported by Khanam et al. 2022. To verify the precision of the analysis, certified standard reference materials (SRMs) (NIST, USA), including 1568a rice flour and 1573a tomato leaf, were utilized. (Supplementary Table S4).

2.6 DCB-extractable root Fe

To extract the iron plaques deposited on the rice root surface, a modified cold dithionite-citrate-bicarbonate (DCB) procedure (Liu et al. 2004) was employed. The root system was uprooted using a root-sampler and collected separately at each of the three growth stages for each treatment. The root systems were then gently washed with deionized water to remove dirt and debris. The root samples were incubated in a DCB solution containing sodium citrate, sodium bicarbonate, and sodium dithionite at 20°C for 2 hours to extract Fe plaque from the entire root system. The detailed methodology for extracting Fe plaque was described in Khanam et al. (2022).

2.8 Indices for arsenic transfer and risk assessment

2.8.1 Bioaccumulation factor (BAF)

The BAF refers to the proportion of As content in the roots of the plants relative to that in the soil (Arumugam et al. 2018). It was calculated by the following equation:

$$\text{BAF} = \frac{\text{Concentration of As in rice root}}{\text{Concentration of As in soil}}$$

2.8.2 Translocation factor (TF)

To calculate the relative translocation of As from rice plant roots to other plant components (shoot or grain), the following formula was employed (Arumugam et al. 2018):

$$\text{TF root to shoot (TFr-s)} = \frac{\text{Concentration of As in rice shoot}}{\text{Concentration of As in rice root}}$$

$$\text{TF shoot to grain (TFs-g)} = \frac{\text{Concentration of As in rice grain}}{\text{Concentration of As in rice shoot}}$$

2.8.3 Average daily intake (ADI)

The potential ingestion exposure to As from rice was evaluated by computing the Average Daily Intake (ADI) using the subsequent equation: ADI = (CiAs × IR) / bw × (EF × ED) / AT × BCF; where ADI denotes the mean daily intake of As (μg kg-1bw day^{-1}), CiAs is the concentration of inorganic As [μg kg^{-1}, with 86% of the total as inorganic, IR is the ingestion rate (0.4 kg day^{-1}), bw represents body weight (60 kg), EF is the frequency of exposure (365 d yr^{-1}), ED is the duration of exposure (70 yr), AT is the averaging time (25550 day) (USEPA 2011), and BCF is the bio-accessibility factor [taking 97% of As in cooked rice as bio-accessible to humans (Signes-Pastor et al. 2012)]. The calculated ADI values were subsequently employed to estimate both the carcinogenic and non-carcinogenic risks. (see 2.8.5 and 2.8.6).

2.8.4 Risk thermometer

Assessment of As toxicity exposure level was carried out through a risk thermometer taking into account the ADI values. The Swedish National Food Agency claims that a risk thermometer can demonstrate a risk prediction assessment procedure (Sand et al. 2015). The risk thermometer evaluates the exposure to a toxic element in food by comparing it with the material's health-based reference value, also known as the Tolerable Daily Intake (TDI), using the following equation: SAMOE (Severity Adjusted Margin of Exposure) = TDI / (AF_{BMR}×AF×SeF×E); Where, TDI (Tolerable Daily Intake) = 3.0 μg kg^{-1}bw day^{-1}; AF_{BMR} = Non-linear relation in dose range (1/10; BMR - Benchmark response); AF (Assessment factors) = A factor 10 (conservative assessment); SeF (Severity factor) = A factor 100; E= Different exposure factor.

2.8.5 Hazard quotient (HQ)

To estimate chronic-toxic risk, the hazard quotient (HQ) was computed using the subsequent equation: HQ = ADI / Rfd; where ADI represents the average daily intake of As (μg kg^{-1}bw day^{-1}) and Rfd is the reference dose of 0.3 μg kg^{-1}bw day^{-1} (US EPA, 2011).

2.8.6 Incremental Lifetime Cancer Risk (ILCR)

To determine the Incremental Lifetime Cancer Risk (ILCR) associated with ingestion exposure, the following formula was utilized: ILCR = ADI x SF; where ADI represents the average daily intake of As (μg kg^{-1}bw day^{-1}) and SF stands for the slope factor of As (per mg $kg^{-1}day^{-1}$). In this study, the SF value used was 1.5 (per mg $kg^{-1}day^{-1}$) (US EPA, 2011).

2.9 Random Forest Model

The Random Forest is a supervised machine learning algorithm that utilizes the principle of recursive partitioning (Breiman, 2001) for classification and regression tasks. It operates independently of the assumption of functional relationships between the predictor and response variables. The random forest model was run considering rice grain arsenic (GrainAs) as the dependent variable and the soil parameters namely available soil arsenic (SoilAs), available soil phosphorus (SoilP), available soil silicon (SoilSi), available soil iron (soilFe) and iron plaque (Feplaque) as the predictor variable was developed using the '*randomForest' (version 4.6-14) package* with a *ntree=1000* and *mtry*=5. The preparation of the partial dependence plot from the random forest was conducted using the 'pdp' package (version 0.7.0).

2.10 Statistical analysis

The data corresponding to each characteristic were analyzed using IBM SPSS Statistics v.26 software and R-Studio (Version 1.3.1093 2.3.1). The Least Significant Difference (LSD) was computed for each parameter at a significance level of 5% and 1% and Duncan's multiple regression test (at P< 0.05) was also carried out. The data presented in the tables show the average values from four replications conducted at various crop growth stages.

3. Result

3.1 Bioavailability of soil As, Fe, Si, P and S

The application of the silicon sources both individually and in combination resulted in significant reduction in bioavailable As ($NaHCO_3$ extractable) content as compared to the control. The application of four different amendments resulted in varying degrees of reduction and followed the order of RSC (32.5%) > CS (18.8%) > Si NPs at RD (16.1%) > Si NPs at 50% RD (13.4%)> SSB (8.6%) over control (Table 1). The combination of SSB+RSC caused a further reduction by 38.7%. The inclusion of RSC thus outperformed others in the reduction of bioavailable As. Irrespective of amendments applied, the Si concentration of amended soil increased sharply to 20.59% (mean) over control and followed the sequence: CS (34.87%)>Si NP at RD (29.69%)> SSB+RSC (21.99%) >Si

NPs at ½ RD (13.21%)> SSB (12.61%) >RSC (11.61%) (Fig. 1& 2). On average, the increase in available Fe, P and S was found highest with the application of SSB+RSC (57.87, 37.57 and 52.7%) followed by the single application of RSC (33.88, 29.88 and 37.2%) and CS (22.3, 14.79 and 15.2%), respectively (Fig. 1& 2).

3.2 Arsenic concentration in different plant plants at different growth stages

On average, the performance of the individual application of the amendments in reducing As (-Δ As, %) content in roots and shoots at maturity followed the order: RSC (45.5, 35.2 and 48.6) > CS (39.0, 50.3 and 44.6) > Si NP at RD (36.4, 39.1 and 42.6) > Si NP at 50% RD (32.0, 33.1 and 33.6), respectively compared to the control (Fig. 3). It is evident from the findings that the application of SSB+ RSC in combinations resulted in a greater reduction (-Δ As, %) in roots, shoot, and grains with the value of 49.4%, 34.2%, and 53.2%, respectively. The same trend was observed throughout growth stages, but more at the maturity stage than tillering and PI. The effectiveness of the amendments in reducing As in root, shoot and grain (maturity) increased at tillering (15.0 and 17.0%, respectively), PI (21.2 and 32.4%) and maturity (41.3, 39.9 and 38.4%, respectively) over control. This was more with SSB+ RSC treatment compared to the others.

3.3 Root biomass, Fe-plaque, number of nodes, plant height and translocation of arsenic in soils to plants

The highest amount of root biomass was found in the SSB+RSC treatment, with a value of 45.2 g per hill, followed by the RSC (41.3 g $hill^{-1}$), Si NP at RD (41.3 g $hill^{-1}$) and SSB (34.8 g $hill^{-1}$). The SSB+RSC treatment resulted in the highest Fe plaque (DCB Fe) with a concentration of 3140 mg kg^{-1}, followed by the RSC (2911 mg kg^{-1}). Compared to the control, the addition of amendments has a significant effect (P > 0.05) on plant height, no of nodes and tiller numbers. The maximum plant height (cm), no of nodes and tiller number were found with SSB+RSC (107.3, 6, 18.7, respectively) followed by CS (98.2, 5.3, 16.3), and Si NP at RD (93.8, 4.3 and 17.2), respectively. Interestingly, the magnitude of increases in Fe plaque formation per unit of root biomass (DCB-Fe/root biomass) was found highest with SSB+RSC (78.12) closely followed by RSC (77.30) and CS (74.60). Irrespective of the amendments, the grain As concentration showed a strong negative relationship with DCB Fe (r = -0.742**, P<0.001); whereas, a positive relationship was observed between DCB Fe and root biomass (r = 0.624*, P<0.005) (Fig. 4).

The average transfer rates of As from soil to root (BAF) and from shoot to grain (TFs-g) were found to be the lowest (3.4 and 0.16, respectively) with SSB+RSC followed by RSC (4.0, 0.20) and CS (4.1, 0.22), compared with the control (5.35, 0.31), respectively (Table 2); on the other hand, the transfer rate of As from root to shoot (TFr-s) was found to be the lowest when using CS (0.29), and was followed by Si NP at RD (0.32) and SSB+RSC (0.35). The concentration of As in the grain was found to have a strong positive correlation with the BAF (r = 0.901**, P<0.001) and TFs-g (r = 0.802**, P<0.001) (Fig. 4).

3.4 Arsenic partitioning in rice grain and cooked rice

The order of efficacy for the various amendments in terms of reducing the levels of arsenic (As) in the endosperm (-ΔAs polished rice, %), bran (-ΔAs bran, %), and husk (-ΔAs husk, %) of the grains, on average, was as follows: SSB+ RSC (46.9, 51.9 and 57.5) > CS (43.8, 46.2 and 50.0) > RSC (39.1, 42.3 and 45) > Si NP at RD (35.9, 36.5 and 42.5), respectively compared with the control. Interestingly, SSB+ RSC caused the highest percent accumulation of As in husk (45%) compared with others (37%). This caused the entry of a lower amount of As in endosperm with SSB+ RSC (19%) compared with others (25%). Irrespective of amendments applied, As content of cooked rice reduced by 31-39% of raw rice when cooked with excess water (water: rice = 6:1) (Fig. 5). However, cooking with contaminated limited water (water: rice = 2.5:1) caused, on average, 17.8 % increase in

As in cooked rice.

3.5 Dietary exposure to arsenic through rice consumption

The consumption of rice cultivated with various amendments resulted in considerably lower levels of average daily intake (ADI) of arsenic (As), with the lowest levels observed in rice grown with SSB+RSC (0.25 μg kg^{-1} BW) followed by RSC (0.36 μg kg-1 BW), CS (0.47 μg kg^{-1} BW), SSB (0.50 μg kg^{-1} BW), Si NP at RD (0.51 μg kg^{-1} BW), and control (0.88 μg kg^{-1} BW), as outlined in Table 3. The hazard quotient (HQ) was also found to differ across the amendments, with values of 0.81, 0.98, 1.56, and 2.92 recorded for SSB+RSC, RSC, CS, and control, respectively (Table 3). Similarly, the incremental lifetime cancer risk (ILCR) for As intake through consumption of cooked rice grown with amendments ranged from 0.44×10^{-3} to 0.86×10^{-3}, whereas the corresponding value for the control was 1.31×10^{-3}.

The 'Risk thermometer' and the calculated 'Severity Adjusted Margin of Exposure' (SAMOE) value for As toxicity of rice raised with different treatments and cooked in different types (contaminated and non-contaminated) and amounts of water (limited and excess) showed separate concern levels of risk from class 3 to class 5 (Fig. 6). Irrespective of cooking method, rice grains raised under control treatment always showed lowest SAMOE values (0.06 to 0.1), followed by SSB (0.07 to 0.2) and RSC (0.1 to 0.22). Whereas, SSB+RSC treatment showed the highest SAMOE values ranging from 0.14 to 0.5. Thus, SSB+RSC showed a concerning level of low risk (class 3), whereas the RSC and SSB showed moderate (class 4) and control showed high risk (class 5) based on the quality of water used for cooking.

3.7 Rice grain arsenic based on *Random forest model*

Random Forest is a supervised machine learning technique that employs recursive partitioning as its fundamental principle for classification and regression tasks. Unlike conventional statistical methods, it does not require any preconceived functional relationships between the response and predictor variables. The variable importance plot from the random forest model for rice grain As content is furnished in figure 7a. The random forest model analysis showed that change in bioavailable soil As (Δ Soil As) explained 48.5% of the variation in the grains As (Fig. 7a). Whereas, change in soil P (Δ Soil P) is the second most important factor in determining grain As content explaining 7.7% variation in grain As content. Change in soil Fe (Δ Soil Fe), soil Si (Δ Soil Si) and Fe plaque (Δ DCB Fe) on rice roots were also the major factors affecting grain As, explaining 5.07%, 1.6% and 1.5% of the variation in the rice, respectively in terms of percentage increase of mean square error (% Inc MSE). (Fig. 7a). Whereas, in terms of increase of node purity (Inc Node Purity), the sequence of importance of the factors in determining grain As content is Δ Soil As> Δ Soil P> Δ Soil Fe > Δ DCB Fe> and Δ Soil Si. The strength of the relationships between predictor variables (soil As, soil P, soil Fe, DCB Fe and soil Si) and grain As concentrations were shown using the partial dependence plots (PDPs). The utilization of partial dependence plots facilitates the visualization of the interrelation between a specific subset of predictors and the response variable, while simultaneously accounting for the average impact of other covariates in the model. The Partial dependence plots (Fig. 7) showed that irrespective of soil Fe and Si, grain As is found to be lowest (0.8-0.9 mg kg^{-1}) when soil As content is <2.5 mg kg^{-1} and soil available P is high (> 30 kg ha^{-1}). Further, figure 8 explains if bioavailable soil As is > 3 mg kg^{-1} and available soil Fe is < 12 mg kg^{-1}, then grain As will be above the safer limit (i.,e > 1 mg kg^{-1}).

4.0 Discussion

In this study, seven sub-systems (or treatments) were utilized, each using different combinations of four sources of Si. The results showed a discrepancy between the amount of Si in the soil and the loading of As in various plant parts, including roots, shoots, and grains. This suggests that other factors, such as P, Fe, and S supplied by the amendment sources, also play an important role in the movement of As from soil to plants. The study aimed to uncover the influence of these determinants on the loading of As not only in rice plants but also in cooked rice, in order to comprehensively assess the impact of amendments on As loading and potential human poisoning.

4.1 Arsenic absorption by roots

Factors affect the transfer of As from soil to rice roots, such as: (i) the decrease in bioavailable As content, (ii) changes in soil parameters such as bioavailable Fe, P, Si, and S, (iii) variations in the amount of root biomass produced by the rice plant, (iv) the extent of Fe plaque formation in the rice rhizosphere, and (v) the presence of As uptake transporters in rice roots when amendments are present. All of the amendments, whether used alone or in combination, significantly reduced the bioavailability of As in the soil. Performance of the amendments in reducing As in root was: SSB+RSC (38.7%)> RSC (32.5%) > CS (18.8%) > Si NPs at RD (16.0%) > Si NPs at 50% RD (13.4%)> SSB (8.6%) over control (Fig. 2). The application of amendments increases bioavailable Si in soil with the maximum increase with CS (34.87%) followed by Si NP at RD (29.69%). Arsenic is able to enter rice roots through the Si influx transporter OsLsi1, as well as through certain plasma membrane intrinsic proteins (PIPs), including *OsPIP2; 4*, *OsPIP2; 6*, and *OsPIP2; 7*, as identified by Kumarathilaka et al. (2019). However, when the soil has an increased level of silicic acid resulting from the application of Si sources, there is competition between silicic acid and As (III) to enter the roots, which leads to a significant reduction in the accumulation of Arsenic in rice roots. The Si/As ratio in soil solution was observed highest (46.2) with RSC+SSB followed by RSC (36.8) which signifies higher competition between As and Si for uptake via same transporters. These higher competitions between Si and As under RSC+SSB treatment reduced the uptake of As in root. Further, higher concentration of Si in soil solution suppresses the expression of the transporter's gene (i.e., *OsLsi1*) (Ma et al. 2008). Silicic acid has been shown to boost the bioavailability of arsenic by displacing both As (III) and As (V) from exchange sites in soil. Furthermore, the higher presence of silicic acid in soil solution also restricts the absorption of As (III) and As (V) onto Fe plaques, as it takes up space on the adsorption sites. The occurrence of these processes simultaneously could potentially clarify the observed lesser reduction in root As levels, particularly when higher Si concentrations are applied using CS (34.87%) and Si NP at RD (29.69%) compared to SSB+RSC (21.99%) and SSB (12.61%) treatments. (Fig. 1). Although, the increase in bioavailable Si was highest with the application of CS (34.87%) followed by Si NP at RD (29.69%), but, the net drop in root As concentration did not show the same trend and found highest with RSC+SSB (53.2%) followed by RSC (46.2%). The unusual higher effectiveness of these amendments may explain by the change in Fe, P and S in soil solution. Change in bioavailable Fe, P and S was found to be highest with SSB+RSC (57.87, 37.57 and 52.7%) followed by RSC (33.88, 29.88 and 37.2%) treatments (Fig. 2), which, may further contribute to the drop in As entry into the roots. The iron (Fe) provided by the amendments contributes to the growth and inherent formation of Fe oxy-hydroxides over a span of several weeks of submergence in paddy rice, which in turn provides an additional surface area for the attraction and adsorption of arsenic (As) (III) and As (V) in the form of inner-sphere complexes, thereby reducing their solubility and bioavailability (Khanam et al. 2022; Mishra et al. 2021). The augmentation of soluble iron (Fe) levels has been reported to facilitate the formation of Fe-plaques on both roots and rhizosphere (Awasthi et al. 2017). These Fe-plaques exhibit a high degree of binding affinity toward both As (III) and As (V), thus acting as an effective buffer against As uptake into the roots. The supplementation of ferrous ions (Fe^{2+}) via amendments has been shown to promote the formation of Fe plaques, thereby impeding the influx of As into rice roots (Table 2). Existence of a significant and negative correlation between DCB Fe (Fe plaque) and root As level

(R^2 = -0.663**, P< 0.005) supports the contention. Further, the higher root biomass with RSC+SSB and RSC treatments (Table 3) confirms more ROL in rhizosphere (Wu et al. 2011) accelerating deposition of Fe-plaques onto root surfaces (Table 2). These findings are reinforced by the presence of a significant positive correlation between root biomass and the formation of Fe-plaques (R2 = 0.777**). In rice, AsV is absorbed by phosphate transporters such as *OsPht1;8* (*OsPT8*) as it resembles phosphate as an analog (Wang et al. 2016). The concentration of bioavailable P was found highest with the application of SSB+RSC (37.57 %) followed by the single application of RSC (29.88 %) and CS (14.79%). The higher content of bioavailable P may suppress the expression of the transporters genes and reduce the uptake of As in rice root. Wang et al. 2016 showed knocking down of *OsPht1;8* could decrease AsV uptake by 33–57% in rice. These findings suggest that the uptake of As by the roots is primarily influenced by the alteration in soil bioavailable As (ΔAs Soil) and change in bioavailable soil Fe (Δ Fe $_{Soil}$), and change in bioavailable soil P (Δ P $_{Soil}$), change in bioavailable soil S (Δ S $_{Soil}$) and change in bioavailable soil Si (Δ Si $_{Soil}$).

4.2 Arsenic translocation from roots to polished rice

The movement of As from roots to shoot and then to grains is mainly influenced by the presence of transporters, the storage of As in vacuoles, the production of phytochelatins, the morpho-physiological traits of plants, and competition among ions (such as As vs Si and As vs P). All the influencing factors are further influenced by the amendments used.

The relative translocation factors of As from root to shoot with the application of amendments was: Control (0.43)> SSB (0.39)> RSC (0.37)> Si NP at 50% RD (0.37)> SSB+RSC (0.35) > Si NP at RD (0.32)> CS (0.29) (Table 3). Therefore, the use of CS proved to be more effective in reducing the translocation of As from roots to shoot compared to the other treatments. Among the 7 treatments compared, we observed lower translocation rates (TFr-s values) of As in treatments with higher bioavailable Si (CS and Si NP at RD), suggesting the potential role of Si in reducing As translocation. Studies have reported that a silicic acid transporter (OsLsi2) is involved in the transport of As (III) from roots to shoots (Suriyagoda et al. 2018; Khanam et al. 2022). A distinct pattern was noted when tracking the movement of As from the shoot to the grains: Si NP at 50% RD (0.29) > Si NP at RD (0.27) > CS (0.24) > RSC (0.20)> SSB+RSC (0.16). This trend of TFs-g signifies, that wherever bioavailable S and P were higher in treatments (SSB+RSC and RSC), there was always a lower TFs-g. When As (III) enters the shoot from the roots, a significant proportion of it is bound with phytochelatins (PCs) and accumulated in the cell vacuoles and nodes, leading to limited transport into the grains. The ability to sequester As is improved by the production of GSH, which is stimulated by SO_4^{2-} provided through the application of SSB+RSC (12.67%) and RSC (11.3%) in the current study (Zou et al. 2018). As previously mentioned, nodes and internodes play a crucial role in storing As and act as a barrier to prevent As transfer to the grains. The number of nodes and internodes were found more with SSB+RSC (6.3) (Table 3) and it is expected that the sequestration in vacuoles will be greater with this treatment. Further loading of As into rice grain mainly carried out by phosphate transporters viz., *OsPT2*, *OsPT8*. Rice straw compost increase the available P in soil by decreasing its adsorption, exhibiting the competition for adsorption sites by organic anion. Further, RCS release low molecular weight organic acids, which dissolves the mineral associated P and increase its availability. Thus, the higher concentration of bioavailable P with the application of amendments may restrict As loading in grain by giving inhibitory competition for transporters (*OsPT2*, *OsPT8*) (Pan et al. 2020; Mishra et al. 2022). Interestingly, only 4.2 % (Table 2) of root As was transferred to grains, with the application of SSB+RSC followed by RSC (5.8%) and CS (6.01) showing almost the double in case of control (10.9%).

The distribution of As in different grain parts (i.e., husk, bran and polished rice) was also influenced by the amendment applied. The lowest amount of As found in the edible portion (polished rice) resulting from SSB+RSC (19%) followed by RSC (22%), CS (24%) and SSB (25%) treatments; whereas, the allocation in husk was the highest with SSB+RSC (45%). This indicated 43% reduction in polished rice As with the application of SSB+RSC.

The concentration of arsenic in cooked rice is not only dependent on the amount of arsenic present in polished rice but also on the method of rice preparation (Khanam et al. 2022). Cooking rice with excess water (water:rice = 6:1) and discarding the resultant gruel can decrease the concentration of arsenic in cooked rice by 31-39%, while cooking with limited water contaminated with arsenic (water:rice = 2.5:1) can increase the arsenic concentration in cooked rice by 17.8%. The extent of depletion of arsenic in cooked rice due to cooking methods did not show significant variation with the amendments, but rather depended on the concentration of arsenic in polished rice. During cooking with excess water, some water-soluble arsenic may be released from the raw rice to the cooking water due to irregular voids and channels created by high temperature (100°C). On the other hand, cooking rice with contaminated water may cause some arsenic to permeate from the cooking water to the cooked rice (Khanam et al. 2022).

4.3 Arsenic risk assessment

In this study, we evaluated potential risks by calculating several risk indicators, including ADI, SAMOE, HQ, and ILCR. To estimate the ADI of As from rice consumption, we assumed that 97% of As in cooked rice is bio-accessible in the human bloodstream (Signes-Pastor et al. 2012). Our results showed that the intake of As (μg kg-1 bw day-1) was 0.25, 0.30, 0.47, 0.50, and 0.51 times lower with SSB+RSC, RSC, CS, SSB, and Si NP at RD, respectively, compared to the control (Table 3). The hazard quotient (HQ) values for Si NP at RD (1.53), SSB (1.66), and control (2.93) exceeded the safe limit (HQ=1) for non-carcinogenic health risk, as shown in table 3. However, in section 3.5, we observed that polished rice obtained with RSC alone or in combination with SSB had As content below the permissible limit (0.20 mg kg-1). Additionally, the HQ values derived from the consumption of cooked rice prepared from the polished rice produced with these treatments (RSC and RSC+SSB) did not exceed the stipulated value (HQ=1), as depicted in figure 6. Similarly, the carcinogenic risk (ILCR) was reduced by 3.2 to 1.9 folds with the application of amendments compared to control.

The lower BAF, TF, and maximum depletion during processing of the polished rice was attributed to the lower ADI, HQ, and ILCR values with the application of amendments (Khanam et al. 2022). According to Khanam et al. 2022, BAF had the greatest impact in reducing ADI, followed by the transferability of As from shoot to grain (TFs-g). It is significant to notice that the quality of the cooking water utilized had an impact on both the SAMOE value and the risk level of As for cooked rice. The SAMOE value for RSC and SSB treatments increased when rice was cooked with non-contaminated extra water, which might put those treatments in the lower-level category of health threat. (Fig. 6).

4.4 Prediction of grain arsenic based on *Random forest model*

The Random Forest is a supervised machine learning algorithm that can be used for classification and regression. It is based on the principle of recursive partitioning and is independent of any assumptions about functional relationships between the response and predictor variables (Breiman, 2001). Briefly, Random Forest analysis ensembles numerous regression trees following a process called “bootstrap aggregation” or “bagging.”. In this investigation we made an attempt to identify the most important factors influencing the grain As content using *random forest model*. The factors considered were: ΔAs_{soil}, ΔFe_{soil}, ΔP_{soil}, ΔSi_{soil} and ΔDCB_{Fe} caused by the management practices followed, and the results explained 70% variability in the grain As. The maximum importance in determining grain As content was explained by ΔAs_{soil} (48.5%) followed by ΔP_{soil} (7.7%), ΔFe_{soil} (2.5%) and ΔSi_{soil}. In another study soil As, P and Fe also proved to be the important variables by random forest model as reported by Sengupta et al. (2021) and Mandal et al. (2023). The three dimension (3D) partial dependence plot (PDP) computed through random forest on variation of rice grain As content with the selected variables (ΔAs_{soil}, ΔFe_{soil}, ΔP_{soil}, ΔSi_{soil} and ΔDCB_{Fe} is a graphical representation of the impact of one or two input variables on the predicted output (grain As content) of a machine learning algorithm, such as a random forest in this case. It provides insight into whether the relationship between the target variable and a feature is linear, monotonic, or nonlinear as previously reported by Mandal et al. (2023). The PDP produced in this investigation, showed linear positive dependence between grain As and soil As, whereas, ΔFe_{soil}, ΔP_{soil}, ΔDCB_{Fe} had linear negative dependence. However, ΔSi_{soil} showed a monotonic or more complex relationship with grain As (Fig 7a, b). In the previous section (3.1), the dual effect of Si (increase soil As concentration but reduce As uptake) explains the monotonic relationship. The model further anticipates that to produce As benign rice grain (<1 mg kg^{-1}), soil P and soil Fe should be > 30 kg ha^{-1} and >12 mg kg^{-1}, respectively if the available soil As is >2.5 mg kg^{-1} (Fig. 8). Based on all these results we anticipated that reduction in grain As could be achieved only on maneuvering in the bottom-line supply chain of As in soil or its transport from shoot to grain. It may be attained by either reducing available soil As content or enhancing P and Fe concentration in soil solution. Further, changing the Fe and Si concentration may also reduce As content in rice grains.

5.0 Conclusion

Despite the management strategies proposed by several researchers at regular intervals, human exposure to As through rice consumption persists. The partial dependent plots from the machine learning technique for identifying the most important factor showed a change in soil P and Fe levels is more strongly related to grain As accumulation rather than a change in Si concentration in soil. This assessment would help farmers and researchers to choose the management options to reduce As concentration in food. Our findings indicate that applying one or two amendments may not be sufficient to directly reduce As accumulation in rice grain to a desired level (HQ < 1). However, these amendments can modify the regulating factors in the soil (such as pH, Soil As, Soil P, Soil Fe, S, Soil Si) and plant morpho-physiological characteristics, leading to the production of As-benign rice grain. This investigation represents one of the most complete works to date on the search for remediation in dietary As exposure through rice consumption dealing with all the possible interplays of soil and plant factors.

Authors' Contributions

RK: Experimental Work, Analysis, Writing – original draft, Reviewing; AKN: Conceptualization, Supervision, Reviewing, Editing; PGPSK: Modeling, Statistical analysis, Reviewing, Editing; JM: Modeling, Reviewing, Editing; MS: Reviewing, Editing, RT: Reviewing, Editing, PB: Supervision, Reviewing, PS: Analysis, SM: Reviewing, Editing, SSM: Analysis, MD: Analysis, RGB: Reviewing, Editing

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Data availability statement

The data that support the findings of this study are available on request from the corresponding author, [Dr A K Nayak]. The data are not publicly available due to their containing information that could compromise the privacy of research participants.

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Ethical Approval

Not applicable

Consent to Publish

The authors declare that all the authors agreed to publish the work in this journal.

Consent to participate

Not applicable

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Table 1. Changes in arsenic content (−Δ As, %) of soil and parts of rice plants and grains, on the application of the soil amendments, compared in the experiment. All the values are means ± standard error of three replications of the experiment. Values with different letter(s) within the same column show significant differences at P ≤ 0.05 level between treatments according to the Duncan's multiple range test (DMRT)

Treatment details		**Tillering**			**Panicle initiation**		**Maturity**		
		Soil	Root	Shoot	Root	Shoot	Root	Shoot	Grain
T2	SSB	8.6d	14.5c	23.5d	20.5ab	27d	27.3d	27.6d	25.1d
T3	RSC	32.5ab	17.5b	27.4c	24.6ab	31.6c	45.5a	35.2c	48.6ab
T4	SSB + RSC	38.7a	22.6a	29.7bc	32a	39.4a	49.4a	34.2c	53.2a
T5	$CaSiO_3$	18.8c	17.4b	31.8a	24.7ab	34.2e	39cd	50.3a	44.6b
T6	Si Np at RD	16.1cd	10.6d	27.2c	15b	27.8d	36.4c	39.1bc	42.6b
T7	Si Np at 50% RD	13.4d	7.4e	21.6d	10.5b	24.7e	32cd	33.1c	33.6c

RD: Recommended dose; **NP**: Nanoparticle; **SSB**: Silicate solubilizing bacteria; **RSC**: Rice straw compost

Table 2. The values of plant height (cm), number of tillers, number of nodes, root biomass (g $plant^{-1}$), Fe plaque (mg kg^{-1}), bioaccumulation factor (BAF), transfer factor (TF), and percent transfer of As with different soil amendments. All the values are means ± standard error of three replications of the experiment. Values with different letter(s) within the same column show significant differences at P ≤ 0.05 level between treatments according to the Duncan's multiple range test (DMRT)

Treatments	Treatment details	Plant height	Number of tillers	No of Nodes (*NS)	Root biomass	Fe plaque	BAF	TFr-s	TFs-g	Percent transfer from root to grain
T1	Control	73.3d	12.3d	3.7	32.4d	2321d	5.35	0.43	0.31	10.92
T2	Silicate solubilizing bacteria (SSB)	86.1c	14.0cd	3.7	34.8cd	2690bc	4.39	0.39	0.25	8.14
T3	Rice straw compost (RSC)	89.8bc	16.3b	4.0	41.3b	2911b	4.09	0.37	0.2	5.82
T4	SSB + RSC	107.3a	18.7a	6.3	45.2a	3531a	3.45	0.35	0.16	4.25
T5	$CaSiO_3$	98.2a	16.3b	5.3	36.3c	2708bc	4.08	0.29	0.22	6.01
T6	Si Np at RD	93.8bc	17.2b	4.3	41.5b	2755bc	4.10	0.32	0.27	6.56
T7	Si Np at 50% RD	91.3bc	15.0cd	4.0	34.2cd	2660c	4.20	0.37	0.29	8.91

*Non-significant

Table 3. The values of the estimated average daily intake (ADI, µg kg BW^{-1}), hazard quotient (HQ), incremental lifetime cancer risk (ILCR) and Severity Adjusted Margin of Exposure (SAMOE) of As from cooked rice prepared out of rice grains raised with different soil amendments

				Severity Adjusted Margin of Exposure			
				Non-contaminated water		Contaminated water	
Treatments details	ADI[a]	HQ	ILCR[b]	Limited	Traditional	Limited	Traditional
Control	0.88	2.93	1.31	0.083	0.123	0.065	0.074
Silicate solubilizing bacteria (SSB)	0.50	1.66	0.75	0.105	0.152	0.079	0.092
Rice straw compost (RSC)	0.30	0.98	0.55	0.157	0.217	0.113	0.134
SSB + RSC	0.25	0.83	0.44	0.207	0.571	0.143	0.174
$CaSiO_3$	0.47	1.56	0.70	0.169	0.231	0.124	0.147
Si Np at RD	0.51	1.53	0.76	0.147	0.213	0.111	0.128
Si Np at 50% RD	0.57	1.92	0.86	0.158	0.188	0.111	0.118

Average daily intake of rice (kg day^{-1}) = 0.4; [a]An adult's body weight of 60 kg was used; [b]ILCR = ADI x CSF x RBA, where ADI - average daily intake, CSF - cancer slope factor and RBA - relative bioavailability; RD: Recommended dose, NP: Nanoparticle

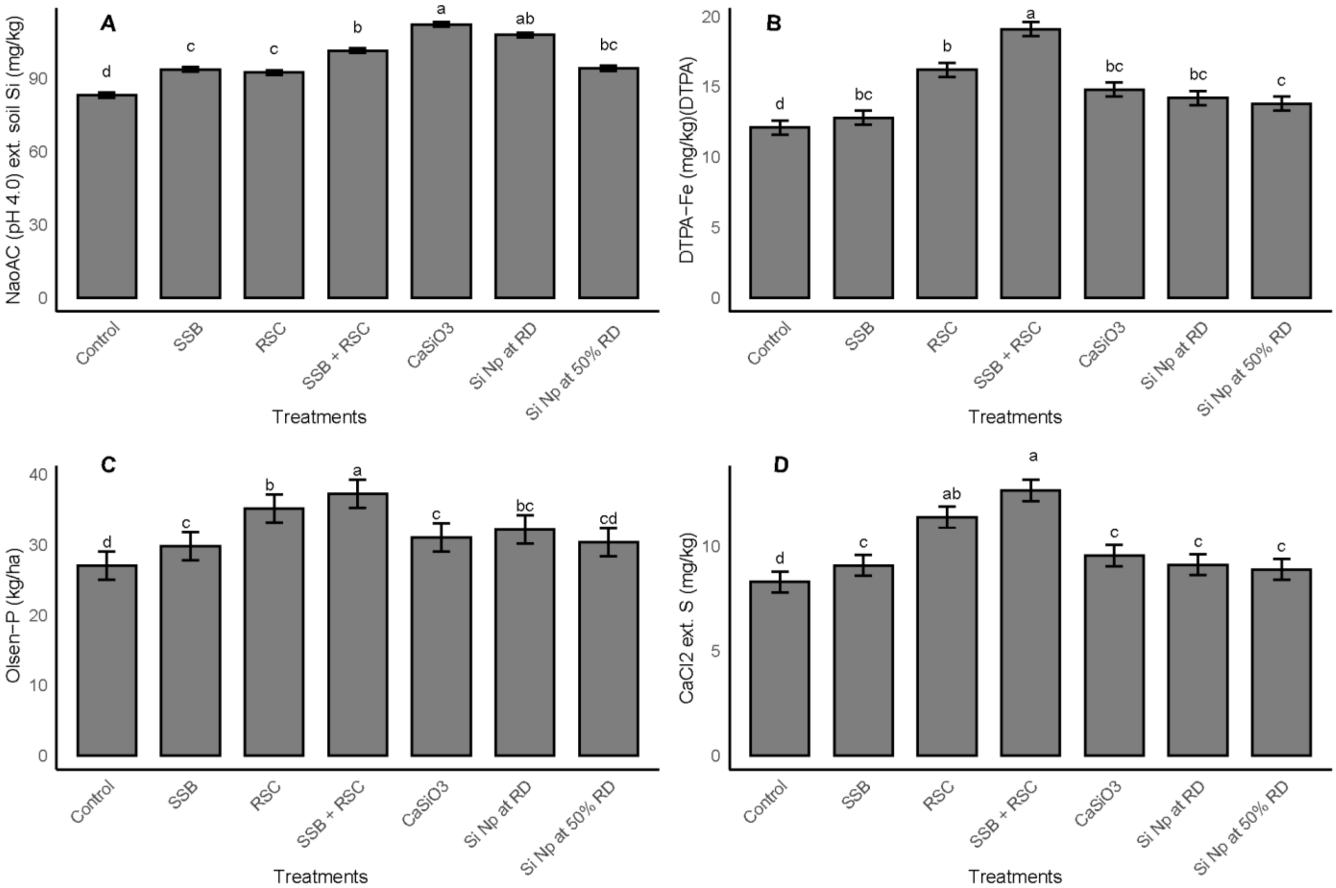


Fig. 1 Changes in levels of bioavailable NaOAc extractable Si (A), DTPA-Fe (B), Olsen- P (C) and $CaCl_2$- S (D) in response to different treatments at the grain filling stage (80 days after rice transplanting). All the values are means ± standard error of three replications of experiment. Bars having the same letter(s) are not significantly different according to DMRT at $P \leq 0.05$ significance level. **SSB**: Silica solubilizing bacteria; **RSC**: Rise straw compost; **Si Np at RD**: SiO_2 Np at 80 mg kg^{-1} soil; **Si Np at 50% RD**: SiO_2 Np at 40 mg kg^{-1} soil

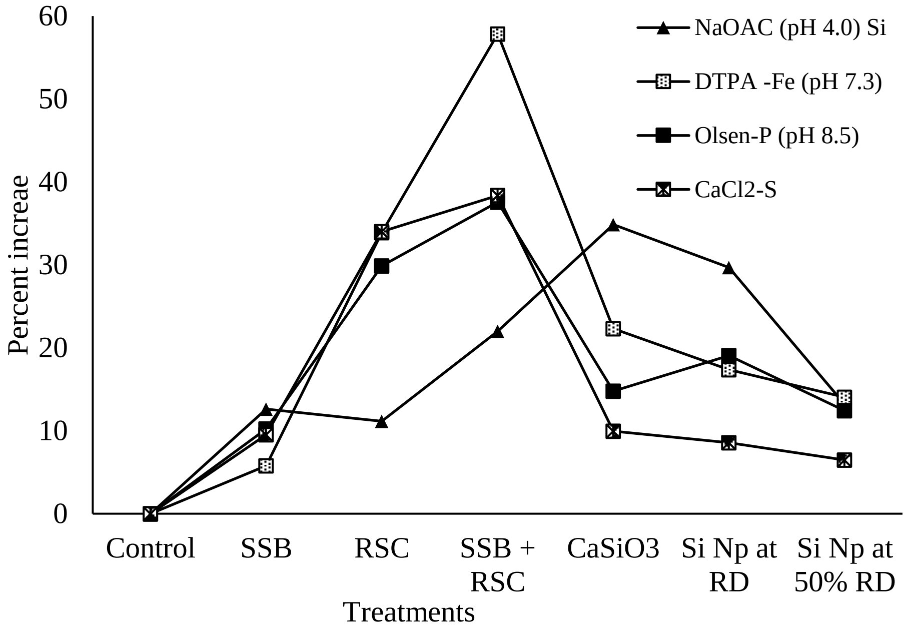


Fig. 2 Percent change in levels of bioavailable NaOAc extractable Si, DTPA-Fe, Olsen- P and $CaCl_2$- S in response to different treatments at the grain filling stage (80 days after rice transplanting).

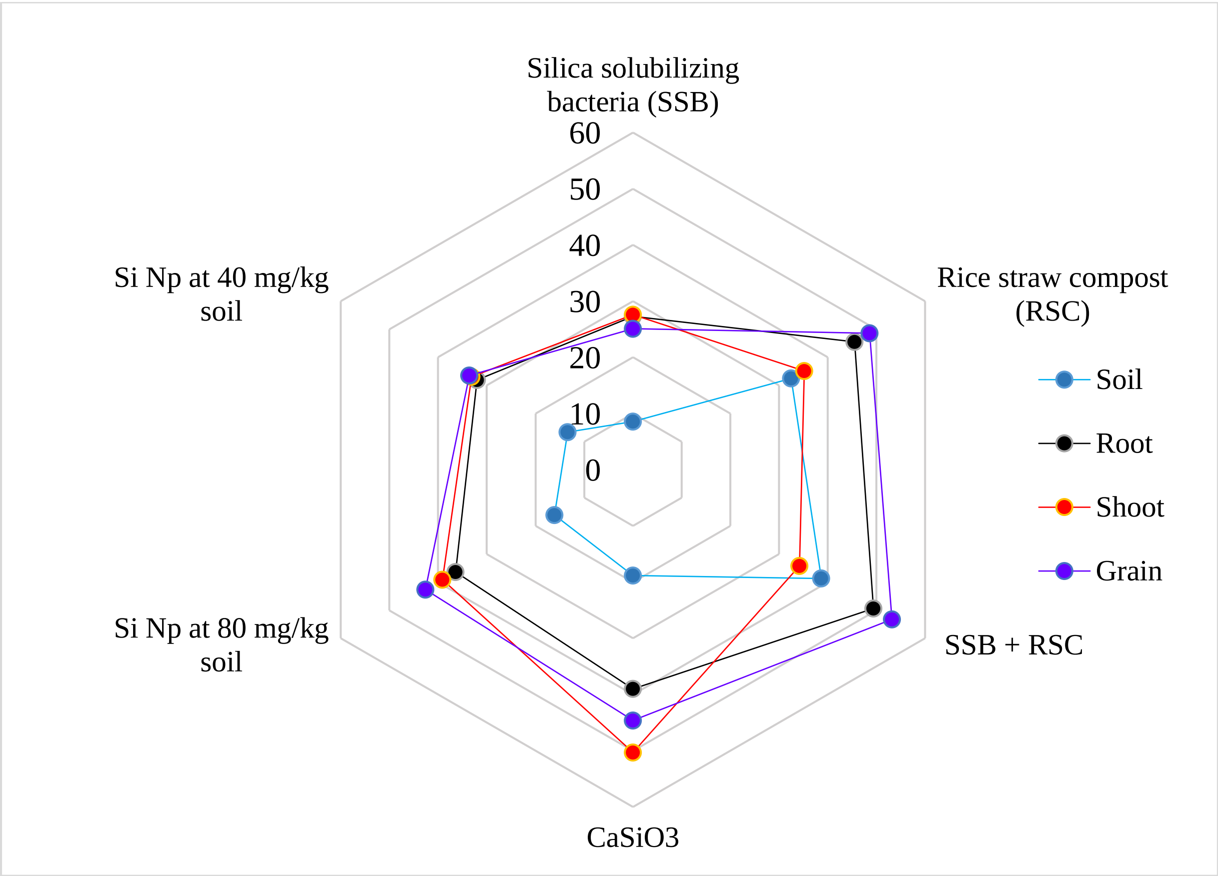


Fig. 3 Efficiency of the soil amendments (singly or in combinations) in causing reduction (% over the control) in arsenic contents in soil, root, shoot and grain at maturity.

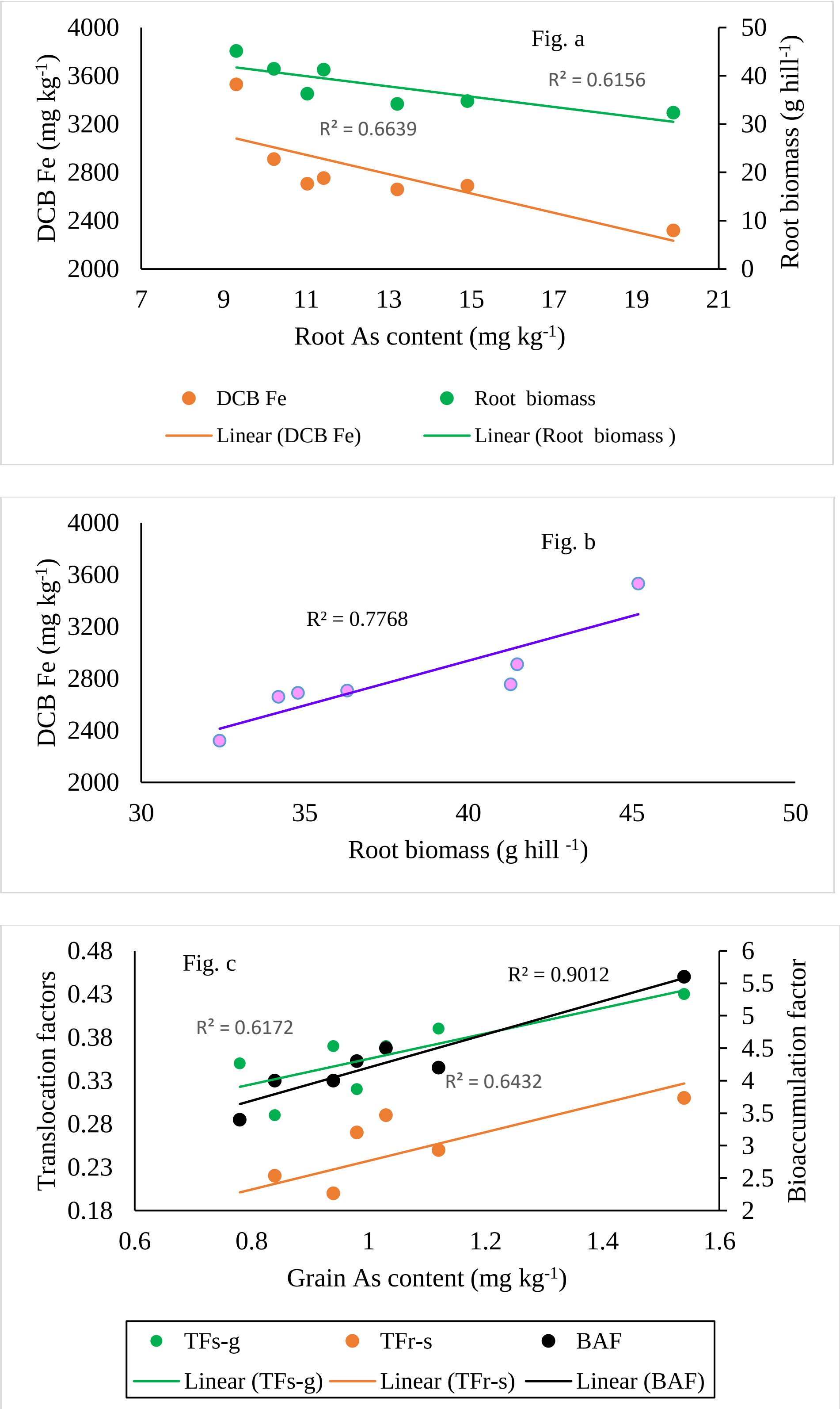
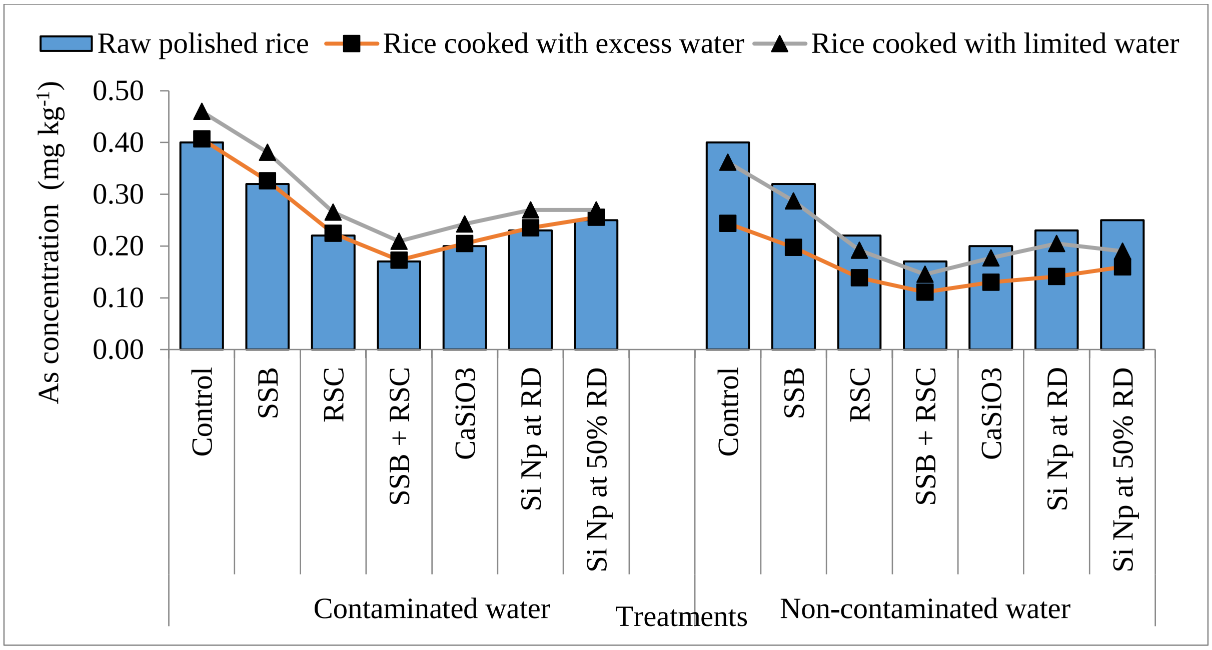


Fig. 4 Relationships of the root As concentration (ΔAs_{root}) with changes in root biomass (Δ Root biomass) and Fe plaque (Δ DCB Fe) formation (a & b) and Translocation factors (TFr-s, TFs-g) and Bioaccumulation factor (BAF) and the changes in grain As content with (ΔAs_{grain}) (c) on imposition of amendments



SSB: Silica solubilizing bacteria; RSC: Rise straw compost; Si Np at RD: SiO_2 Np at 80 mg kg^{-1} soil; Si Np at 50% RD: SiO_2 Np at 40 mg kg^{-1} soil

Fig. 5 Change in arsenic concentration in cooked rice prepared from the polished rice with different proportions of contaminated and non-contaminated water

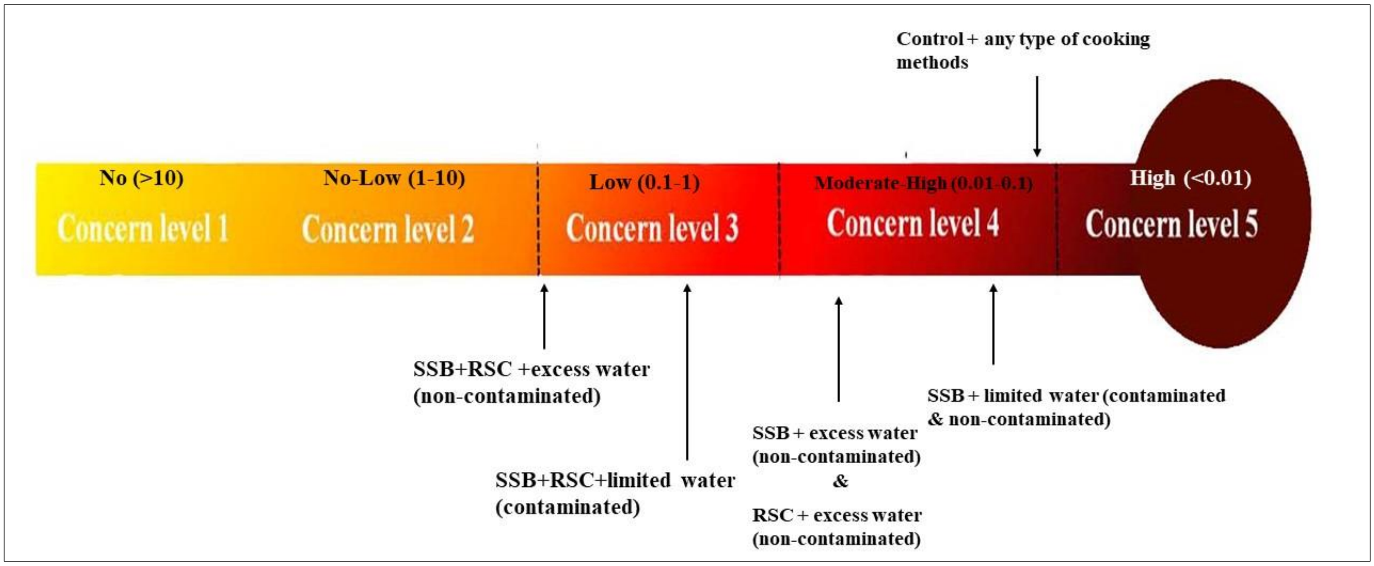


Fig. 6 Risk thermometer scale showing the class of As toxicity due to consumption of cooked rice prepared through different methods using rice grains raised under different treatments

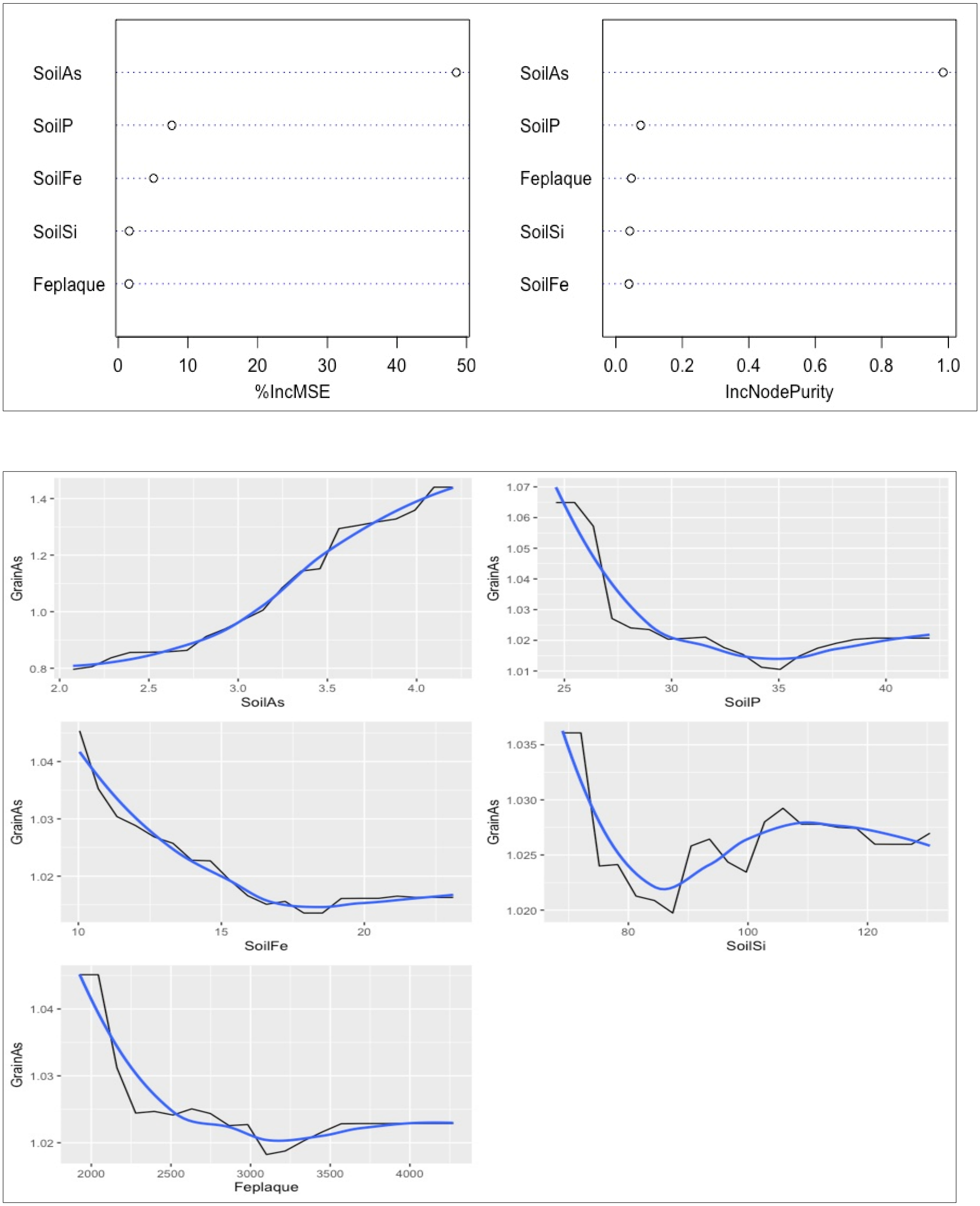


Fig. 7 a & b. Variable importance plots for predicting grain arsenic (6a) and their relationship with grain arsenic with partial dependence plots (6b) computed through the *Random forest model*

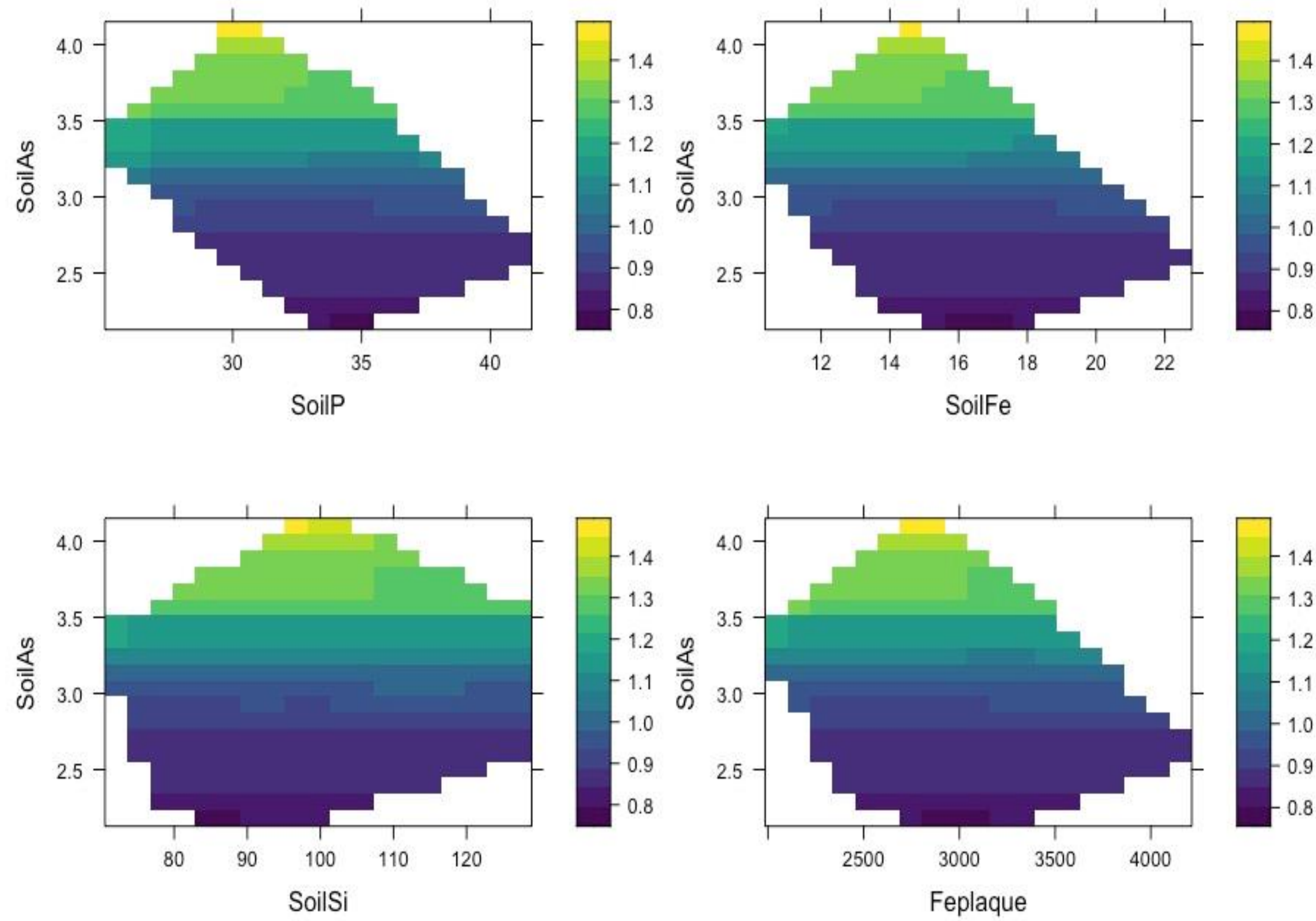


Fig. 8 Partial dependence plots of predictor variables (soil As, soil P, soil Fe, soil Si and Fe plaque) with respect to grain As content computed through the *Random forest model*

Supplementary Table S1 Physico-chemical properties of the experimental soil, arsenic content of externally added soil amendments and fertilizers and analytical methods used for analysis

Particulars		Method	Reference
Mechanical composition		International pipette	Piper (1966)
Sand (g kg^{-1})	171		
Silt (g kg^{-1})	548		
Clay (g kg^{-1})	281		
Textural class	silty clay loam		
Chemical characteristics			
Soil reaction (pH)	7.02	pH meter (1:2.5 soil water suspension)	Jackson (1973)
Electrical conductivity (EC) (dSm^{-1})	0.18	Wheatstone Conductivity Bridge (1:2.5 soil water suspension)	Jackson (1973)
Cation exchange capacity (CEC) [c mol $(p)^+$ kg^{-1}]	16.1	NH_4^+ displacement by using 1.0N NH_4OAc (pH 7.0)	Schollenberger and Dreibelbis (1930)
Organic carbon (g kg^{-1})	4.9	Chromic acid wet digestion	Walkley and Black (1934)
$KMnO_4$-N (kg ha^{-1})	227.0	Alkaline permanganate method	Subbiah and Asija (1956)
Olsen P (kg ha^{-1})	26.3	Colorimetric with ascorbic acid reduction	Olsen et al (1982)
Available K (kg ha^{-1})	118.4	1.0N NH_4OAc (pH 7.0) extractable K in flame photometer	Standford and English (1949)
DTPA - Iron (mg kg^{-1})	24.1	DTPA extractable micronutrient analysis using Atomic Absorption Spectrophotometer (AAS)	Lindsay and Norvell (1978)
DTPA-Zinc (mg kg^{-1})	0.61		
Olsen extractable soil arsenic (As) (mg kg^{-1})	3.03	Olsen reagent (0.5M $NaHCO_3$, soil: extractant of 1: 10 w/v) using AAS	Schmidt et al. (2004)
Total soil As (mg kg^{-1})	15.83	Using AAS, See section 2.5	Rahman et al. (2007)
Total As in amendments and fertilizers (mg kg^{-1})			
Rice straw compost	1.03	Using AAS, See section 2.5	Rahman et al. (2007)
$CaSiO_3$	6.7		
Urea	1.02		
DAP	12.3		
MOP	1.09		

Supplementary Table S2 Details of soil amendments used and their costs

Treatment details	Symbol	Dose (kg ha^{-1})	Reference	Cost ($ ha^{-1})
Control	-	-		
Silicate solubilizing bacteria	SSB	2	(Ghouse et al., 2011)	16
Rice straw compost	RSC	500	(Ghouse et al., 2011)	31
$CaSiO_3$	CS	400		76
SiO_2 nanoparticles at 80 mg kg $soil^{-1}$	Si NP at RD	179	Alvarez et al., 2018; Mustafa et al., 2019	115
Silicate solubilizing bacteria + Rice straw compost	SSB+RSC	2+500		47
SiO_2 nanoparticles at 40 mg kg $soil^{-1}$	Si NP at 50% RD	89.5	Alvarez et al., 2018; Mustafa et al., 2019	57

Recommended dose of Si for rice cultivation: 200 kg ha^{-1}

Supplementary Table S3. Elemental composition of rice straw compost used in the experiment

Elements	
C (%)	29.2
Cellulose (%)	16.5
Hemicellulose (%)	18.3
Lignin (%)	7.5
Total Si (%)	4.5
Total P (%)	0.25
Total Fe (mg kg^{-1})	835
Total Zn (mg kg^{-1})	34.2

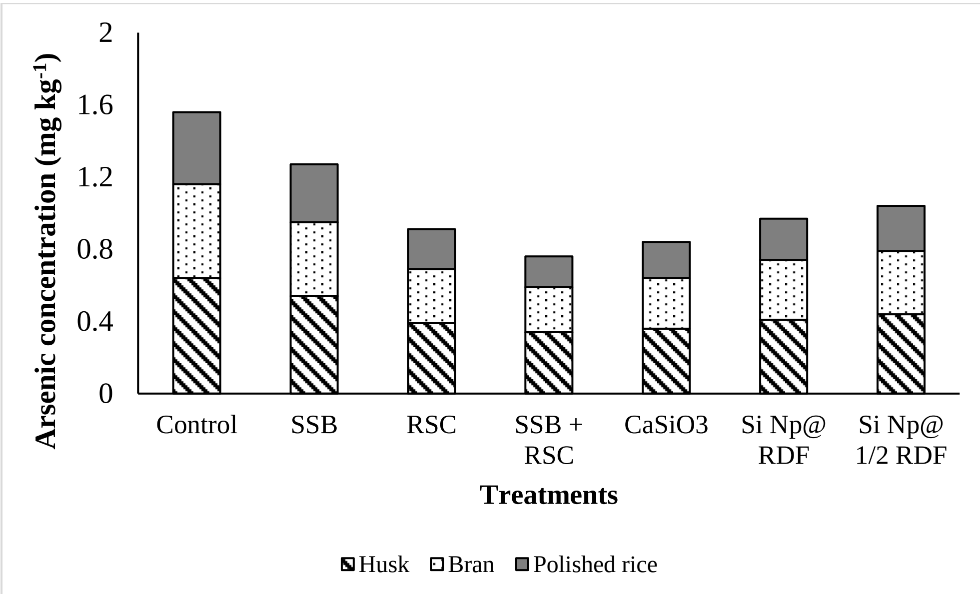
Supplementary Table S4 Analysis of standard reference materials for As by Atomic Absorption Spectrophotometer (AAS)

Certified standard reference material (SRM)	*n*	Certified value (µg g^{-1})	Observed values (µg g^{-1})
1568a rice flour	6	0.112±0.0024	0.122±0.004
1573a tomato leaf	6	0.285±0.014	0.296±0.011

Supplementary Table S5 Relationships of the grains As content with root biomass, Fe-plaque, plant height and bioavailable As, Si, Fe and P

	Grain As	Soil_As	Soil_Si	Soil_Fe	Soil_P	Plnat height	No of Nodes	Rooot biomass
Grain As	1							
Soil_As	0.823	1.000						
Soil_Si	-0.815	-0.529	1.000					
Soil_Fe	-0.772	-0.968	0.547	1.000				
Soil_P	-0.807	-0.985	0.582	0.957	1.000			
Plnat height	-0.945	-0.809	0.858	0.839	0.807	1.000		
No of Nodes	-0.716	-0.693	0.688	0.812	0.659	0.869	1.000	
Root biomass	-0.716	-0.889	0.678	0.884	0.942	0.754	0.640	1.000
Fe plaque	-0.769	-0.912	0.617	0.957	0.937	0.858	0.775	0.878

** Correlation is significant at the 0.01 level, *Significant at the 0.05 level



Supplementary Fig. S1 Distribution of As (as per cent of total) in different fractions of rice grain such as husk, bran and polished rice with different soil amendments.