PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol x(x): xx–xx
https://doi.org/10.14719/pst.4344

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RESEARCH ARTICLE

Soil zinc dynamics over time on applied organics, bacterial inoculant and zinc fertilizers in calcareous soil of southern India

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OPEN ACCESS

ARTICLE HISTORY
Received: 16 July 2024
Accepted: 24 September 2024
Available online
Version 1.0 : 10 November 2024

Check for updates

Additional information
Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

CITE THIS ARTICLE
Bose KSC, Selvi D, Vinothini R, Balaganesh B, Vinoth Kumar V, Venkatesh V, Kumaran VRS. Soil zinc dynamics over time on applied organics, bacterial inoculant and zinc fertilizers in calcareous soil of southern India. Plant Science Today (Early Access). https:/doi.org/10.14719/pst.4344

Abstract

Bioavailability of zinc was impacted by soil properties, externally applied sources, time, and various fractions of zinc. An experiment was conducted to investigate the bioavailable and other zinc fractions in calcareous soil and the efficiency of organic and inorganic sources on bioavailability with the presence and absence of zinc solubilizing bacteria (ZSB). The sequential extraction procedure followed at every interval of the incubation period for all forms of zinc was studied. Externally added inorganic sources significantly affected all fractions of zinc compared to untreated soils. Among them, $ZnSO_4$ influences all forms of zinc, mainly Ws+Ex (water soluble+exchangeable) and carbonate-bound zinc, whereas Zn-EDTA maintains a high status of bioavailable zinc throughout the experimental period. On the 60th day of incubation, Zn-EDTA and $ZnSO_4$ applied to soil maintained bioavailable zinc content of 3.71 and 2.94 times higher than that of control. Irrespective of sources, the available zinc of Zn fertilizers applied to soils was reduced with an increase in incubation days. Organic and microbial addition effects solely or combined increase the soil zinc content significantly in both fertilized and unfertilized soils. Among two different organic sources, the zinc solubility performance of farmyard manure was higher than that of vermicompost. In untreated soil, residual and carbonate-bound fractions contribute a major portion towards total zinc based on quantity. The bioavailable fraction mainly the Ws+Ex and organically bound fractions were markedly influenced in all treatments.

Keywords

Bioavailability; calcareous soil; Zn fractions; inorganic Zn sources; organics; ZSB

Introduction

The solid solution interplay imparts the ultimate role in the concentration of plant available zinc on the dynamic soil body (1). Solid (quantity) form dominates most of the agricultural soil, which impedes the solution (intensity) zinc. Factors like pH, organic matter, clay content, calcareousness, and external fertilization regulate the diverse phase of zinc (2-4).The proportion rate of influence of the above factors varies among the soils. Irrespective of all factors, the maximum soil residual form of Zn dominates other forms (5, 6). The distribution of zinc from solution to solid phase is expeditious where it slows down while vice-versa. In calcisol, carbonate's role

as the main scavenger of bioavailable zinc (BAZ) leads to an increase in the inaccessibility of plants to forage.

By altering the zinc driving factors to the maximum possible extent, the bioavailability of zinc can be shoot-upped. To sustain or increase the released zinc, there is a continuous supply of solubilizing agents needed, which only comes through biomatter. Time plays a pivotal role in BAZ concentration in soil solution. The extractability of recently added zinc is effortless, which decreases over time mainly through micropore diffusion (7). The diverse form of solid zinc ascents or descents over time (8). Zinc solubilizing bacteria help to reduce the fixation and increase the availability of zinc that has already been fixed (9).

In that way, the paper discusses the soluble, insoluble, and chelated sources of Zn and its reaction in a calcareous soil for some time with the integration of organics and microbes. To evaluate the consequences of the above external load factorized as Zn sources, organics, and microbes. It will also acquaint the most labile form, which contributes to the BAZ on soil. The current work extracts quantitative data about different forms of zinc.

Materials and Methods

Experimental soil

The surface soils (0-15 cm) for the incubation experiment were collected from the eastern block farm of the Tamil Nadu Agricultural University of Coimbatore district with a latitude and longitude of 11°0'35" N 76°56'26" E and 411 m above mean sea level. The experimental soil comes under semi-arid agro-climatic regions of Tamil Nadu with annual precipitation of 695.8 mm. The soil belonged to the (*Vertic Ustropept*) Periyanayakkanapalayam series. The soil samples were air-dried, sieved through a 2 mm sieve, and thoroughly mixed. Soil analysis was carried out for physical and chemical parameters namely pH, EC, organic carbon, bulk density, soil texture, cation exchange capacity (CEC), available phosphorus, calcium carbonate equivalent (CCE), total and available zinc (Table. 1). Farmyard manure and vermicompost were shade dried and passed through 2 mm sieve and analysed for chemical properties namely, pH, EC, organic carbon, total micronutrients and total nitrogen and phosphorus were analysed (Table 2).

Table 1. Characterization of experimental calcareous soil.

S.No	Soil Properties	Value
1.	pH	8.37
2.	EC (dS m^{-1})	0.49
3.	Organic carbon (g kg^{-1})	4.6
4.	CCE (%)	11.5
5.	Soil texture	Sandy Clay Loam
6.	Clay (%)	27.6
7.	CEC c mol (+) Kg^{-1}	19
8.	P (ppm)	8.94
9.	Zn^a (mg kg^{-1})	0.534
10.	Zn^t (mg kg^{-1})	34.27

[a]indicates available Zn

[t]indicates total zinc

Table 2. Properties of organic sources added in the experiment.

Properties	Farmyard Manure	Vermicompost
pH	7.26	7.73
EC (dS m^{-1})	0.54	2.85
OC (%)	0.49	0.43
Total Zn (mg kg^{-1})	112	167
Total Fe (mg kg^{-1})	584	718
Total Cu (mg kg^{-1})	32.5	58.3
Total Mn (mg kg^{-1})	234.1	296.5
Total P (%)	0.36	0.72
Total N (%)	0.84	1.32

Experimental details

The incubation experiment was framed to know the sole and interaction effects of inorganic zinc sources (Control, $ZnSO_4$, Zn_3PO_4, ZnO and Zn-EDTA), organics (FYM and VC) and microbe (ZSB - *Pseudomonas chlororaphis*). This experiment was conducted in a completely randomized design (CRD) (5 × 3 × 2 - Inorganic zinc sources × Organics × Microbes) replicated thrice.

An exact quantity of 200 g soil was taken in each incubation bottle with the zinc equivalent of 5 kg ha^{-1} and added through different sources as mentioned above. Organic matter namely, FYM (12.5 t ha^{-1} equivalent) and vermicompost (5 t ha^{-1} equivalent), were added with respective quantities. Zinc solubilizing bacterial strain *P. chlororaphis* was grown in Luria Bertani medium (500 mL ha^{-1} equivalent, Cfu - 10^{12} per mL) infused with organics and applied to respective treatments. Distilled water was applied uniformly for all treatments and replications to maintain 80 % field capacity moisture. Incubation bottles were kept under controlled conditions to impede the rapid evaporation and influences of other external factors. Soil samples were collected periodically on the 1st, 15th, 30th, 45th and 60th days after incubation. At each time interval, the soil samples were collected, shaded, and analysed for pH, EC, available phosphorus and fractions of zinc.

Fractionation

The different fractions of zinc are determined by consecutive extraction of periodically collected soil samples from the incubation experiment following the fractionation procedure (10). The chemical forms of zinc are water soluble+exchangeable (Ws+Ex-Zn), carbonate bound (Carb-Zn), organically complexed (OC-Zn), manganese oxide bound (Mn Ox-Zn), amorphous (Am Fe-Zn) and crystalline (Cry Fe-Zn) oxide bound zinc and residual (Res-Zn) were sequentially quantified. The residual fraction was computed by the difference between total zinc and the sum of all other fractions. 5 g of air-dried soil samples into a 100 mL shaking bottle and extractant were added (for each fraction sequentially). Shaking was done by rotary shaker at 25°C and to get a clear solution, samples were centrifuged (4000 rpm, 10 min) and filtered (Whatman No. 42 filter paper) (Table 3).

$$\text{Percent transformation (\%)} = \frac{\text{Treated soil Zn (mg kg}^{-1}\text{) - Untreated soil Zn (mg kg}^{-1}\text{)}}{\text{Applied Zn (mg kg}^{-1}\text{)}} \times 100 \quad \text{(Eqn.1)}$$

Table 3. Procedure for sequential extraction of zinc on experimental soil (10).

No	Fractions	Extractant	Soil (g): Solution (mL)	Shaking Time (hr)
1	Water soluble (Ws) + Exchangeable (Ex)	1M Mg (NO3)2	5:20	2 hr
2	Carbonate bound (Carb-F)	1M NaOAc (pH = 5, CH3COOH)	5:20	5 hr
3	Organically complexed (OC-F)	0.7 M NaOCl (pH = 8.5)	5:20	0.5 hr in water bath
4	Manganese oxide bound (Mn Ox-F)	0.1 M NH2OH.HCl (pH = 2, HNO3)	5:50	0.5 hr
5	Amorphous Fe and Al oxides bound (Am Fe-F)	0.25 M NH2OH.HCl + 0.25 M HCl	5:50	0.5 hr in water bath at 50°C
6	Crystalline Fe and Al oxides bound (Cry Fe-F)	0.2 M(NH4)2C2O4 + 0.2 M H2C2O4 + 0.1 M C6H8O6	5:50	0.5 hr in water bath at 50°C
7	Residual Zn (RES-F)	Total zinc - Sum of all fractions		
8	Total	HCl + HNO_3	1:15	Microwave digestion

Statistical analysis

Statistical analysis was done on recorded data analysis of variance (ANOVA) and weigh-up means by least significance difference (LSD) at a probability level of 5 % by AGRES. The multiple regression equation was computed on DTPA Zn with other fractions as done by SPSS 16.0 statistical software.

Results

The zinc fraction series on the initial day of incubation in untreated soil was in the order of residual>> carbonate> crystalline Fe oxide >amorphous Fe oxide> organically bound >manganese oxide bound> water soluble and exchangeable zinc (Fig. 2-5). This was in line with the previous study, that recorded the order of zinc forms in calcareous soil as Res F>>Carb-F>Crys Fe Ox-F>Am Fe Ox-F>OC-F>Mn Ox-F>Ws+Ex-F in untreated soil (11).

Regardless of organics (FYM and VC) and microbes (ZSB), application of $ZnSO_4$ and Zn-EDTA treatments significantly enhanced the Ws+Ex fraction compared to other zinc sources (Fig. 2). At 1st day of incubation, the percentage of Ws+Ex fraction of zinc to total zinc was 4.33>4.12>1.97 >1.86> and 1.28 for Zn-EDTA >$ZnSO_4$> ZnO >$Zn_3(PO_4)_2$> control, respectively. The same sequence was upheld up to the end of the experiment: Zn-EDTA (2.04 %) >$ZnSO_4$ (1.77 %)> ZnO (1.39 %)> $Zn_3(PO_4)_2$ (1.33 %)> control (1.06 %).

A significant reduction was observed in DTPA-Zn, Ws+Ex-Zn and OC-Zn (Fig. 1-3) on the 60th day of incubation on $ZnSO_4$ and Zn-EDTA treatments. Irrespective of organics, there is a subsequent increase in carbonate (Fig. 4), amorphous, crystalline and manganese oxide fractions with proceeding incubation days.

Zn-EDTA application showed a maximum bio-available Zn (DTPA) at all stages of incubation, starting from 1st day to the 60th day with and without ZSB and organics, followed by $ZnSO_4$ (Fig. 1). The final day results show Zn-EDTA and $ZnSO_4$ applied soil maintained bioavailable zinc content of 3.71 and 2.94 times higher than that of control. The results of some researchers indicated that the application of Zn-EDTA (Chelated zinc) fraction maintains a higher concentration of bio-available zinc compared to other fertilizer sources, especially in calcareous soils (12-14).

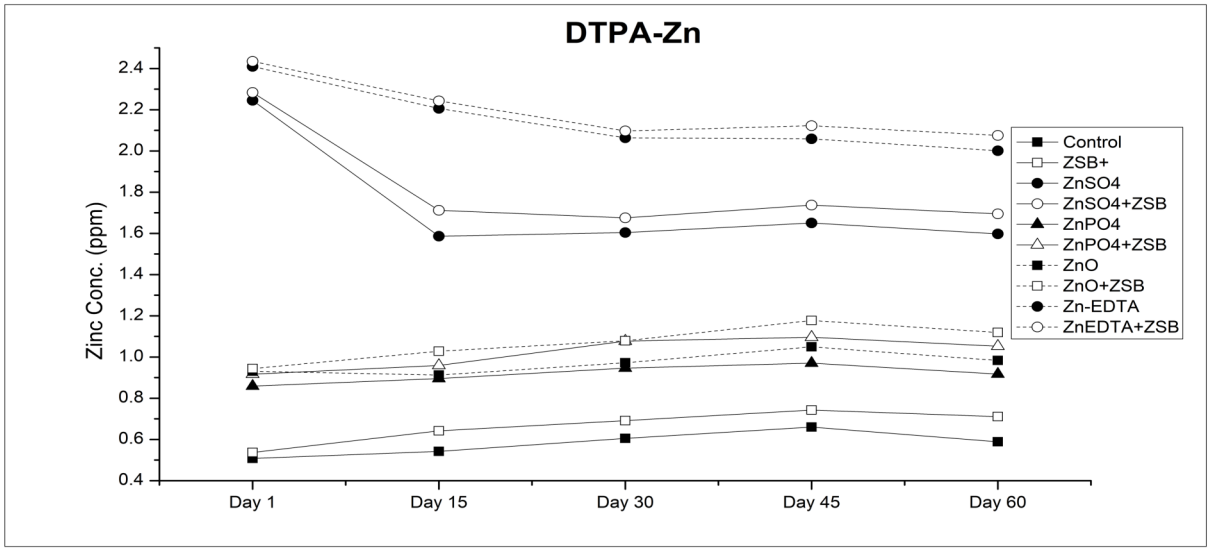

Fig. 1. Zinc sources effect on DTPA-Zn with presence and absence of ZSB.

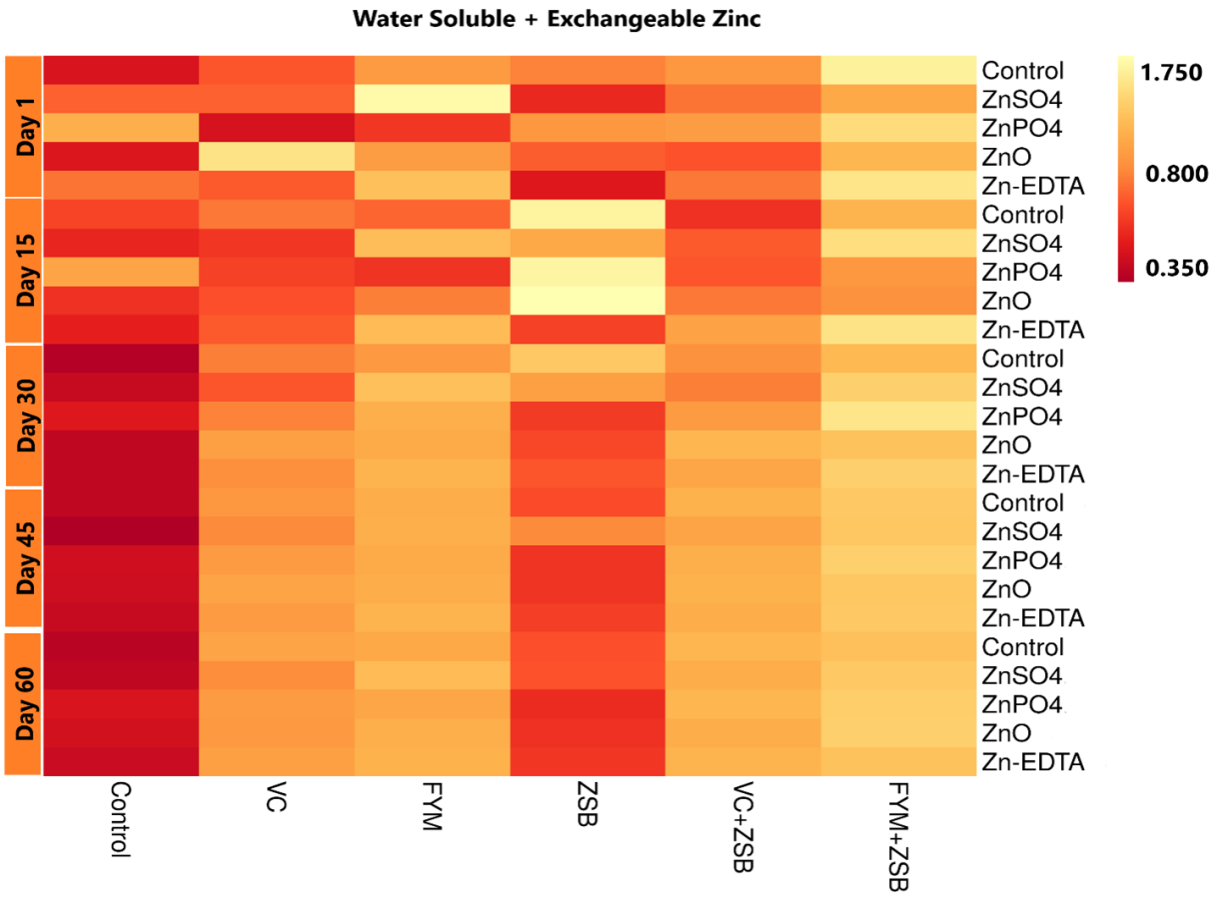

Fig. 2. The heat map illustrates the concentrations of Ws+Ex - Zn at intervals of 1, 15, 30, 45, and 60 days following the incubation period. The colour spectrum spans from deep red to pale yellow on the heat map. Deep red signifies the lowest concentration of water-soluble and Ws+Ex - Zn, while pale yellow represents the highest concentration. An intermediate concentration of Ws+Ex - Zn is denoted by the colour orange.

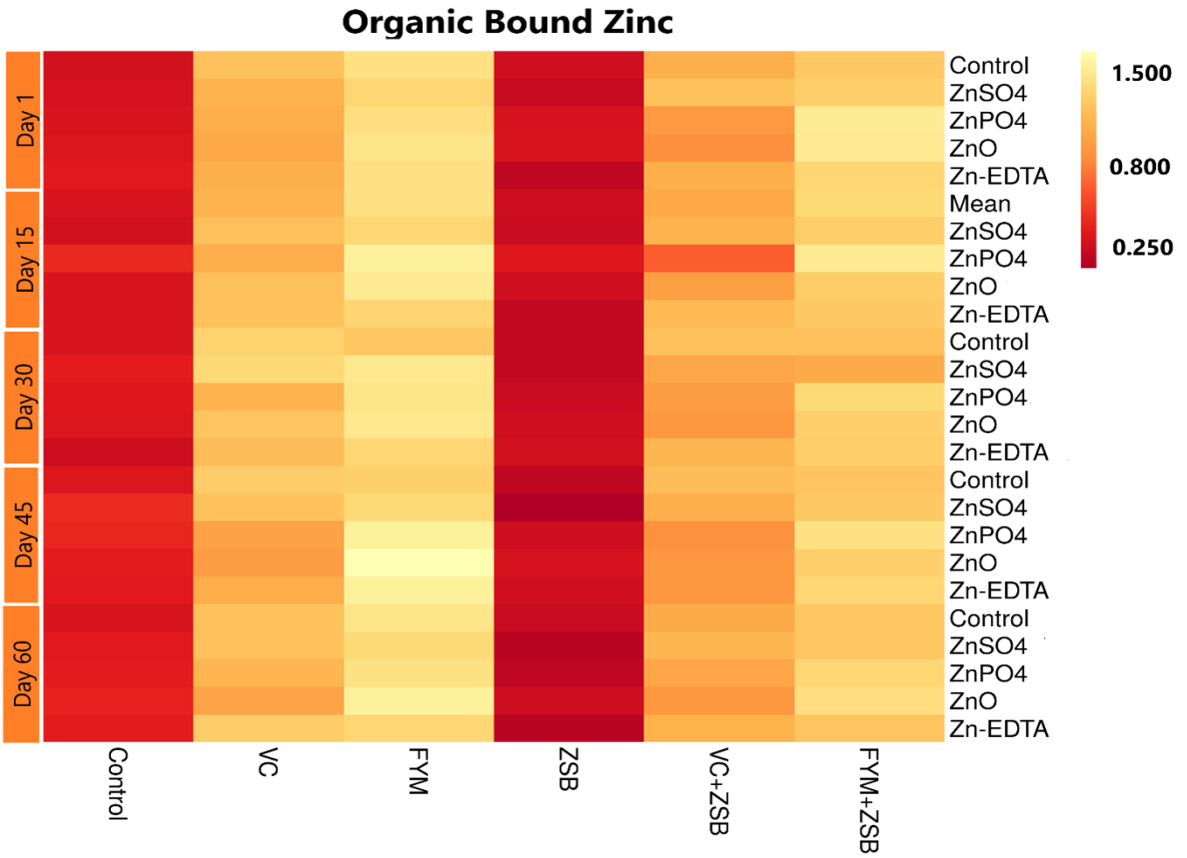

Fig. 3. The heat map illustrates the concentrations of OC-Zn at intervals of 1, 15, 30, 45, and 60 days following the incubation period.. The colour spectrum spans from deep red to pale yellow on the heat map. Deep red signifies the lowest concentration of OC-Zn, while pale yellow represents the highest concentration. An intermediate concentration of OC-Zn is denoted by the colour orange.

$ZnSO_4$ recorded the highest conversion towards the carbonate bound zinc. In $ZnSO_4$ alone percent treatment, 54.04 % of applied zinc was turned into carbonate bound zinc on the 60th day after incubation. At the same time, the $ZnSO_4$+FYM+ZSB recorded only 32.58 % conversion (Fig. 4). Zn-EDTA recorded the highest conversion over Ws+Ex zinc with a maximum of 28.53 % with EDTA+ZSB+FYM combinations. In $ZnSO_4$ and Zn-EDTA added treatments, water soluble and exchangeable zinc were decreased with an increase in incubation days and the lowest was recorded at the final (60th) day of incubation (Fig. 2). The carbonate bound zinc increased with an increase in incubation days in all treatment combinations. Similar day, $ZnSO_4$ recorded the highest carbonate bound zinc (8.60 % of total zinc), and the lowest was observed in the control+ZSB treatment (5.06 %) (Fig. 4).

The dissolution efficiency of ZSB in the decreasing order was zinc oxide > zinc phosphate > zinc sulphate > Zn-EDTA treatment in ZSB alone and organic combination treatments. The solubilisation potential of ZSB was markedly increased when applied with both organic matters (FYM and VC). The highest solubilisation potential was recorded in ZnO+ZSB+FYM (186.4 %) and it was followed by $Zn_3(PO_4)_2$+FYM+ZSB (180.5 %), whereas the lowest solubilisation potential was observed in Zn-EDTA treatment.

In amorphous and crystalline sesquioxide fractions of zinc, increase with an increase in incubation days, compared to crystalline bound zinc, amorphous zinc rapidly adsorbs solution zinc. On the final day of the experiment, the lowest quantity of amorphous (0.598 mg kg^{-1}) and crystalline (1.259 mg kg^{-1}) zinc was in the control + ZSB alone treatment. Previous studies have recorded that the external application of zinc increases amorphous and crystalline iron oxide bound zinc (7, 15).

The residual fraction of zinc (an unavailable fraction for plants) contributed on 1st day of the incubation experiment was 86.2 to 78.0 %, whereas at the end (60th) of the experiment, a slight micro-scale decrease was observed from 85.4 to 77.0 %. On the 60th day, after residual zinc, the carbonate bound fraction contributed the highest percentage of total zinc, ranging from 4.95 to 7.76 % (Fig. 5). An increase in residual Zn was observed in all treatments with added Zn sources. The highest increase was recorded with the ZnPO4 treatment, showing increments of 7.2 %, 6 %, 4.2 %, 4.3 % and 5.6 % over the control at 1st, 15th, 30th, 45th and 60th days after incubation, respectively. There is no significant difference observed with other treatment combinations. The results of (16) support the present finding that the major forms of zinc observed in calcareous soil were found in residual and carbonate forms. Significantly higher residual zinc was observed in inorganic sources applied to soils compared to control.

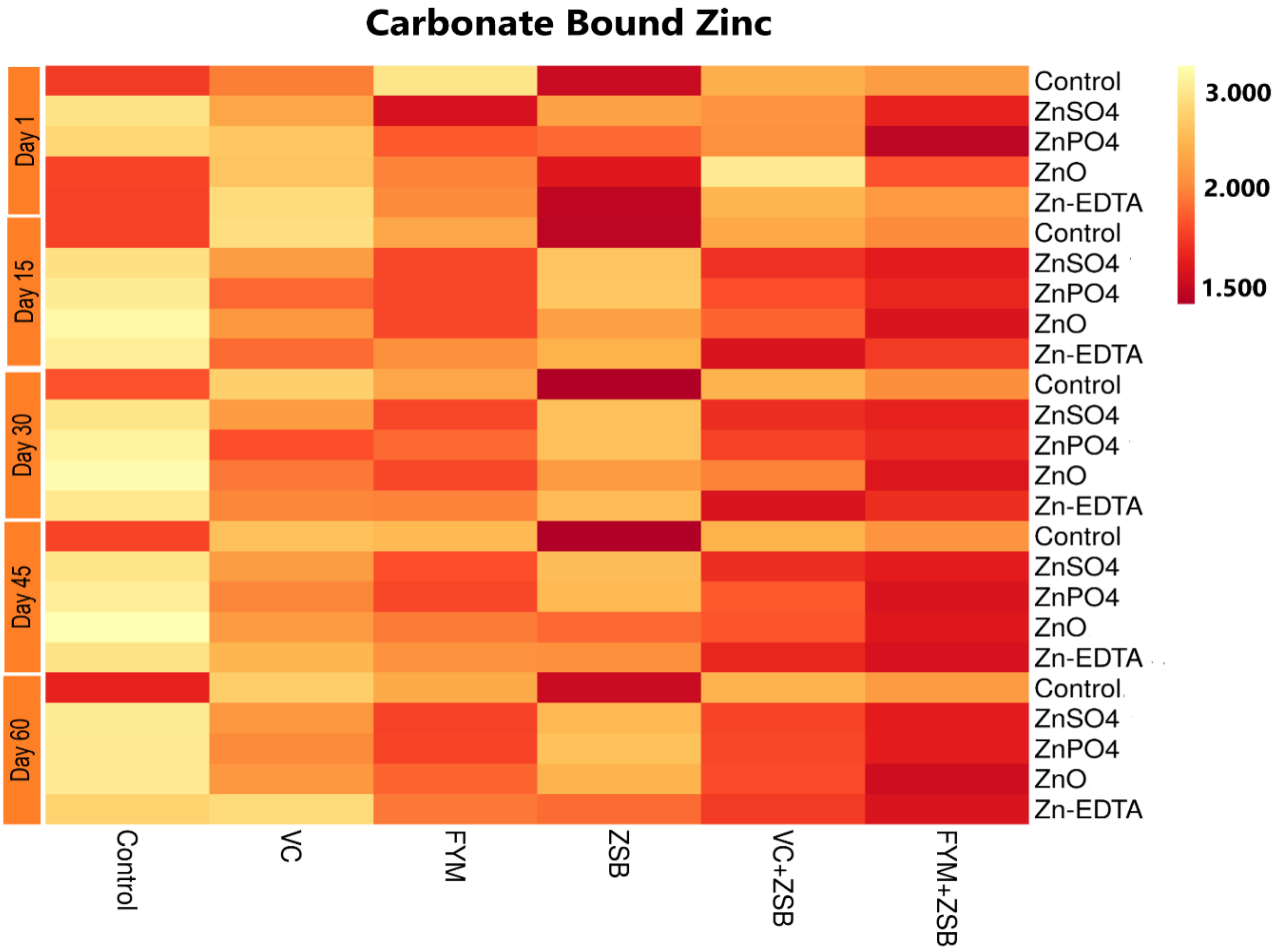

Fig. 4. The heat map illustrates the concentrations of Carb-Zn at intervals of 1, 15, 30, 45, and 60 days following the incubation period. The colour spectrum spans from deep red to pale yellow on the heat map. Deep red signifies the lowest concentration of Carb-Zn, while pale yellow represents the highest concentration. An intermediate concentration of Carb-Zn is denoted by the colour orange.

The multiple regression equation works to find the interrelationship between DTPA with other fractions of zinc (Table 4). The Ws+Ex zinc showed a significant positive coefficient with DTPA-Zn at all intervals of incubation. At the same time, organically bound zinc showed a significantly negative coefficient with DTPA-Zn in all stages.

Discussion

Organically treated soils recorded significantly higher DTPA Ws+Ex and organic zinc in the later stages of incubation. The investigation of few researchers reported that after completion of the experiment, Ws+Ex and organic matter fraction decreased, whereas carbonate, crystalline Fe oxide, amorphous Fe oxide, and Mn oxide fractions increased in calcareous soil (11, 12). A quick fixation of zinc was observed immediately after substitution in calcareous soil. An abrupt decrement in Ws+Ex zinc was observed over $ZnSO_4$ and Zn-EDTA treatments on the 15th day after the initiation of the experiment and a gradual decrement was observed in subsequent days.

The upshot of (17) clutches the proof of 30 % of the applied non-chelated form of zinc fixed in soil on an unavailable form within 14 days of incubation. The solubility of $ZnSO_4$ and Zn-EDTA was higher than other fertilizer sources, which may lead to a higher concentrate solution and exchangeable zinc over other sources (18). The Ws+Ex and organic matter fractions were labile bound and freely mobile, but the carbonate and metal oxide fractions were firmly bonded, whose concentrations were always in ascending order unless there was an interference on soil properties (8). When compared to vermicompost, FYM added soils recorded the highest organically bound zinc fraction at all stages of the incubation experiment. The zinc concentration was higher in vermicompost than in FYM, but the quantity of FYM applied was on average double the time of vermicompost, which leads to higher zinc concentrations being observed in FYM applied soils. The highest bioavailability was observed in FYM+ZSB with all zinc sources including the control throughout the experiment**.** It may be due to the higher reduction of pH by farmyard manure than vermicompost since the applied organics quantitatively differ (FYM-12.5 t ha^{-1} and VC-5 t ha^{-1}), which caused a higher gain in organic carbon in FYM treatment.

The chelated form of zinc has more stability to sustain in solution form than zinc sulphate, which leads to higher bioavailability. Moreover, in artificially chelated zinc, various sources of fixation were restricted by the chelation complex (14, 19).

The carbonate-bound zinc was significantly higher in $ZnSO_4$ treatments in the experiment conducted by some researchers reported, a 6.6 fold increase in carbonate-bound zinc (16, 20). Due to the calcareousness, optimum pH and higher solubility of the applied source ($ZnSO_4$), it resulted in a higher solution concentration of zinc, which accumulated over carbonate and other fractions of zinc (17). Some of the chelated forms are also fixed due to the replacement of Zn by calcium present in the soil and also due to the formation of calcium zincate (21).

Table 4. Multiple regression equation of DTPA Zn with other fractions on different intervals.

DTPA (Days)	Equation	R^2
1	1.816 (ZF1) - 0.595 (ZF2) - 0.054 (ZF3) + 0.226 (ZF4) + 0.155 (ZF5) - 0.176 (ZF6) - 0.032 (ZF7) +1.386^X	**0.995**
15	2.080 (ZF1) - 0.280 (ZF2) - 0.083 (ZF3) -1.108 (ZF4) + 2.579 (ZF5) -1.291 (ZF6) - 0.014 (ZF7) -1.272^X	**0.949**
30	2.177 (ZF1) + 0.224 (ZF2) - 0.251 (ZF3) -1.052 (ZF4) + 0.844 (ZF5) - 0.064 (ZF6) + 0.030 (ZF7) -1.887^X	**0.936**
45	2.019 (ZF1) + 0.369 (ZF2) - 0.027 (ZF3) - 0.644 (ZF4) - 0.646 (ZF5) + 0.662 (ZF6) - 0.015 (ZF7) + 0.402^X	**0.915**
60	2.208 (ZF1) + 0.136 (ZF2) - 0.866 (ZF3) + 0.119 (ZF4) + 0.595 (ZF5) - 0.566 (ZF6) - 0.148 (ZF7) + 3.554^X	**0.934**

ZF1 - Ws+Ex+Zn; ZF2 - Carbonate Zn; ZF3 - Organic Zn; ZF4 - Am Fe Ox Zn; ZF5 - Cry Fe Ox Zn; ZF6 - Mn Ox Zn; ZF7- RES-Zn.; X = Denotes constant

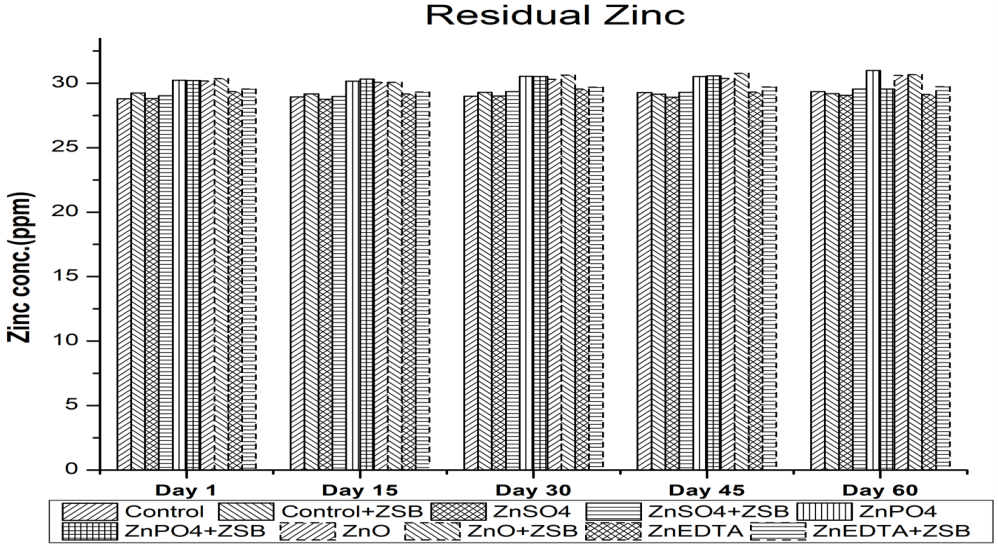

Fig. 5. Zinc sources effect on residual zinc with presence and absence of ZSB.

Significantly higher residual zinc was observed in inorganic sources applied to soils compared to control. The relative proportion of residual zinc with other fractions was decreased in zinc-applied soils with an increase in incubation days; a slight decrement in residual zinc was observed in the percentage residual zinc fraction on all intervals. Previous study on heavy metal fractionation on compost-amended soil showed a reduction in the residual form of zinc concluded due to organic matter zinc adsorption by uptake (22).

The higher solubilisation of zinc was recorded in zinc oxide treatment. The *Pseudomonas spp.* solubilisation is of higher efficiency with zinc oxide medium compared to zinc phosphate (9). $ZnSO_4$ and Zn-EDTA yield a higher concentration of bio-available zinc, which diminishes the growth and solubilisation efficiency of ZSB and the production of 2-ketogluconic acid and gluconic acid produced by *Pseudomonas spp.* (23-25). Among the fertilizers applied to soils, zinc phosphate recorded the lowest contribution towards carbonate, Cry Fe-Ox and Am Fe-Ox zinc compared to other fertilizers. It may be due to the presence of phosphorus in zinc phosphate fertilizer that increases the competition for fixation over free carbonates and adsorption sites of iron and aluminium oxides, which reduce the lower contribution of adsorbed zinc towards respected fractions (26, 27).

Conclusion

Fertilization has a greater impact on all forms of zinc, mainly water-soluble + exchangeable and carbonate-bound zinc. The presence of free $CaCO_3$ significantly influences the plant availability of Zn in calcareous soil. A consistent supply of reclamation buffers is needed to solubilize or protect bioavailable zinc in its form. Applied EDTA-Zn served as a suitable inorganic source for calcareous soil to maintain a solution zinc concentration. However, its durability was greatly affected by the properties of calcareous soil. In the case of $ZnSO_4$, it showed greater fixation and was economically costlier than other sources. Considering the cost economy, the zinc compounds like ZnO and $Zn_3(PO_4)_2$ were a little lower and involved greater solubilisation efficiencies with organic and microbes than preceding Zn sources. In untreated calcareous soil, the residual and carbonate fractions of zinc were predominantly present. The low recovery of soluble zinc fertilizers like $ZnSO_4$ in calcareous soils is mainly due to the conversion of zinc to a less available form. However, the bioavailability of zinc was increased with the application of organic materials.

Acknowledgements

The authors are thankful to Tamil Nadu Agricultural University for facilitating needed laboratory and miscellaneous resources.

Authors' contributions

KS conducted the laboratory experiment; DS participated in hypothesis formulation and design of the experiment; RV helped in Zn fraction analysis; BB worked on statistical analysis; VV carried out graphical representation of data; VV carried out grammatical checking; VRS participated in the lab experiment.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

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