
*International Journal of Plant & Soil Science*
*11(2): 1-12, 2016; Article no.IJPSS.25790*
*ISSN: 2320-7035*

SCIENCEDOMAIN *international*
*www.sciencedomain.org*

# Zinc Solubilizing Potential of *Enterobacter cloacae* Strain ZSB14 in Three Different Semi-arid Tropical Soils

**S. Krithika[1], G. Prasad[1] and D. Balachandar[1*]**

*[1]Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore, 641003, India.*

***Authors' contributions***

*This work was carried out in collaboration between all authors. Author DB designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author GP managed the literature searches and improved the manuscript. Author SK managed the analyses of the study and performed the statistical analysis. All authors read and approved the final manuscript.*

***Article Information***

DOI: 10.9734/IJPSS/2016/25790
Editor(s):
(1) Fatemeh Nejatzadeh, Department of Horticulture, Faculty of Agriculture, Khoy Branch, Islamic Azad University, Iran.
(2) A. Mujib, Department of Botany, Hamdard University, India.
Reviewers:
(1) Aysen Akay, Selcuk University, Turkey.
(2) Fernando José Cebola Lidon, New University of Lisbon, Portugal.
(3) K. Narasimha Swamy, Indian Institute of Rice Research, India.
(4) Rachmawati Hasid, Halu Oleo University, Indonesia.
(5) Regina Teresa Rosim Monteiro, University of Sao Paulo, Brazil.
(6) Anonymous, University of Idaho, USA.
Complete Peer review History: http://sciencedomain.org/review-history/14570

***Original Research Article***

***Received 20th March 2016***
***Accepted 5th May 2016***
***Published 11th May 2016***

## ABSTRACT

Microbial transformation of soil unavailable zinc (Zn) into available form is an important agronomical approach not only to alleviate the Zn deficiency of stable food crops but also to improve the nutritional quality of human diet. Zinc solubilizing bacteria (ZSB) as an inoculant to the crop plants could be a sustainable input for Zn-fertilization as well as for Zn-biofortification. In order to explore these bacteria, we have conducted a microcosm study to assess its potential in terms of Zn availability in three different soils of semi-arid tropics and its interaction effects with different insoluble Zn amendments. The ZSB strain, *Enterobacter cloacae* ZSB14 was inoculated to semi-arid tropical red lateritic, wetland and calcareous soils with or without zinc amendments *viz,* zinc oxide (ZnO), zinc carbonate ($ZnCO_3$) and zinc phosphate ($Zn_3(PO_4)_2$) under controlled condition

**Corresponding author: E-mail: dbalu@tnau.ac.in;*

and assessed for its Zn-releasing potential. The incubation study conducted up to 40 days revealed that the ZSB inoculation alone could not increase the Zn availability significantly in any of the three tested soils and the inoculation should be supplemented with insoluble Zn amendments. The preferred order of Zn amendments for ZSB-bound Zn release in all the three tested soils was $ZnO>ZnCO_3>Zn_3(PO_4)_2$. The results also revealed that the ZSB inoculation with Zn amendment was more effective for increasing the Zn availability of red lateritic (8.57 mg/kg) and wetland (6.35 mg/kg) soils than calcareous soil (4.47 mg/kg). ZSB inoculation alone and with zinc phosphate amendment increased the soil available potassium and phosphorus contents, respectively in all the soils. From the results, it is evident that pH and calcium carbonate contents of the soil are the major drivers of ZSB-bound Zn release in these soils. This study made progress to understand the efficacy of zinc solubilizing bacteria and its interaction with Zn amendments in different semi-arid tropical soils, which could be used as inoculant for Zn-fertilization.

*Keywords: Micronutrient; semi-arid tropical soils; zinc amendments; zinc solubilizing bacteria.*

## 1. INTRODUCTION

Zinc (Zn) is now being considered as the fourth plant nutrient that limits the yield of several staple food crops like rice and maize after nitrogen, phosphorus and potassium, respectively [1]. Nearly 30% of the world agricultural soils are Zn-deficient (<1 mg/kg of available Zn) [2]. Soil zinc deficiency not only reduces the yield but also the grain zinc content in food crops and subsequently causes malnutrition [3]. For example, Zn-deficient wheat stored only 16 mg/kg of Zn in its grain, while Zn-sufficient wheat had >40 mg/kg of grain-Zn [4]. Though the soil inherent characteristics such as alkalinity, calcareous nature and low soil organic carbon (SOC) primarily limit the Zn availability, agricultural intensification and imbalanced nutrient managements further worsen the situation [5]. Application of Zn-fertilizers such as soluble zinc sulphate or sparingly-soluble zinc oxide is the common practice being adopted to correct the Zn deficiency [6]. The applied Zn precipitated immediately in soil as insoluble forms viz, hydroxides, carbonates, phosphates and sulphides as dictated by physico-chemical properties. Since the tropical and sub-tropical soils are relatively low in SOC, further fixation ended with non-exchangeable forms such as metal oxides or clay sorbed minerals. Thus, the Zn-fertilizers had only 1-5% use efficiency for most of the crops [1]. Alternatively, exploring the soil bacteria, able to solubilize the inorganic Zn and thereby increase the bioavailability for crop assimilation, is a viable option to achieve the objective of correcting the Zn deficiency and thereby overcoming the zinc malnutrition in human [7,8].

Several soil bacteria such as *Pseudomonas, Bacillus, Gluconacetobacter, Burkholderia, Acinetobacter, Serratia, Flavobacter* and *Enterobacter* were already reported as zinc solubilizing bacteria (ZSB) [9-13]. They produce variety of low molecular weight organic acids, dissolute Zn from insoluble forms; reduce the pH of the soil solution and thereby increase the plant available zinc ($Zn^{2+}$) [11,14]. Inoculation of these bacteria enhanced the Zn uptake of rice [15], maize [16], wheat [12,17], green gram [14] and soybean [12,18]. Their Zn biofortification potential in grains was also reported in rice [15] and wheat [12,19]. These plant growth promoting bacteria, when colonize the roots of plants, increased the soil available Zn along with other nutrients such as phosphorus and iron non-specifically in the rhizospheric region through acidification, bacterial siderophores and organic acids [20]. Though there are several reports available on zinc solubilizing bacteria [8], its agronomical potential is not yet fully-explored for zinc management of crops.

The semi-arid tropical soils are characterized by low SOC with limited nutrient carrying capacity, especially for nitrogen, phosphorus, sulphur, zinc and boron [21]. As the physico-chemical properties of the soil is most responsible factor decides the Zn availability, it is essential to know how ZSB could effectively release the Zn in these soils to ensure the Zn fertilization. Likewise, few studies revealed that the ZSB-released Zn in the soil was trivial, when no external Zn source was applied along with ZSB [12,13]. Hence it is also important to know whether the ZSB inoculation needs Zn amendment or not for different soil types especially for those nutritionally-poor soils of semi-arid tropics. To address these concerns in order to exploring the ZSB as potential Zn-fertilizing and Zn-fortifying agent for food crops, in the present work, we have assessed the interaction effects of zinc amendments and ZSB inoculant in different semi-arid tropical soils.

## 2. MATERIALS AND METHODS

### 2.1 Bacterial Strain and Culture Condition

*Enterobacter cloacae* strain ZSB14, capable of solubilizing insoluble ZnO, $ZnCO_3$ and $Zn_3(PO_4)_2$, isolated and characterized from rhizosphere of rice [13] was used for this study. The strain was routinely grown in Bunt and Rovira medium containing 0.1% ZnO with and without agar (1.5%) [22] at 30℃ in an incubator (Lab Companion, USA).

### 2.2 Soil Samples

Three different semi-arid tropical soils viz., red lateritic soil, wetland soil and calcareous soil were collected from Tamil Nadu Agricultural University research farms and three independent samples were maintained per soil. Soil samples from 0-30 cm collected from 10 different locations of a field, pooled, removed from stones and stubbles were powdered, packed in water and air tight plastic bags and stored at 4℃ for all the analyses. Soil pH and electrical conductivity were estimated with a glass electrode using a soil to water ratio of 1:1. Soil organic carbon was determined by dichromate oxidation [23]. Soil available N was extracted with 2 M KCl for 1 h and determined by Kjeldahl method [24]. Available P was extracted with Olsen reagent [0.5 M $NaHCO_3$ (pH 8.5)] at soil-extractant ratio of 1:20, shaken for 30 min and quantified by molybdenum – blue colorimetry [25]. Available K was extracted with neutral normal ammonium acetate (pH 7.0), shaken for 25 min and measured by flame photometry [26]. Soil available micronutrients viz, copper (Cu), manganese (Mn), iron (Fe) and zinc (Zn) were analyzed by atomic absorption spectroscopy after extraction with diethylene triamine penta acetic acid (DTPA) [27]. Calcium carbonate ($CaCO_3$) content in the soils was determined by dissolution with 1N hydrochloric acid in a volumetric calcimeter calibrated against analytical $CaCO_3$ [28]. The soil microbial biomass carbon (MBC) was quantified by fumigation extraction method [29]. The physico-chemical properties of soils and geographical coordinates of soil collection sites were presented as Table 1.

### 2.3 ZSB Inoculum

The strain ZSB14 was cultivated in one litre of Bunt and Rovira medium supplemented with ZnO to achieve a final Zn concentration of 0.1% at 30℃ in an incubator till reached a final concentration of approx. $10^9$ colony forming units (cfu) per ml. The bacteria were pelletized by centrifugation at 5000 g for 20 min at room temperature and cell pellets were re-suspended in 100 ml of 0.1 x phosphate buffered saline (PBS) and centrifuged. This operation was repeated and afterwards the cell pellets were re-suspended in 100 ml of PBS and measured the cell concentration by serial-dilution and plating on Bunt and Rovira medium.

**Table 1. Physico-chemical properties of soils and geographical coordinates of the soil collection sites used in the present study**

| Physico-chemical properties | Red lateritic soil | Wetland soil | Calcareous soil |
|---|---|---|---|
| Texture | Clay | Clay-loam | loam |
| pH | 5.97 (±0.03)$^a$ | 8.74 (±0.04)$^b$ | 8.81 (±0.03)$^b$ |
| Electrical conductivity ($dSm^{-1}$) | 0.24 (±0.01)$^a$ | 0.23 (±0.01)$^a$ | 0.63 (±0.08)$^b$ |
| Soil organic carbon (%) | 0.65 (±0.12)$^a$ | 0.45 (±0.07)$^a$ | 0.53 (±0.06)$^a$ |
| Available N (mg/kg) | 131.23 (±2.94)$^a$ | 145.30 (±1.87)$^{ab}$ | 151.20 (±4.67)$^{ab}$ |
| Available P (mg/kg) | 15.78 (±0.71)$^a$ | 25.10 (±0.70)$^b$ | 34.17 (±1.07)$^c$ |
| Available K (mg/kg) | 135.78 (±5.77)$^a$ | 198.61 (±16.67)$^c$ | 150.86 (±8.13)$^b$ |
| Available Zn (mg/kg) | 1.73 (±0.02)$^a$ | 1.91 (±0.04)$^a$ | 1.84 (±0.02)$^a$ |
| Available Fe (mg/kg) | 4.43 (±0.05)$^a$ | 2.18 (±0.03)$^b$ | 0.43 (±0.01)$^c$ |
| Available Mn (mg/kg) | 10.33 (±0.20)$^a$ | 6.19 (±0.29)$^b$ | 8.54 (±0.18)$^{ab}$ |
| Available Cu (mg/kg) | 0.46 (±0.00)$^a$ | 1.01 (±0.04)$^a$ | 0.94 (±0.04)$^a$ |
| Calcium carbonate (%) | 4.67 (±0.67)$^a$ | 7.22 (±0.33)$^b$ | 17.67 (±0.88)$^c$ |
| Microbial biomass carbon (mg/g) | 0.18 (±0.01)$^a$ | 0.15 (±0.02)$^a$ | 0.18 (±0.02)$^a$ |
| Geographical coordinates of sampling site | 10.4℃N latitude; 78.82℃E longitude; 102 m altitude | 11.12℃N latitude; 76.99℃E longitude; 426 m altitude | 11.00℃N latitude; 76.93℃E longitude; 426 m altitude |

*Values are mean (±standard error) (n=6) and values followed by the same letter in each row are not significantly different from each other as determined by DMRT (P = 0.05)*

### 2.4 Soil Microcosm Study

Unsterilized soil samples were adjusted to nearly 50% moisture holding capacity with sterile distilled water. The ZSB strains prepared as inoculum was thoroughly mixed with soil with a final concentration of about $10^7$ cfu per g along with three different insoluble zinc sources [zinc oxide (ZnO), zinc carbonate ($ZnCO_3$) and zinc phosphate ($Zn_3(PO_4)_2$)] as amendments at the rate of 0.1% Zn. From this, a quantity of 500 g of the inoculated soil was transferred to sterile containers (HiMedia, India) with perforation for air exchange. Unamended but ZSB inoculated controls were maintained. The same amount of soil (with and without Zn amendments) added with PBS instead of cell suspension was maintained as uninoculated controls. The moisture per cent was maintained during the course of experiment by adding additional sterile deionized water weekly to maintain the original weight of the container. Three replicates were maintained per sample. The inoculation was done in Biological safety cabinet (Nuaire, USA) and the containers were incubated at temperature-controlled incubator (Lab Companion, USA) at 30℃. Soil samples were withdrawn on every 10 days after inoculation and assessed for pH, available zinc, iron, phosphorus, potassium and MBC as described earlier.

### 2.5 Statistical Analysis

All the data were subjected to statistical analysis with software, Microsoft Excel for Windows 2007 add-in with XLSTAT Version 2010.5.05 [30]. Statistically significant differences between the treatments were analyzed using analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) at 5% significance level. To group the soil samples with similar nutrient flux due to ZSB inoculation and Zn amendments, a cluster analysis was performed with Bray-Curtis similarity [31] using PRIMER 7 software (version 7.0.9, Plymouth Routines in Multivariate Ecological Research, v.6.1.13; PRIMER-E, Plymouth, UK). All the environmental data were log-transformed and normalized prior to analysis. To confirm the clustering, non-metric multidimensional scaling (MDS) was performed to ordinate the similarity data. Besides, one-way analysis of similarities (ANOSIM) test was also conducted to detect the significant difference between the clustered groups.

## 3. RESULTS

### 3.1 Soil pH

Addition of Zn amendments influenced the pH of all the three soils, but not due to ZSB inoculation. The ZnO, $ZnCO_3$ and $Zn_3(PO_4)_2$ increased the pH of red lateritic soil from 5.70 to 6.13, 6.33 and 5.93, respectively immediately after amended ($0^{th}$ day) (Fig. 1A, D, G). In wetland soil, the pH was raised from 8.03 to 8.23, 8.23 and 8.06 for the above amendments (Fig. 1B, E, H) and for calcareous soil, the values were 8.56, 8.5 and 8.03, respectively for those Zn amendments (Fig. 1C, F, I). The soil pH raised after the Zn amendment had little flex (±0.30) throughout the experimental period. All the ZSB inoculated controls maintained their inherent pH for all the three soils assessed.

### 3.2 Soil Available Zinc

The available Zn (DTPA-Zn) content of three soils tested was significantly increased due to ZSB inoculation with Zn amendments. The red lateritic soil recorded the mean available Zn level of 8.57 mg/kg, while the wetland and calcareous soil recorded 6.35 mg/kg and 4.47 mg/kg, respectively for ZSB + Zn amendment after 40 days of incubation. In red lateritic soil, ZnO and $ZnCO_3$ facilitated mean ZSB-mediated Zn levels of 9.04 and 9.02 mg/kg, respectively while for $Zn_3(PO_4)_2$, it was 7.63 mg/kg. The unamended but ZSB inoculated treatment recorded a mean Zn of 1.86 mg/kg and the Zn-amended, but uninoculated control recorded a mean of 1.62 mg/kg (Fig. 2A, D, G).

In wetland soil, the mean available Zn was increased from a base level of 1.56 mg/kg to 6.83, 6.89, 5.34 and 2.13 mg/kg for ZnO, $ZnCO_3$, $Zn_3(PO_4)_2$ and unamended control, respectively when incubated along with ZSB inoculation. The Zn-amended uninoculated control recorded a mean value of 1.83 mg/kg DTPA-Zn in this soil (Fig. 2B, E, H). In calcareous soil, the addition of ZnO, $ZnCO_3$, $Zn_3(PO_4)_2$ enhanced the mean available Zn levels of 4.87, 4.31 and 4.24 mg/kg, respectively for ZSB inoculation. The unamended ZSB inoculated and Zn-amended but uninoculated controls recorded insignificant mean Zn concentrations of 1.70 and 1.83 mg/kg, respectively (Fig. 2C, F, I). Irrespective of soil types, addition of ZnO or $ZnCO_3$ gradually increased the Zn levels up to 20 days and then declined. Whereas for zinc phosphate amendment, the soils reached maximum Zn concentration on $10^{th}$ day, which were lower than

ZnO and $ZnCO_3$ and maintained the same level till the experimental period (Fig. 2).

### 3.3 Soil Available Phosphorus

Soil available P was significantly influenced by Zn amendments, with added effect of ZSB inoculation. The addition of zinc phosphate with or without ZSB inoculation significantly increased the mean available P of all the three soils than zinc oxide and carbonate (Fig. 3). The ZSB inoculated and zinc phosphate amended soil recorded 146.25 mg/kg of mean available P in red laterite soil, which was 354% more than unamended control (Fig. 3G). The same treatment recorded 177.14 mg/kg and 143.65 mg/kg of mean available P for wetland and calcareous soils, respectively, which were 211 and 298% higher than their respective unamended controls (Fig. 3H and Fig. 3I). Zinc phosphate amended but unioculated controls also recorded significantly higher mean available P in all the three soils (113.57, 124.54 and 127.03 mg/kg for red lateritic, wetland and calcareous soil, respectively) than their respective unamended controls. ZnO and $ZnCO_3$ amended soils had more significant influence of ZSB inoculation than Zn amendments. Unamended but ZSB inoculated red lateritic and wetland soils recorded a mean percent increase of 41.9 and 96.5% than control. However, in calcareous soil, both Zn amendments (ZnO and $ZnCO_3$) and ZSB inoculation had no effect for available P. An average of 10-25% less available P was recorded in those treatments than their respective controls.

### 3.4 Soil Available Potassium

ZSB inoculation with or without Zn amendments significantly increased the available K content of all the soils tested (Fig. 4). In red lateritic soil, the mean available K was increased from 147 mg/kg to 175.38 and 170.50 mg/kg due to ZSB + Zn and ZSB alone, respectively (Fig. 4A, D, G). In wetland soil, for the same treatments, the mean available K contents were 252.79 and 247.79 mg/kg, respectively (Fig. 4B, E, H). ZSB+Zn amendment increased the mean available K to 255.11 mg/kg and ZSB inoculation alone reported to 268.19 mg/kg in calcareous soil (Fig. 4C, F, I).

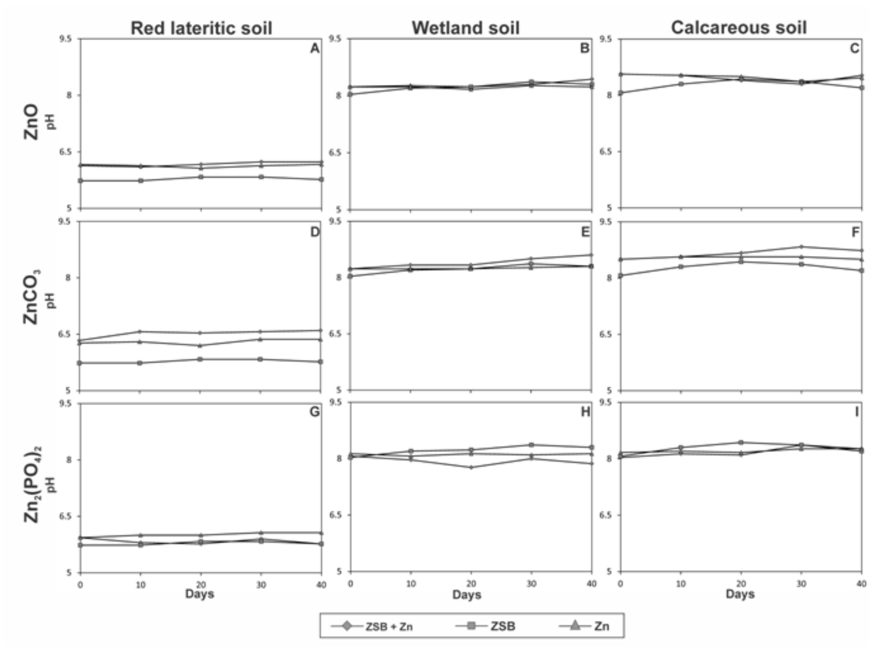


**Fig. 1. Interaction effect of ZSB inoculation and Zn amendments on changes in pH of red lateritic soil (A,D,G), wetland soil (B,E,H) and calcareous soil (C,F,I). The Zn amendments viz., ZnO, $ZnCO_3$, $Zn_3(PO_4)_2$ applied at 0.1% Zn equivalent in respective treatment. Means of three replicate values plotted, errors bars indicate the standard error. (♦) ZSB inoculation with respective Zn amendment; (■) ZSB inoculation alone; (▲) Zn amendment alone**

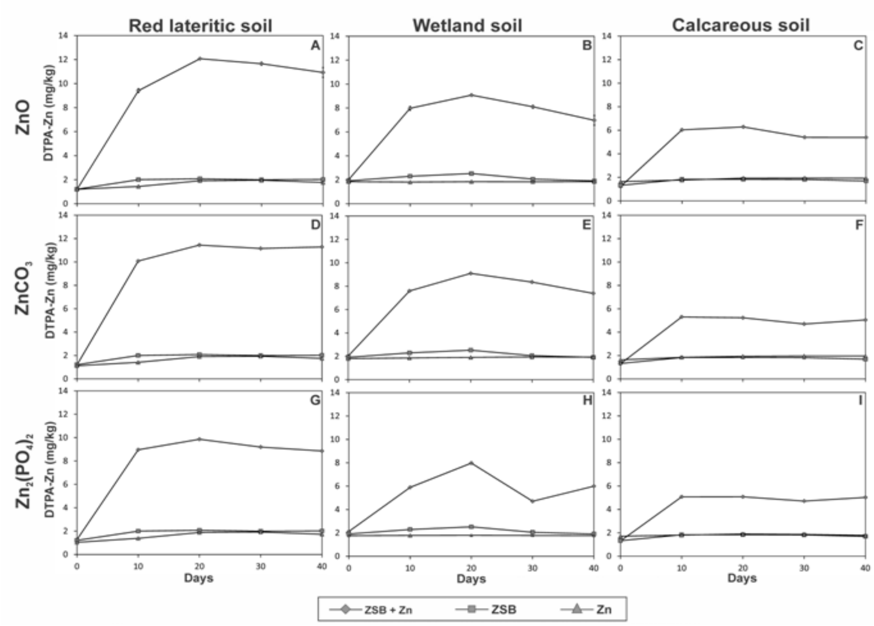


**Fig. 2. Interaction effect of ZSB inoculation and Zn amendments on changes in DTPA extractable Zn content of red lateritic soil (A,D,G), wetland soil (B,E,H) and calcareous soil (C,F,I). The Zn amendments viz., ZnO, $ZnCO_3$, $Zn_3(PO_4)_2$ applied at 0.1% Zn equivalent in respective treatment. Means of three replicate values plotted, errors bars indicate the standard error. (♦) ZSB inoculation with respective Zn amendment; (■) ZSB inoculation alone; (▲) Zn amendment alone**

The Zn amendments alone did not cause any significant effect on available K content of soils. In combination with ZSB, no significant difference among the Zn amendments was found in any of the soil for increase of available K. In red laterite, available K level reached its maximum on $10^{th}$ day itself due to ZSB inoculation and maintained the same up to 40 days of incubation. However, in wetland soils, the maximum K was reached on $20^{th}$ day and for calcareous soil, it was on $30^{th}$ day for the same treatment.

### 3.5 Soil Microbial Biomass Carbon

ZSB inoculation with or without Zn amendment increased the soil microbial biomass carbon significantly than their unioculated controls in all the tested soils (Fig. 5). Among the three soils, the response of ZSB inoculation in terms of MBC increase was more significant in calcareous soil (Fig. 5C, E, I) than red laterite (Fig. 5A, D, G) and wetland soil (Fig. 5B, E, H). The mean MBC increase in calcareous soil for ZSB and ZSB + Zn treatments were 0.58 and 0.51 mg/g of soil, respectively, while the values for red lateritic and wetland soils were 0.38 and 0.44 mg/g and 0.25 and 0.34 mg/g, respectively. None of the Zn amendments significantly increased the MBC of any of the soils tested. The time course increase of MBC was in positive for red lateritic soil, while in wetland soil, the gradual increase of MBC up to 20 days and then declined in later days. In calcareous soil, the flex in the MBC build up was noticed in time course of incubation.

### 3.6 The Interaction between ZSB and Zn Amendments

The MDS plot relating the soil samples amended with different Zn sources and ZSB inoculation discriminated the samples based on the similarities of assessed soil variables (Fig. 6).

The Kruskal's stress value reported (0.06) for MDS indicates good ordination of samples (less than 0.1) i.e., with little risk of misinterpreting patterns. Likewise, the ANASIM also showed significant difference ($r$ = 0.54; $P$ = 0.0021) among the treatment enforced soil types. The red lateritic soil samples are discriminated from wetland and calcareous soil samples, irrespective of the treatments enforced. The ZnO or $ZnCO_3$ amended and inoculated with ZSB

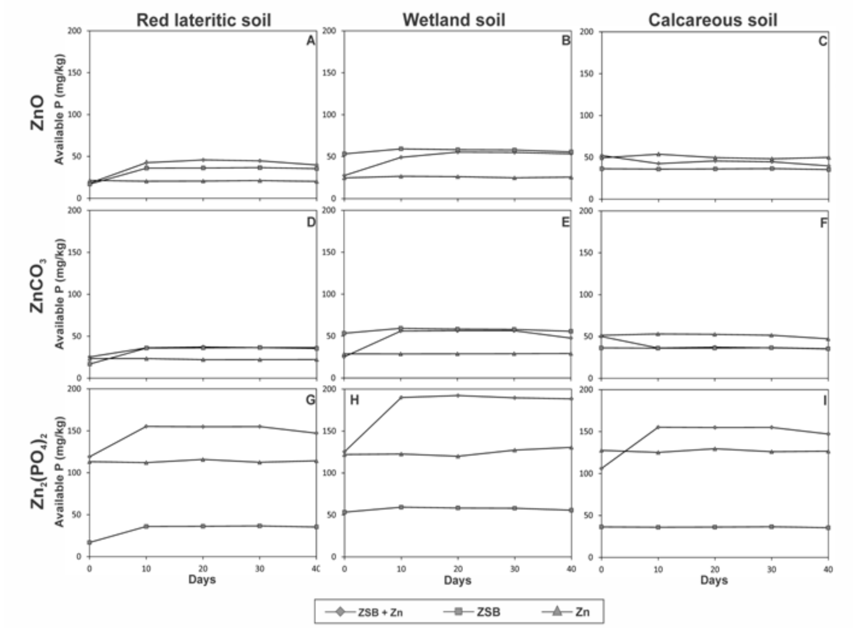


**Fig. 3. Interaction effect of ZSB inoculation and Zn amendments on changes in available phosphorus content of red lateritic soil (A,D,G), wetland soil (B,E,H) and calcareous soil (C,F,I). The Zn amendments viz., ZnO, $ZnCO_3$, $Zn_3(PO_4)_2$ applied at 0.1% Zn equivalent in respective treatment. Means of three replicate values plotted, errors bars indicate the standard error. (♦) ZSB inoculation with respective Zn amendment; (■) ZSB inoculation alone; (▲) Zn amendment alone**

wetland and calcareous soils had more than 90% similarity and tightly clustered in MDS plot. The unamended but inoculated controls of those soils were also in the same group. The ZSB inoculated and ZnO or $ZnCO_3$ amended red lateritic soil samples were discriminated with less than 80% Bray – Curtis similarity and out-grouped with wetland and calcareous soils having the same treatmental effects. The ZnO or $ZnCO_3$ amended but unioculated samples of all the soils were also in the same group revealed that the ZSB inoculation had significant effect on the changes in the observed variables. When the soils were amended with zinc phosphate alone and with ZSB inoculation (ZP and ZP+ZSB), all the three soil types clustered together with 80% similarity in MDS plot. The results clearly revealed that the response of wetland and calcareous soils to ZSB inoculation was different from red lateritic soil and $Zn_3(PO_4)_2$ differed from ZnO and $ZnCO_3$ amendments.

## 4. DISCUSSION

Use of microbial resources to increase the soil Zn availability to the crop plants is one among the sustainable approaches to lessen the Zn deficiency problems as well as to improve the grain-Zn content of major food crops [12,19,20]. Though this microbial-mediated agronomical approach has enough potential for zinc fertilization and fortification, there are several basic informations have to be addressed such as survival and performance of ZSB under different soil types, suitable Zn amendments for effective Zn solubilization, interaction with other nutrients of soil and so on. Through the present work, we reported that the Zn solubilizing potential of ZSB mainly depends on the pH of the semi-arid tropical soils and the bacterial inoculation should essentially be coupled with external Zn amendments for effective release of Zn to the soil solution.

### 4.1 Soil PH Regulates the Zn Solubilizing Potential of Bacterial Strain

The soils used in the present work are predominant to Southern India, in which rice, maize, sorghum, groundnut like crops are being cultivated. The major attributes differed among those soils are pH, available phosphorus, potassium and iron contents. Apart from these, higher level of calcium carbonate was noticed in calcareous soil than wetland and red lateritic soils (Table 1 of the present work). When ZSB was inoculated to these soils with or without Zn amendments, the ZSB-bound Zn release significantly differed due the physico-chemical properties. However, irrespective of soils, ZSB increased the available Zn to a concentration that is in the range of Zn-sufficient condition (3-10 mg/kg; Fig. 2 of the present work) implies that the ZSB strain can perform well in all these soils. Low pH of red lateritic soil favoured ZSB to solubilize and release more Zn than in wetland and calcareous soils (Fig. 1 and 2 of the present work). As Lindsay [32] pointed out, Zn solubility is highly pH-dependent and decreases 100-fold with increase of each unit of PH. The predominant available forms of Zn in soil solution are $Zn^{2+}$ at pH <7.5; Zn (OH) between pH 7.5 and 9.0; $Zn(OH)_2$ at pH 9.1 and above [32]. In general, Zn deficiency is widespread in alkaline calcareous soil [33] and in the present work too, the ZSB inoculation was less-effective to calcareous soil in terms of increase of available Zn. The wetland and calcareous soils used in the present work had similar physico-chemical attributes including pH except the calcium carbonate content. The least Zn release by ZSB in calcareous soil might be due to the excessive calcium carbonate content of calcareous soil which reduces the availability. When excess calcium carbonate is present in the soil, it increases the soil pH; sorbs the Zn on its precipitates and calcium forms calcium zincate and subsequently reduces the availability [1].

### 4.2 ZSB Needs Amendments for Effective Zn Release

Soil Zn is in number of chemical forms with varying solubility which include soluble Zn (water soluble), adsorbed on exchange site (exchangeable), organic matter-bound Zn, secondary mineral zinc precipitates, amorphous sesquioxide-bound Zn and as structural part of primary minerals [34,35]. Water soluble Zn is readily available to plant and is in equilibrium with adsorbed or exchangeable forms controlling the Zn availability by adsorption and desorption reactions [36]. The ZSB inoculants are being developed in the present work or elsewhere with an objective to increase the adsorbed Zn proportions of soil by dissolving the Zn precipitates through organic acid production and subsequently to increase the adsorption-desorption reactions so as to increase the solution Zn. However, the present work and previous reports revealed that the ZSB inoculants could not use the native Zn forms for their dissolution. Inoculation of ZSB (*Bacillus aryabhattai* strains) to soybean and wheat increased the soil available Zn concentrations significantly than uninoculated control, from 0.62 mg/kg to 0.98 and 0.75 mg/kg, respectively. However, the increase of Zn availability due to bacterial inoculant was trivial [12]. In the present study too (Fig. 2), it is obvious that ZSB inoculation alone could not increase the available Zn in any of the soils tested which is in accordance with previous reports [12,13,37]. Since all these semi-arid tropical soils are low in organic carbon, the Zn is transformed into non-exchangeable forms such as secondary mineral precipitates, amorphous sesquioxide bound forms etc [5]. Continuous flooding of wetland soil reduced the redox potential and favoured to precipitate the Zn as ZnS [38]. Hence, it is clear from these findings that the bacterial inoculation alone may not be sufficient to improve the Zn availability, especially those soils suffering with Zn deficiency. Hence, in the present work, we have assessed three Zn amendments [ZnO, $ZnCO_3$ and $Zn_3(PO_4)_2$] to increase the efficiency of ZSB and among them, ZnO and $ZnCO_3$ recorded maximum ZSB-bound Zn release, while the zinc phosphate had least response in all the soils. However, the zinc phosphate amendment also helped ZSB to increase the Zn availability within the range of Zn sufficient conditions in all the three tested soils (5-7 mg/kg of soil). Comparing the three amendments, ZnO is sparingly soluble followed by zinc carbonate and least soluble is zinc phosphate. As the Zn solubilizing efficiency of ZSB is directly proportional to the solubility index of these compounds, it is clear that $ZnO>ZnCO_3>Zn_3(PO_4)_2$ is the order of preferred Zn amendments for effective ZSB-bound Zn release [11,13]. As the ZSB strain used in the present work did not have any marker character to assess its population in soil with time course, we used the soil microbial biomass carbon as indirect measure to monitor its fate. The MBC of the ZSB inoculated soils significantly increased than uninoculated soils and stabilized throughout the experimental period implies that the zinc solubilizing activity is induced significantly by the Zn amendments (Fig. 5 of the present work). The present MBC results also suggest that the dosage of Zn amendments (0.1% Zn equivalent) was toxic to neither the ZSB inoculant nor the native microbial communities of the tested soils. From the present experiment, it is also confirmed that native soil microflora could not use the amended Zn and ZSB inoculation is essential for increasing the availability of Zn in all these semi-arid tropical soils.

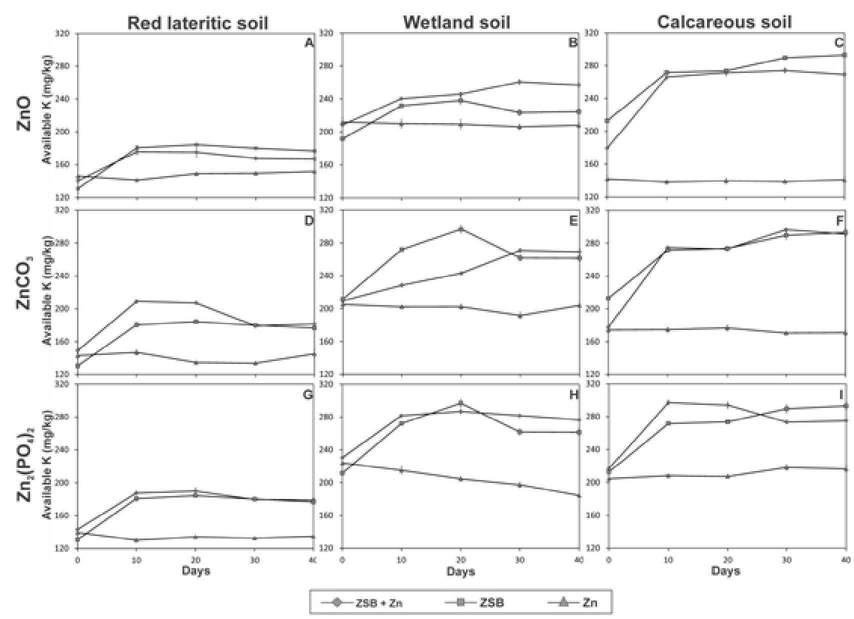


**Fig. 4. Interaction effect of ZSB inoculation and Zn amendments on changes in available potassium content of red lateritic soil (A,D,G), wetland soil (B,E,H) and calcareous soil (C,F,I). The Zn amendments viz., ZnO, $ZnCO_3$, $Zn_3(PO_4)_2$ applied at 0.1% Zn equivalent in respective treatment. Means of three replicate values plotted, errors bars indicate the standard error. (♦) ZSB inoculation with respective Zn amendment; (■) ZSB inoculation alone; (▲) Zn amendment alone**

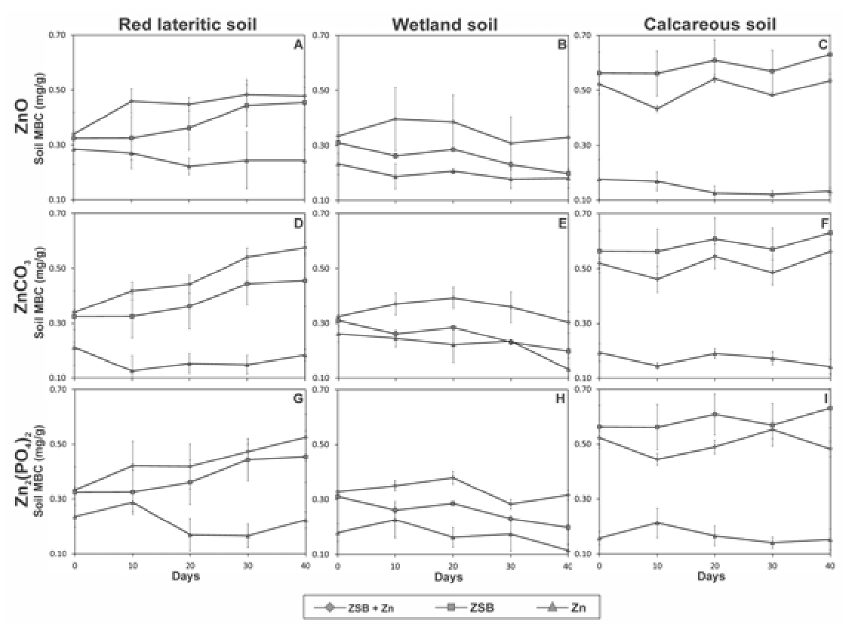


**Fig. 5. Interaction effect of ZSB inoculation and Zn amendments on changes in microbial biomass carbon content of red lateritic soil (A,D,G), wetland soil (B,E,H) and calcareous soil (C,F,I). The Zn amendments viz., ZnO, $ZnCO_3$, $Zn_3(PO_4)_2$ applied at 0.1% Zn equivalent in respective treatment. Means of three replicate values plotted, errors bars indicate the standard error. (♦) ZSB inoculation with respective Zn amendment; (■) ZSB inoculation alone; (▲) Zn amendment alone**

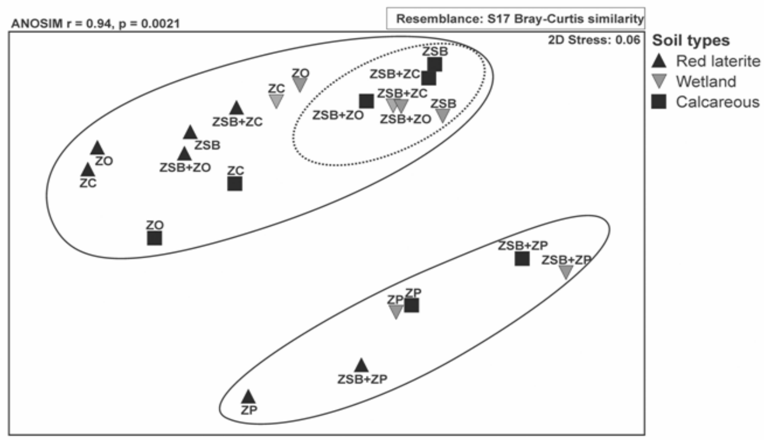


**Fig. 6. Non-metric multidimensional scaling (MDS) ordination of similarity (Bray-Curtis) of three different soils as influenced by ZSB inoculation and Zn amendments. (▲) red lateritic soil; (▼) wetland soil; (■) calcareous soil. ZSB – Zinc solubilizing bacterial inoculation; ZO – Zinc oxide amendment; ZC – Zinc carbonate amendment; ZP – Zinc phosphate amendment. All the assessed soil physico-chemical variables (detailed in materials and methods) were log-transformed and normalized for assessing the similarity. Continuous lined circles engulfing the soil samples based on 80% similarity clustering and dotted line circle was based on grouping with 90% similarity**

### 4.3 ZSB Increases Soil Available Phosphorus and Potassium

The present soil incubation study conducted in three different semi-arid tropical soils revealed that apart from Zn, the availability of phosphorus and potassium contents of the soils was also influenced by ZSB and or Zn amendments. As organic acid production (especially gluconic acid) is the primary mode of action for Zn dissolution, these low-molecular weight acids can non-specifically solubilize phosphorus, potassium, calcium and manganese from their respective minerals or from insoluble precipitates depending upon the physico-chemical properties of the soil [39]. The organic acids can adhere to mineral surface and extract the nutrients non-specifically from the mineral particles through electron transfer; break the oxygen links in the minerals and release the nutrients and chelate ions present in the solution through carboxyl and hydroxyl groups and thereby indirectly accelerating the dissolution rate of minerals [40]. In the present investigation, the ZSB alone could not increase the P availability but can increase K availability implies that the acidity produced by ZSB had the potential to release the native K-minerals but not from P-minerals. However, when zinc phosphate was used as Zn-amendment, ZSB indirectly increased the P availability of all the soils tested and ZnO and $ZnCO_3$ had no role in it. Fe toxicity and low pH of red lateritic soil [41] and high calcium content of calcareous soil [42] are the reasons for relatively low ZSB + zinc phosphate effect in terms of P availability in the present study.

## 5. CONCLUSIONS

This study made progress towards the understanding of microbial mediated Zn availability in three different soils of semi-arid tropics as influenced by their physico-chemical properties. The soil pH and calcium carbonate contents are the major drivers of ZSB-bound Zn availability in those soils. ZSB inoculation could be more effective for Zn-fertilization of crops growing in acidic and wetland soils than those of calcareous soil. We also showed that the ZSB inoculation alone could not dramatically increase the $Zn^{2+}$ in these soils, as it should be coupled with Zn amendments as supplementary to achieve the Zn availability levels that could be effectively uptake by the crop plants. ZSB inoculation with and without Zn amendments also improved the P and K availability of the soils, respectively. The over-all results of the present study suggest that ZSB inoculation with zinc phosphate would be a better alternative for the present Zn-fertilization, which could also increase the phosphorus and potassium availability in all these semi-arid tropical soils as additional benefit.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES


1. Alloway BJ. Zinc in soils and crop nutrition: International zinc association Brussels. Belgium. 2008;135.
2. Hacisalihoglu G, Kochian LV. How do some plants tolerate low levels of soil zinc? mechanisms of zinc efficiency in crop plants. New Phytol. 2003;159(2):341-350.
3. Cakmak I, Kalayci M, Ekiz H, Braun HJ, Kilinc Y, Yilmaz A. Zinc deficiency as a practical problem in plant and human nutrition in Turkey: A NATO-science for stability project. Field Crops Res. 1999; 60(1–2):175-188.
4. Cakmak I. Enrichment of cereal grains with zinc: Agronomic or genetic biofortification? Plant Soil. 2008;302(1-2):1-17.
5. Rehman H-U, Aziz T, Farooq M, Wakeel A, Rengel Z. Zinc nutrition in rice production systems: A review. Plant Soil. 2012;361(1-2):203-226.
6. Rengel Z, Batten GD, Crowley DE. Agronomic approaches for improving the micronutrient density in edible portions of field crops. Field Crops Res. 1999;60(1–2): 27-40.
7. Saravanan VS, Kumar MR, Sa TM. Microbial zinc solubilization and their role on plants, in Maheshwari DK. Editor. bacteria in agrobiology: Plant nutrient management, Springer Berlin, Heidelberg; 2011.
8. Hafeez F, Abaid-Ullah M, Hassan M. Plant growth-promoting rhizobacteria as zinc mobilizers: A promising approach for cereals biofortification, in Maheshwari DK, Saraf M, and Aeron A, Editors bacteria in agrobiology: Crop Productivity, Springer Berlin Heidelberg: Berlin Heidelberg; 2013.
9. Di Simine C, Sayer J, Gadd G. Solubilization of zinc phosphate by a strain of *Pseudomonas fluorescens* isolated from a forest soil. Biol Fert Soils. 1998;28(1): 87-94.
10. Fasim F, Ahmed N, Parsons R, Gadd GM. Solubilization of zinc salts by a bacterium isolated from the air environment of a tannery. FEMS Microbiol Lett. 2002;213(1): 1-6.
11. Saravanan VS, Osborne J, Madhaiyan M, Mathew L, Chung J, Ahn K, Sa T. Zinc metal solubilization by *Gluconacetobacter diazotrophicus* and induction of pleomorphic cells. J Microbiol Biotechnol. 2007;17(9):1477-1482.
12. Ramesh A, Sharma SK, Sharma MP, Yadav N, Joshi OP. Inoculation of zinc solubilizing *Bacillus aryabhattai* strains for improved growth, mobilization and biofortification of zinc in soybean and wheat cultivated in vertisols of central India. Appl Soil Ecol. 2014;73:87-96.
13. Krithika S, Prasad G, Balachandar D. Zinc solubilization potential of bacterial isolates from rice rhizosphere. Geoderma; 2016.
14. Iqbal U, Jamil N, Ali I, Hasnain S. Effect of zinc-phosphate-solubilizing bacterial isolates on growth of *Vigna radiata.* Ann Microbiol. 2010;60(2):243-248.
15. Vaid S, Kumar B, Sharma A, Shukla A, Srivastava P. Effect of zn solubilizing bacteria on growth promotion and Zn nutrition of rice. J Soil Sci Plant Nutr. 2014;14(4):889-910.
16. Goteti PK, Emmanuel LDA, Desai S, Shaik MHA. Prospective zinc solubilising bacteria for enhanced nutrient uptake and growth promotion in maize (*Zea mays* L.). Int J microbiol. 2013;1-7.
17. Rana A, Saharan B, Nain L, Prasanna R, Shivay YS. Enhancing micronutrient uptake and yield of wheat through bacterial PGPR consortia. Soil Sci Plant Nutr. 2012; 58(5):573-582.
18. Sharma SK, Sharma MP, Ramesh A, Joshi OP. Characterization of zinc-solubilizing *Bacillus* isolates and their potential to influence zinc assimilation in soybean seeds. J Microbiol Biotechnol. 2012;22(3): 352-359.
19. Sirohi G, Upadhyay A, Srivastava PS, Srivastava S. PGPR mediated zinc biofertilization of soil and its impact on growth and productivity of wheat. J Soil Sci Plant Nutr. 2015;15(1):202-216.
20. Sessitsch A, Kuffner M, Kidd P, Vangronsveld J, Wenzel WW, Fallmann K, Puschenreiter M. The role of plant-associated bacteria in the mobilization and phytoextraction of trace elements in contaminated soils. Soil Biol Biochem. 2013;60:182-194.
21. Sahrawat KL, Wani SP. Soil Testing as a tool for on-farm fertility management: Experience from the semi-arid zone of India. Commun Soil Sci Plant Anal. 2013; 44(6):1011-1032.

22. Bunt J, Rovira A. Microbiological studies of some subantarctic soils*.* J Soil Sci. 1955; 6(1):119-128.
23. Walkley A, Black IA. An examination of the degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method*.* Soil Sci. 1934;37(1):29-38.
24. Waring SA, Bremner JM. Ammonium production in soil under waterlogged conditions as an index of nitrogen availability*.* Nature. 1964;201(4922):951-952.
25. Olson SR. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. US Department of Agriculture Circular 939: Washington DC, USA; 1954.
26. Hanway JJ, Heidel H. Soil analyses methods as used in Iowa state college soil testing laboratory*.* Iowa Agriculture. 1952; 57:1-31.
27. Lindsay WL, Norvell WA. Development of a DTPA soil test for zinc, iron, manganese and copper*.* Soil Sci Soc Am J. 1978; 42(3):421-428.
28. Nelson R. Carbonate and gypsum, in A. Page, Editor. methods of soil analysis. Part 2 chemical and Microbiological Properties Soil Science Society of America: Madison, WI; 1982.
29. Jenkinson DS, Ladd JN. Microbial biomass in soil, measurement and turn over, in Paul EA, Ladd JN, editors. Soil Biochemistry, Marcel Dekker: New York; 1981.
30. XLSTAT. XLSTAT; 2010, 2010. Available from: http://www.xlstat.com.
31. Clarke KR. Non-parametric multivariate analyses of changes in community structure*.* Aust J Ecol. 1993;18(1):117-143.
32. Lindsay WL. Inorganic equilibria affecting micronutrients in soils in Mortvedt JJ, et al. editor. Micronutrients in Agriculture Soil Science Society of America: Madison, Wisconsin; 1991.
33. Alloway BJ. Soil factors associated with zinc deficiency in crops and humans*.* Environ Geochem Health. 2009;31(5):537-548.
34. Shuman ML. Chemical forms of micronutrients in soil, in Mortvedt JJ, et al. editors. Micronutrients in Agriculture, Soil Science Society of America: Madison, Wisconsin; 1991.
35. Broadley M, Brown P, Çakmak İ, Rengel Z, Zhao F. Marschner's function of nutrients: Micronutrients, in Marschner H, editor. Mineral Nutrients of Higher Plants, Academic Press: New York, US; 2011.
36. Takkar PN, Sidhu BS. Kinetics of zinc transformation in submerged alkaline soils in the rice growing tracts of Punjab*.* J Agric Sci. 1979;93(02):441-447.
37. Sarathambal C, Thangaraju M, Paulraj C, Gomathy M. Assessing the zinc solubilization ability of *Gluconacetobacter diazotrophicus* in maize rhizosphere using labelled $^{65}Zn$ compounds*.* Indian J Microbiol. 2010;50(1):103-109.
38. Izquierdo M, Impa SM, Johnson-Beebout SE, Weiss DJ, Kirk GJD. Measurement of isotopically-exchangeable Zn in Zn-deficient paddy soil*.* Euro J Soil Sci. 2015; 67(1):51-59.
39. Uroz S, Calvaruso C, Turpault M-P, Frey-Klett P. Mineral weathering by bacteria: Ecology, actors and mechanisms*.* Trends Microbiol. 2009;17(8):378-387.
40. Welch SA, Taunton AE, Banfield JF. Effect of microorganisms and microbial metabolites on apatite dissolution*.* Geomicrobiol J. 2002;19(3):343-367.
41. Oelkers EH, Valsami-Jones E. Phosphate mineral reactivity and global ustainability*.* Elements. 2008;4(2):83-87.
42. Von Wandruszka R. Phosphorus retention in calcareous soils and the effect of organic matter on its mobility*.* Geochem T. 2006;7(6):1-8.




*Peer-review history:*
*The peer review history for this paper can be accessed here:*
*http://sciencedomain.org/review-history/14570*