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Effectiveness of *Bacillus paramycoides* for Improving Zinc Nutrition of Rice Irrigated with Alkali Water

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Abstract: Worldwide zinc deficiency in the soil under cereal production is a common problem affecting the yield and nutritional value of several crops. Bioaugmentation of soil zinc with zinc-solubilizing bacteria can be a promising option for increasing the zinc nutrition to crops. The objectives of the study were to evaluate *Bacillus paramycoides* for improving yield, zinc nutrition, and zinc availability in rice grown under sodicity stress caused by alkali water irrigation. Treatments included T_1: control, T_2: substrate, T_3: *Bacillus paramycoides*, T_4: control (T_1) + zinc sulphate, T_5: substrate (T_2) + zinc sulphate, and T_6: *Bacillus paramycoides* (T_3) + zinc sulphate. Rice yield, zinc content, and uptake, and apparent zinc recovery were not altered by *Bacillus paramycoides*. The different fractions of zinc measured after 30 and 60 days after transplanting of the rice remain unaffected by the inoculation of *Bacillus paramycoides*. Further, an equal number of zinc-solubilizing bacteria present in the rice rhizosphere of control plots after 30 days of transplanting suggests the importance of the native rhizospheric microbiome in zinc nutrition. It is concluded that the application of *Bacillus paramycoides* in sodicity-stressed rice did not provided additional benefits in terms of zinc nutrition and yield. Further investigation will be required to improve the apparent zinc recovery of crops in those areas, where alkali water is continuously utilized for irrigation.

Keywords: rice; Zn fraction; Zn nutrition; Zn-solubilizing bacteria; alkali water irrigation

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1. Introduction

The soil is a vast reservoir of nutrients required for plant growth as well as a vital nutrient source for human beings. Globally, ~50% of soils under cereal cultivation revealed low levels of plant-available zinc (Zn) [1]. In India, ~49% of the soil samples analyzed across the country indicated deficiency (<0.6 mg kg^{-1}) of DTPA Zn [2]. Among various cereal crops, rice is essential internationally as it provides the staple food to more than half of the world's population [3]. Moreover, India is the largest producer of rice in the world with an output of 129 million metric tons in 2021–2022 [4]. Inherently, the Zn content found in rice is too low to meet human requirements [5,6]. Nowadays, increasing Zn content in rice is a priority of researchers worldwide to address Zn malnutrition. The Zn deficiency found in plants is mainly because of its little solubility instead of its low content in soils [7,8]. The bioavailability of Zn in soils is a consequence of its solubility from various fractions (found in water soluble + exchangeable Zn; organically complexed Zn; amorphous sesquioxide Zn; and crystalline sesquioxide-bound Zn) of Zn [9]. The bioavailable Zn in soil solution remains in dynamic equilibrium with these forms. The fertilizer Zn applied to soil also undergoes various transformations in different fractions depending upon its

relative preponderance in native soils [9,10]. Moreover, soil properties (soil texture, soil moisture content, soil reaction, and soil organic matter), quality of irrigation water, types of crops and their genotypes, rhizospheric microbiomes, and root structure influence Zn availability [11].

Groundwater with poor quality has been continuously utilized for irrigation to several crops throughout the world [12]. Low-quality groundwater, especially saline and alkali/sodic groundwater, is widely utilized for irrigation in arid to semi-arid regions [13]. The quality of groundwater in the North–West states (especially in Uttar Pradesh, Haryana, Punjab, and Rajasthan) of India is mostly alkaline in nature [14]. Consequently, irrigation with groundwater having saline and alkali/sodic nature increases land degradation and degrades crop productivity and agro-ecosystems drastically [15]. Further, the soils of the Indo-Gangetic plains undergoing sodification through alkali water irrigation restrict root development due to soil compaction on drying. An anoxic environment under wetting conditions was more critical for crop Zn nutrition [16].

Addressing Zn nutrition of the plant–human chain through genetic approaches like developing cultivars with better Zn acquisition and partitioning in plant parts suitable for human consumption is a long-term process [17]. However, agronomic approaches can be an effective short-term solution by improving nutrient acquisition and facilitating transport from the soil solution/matrix to the plant. Although, the addition of Zn through fertilizer is an extensively accepted practice by farmers to counteract such types of problems. However, the recovery of this applied Zn rarely goes beyond 2% [17,18], and the remaining Zn is transformed into relatively insoluble Zn forms or lost from the soils through erosion and surface runoff [19]. Utilization of the rhizospheric Zn-solubilizing microbes, with the established potential of solubilizing insoluble Zn pools of soil, can be a pragmatic complementary strategy for improving Zn nutrition to several crops [20]. The Zn-solubilizing bacteria (ZnSB) can solubilize insoluble Zn by reducing the rhizospheric pH by producing organic anions. The changes in root rhizospheric pH as well as organic acid secretion support Zn mobilization [21]. It is facilitated by a decrease in the sorption of Zn ions by changing the surface chemistry of soil colloids and desorption of Zn from the sorption sites [22]. The exudate secretions from the root rhizosphere also facilitate the mineralization of organically complexed as well as bound Zn [23] and also solubilize the recalcitrant sources of Zn. Alkaline soils under rice cultivation are especially plagued with Zn deficiency throughout Asia [24,25]. Several reports endorse the use of ZnSB for improving Zn content in plants [20,26–29]. However, most of the studies have been performed in normal soils using pot culture/controlled conditions. Moreover, the evaluation of ZnSB is completely missing regarding Zn nutrition and the apparent recovery of Zn in soils going under sodification with alkali water irrigation. Therefore, this investigation was carried out under field conditions to (i) assess the effectiveness of *Bacillus paramycoides* on the yield of sodicity-stressed rice, content as well as uptake of Zn, and apparent Zn recovery, and (ii) study the effectiveness of *Bacillus paramycoides* on various fractions of Zn during growth as well as after rice harvesting.

2. Materials and Methods

2.1. Collection of Rhizospheric Soil and Isolation of Zinc-Solubilizing Bacteria

Rhizospheric soils were collected from (latitude—29°42′27″; longitude—76°57′07″) wheat plants grown under sodic soils (soil texture: sandy loam; soil pH_2: 9.3 ± 0.02; EC_2: 1.24 ± 0.01 dS m^{-1}; available nitrogen: 160 ± 3.1 kg ha^{-1}; available phosphorous: 14.6 ± 0.8 kg ha^{-1}; available potassium: 225 ± 4.2 kg ha^{-1}; and DTPA Zn: 2.34 ± 0.06 mg kg^{-1}). Bacterial isolation was carried out from the collected rhizospheric soil samples on Pikovskaya (modified) agar medium (agar = 20.0 g; glucose = 10.0 g; yeast extract = 5.0 g; K_2HPO_4 = 2.0 g; $(NH_4)_2SO_4$ = 1.0 g; KCl = 0.20 g; and $MgSO_4 \cdot 7H_2O$ = 0.10 g were added in 1.0 L of distilled water having a pH of 9.0) [30]. Again, 0.1% ZnO (*w*/*v*) was added in the agar medium. The bacterial culture was identified through the 16 S rDNA technique, and based on the nucleotide homology as well as phylogenetic analysis, this culture showed

high similarity with *Bacillus paramycoides*. More details of the molecular identification and biochemical characterization of these bacterial isolates (*Bacillus paramycoides* strain−1) are mentioned in the previous study [21].

2.2. In Vitro Zn Solubilization of Bacillus paramycoides

The zinc solubilization potential of the culture was studied in a Pikovskaya (modified) agar medium. Freshly grown bacterial isolates were spot inoculated on the Pikovskaya (modified) agar medium by sterilized toothpicks using a laminar air flow cabinet. The spot-inoculated petri plates (diameter: 90 mm) were incubated at 28 ± 2 °C for 48 h to find clear halo zone formation around the bacterial colonies. The size of the diameter of the halo zone around the colonies and the size of the diameter of the colonies were recorded. The Zn-solubilizing efficiency (ZSE) of bacterial isolates was calculated using the following formula [31]:

$$\mathrm{ZSE}(\%) = \frac{[\mathrm{diameterofthehalozone\ (mm)}]}{\mathrm{diameterofthecolony\ (mm)}} \times 100$$

Moreover, Zn solubilization can be quantified by the bacterial culture in a liquid medium. In brief, 50 mL of Pikovskaya (modified) broth (glucose = 10.0 g; yeast extract = 5.0 g; K_2HPO_4 = 2.0 g; $(NH_4)_2SO_4$ = 1.0 g; KCl = 0.20 g; and $MgSO_4 \cdot 7H_2O$ = 0.10 g were added in 1.0 L of distilled water having a pH 9.0). Again, 0.1% zinc oxide (*w*/*v*) was added in the broth. Further, broth was added in an Erlenmeyer flask of 100 mL capacity. After broth sterilization, a 1 mL aliquot of bacterial culture having 10^8 cfu (colony forming units) mL^{-1} was added in the flask and kept (at 28 ± 2 °C) in an orbital shaking incubator at 120 rpm. After ten days of incubation, the broth was centrifuged for 10 min at 6100 rpm; subsequently, the supernatant was filtered through Whatman No. 42 filter paper. Zn concentration in the supernatant was measured by an atomic absorption spectrophotometer (AAS) (ZEENIT 700P, Analytik Jena, Rostock, Germany). The production of organic acid in the broth was measured by a titration method with 0.1 N NaOH taking the phenolphthalein indicator [23]. Further, the pH of the supernatant was measured by a pH meter (Eutech Instruments, pH 510, Singapore, Singapore).

2.3. Field Experiment

Bacillus paramycoides samples were evaluated at two different field sites during the *Kharif* seasons of 2020 at Jodhpur (site-I, latitude—30°5′31.9″, longitude—76°32′39.1″, and area: 8000 M^2) and Budhmore (site-II, latitude—30°5′10″, longitude—76°31′45″, and area: 4000 M^2) village, Patiala, Punjab (Figure 1). The groundwaters of these areas are alkaline in nature and continuously utilized for the irrigation of rice–wheat systems.

The soils belong to loamy textures which were being sodicated through alkali groundwater irrigation (RSC (residual sodium carbonate), SAR (sodium adsorption ratio), and EC (electrical conductivity) as 4.2 me L^{-1}, 8.2, and 0.92 dS m^{-1}, respectively at site-I, whereas the values found were 3.5 me L^{-1}, 10.6, and 0.89 dS m^{-1}, respectively at site-II). Comparatively, site-I was more alkaline in nature and the groundwater utilized for irrigation had more RSC as compared to site-II. Moreover, the farmers of site-II applied gypsum amendments to counteract the sodicity stress created by alkali water irrigation. For several years, the land use of both sites was rice–wheat systems. Treatments included T_1: control (substrate as well as *Bacillus paramycoides* was not inoculated (other agronomic management practices remained same)), T_2: substrate (quantity (farm yard manure: 100 kg and jaggery: 1 kg for one-acre area) required for *Bacillus paramycoides* inoculation in T_3 treatment), T_3: *Bacillus paramycoides*, T_4: control (T_1) + zinc sulphate ($ZnSO_4 \cdot 7H_2O$) (5 kg Zn ha^{-1}: soil application), T_5: substrate (T_2) + zinc sulphate, and T_6: *Bacillus paramycoides* (T_3) + zinc sulphate. The above-mentioned six treatments were evaluated at site-I, while the first three treatments were assessed at site-II. Rice varieties PR-126 (short duration) and CSR 30 Basmati (long duration) were cultivated at site-I and site-II, respectively. Recommended doses of fertilizers (NPK = 150:60:60 and 80:60:00 kg ha^{-1} for PR-126 and CSR 30, respectively) and other recommended agronomic packages of practices were adopted for the respective

rice varieties. The rice varieties PR-126 and CSR 30 were transplanted on 27 June and 4 July, 2020, respectively, and harvesting was conducted on 12 October and 9 November, 2020, respectively. Based on the laboratory results, the bacterial culture was tested in rice under field conditions. For bacterial culture preparation, the loop full of culture was inoculated into the broth and kept at 28 ± 2 °C in an orbital shaker for 24 h at 120 rpm. This freshly grown culture was mixed with autoclaved farm yard manure (carrier) and kept for 24 h under sterilized conditions. The inoculated carrier was diluted and spread on nutrient agar medium and incubated at 28 ± 2 °C. This carrier showed 42 × 10^7 cfu g^{-1} after 24 h. This carrier (5 kg) was mixed into 100 kg FYM + 1 kg jaggery (substrate) for a one-acre area, kept overnight, and applied to soils the same day before the transplanting of rice seedlings.

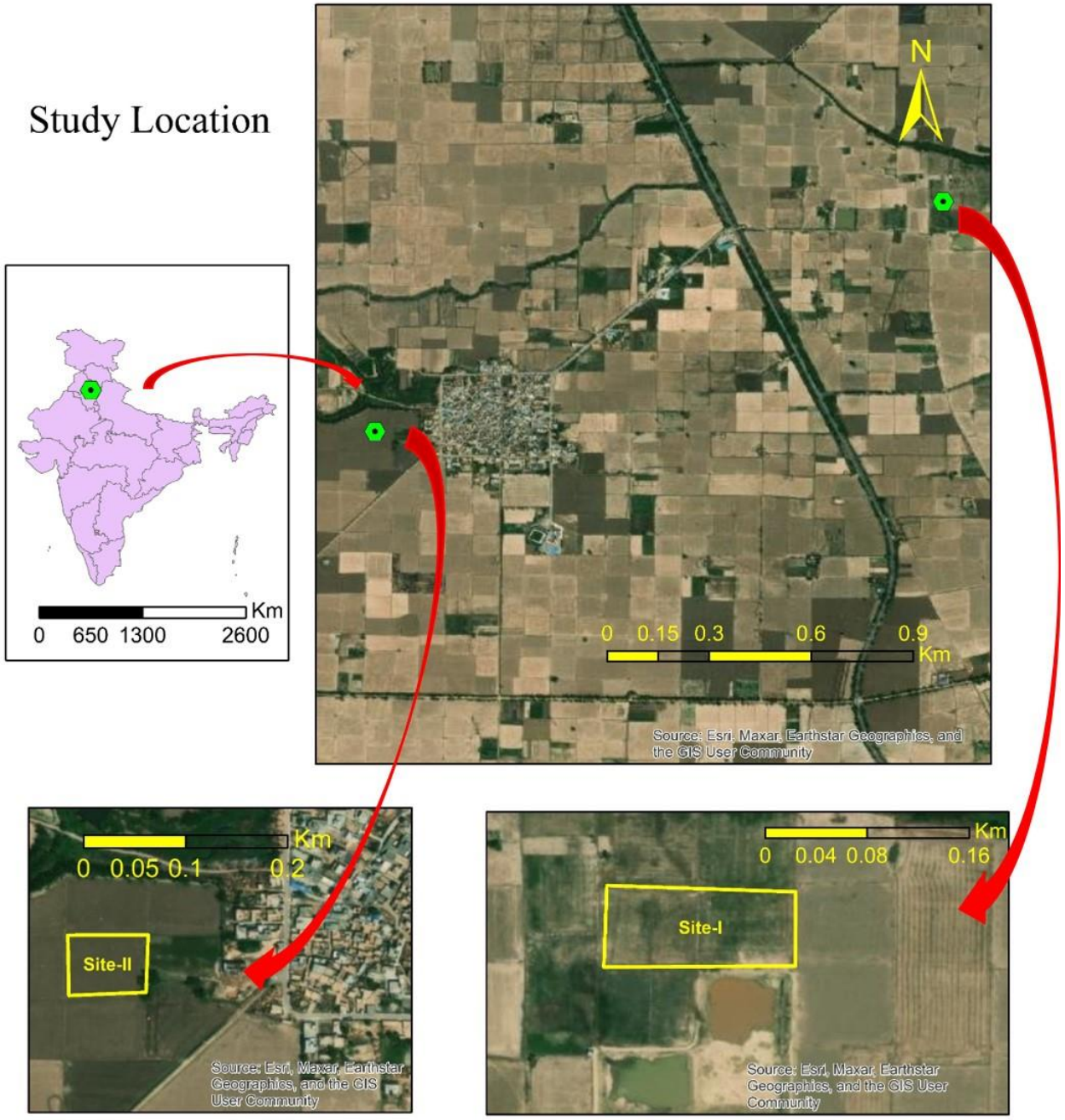

Figure 1. Location of field (experimental) sites.

2.4. Rice Yield

The biological yield was measured after manually harvesting the rice at physiological maturity. After recording the fresh weight, the whole plants were air dried and subsequently, the plants were threshed and grain yields were recorded. After that, samples of the grains were dried at 60 °C to a constant weight using a hot air oven. Grain yield was adjusted to 14% moisture content for minimizing the error arising due to variation in the moisture content of grains. Straw yield was presented after being sundried.

2.5. Zinc Content, Zinc Uptake, and Apparent Zinc Recovery

Grain and straw samples were processed (dried and ground) and consequently digested with the HNO_3 and $HClO_4$ (di-acid mixture prepared in 3:1 ratio) using the hot plate technique [32]. After making the digest volumes with double distilled water, the samples were filtered. Zn concentration was determined using AAS (ZEENIT 700P, Analytic Jena, Rostock, Germany). Zn uptake and apparent Zn recovery are calculated as

$$\text{Zn uptake in grain } \left(\text{gha}^{-1}\right) = \frac{\left[\text{Zn concentration in grain } \left(\text{mgkg}^{-1}\right) \times \text{Grain yield } \left(\text{kgha}^{-1}\right)\right]}{1000}$$

$$\text{Apparent Zn Recovery } (\%) = \frac{\left[\text{Total Zn uptake from fertilized plot } \left(\text{kgha}^{-1}\right) - \text{Total Zn uptake from unfertilized plot } \left(\text{kgha}^{-1}\right)\right]}{\text{Amount of Zn applied through fertilizer } \left(5\text{kgha}^{-1}\right)} \times 100$$

2.6. Analysis of Zinc Fractionation and Other Soil Properties

The Zn fractionation (water soluble + exchangeable Zn (targeted in 1st step), organically complexed Zn (targeted in 2nd step), amorphous sesquioxide-bound Zn (targeted in 3rd step), and crystalline sesquioxide-bound Zn (targeted in 4th step of sequential fractionation)) study in soil was conducted at 30 and 60 days after transplanting (DAT) and after rice harvesting following the standard method [9,10]. In this method, 5 g soil was sequentially extracted with different reagents (Figure 2) and determined on AAS.

The initial properties of soils and standard procedures used for their determination are mentioned in Table 1.

Table 1. Physical and chemical properties of soils before transplanting of rice at both sites.

Parameters	Unit	Site-I	Site-II	Methodology Followed
pH_2		9.2	8.3	[33]
EC_e	dS m^{-1}	2.9	0.83	
CEC	[cmol (p+) kg^{-1}]	15.43	12.30	
ESP	%	36	17	[16]
OC		0.39	0.48	[34]
Available N	(kg ha^{-1})	141.3	184.2	[35]
Available P		19.4	18.9	[36]
Available K		291.1	276.8	[37]
DTPA Zn	(mg kg^{-1})	2.57	1.41	[38]
Water soluble plus exchangeable Zn fraction		0.07	0.12	[9,10]
Organically complexed Zn fraction		1.1	0.84	
Amorphous sesquioxide-bound Zn fraction		3.6	2.52	
Crystalline sesquioxide-bound Zn fraction		2.36	3.79	

Note: pH_2 represents the pH of soil:water suspension in 1:2 (w/v) ratio and ECe represents the electrical conductivity of the soil water saturation extract.

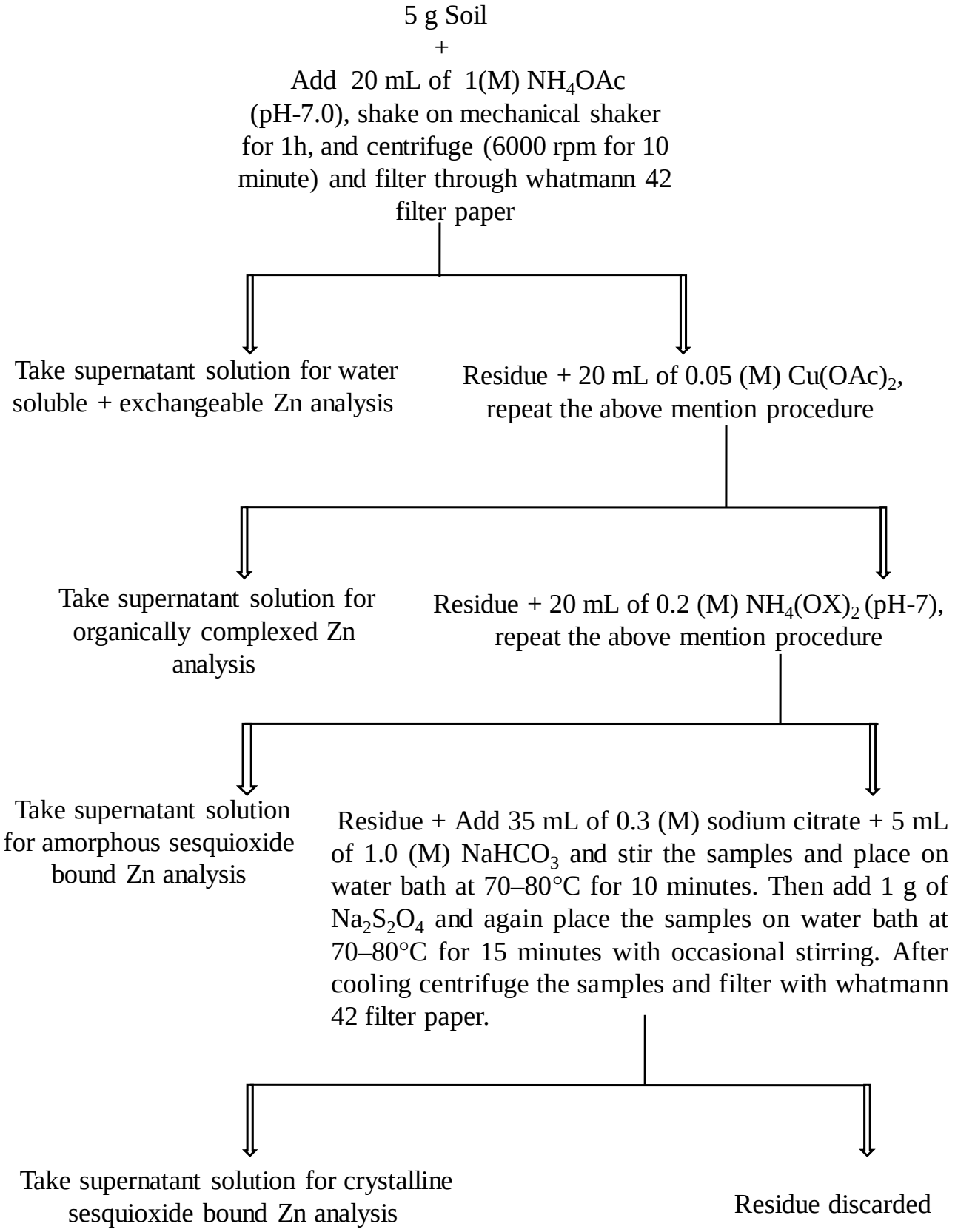

Figure 2. Methodology of Zn fractionation study.

2.7. Enumeration of Zn-Solubilizing Bacterial Population

The rhizospheric soil sample was collected at 30 DAT to enumerate ZnSB. The ZnSB were enumerated through spread plate techniques (one mL of soil suspension from appropriate dilution was used for the study) [39] using a Pikovskaya (modified) agar medium (agar = 20.0 g, glucose = 10.0 g, yeast extract = 5.0 g, K_2HPO_4 = 2.0 g, $(NH_4)_2SO_4$ = 1.0 g, KCl = 0.20 g, and $MgSO_4 \cdot 7H_2O$ = 0.10 g in 1.0 L distilled water having a pH 9.0) [30]. Again, 0.1% ZnO (*w*/*v*) was added in the agar medium. Bacterial colonies making clear halo zones were designated as Zn solubilizers. The culturable ZnSB population was enumerated after incubating the plates at 28 ± 2 °C for 48 h.

2.8. Statistical Analysis

For statistical analysis, one-way analysis of variance (ANOVA) was conducted in randomized complete block design (RCBD) using seven replications on SAS (9.4). Means were separated through Duncan's multiple range test at *p* value of 0.05.

3. Results

3.1. In Vitro Assessment of Bacillus paramycoides

The diameter of Zn solubilization and solubilization efficiency shown by *Bacillus paramycoides* was 14 and 169 mm, respectively. With the inoculation of *Bacillus paramycoides* under the broth assay, Zn solubilization was 228.4 ± 3.6 µg Zn mL^{-1}, while the pH and organic acid in the broth were 5.16 and 33.50 ± 0.5 mol m^{-3}, respectively. Zn solubilization, pH reduction, and organic acid production significantly differed (p = 0.05) with the uninoculated broth (Table 2).

Table 2. Effect of *Bacillus paramycoides* on Zn solubilization efficiency, production of organic acid, and pH of the broth after laboratory incubation for 10 days.

Treatments/Parameters	Diameter of Zn Solubilization	Zn Solubilization Efficiency [a] (ZSE) (%)	Zn Solubilization (µg Zn mL^{-1})	pH	Organic Acid [b] (mol m^3)
Inoculated broth with *Bacillus paramycoides*	14 ± 0.3	169 ± 6.1	228.4 ± 3.6 [a]	5.16 ± 0.0051 [a]	33.50 ± 0.5 [a]
Uninoculated broth	-	-	17.69 ± 0.4 [b]	7.16 ± 0.07 [b]	13.00 ± 0.5 [b]

Note: ±: Depicts the standard error of mean and values indicated by different lower case letters are significantly different at a *p* value of 0.05. [a] Denotes the petri plates were incubated at 28 ± 2 °C for 48 h and [b] denotes the broths were incubated for 10 days at 28 ± 2 °C in an orbital shaking incubator at 120 rpm.

3.2. Yield and Zinc Nutrition

Bacillus paramycoides did not show significant effects on the grain and straw yield of rice over the substrate and control at both sites (Figure 3).

Furthermore, the application of Zn sulphate (T_4, T_5, and T_6) was not effective for enhancing grain and straw yield compared with treatments where Zn sulphate was not applied (T_1, T_2, and T_3). In Zn nutrition, *Bacillus paramycoides* was ineffective in improving Zn content and uptake in grain as well as in straw produced at both sites (Table 3). Apparent Zn recovery was very low and remained unaffected among treatments at site-I. Application of Zn sulphate significantly enhanced the Zn content (21%) and uptake (25%) in grain over treatments where Zn sulphate was not applied. However, Zn content and uptake were very low at both sites.

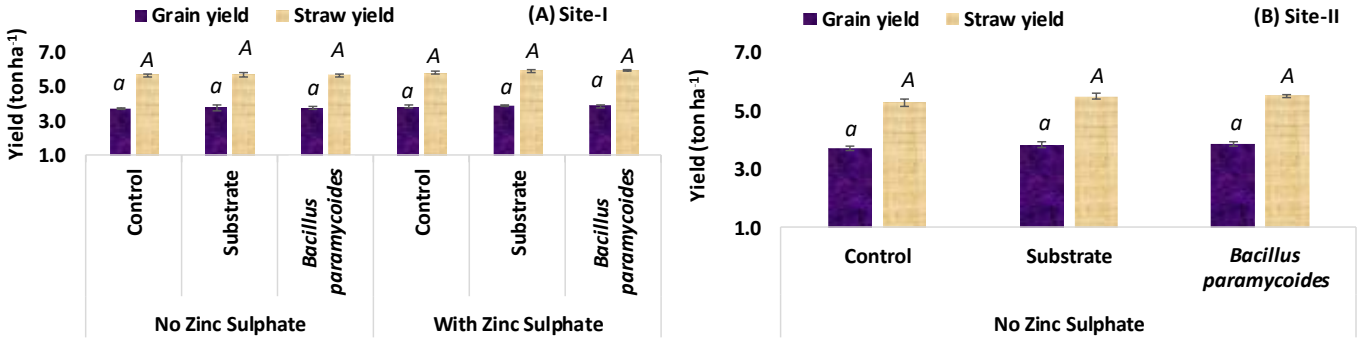

Figure 3. Effect of *Bacillus paramycoides* and zinc sulphate on rice yield at both sites. Note: Error bar depicting standard error of mean. Different lowercase and uppercase letters, presenting grain and straw yield, respectively, mentioned on the values of each of the treatments were significantly different from each other as per DMRT (0.05).

3.3. Zinc Fractionation and Population of ZnSB

Different fractions of Zn were analyzed at 30 and 60 DAT and at rice harvesting (105 and 125 DAT for the PR-126 and CSR 30 varieties, respectively). A significant difference was not observed among treatments of *Bacillus paramycoides*, substrate, and control at both sites (Table 4). Zn sulphate application significantly enhanced the water soluble + exchangeable Zn fraction, amorphous sesquioxide-bound Zn fraction, as well as the crystalline sesquioxide-bound Zn fraction at 30 DAT over an unfertilized plot. However, at 60 DAT and at harvesting, the Zn fraction was not significantly affected by Zn fertilization. Organically complexed Zn was not affected by Zn fertilization even at 30 DAT of rice. At site-I, the values of the water soluble + exchangeable Zn fraction, organically complexed Zn fraction, and amorphous sesquioxide-bound Zn fraction were higher at 30 and 60 DAT compared with the Zn values of the respective fractions before rice transplanting; however, the opposite was the case for the crystalline sesquioxide-bound Zn fraction. At site-II, the values of the water soluble + exchangeable Zn fraction, organically complexed Zn fraction, and amorphous sesquioxide-bound Zn fraction were higher at 60 DAT and at harvesting compared with 30 DAT and before transplanting of rice. The Zn content of grain showed a positive relation to water soluble + exchangeable Zn (R^2: 0.8388), organically complexed Zn (R^2: 4398), and amorphous sesquioxide-bound Zn fraction (R^2: 5217), and negative relation to crystalline sesquioxide-bound Zn (R^2: 4289) at site-I (Figure 4A–D). However, at site-II, only the first two fractions (water soluble + exchangeable Zn (R^2: 0.9353) and organically complexed Zn (R^2: 9672)) showed a positive relation to Zn content in grain (Figure 5A–D). The ZnSB population was similar under all the treatments analyzed after 30 DAT at both sites (Figure 6).

Table 3. Effect of *Bacillus paramycoides* and zinc sulphate on zinc content and uptake in rice.

Treatments		Site-I					Site-II			
		Zn Content in Rice Grain (mg kg^{-1})	Zn Content in Rice Straw (mg kg^{-1})	Zn Uptake in Rice Grain (g ha^{-1})	Zn Uptake in Rice Straw (g ha^{-1})	Apparent Zn Recovery (%)	Zn Content in Rice Grain (mg kg^{-1})	Zn Content in Rice Straw (mg kg^{-1})	Zn Uptake in Rice Grain (g ha^{-1})	Zn Uptake in Rice Straw (g ha^{-1})
No Zinc Sulphate	Control	4.30 ± 0.13 ^{b}	28.05 ± 4.52 ^{a}	14.00 ± 0.48 ^{b}	117.2 ± 17.12 ^{a}	-	10.01 ± 1.33 ^{a}	40.06 ± 3.60 ^{a}	32.54 ± 4.06 ^{a}	208.1 ± 16.13 ^{a}
	Substrate	4.51 ± 0.15 ^{b}	29.33 ± 4.02 ^{a}	14.90 ± 0.51 ^{b}	126.8 ± 19.81 ^{a}	-	9.65 ± 0.85 ^{a}	44.97 ± 2.71 ^{a}	32.45 ± 2.91 ^{a}	230.4 ± 15.40 ^{a}
	Bacillus paramycoides	4.48 ± 0.16 ^{b}	30.41 ± 4.19 ^{a}	14.80 ± 0.65 ^{b}	130.5 ± 17.03 ^{a}	-	9.83 ± 1.24 ^{a}	45.09 ± 3.91 ^{a}	33.13 ± 4.18 ^{a}	226.9 ± 18.72 ^{a}
With Zinc Sulphate	Control	5.38 ± 0.23 ^{a}	31.51 ± 3.41 ^{a}	18.20 ± 0.99 ^{a}	137.4 ± 15.10 ^{a}	0.49 ± 0.14 ^{a}	-	-	-	-
	Substrate	5.27 ± 0.22 ^{a}	31.09 ± 3.92 ^{a}	17.89 ± 0.82 ^{a}	140.4 ± 18.23 ^{a}	0.54 ± 0.08 ^{a}	-	-	-	-
	Bacillus paramycoides	5.41 ± 0.30 ^{a}	30.92 ± 4.41 ^{a}	18.35 ± 1.02 ^{a}	137.3 ± 18.23 ^{a}	0.49 ± 0.17 ^{a}	-	-	-	-

Note: ±: Depicts the standard error of mean and values indicated by different lower case letters are significantly different at a *p* value of 0.05.

Table 4. Effect of *Bacillus paramycoides* and zinc sulphate on zinc fractions of soil at different stages of rice.

Treatments		At 30 DAT				At 60 DAT				After Harvesting of Rice			
		Water Soluble + Exchangeable Zn	Organically Complexed Zn	Amorphous Sesquioxide-Bound Zn)	Crystalline Sesquioxide-Bound Zn	Water Soluble + Exchangeable Zn	Organically Complexed Zn	Amorphous Sesquioxide-Bound Zn)	Crystalline Sesquioxide-Bound Zn	Water Soluble + Exchangeable Zn	Organically Complexed Zn	Amorphous Sesquioxide-Bound Zn)	Crystalline Sesquioxide-Bound Zn
Site-I		mg kg^{-1}											
No Zinc Sulphate	Control	0.20 ± 0.02 ^{b}	1.79 ± 0.10 ^{a}	3.91 ± 0.67 ^{b}	1.57 ± 0.28 ^{b}	0.12 ± 0.01 ^{a}	2.15 ± 0.21 ^{a}	5.73 ± 0.41 ^{a}	1.99 ± 0.27 ^{a}	0.17 ± 0.04 ^{a}	1.64 ± 0.09 ^{a}	4.41 ± 0.13 ^{a}	2.02 ± 0.12 ^{a}
	Substrate	0.18 ± 0.04 ^{b}	1.80 ± 0.09 ^{a}	3.91 ± 0.76 ^{b}	1.68 ± 0.25 ^{b}	0.13 ± 0.01 ^{a}	2.21 ± 0.21 ^{a}	5.74 ± 0.22 ^{a}	2.05 ± 0.34 ^{a}	0.18 ± 0.04 ^{a}	1.59 ± 0.09 ^{a}	4.29 ± 0.16 ^{a}	2.03 ± 0.13 ^{a}
	Bacillus paramycoides	0.17 ± 0.04 ^{b}	1.79 ± 0.10 ^{a}	3.97 ± 0.68 ^{b}	1.65 ± 0.17 ^{b}	0.11 ± 0.01 ^{a}	2.02 ± 0.10 ^{a}	5.91 ± 0.41 ^{a}	2.04 ± 0.23 ^{a}	0.17 ± 0.03 ^{a}	1.70 ± 0.06 ^{a}	4.30 ± 0.08 ^{a}	1.92 ± 0.22 ^{a}
With Zinc Sulphate	Control	0.28 ± 0.02 ^{a}	1.84 ± 0.08 ^{a}	4.76 ± 0.56 ^{a}	2.33 ± 0.15 ^{a}	0.12 ± 0.00 ^{a}	1.87 ± 0.14 ^{a}	5.83 ± 0.57 ^{a}	2.08 ± 0.29 ^{a}	0.21 ± 0.04 ^{a}	1.81 ± 0.07 ^{a}	4.65 ± 0.20 ^{a}	1.94 ± 0.10 ^{a}
	Substrate	0.31 ± 0.04 ^{a}	1.80 ± 0.09 ^{a}	4.90 ± 0.57 ^{a}	2.21 ± 0.17 ^{a}	0.14 ± 0.02 ^{a}	2.15 ± 0.17 ^{a}	5.65 ± 0.20 ^{a}	2.06 ± 0.30 ^{a}	0.21 ± 0.03 ^{a}	1.71 ± 0.09 ^{a}	4.48 ± 0.21 ^{a}	1.89 ± 0.13 ^{a}
	Bacillus paramycoides	0.30 ± 0.02 ^{a}	1.82 ± 0.14 ^{a}	4.91 ± 0.73 ^{a}	2.11 ± 0.06 ^{a}	0.13 ± 0.01 ^{a}	1.92 ± 0.19 ^{a}	5.64 ± 0.65 ^{a}	2.09 ± 0.21 ^{a}	0.20 ± 0.02 ^{a}	1.68 ± 0.06 ^{a}	4.43 ± 0.15 ^{a}	1.94 ± 0.24 ^{a}
Site-II													
No Zinc Sulphate	Control	0.13 ± 0.03 ^{a}	1.04 ± 0.03 ^{a}	2.66 ± 0.08 ^{a}	2.94 ± 0.34 ^{a}	0.18 ± 0.01 ^{a}	1.44 ± 0.13 ^{a}	3.34 ± 0.22 ^{a}	2.68 ± 0.42 ^{a}	0.16 ± 0.05 ^{a}	0.78 ± 0.04 ^{a}	2.50 ± 0.09 ^{a}	2.21 ± 0.18 ^{a}
	Substrate	0.11 ± 0.02 ^{a}	0.98 ± 0.03 ^{a}	2.53 ± 0.19 ^{a}	2.94 ± 0.24 ^{a}	0.18 ± 0.03 ^{a}	1.52 ± 0.17 ^{a}	3.39 ± 0.34 ^{a}	2.56 ± 0.34 ^{a}	0.12 ± 0.03 ^{a}	0.76 ± 0.04 ^{a}	2.50 ± 0.11 ^{a}	2.07 ± 0.08 ^{a}
	Bacillus paramycoides	0.14 ± 0.03 ^{a}	0.99 ± 0.04 ^{a}	2.60 ± 0.05 ^{a}	2.55 ± 0.07 ^{a}	0.16 ± 0.02 ^{a}	1.38 ± 0.16 ^{a}	3.13 ± 0.17 ^{a}	2.42 ± 0.38 ^{a}	0.15 ± 0.02 ^{a}	0.77 ± 0.05 ^{a}	2.43 ± 0.10 ^{a}	1.88 ± 0.19 ^{a}

Note: ±: Depicts the standard error of mean and values indicated by different lower case letters are significantly different at a *p* value of 0.05.

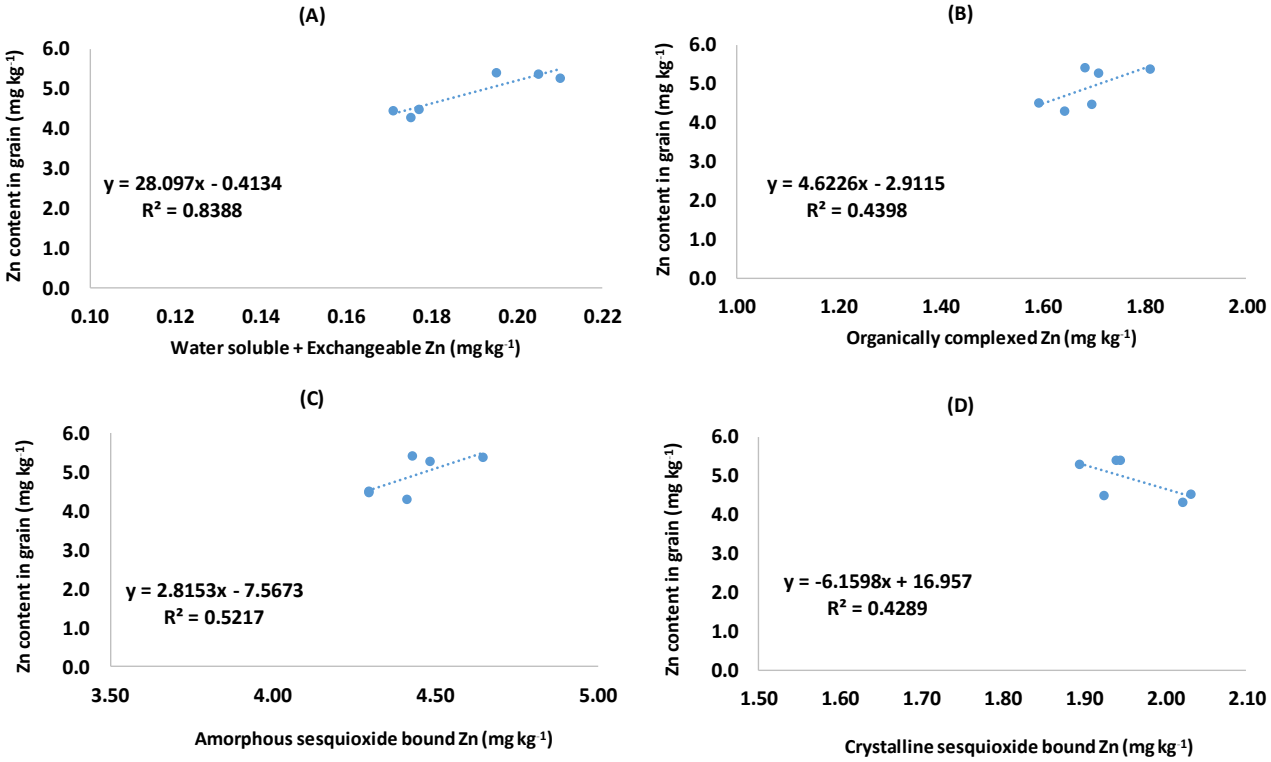

Figure 4. Relationship between Zn content of grain and (**A**) water soluble + exchangeable Zn, (**B**) organically complexed Zn, (**C**) amorphous sesquioxide Zn, and (**D**) crystalline sesquioxide Zn at site-I.

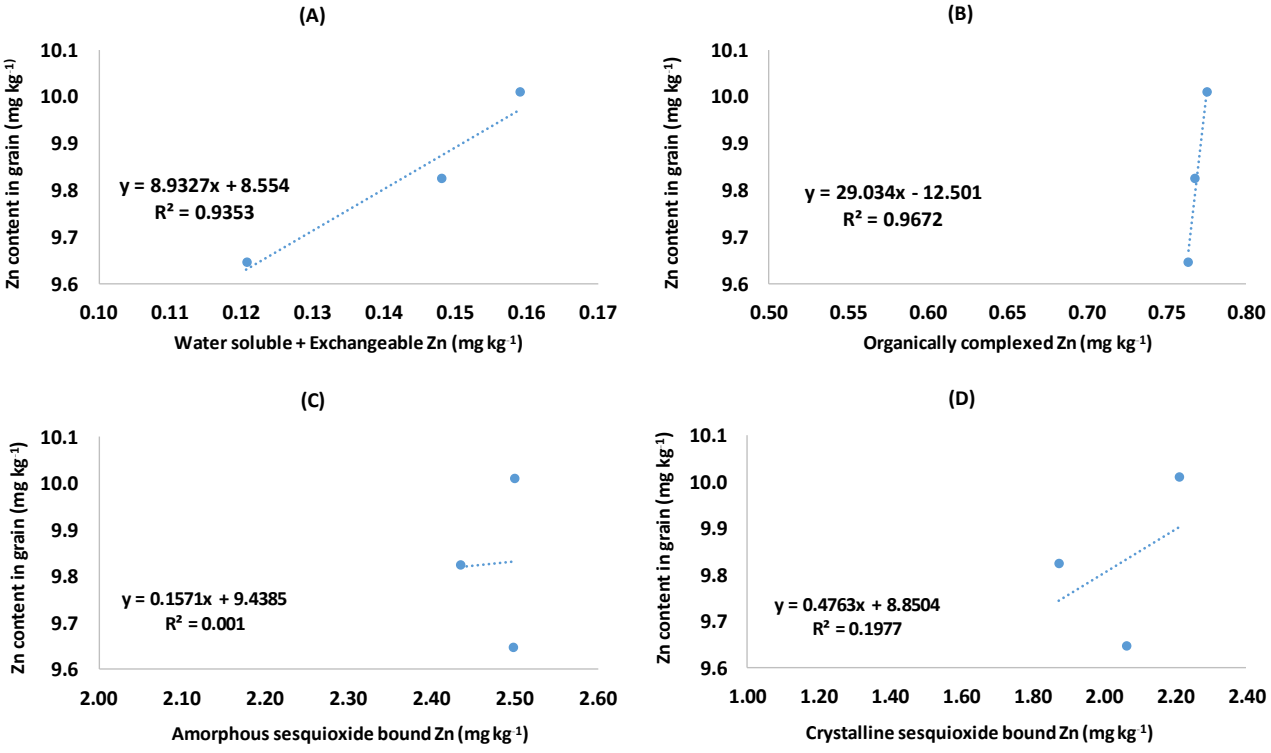

Figure 5. Relationship between Zn content of grain and (**A**) water soluble + exchangeable Zn, (**B**) organically complexed Zn, (**C**) amorphous sesquioxide Zn, and (**D**) crystalline sesquioxide Zn at site-II.

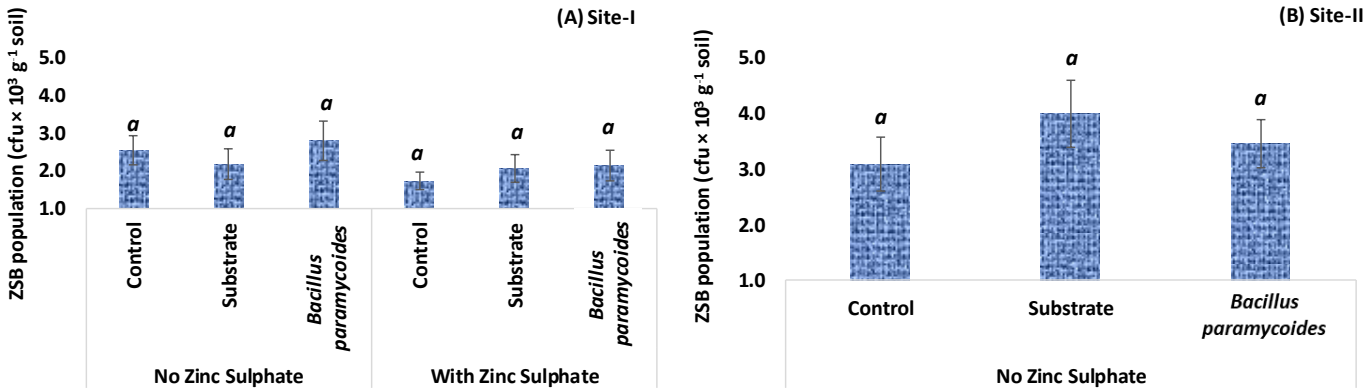

Figure 6. Effect of *Bacillus paramycoides* and zinc sulphate on population of ZnSB after 30 days of planting. Note: Error bar depicts the standard error of mean. Different lowercase letters mentioned on the value of each treatment were significantly different from each other as per DMRT (0.05).

4. Discussion

Bacillus paramycoides solubilized Zn from the ZnO compound in the broth, which was mainly due to a reduced pH and the generation of organic acid in the broth. Microorganisms produce organic acids, which was noticed in the present investigation, as well as exudates with chelating agents. However, the excretion of metabolites, siderophores, and CO_2 evolved during respiration also made significant contributions to Zn solubilization. The importance of all the processes are different and mainly depend upon the growth conditions and nature of the microorganisms [23].

Although many researchers observed the positive effect of Zn-solubilizing strains on crop yield and Zn nutrition in different crops from Asian countries [24,25,28,40–43], most of the studies were conducted using controlled/pot culture conditions. The bacterial isolates used in this study significantly improved Zn nutrition in rice under pot culture using low-zinc soils [21]. However, in this study, rice yield, Zn content, Zn uptake, and apparent Zn recovery were not affected significantly by *Bacillus paramycoides* application. The possible reasons may be (i) a similar amount of Zn solubilized under treatments of substrate and control by a combined and interactive effect of native rhizospheric microflora, soil pH, and soil Eh; and (ii) adaptations of *Bacillus paramycoides* in the rhizospheric microbiome of rice were complex under sodicity stress.

Moreover, the nature and the abundance of the native microbiome of the soil can be a major hurdle to the freshly introduced microbial cells. It is sometimes difficult for newly introduced cells to survive in the stressed conditions, as well as the challenge to compete with the indigenous, better-adapted microbial populations for nutrients under a stressed environment [44].

The application of Zn sulphate enhanced the Zn content and uptake in grain due to a significant increase in the water soluble + exchangeable Zn fraction at 30 DAT in the present investigation. However, at 60 DAT and at harvesting, this fraction of Zn was not altered significantly, potentially due to a practice of water draining from the rice field after 24–48 h of irrigation or after rainfall to prevent crops from the toxic effects of CO_3^{-2} and HCO_3^{-}, which might be the possible reason for the loss of soluble Zn from fields.

DTPA-extracting reagents have proved to be successful in assessing the readily available Zn in upland soils. Still, they may not be so effective in wetland rice soils because of the dynamic and complex environment in such soils [9]. Therefore, different forms of Zn were analyzed at various growth stages of rice and at harvesting. *Bacillus paramycoides* does not alter values of other forms of Zn at any stages, potentially due to a similar number of ZnSB present in rhizospheric soils of all the treatments analyzed after 30 DAT of rice, which suggests that the native microflora in the rice rhizosphere equally contributes to Zn solubilization in other treatments where *Bacillus paramycoides* was not applied. Under Zn fertilization, the increases in the water soluble + exchangeable zinc fractions, amorphous

sesquioxide-bound Zn fractions, and crystalline sesquioxide-bound Zn fractions at 30 DAT were due to the addition of an external source of Zn. However, at later stages, all the fractions were comparable among treatments, which might be due to Zn losses through regular water draining on irrigation/rainfall events. The Zn content in grain showed a better relation to the water soluble + exchangeable Zn fraction as well as the organically complexed Zn fraction, potentially due to a significant contribution of these two fractions for Zn acquisition in plants [45]. An increase in the first three fractions of Zn after 30 and 60 DAT and even at harvesting compared with the initial status of the respective fractions might be due to an alteration in the activity of rhizospheric microbiomes, soil pH, and Eh equilibrium during the crop growth.

5. Conclusions

Bacillus paramycoides has the potential to solubilize zinc under broth culture with a decrease in pH and generation of titratable acidity. However, under field conditions, the application of *Bacillus paramycoides* has no potential to improve rice yield, zinc content, zinc uptake, apparent zinc recovery, and different fractions of zinc in soils continuously irrigated through alkali waters. The application of Zn fertilizer has proved to be beneficial for improving Zn nutrition to rice under sodicity stress created by alkali water. Further investigation will be required to improve the apparent zinc recovery of rice for minimizing zinc losses in those areas, where only alkali water is the source of irrigation. An equal number of zinc-solubilizing bacteria present in the rice rhizosphere suggests the importance of native rhizospheric microbiomes and their dynamics in zinc nutrition to rice. Further investigation will be required to assess whether the external application of zinc-solubilizing bacteria is compatible in the rhizospheric niche under different agro-ecology scenarios and genotypes for zinc biofortification in rice under an alkaline environment.

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