THEORETICAL BIOLOGY FORUM

TBF: 355–364 (2023)
Research Article

Effect of different sources, levels and methods of zinc application with bio inoculant on zinc transformation and soil chemical properties of maize in *Alfisols*

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ABSTRACT

Zinc is a crucial micronutrient that serves an essential role in many biological processes. However, its insufficiency is prevalent in various global regions, particularly in developing nations where maize is a primary dietary staple. The purpose of this study was to investigate the "Effect of different sources, levels, and methods of zinc application with bio inoculant on zinc transformation and soil chemical properties of maize in Alfisols". The field experiment was done with nine treatments replicated thrice using a randomized block design during the Rabi season of 2021-22 at the College of Agriculture in Hassan, Karnataka. These treatments included NPK + foliar application of Nano Zn (2 ml L^{-1} and 4 ml L^{-1}) and NPK + $ZnSO_4$ (0.25 and 0.5 % at 45 DAS and silking stage), NPK + seed treatment of Zn solubilizer alone, and various combinations of soil application of $ZnSO_4$ @ 5 and 10 kg ha^{-1}, NPK + $ZnSO_4$ @ 10 kg ha^{-1}, and only NPK. Water soluble + exchangeable, organically complexed, manganese oxide bound, amorphous and crystalline sesquioxide bound zinc fractions, available nutrients (N, K_2O, Ca, Mg, S, and Zn) and dehydrogenase activity were higher in NPK + soil application of $ZnSO_4$ @ 10 kg ha^{-1} + Zn solubilizer at harvest. The use of zinc solubilizing bacteria represents a cost-effective and sustainable strategy for zinc supplementation where zinc deficiency in the soil is a common issue in many regions.

Keywords- *Zinc fractions, Zn-solubilizing bacteria, Soil available primary, secondary and micronutrients.*

ARTICLE HISTORY
02 November 2022: Received
21 February 2023: Revised
09 May 2023: Accepted
26 July 2023: Available Online

DOI:
https://doi.org/10.61739/TBF.2023.12.2.355

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1. Introduction

Maize is an important grain crop for human and animal consumption. It is called the "Queen of Cereals" for its tremendous production potential. Maize is nutrient-responsive and adaptable to varied soil and climate conditions. About 66% of maize production is used as feed, 25% as food and industrial products, and the rest as seed, *etc.* [1]. It is becoming more important in cropping systems due to its higher yield potential, short growing season, high-value food, forage, and feed for livestock and poultry, and cheaper raw material for agro-based industries. Unfortunately, soil micronutrient deficits, notably zinc, are reducing maize production potential.

Zinc (Zn) deficiency impacts agricultural plant growth, metabolism, and reproduction. Zinc aids plant disease resistance, photosynthesis, cell membrane integrity, protein synthesis, and pollen production [2]. About 30% of the world's population is zinc deficient, and 50% of important grain-growing soils lack plant-available zinc [3]. Zinc deficiency was identified in 40% of soil samples in India and 75% in Karnataka [4]. The use of a cereal-based diet low in zinc can lead to development retardation, infectious illness susceptibility, iron-deficient anemia, and other health issues [5]. Currently, the increasing Zn concentration in cereals is a major global challenge. In the Indian scenario, Zn deficiency is usually corrected by application of $ZnSO_4$, Zn chelates like Zn-EDTA, and ZnO, which can be applied to crops via different methods *viz.* seed treatment, soil application, foliar application, *etc.*

Soil application is most successful for the continuous supply of nutrients, which are required in greater amounts. The efficiency of soil-applied $ZnSO_4$ is only 1 to 4 percent, and the majority of applied zinc is rendered unavailable to plants due to a variety of factors including soil pH, high levels of available phosphorus, interaction with other nutrients, leaching, fixation, and conversion to unavailable forms such as $ZnCO_3$, $ZnFe_2O_4$, $ZnSiO_4$ and [6]; [3]. Microbial transformation of Zn from unavailable to available form is an important approach contributing to plant Zn nutrition. There are several studies conducted globally where Zn solubilizers proved to be a good alternative to chemical fertilizers and increased plant growth and yield markedly. Microbes like bacteria and fungi are potential alternatives that could meet plant zinc requirements by solubilizing the complex zinc in soil. They are capable of colonizing the rhizosphere, root surface, and internal tissue of the plant and subsequently increasing the availability of Zn to the plant. The bacterial genus *Pseudomonas* harbors several species with acknowledged activity as PGPB [7]. The Zn-solubilizing *Pseudomonas* strains solubilize unavailable zinc through the production of chelating ligands, secretion of organic acids, amino acids, vitamins and phytohormones and through oxidoreductase systems and proton extrusion. Apart from solubilizing Zn, they also play other functions like enhancing soil physical, chemical, and biological properties [8].

The availability of zinc to plants in soil has been observed to vary with different zinc fractions and it contributes 5 percent or less of the total zinc present. Zinc exists in five distinct pools in soils, which include water-soluble plus exchangeable, organically complexed forms and amorphous sesquioxide bound as available form; crystalline sesquioxide bound and residual zinc

as unavailable form to plants [9]. A proper understanding of the fractions that control the distribution of Zn between active soil constituents and soil solution is fundamental to a proper understanding of the chemistry of zinc in soil [10].
In light of the aforementioned considerations, the study aims to Effect of different sources, levels, and methods of zinc application with bio inoculant on zinc transformation in soil and soil properties of maize in *Alfisol* by elucidating the intricate relationships between soil zinc chemistry, microbial activity, and soil nutrients after harvest of maize crop.

2. Material and Methods

2.1. Description of the study site

A field experiment was conducted during the *rabi* season of 2021-2022 to study the "Effect of different sources, levels, and methods of zinc application with bio inoculant on zinc transformation in soil and soil properties of maize in *Alfisol*" at College of Agriculture, Hassan, UAS, Bengaluru, Karnataka, which comes under Agro Climatic Zone-7 (Southern Transitional Zone) of Karnataka which has 12^0 57' North, latitude and 75^0 58' East, longitude with an altitude of 950 meters above mean sea level. The soil was sandy loam in texture with 68.10, 14.90, and 17.00 percent sand, silt, and clay, respectively. The soil was slightly acidic in pH (6.45) and normal in soluble salts (0.47 dS m^{-1}). The soil was low in available nitrogen (270.04 kg ha^{-1}), medium in organic carbon (5.27 g kg^{-1}), available P_2O_5 (43.59 kg ha^{-1}), available K_2O (170.08 kg ha^{-1}) and available sulfur (13.7 mg kg^{-1}) content. The exchangeable calcium and magnesium content of the soil was 5.8 and 2.4 mol kg^{-1}, respectively. The DTPA extractable micronutrient content *viz.,* iron, copper, and manganese were in the medium range (7.16, 0.66, and 8.18 mg kg^{-1}, respectively) and DTPA extractable zinc was (0.64 mg kg^{-1}). The crops received the highest rainfall on average per month in March 2022 (238 mm) and April 2022 (397 mm), the maximum temperature in April 2022 (37.61 C), and the minimum temperature in December 2021 (13.5 °C).

2.2. Treatment details

The experimental crop selected for this study is maize, specifically the Hybrid 5109 variety. The experiment consists of nine different treatments, each of which has been reproduced three times. The experimental methodology employed for this study is a randomized full-block design. The seed inoculant was administered directly onto the seeds, namely through seed treatment with *Pseudomonas florescence* at a rate of 350g per hectare, as per the prescribed dosage indicated by the producers. The process of seed inoculation was conducted under shaded conditions, soon before the act of sowing. The fertilization of the crop was supplied as per the below-mentioned treatment details as shown in Table 1.

Table 1: Treatment details of the research are as follows:

Treatments	Details
T_1	RDF (Soil application of $ZnSO_4$ @ 10 kg ha^{-1})
T_2	NPK (without zinc)
T_3	NPK+ Foliar spray of Nano Zinc @ 2 ml per liter two sprays at 45 DAS and at the silking stage
T_4	NPK+ Foliar spray of Nano Zn @ 4 ml per liter two sprays at 45 DAS and at silking stage
T_5	NPK+ Foliar spray of $ZnSO_4$ @ 0.25 % (two sprays at 45 DAS and at the silking stage)
T_6	NPK+ Foliar spray of $ZnSO_4$ @ 0.5 % (two sprays at 45 DAS and at the silking stage)
T_7	NPK+ Soil application of $ZnSO_4$ @ 5 kg ha^{-1} + Zn Solubilizer
T_8	NPK+ Soil application of $ZnSO_4$ @ 10 kg/ha + Zn Solubilizer
T_9	NPK+ Zn Solubilizer (without $ZnSO_4$ application)

Note: **FYM (12.5 t ha^{-1}) and fertilizers like Urea, DAP and MOP were applied to all treatments. *Recommended dose of fertilizers (RDF) includes 100: 75: 40:10 (N, P_2O_5, K_2O, $ZnSO_4$) kg ha^{-1}*

2.3 Soil sampling and analysis

Soil samples were obtained from the experimental site at a depth of 0-15 cm before land preparation. These samples were then subjected to analysis to determine their varied qualities. Table 2 presents the analytical techniques utilized for the determination of physical, chemical, and biological characteristics of soil. Similarly, soil samples were obtained from each treatment by collecting samples from three distinct locations after the crop's harvest. The samples underwent shade drying, while the air-dried samples were treated by a gentle pounding method utilizing a wooden pestle and mortar. The soil sample was subjected to sieving using a 2 mm sieve and thereafter stored for subsequent examination.

Table 2: Methods followed for the analysis of soil samples

Parameters	Methods	References
Soil texture (%)	International pipette method	[11]
Bulk density (Mg m^{-3})	Keen Raczkowski cup method	[11]
pH (1:2.5)	Potentiometric method	[12]
EC (1:2.5)	Conductometric method	[12]
Organic carbon (g kg^{-1})	Wet oxidation method	[13]

Available nitrogen (kg N ha^{-1})	Alkaline potassium permanganate Method	[14]
Available phosphorous (kg P_2O_5 ha^{-1})	Bray's extractant using a spectrophotometer	[12]
Available potassium (kg K_2O ha^{-1})	Ammonium acetate extractant using Flame photometer	[12]
Available sulphur (mg kg^{-1})	Turbidimetric method	[12]
Exchangeable calcium [cmol (p+) kg^{-1}]	Complexometric titration	[12]
Exchangeable magnesium [cmol (p+) kg^{-1}]	Complexometric titration	[12]
DTPA extractable Fe, Mn, Zn and Cu (mg kg^{-1})	Atomic absorption spectrophotometry	[15]

2.4 Estimation of dehydrogenase activity ((µg of TPFg^{-1} of soil 24 h^{-1})

The dehydrogenase activity in the soil was determined by the procedure given by [16]. Five grams of soil was taken in a screw cap tube (treatment-wise). To each tube, 1 ml of 3 per cent solution of 2, 3, 5 Triphenyl tetrazolium chloride (TTC) and 2.5 mL of distilled water were added and were mixed using a glass rod. The screw caps were closed and incubated at 30°C for 24 hours. Then 10 mL of methanol was added to the flask, and were shaken for a minute, and filtered using Whatman No. 1 filter paper into 50 mL volumetric flask. Additional methanol (10 mL) was added to the flask until no reddish colour appeared in the methanol extract. The extract was pooled and volume was made to 50 mL using methanol. The intensity of the red colour was determined using a spectrophotometer with methanol as blank. The amount of TPF (2, 3, 5-triphenyl formazon) produced was calculated using a standard graph prepared by using different concentrations of TPF standards.

2.5 Fractionation of zinc in soils

The zinc present in soils was separated into various forms, including water-soluble + exchangeable zinc (WSEX-Zn), organically complexed zinc (OM-Zn), crystalline sesquioxide bound zinc (CRYOX-Zn), amorphous sesquioxide bound zinc (AMOX-Zn), manganese oxide bound zinc (MnOX-Zn), and residual zinc (RES). The operational definition of each fraction is provided in Table 3, following the guidelines outlined by [17] and subsequently updated by [20] and [18]. The extractions were taken in 50 ml polypropylene centrifuge tubes with suitable weight of soil. Between each successive extraction, the supernatant was obtained by centrifuging for 15 min (3000 rpm) and filtering. The concentration of zinc in the centrifugate was determined by an Atomic Absorption Spectrophotometer [19] and expressed in mg kg^{-1}.

Total zinc was determined by digesting the soil samples with hydrofluoric acid in closed polypropylene bottles. Hundred mg of soil sample was transferred into a 250 ml polypropylene bottle, 2 ml of aquaregia was added to disperse the sample. Later, exactly 10 ml of hydrofluoric acid was added and the contents were shaken to dissolve the sample for a period of 2 to 8 hrs. The residue present after the treatment was dissolved using a saturated solution of boric acid and subsequently used for the determination of total Zn by atomic absorption spectrophotometry [19].

Table 3. Sequential extraction of zinc fractions.

Extractant	Step	Fraction	Conditions Solution Soil (g): Solution (ml)
1M $(NH_4)OAc$ (pH 7.0)	1	Water soluble + exchangeable (WSEX-Zn)	5: 20; shake for 1 hour
0.05 M $Cu(OAc)_2$	2	Organically complexed (OM-Zn)	5: 20; shake for 1 hour
0.3 M Sodium citrate + 1.0 M $NaHCO_3$ + 1 g $Na_2S_2O_4$ [Citrate-Bicarbonate- Dithionite (CBD)]	3	Crystalline sesquioxide bound (CRYOX-Zn)	Boiling water bath, 10 min, stir occasionally, keep on water bath (70 – 80°C), 15 min, stir occasionally
0.2 M $(NH_4)_2C_2O_4.H_2O$ + 0.2 M $H_2C_2O_4$ (pH 3.0)	4	Amorphous sesquioxide bound (AMOX-Zn)	5: 20 shake 1 hour
0.1 M NH2OH.HCl (pH 2.0)	5	Manganese oxide bound (MnOX- Zn)	Shake 30min
HCl+ HNO_3 conc	6	Residual (RES) zinc	1: 08 Aqua regia
Sum of the extractable amounts	7	Total	

3. Results and discussion

3.1 Effect of different sources, levels and methods of zinc application with bio inoculant on zinc fractions in soil

The distribution of zinc fractions in the soil following the harvest of a maize crop is depicted in Table 4. The zinc fractions, including water-soluble + exchangeable-Zn (WsEx-Zn), manganese oxide-Zn (Mn-Zn), organically bound zinc (Oc-Zn), amorphous Fe oxide Zn (AmOx-Zn), crystalline Fe oxide Zn (CryOx-Zn), residual zinc (RES-Zn), and total zinc, exhibited significant variations as a result of different sources, levels and application techniques of zinc.

3.1.1 Water soluble + exchangeable and organically bound zinc fractions (mg kg^{-1})

The findings from Table 4 indicate that the levels of water-soluble + exchangeable and organically bound zinc was significantly higher in the treatment that received NPK+ Soil application of ZnSO4 @ 10 kg ha^{-1} + Zn Solubilizer (1.83 and 2.11 mg kg^{-1}, respectively) compared to the T_7 treatment (1.05 and 1.96 mg kg^{-1}, respectively). These results suggest that the aforementioned treatments were more effective than the other treatments evaluated. The NPK treatment exhibited lower concentrations of water-soluble, exchangeable, and organically bound zinc, with observed values of 0.29 mg kg^{-1} and 0.89 mg kg^{-1}, respectively.

Zinc plays a significant role in the nutrition of maize. Application of $ZnSO_4$ at 5 and 10 kg ha^{-1} recorded higher organic bound zinc because of higher availability for adsorption compared to without zinc treatment. It causes an increase in the organically complex zinc forms of native soil Zn with a constant decrease in other forms, suggesting equilibrium of this form in soil (Mandal and Mandal, 1986)[20]. Similar values of organically complex Zn have been reported by [21]. The organically complex zinc fraction of Zn varied directly with the organic carbon content of the soils (Mandal and Mandal, 1986)[20]. These results were in line with those of [22] and [23].

3.1.2 Crystalline Fe oxide, amorphous Fe oxide, and manganese zinc (mg kg^{-1})

Upon examining the data presented in Table 4, it was observed that the treatment involving the application of NPK fertilizer along with soil application of $ZnSO_4$ at a rate of 10 kg ha^{-1}, along with the addition of Zn Solubilizer, resulted in significantly higher values of crystalline Fe oxide, amorphous Fe oxide, and manganese bound zinc fraction (6.51, 4.75, and 7.23 mg kg^{-1}, respectively). This was followed by the treatment involving the application of NPK fertilizer along with soil application of $ZnSO_4$ at a rate of 5 kg ha^{-1}, along with the addition of Zn Solubilizer, which recorded slightly lower values (6.22, 4.44, and 6.58, respectively). Lower levels of crystalline sesquioxide zinc (2.18, 1.03, and 3.08 mg kg^{-1}, respectively) were observed in NPK fertilizers without zinc.

3.1.3 Residual zinc (mg kg^{-1})

Scrutiny of data in Table 4 showed that residual zinc fraction was significantly superior over all other treatments. Significantly higher values (135.46 mg kg^{-1}) in treatment RDF. Lower residual zinc was recorded in T_9 treatment NPK+ Zn Solubilizer (without $ZnSO_4$ application) of 101.25 mg kg^{-1}.

Table 4: Effect of different sources, levels and methods of zinc application with bio inoculant on zinc fractions in soil.

Treatment	WSEX Zn	OC Zn	CRYOX Zn	AMOX Zn	Mn Zn	RES Zn	Total Zn
	mg kg^{-1}						
T_1: RDF	0.96	1.79	5.41	3.91	5.75	135.46	153.28
T_2: NPK (without zinc)	0.29	0.89	2.18	1.03	3.08	110.68	118.15
T_3: NPK+ Foliar spray of Nano Zn @ 2 ml per lit	0.55	1.32	3.91	2.94	4.40	123.00	136.12
T_4: NPK+ Foliar spray of Nano Zn @ 4 ml per lit	0.56	1.34	3.93	2.93	4.43	124.19	137.38
T_5: NPK+ Foliar spray of $ZnSO_4$ @ 0.25 %	0.57	1.33	3.90	2.94	4.44	123.78	136.96
T_6: NPK+ Foliar spray of ZnSO4 @ 0.5 %	0.56	1.35	3.94	2.96	4.46	120.87	134.14
T_7: NPK+ Soil application of $ZnSO_4$ @ 5 kg ha^{-1} + Zn Solubilizer	1.05	1.96	6.22	4.44	6.58	118.88	139.13
T_8: NPK+ Soil application of $ZnSO_4$ @ 10 kg ha^{-1} + Zn Solubilizer	1.83	2.11	6.51	4.75	7.23	131.69	154.12
T_9: NPK+ Zn Solubilizer	0.63	1.68	3.43	2.46	3.95	101.25	113.40
S.Em±	**0.030**	**0.050**	**0.150**	**0.110**	**0.170**	**4.040**	**4.550**
C.D. at 5 %	**0.080**	**0.160**	**0.460**	**0.330**	**0.510**	**12.120**	**13.630**
Initial	**0.26**	**0.81**	**2.04**	**0.99**	**3.01**	**107.54**	

The content of different fractions of zinc was following order residual > Mn oxide > crystalline Fe > amorphous Fe. Higher crystalline and amorphous Fe and Mn bound zinc and residual zinc were recorded in all the treatments compared to water soluble + exchangeable zinc, which might be due to the pH of the soil being acidic in nature and containing more crystalline and amorphous Fe and Mn oxides and hydroxides, which increase the adsorption and precipitation of available zinc and convert it into unavailable Fe and Mn bound zinc and residual zinc. The above findings indicated that the residual Zn was the dominant fraction among all the Zn fractions studied, and the residual form of Zn is associated with the mineral fraction of soil and agrees with the findings of [24] and [25].

The results showed a marked increase in the residual Zn in soil, which indicates considerable mobilisation of zinc to the residual fraction. Comparable results were also observed by [9]. Many researchers reported that a large portion of Zn was in the residual fraction, with very little effect on the extraction plant uptake [26], [27] and [28].

3.1.4 Total zinc (mg kg^{-1})

The data in Table 4 indicated that total zinc content in soil was enhanced significantly due to imposed treatments. Significantly higher total zinc was observed in T_8 (153.28 mg kg^{-1}) and lower total zinc was recorded in T_9 of 113.40 mg kg^{-1}.

The results (Table 4) indicated that except for residual Zn (Res-Zn), all the fraction of zinc of soil after harvest of crop was increased significantly with the application of zinc solubilizer in combination with different levels of $ZnSO_4$ *i.e.,* NPK + soil application of @ 10 kg ha^{-1} + Zn solubilizer, which was significantly superior over all other treatments. Among these fractions in treated plots, the least fraction was water soluble and exchangeable fraction and highest fraction was residual zinc. All these fractions of zinc increased with an increase in levels of combination along with NPK + FYM and application of zinc solubilizer. Around 90-95 per cent of total Zn in the soil is constituted by residual-Zn and was found to significantly decrease with the application of zinc solubilizing bacteria. This indicates that a portion of residual-Zn got transformed and redistributed into other fractions due to solubilization of the fixed form of zinc into a labile form. An increase in concentration of all the fractions except residual-Zn due to the applied ZnSB, which has high solubility and mobility in soil, got distributed into soluble and sparingly soluble fractions of zinc such as exchangeable-Zn, Manganese oxide-Zn, organically bound zinc, amorphous Fe oxide Zn and crystalline Fe oxide Zn, which in turn had a positive and significant correlation with the DTPA Zn in soil. This shows that applied zinc sources in soil are mainly concentrated in easily and sparingly soluble Zn fractions and that an increase in its availability in soil was noticed due to their contribution to the soil available pool. [29], [30], and [31].

The availability of zinc for crops was reported to exist in association with the distribution of this nutrient among soil fractions. It will help to specify the chemistry of zinc in soils and perhaps its availability for the crop's uptake. Oxide bound and residual Zn is well known to be more stable whereas exchangeable and water-soluble zinc fraction are more soluble [32]. There is an increase in exchangeable-Zn by an increase in the mineralization of organically bound zinc or solubilization of zinc from recalcitrant sources such as oxide-bound zinc into exchangeable -Zn [33]. The results are in conformity with the findings of [34].

3.2 Effect of different sources, levels and methods of zinc application with bio inoculant on soil properties.

3.2.1 Soil pH, EC and OC

Data pertaining to different sources, levels and methods of zinc application on soil pH, electrical conductivity and organic carbon content of soil after harvest of maize crop are presented in Table 5.

There was no significant difference among soil properties concerning soil pH, EC, and OC at harvest. However, higher soil pH (6.38) was recorded in treatments that received only NPK (without zinc). A lower pH (6.09) was noticed in NPK+ soil application of $ZnSO_4$ @ 10 kg ha^{-1} + Zn Solubilizer. The decrease in pH noticed in plots treated with zinc sulfate and zinc solubilizers might be due to the production of organic acids by zinc solubilizers and increased sulfur content after the dissolution of zinc sulfate in soil. Similar results were reported [35] and [36].

Table 5: Soil pH, EC, and Organic Carbon content in soil at harvest of maize as influenced by different sources, levels, and methods of zinc application

Treatments	pH	EC (dS m^{-1})	OC (g kg^{-1})
T_1: RDF	6.21	0.42	5.24
T_2: NPK (without zinc)	6.35	0.40	5.14
T_3: NPK+ Foliar spray of Nano Zn @ 2 ml l^{-1}	6.30	0.42	5.20
T_4: NPK+ Foliar spray of Nano Zn @ 4 ml l^{-1}	6.27	0.43	5.22
T_5: NPK+ Foliar spray of $ZnSO_4$ @ 0.25 %	6.31	0.41	5.21
T_6: NPK+ Foliar spray of ZnSO4 @ 0.5 %	6.28	0.42	5.23
T_7: NPK+ Soil application of $ZnSO_4$ @ 5 kg ha^{-1} + Zn Solubilizer	6.12	0.42	5.31
T_8: NPK+ Soil application of $ZnSO_4$ @ 10 kg ha^{-1} + Zn Solubilizer	6.09	0.45	5.41
T_9: NPK+ Zn Solubilizer	6.17	0.41	5.28
S.Em±	0.07	0.02	0 .08
C.D. at 5 %	NS	NS	NS
Initial values	6.45	0.47	5.27

NS – Nonsignificant

Electrical conductivity and organic carbon content did not show any significant change. However, NPK+ soil application of $ZnSO_4$ @ 10 kg ha^{-1} + Zn solubilizer had higher EC and organic carbon content (0.4 dS m^{-1} and 5.41 g kg^{-1}, respectively). Lower EC and organic carbon content were recorded in NPK (without zinc). The slight increase in EC in T_8 might be due to the addition of salts through zinc sulfate and the solubilization of native minerals by zinc-solubilizing bacteria. These results were corroborated by the findings of [37], [38] and [39]. Organic carbon is an important indicator of soil fertility, which sequentially reflects soil microbes and enzyme activities. The maximum organic carbon content was recorded in treatment T_8. It might be due to the inoculation of zinc-solubilizing bacteria with Zn fertilization intensifying the root morphological characteristics such as root length, volume, and spread, resulting in superior crop growth with higher root biomass generation, which might be the probable cause of the improvement in organic carbon content in the soil. These findings are similar to those of [40].

3.2.2 Available major nutrient status (N, P_2O_5 and K_2O)

The available major nutrient status of soil after harvest of maize as influenced by different sources, levels, and methods of zinc application is furnished in Table 6. The data clearly showed that there was a significant variation in the available nitrogen, phosphorous, and potassium status of soil maize at harvest due to the combined application of FYM and fertilizers along with zinc solubilizers in the soil.

Table 6: Available N, P_2O_5, and K_2O content of soil (kg ha^{-1}) at harvest of maize as influenced by different sources, levels, and methods of zinc application

Treatments	**Avail. N (kg ha^{-1})**	**Avail. P_2O_5 (kg ha^{-1})**	**Avail. K_2O (kg ha^{-1})**
T_1: RDF	322.01	54.87	205.06
T_2: NPK (without zinc)	282.53	58.69	184.67
T_3: NPK+ Foliar spray of Nano Zn @ 2 ml l^{-1}	299.12	58.25	197.98
T_4: NPK+ Foliar spray of Nano Zn @ 4 ml l^{-1}	301.04	58.56	193.66
T_5: NPK+ Foliar spray of $ZnSO_4$ @ 0.25 %	297.14	58.44	195.34
T_6: NPK+ Foliar spray of ZnSO4 @ 0.5 %	298.68	58.61	199.2
T_7: NPK+ Soil application of $ZnSO_4$ @ 5 kg ha^{-1} + Zn Solubilizer	317.29	52.03	203.66
T_8: NPK+ Soil application of $ZnSO_4$ @ 10 kg ha^{-1} + Zn Solubilizer	326.64	50.39	207.04
T_9: NPK+ Zn Solubilizer	312.77	56.91	202.67
S.Em±	8.17	1.65	4.53
C.D. at 5 %	24.50	4.91	13.58
Initial Values	270.04	43.59	170.08

The soil nitrogen and potassium content was significantly higher in NPK+ soil application of $ZnSO_4$ @ 10 kg ha^{-1} + Zn solubilizer (326.64 and 207.04 kg ha^{-1}, respectively) and comparable to soil application Zn @10 kg ha^{-1} (322.01 and 205.06 kg ha^{-1}, respectively) at harvest. The treatment NPK (without zinc) had substantially lower available nitrogen and potassium than other treatments (282.53 kg ha^{-1} and 184.67 kg ha^{-1}, respectively). Significantly higher available phosphorus (58.69 kg ha^{-1}) was recorded in the NPK (no zinc) treatment, which was comparable to the zinc-sprayed treatments T_3, T_4, T_5, and T_6. The treatment that received NPK+ soil application of $ZnSO_4$ at 10 kg ha^{-1} + Zn solubilizer had the lowest available phosphorus content in the soil (50.39 kg ha^{-1}).

The present investigation revealed that the highest available nitrogen and potassium after harvest of a maize crop was observed under the application of NPK+ soil application of $ZnSO_4$ @ 10 kg ha^{-1} + Zn Solubilizer. The available N and K content in the soil was significantly enhanced by the zinc along with zinc solubilizer application that caters to the increased availability of zinc and attributed to the synergistic effect of Zn in influencing nitrogen (N) transformation reaction and solubilization of potassium (K) from micaceous minerals by microbial inoculation. This result agrees with the reports of [41] and [42]. Decreased values of available phosphorus content in soil were observed in the treatments that received zinc fertilizers along with zinc solubilizers when compared to the treatments that received zinc fertilizers alone. The decrease in available phosphorus content is due to the antagonistic effect of phosphorus and zinc. Similar results were reported by [38] Durgude *et al.* (2014).

3.2.3 Secondary nutrients (Ca, Mg and S)

Application of different sources, levels, and methods of zinc application had a significant effect on exchangeable calcium, magnesium, and available sulfur status at harvest (Table 7). Further, higher exchangeable calcium, magnesium, and available sulfur content of 5.91, 2.55 col [p+] $kg^{-1,}$ and 25.42 mg kg^{-1} were noticed in NPK+ soil application of $ZnSO_4$ @ 10 kg ha^{-1} + Zn Solubilizer and it was on par with RDF. Treatment NPK (no zinc) resulted in lower exchangeable Ca, Mg, and available sulfur content (5.51, 2.39 cmol [p+] kg^{-1} and 14.79 mg kg^{-1}, respectively).

The application of FYM in conjunction with zinc solubilizer resulted in increased exchangeable calcium and magnesium, which may have been caused by the release of calcium from added FYM after mineralization and the release of calcium and magnesium from soil exchange sites upon decomposition of organic materials.

Table 7: Exchangeable Ca and Mg [cmol kg^{-1}] and available sulfur (mg kg^{-1}) content of soil at harvest by different sources, levels, and methods of zinc application

Treatments	**Exch. Ca [cmol (p+) kg^{-1}]**	**Exch. Mg [cmol (p+) kg^{-1}]**	**Avail, S (mg kg^{-1})**
T_1: RDF	5.79	2.54	23.05

T_2: NPK (without zinc)	5.51	2.39	14.79
T_3: NPK+ Foliar spray of Nano Zn @ 2 ml l^{-1}	5.62	2.47	19.34
T_4: NPK+ Foliar spray of Nano Zn @ 4 ml l^{-1}	5.64	2.46	17.34
T_5: NPK+ Foliar spray of $ZnSO_4$ @ 0.25 %	5.63	2.44	19.05
T_6: NPK+ Foliar spray of ZnSO4 @ 0.5 %	5.65	2.49	18.18
T_7: NPK+ Soil application of $ZnSO_4$ @ 5 kg ha^{-1} + Zn Solubilizer	5.84	2.51	22.25
T_8: NPK+ Soil application of $ZnSO_4$ @ 10 kg ha^{-1} + Zn Solubilizer	5.91	2.55	25.42
T_9: NPK+ Zn Solubilizer	5.74	2.53	19.95
S.Em±	0.13	0.09	0 .97
CD (P=0.05)	0.39	0.27	2.92
Initial values	5.8	2.4	13.7

Similar results were found by [43] and [44] under the lettuce crop and in the pearl millet-wheat cultivation system, respectively. The available sulfur (S) was substantially affected by the zinc application sources, levels, and methods. Significantly more available sulfur was present in the $ZnSO_4$-treated plots than in those that were not treated with $ZnSO_4$. The discharge of sulfur from $ZnSO_4$ into the soil may account for this. After a portion of the released S from $ZnSO_4$ was utilized, the remainder may have contributed to the soil's available pool. The application of zinc sulfate and zinc solubilizers increased microbial activity, which in turn caused heterotrophic bacteria to oxidize sulfide minerals to sulfate, thereby rendering them available [45]. Zinc fertilization increases the availability; [46] supported these findings.

3.2.4 DTPA extractable micronutrients (Cu, Fe, Mn, and Zn)

The data on iron, manganese, and copper concentrations in crop soil did not change significantly as a result of the use of different zinc sources, levels, and methods of application (Table 8). A numerically higher amount of iron, manganese, and copper content was recorded in T_8 treatment, which received NPK+ soil application of $ZnSO_4$ @ 10 kg ha^{-1} + Zn Solubilizer and the lowest iron, manganese, and copper content was recorded in treatment NPK (without zinc).

Table 8: DTPA extractable Fe, Mn, Zn, and Cu content of soil at harvest of maize as influenced by different sources, levels, and methods of zinc application

Treatments	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})	Cu (mg kg^{-1})
T_1: RDF	7.64	8.23	2.56	0.691
T_2: NPK (without zinc)	7.06	8.15	0.59	0.569
T_3: NPK+ Foliar spray of Nano Zn @ 2 ml per lit	7.18	8.22	0.58	0.564
T_4: NPK+ Foliar spray of Nano Zn @ 4 ml per lit	7.07	8.23	0.61	0.563
T_5: NPK+ Foliar spray of $ZnSO_4$ @ 0.25 %	7.26	8.19	0.63	0.563
T_6: NPK+ Foliar spray of ZnSO4 @ 0.5 %	7.16	8.21	0.62	0.562
T_7: NPK+ Soil application of $ZnSO_4$ @ 5 kg ha^{-1} + Zn Solubilizer	7.74	8.23	2.43	0.662
T_8: NPK+ Soil application of $ZnSO_4$ @ 10 kg ha^{-1} + Zn Solubilizer	7.84	8.28	3.58	0.684
T_9: NPK+ Zn Solubilizer	7.69	8.26	2.09	0.651
S.Em±	0.26	0.27	0.04	0.02
C.D. at 5 %	NS	NS	0.12	NS
Initial values	7.16	8.18	0.64	0.61

NS – Nonsignificant

The DTPA extractable zinc content of the soil was significantly higher in NPK+ Soil application of $ZnSO_4$ @ 10 kg ha^{-1} + Zn solubilizer (3.58 mg kg^{-1}) followed by RDF (2.56 mg kg^{-1}) and it is on par with treatment T_7. Treatment NPK (without zinc) recorded a significantly lower zinc status (0.59 mg kg^{-1} at harvest). However, the treatments that received soil application of Zn, (T_1, T_7 and T_8) recorded higher values of available zinc in soil than the treatments that received foliar application of Zn (T_3, T_4 and T_5 and T_6) and the higher availability of Zn, Fe, Mn and Cu in soil after harvest of maize crop was due to application of zinc solubilizer inoculation with different levels of $ZnSO_4$, NPK+ soil application of $ZnSO_4$ @ 10 kg ha^{-1} + Zn Solubilizer. It could be due to zinc solubilizing bacteria (ZnSB) producing organic acids *viz*., gluconic acids, which are identified as strong acids among the mono carboxylic group of acids and are found to be easily biodegradable even as 98 percent are easily biodegraded in two days. Gluconic acids, even as the major anion, might be an important agent that helps in the solubilization of insoluble zinc compounds. It solubilizes the very tightly bound residual form of zinc and enhances the availability of zinc. This result was confirmed with [47], who reported a significant increase in the soil available micronutrient content due to the solubilization of ZnSB.

The observed elevation in zinc concentration within the soil in the RDF treatment can likely be attributed to the introduction of zinc fertilizer into the soil. Furthermore, the availability of zinc within the soil rose proportionally with the higher rates of zinc application. This may be because zinc sulfate is more water-soluble and therefore readily available, causing its effect to be visible in the soil's DTPA-extractable zinc content. According to [48], the solubility of zinc sulfate in water is an essential criterion for the availability of zinc in soil. The availability of zinc would have decreased because of increased moisture, as predicted by Chatterjee *et al.* (1992). The results of this study were consistent with the findings reported by [37], [49], and [50].

3.2.5 Effect of different sources, levels, and methods of zinc application with bio inoculant on biological properties (Dehydrogenase activity) of soil

Treatments varied significantly among different sources, levels and methods of zinc application concerning dehydrogenase activity in the rhizosphere soil of the maize at harvest (Table 9). Dehydrogenase enzyme activity was significantly higher in NPK+ soil application of $ZnSO_4$ @ 10 kg ha^{-1} + Zn Solubilizer (31.02 g TPF g^{-1} soil day^{-1}) than in NPK+ soil application of $ZnSO_4$ @ 5 kg ha^{-1} + Zn Solubilizer (27.21 g TPF g^{-1} soil day^{-1}). The lowest activity was observed in treatment NPK (without zinc) (14.04µg TPF g^{-1} soil day^{-1}). Soil organic carbon level was positively associated with dehydrogenase activity. Dehydrogenase activity may have increased because of the increasing organic matter content of the soil in the 10 kg ha^{-1} NPK+ $ZnSO_4$ soil application solubilizer treatment. Soil enzymatic activity is enhanced when biofertilizers (including Zn-solubilizers) and farmyard manure are applied to maize crops instead of chemical fertilizers. Farmyard manure boosts soil enzymatic activity because it adds organic matter, which is essential for the survival of rhizosphere microbial communities [51]

Table 9: Effect of different sources, levels and methods of zinc application on dehydrogenase activity in soil.

Treatment	**Dehydrogenase activity (µg TPF g^{-1} soil day^{-1})**
T_1: RDF	20.54
T_2: NPK (without zinc)	14.04
T_3: NPK+ Foliar spray of Nano Zn @ 2 ml l^{-1}	16.89
T_4: NPK+ Foliar spray of Nano Zn @ 4 ml l^{-1}	16.67
T_5: NPK+ Foliar spray of $ZnSO_4$ @ 0.25 %	17.54
T_6: NPK+ Foliar spray of $ZnSO_4$ @ 0.5 %	18.68
T_7: NPK+ Soil application of $ZnSO_4$ @ 5 kg ha^{-1} + Zn Solubilizer	27.21
T_8: NPK+ Soil application of $ZnSO_4$ @ 10 kg ha^{-1} + Zn Solubilizer	31.02
T_9: NPK+ Zn Solubilizer	22.64
S.Em±	0.72
C.D. at 5 %	2.15
Initial	17.33

Conclusion

The results of this investigation revealed that, *P. fluorescens* for the inoculation of maize seeds before sowing is capable of promoting available chemical zinc fractions and soil available nutrients in soils. At harvest of maize crop, treatment NPK+ Soil application of $ZnSO_4$ @ 10 kg ha^{-1} + Zn solubilizer, recorded significantly higher water-soluble and exchangeable, organically complexed, amorphous and crystalline sesquioxide bound and manganese oxide bound zinc fractions. Significantly higher residual zinc was recorded in RDF. Significantly higher available nutrients (N, K_2O, Ca, Mg, S, and Zn) and enzymatic activity (Dehydrogenase) were recorded in NPK+ Soil application of $ZnSO_4$ @ 10 kg ha^{-1} + Zn Solubilizer. The findings suggest that optimizing these variables can enhance zinc availability in the soil, improve soil health, and increase maize yield where soil zinc deficiency is a common issue. Overall, this research contributes to our understanding of sustainable farming practices and highlights the potential of using zinc and bio inoculants to improve soil health and crop yield. It underscores the importance of continued research in this area to support sustainable agriculture and food security.

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