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Zinc and iron enrichment of vermicompost can reduce the arsenic load in rice grain: An investigation through pot and field experiments

Sudip Sengupta [a,b], Kallol Bhattacharyya [a], Jajati Mandal [c,*], Parijat Bhattacharya [a,b], Asoke Prasun Chattopadhyay [d]

[a] Department of Agricultural Chemistry and Soil Science, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, 741252, India
[b] School of Agriculture, Swami Vivekananda University, Barrackpore, West Bengal, 700121, India
[c] School of Science, Engineering and Environment, University of Salford, Manchester, M5 4WT, United Kingdom
[d] Department of Chemistry, University of Kalyani, Kalyani, Nadia, West Bengal, 741235, India

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ABSTRACT

The heavy metalloid arsenic (As), occurring in both trivalent and pentavalent is extremely toxic and has detrimental effect on humans through water-soil-crop transfer. Previously organic and inorganic amendments have been used separately for mitigation of As in rice but there exists a research gap regarding use of them simultaneously. In this study, both pot and field scale investigations were undertaken for four consecutive years in As-contaminated locations to assess the efficacy of zinc (Zn) and iron (Fe)-enriched vermicompost in reducing the As uptake in rice grain. Altogether seven types of vermicompost and three application rates were evaluated. The treatment combination V_4D_1 (enriched vermicompost V_4 applied to soil at 3 t/ha) recorded the lowest soil available As (2.525 mg kg^{-1}) and the highest soil available As (2.982 mg kg^{-1}) was observed with V_5D_3 (enriched vermicompost V_5 applied to soil at 1.5 t/ha). Application of enriched and non-enriched vermicompost reduced As in grain by 58.14 and 31.40% respectively over no vermicompost (control). The partial dependence plot from stepwise regression modelling of different fractions of As revealed that an increase in organically bound As resulted in a decrease in the availability of As and hence uptake by rice. Further, Zn and Fe-enriched vermicompost resulted in increase of iron plaque formation on the root. A significant positive relationship ($r = 0.462$) was observed between dithionite-citrate-bicarbonate (DCB) extractable -Fe andAs. A significant negative correlation ($r = -0.410$) between DCB-Fe and grain As, advocates better root plaque formation resulting a higher capacity to sequester As onto the root surface and reducing its's entry into the rice system. The carcinogenic risk somewhat was benign (TCR of 2.69×10^{-3} and SAMOE of 0.101) against no vermicompost (TCR of 6.64×10^{-3} and SAMOE of 0.04). Therefore enriching vermicompost with $ZnSO_4$ and $FeSO_4$ at 10% dry weight basis (V_4) of the composting substrate can lower arsenic build-up in rice grains without affecting yield.

1. Introduction

The trace element arsenic (As), which is really harmful, is a major environmental problem due to its abundance in soil, water, plants, animals, and the human body (Bhattacharyya and Sengupta, 2020; Sengupta et al., 2021). As contamination of groundwater resources is posing a serious threat to the health of millions of people (Mandal et al., 2021) in various parts of the world with the maximum magnitude in Bangladesh followed by West Bengal, India (Laha et al., 2021). Cultivation in arsenic-polluted soil and irrigation with contaminated water increased the arsenic load in edible and other parts of both rice and other arable crops (Sanyal, 2017).

Nearly 90% of the water used in agricultural sector is groundwater more so in the affected West Bengal belt to meet crop irrigation needs (Mandal et al., 2021; Khanam et al., 2022). Although different water treatment techniques have been reported till date (Kenawy et al., 2018; Khan et al., 2020a, 2020b; Alqadami et al., 2023) for purification of metal-contaminated groundwater but having limited applicability for irrigation water in the field conditions. This requires immediate attention because it acts as a diffuse and uncertain source of contamination once As enters the food chain due to prolong use of contaminated water for irrigation purposes (Laha et al., 2021; Mandal et al., 2023). So,

* Corresponding author.
E-mail address: J.Mandal1@edu.salford.ac.uk (J. Mandal).

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low-cost strategies for arsenic mitigation are needed, which at the same time would be adaptable, profiteering and not compromise productivity (Bhattacharyya and Sengupta, 2020).

Moreover, half of the world's population relies on rice as the staple food, particularly in Asian, African, and Latin American nations (Majumder and Banik, 2019). Daily milled rice consumption is high in Bangladesh and India (approximately 103 and 268 kg per capita year^{-1} respectively; FAO, 2017). According to Mwale et al. (2018), rice makes up about 30% of the calorific diet in India and approximately 73% of the calorific intake in Bangladesh (Mandal et al., 2021). However, eating rice may also be a significant way to get exposed to As (Mondal and Polya, 2008; Mondal et al., 2010, 2020; A. Mondal et al., 2020). Under the conditions that rice is grown, As is a substantial sink in soil and is exceedingly available to rice roots (Kumarathilaka et al., 2018). When compared to other cereal crops, rice plants are substantial As accumulators (Awasthi et al., 2017; Sengupta et al., 2021), and irrigation of paddy fields with water contaminated with As raises As concentrations in paddy soil, rice straw, and grain (Panaullah et al., 2009).

The arsenic content of rice and other arable crops was found to get significantly reduced by organic amendments, especially in the case of vermicompost (Mandal et al., 2019a; Bhattacharyya et al., 2021; Sengupta et al., 2021). Arsenic was found to be less available and less readily absorbed by crops and soil when Zn and Fe were applied (Craw and Chappell, 2000; Karmakar et al., 2021; Matsumoto et al., 2015). While the efficacy of Fe was found to be more regarding formation of Fe plaque and subsequent sequestration of As, the role of Zn is also conspicuous in As mitigation in terms of formation of complexes like Zn-arsenate having wide range of chemical stability ensuring that once such complexes are formed, the release and phyto-availability of As is curtailed. Thus enrichment of vermicompost may be made with iron and zinc to evaluate the efficiency of enriched vermicompost in binding soil arsenic (Pigna et al., 2006). The current research group in their earlier study (Sengupta et al., 2022) have established that the humic and fulvic acids extracted from the Zn and Fe enriched vermicompost has better complexation ability than non-enriched vermicompost with As in solution.

However, studies involving the enrichment of vermicompost with Zn and Fe; and its use in As mitigation at the field level have never been attempted in the Indian scenario. Farmers have a general tendency not to apply Zn and Fe as amendments of As mitigation unless its effect is felt in terms of crop yield. On the other hand, the progressive demand for organic manures in farming operations could hardly be met today due to reduced livestock population, lack of awareness to use farm refuse etc. Vermicompost offers an important means of managing natural resources by utilizing crop residues, industrial toxic by-products such as brick kiln coal ashes, and different types of solid wastes in an environmentally friendly manner, as highlighted in previous studies (Goswami et al., 2017; D. Mondal et al., 2020; Sengupta et al., 2020). With the current focus on promoting healthy soil and safeguarding the environment against arsenic contamination in contaminated areas, the availability of organic matter in the form of vermicompost is a top priority. In this study, we hypothesized that combining organic (vermicompost) and inorganic (Zn and Fe) amendments could address the issue of limited availability of farm refuse or dung. Even at lower doses, Zn and Fe enriched vermicompost showed significant efficacy in reducing arsenic in rice grains through better complexation in the soil system, as demonstrated in our laboratory experiments (Sengupta et al., 2022). Therefore, the current study was designed with the following specific objectives in mind: (i) to examine the effectiveness of Zn and Fe-enriched vermicompost in lowering As levels in soil and rice grains even at doses lower than those recommended for regular vermicompost through pot study; (ii) to validate the efficacy of enriched vermicompost through field experiments with rice; and (iii) to assess the treatments' effectiveness in reducing human health risks.

2. Materials and methods

2.1. Production and characterization of Zn and Fe-enriched vermicompost

The details of the production of Zn and Fe-enriched vermicompost, the methods of characterization and the details of physicochemical and biological characters of the enriched vermicomposts are outlined in Sengupta et al. (2022). The different combinations of vermicompost samples prepared and their characteristics can be seen in Tables S1 and S2 respectively (adapted from Sengupta et al., 2022).

2.2. Site features, experimental design, and agronomic management

The experiment location was chosen in the As-contaminated village of Ghentugachi in the Chakdah block of Nadia, West Bengal, India (23°02′N, 88°34′E). According to the Village Summary of Tube-well Test Results under JPOA with UNICEF, available at http://www.dngmresfoundation.org, the location was chosen due to the high As concentration of the groundwater utilized for irrigation. The research area experiences sub-tropical weather, with average annual rainfall ranging from 1200 to 1500 mm, relative humidity ranging from 36 to 85%, and maximum and minimum temperatures, respectively, of about 37.5 °C and 12 °C. The soil is a new alluvial (*Inceptisol*), which is described as having neutral soil pH, silty clay, a moderate amount of available N and K, and a high amount of available P. Elevated concentrations of As have been found in soil and water, particularly in shallow tube well (STW) water (Das et al., 2016; Bhattacharyya et al., 2021).

The experiment was conducted during *rabi* season for four consecutive years including two years of pot study (2018-19 and 2019-20) and two years of field study (2020-21 and 2021-22). Initially, pot experiment was conducted with soils from the location to assess the efficacy of Zn and Fe-enriched vermicompost at varying doses; and then field experiment was conducted to validate the findings and delineate the underlying mechanism of such action.

For the pot experiment, surface soil (0–15 cm) was sampled, and the bigger clods were pulverized. The study comprised 66 pots in total (thrice replicated factorial design) [(7 vermicompost x 3 doses of application x 3 replications) + 3 replications of control (no vermicompost)]. 5 kg of soil sample was filled in each of these pots and two healthy rice seedlings of IET-4786 were transplanted in the last week of January. Pre-prepared vermicompost samples were weighed and applied to the pots prior to sowing at three (3) doses viz. 3, 2.25 and 1.5 t ha^{-1} (D_1, D_2 and D_3) and thoroughly incorporated into the soil. Standard levels of N, P, K fertilizers (130:65:65 kg ha^{-1}) were applied. During the application of fertilizers, initially the N, P and K content of the vermicompost treatment was computed and the remaining amount from the recommended dose was applied as urea, SSP and MOP to eliminate any chance of Piper-Steenbjerg effect (Wikström, 1994). The full doses of P and K and half the quantity of N were applied as basal and the remaining N in two splits at the maximum tillering and panicle initiation stage. Water samples were collected from the site stored in sterile polyethylene bottles, preserved by adding 0.1% v/v concentrated HNO_3 (69%) and stored at 4 °C for analysis (Chowdhury et al., 2020) and their As content was analysed in the laboratory. Thereafter, corresponding to the As content, As solution was prepared in the laboratory from standard As solution (Sigma, 1000 mg L^{-1}) and was mixed with irrigation water and applied to the pots. After the initial crop establishment (about 15 days after transplanting) irrigations were provided with the prepared As solution at 500 ml water/pot/irrigation throughout the crop growth period. To maintain optimum growth and production of the crop, regular weeding and required insect control procedures were implemented. In the final week of April, the crop was harvested, and soil samples from the root zone and plant components were gathered.

Regarding the field experiment, 66 plots, each 3m × 4m in size, were laid in a thrice replicated factorial design. Bunds were set up for water stagnation after three ploughings. The vermicompost treatments at the three doses of application viz. 3, 2.25 and 1.5 t ha^{-1} were added to the plots while puddling for optimal soil mixing. The rice seeds (cv. IET-4786) were planted in a nursery bed in the middle of December and then moved to the main plot in the last week of January, with a spacing of 20 cm × 15 cm and a depth of 3–4 cm, and 2–3 plants per hill. Fertilizer was administered at the prescribed rate (130:65:65 kg ha^{-1} of N, P, and K). Similar to the pot study initially the N, P and K content of the vermicompost treatment was computed and the remaining amount from the recommended dose was applied as urea, SSP and MOP to eliminate any chance of Piper-Steenbjerg effect (Wikström, 1994). The full doses of P and K and half the quantity of N were applied as basal and the remaining N in two splits at the maximum tillering and panicle initiation stage. Irrigations were provided based on crop critical growth stages using As contaminated irrigation water at the site by continuous submergence (*by maintaining* 4 cm *standing water throughout*). Weeding and pest control procedures were implemented. In order to reduce the border effect, the edges of each plot were left when the crop was harvested in the final week of April.

2.3. Collection and preparation of soil and plant samples

From the experimental pots and field locations, the initial and post-harvest soil samples (0–15 cm) were gathered, air-dried, pulverized, sieved (2–mm), and then kept in pre-marked airtight polythene packets. The physicochemical characterization approach was adopted using standard analytical techniques elaborated in Table S3. The plant (rice) samples were collected at harvest and washed with tap water followed by double-distilled water and then diluted hydrochloric acid. The samples were then chopped, divided into the root, shoot, and grain, and dried for 24 h at 55–60 °C in an air oven. Whatman No. 42 filter paper was used to filter the dry materials after they had been crushed and digested with a mixture of acids consisting of HNO_3, $HClO_4$, and H_2SO_4 in a ratio of 10:4:1 (v/v) (Jackson, 1973). Soil available As refers to the As content that is available to the plants from the soil and measured by extracting the soil with 0.5 M $NaHCO_3$ at 1:20 ratio of soil suspension and shaking in a reciprocating shaker for 30 min followed by filtration (Raj et al., 2021). The As content in soil and plant extract was determined by atomic absorption spectrophotometer (AAS).

2.4. Fractionation of soil As pools

The post-harvest soils have been extracted sequentially to determine different arsenic fractions by the standard method (sequentially described in Table S4) as described by Giri et al. (2012). In doing so, 5 g soil was treated sequentially with the series of extractants (1 N NH_4Cl, 0.5 N NH_4F, 0.2 M NH_4-oxalate, 0.2 M NH_4-oxalate + 0.1 M ascorbic acid, 0.5 N H_2SO_4; made by Merck (India) and Merck (Germany)) along with intermittent washing with saturated NaCl. The different inorganic fractions include water-soluble, aluminium-bound, amorphous iron-bound, crystalline iron-bound and calcium-bound arsenic fractions obtained from the sequential extractants used, respectively. From the total arsenic data, the residual fractions i.e. organically bound fractions were determined by subtracting the sum of the inorganic fractions from the total arsenic data of each of the experimental sites. As content in each fraction of soil was determined by atomic absorption spectrophotometer (AAS).

2.5. Iron plaque formation on root in relation to plant growth

Rice root has regular occurrence of iron plaque containing crystalline or amorphous structure of iron oxides and hydroxides. These substances being powerful As sorbents, iron plaque becomes a significant As sink and may sometimes contain even higher levels of As than roots and often provides an important avenue for mitigating As entry in rice grain (Khanam et al., 2022). In the current experiment the treatments contained different treatments of Fe mixed complexed with organic moieties which upon addition to soil will release slowly and under reduced environment of rice ecosystem will get precipitated on the root surface due to the radial oxygen loss (ROL). So, a modified cold dithionite-citrate-bicarbonate (DCB) approach was used to remove the iron plaques that had developed and been deposited onto the roots (Liu et al., 2004). The root sampler was used to gently uproot the plants, and the entire root systems of each treatment were collected separately. The DCB desorbed roots were kept after 4 days of oven drying at 65 °C. On the basis of dry weight, element concentrations in DCB extracts, roots, and shoots were estimated. Following were the calculations for total As (T_{As}), percent of As in DCB extracts, roots, and shoots (Liu et al., 2004):

$$T_{As} = T_{DCB-extract-As} + T_{Root-As} + T_{Shoot-As}; \dots \dots \dots \dots \dots \dots \quad (1)$$

$$T_{DCB-extract-As} = C_{DCB-extract-As} \times \text{root biomass}; \dots \dots \dots \dots \dots \dots \quad (2)$$

$$DCB-As\% = (T_{DCB-extract-As}/T_{As}) \times 100; \dots \dots \dots \dots \dots \dots \quad (3)$$

where $T_{DCB\text{-}extract\text{-}As}$, $T_{Root\text{-}As}$, and $T_{Shoot\text{-}As}$ represent the total As in DCB extracts, roots and shoots, respectively; $C_{DCB\text{-}extract\text{-}As}$ is the As concentration in DCB extracts (all units expressed in mg/kg). The As was measured by AAS. Higher accumulation of Fe and consequent formation of Fe plaque may provide a valuable reason of As mitigation in edible rice grains using Zn and Fe enriched vermicompost necessitating its assessment using DCB extraction.

2.6. Instrumental condition

PerkinElmer (Singapore) made Atomic Absorption Spectrophotometer (AAS) was used to determine the amount of As in plant digest and soil extract after successive dilution with distilled water, reaction with concentrated HCl, KI, and ascorbic acid for 45 min (Sparks et al., 2006). Through the use of rice standard reference material (SRM1568a) established by the National Institute of Standards and Technology (NIST), the analytical methodology of As determination was validated. The current PerkinElmer AAnalyst 200 AAS attached with Flow Injection for Atomic Spectroscopy (FIAS) Systems at λ_{max} = 193.7 nm exhibited As concentration as 287 ± 8 μg kg^{-1}, thus exhibiting good agreement with the certified value of 290 ± 30 μg kg^{-1} for SRM1568a. Every batch of 30 samples included analysis of one standard reference material, two blank reagents, and accuracy validation in triplicates. The method precision was assessed with respect to relative standard deviation (RSD) and minimum detection limit (MDL) of the instrument was determined (0.2 ppb). RSD ranged from 4.1 to 4.3% for As in the current experiment.

2.7. Risk evaluation of As exposure from eating rice grains

2.7.1. Target Cancer Risk (TCR)

TCR is significant in dietary risk assessment since it classifies a person's lifetime exposure to carcinogenic As. It was determined as per the equation (Table S5) outlined by Antoine et al. (2017) and Bhattacharyya et al. (2021).

2.7.2. Risk thermometer and severity adjusted margin of exposure (SAMOE)

The Swedish National Food Agency describes risk thermometer as a well-established, innovative risk characterisation procedure (Sand et al., 2015). The risk thermometer compares the health-based Tolerable Daily Intake and primarily calculates the exposure to As in food (TDI). The equation as proposed by Chowdhury et al. (2020) was used to calculate the human dietary exposure to As from eating rice (Table S4).

2.8. Statistical analysis, computation and modelling

The R-Studio (Version 1.3.1093 2.3.1) was used for descriptive sta-

Table 1
Effect of different vermicompost and doses on arsenic contents of rice (cv. IET-4786) and post harvest soil (pooled estimate of two years of pot and two years of field study).

	Soil Av_As (mg/kg)		Root As (mg/kg)		Shoot As (mg/kg)		Grain As (mg/kg)	
	Pot	Field	Pot	Field	Pot	Field	Pot	Field
Vermicompost								
V_1	2.808bc	2.821c	47.52b	48.84b	5.42bc	5.56b	0.59a	0.59a
V_2	2.806bc	2.958bc	47.45b	48.72b	5.38bc	5.60b	0.59a	0.62a
V_3	2.753c	2.981ab	46.60b	47.73b	5.42bc	5.78ab	0.52b	0.56ab
V_4	2.705e	2.719d	39.65c	40.41c	5.01c	4.88c	0.36c	0.36d
V_5	2.996a	3.128a	50.72a	52.55a	5.78b	5.63b	0.48b	0.48b
V_6	2.901ab	2.964bc	49.12a	50.88a	6.01a	5.96a	0.59a	0.59a
V_7	2.723d	2.896c	46.07b	46.89b	5.45bc	5.44bc	0.44bc	0.43cd
Dose of application								
D_1	2.744b	2.852b	45.43b	46.78b	5.30b	5.27b	0.50ab	0.51b
D_2	2.797ab	2.932ab	46.53b	47.79b	5.42b	5.55ab	0.52a	0.53a
D_3	2.898a	3.024a	48.23a	49.45a	5.76a	5.84a	0.51ab	0.52ab
Interactions								
V_1D_1	2.654de	2.713d	46.20c	47.49b	5.27c	5.41cd	0.57b	0.57c
V_1D_2	2.703cd	2.808c	47.06b	48.37b	5.36c	5.60c	0.59a	0.59b
V_1D_3	2.832ab	2.942b	49.30a	50.67a	5.62b	5.69b	0.61a	0.66a
V_2D_1	2.704cd	2.894bc	47.06b	48.37b	5.36c	5.44cd	0.59a	0.61a
V_2D_2	2.674d	2.862bc	46.55c	47.84b	5.31c	5.54c	0.58b	0.60a
V_2D_3	2.812ab	3.119ab	48.76a	49.93b	5.47c	5.83b	0.61a	0.66a
V_3D_1	2.577e	2.802c	43.80c	44.85c	4.91	5.24d	0.54c	0.59b
V_3D_2	2.703cd	2.999b	46.88b	48.01b	5.34c	5.70b	0.58b	0.63a
V_3D_3	2.830ab	3.141ab	49.13a	50.35a	6.02a	6.41a	0.43e	0.47d
V_4D_1	2.525f	2.562e	37.79e	38.73e	4.89e	4.80e	0.35g	0.36ef
V_4D_2	2.627de	2.733d	40.07d	41.06d	4.99de	4.89e	0.36f	0.37e
V_4D_3	2.749c	2.857bc	41.07d	41.44d	5.14d	4.97de	0.37f	0.37e
V_5D_1	2.851ab	3.085ab	50.06a	51.88a	5.48c	5.16d	0.47d	0.48cd
V_5D_2	2.831ab	3.064ab	49.71a	51.51a	5.44c	5.38c	0.47d	0.47d
V_5D_3	2.982ab	3.228ab	52.38a	54.28a	6.41a	6.35a	0.50d	0.51cd
V_6D_1	2.750c	2.976b	48.28a	50.03a	5.91ab	5.72b	0.54c	0.56c
V_6D_2	2.830ab	3.064ab	49.71a	51.51a	6.08a	6.25a	0.60a	0.64a
V_6D_3	2.812ab	2.843bc	49.35a	51.12a	6.04a	5.90b	0.61a	0.59b
V_7D_1	2.575e	2.896bc	44.83c	46.08c	5.30c	5.13d	0.42e	0.41
V_7D_2	2.674d	2.997b	45.76c	46.27c	5.41c	5.47c	0.43e	0.44d
V_7D_3	2.773bc	3.025b	47.63b	48.34b	5.63b	5.71b	0.45de	0.46d
No vermi	***3.17a***	***3.24a***	***71.68a***	***73.67a***	***9.12a***	***9.35a***	***0.87a***	***0.86a***

V_1 as non-enriched vermicompost; V_2, V_3 and V_4 (includes Zn and Fe enriched vermicompost with 5%, 7.5% and 10% w/w basis of composting substrates applied before application of *Eisenia fetida*) and V_5, V_6 and V_7 (includes Zn and Fe enriched vermicompost with 5%, 7.5% and 10% w/w basis of composting substrates applied at peak stage of *Eisenia fetida* multiplication, 25–30 days after *Eisenia fetida* application). D_1, D_2 and D_3 indicate the doses (3, 2.25, and 1.5 t/ha). Values with different alphabets (to be read vertically) are significantly different from each other according to the DMRT test ($p < 0.05$). Each value is a mean of three replicates and the single effects are derived by factorial ANOVA followed by DMRT of the interaction values.

tistics (mean, standard deviation, etc.), Duncan's multiple range test, stepwise regression (backward), variance inflation factor (VIF) and partial dependence plots (PDPs) from the regression model. Stepwise regression is the iterative process of building a regression model step by step while choosing independent variables to be included in the final model. The backward selection method was used in this study as the number of samples was larger than the number of variables. The grain As content was considered as the dependent variable whereas the $NaHCO_3$ extracted available As (AvAs), water-soluble As (WsAs), aluminium-bound As (AlAs), amorphous iron-bound As (AmFeAs), calcium bound As (CaAs), crystalline iron-bound As (CrysFeAs), organically bound As (OrgComAs) as the independent variables, the data of which were obtained from the sequential As fractionation scheme following Giri et al. (2012). The partial dependence plots (PDPs) shows the dependence between the dependent variable with a set of independent variables (variables of interest), marginalizing over the values of all other independent variables. The presence of multicollinearity in the regression model may jeopardize the PDP assumptions; hence the degree of multicollinearity for each variable was assessed using the variance inflation factor (VIF). Collinearity causes parameter estimates to have higher variances, which results in inferences about the relationship between the dependent and independent variables that are incorrect (Midi et al., 2010). In a regression study, the variance inflation factor gauges how multicollinear the predictor variables are (Mandal et al., 2023)). According to Franke (2010), multicollinearity is high if VIF >10. In our investigation, the VIF were 1.42, 1.60, 2.59, 10.00, 2.33, 6.37 and 8.18 for AvAs, WsAs, AlAs, AmFeAs CaAs, CrysFeAs and OrgComAs respectively. In this study, coefficient of determination (R^2), root mean square error (RMSE), and mean absolute error (MAE) were calculated to assess the performance of the models. The objective is to develop a model with high performance and less error.

$$R^2 = 1 - \frac{\sum (a_i - b_i)^2}{\sum (a_i - \mu_a)^2} \tag{4}$$

Where a denotes the output values, b denotes the real values, and μ_a is the mean value of the a values, ith is the number of observations such as *1, 2, 3 …, n.*

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(a_i - b_i)^2} \tag{5}$$

$$MAE = \frac{1}{n}\sum_{i=1}^{n}|a_i - b_i| \tag{6}$$

3. Results and discussion

3.1. Characteristics of the experimental site and vermicompost treatments

The experimental soil had a neutral pH (7.42), a low soluble salt concentration (EC- 0.35 dS m^{-1}), medium organic carbon content (5.43 g/kg), silty clay texture with 48.5% clay, cation exchange capacity of 16.95 cmol(p+)/kg, low available nitrogen (258 kg ha^{-1}) and available potassium (199 kg ha^{-1}); while the available phosphorus content was high (43.4 kg ha^{-1}). Total and $NaHCO_3$ extractable available As values were somewhat higher at 18.68 and 2.57 kg ha^{-1}, respectively. As concentrations in rice grains range from 0.86 to 0.11 mg kg^{-1}, and in this region, rice is often produced by irrigating with shallow tube-well laden As contaminated subsurface water (0.31 ± 0.03 mg L^{-1}). According to Mukhopadhyay and Sanyal (2004), the research location has been previously identified as an As-contaminated site. Sinha and Bhattacharyya (2014), Chowdhury et al. (2018), and Sengupta et al. (2021) have all confirmed that As uptake through rice has an associated dietary risk.

Further, the vermicompost treatments were characterized (Table S2) for their total C, N, P, K concentrations (per cent dry weight basis), with the results of non-enriched vermicompost and six different Zn and Fe enriched vermicompost having the values of 21.20 ± 1.23, 1.04 ± 0.11, 0.96 ± 0.05, 0.68 ± 0.07 per cent and ranging from 26.60 ± 1.53, 1.41 ± 0.10, 1.05 ± 0.07, 0.91 ± 0.11 per cent respectively. The mean C:N ratios of the non-enriched and enriched vermicomposts were 20.4:1, and 18.8:1 respectively. Prior to field application, the vermicompost treatments employed in the current investigation had their As concentration evaluated. All of the organics were determined to have As concentrations below the detection limit. Higher nutrient content and lower C:N ratio in the case of enriched vermicompost favours growth of *Eisenia fetida*, microbial activity and better quality of the product as reported by Sengupta et al. (2022).

3.2. Effect of vermicompost treatments on soil available arsenic

The changes in the soil available arsenic (As) following the application of seven combinations of different vermicompost samples at different doses of application have been recorded in Table-1 across the two years of pot as well as two years of field study. Results reveal that soil available As varies from 2.525 to 2.982 mg kg^{-1} in both years of pot experiment while for field experiment it ranged from 2.562 to 3.228 mg kg^{-1}; while no vermicompost had higher estimates (3.170 and 3.240 mg kg^{-1}).

The application of vermicompost resulted in a decrease in the soil available As with respect to no vermicompost application in both pot and field experiments regarding all the treatments, which were more conspicuous in the case of enriched vermicompost (except V5 and V6) as compared to non-enriched vermicompost (V1). The lowest available As was associated with the enriched vermicompost V_4. Reducing the dose of application of vermicompost resulted in a significant increase in the soil available As in most cases with the maximum value being associated with D_3 (dose of 1.5 t/ha). The interaction of the different types of vermicompost along with their doses revealed that the lowest soil available As was associated with V_4D_1 (enriched vermicompost V_4 applied to soil at 3 t/ha). The highest soil available As was associated with V_5D_3 (enriched vermicompost V_5 applied to soil at 1.5 t/ha) possibly due to poor complexation, while non-enriched vermicompost (V_1), also had higher levels of soil As.

Arsenic is released into the environment in both inorganic and organic forms. Arsenate (As V) exists in four forms in aqueous solution based on pH: H_3AsO_4, $H_2AsO_4^-$, $HAsO_4^{2-}$ and AsO_4^{3-}. Similarly, As (III) exists in five forms: $H_4AsO_3^+$, H_3AsO_3, $H_2AsO_3^-$, $HAsO_3^{2-}$ and AsO_3^{3-} (Sanyal, 2017; Sengupta et al., 2023). The ionic forms of As (V) dominate at pH > 3, and As (III) is neutral at pH < 9 and ionic at pH > 9. At pH 6–8, in most aquatic systems, both $H_2AsO_4^-$ and $HAsO_4^{2-}$ ions occur in considerable proportions in an oxidized environment (redox potential, Eh = 0.2–0.5V), while the aqueous acid, H_3AsO_3, is the predominant species under reduced conditions (Eh = 0–0.1V) (Sanyal, 2017; Sengupta et al., 2023). Studies have shown that organic manure affects the presence, availability, and mobility of As by immobilising, adsorbing, binding, or co-precipitating it (Sengupta et al., 2021). The variation and modification of soil pH may also favour such complexation. By producing negatively charged adducts, the complexation of arsenate and arsenite with humic acid (HA) via phenolic, carboxylic, amino, and sulfhydryl functional groups may act as the binding sites for As (Mandal et al., 2019a; Kumar et al., 2021). The strength of the direct connection of As with these functional moieties may vary, representing various binding mechanisms. Furthermore, Dissolved Organic Matter (DOM)--cation-As complexes may be formed by the functional groups in HA to bind As via a cation (such as Fe) bridge binding mechanism (Ritter et al., 2006). The better stability of organo-As complexes in the case of enriched vermicompost (Sengupta et al., 2022) may also be the reason for soil As reduction.This better complexation can be attributed to the log K or stability constant pattern of FA-V_4 > FA- V_7 > HA-V_4 > FA-V_2 > FA-V_6 > FA-V_5 > FA-V_3 > HA-V_7 > FA-V_1 > HA-V_2 > HA-V_3 > HA-V_6 > HA-V_5 > FA-soil > HA-V_1 > HA-soil (Sengupta et al., 2022). Therefore application of such vermicomposts at different doses resulted in variation in the levels of soil As.

3.3. Arsenic accumulation in rice plant parts under vermicompost amendments

The accumulation of arsenic in different plant parts like root, shoot and grain under the influence of the application of seven combinations of vermicompost at varying doses has been recorded in Table 1. The pooled estimates of two years of pot study reveal that the As content of rice root, shoot and grain varies from 37.79 to 52.38, 4.89–6.41 and 0.35–0.61 mg kg^{-1} respectively; while for the two years of field study the respective contents were 38.73–54.28, 4.80–6.41 and 0.36–0.66 mg kg^{-1}. When no vermicompost was applied the As content of root, shoot and grain had a mean estimate of 71.68 and 73.67; 9.12 and 9.35; 0.87 and 0.86 mg kg^{-1} respectively for pot and field experiments.

The applications of vermicompost were responsible for the decrease in the root, shoot and grain arsenic content, while there was a conspicuous effect in the case of enriched vermicompost as compared to non-enriched vermicompost. Among the treatments the higher estimates for root arsenic were associated with the treatment V_5 at par with V_6; while the highest estimate of the shoot and grain arsenic was observed in the case of V_6 at par with V_1. The lowest estimate for root, shoot and grain arsenic was associated with V_4. Reducing the dose of application of vermicompost significantly increased the root, shoot and grain arsenic content with the lowest value being associated with D_1 (dose of 3 t/ha). The interaction of different vermicompost treatments along with their doses of application revealed that the lowest content of root, shoot and grain arsenic was associated with V_4D_1 (enriched vermicompost V_4 applied to the soil at 3 t/ha). The highest arsenic content for root and shoot was associated with V_5D_3 (enriched vermicompost V_5 applied to the soil at 1.5 t/ha); while for grain arsenic, V_2D_3 (enriched vermicompost V_2 applied at 1.5 t/ha) assumed the highest value. In all cases the arsenic content in root, shoot and grain were at par with V_1D_3 (non-enriched vermicompost applied at 1.5 t/ha).

The results revealed that arsenic accumulations by the rice cultivar were highest in root followed by stem > grain irrespective of the types and doses of vermicomposts. In rice, arsenic often moves from the roots to the tiller. There are variations in As concentration in plant components, with some studies using several varieties of rice demonstrating a reduction in As concentration from root to grain (Welna et al., 2015). According to Khanam et al. (2022), after entering through the roots, As goes into the shoots before ending up in the grains. It experiences second sieving while in shoots because of sequestration in stem cell nodes, internodes, and vacuoles. According to them, the number of such sites in the shoots, As transporters, and its chelating chemicals (such as phytochelatines), determine the translocation of As from roots to shoot and from shoot to grains.

The effect of As reduction in rice grain under the application of best treatment of enriched vermicompost (V_4) and non-enriched

Table 2
Effect of different vermicomposts and doses on arsenic fractions (mg kg^{-1}) in post harvest paddy soil (IET-4786) (pooled estimate of two years of field study).

	Ws_As	Al_As	Am Fe_As	Crys Fe_As	Ca_As	Org_As
No vermicompost	1.163a	3.211a	12.167f	6.910d	1.776a	16.458d
V_1D_1	0.767c	2.921b	12.836a	7.205b	1.652c	17.578c
V_1D_2	0.788c	2.919b	12.767b	6.815d	1.670b	17.988c
V_1D_3	0.810b	2.920b	12.571c	6.994c	1.687b	17.952c
V_2D_1	0.768c	2.983b	12.871a	7.100c	1.670b	17.939c
V_2D_2	0.739c	2.937b	12.767b	7.047c	1.634d	17.785c
V_2D_3	0.723c	2.977b	12.663b	6.910d	1.626d	17.986c
V_3D_1	0.754c	2.800c	12.571c	7.426a	1.617d	17.792c
V_3D_2	0.790c	2.917b	12.294	7.310b	1.573e	18.063b
V_3D_3	0.817b	2.924b	12.224e	7.216b	1.643c	18.110b
V_4D_1	0.564e	2.927b	12.478d	7.152c	1.626d	18.508a
V_4D_2	0.576e	2.993b	12.386d	7.110c	1.634d	18.210b
V_4D_3	0.631d	2.937b	12.213e	6.815d	1.670b	18.681a
V_5D_1	0.737c	2.917b	12.271e	7.258b	1.687b	18.089b
V_5D_2	0.758c	2.921b	12.259e	7.068c	1.696b	18.245b
V_5D_3	0.767c	2.947b	12.224e	6.794d	1.661c	18.540a
V_6D_1	0.723c	2.936b	12.398d	7.258b	1.670c	18.532a
V_6D_2	0.726c	2.947b	12.340d	7.100c	1.687b	18.531a
V_6D_3	0.737c	2.940b	12.282e	7.047c	1.696b	18.417a
V_7D_1	0.610d	2.922b	12.836a	7.510a	1.643c	17.487c
V_7D_2	0.626d	2.936b	12.709b	7.395b	1.652c	17.616c
V_7D_3	0.654d	2.924b	12.675b	7.352b	1.643c	17.833c

V_0 refers to no vermicompost application. V_1 as non-enriched vermicompost; V_2, V_3 and V_4 (includes Zn and Fe enriched vermicompost with 5%, 7.5% and 10% w/w basis of composting substrates applied before application of *Eisenia fetida*) and V_5, V_6 and V_7 (includes Zn and Fe enriched vermicompost with 5%, 7.5% and 10% w/w basis of composting substrates applied at peak stage of *Eisenia fetida* multiplication, 25–30 days after *Eisenia fetida* application). D_1, D_2 and D_3 indicate the doses (3, 2.25, and 1.5 t/ha). Values with different alphabets (to be read vertically) are significantly different from each other according to the DMRT test ($p < 0.05$). Each value is a mean of three replicates.

vermicompost (V_1) was conspicuous having a mean value of 58.14 and 31.40% respectively over no vermicompost application. Even interaction with doses revealed that application of enriched vermicompost (V_4) at half of the recommended dose (1.5 t ha^{-1}; D_3) reduced As in grain by 57.47% as compared to non-enriched vermicompost at the recommended dose (V_1D_1) having a capacity of reducing As by 34.48% over no vermicompost situation.

Previous research has shown that different kinds of organic amendments can effectively lower the amount of As in plant edibles. Similar occurrences of As decrease in wheat and maize grain were discovered by Mandal et al. (2019b) using sugarcane bagasse, paddy husk, rice straw, vermicompost, and FYM. In the trend of vermicompost > mustard cake > FYM, organic treatments were also successful in reducing the amount of As in cauliflower, spinach, and tomato (Bhattacharyya et al., 2021). In each case, the fundamental cause of the decreased uptake in plant was hypothesized to be an organo- As complexation in the soil (Sengupta et al., 2021). The better stability of organo- As complexes involving the enriched vermicompost samples might well be the underlying reason that they sequester a greater portion of arsenic (Sengupta et al., 2022). Further possible mechanisms of As reductions were studied during the field experiment as elucidated in the succeeding sections.

3.4. Arsenic fractions and their relationship with grain As content

The experimental soils were characterized for different fractions of arsenic (Table 2). The recoveries of water-soluble arsenic (WsAs) for pooled data across the two years of field study ranged from 0.564 to 1.163 mg kg^{-1}, with the highest estimate associated with no vermicompost (V_0) application and the lowest value for V_4D_1. Similarly, the recoveries of aluminium-bound arsenic (AlAs) ranged from 2.800 to 3.211 mg kg^{-1}, with the highest estimate associated with V_0 and the lowest for V_3D_1. The higher contribution of As was associated with Fe-bound fractions, of which the recoveries of amorphous iron-bound arsenic (AmFeAs) ranged from 12.167 to 12.871 mg kg^{-1}, with the lowest estimate associated with V_0 and the highest value for V_2D_1; while the crystalline iron bound arsenic (CryFeAs) ranged from 6.815 to 7.510 mg kg^{-1}, with the lowest estimate associated with V_1D_2 and highest value for V_7D_1. The recoveries of calcium-bound arsenic (CaAs) were in the range of 1.573–1.776 mg kg^{-1}, with the highest estimate associated with V_0 and the lowest value for V_3D_2. The organically bound content (OrgComAs) obtained by subtracting the sum of the preceding pools from total As for pooled data ranged from 16.458 to 18.681 mg kg^{-1}, with the lowest estimate in the case of V_0 and highest content for V_4D_3.

The per cent contribution of the different fractions of arsenic towards the total arsenic content was computed and has been presented in Fig-1. For all the different types of soil samples, including those where no vermicompost was applied, as well as the application of both non-enriched and enriched vermicompost, the relative abundance of different arsenic fractions followed the order of WsAs < CaAs < AlAs < CryFeAs < Am FeAs < OrgComAs. Such observations, however, are in quite good agreement with the findings reported by earlier workers regarding the predominance of soil arsenic in iron-bound forms in the Bengal-delta basin (Sanyal, 2017). Giri et al. (2012) however showed a slight variation in the relative abundance of As fractions with the order of water-soluble As < Ca As < Al As < amorphous Fe As < crystalline Fe As. Kumari et al. (2021) also reported that in inceptisols of Bihar, India the different fractions of arsenic in terms of its abundance followed the order crystalline iron bound arsenic > aluminum bound arsenic > calcium bound arsenic > amorphous iron bound arsenic > water soluble across all the doses of As application.

Further comparison among the vermicompost samples regarding contribution to the organic bound forms, followed the trend of $V_4 > V_6 > V_5 > V_3 > V_2 > V_1 > V_7 > V_0$. The reverse trend was followed in the case of water-soluble arsenic which emphasizes the fact that enriched vermicompost has an edge in reducing the soil As bio-availability in the soil-plant system (Sengupta et al., 2021). The better complexation of humic and fulvic acid samples of these enriched vermicompost samples with arsenic (Sengupta et al., 2022) further explains the predominance of the arsenic association with organic pools.

Table 3 depicts the summary of the stepwise regression model. The final model (Grain As = −3.97 + 0.26AvAs + 0.255WsAs + 0.19AmFeAs + 0.93 CrysFeAs – 0.23 OrgComAs) from stepwise regression identified the AvAs, WsAs, AmFeAs, CrysFeAs, and OrgComAs as the significant variables ($p < 0.01$) having either positive or negative relationship contributing towards the grain As content with an R^2 of 0.54 and Adj-R^2 of 0.52. The AvAs and all the soil As fractions have

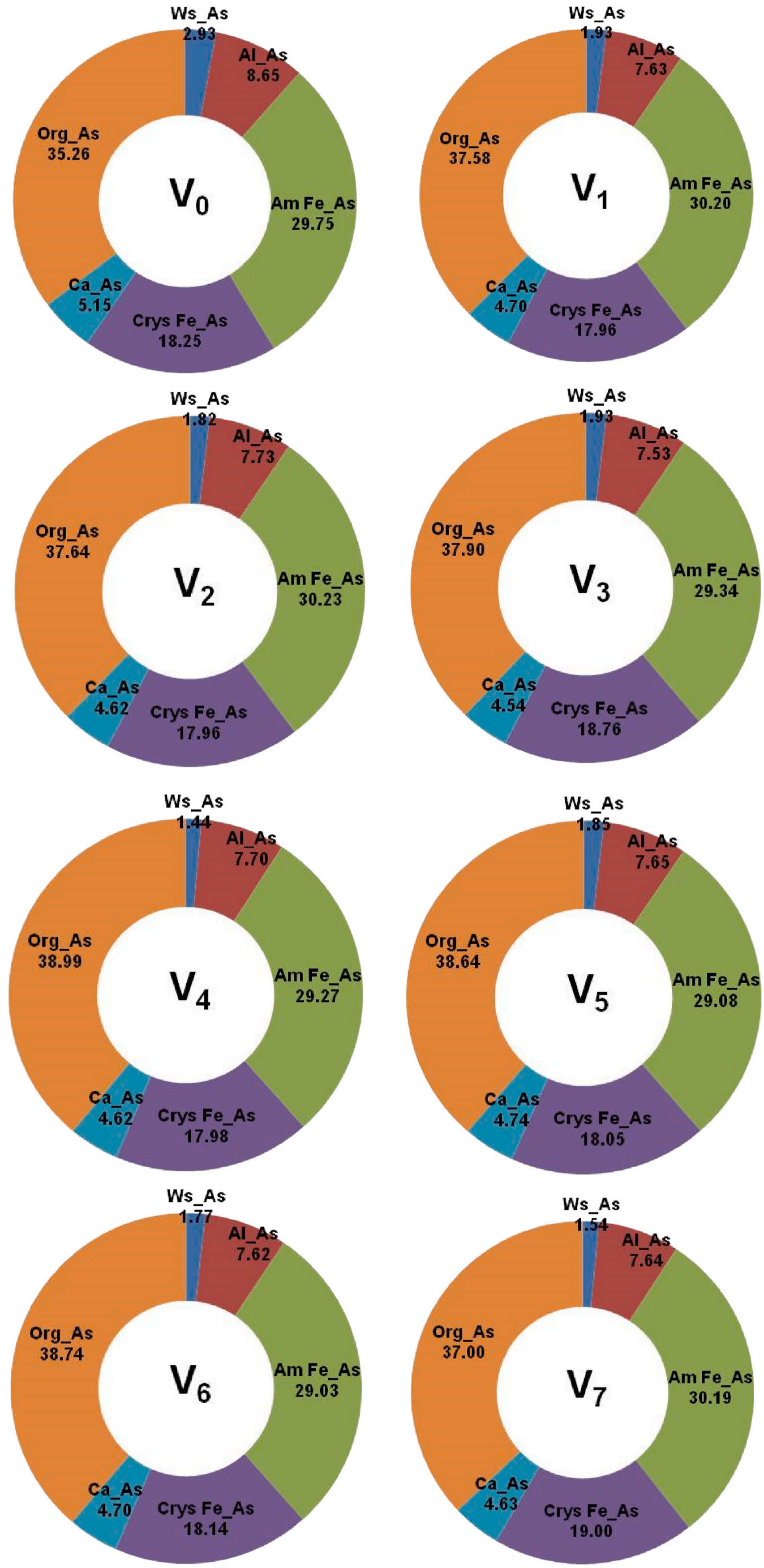

Fig. 1. Percentage change in arsenic fractions under the influence of applied vermicompost treatments in paddy (IET-4786) soil (pooled data of two years of field study).

Table 3
Stepwise regression of grain As with soil As fractions and available As (n = 132).

Model Summary			
R	0.737	Root Mean Square Error (RMSE)	0.081
R-Squared	0.543	Coefficient of Variation	15.186
Adjusted R-Squared	0.525	Mean Square Error (MSE)	0.007
Predicted R-Squared	0.486	Mean Absolute Error (MAE)	0.063

ANOVA	Sum of Squares	DF	Mean Square	F	Sig.
Regression	0.980	5	0.196	29.939	0.0000
Residual	0.825	126	0.007		
Total	1.805	131			

Parameter Estimates: Model	Beta	Std. Error	Std. Beta	t	Sig	Lower	Upper
(Intercept)	−3.970	0.524		−7.582	0.000	−5.007	−2.934
Av_As	0.261	0.043	0.433	6.055	0.000	0.176	0.346
Ws_As	0.255	0.065	0.254	3.839	0.000	0.125	0.384
Am Fe_As	0.193	0.028	1.291	6.932	0.000	0.138	0.248
Crys Fe_As	0.938	0.165	0.808	5.679	0.000	0.611	1.265
Org_As	−0.023	0.006	−0.628	−3.832	0.000	−0.034	−0.011

Elimination Summary: Step	Variable removed	R-square	Adj R-Square	C(p)	AIC	RMSE
1	Al_As	0.5452	0.5233	6.7028	−279.9953	0.0810
2	Ca_As	0.543	0.5248	5.3022	−281.3624	0.0809

a positive relationship with the grain As content except the organically bound As. This implies the fact that the more organic fractions, the less will be the availability of As and hence the lower uptake of As by rice. The PDP from the regression model in Fig. 2 gives us an insight into the grain As content in presence of AvAs and other fractions. It can be observed that an increase in the fractions resulted in a simultaneous increase of the AvAs and in turn the grain As content. On the other hand, the organically bound As have an inverse relationship with the AvAs. An increase in organically bound As resulted in a reduction of the AvAs and in turn reduced the uptake of As rice. This justifies the efficacy of the organic amendments in reducing the grain As content as previously reported (Mandal et al., 2019b; Sengupta et al., 2022).

3.5. Dry matter and grain yield under vermicompost amendments

The dry matter and grain yield of rice following the application of seven combinations of vermicompost samples at different doses have been recorded in Table 4 as pooled data for two consecutive years of the field experiment. Results revealed that the dry matter yield of rice varied from 6.98 to 10.41 t/ha while the grain yield showed a range of 2.53–3.77 t/ha. When no vermicompost was applied, the dry matter and grain yields were 6.58 and 2.19 t/ha, respectively.

The applications of vermicompost were responsible for the increase in dry matter, and grain yield, while there was a conspicuous effect in the case of enriched vermicompost as compared to non-enriched vermicompost. The highest estimates for both dry matter and grain yield were associated with V_4 while the lowest estimate was associated with V_1. Reducing the dose of application of vermicompost significantly decreased the dry matter, and grain yield of rice. The interaction of different types of vermicompost treatments along with doses revealed that the maximum estimate of dry matter and grain yield was associated with V_4D_1; while V_1D_3 assumed the lowest dry matter and grain yield.

Vermicompost-mediated yield improvement of rice has been reported by Sengupta et al. (2021). The soil's high porosity, aeration, drainage, water-holding capacity, presence of beneficial microflora, nutrients like nitrates, phosphates, exchangeable calcium and potassium, and plant growth regulators all contribute to the increased growth and yield of different plants when vermicompost is applied to the area (Bejbaruah et al., 2013). Nitrogen availability boosts plant development and the leaf area index, which in turn boosts light absorption and results in increased dry matter and yield (Joshi et al., 2013). The application of vermicompost improved root growth in terms of root length, surface area, mean diameter, root volume, and the number of root tips, according to Ruan et al. (2021). Vermicompost incorporation may be the primary cause of the enhanced availability of nutrients in the rhizosphere matrix as it is directly associated with a wide scale of nutrient cycling and also acting as an avenue for replacement of chemical fertilization under rice system and sustaining soil health and organic C pool (Sahariah et al., 2020). The root system's physical traits and activity can also have a substantial impact on the growth and development of plants. The root system is the organ that plants use to receive water and nutrients from the soil (Pan et al., 2017). A stronger root system will encourage rice growth and development as well as biomass build-up during the growing season following transplanting, having a significant impact on rice yield (Chen et al., 2021). The total and accessible nutrient content in the soil-plant system increased after iron and zinc were added to vermicompost. The added Zn and Fe, along with other nutrients like P, can improve chlorophyll content and photosynthetic efficiency, assimilate partitioning, and increase growth and yield (Sengupta et al., 2020).

3.6. Root iron plaque formation and its significance

The DCB-extractable arsenic and iron in roots of the rice plants (i.e. iron plaques) under the influence of the application of seven different vermicompost combinations at different doses have been recorded in Table 4 as pooled data of two consecutive years of field study. Results reveal that the DCB-extractable arsenic content of rice root varies from 45.03 to 76.45 mg kg^{-1} while the DCB-extractable iron content ranged from 2964.11 to 4136.61 mg kg^{-1} under vermicompost treatment. When no vermicompost was applied the values of DCB-extractable arsenic and iron were 27.61 and 1132.90 mg kg^{-1} respectively.

The applications of vermicompost were responsible for the increase in the DCB-extractable arsenic and iron in the root, while there was a conspicuous effect in the case of enriched vermicompost as compared to non-enriched vermicompost. The highest contents for DCB-extractable arsenic and iron in the root were associated with V_4 while the lowest estimate of both was observed in the case of V_1. Reducing the dose of

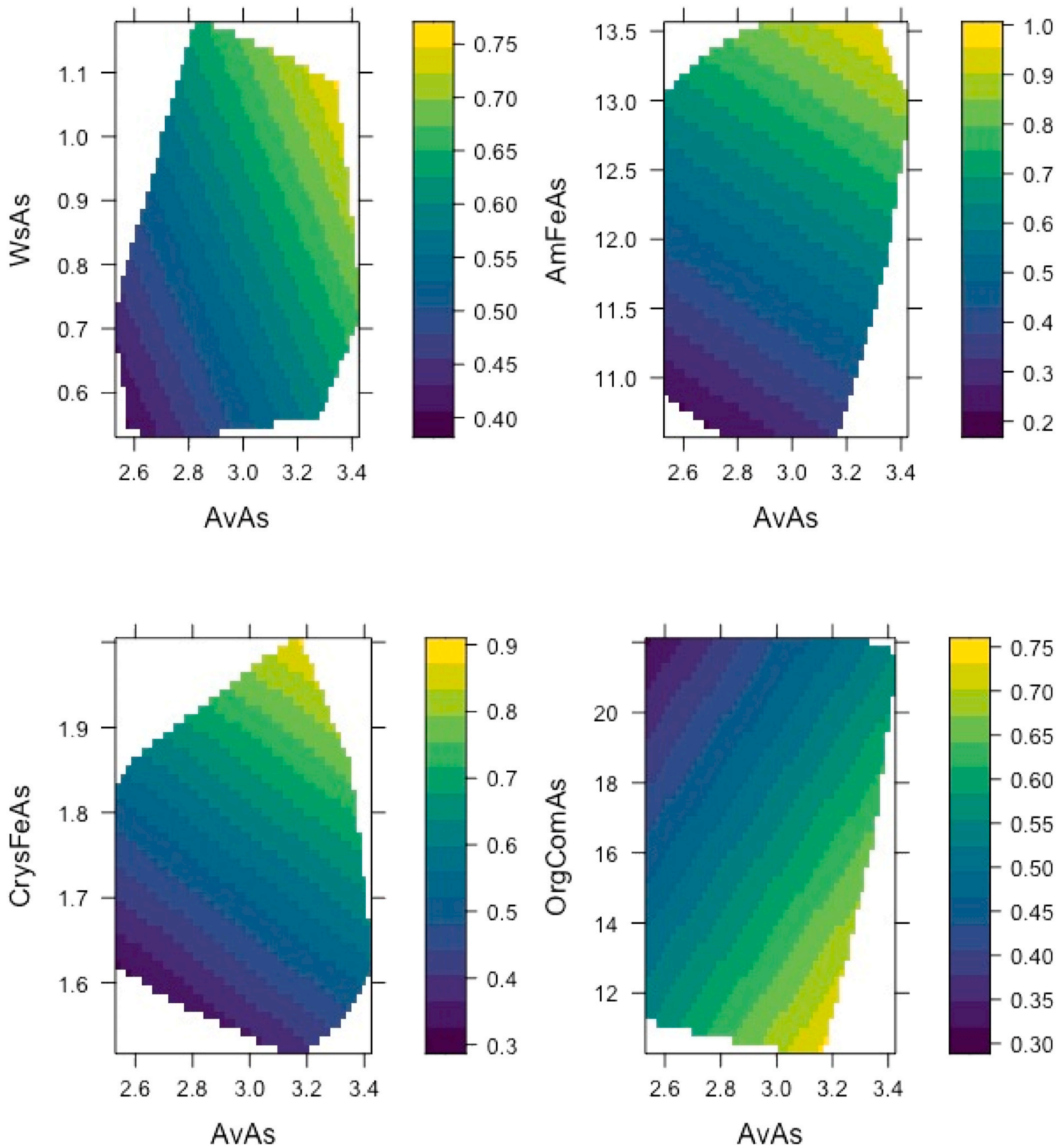

Fig. 2. Partial dependence plots (PDPs) from the stepwise regression model show the marginal effect of the soil arsenic (As) fractions (WsAs: water-soluble As, AmFeAs: Amorphous iron bound As, CrysFeAs: crystalline iron bound As and OrgComAs: organically bound As) with available As (AvAs) affecting the content of the As in rice grain (represented by values and colour intensity at the right side of each plot). All the parameters have the unit of mg kg^{-1}. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

vermicompost application significantly decreased the DCB-extractable arsenic and iron in the root, with the lower value being associated with D_2 and D_3. The interaction of vermicompost treatments with their doses revealed that the highest content of DCB-extractable arsenic and iron in the root was associated with V_4D_1. The lowest DCB-extractable arsenic was associated with V_3D_2 at par with V_1D_3; while for DCB-extractable iron, V_1D_3 assumed the lowest value. The percentage contribution of DCB-extractable arsenic to account for the total arsenic content in root and shoot was computed and represented in Fig-3a. The results reveal that the application of vermicompost is associated with an increment in iron plaque formation. Moreover, elevated levels of application of vermicompost enriched with Fe and Zn further increased the Fe plaque formation and reduced arsenic accumulation in plant edibles (see Fig. 4).

On the surface of the roots of aquatic and wetland plants, such as rice, iron plaque (IP) production is a regular occurrence. It has a crystalline or amorphous structure and contains lepidocrocite, goethite, and iron hydroxides (Fe). Numerous studies demonstrate that IP contains mixed-phase Fe hydroxides; on aquatic roots, the crystalline and weakly crystalline phases are frequently found close together. The local biogeochemical factors at particular places lead to the distinct phases that are present. Lepidocrocite [g-FeO(OH)] and siderite ($FeCO_3$) have been discovered in numerous studies, although ferrihydrite [$(Fe^{3+})_2O_3.5H_2O$] and goethite [a-FeO(OH)] appear to be present in all

Table 4
Effect of different vermicompost samples and doses on iron plaque formation, dry matter, and grain yield of rice (cv. IET-4786) (pooled estimate of two years of field study).

	DCB_As (mg/kg)	DCB_Fe (mg/kg)	Dry matter yield (t/ha)	Grain yield (t/ha)
Vermicompost				
V_1	52.16c	3125.39c	7.06d	2.56d
V_2	53.25c	3542.39b	8.37b	3.03c
V_3	52.84c	3592.23b	8.69b	3.15b
V_4	72.07a	3685.50a	10.31a	3.74a
V_5	63.19a	3271.07d	9.83a	3.56a
V_6	56.01b	3325.75d	8.94b	3.24b
V_7	58.96b	3396.05c	7.94c	2.88c
Dose of application				
D_1	61.23a	3746.42a	8.98a	3.25a
D_2	56.41b	3415.83b	8.65b	3.14b
D_3	57.44b	3139.91b	8.56b	3.10b
Interactions				
V_1D_1	55.72d	3726.58a	7.16d	2.59e
V_1D_2	51.39d	3285.50c	7.04e	2.55ef
V_1D_3	49.36e	2937.73e	6.98e	2.53ef
V_2D_1	57.20c	3900.67a	8.34c	3.02d
V_2D_2	51.22d	3429.17b	8.45c	3.06d
V_2D_3	51.33d	3297.34c	8.34c	3.02d
V_3D_1	61.31b	3992.49a	8.80c	3.19c
V_3D_2	45.03e	3421.04b	8.66c	3.14c
V_3D_3	62.19b	3363.17b	8.62c	3.12c
V_4D_1	76.45a	4136.61a	10.41a	3.77a
V_4D_2	73.73a	3616.05b	10.28a	3.73a
V_4D_3	66.04b	3303.86b	10.24a	3.71a
V_5D_1	61.98b	3417.34b	10.15a	3.68a
V_5D_2	63.04b	3247.43c	9.73b	3.53b
V_5D_3	64.57b	3148.46c	9.60b	3.48b
V_6D_1	55.79d	3421.53b	9.10b	3.30c
V_6D_2	61.64b	3290.98c	8.91c	3.23c
V_6D_3	50.59d	2964.75d	8.82c	3.20c
V_7D_1	60.17b	3629.77b	8.93c	3.24c
V_7D_2	58.76c	3620.65b	7.51d	2.72de
V_7D_3	58.03c	2964.11d	7.39d	2.68de
No vermicompost	***27.61f***	***1132.90f***	***6.58f***	***2.19f***

V_1 as non-enriched vermicompost; V_2, V_3 and V_4 (includes Zn and Fe enriched vermicompost with 5%, 7.5% and 10% w/w basis of composting substrates applied before application of *Eisenia fetida*) and V_5, V_6 and V_7 (includes Zn and Fe enriched vermicompost with 5%, 7.5% and 10% w/w basis of composting substrates applied at peak stage of *Eisenia fetida* multiplication, 25–30 days after *Eisenia fetida* application). D_1, D_2 and D_3 indicate the doses (3, 2.25, and 1.5 t/ha). Values with different alphabets (to be read vertically) are significantly different from each other according to the DMRT test ($p < 0.05$). Each value is a mean of three replicates and the single effects are derived by factorial ANOVA followed by DMRT of the interaction values.

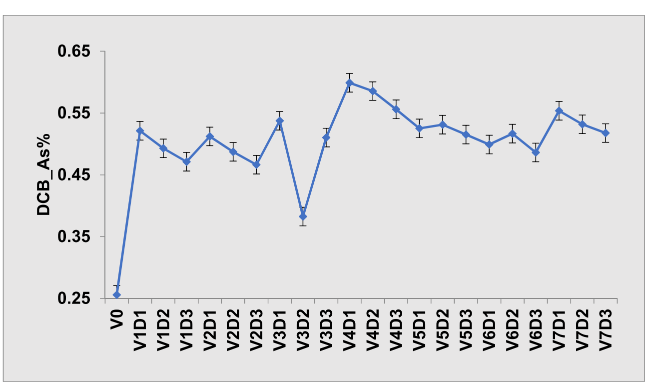

Fig. 3a. Percent contribution of DCB extractable As (associated with root iron plaque) to total As under the influence of different vermicompost and their dose of application. Results represented as the mean of three observations± standard deviations of pooled data of two years of field study.

IP measured so far (Tripathy et al., 2014). Since rice is a semi-aquatic plant, its extensive root aerenchyma permits oxygen to enter from the shoot to root for respiration. A portion of the oxygen is released from the aerenchyma of rice roots to the rhizosphere to combat the anaerobic conditions in submerged soil. Referred to as radial oxygen loss (ROL), it varies from genotype to genotype and is influenced by soil waterlogging and/or O_2 availability (Colmer et al., 2006). The IP, a precipitate of iron oxides and hydroxides with a distinctive orange colour, is formed on the root surface when released O_2 oxidises Fe^{2+} to Fe^{3+}. Because iron oxides and hydroxides are powerful As sorbents, iron plaque becomes a significant As sink and contains even higher levels of As than roots (Awasthi et al., 2017).

The relationship of DCB-extractable Fe (Fe plaque) with the dry matter yield of rice, DCB-extractable arsenic and grain arsenic was determined and recorded in Fig. 3b. Results reveal a positive relationship ($r = 0.205$) between dry matter and DCB-Fe, which suggests that a plant which is well-developed with elevated dry matter yield would also preferentially improve the root biomass and enable iron plaque formation. The higher root biomass ensures higher radial oxygen loss (ROL) around its rhizosphere (Zhu et al., 2015) favouring the formation and deposition of an increased amount of Fe-plaques in the vicinity of their roots and onto root surfaces. Similar to our findings, the existence of a positive relation between root biomass and Fe-plaque was reported by earlier researchers (Li and Wang, 2013; Khanam et al., 2022).

Further, there exists a higher positive relationship ($r = 0.462$) among DCB-Fe and DCB-As; and a higher negative relationship ($r = -0.410$) among DCB-Fe and grain As, which suggests better root plaque formation supports a higher capacity to sequester As onto the root surface and curtail the entry of the metalloid into the rice system. It was reported that 75–89% of As potentially available for intake was concentrated in iron plaques (Awasthi et al., 2017). The use of enriched vermicompost favours Fe load in the rhizosphere and thus enables Fe-plaque formation

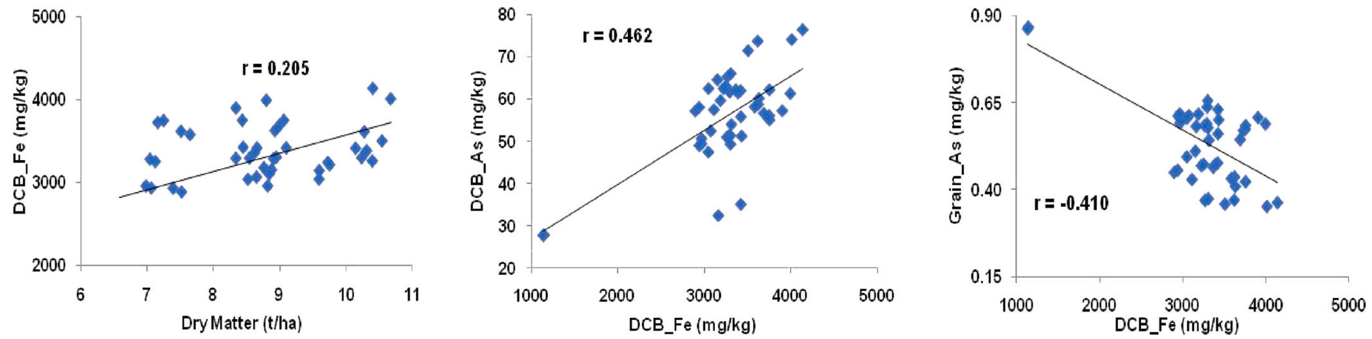

Fig. 3b. Relationship between DCB-Fe and Dry Matter, DCB-Fe and DCB-As, DCB-Fe and Grain-As of rice under the influence of different vermicompost samples and their dose of application.

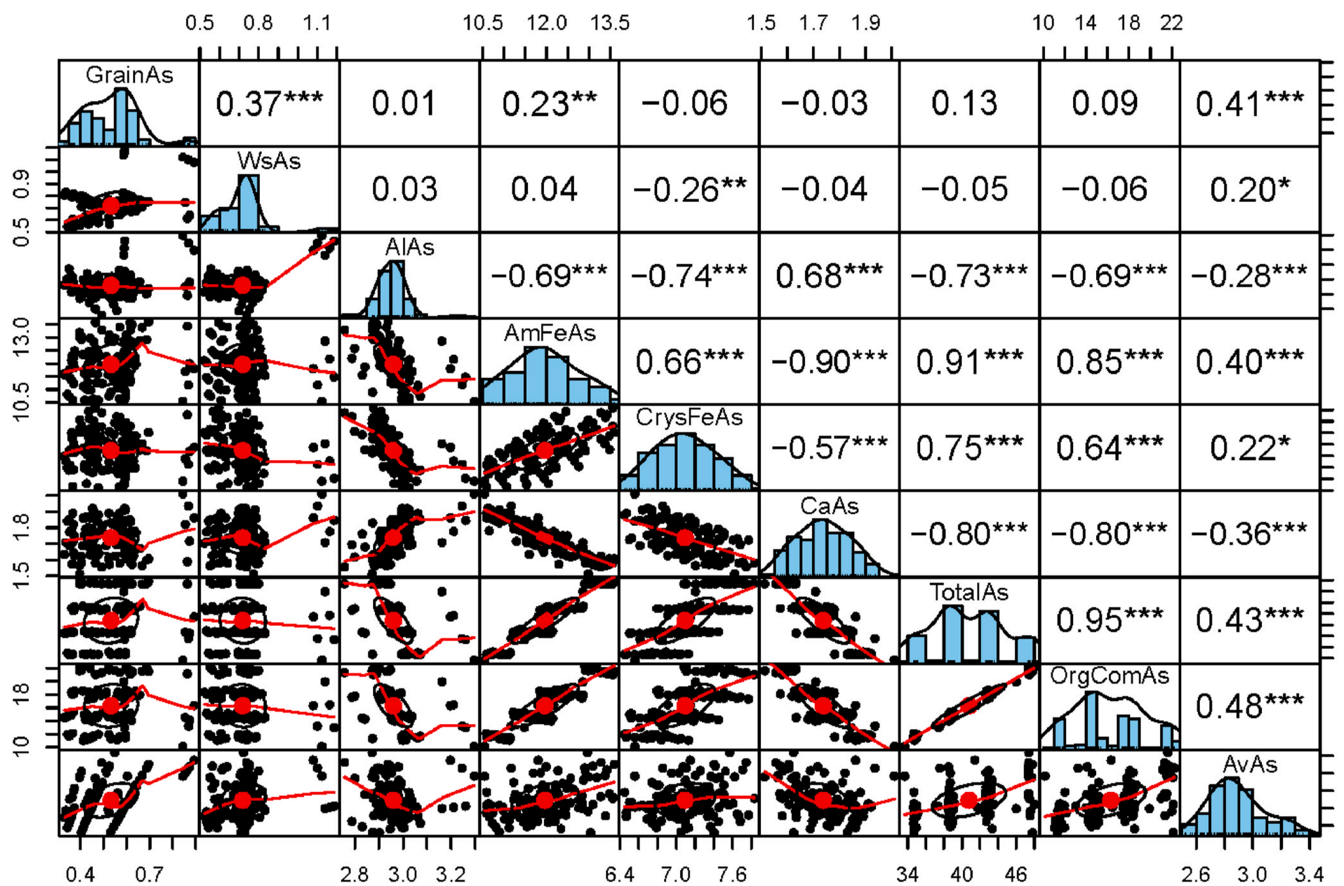

Fig. 4. Spearman correlation of rice grain As and soil As with As fractions (n = 132).

and lower As uptake. A similar increase in Fe content mediated plaque formation in the studies involving Fe-rich organic amendments have reported by Yin et al. (2017) and Sui et al. (2021), who suggested elevated Fe content induces a reduction/oxidation reaction cycle that results in co-deposition of heavy metals subsequently.

3.7. Dietary risk to humans associated with the consumption of arsenic-contaminated rice grain

A critical assessment of its transit through the food chain is required due to the growing concern posed by the toxicity found in places with no background of arsenic contamination. In the research area, rice is the main dietary staple and is often consumed three times per day with vegetables (Signes-Pastor et al., 2008). When cooked with contaminated water, the presence of As in food from the West Bengal region, particularly in rice, and its effects on health had already been predicted (Upadhyay et al., 2019). With inorganic As (iAs) concentration, which is dependent on variety and location, the risk of exposure to arsenic through diet remained more serious. The same research team (Sengupta et al., 2021) stated that the present variety IET-4786 contained 86.6% iAs (out of total As) in the study area, and the same data was used here to construct holistic expressions of human dietary risk from rice grain consumption. The study was mostly conducted on brown rice, hence all risk factors were obtained using the recommended maximum level of 0.4 mg kg^{-1} by the Joint FAO/WHO Expert Committee on Food Additives.

3.7.1. Target Cancer Risk (TCR) of as through rice grain

The results of the risks of cancer through consumption of contaminated rice grain suggest that in all cases the cancer risk probabilities are

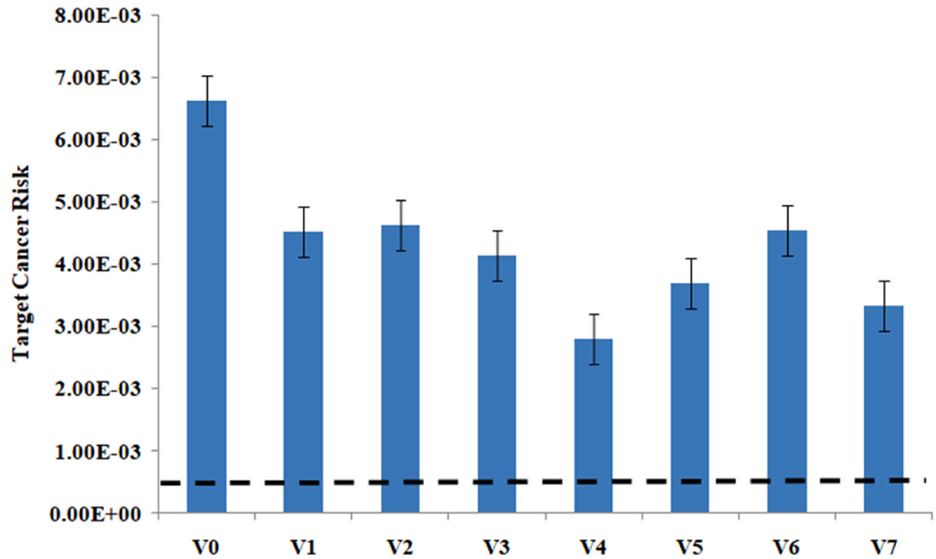

Fig. 5a. Illustration of Target Cancer Risk of dietary exposure to As through consumption of contaminated grains under the influence of applied vermicompost treatments. The dotted line indicates the threshold value of exposure (1xE-04 *i.e.* 10^{-4}) for the occurrence of cancer in human beings. The value of exposure below the line is considered safe, while values above the line are unsafe.

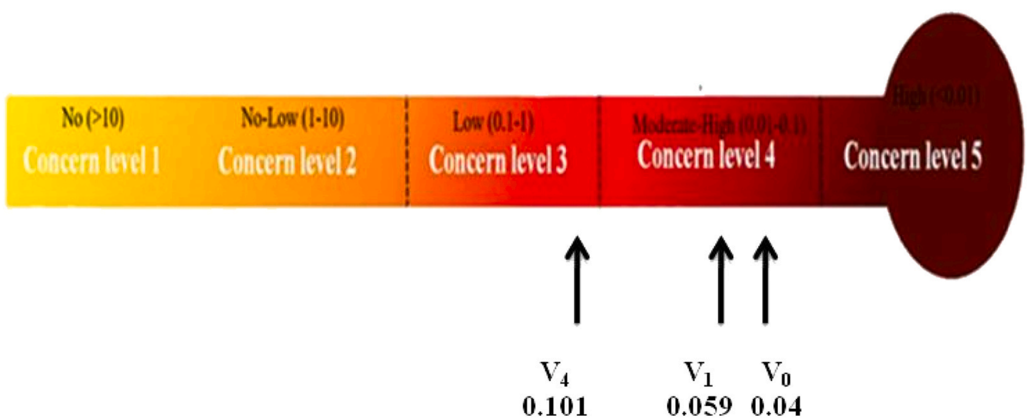

Fig. 5b. Risk thermometer scale showing the class of arsenic toxicity through intake of rice cultivated under different vermicompost treatments.

high. The TCR is quite high (6.60×10^{-3} to 6.64×10^{-3}), much higher than the tolerable limit of 10^{-4} (Shaheen et al., 2016) without vermicompost application. Even after using organic amendments (non--enriched and enriched vermicomposts), the lowest TCR is 2.69×10^{-3} at V_4D_1 still higher than the tolerable limit. Nevertheless, vermicompost application could ensure a reasonable curtailment against the carcinogen and such effects were even more pronounced with enriched vermicompost samples (Fig-5a). There have previously been reports of elevated cancer risk factors associated with dietary As intake (Mondal et al., 2010; Halder et al., 2014; Sengupta et al., 2021).

3.7.2. Risk thermometer and SAMOE (severity adjusted margin of exposure) through rice grain

The calculated 'SAMOE' value for As toxicity through dietary exposure to contaminated rice in the present study, displayed in terms of risk thermometer showed varying concern levels of risk from class 4 (moderate-high) to class 3 (low risk) depending on As concentration (Fig. 5b). The highest SAMOE (0.04) is associated with no vermicompost, while application of non-enriched and enriched vermicompost had much lower SAMOE (0.059 and 0.101 respectively). The lowest estimate of SAMOE has been observed at V_4D_1. When diverse actions result in variable quantities and origins of As in rice grains, the hazardous load might be exacerbated when the grains are consumed cooked, parboiled, or even raw (Chowdhury et al., 2020). Extended use of contaminated rice grain as a main source of food together with other dietary components results in substantial As poisoning (Bhattacharyya and Sengupta, 2020; Sengupta et al., 2021).

4. Conclusion

Arsenic in soil-water-plant systems has been thoroughly characterized and the efficiencies of organic (FYM, composts etc) and inorganic (Zn, Fe, Si, P etc) amendments in arsenic mitigation had been established conspicuously. The hindrance of inorganic (Zn, Fe) over-application/toxicity and the inadequacy of bulk organic matter (like vermicompost, compost, cow dung manure etc) proclaimed the idea of enrichment of organic tools with inorganic moieties. The present study has made a modest attempt to devise a strategy of using both the organic and inorganic tools together in a much more efficient, sustainable and eco-friendly approach. The enrichment of vermicompost by $ZnSO_4$ and $FeSO_4$, even at lower doses of application ensured sufficient As reduction in rice grain and soil, optimized grain yield, and even made risk parameters (TCR, SAMOE) somewhat benign. It was found that the Zn and Fe-enriched vermicompost augmented Fe plaque formation in the root that arrests upward translocation of As, reduced water-soluble arsenic fraction and increased Fe bound and organically bound As fractions in soil which are not readily bio-available. Thus based on the comparative advantage, we may propose that enrichment of vermicompost by $ZnSO_4$ and $FeSO_4$ @ 10% w/w dry weight basis of composting substrate, prior to application of *Eisenia fetida*, can significantly reduce arsenic accumulation in rice grains without compromising yield.

Research involving human participants and/or animals

This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent

Informed consent was obtained from all individual participants included in the study.

Author contributions

Sudip Sengupta: Methodology, Investigation, Data curation, Software and Writing- Original draft preparation; Kallol Bhattacharyya: Conceptualization, Methodology, Supervision; Jajati Mandal: Data curation, Visualization, Software, Writing- Reviewing and Editing; Parijat Bhattacharya: Investigation, Data curation; Asoke Prasun Chattopadhyay: Methodology, Validation, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jclepro.2023.138267.

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