SOIL PHYSICO-CHEMICAL PROPERTIES AND *IN-VITRO*ZINC SOLUBILIZATION OF RHIZO-MICROBES FROM THE DIFFERENT RHIZOSPHERIC SOIL SAMPLES OF HARSH ENVIRONMENT

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Abstract
In our study, we have isolated seventy-five rhizobacteria (Pseudomonas sp., Bacillus sp., Azotobacter sp., and Rhizobium sp.), six fungi (Aspergillus sp.) and actinomycetes (Streptomyces sp. and Nocardia sp.) from the different harsh environmental rhizospheric soil samples of Karnataka, India. Isolates have been screened and estimated for qualitative and quantitative zinc solubilization (incorporated with ZnO, $Zn_3(PO4)_2$ and $ZnCO_3$) by atomic absorption spectrophotometer analysis. Other than, the physico-chemical properties (pH, EC and CHNS analysis) of all soil samples was done. This work was carried out to explain the isolates Bacillus sp. R10A, Bacillus sp. R19E, and Pseudomonas sp. KB9 were able to dissolve 6.124, 6.127, and 6.220 (Zn) mg l^{-1}, respectively and its effect on plant growth. Other qualitative study showed that the isolates could solubilized Zn compounds in plate assay in the range with $Zn_3(PO_4)_2$: (3 -10mm), ZnO (2 - 10mm) and $ZnCO_3$: (3 - 5mm). This study suggested that the Bacillus sp. R10A, Bacillus sp. R19E, and Pseudomonas sp. KB9 has a potential role to play in the management of Zn nutrients in harsh climate.Findings suggest that the existence of this potential Zn solubilizer may play a crucial role in the mobilization of nutrients and the management of plant survival in harsh environments and nutrient deficiency.

Keywords: Rhizo-microbes, soil, physico-chemical analysis, zinc solubilization, AAS.

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INTRODUCTION

Zinc (Zn) is one of the most essential micronutrients required in the range of 5 to 100 mg. kg^{-1} concentrations in tissues for the management of nutrients, growth and reproduction of plants (Samreen *et al.*, 2017; Sharma *et al.*, 2013; Goteti *et al.*, 2013). Zn deficiency in plants leads to reduced membrane integrity, increased rate of many important metabolic reactions and synthesis of carbohydrates, auxins, nucleotides, cytochromes, chlorophyll, (Samreen *et al.*, 2017) and increased susceptibility to heat stress (Dimkpa and Bindraban, 2016; Goteti*et al.*, 2013). The rhizosphere is the place where very crucial and intensive interactions take place between microorganisms, soil, plants, and soil rhizo-microbes, influenced by compounds exuded by roots, and microorganisms feeding on its compounds (Hassan et al., 2019; Dimkpa and Bindraban, 2016; Jacoby et al., 2013; McNear, 2013; Antoun and Prevost, 2006). Inorganic composts are prescribed as a potential wellspring of Zn micronutrient however they are immediately settled on soil, making poor accessibility for plants (Palanog *et al.*, 2019; Hefferon, 2019; Zia *et al.*, 2000). Zinc solubilizing microbes are the most potential alternates for zinc nutrient supplement (Zaheer *et al.*, 2019; Goteti*et al.*, 2013). The application of zinc sulfate ($ZnSO_4$) in the form of bio-fertilizer might increase crop yield and decrease Zn deficiency in the rhizosphere (Hafeez *et al.*, 2013). However, the soil-applied ZnO, $ZnCO_3$, and $ZnSO_4$ transformed into different insoluble forms depending on the type of soil and entirely become unavailable in the environment within few days of processing (Rakshit *et al.*, 2015; Rattan and Shukla, 1991). In rhizospheric soils, up to 90% of connected Zn containing microbial compost or plant growth promoters is adsorbed on soil colloids and hastened (Broadley *et al.*, 2007; Saeed and Fox, 1977). Some fungi (*A.niger*), bacteria

(*Pseudomonas* spp., *Azotobacter* spp., and *Bacillus* spp.) and actinomycetes (*Streptomyces* spp. and *Nocardia* spp.) help in the formation of pigments in Zn deficiency (Chernavina, 1970). In this study, we have shown that twenty-nine rhizo-microbes could contribute to plant growth and the management of nutrient deficiency.

MATERIALS AND METHODS

Soil samples collection

Twenty rhizospheric soil samples were collected from Chikballapura, Bangalore Rural, Bengaluru and Kolar district of Karnataka, India. All soil samples collection sites and plant spp. name are graphically represented by Mitra*et al.*,(2019).

Physico-chemical analysis of soil samples

Theelectrical conductivity(EC) and pH of the suspension (soil) were measured with digital pH meter (EUTECH-250, India) followed by the Kasa*et al.*, 2015. Essential examinations of aggregate carbon, hydrogen, nitrogen, and sulfur is performed to give carbonate, natural carbon and to get some thought of the creation of the dirt samples. For the CHNS analysis (Elementer-CUBE), freeze-dried and crushed soil samples were weighed (5-10 mg) and mixed with an oxidizer [vanadium pentoxide (V_2O_5)] in a tin capsule, which is then combusted in a reactor at 1000°C and quantified with a TCD (Meyers, 1994). The results of the CHNS analysis are recorded.

Isolation of microbes

The soil stuck around the roots was removed by washing the roots and diluted soil samples were inoculated on different media plates (Table 1) for the isolation ofplant growth promoting(PGP)microorganisms (Islam*et al.*, 2015; Mitra *et al.*, 2019; Devi *et al.*, 2016; Islam*et al.*, 2015). The selected cultures were incubated for 24h at 30^0C. The pure cultures were stored at -20^0C and re-cultured for the qualitative and quantitative estimation of Zn solubilization.

Qualitative estimation of Zn solubilization

In the qualitative study, all the isolates (bacteria, fungi, and actinobacteria) were tested for Zn solubilization by plate assay using modified liquid mineral salts medium ($g \cdot lit^{-1}$) specified by (Saravanan *et al.*, 2006), containing dextrose: 10.0g; $(NH_4)_2SO_4$: 1.0g; KCl: 0.2g; K_2HPO_4: 0.1g; $MgSO_4$: 0.2g; pH: 7.0 and insoluble Zn compound (ZnO, $Zn_3(PO_4)_2$ and $ZnCO_3$: 0.1%; Agar: 15.0g) and autoclaved at 121^0C for 20min. Actively growing cultures of each strain were spot-inoculated (2μL) onto the plates and plates were incubated at 28^0C for 48h. Zn solubilization of isolates was determined by measuring the diameter of the clear zone and colony growth (Sunithakumari *et al.*, 2016).

Quantitative estimation by AAS

The quantitative study of zinc solubilization was studied in 150mL conical flasks containing 50mL of liquid mineral salt medium supplemented with Zn compounds. Appropriate uninoculated controls were maintained. The broth was inoculated with 100μL of overnight grown bacterial inoculum and incubated for 72h at 160 rpm in an incubator shaker at 28^0C.

Table 1 Media used for theisolation of Zn solubilizing microorganism

Media Name	Isolation of PGP microorganism
Nutrient Agar and Nutrient Broth (HiMedia, India)	General microbes (*Bacillus* sp.)
King's B agar (Himedia, India)	*Pseudomonas spp.*
Jensen's medium	*Azotobacter spp.*
Yeast Extract Mannitol agar, (HiMedia, India)	*Rhizobium spp.*
Potato Dextrose Agar (HiMedia, India)	Fungus (*Aspergillus spp.*)
Modified Nutrient Agar (MNA)	Actinobacteria
Kenknight's Media (HiMedia, India)	Actinobacteria
Basal media	Zinc solubilizing bacteria

The isolates were centrifuged at 15000 rpm for 15 min and the supernatant was passed through 0.25µm membrane filter so as to obtain the culture filtrate containing only the soluble forms of Zn metal (Francis *et al.*, 1988). Then the filtrate sample was estimated to an atomic absorption spectrometer (AAS-900H) to find the concentration of available zinc present in the sample.

RESULTS AND DISCUSSION

Soil physico-chemical properties

Soil samples collected from the rhizosphere of the plant belonging to the different harsh environment of Karnataka were brought to the laboratory and kept on a polyethylene bag and stored in a refrigerator at 4^0C. The most results of soil sample pH and EC are normal (Table 2). The C/N value of soil sample RS1, RS2, RS5, and RS10 are 0.0000. The C/N ratio of soil sample RS4, RS11, RS12, RS14, RS16, RS18, RS19, and RS20 showed that the soil samples contain high organic matter and the C/N value of soil sample RS6 and RS8 is out of the normal range (Table 2). The soil samples ID 3 (21.5), 7 (22.7) and 9 (25.8) shows normal C/N ratio and the rest of the soils represent very harsh environment deviating wide apart from the normal agricultural soils. All the soils were acidic to near neutral except sample RS20 which is neutral (7.02).

Qualitative estimation: Zinc solubilization by isolates

The qualitative Zn solubilization of seventy-five bacterial isolates (A, B and C), six fungi (D) and actinomycetes (E) results are shown in Fig. 1 and analyzed by R programming - Heat-map plot namely, $ZnCO_3$, $Zn_3(PO_4)_2$ and ZnO, under the assay conditions. Among the cultures, *Bacillus* sp. R4B, R10B, and R20B showed the highest solubilization zone in $Zn_3(PO_4)_2$, whereas R17C and R10B showed 10mm zone in ZnO amended medium (Fig. 2).

Table 2 CHNS analysis data of harsh environment soil sample

Soil Sample ID	C%	H%	N%	S%	C/N Ratio	pH	EC ($ds.m^{-1}$)
RS1	0.85	0.310	0.00	0.027	0.0000	5.26	0.1103
RS2	0.86	0.228	0.00	0.008	0.0000	5.36	0.1288
RS3	1.42	0.772	0.07	0.013	21.4811	5.87	0.1674
RS4	1.02	0.762	0.06	0.010	18.1339	5.35	0.1156
RS5	0.49	0.868	0.00	0.006	0.0000	5.79	0.1145
RS6	0.69	0.639	0.01	0.007	116.5552	5.13	0.1648
RS7	0.97	0.673	0.04	0.010	22.6755	4.60	0.0927
RS8	0.74	1.394	0.00	0.010	183.9937	5.48	0.1162
RS9	1.38	1.055	0.05	0.014	25.7994	5.88	0.1452
RS10	0.00	0.008	0.00	0.004	0.0000	4.59	0.1091
RS11	0.28	0.556	0.02	0.040	11.6841	6.18	0.1166
RS12	1.01	0.671	0.09	0.024	11.0845	6.17	0.1772
RS13	1.20	0.794	0.12	0.018	10.3282	5.67	0.1144
RS14	0.50	0.394	0.05	0.009	9.4342	6.59	0.1602
RS15	0.75	0.387	0.07	0.012	11.2897	5.91	0.1654
RS16	0.68	0.472	0.08	0.009	8.0863	5.33	0.1867
RS17	1.28	0.427	0.12	0.013	10.7216	5.49	0.1907
RS18	0.85	0.916	0.10	0.010	8.6915	5.48	0.1181
RS19	2.20	0.553	0.20	0.020	11.0151	5.92	0.3780
RS20	0.58	0.787	0.05	0.008	12.4970	7.02	0.2070

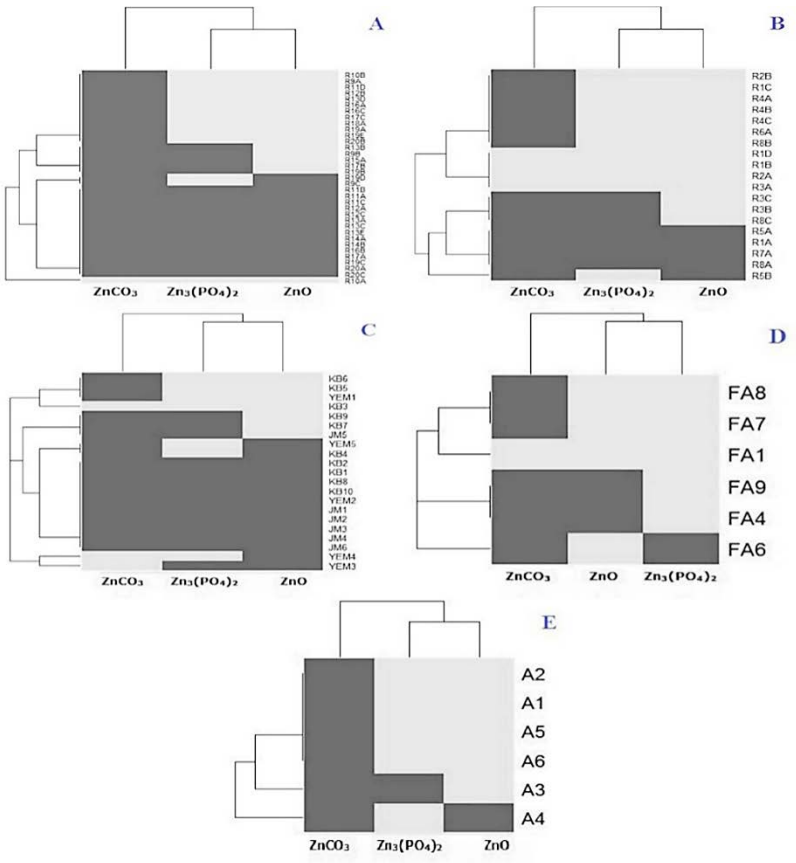

Figure 1.Qualitative Zn solubilization of isolates analyzed by Heat-map plot where gray colour-positive and blue-negative solubilizer of Zn

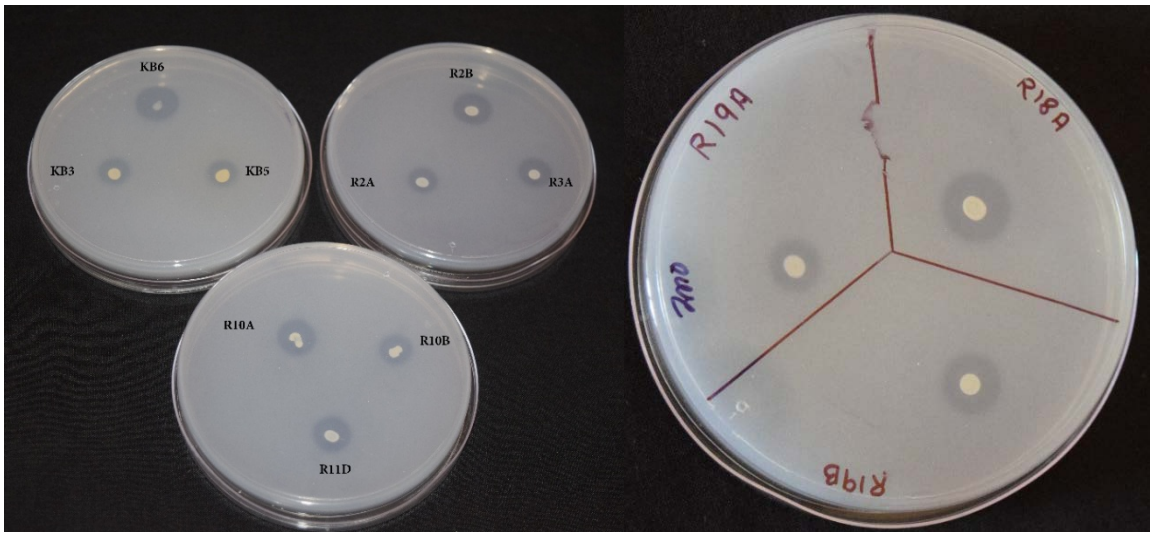

Fig. 2. Zinc solubilization by potential bacterial isolates

The zone of solubilization was comparatively high in ZnO amended medium as compared to $ZnCO_3$ and $Zn_3(PO_4)_2$. Size of the solubilization zone ranged from 3 to 10mm in $Zn_3(PO_4)_2$, from 2 to 10mm in ZnO and from 3 to 5mm in $ZnCO_3$ incorporated medium.Similarly, Khanghahi *et al.*, 2018 reported that prospective implementation as bio inoculants to overcome Zn's unavailability in soil, assess zinc solubilizing capacity in zinc oxide, zinc carbonate, and zinc phosphate supplement (0.1%) of 80 (effective strain: *Agrobacterium tumefaciens* and *Rhizobium* sp.)

PGPB strains from barley and tomato plants rhizosphere.

Quantitative estimation: Zn solubilization by isolates

Quantitative study of twenty-nine active zinc solubilizer was studied in 150mL conical flasks containing 50mL of a liquid mineral salt medium. The concentration of solubilizing zinc was estimated using atomic absorption spectrophotometer. The results revealed that *Bacillus*sp. R10A, R19E, and *Pseudomonas*sp. KB9 were able to dissolve 6.124, 6.127, and 6.220 $mg.l^{-1}$ from $ZnCO_3$, respectively, in a liquid medium and they were consistent with the observations on solid medium (Fig. 3). Gotet*i et al.*,(2013) reported that potential ten strains screened for Zn solubilization, and P33, P29 and B40 produced 22mm clear zones on medium incorporated with $ZnCO_3$; P17 and B40 showed 31.0mm in ZnO incorporated medium. Others results of Zn solubilization by P29 and B40 in broth amended with ZnO (18 and 17 ppm) and $ZnCO_3$ (17 and 16.8 ppm) respectively (Gotet*i et al.*, 2017).

However, R10A and KB9 which was found to be the leading solubilizer on plate agar did not imitate the result in broth amended with ZnO though significant fall in pH (4.31) was noted. Significant reduction of pH was observed in the broth cultures amended with ZnO (pH 4.18 - 4.72) (Fig. 4).

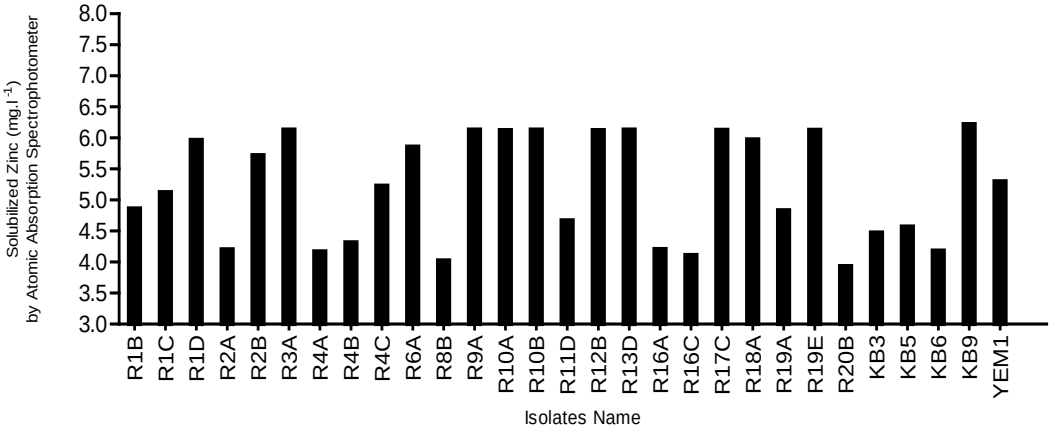

Figure 3. Quantity of Zn solubilization by isolates using AAS analysis

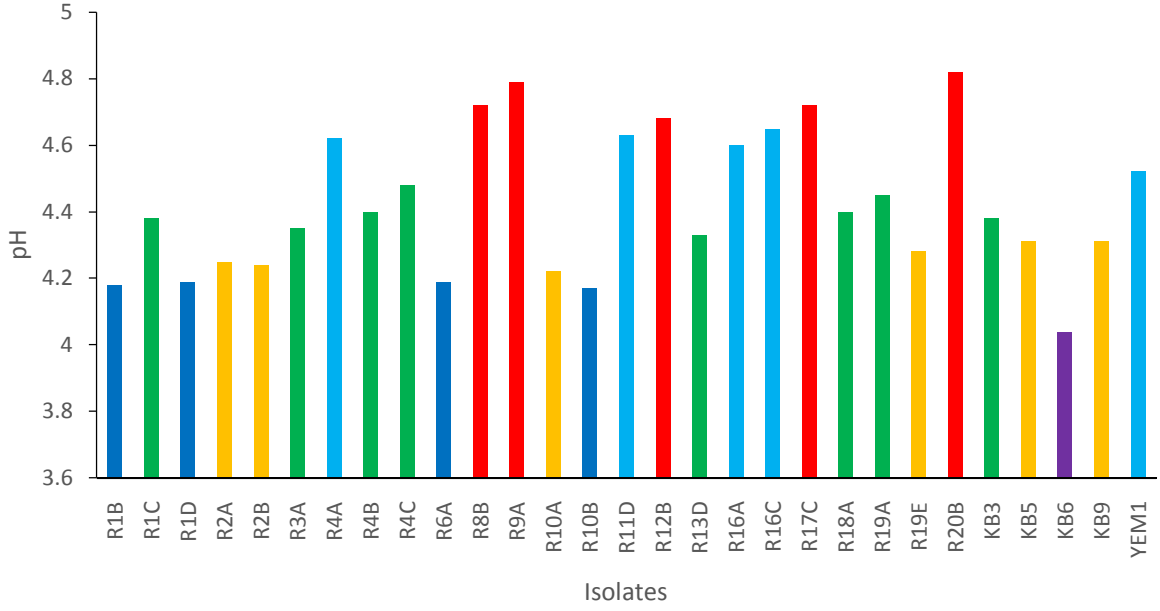

Figure 4. pH increase and decrease in zinc solubilization medium

Sunithakumari*et al.*,(2016)reported that *P. aeruginosa*, *Stenotrophomonasmaltophilia*, *Enterobacter aerogenes*, *Mycobacterium brisbanense*, and *Xanthomonas retroflexus*, highly Zn solubilizer isolated from eight different agricultural fields (ground nut, banana, maize,chilli, sorghum, tomato sugarcane, and field bean) from Coimbatore district of Tamil Nadu and *P. aeruginosa* showed highest solubilization of Zn in the broth and also maximum decrease in the pH from 7 - 3.3 and noted maximum IAA production.

CONCLUSION

In our experiment, Zn solubilization and quantification of some rhizo-microbes using AAS analysis and estimated physico-chemical characteristics of soil samples using the CHNS & chemical method. Study results revealed that the isolates R4B, R10B, and R20B showed their efficiency in Zn solubilization and we can use these cultures for the enhancement of plant nutrient (Zn) management in sustainable farming.

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