

Article

Zerovalent Iron Modulates the Influence of Arsenic-Contaminated Soil on Growth, Yield and Grain Quality of Rice

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Abstract: This study aimed to investigate the effects of zerovalent iron (ZVI/Fe₀) on growth, yield and grain quality of rice (*Oryza sativa* L.) cv. BRRI dhan49 in arsenic (As)-contaminated soils. The pot experiment was arranged in a complete randomized design (CRD). The treatments on rice applied were As in soils at As₀ (0 mg kg⁻¹), As₂₀ (20 mg kg⁻¹), and As₄₀ (40 mg kg⁻¹) with a combination of ZVI at ZVI₀ (0%), ZVI_{0.5} (0.5%), ZVI_{1.0} (1.0%), and ZVI_{1.5} (1.5%) with three replications. Contents of phosphorus (P), potassium (K), manganese (Mn), zinc (Zn), iron (Fe), and As in grains of rice; and Fe and As content in cultivated soils were determined. The application of ZVI had negative or no effect on shoot weight, tiller number, and grain yield. Although application of ZVI had little or no effect on thousand grain weight, P, K, Zn, and Mn of rice grains, Fe content in rice grains was increased by ZVI treatments in a dose-dependent manner. The grain As content was non-significantly reduced by the ZVI application. Soil bacterial population was negatively influenced by the ZVI in a dose-dependent manner which might be linked with As content in the soils. Therefore, a further elaborative study is needed to elucidate the mechanisms of the effects of ZVI and soil As on rice and rhizosphere soil microorganisms.

Keywords: metalloid; plant-microbe interactions; phytoremediation; soil contamination; soil hazardous materials



Citation: Akter, S.; Rahman, G.K.M.M.; Hasanuzzaman, M.; Alam, Z.; Watanabe, T.; Islam, T. Zerovalent Iron Modulates the Influence of Arsenic-Contaminated Soil on Growth, Yield and Grain Quality of Rice. *Stresses* **2021**, *1*, 90–104. <https://doi.org/10.3390/stresses1020008>

Academic Editor: Luz E. de-Bashan

Received: 21 December 2020

Accepted: 26 April 2021

Published: 28 April 2021

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1. Introduction

A naturally occurring element, arsenic (As) is considered as a pollutant in soil, water, and air. Arsenic is one of the most poisonous elements on the earth's surface. The inorganic form of As is more hazardous to living organisms than the organic form. The inorganic form of As arises mainly through anthropogenic activities and is associated with major human health problems including cancer [1]. Many river basins and the deltaic regions of the world (mainly in the tropical regions) have a serious problem of As contamination in groundwater [2]. Drinking water containing excess amount of As has been reported from several parts of the world including Bangladesh, India, China, Nepal, Mexico, Argentina, Chile, Hungary, Taiwan, and USA. There is a higher As concentration in paddy cultivated soils than in non-paddy soils. As concentration is also higher in groundwater-irrigated soil than in surface-water irrigated soil [3]. During the dry (Robi) season in Bangladesh, most of the cultivable land are irrigated by ground water and 77% of that area are covered by

rice cultivation alone [4]. Arsenic accumulation in soils through groundwater utilization is also a major threat for rice cultivation.

Arsenic-contaminated irrigation water causes high levels of As in paddy soil [5] and decreases the yield of rice [5,6]. Rice plants grown on soils with elevated As have increased As content in the grains [7]. The As uptake by plants is directly influenced by the As concentration in the soil. Normally, As concentration of rice grains is $<1.0 \text{ mg kg}^{-1}$ (dry weight basis). Absorption of As varies among plant species and even among paddy varieties when they are grown in As-contaminated soils [8–10]. As is transported to the plant root through phosphate transporters and nodulin 26-like intrinsic channels. The silicic acid transporter may have a vital role in the entry of methylated As, dimethylarsinic acid, and monomethylarsonic acid into the root. Among all As species, dimethylarsinic acid is mobile in plants and can easily transfer from root to shoot. In addition, the *OsPTR7* gene has a key role in moving dimethylarsinic acid in the xylem or phloem [11]. High levels of As in food grains poses a serious threat to human health in Bangladesh and some other countries having high As-contaminated agricultural soils. Therefore, remediation of As-contaminated soils and reduction of As levels in agricultural products by the application of novel approaches are important for sustainable agricultural practices in the As-contaminated areas.

Iron is found as one of the most abundant elements in the earth's crust. Over the last decade, many researchers have investigated the removal of soil heavy metal (HM) contaminants through the utilization of zerovalent iron (ZVI or Fe_0). The ZVI has been widely used to remediate many soil contaminants, HMs, and As compounds [12]. Zerovalent iron is a reactive material with reducing power that is effective to stabilize the toxic elements in a solution [13]. The ZVI-based filtration technology has been shown as an affordable, easily applied, and most efficient water treatment system. Generally, ZVI can be injected into the immediate vicinity of contaminant sources which replaces toxic materials with Fe [14]. It reacts with oxidized contaminants such as chromium, cadmium, and selenium as an effective reductant. The contaminant removal mechanism of ZVI is that it directionally shifts its own electrons to the contaminants and changes the contaminant into a non-toxic or less toxic species [15–17]. The ZVI can potentially stabilize contaminated soil elements through the oxidation processes, which changes soil pH, and delivers an effective surface for absorption of both anions and cations. The mechanism of As remediation by ZVI is adsorption, surface precipitation, and then redox reaction [18]. Sorption of As species greatly affects by the ZVI coated with stabilizers, which alters the surface potential [19]. The ZVI can reduce cadmium in the soil thereby making it less biologically active and lowering cadmium levels in the rice grains through its reducing power [13]. The hypothesis of our study was that ZVI remediates As from soil and increases plant growth as well as reducing the As uptake by grains. Considering the reducing power of ZVI against the HMs, we evaluated the effects of ZVI on growth, yield, and grain As content in rice cultivated in varying levels of As-contaminated soils. Additionally, we examined the effect of ZVI on grain quality of rice and bacterial population in the cultivated soils.

2. Results

2.1. Effect of Zerovalent Iron on Plant Growth

The present investigation included the effects of ZVI on growth, yield, and As contents in the rice plant, and also on soil quality and bacterial population in the cultivated soils. There were considerable effects of ZVI and As doses on plant growth attributes. Plant height at 50 days after planting (DAP) was influenced by soil As content in a dose-dependent manner (Table 1). The tallest plants were recorded in $\text{As}_0\text{ZVI}_{0.5}$, while the shortest plants were found in $\text{As}_{40}\text{ZVI}_0$. It was revealed that plant height reduced by 9.93% in As_{40} compared control (As_0).

Table 1. Effects of varying doses of zerovalent iron and arsenic contents in soils on growth parameters of rice plant.

Treatment		Plant Height (cm)	Shoot Dry wt. (g) after Harvesting	Root Dry wt. (g) after Harvesting	No. of Tiller per Hill at 50 DAP	No. of Effective Tiller per Hill at 50 DAP
As ₀	ZVI ₀	85.60 ± 1.33a	47.33 ± 1.66a	15.67 ± 0.76ab	27.67 ± 1.78a	27.33 ± 1.19a
	ZVI _{0.5}	85.77 ± 1.33a	46.33 ± 3.78a	20.00 ± 1.41ab	25.00 ± 2.83ab	25.00 ± 2.83ab
	ZVI _{1.0}	84.33 ± 1.34ab	44.67 ± 2.13ab	29.00 ± 1.25a	24.67 ± 0.72ab	24.67 ± 0.72abc
	ZVI _{1.5}	83.00 ± 1.27abc	36.67 ± 3.53abc	18.00 ± 1.25ab	18.67 ± 2.23a–d	18.33 ± 2.33a–d
As ₂₀	ZVI ₀	83.00 ± 1.31abc	32.67 ± 1.09bcd	11.67 ± 0.72b	20.33 ± 0.98a–d	20.33 ± 0.98a–d
	ZVI _{0.5}	82.73 ± 1.21abc	28.33 ± 3.60cd	12.67 ± 0.72ab	16.33 ± 3.14a–d	16.00 ± 2.94a–d
	ZVI _{1.0}	82.23 ± 0.74abc	25.33 ± 1.19cd	15.00 ± 0.94ab	13.33 ± 2.76bcd	13.33 ± 2.76bcd
	ZVI _{1.5}	83.73 ± 0.71ab	22.33 ± 0.98d	12.00 ± 0.47ab	12.00 ± 2.94d	12.00 ± 2.94d
As ₄₀	ZVI ₀	77.10 ± 0.68c	20.67 ± 0.27d	6.00 ± 0.47b	16.33 ± 1.66a–d	16.33 ± 1.66a–d
	ZVI _{0.5}	78.53 ± 0.24bc	24.33 ± 1.44cd	7.00 ± 0.47b	12.33 ± 0.27cd	12.33 ± 0.27cd
	ZVI _{1.0}	81.33 ± 0.36abc	22.00 ± 1.41d	9.00 ± 0.47b	10.33 ± 0.54d	10.33 ± 0.54d
	ZVI _{1.5}	81.33 ± 0.36abc	19.33 ± 0.98d	8.00 ± 0.47b	10.00 ± 1.25d	9.67 ± 1.89d

Mean was calculated based on three replications of each treatment ± SE. Values in a column with different letter(s) are significantly different at $p \leq 0.05$ applying Tukey's HSD test.

The application of ZVI at As₀ in soil significantly influenced the shoot and root weight of rice (Table 1). The effect of ZVI and As reduced the shoot dry weight by up to 59%. The highest and lowest growth of rice were recorded in control (As₀ZVI₀) and As₄₀ZVI_{1.5} treatments, respectively. On the other hand, ZVI and As treatments also reduced root dry weight by up to 62% compared to the control. The highest and lowest root dry weight were obtained from As₀ZVI_{1.0} and As₄₀ZVI₀, respectively. However, there was no significant effect of ZVI on root dry weight at As₂₀ and As₄₀. The ZVI and As negatively influenced tiller number at 50 DAP (Table 1). Tiller number at 50 DAP was the highest in As₀ZVI₀ and the lowest tiller number was in As₄₀ZVI_{1.5}. The effect of ZVI and As reduced the tiller number by 64% compared to the control. Effective tiller number was also affected by ZVI in the same changing trend of tiller number at 50 DAP (Table 1). Effective tiller number reduced to by 4.83% compared to the control, where the highest effective tiller was in As₀ZVI₀ and the lowest number was in As₄₀ZVI_{1.5}.

2.2. Effect of Zerovalent Iron on Grain Yield

Effect of ZVI played a considerable role on grain dry weight (Table 2). The highest and the lowest grain dry weights of rice were produced by the treatments of As₀ZVI_{0.5} and As₄₀ZVI_{1.5}, respectively. Grain dry weight of rice was reduced by the effects of As and ZVI by 76% compared to the control. The ZVI played no effect on thousand grain weight (Table 2). Thousand grain weight was highest in As₄₀ZVI_{1.0} and lowest in As₂₀ZVI_{1.0}.

Table 2. Effect of varying doses of zerovalent iron on grain yield of rice.

Treatment		Grain Dry Weight (g plant ⁻¹)	Thousand Grain Weight (g)
As ₀	ZVI ₀	64.25 ± 1.27ab	12.25 ± 0.27a
	ZVI _{0.5}	71.43 ± 5.24a	13.02 ± 0.27a
	ZVI _{1.0}	52.53 ± 5.12abc	12.83 ± 0.44a
	ZVI _{1.5}	49.53 ± 10.11abc	13.55 ± 0.56a
As ₂₀	ZVI ₀	42.47 ± 1.19bcd	11.13 ± 0.77a
	ZVI _{0.5}	42.82 ± 2.37bcd	13.00 ± 0.52a
	ZVI _{1.0}	37.12 ± 3.18cde	10.78 ± 1.27a
	ZVI _{1.5}	24.05 ± 0.35de	11.23 ± 0.47a
As ₄₀	ZVI ₀	34.42 ± 3.21cde	10.98 ± 1.20a
	ZVI _{0.5}	30.00 ± 1.07cde	12.10 ± 0.19a
	ZVI _{1.0}	22.58 ± 0.86de	13.60 ± 0.17a
	ZVI _{1.5}	17.07 ± 1.82e	12.83 ± 0.31a

Mean was calculated based on three replications of each treatment ± SE. Values in a column with different letter(s) are significantly different at $p \leq 0.05$ applying Tukey's HSD test.

2.3. Effect of Zerovalent Iron on Grain Phosphorus, Potassium, Zinc, Manganese, and Iron Content

The effects of ZVI on grain phosphorus (P) and potassium (K) contents are shown in Figure 1. The highest grain P content was found in As₂₀ZVI_{1.0} and the lowest in As₀ZVI_{1.0}, As₂₀ZVI₀, and As₄₀ZVI₀ treatments (Figure 1A). On the other hand, the highest and the lowest rice grain K contents were found in As₀ZVI₀ and As₂₀ZVI₀ treatments, respectively, (Figure 1B). The effect of ZVI on grain zinc, manganese, and iron content are shown in Figure 2. The highest Zn content in rice grain was found in As₀ZVI_{1.5} and As₂₀ZVI_{1.5} and the lowest Zn content was in As₀ZVI_{0.5} and As₂₀ZVI₀ treatments (Figure 2A). The treatments As₄₀ZVI_{1.5} and As₂₀ZVI_{0.5} resulted in the highest and the lowest grain manganese contents in rice (Figure 2B). Grain Fe content increased with the increasing doses of ZVI (ZVI₀ < ZVI_{0.5} < ZVI_{1.0} < ZVI_{1.5}) (Figure 2C). The treatments As₀ZVI_{1.5} and As₄₀ZVI_{1.5} gave the highest grain Fe content, while the lowest grain Fe was obtained in treatments of As₂₀ZVI₀ and As₄₀ZVI₀.

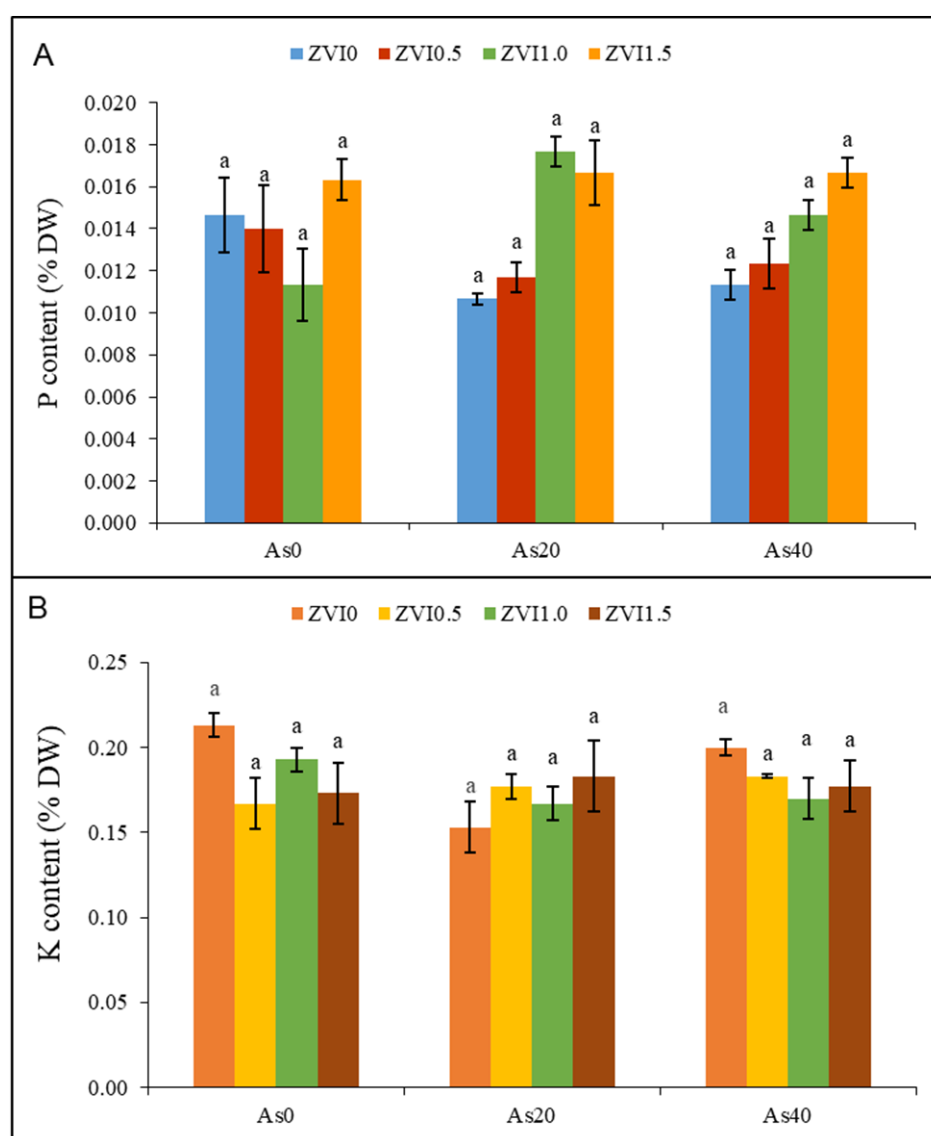


Figure 1. Effect of zerovalent iron on grain phosphorus and potassium contents in rice plants: (A) phosphorus content (%) in rice grain, and (B) potassium content (%) in rice grain. Mean was calculated based on three replications of each treatment \pm SE. Values in a column with different letter(s) are significantly different at $p \leq 0.05$ applying Tukey's HSD test.

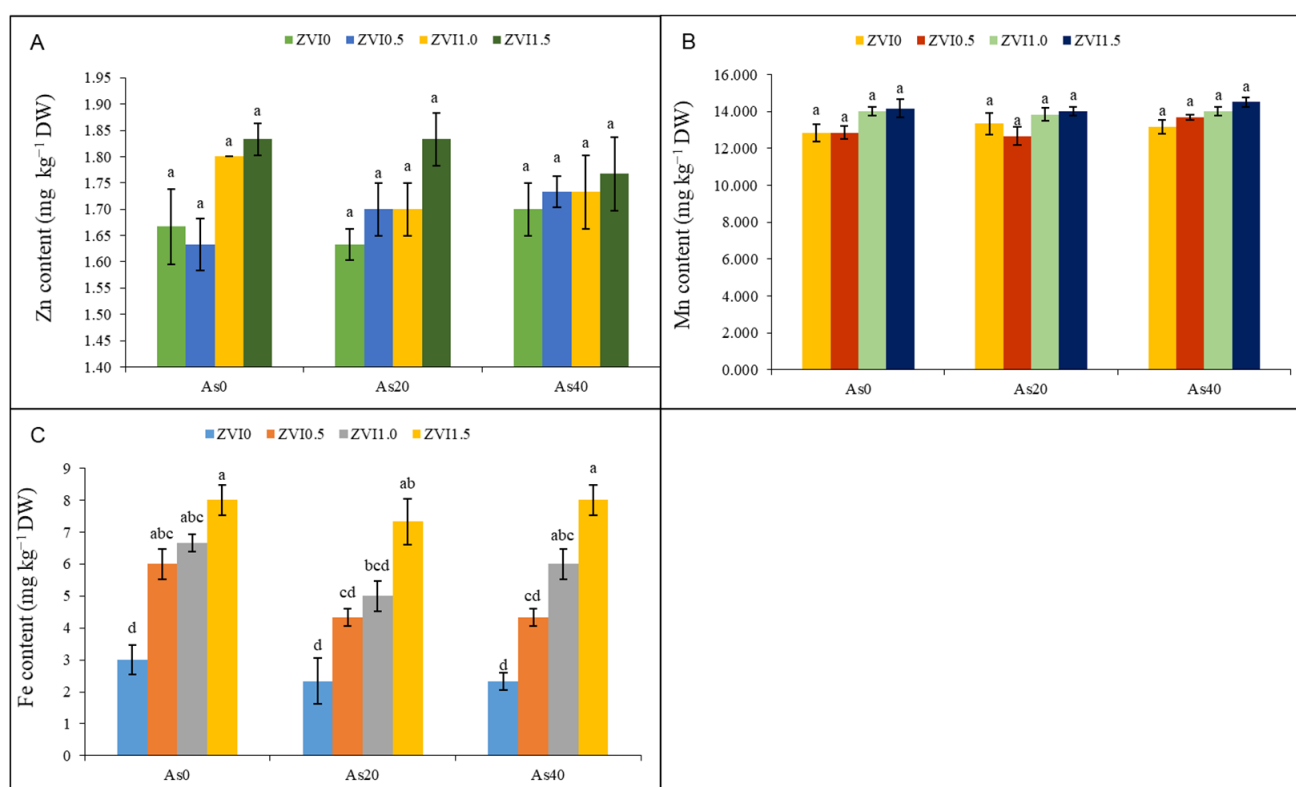


Figure 2. Effect of zerovalent iron on grain mineral contents in rice plants: (A) zinc content (mg kg^{-1}) in rice grains, (B) manganese content (mg kg^{-1}) in rice grains, and (C) iron content (mg kg^{-1}) in rice grains. Mean was calculated based on three replications of each treatment \pm SE. Values in a column with different letter(s) are significantly different at $p \leq 0.05$ applying Tukey's HSD test.

2.4. Effect of Zerovalent Iron on Grain Arsenic

The effect of ZVI played no significant role in grain As content of rice (Figure 3A). The treatment As₀ZVI_{0.5} gave the highest As content in rice grains, however, both As₀ZVI_{1.0} and As₀ZVI_{1.5} treatments resulted the lowest grain As contents among As₀ treatment in rice. Among the treatment As₂₀ with varying doses of ZVI, the highest and lowest grain As contents were found in As₂₀ZVI₀ and As₂₀ZVI_{1.0}, respectively. On the other hand, the highest and lowest grain As contents resulted from As₄₀ZVI₀ and As₄₀ZVI_{1.5} among As₄₀ treatments, respectively. The effects of ZVI on grain As uptake are shown in Figure 3B. The highest and the lowest As uptake by rice were shown by As₀ZVI_{0.5} and As₀ZVI_{1.5} among As₀ variables, respectively. Among the As₂₀ variables, the highest and lowest As uptake was obtained in As₂₀ZVI_{0.5} and As₂₀ZVI_{1.5} treatments. However, at high soil As concentration (As₄₀), the highest and the lowest As uptake by rice was found in As₄₀ZVI_{0.5} and As₄₀ZVI_{1.5} treatments, respectively.

2.5. Effect of Zerovalent Iron on Soil Iron and Arsenic Concentration

The ZVI increased the available Fe content of in the cultivated soil (Figure 4). In non-As (As₀)-contaminated soils, the highest and lowest soil Fe contents were found in As₀ZVI_{1.5} and As₀ZVI₀ treatments, respectively. However, in soil As at As₂₀, the highest and lowest Fe contents were recorded in As₂₀ZVI_{1.0} and As₂₀ZVI₀ treatments, respectively. When soil As concentration was at As₄₀, the highest and lowest Fe contents in rice grain were recorded in the As₄₀ZVI_{1.0} and As₄₀ZVI₀ treatments, respectively. The effect of ZVI on total As content in soil is presented in Figure 5. As expected, the lowest soil As content was found in As₀ZVI₀. However, at As₂₀ and As₄₀, ZVI treatments at As₂₀ZVI_{0.5} and As₄₀ZVI_{1.0} resulted in the lowest As contents in the pre-transplanting soils. At zero-As-containing

soils, the lowest As content was found in $As_0ZVI_{0.5}$. In soil As at As_{20} and As_{40} , $As_{20}ZVI_{0.5}$ and $As_{40}ZVI_{1.0}$ treatments resulted in the lowest As contents in the post-harvest soils.

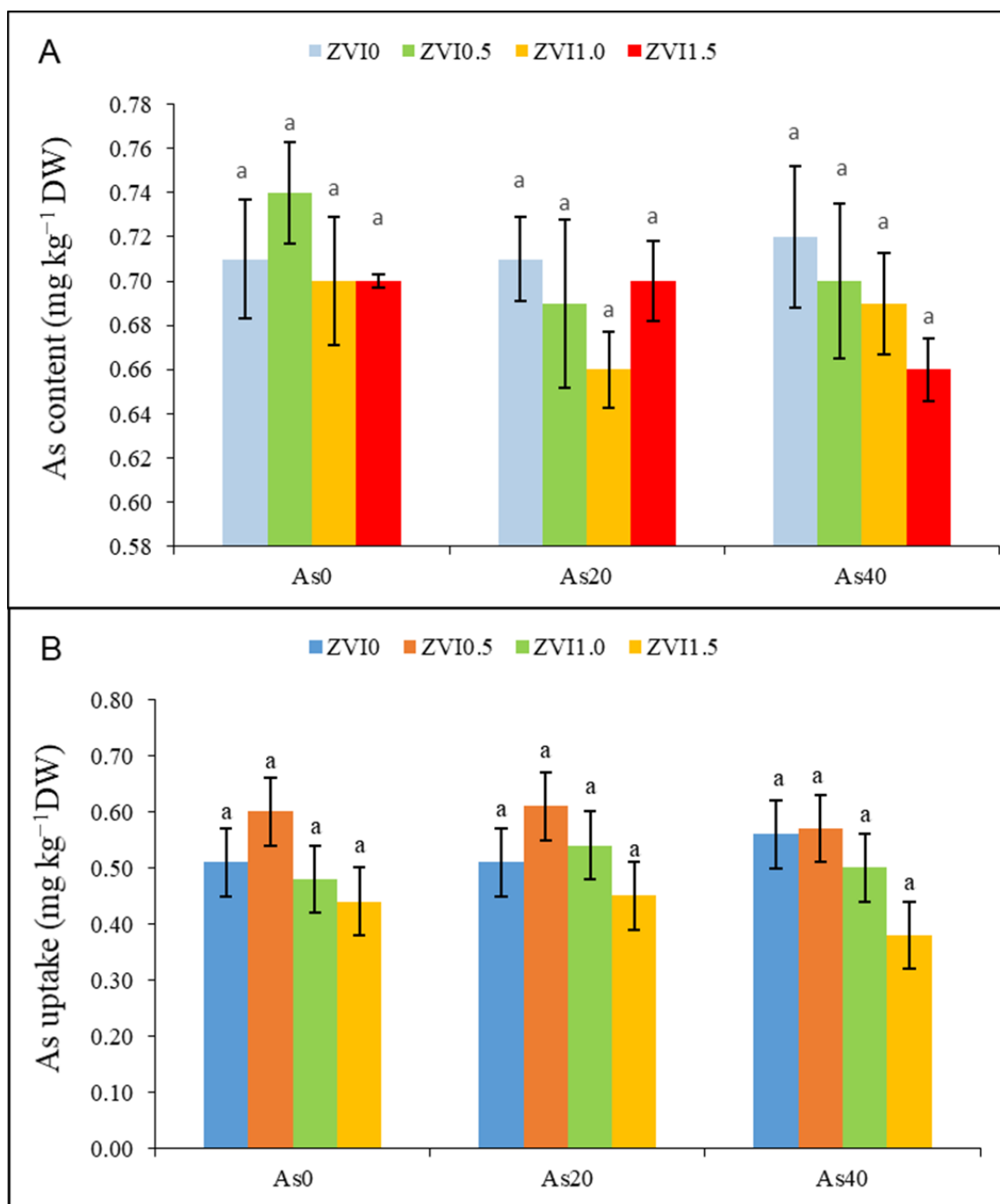


Figure 3. Effect of zerovalent iron on grain arsenic content and As uptake by rice. (A) Total arsenic content (mg kg^{-1}) in grain, and (B) available arsenic uptake (mg kg^{-1}) by rice. Mean was calculated based on three replications of each treatment \pm SE. Values in a column with different letter(s) are significantly different at $p \leq 0.05$ applying Tukey's HSD test.

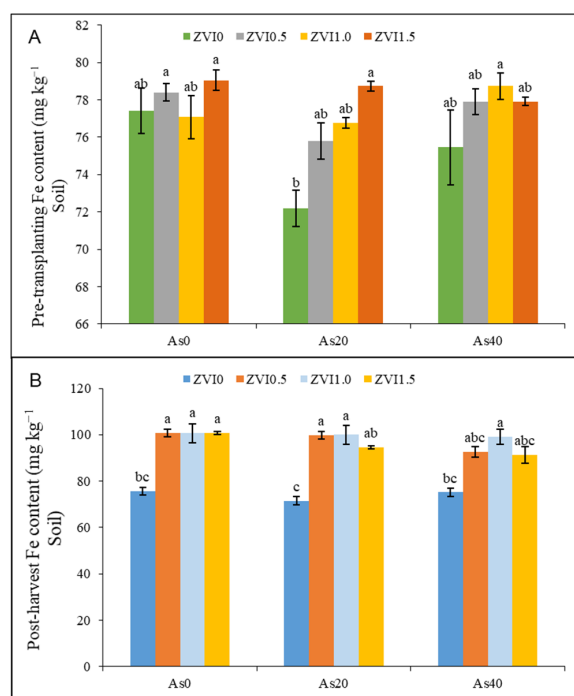


Figure 4. Effect of zerovalent iron on total iron content in soils. (A) Total iron content in pre-transplanting soil, and (B) total iron content in post-harvest soil. Mean was calculated based on three replications of each treatment \pm SE. Values in a column with different letter(s) are significantly different at $p \leq 0.05$ applying Tukey's HSD test.

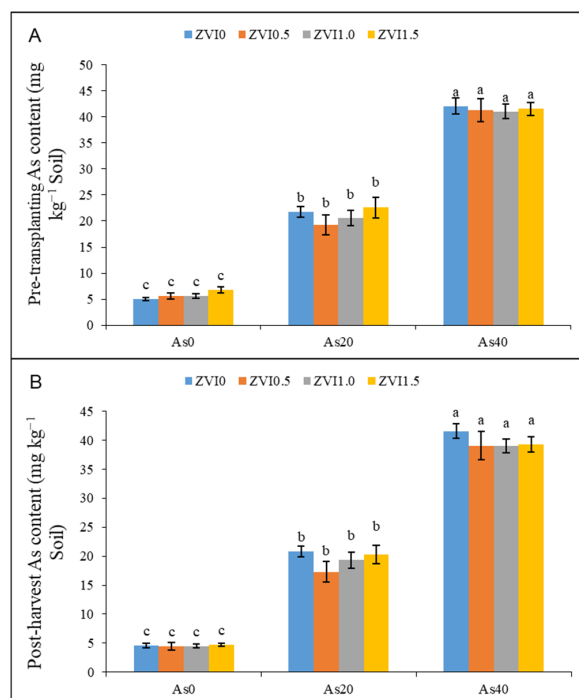


Figure 5. Effect of zerovalent iron on total arsenic content in soils. (A) Total arsenic content in pre-transplanting soil, and (B) total arsenic content in post-harvest soil. Mean was calculated based on three replications of each treatment \pm SE. Values in a column with different letter(s) are significantly different at $p \leq 0.05$ applying Tukey's HSD test.

2.6. Effect of Zerovalent Iron on Soil Bacterial Colony-Forming Unit

Higher concentrations of both ZVI and As reduced bacterial colony-forming unit (CFU; Figure 6). The highest bacterial colonies were recorded in As₂₀ZVI₀ treatment and the lowest in As₄₀ZVI_{1.5} treatment. The bacterial colonies reduced with the increase of both ZVI and As, among other variables.

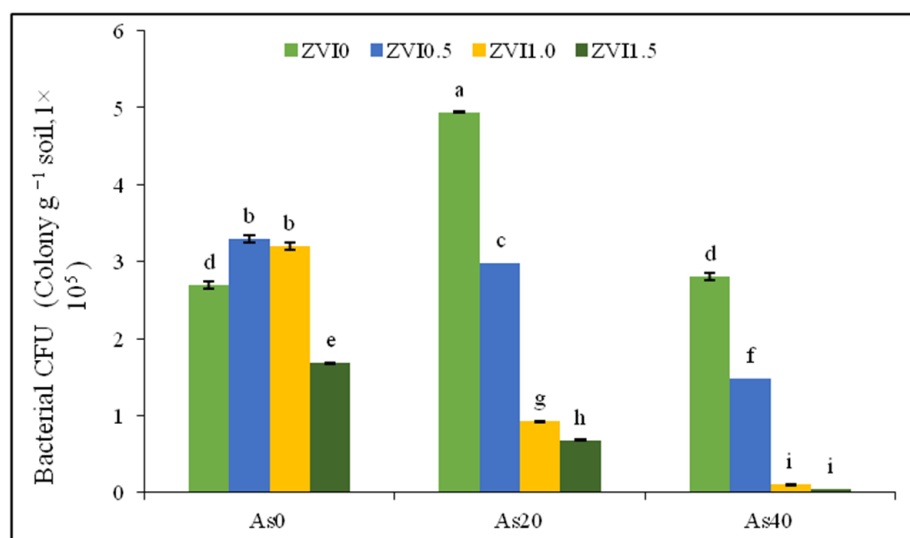


Figure 6. Effect of zerovalent iron on bacterial colony-forming unit (CFU) in cultivated soils. Mean was calculated based on three replications of each treatment \pm SE. Values in a column with different letter(s) are significantly different at $p \leq 0.05$ applying Tukey's HSD test.

3. Discussion

In the current study, application of ZVI had negative effects on shoot weight, tiller number, and grain yield of rice. The ZVI application exhibited no considerable effects on P, Mn and Zn contents in rice grain. However, ZVI application increased Fe contents in rice grains. The population of rhizospheric bacteria was significantly decreased by the application of higher levels of ZVI and As. Abbas et al. [20] also found no significant effect of Fe on the P uptake by wheat crops. In a study with *Arabidopsis*, ZVI application did not have any clear influence on Mg uptake, although it increased P content in the plant shoot, whereas Mn and Zn content of the plant shoots decreased due to application of ZVI [21]. Yoon stated that proton secretion owing to the application of ZVI in soil activates H⁺-ATPase that may acidify rhizospheric soil which increase P availability. In addition, active As and available P content of soil as well as grain As content can be reduced by ZVI. The reduction of As in rice plants by ZVI occurred due to active As stabilization by ZVI in soil and increasing Fe plaque quantity in soils. The ZVI is a barrier to transporting As from soil to rice plant, and Fe adsorbs the P that reduces As into the rice root by the competitive mechanism between P and As. Active As in soil is decreased by the stabilization effects of ZVI, which probably plays the main role to reduce As content in soil and grains [21].

The higher amount of As uptake as well as the Fe concentration in soil can reduce the plant height considerably due to As toxicity which is clear from the outcome of our current study on the ZVI-As effect on the soil–plant system. Speciation of As uptake by plants (e.g., As^{III}, As^V), types of plant species and some soil factors control As accumulation, which cause As toxicity in plant tissue. Toxicity, detoxification, and As (III and V) uptake differ due to different plant species as they have different adaptive mechanisms [22]. Maximum plant growth occurs when Fe concentration remains between 10 and 50 mg L^{−1} and the growth reduces due to Fe toxicity amending with Fe at 250 and 500 mg L^{−1} [23]. The ZVI reduces the total and inorganic As content in the root and grain without showing any significant effect on the straw [24]. However, plant growth is inhibited by Fe concentration at 24.6 μ mol

Fe L^{-1} [25]. The highest (at 1000 mg kg^{-1}) suppression rate of shoot length reached by ZVI which was 57.5% [26], but there were no significant effects on seedling elongation in some plants (*Lepidium sativum*, *Sorghum saccharatum*, and *Sinapis alba*) due to Fe particles. [27]. Biomass production and biostimulation effects (like increased seedling length) are detected at exposure to the highest Fe concentrations. Resistant varieties may have a partitioning Fe mechanism (which is absent in susceptible varieties) in plant tissues (the shoot system) without causing any cell damage. There may have a link between leaf symptoms and the chemical signal which is transmitted by the plant root system. The root system of susceptible varieties may transmit stronger signals when grown in higher-Fe-containing media than the resistant variety with low-Fe-containing media. Higher As concentration reduces the shoot dry weight, root dry weight, and seedling emergence [28]. Excess Fe application in plants can reduce the root and shoot dry weight [29]. With the gradual increasing of Fe concentrations in soil, total As content of the rice shoot also increases steadily when rice seedlings are grown with As^{V} [30]. In addition, ZVI can inhibit the growth of rice seedlings in higher concentrations ($>500 \text{ mg kg}^{-1}$) in soil though it did not show any effect on seedling germination [26]. However, if the As is in the dimethylarsinate form, the total As content in plant shoot is independent of Fe concentrations in the soil. With the increasing of exposure time for As^{V} and dimethylarsinate, total As concentration also increases in rice shoots. The yields of brown rice and straw reduce slightly and insignificantly due to application of Fe-bearing materials [31]. There is also evidence that plant growth inhibition is the result of Fe toxicity [32]. The ZVI (20 mg kg^{-1}) can increase the seedling vigor (shoot and root length, photosynthetic pigment content, and biomass) by the way of increasing the water uptake capacity of rice plant [33]. Under the Fe(−) condition, ZVI improves rice growth at 50 mg L^{-1} concentration but in the Fe(+) condition ZVI does not exhibit any positive effect, in fact plant growth is inhibited at 500 mg L^{-1} concentration. In addition, ZVI at 500 mg L^{-1} concentration reduces root volume and leaf biomass and enhances oxidative stress in the plant [34]. The micro-sized Fe particle has some negative effect on the germination percentage of *Sinapis alba*, *Sorghum saccharatum*, and *Lepidium sativum* plants [27]. Fe particle application in soil has some significant effect on grain yield and harvest index. Factors such as the genetic structure of the plant, the way of utilizing the metabolic products, and the effect of high pH that hinder Fe availability to calcareous soil, affect the yield and harvest index [35]. Interestingly, findings of some studies reveal that ZVI exhibits a negative effect on polluted soils [36].

Supplementary K nutrition is unable to reduce the effect of Fe stress on plant growth. In addition, it also not affect the Fe accumulation in plants [23]. There is no significant effect of Fe on K uptake by wheat crops [20]. The ZVI application efficiency is strongly associated with soil pH and contaminant type as well as with the presence of organic matter, clay minerals, Fe, and manganese oxyhydroxides [37]. Although total Zn uptake and Zn translocation decrease with increasing concentration of Fe [38]. In Fe-deficiency, the transformed high Fe significantly increases grain Zn concentration [39].

One of the important findings of this study is that ZVI significantly increases grain Fe content in a dose-dependent manner. There is a negative correlation of root and leaf Fe concentrations with Mn, whereas stem Fe concentrations are positively correlated with Mn [40]. The higher dose of ZVI increases the Fe content in leaves and stems [41]. On the contrary, an experiment carried by Wang et al. [26] revealed that higher concentrations of ZVI resulted Fe-deficiency symptoms in plants. In addition, in the shoots, the active Fe content decreased but it did not decrease in the roots. Interestingly, available and total Fe content in soil were not less than the control. This might be due to blocking the transport of active Fe from root to shoot of rice seedlings by ZVI. Arsenic removal capacity of ZVI is approximately $7.5 \text{ mg As per gram Fe}$ [42] and ZVI can reduce total As in aqueous solution [43,44]. Arsenic concentrations can be reduced substantially by granular ZVI [45].

Uptake of As by rice grains clearly manifests a positive response to Fe particles (100 μm), which significantly reduces As concentrations in grain [46]. In the current study, application of ZVI reduced the uptake of As in rice grain though it was non-significant. However, application of ZVI significantly increased the grain Fe contents of rice.

The application of ZVI effectively minimized the uptake of all target risk elements (As, Zn, Pb, and Cd) into plant tissues [47]. Fe powder application lessens the As accumulation in rice grain effectively [48] and this will be auspicious practice to reduce As accumulation, although the mechanism of the reduction of As content in rice grain by the application of ZVI is not clear from our current study. However, it might be a reason for the activities of rhizobacteria and the interaction of ZVI with other soil particles and ions. The effect of ZVI depends on the bacterial genus and strain along with the bacterial phase. In lag and stationary phases, bacterial cells show strong resistance to ZVI, whereas in exponential and decline phases bacterial cells are less resistant and become inactive rapidly with increasing the concentration of ZVI [49]. Limitation of Fe particles reduces the bacterial colony which could be due to reduction of bacterial motility. The bacterial cell interaction reduces in some cases as the result of reducing bacterial motility and colonization with the limitation of Fe particle in the ocean [50]. In our study, both As concentration in soils and ZVI doses decreased the soil bacterial population in a dose-dependent manner. The ZVI has a strong bactericidal effect on *Escherichia coli* under deaerated conditions [51]. The ZVI exhibits a toxic impact on soil organisms and the impact variability according to soil type. The toxic effect on soil organism due to ZVI was lower than in in vitro assay [52]. El-Temsah et al. [53] revealed that oxidation of ZVI may be caused by O_2 consumption and excess Fe being available in water and soil, which affects the organisms negatively. In addition, ZVI stabilized with sodium carboxymethyl cellulose significantly reduces soil bacterial biomass [54].

4. Materials and Methods

4.1. Experimental Materials and Treatments

Our experiment was carried out at the experimental net house of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), located at the center of Madhupur Tract (AEZ-28) at about $24^{\circ}05'$ north latitude and $90^{\circ}25'$ east longitude having a mean elevation of 8.4 m above the sea level. The soil used for the experiment belongs to Salna series representing shallow red-brown terrace soil type. As per USDA soil classification, the experimental soil is classified as Typic Paleudults under Ochrept sub-order of Inceptisol order [55]. The soil was generally characterized by heavy clays within 50 cm from the surface and was acidic in nature. The pot experiment was laid out in a complete randomized design (CRD) with three replications. A total of 36 pots were used for 12 treatments with As and ZVI. Sodium arsenate ($\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$) doses with 0, 20, and 40 mg kg^{-1} , and the ZVI (ca. 100 μm) doses with 0, 0.5, 1.0, and 1.5%. As doses were As_0 for control, As_{20} for arsenic 20 mg kg^{-1} , and As_{40} for arsenic 40 mg kg^{-1} , and ZVI doses were ZVI_0 for control, $\text{ZVI}_{0.5}$ for ZVI 0.5%, $\text{ZVI}_{1.0}$ for ZVI 1%, and $\text{ZVI}_{1.5}$ for ZVI 1.5% of total soil volume (0, 1400, 2800, and 4200 kg ha^{-1} , respectively, considering soil depth of 0.2 m). *Oryza sativa* L. cv. BRRI dhan49 was used as the experimental rice variety. Each pot contained 5 kg of dried soil. Sodium arsenate was mixed with every pot according to dose and kept in standing water for 3 days. Seeds were sown directly in the nursery bed (non As-contaminated soil) at the BSMRAU Farm. Thirty-day-old seedlings of rice were transplanted into the pots. A single healthy seedling was planted in each pot and then ZVI was applied according to treatment dose. Tagging was also done at the same day. A spacing of pot-to-pot distance of 45 cm was maintained. Weeding was done to keep the crop free from weed. Sufficient irrigation was maintained using tap water as it was a pot experiment. The crop was kept insect and disease free. Rats and other pests were controlled by regular monitoring. Other intercultural operations were carried out accordingly. Harvesting was done at 145th day, after full ripening of the crop.

4.2. Crop Husbandry

Chemical fertilizers were applied at recommended doses for the BRRI dhan49 rice at the rate of 195, 60, 105, 67 and 10 kg/ha of urea, triple super phosphate, muriate of potash, gypsum, and zinc sulphate, respectively. The required doses of all fertilizers except urea were applied as basal dose 3–4 days before transplanting the rice seedlings. The entire dose of urea was applied in three installments. The first installment of one third of urea was applied at basal dose followed by second top dressing comprising one third of urea, at the time of panicle initiation followed by a last top dressing comprising the rest of the urea at the time of flowering [56]. As was added to the soil at 0, 20, and 40 mg kg⁻¹; and 0, 25 g, 50 g, and 75 g of ZVI per pot (0, 1400, 2800, and 4200 kg ha⁻¹, respectively, considering soil depth of 0.2 m) was added to the soil. Irrigation and weeding were done as when necessary.

4.3. Observation of Morphological Parameters

Plant height (at 50 DAP), shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, grain fresh weight, grain dry weight, and thousand grain weight were measured by a weighing machine. Tiller number and effective tiller number were measured by the direct counting method.

4.4. Plant Sampling and Chemical Analysis of Plant Samples

4.4.1. Plant Sampling

Plant samples were collected from each treatment of the pot after harvesting. The samples were air dried until properly dried in room temperature. Then straws were chopped into smaller sizes (5–7 cm). Dried plant (straw, root, and rice grain) samples were again dried in oven for at least 72 h at 60 °C. Then all plant samples were ground and 2 g samples of the dried plant material were used for determination of mineral content and total As content.

4.4.2. Chemical Analysis of Grain Samples

Phosphorus and Potassium in Grain

For P determination, 1 g of dried plant sample was taken for wet digestion (The P content of the plant sample was converted to orthophosphates by digestion with HNO₃ and HClO₄ mixture) and 5 mL of digest was taken in a 50 mL volumetric flask. Then 10 mL of vanadomolybdate reagent was added. The rest of the volume made up with distilled water and shaken thoroughly. After 30 min, a yellow-colored complex developed (when orthophosphates are made to react with molybdate and vanadate, a yellow-colored vanadomolybdophosphoric heteropoly complex is formed), which was stable for days. The intensity of the yellow color is directly proportional to the concentration of P present in the sample, which was read on the atomic absorption spectrophotometer (Model No 170- 30, HITACHI, Japan) [57–59]. For K determination, acid (KCl) digestion of 1 g plant sample was taken and volumed up to 100 mL in a volumetric flask. The sample was used for estimation in the range 5–10 mg K/kg (5–10 µg K/mL) by further diluting as appropriate. A blank was prepared in the same way without adding plant digested material. An aliquot of 5 mL was taken for estimation and volumed up to 100 mL and atomized at 766.5 nm on the calibrated atomic absorption spectrophotometer (Model No 170- 30, HITACHI, Japan), on which the standard curve had also been prepared. The absorbance was recorded against each sample. The concentration of K for the particular absorbance was observed for the sample from the standard curve [57,59].

Determination of Zinc, Manganese and Iron in Grain

Determination of Zn, Mn, and Fe was done as the same manner of K. Samples were digested with concentrated HNO₃ and HClO₄ mixture [57] for determination of total Zn, Mn, and Fe content with the help of an atomic absorption spectrophotometer (Model No 170-30, HITACHI, Japan).

Determination of Arsenic in Grain

Dried rice grain was homogenized with a vibrating sample mill (HEIKO TI-200). Samples were digested by nitric (HNO₃)-perchloric (HClO₄) acid digestion, with the help of block digester (behrotest K24 Digestion Unit). To maintain the analytical quality of rice flour digestion-certified reference material (NIST 1568a) was run with each set of samples. All samples were pre-reduced with potassium iodide and ascorbic acid to reduce As (V) to As (III) before determination of As by hydride generation atomic absorption spectrophotometer (Buck Scientific 210 VGP) with continuous flow of hydride generation system (HG-AAS). The standards were prepared following the same analytical matrix as followed for grain sample preparation. Desired and reasonable standard solutions were prepared for preparing the standard calibration curve.

4.4.3. Measurement of Arsenic Uptake

Arsenic uptake by grains from soil was calculated using the following formula-

$$\text{Arsenic uptake} = A \times Y \quad (1)$$

where, A = arsenic content of grain (mg kg⁻¹) and Y = dry matter production of grain

4.5. Soil Sampling and Chemical Analysis of Soil Samples

4.5.1. Soil Sampling

Soil samples were collected from each treatment before ZVI application in the pot and after harvesting of plant. The samples were air dried until properly dried. Dried soil samples were used for determination of ZVI and As content.

4.5.2. Chemical Analysis of Soil Samples

Determination of Iron

The air-dried soil samples were ground to pass through 2 mm sieve. Ammonium oxalate extractable Fe was determined by the method as described by Schwertmann [60] and McKeague and Day [61]. The extractions were carried out in triplicate. Iron in filtrates was determined by atomic absorption spectrophotometry (Model No 170-30, HITACHI, Japan) with air acetylene flame.

Determination Total Arsenic

Hydride generation atomic absorption spectrophotometer (HG-AAS) was used to determine total As described by Jacobs et al. [62] and Loeppert and Biswas [63]. For analysis, plant tissue samples were prepared by grinding followed by drying at 60° C and the samples were washed before drying. In case of soil As, air dried and sieved (<2 mm) samples were used. The HNO₃-HClO₄ procedure applied for digestion of plant tissue samples utilizes an aluminum heating block (behrotest K24 Digestion Unit) and 50 mL graduated test tubes, and was adopted because it allowed a large number of samples to be digested simultaneously. Digestion of soil samples by H₂SO₄-HClO₄ was done using the heating block and test tubes used for plant tissue analysis. Ammonium acetate (1 N; pH-7.0) was used to extract As-treated soil samples. The extraction procedure was performed using 10 g of soil and 50 mL of extractant in a 100 mL plastic centrifuge tube. After being shaken on an electrical shaker (30 min) the extract was collected by centrifugation. Reduction distillation method was used to determine As content. Two volumes of absorbent and two wavelengths were used to measure arsenomolybdate color in the optimum transmission range (20 to 70% T).

4.6. Microbial Population Estimation

Soil samples collected just after harvesting rice were used for counting bacterial populations. One gram of soil sample was taken from a treatment and vortexed for 1 min in 100 mL distilled water in a sterile test tube to make an homogenous mixture. Then, a

dilution series made up to 1×10^{-9} . 100 μL aliquots of each sample (1×10^{-9} dilution series) was spread on petri dishes containing nutrient agar media and incubated at 25°C for 48 h [64]. Finally, bacterial colonies were counted depending on morphologically distinct character (color, size, and shape) as bacterial colony forming unit (CFU), grown in nutrient broth medium with the help of stereo binocular microscope as described in Sarker et al. [65].

4.7. Statistical Analysis

The statistical analysis was done using computer program CoStat v.6.400 [66] and the data were analyzed by one-way analysis of variance (ANOVA) and then differences were compared by Tukey's honestly significant difference (HSD) post-hoc analysis with significance set at $p \leq 0.05$. All the associated graphs were prepared by MS Excel 2016.

5. Conclusions

Our present findings revealed that the application of ZVI had effects on plant growth attributes, soil mineral content, soil HMs, grain As content, and bacterial population in the rhizospheric soils. The ZVI had negative effects on shoot growth of rice plants and dry weight of the root. Application of ZVI had no influence on contents of grain potassium, manganese and zinc. However, the ZVI application increased the content of iron in rice grain. Soil bacterial population was negatively influence by the ZVI, which might be linked with As contents in the cultivated soils. The ZVI utilization in As-contaminated soil had no effect on arsenic content in the soil or on the uptake of arsenic by the grain. The effects of ZVI on plant growth, yield and nutrient uptake in As contaminated areas are needed to be explored elaborately. Therefore, a further study is needed to elucidate the mechanism of As reactions in soil as well as plants by the application of ZVI under field conditions.

Author Contributions: Conceptualization, T.I. and T.W.; methodology, G.K.M.M.R.; experiment conduct and formal analysis, S.A., Z.A., and G.K.M.M.R.; investigation, S.A.; resources, T.I.; data curation, T.I.; writing—original draft preparation, S.A. and M.H.; writing—review and editing, M.H. and T.I.; visualization, S.A. and M.H.; supervision, T.I. and G.K.M.M.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data may be available upon request.

Acknowledgments: We are thankful to Mitsuru Osaki of Graduate School of Agriculture of Hokkaido University, Japan for generously providing the zerovalent Fe used in this research.

Conflicts of Interest: The authors declare no conflict of interest.

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