International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(3): 1092-1097

Received: 13-03-2019
Accepted: 15-04-2019

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Influence of moisture on zinc fractions and its association with soil properties in two different types of soils of Karnataka

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Abstract
An incubation study was conducted to be acquainted with the distribution of different forms of soil Zn in two different types of soils under three different moisture regimes (submergence, saturation and field capacity) for 90 days period. The results revealed that various chemical pools of Zn in soils treated with graded levels of Zn and N fertilizers application (0, 37.5 and 50 kg ha^{-1} Zn as $ZnSO_4$ and 0, 125 and 150 kg ha^{-1} N as Urea) varied significantly across the moisture regimes and at different incubation periods. The water soluble and exchangeable zinc (WSEX-Zn) fraction reached the maximum content (2.75 mg kg^{-1}) in M1N4 at 90 days after incubation (DAI) whereas M1N1 at 30 DAI recorded the lowest value (0.08 mg kg^{-1}). Organic carbon content exhibited a significant positive correlation with water soluble and exchangeable zinc, Organic matter and Manganese oxide bound zinc in normal soil at 0 DAI. The mobility factor percentage at 60 DAI followed the order (13.5) >15 DAI (12.8)> 90 (11.8)> 30 (11.6) > 45 (7.5) in normal soil, whereas the mobility factor in saline soil reduced in the order: 15 DAI (10.1)>30 DAI (9.8) > 60 DAI (6.1)>45 DAI (4.3) > 90 DAI (2.8). Among the different moisture levels, field capacity (M3) level significantly increased WSEX-Zn (0.94 and 0.97) as compared to M1 and M2 levels in both soils. These results indicated that while added Zn and N significantly increased the WSEX- Zn fraction of soil, the latter is adversely affected due to longer period of submergence thereby causing a reduction in the Zn availability compared to soils at saturation and field capacity.

Keywords: Added Zn and N, mobility factor, Zn fractions, submergence condition

Introduction

Zinc is known to occur in soil in a number of discrete chemical forms differing in their solubility and thus availability to plants (Sarkar and Deb, 1982) [17]. Zinc exists in five distinct pools in soils viz., water soluble, exchangeable, adsorbed, chelated or complexed zinc. These pools differ in strength (or reversibility) and therefore in their susceptibility to plant uptake, leaching, and extractability. The equilibrium among the different pools is influenced by pH, Eh, and concentration of zinc and other cations, particularly iron and manganese. The readily available zinc forms *viz.*, water soluble, exchangeable and chelated zinc forms are in reversible equilibrium with each other (Viets, 1962) [20]. Zinc in soluble organic complexes and exchange positions is responsible for the maintenance of sufficient zinc level for wetland rice (Murthy, 1982) [11]. Among the different Zn- fractions, the water soluble plus exchangeable zinc, organically complex zinc, amorphous sesquioxide bound zinc and crystalline sesquioxide bound zinc could explain 95.2 per cent of the variability of zinc concentration in wetland rice (Mandal and Mandal, 1986) [9]. Water soluble plus exchangeable and organically complexed forms are considered to be available, amorphous sesquioxide bound form is potentially available and crystalline sesquioxide bound and residual Zn forms are unavailable to plants (Mandal *et al.* 1992) [10]. About five or less than five per cent of total zinc present in soil is available to plants at any given time. The application of Zn at different levels is known to increase the various fractions of Zn in soil (Talukder *et al*. 2011) [18]. Typically soil total Zn ranges between 10-300 mg kg^{-1} with a mean value of 55 mg kg^{-1} (Kiekens, 1995), however knowledge of total Zn provides only limited information about its transformation and bio-availability. Although total soil Zn concentration may be high, deficiencies arise because Zn availability depends on the chemical forms of soil Zn. Application of Zn fertilizers can temporarily help offset plant Zn deficiency symptoms. Hence, it is important to have a better insight into the transformation of soil zinc fractions, which in turn will help in understanding the contribution of individual zinc form to its availability to plants.

Antagonistic and synergistic effects between applied nutrients have been studied in various crops and soils. But, the interaction effect between applied zinc and nitrogen on different zinc fractions has not been studied extensively. Keeping this in view, the objectives of the present investigation were a) to have a better understanding of the transformation of soil zinc fractions by the integrating theapplication of N with Zn in two different types of soils under various moisture regimes and b) also to correlate soil properties with various zinc fractions.

Materials and Methods

An incubation study was conducted at 28°C during 2016to study the release pattern of Zn fractions following the external application of Zn through $ZnSO_4$. The experiment was conducted with normal soil (EC <4 ds m^{-1}) and saline soil (EC >4 ds m^{-1}) collected from the surface layer (0 – 20 cm) of a TypicVertisol (Table 1). The soil samples were air-dried, powdered and sieved through 2 mm nylon sieve. There were 27 treatments consisting of 3 levels of moisture regimes (submergence, saturation and field capacity) and 3 levels of Zn (0, 37.5 and 50 kg Zn $ha^{-1}$and 3 levels of N (0, 125 and 150 kg N ha^{-1}) in the form of zinc sulphate and Urea, respectively imposed on two different kinds of soils. The incubation study was continued up to 90 days. The experiment was laid out in Completely Randomized Design with three replications. Measurements for different fractions of soil Zn were done at six periods i.e. 0, 15, 30, 45, 60 and 90 days after incubation (DAI).

Incubation Method

One hundred g of air-dried 2 mm sieved soil sample was weighed into an incubated polythene bottle (125 ml). Different amounts of Zn as $ZnSO_4$ were added as per treatment combinations and mixed thoroughly by glass rod after adding water. To maintain different moisture conditions in the incubated bottles, water was added as per requirement of the soil. The soils were incubated at about 28˚C, and maintained for 0, 15, 30, 45, 60, 75 and 90 days under incubated condition. All the soils were treated with basal dose of 100% RDF (60: 60 – P_2O_5:K_2O) in the form of SSP and MOP and FYM at the rate of 10 tonnes per ha was mixed with the soil. Zinc treatments were added in the form of solution, which was mixed uniformly with soil. Each incubated soil sample was analyzed for pH, EC and OC.

Soil Zn fraction (fertility fractions) were determined using a modified sequential extraction procedure of Murthy (1982) [11] and adopted by Mandal *et al*. (1992) [10]. Total Zn in soils has beendivided into seven fractions (Table 1). Initially, Zn was extracted from soils using 50 mL plastic centrifuge tubes with individual extracting solutions (see Table 1) and shaken in a rotary shaker at room temperature (25 ± 2 °C). The supernatant solutions obtained from each successive stage of extraction were centrifuged at 3,500 rpm for 15 min, decanted and filtered through Whatman No. 42 filter paper. It was ensured that there was no time lag between addition of extractant and shaking of the tubes. Total Zn (T Zn) was calculated as the sum of all the fractions determined. Three replicates of all sequential extractions and analysis were performed for each soil sample (Table 2).

Table 1: Physical and chemical properties of soils used for incubation experiment

S. No	Parameters	Value	
		Normal soil	Saline soil
1.	pH (1: 2.5)	7.96	8.33
2.	Electrical conductivity (dS m^{-1})	1.7	7.0
3.	Organic carbon (g kg^{-1})	5.3	2.4
4.	Available Nitrogen (Kg ha^{-1})	420	194
5.	Available Phosphorous (Kg ha^{-1})	43	20.2
6.	Available Potassium (Kg ha^{-1})	599	414
7.	Calcium (meq per 100g soil)	7.3	8.34
8.	Magnesium (meq per 100g soil)	2.74	1.22
9.	Zinc (ppm)	0.60	0.46
10.	Copper (ppm)	1.64	0.92
11.	Iron (ppm)	6.58	6.10
12.	Manganese (ppm)	9.36	8.91
13.	ESP (%)	21.4	67.4
14.	SAR	5.8	42.4
15	CEC (meq per 100 g soil)	65.7	48.9

Table 2: Sequential extraction procedure for zinc in soil

Fractions	Extractant	Conditions Solution Soil (g): Solution (ml)	Reference
Water soluble + Exchangeable (WSEX-Zn)	1M $(NH_4)OAc$ (pH 7.0)	5: 20; shake for 1 hour	Murthy (1982) [11] modified by Mandal & Mandal (1986) [9]
Organically complexed (OM- Zn)	0.05 M $Cu(OAc)_2$	5: 20; shake for 1 hour	Murthy (1982) [11] modified by Mandal & Mandal (1986) [9]
Crystalline sesquioxide bound (CRYOX-Zn)	0.3 M Sodium citrate + 1.0 M $NaHCO_3$ + 1 g $Na_2S_2O_4$ [Citrate-Bicarbonate- Dithionite (CBD)]	Boiling water bath, 10 min, stir occasionally, keep on water bath (70 – 80°C), 15 min, stir occasionally	Murthy (1982) [11] modified by Mandal & Mandal (1986) [9]
Amorphous sesquioxide bound (AMOX-Zn)	0.2 M$(NH_4)_2C_2O_4.H_2O$ + 0.2 M $H_2C_2O_4$ (pH 3.0)	5: 20 shake 1 hour	Murthy (1982) [11] modified by Mandal & Mandal (1986) [9]
Manganese oxide bound (MnOX- Zn)	0.1 M $NH_2OH.HCl$ (pH 2.0)	Shake 30min	Chao (1972)
Res* (Residual)	HCl+ HNO_3conc	1: 08 Aqua regia	

*Samples are dried and finely grounded

Results and Discussions

Water soluble and exchangeable zinc fractions (WSEX-Zn)

The WSEX-Zn fraction showed an increasing trend for added levels of Zn and N as compared to control (Table 3). Across different incubation time intervals and moisture levels, the WSEX-Zn fraction reached the maximum content (2.75 mg kg^{-1}) under M1N4 at 90 DAI whereas M1N1 at 30 DAI recorded the lowest value (0.08 mg kg^{-1}).Combined application of Zn and N significantly increased WSEX- Zn fractions under all three moisture regimes to a maximum value of 1.83 mg kg^{-1} in N8 treatment under M1 (continuously submergence). Further, the lowest value (0.69 mg kg^{-1}) was reported under M1 treatment combined with no addition of Zn and N. Among the various moisture regimes, M2 (1.26 mg kg^{-1}) recorded maximum WSEX- Zn fraction during the study period.

Table 3: Effect of different moisture and nutrient levels (Zn and N) on Water soluble and exchangeable Zn (WSEX- Zn) fraction in normal soils during the incubation period

Normal soil	M1					M2						M3			
	15 DAI	30 DAI	60 DAI	90 DAI	Mean	15 DAI	30 DAI	60 DAI	90 DAI	Mean	15 DAI	30 DAI	60 DAI	90 DAI	Mean
N1	0.91	0.08	0.42	1.33	0.69	0.38	0.23	0.67	2.17	0.86	0.97	0.72	0.49	1.92	1.03
N2	0.90	0.15	0.24	1.58	0.72	1.24	0.34	0.61	1.92	1.03	1.00	0.38	0.55	2.00	0.98
N3	0.86	0.23	0.36	1.50	0.74	0.76	0.15	0.42	1.58	0.73	0.81	0.42	1.15	1.92	1.08
N4	0.90	0.53	0.65	2.00	1.02	0.95	0.80	1.70	2.58	1.51	1.76	0.30	1.03	2.00	1.27
N5	1.21	1.30	1.03	2.75	1.57	1.90	1.68	0.85	2.33	1.69	1.81	0.57	1.03	2.42	1.46
N6	1.57	0.42	0.79	2.00	1.20	1.00	1.90	1.03	1.75	1.42	1.67	0.37	1.09	2.00	1.28
N7	1.29	0.72	0.79	2.00	1.20	2.33	1.10	0.73	2.25	1.60	1.48	0.11	1.52	2.00	1.28
N8	1.95	1.49	1.94	1.92	1.83	1.43	0.50	0.97	2.25	1.29	1.33	0.80	0.97	2.50	1.40
N9	2.10	1.03	0.91	1.87	1.48	0.62	1.07	1.09	2.00	1.20	2.10	0.69	0.61	2.17	1.39
Mean	1.30	0.66	0.79	1.88	1.16	1.18	0.86	0.90	2.09	1.26	1.44	0.48	0.94	2.10	1.24

At 15 DAI;	At 30 DAI	At 60 DAI	At 90 DAI
MSEd=0.02CD= 0.04	MSEd=0.01 CD= 0.012	MSEd=0.01CD= 0.013	MSEd=0.02CD= 0.032
NSEd= 0.03CD=0.06	NSEd= 0.01 CD=0.021	NSEd= 0.012CD=0.023	NSEd= 0.03CD=0.06
MN SEd=0.05CD= 0.11	MN SEd=0.02 CD=0.034	MNSEd=0.02CD=0.04	MN SEd=0.05CD=0.09

The WSEX-Zn fraction in saline soils ranged from 0.03-2.08 mg kg^{-1} with an average value of 0.45 mg kg^{-1} across the various moisture, nutrient levels as well as incubation periods. Irrespective of incubation period and moisture levels, N6 treatment recorded the highest mean value (0.73 mg kg^{-1}). As in case of normal soils, the WSEX-Zn fraction showed an increasing trend for added levels of Zn and N as compared to control (Table 4) Among different incubation time intervals and moisture levels, the WSEX-Zn fraction reached the maximum content at M2N7 (2.08 mg kg^{-1}) at 60 DAI, whereas M2N9 at 30 DAI reported the lowest value (0.03 mg kg^{-1}). Among the various moisture regimes, M3 (0.50 mg kg^{-1}) recorded maximum WSEX- Zn fraction. Continuous submergence recorded minimum WSEX-Zn content in both soils at different incubation periods. These findings are in line with Hazra *et al*. 1987 [6].

Table 4: Effect of different moisture and nutrient levels (Zn and N) on Water soluble and exchangeable Zn (WSEX- Zn) fraction in saline soils during the incubation period

Nutrient levels	M1					M2					M3				
	15 DAI	30 DAI	60 DAI	90 DAI	Mean	15 DAI	30 DAI	60 DAI	90 DAI	Mean	15 DAI	30 DAI	60 DAI	90 DAI	Mean
N1	0.12	0.06	0.11	0.44	0.18	0.16	0.55	0.90	0.20	0.45	0.66	0.06	0.56	0.12	0.35
N2	0.16	0.20	0.23	0.44	0.26	0.04	0.41	0.79	0.20	0.36	0.33	0.12	0.56	0.08	0.27
N3	0.25	0.20	0.23	0.53	0.30	0.04	0.38	0.73	0.36	0.38	0.37	0.12	0.62	0.24	0.34
N4	0.41	0.29	0.45	0.48	0.41	0.08	0.29	0.62	0.53	0.38	0.37	0.17	0.79	0.40	0.43
N5	0.41	0.32	1.30	0.32	0.59	0.25	0.26	0.45	0.57	0.38	0.45	0.23	1.69	0.53	0.73
N6	0.45	0.38	0.68	0.20	0.43	0.33	0.26	0.96	0.57	0.53	0.66	0.29	0.85	0.53	0.58
N7	0.45	0.41	0.96	0.08	0.47	0.33	0.09	2.08	0.40	0.73	0.25	0.35	1.13	0.44	0.54
N8	0.33	0.38	0.79	0.12	0.40	0.58	0.06	0.56	0.32	0.38	0.25	0.38	0.96	0.57	0.54
N9	0.33	0.55	0.68	0.16	0.43	0.54	0.03	1.18	0.12	0.47	0.16	0.32	1.58	0.81	0.72
Mean	0.33	0.31	0.60	0.31	0.39	0.26	0.26	0.92	0.36	0.45	0.39	0.23	0.97	0.41	

At 15	At 30 DAI	At 60 DAI	At 90 DAI
MSEd=0.003CD= 0.006	MSEd=0.002 CD= 0.004	M SEd=0.01CD= 0.014	M SEd=0.003CD=0.005
NSEd= 0.005CD=0.010	NSEd= 0.004CD=0.008	NSEd= 0.012CD=0.024	N SEd= 0.005CD=0.009
MNSEd= 0.009CD=0.017	MN SEd=0.006CD=0.013	MN SEd=0.02CD=0.042	MN SEd=0.008CD=0.015

M1: Continuous flooding, M2: Safe AWD, M3: Saturated soil culture, N1: Zn_0N_0; N2: Zn_0N_{125}; N3: Zn_0N_{150};
N4: $Zn_{37.5}$ N_0; N5: $Zn_{37.5}$ N_{125}; N6: Zn $_{37.5}$ N_{150}; N7: Zn_{50} N_0; N8: Zn_{50} N_{125}; N9: Zn_{50} N_{150}

Significance of moisture on mobility factor of zinc in two types of soils

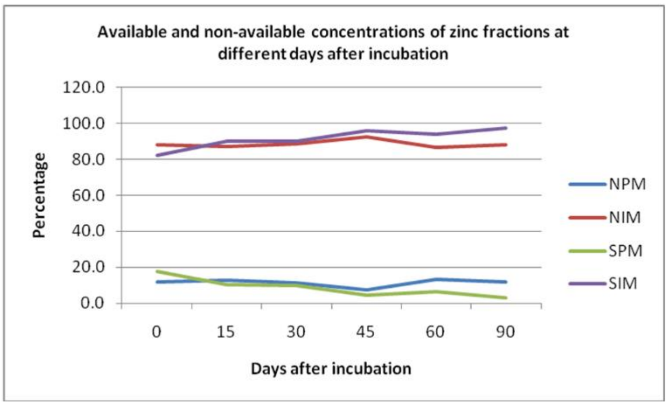

Fig 1: Available and non-available concentrations of zinc fractions at various interval periods

Mobility factor of zinc in soils under study

The mobility factor value determines the Zn present in mobile form or those which are biologically available to the plants. High mobility factor (MF) values have been reported or interpreted as evidence of relatively high reactivity, high liability and high biological availability of heavy metals in soil (Kabala and Singh, 2001; Ramzan *et al.* 2014) [7, 15]. Mobility factor was calculated as the sum of all the mobile fractions of Zn, viz., WSEX, OMx, AMOX and CRYOX (Tessier *et al.* 1979; Kabala and Singh, 2001) [19, 7]. The mobility factor percentage was observed to be maximum (Fig 1) in 60 DAI (13.5) >15 DAI (12.8)> 90 (11.8)> 30 (11.6) > 45 (7.5) in normal soil whereas in saline soil the order was 15 DAI (10.1)>30 DAI (9.8) > 60 DAI (6.1)>45 DAI(4.3) > 90 DAI (2.8).It clearly showed that plant available zinc is limited to the crops grown in saline soil even after application of nutrients (Zn and N). It is attributed to low levels of labile or bio available Zn, the low levels of native Zn, slow solubilization Zn in soil, strong adsorption of Zn and leaching of Zn dissolved with OM.Similar results were reported by Rieuwerts *et al.* (2006) [16].

Correlation Coefficient of Different forms of Zn with soil properties

Data on correlation coefficient between selected soil properties with different forms of zinc for two soils are presented in Table 5. The results revealed that almost all the fractions show more or less similar correlation trend with pH and electrical conductivity at 0 and 90 DAI. Significant and negative correlation between OM- Zn fractions with pH at 0 DAI and between OC- Zn fraction and EC at 90 DAI were observed. Soil organic carbon content showed a significant positive correlation with water soluble and exchangeable zinc, OM bound Zn and Manganese oxide bound zinc in normal soil at 0 DAI whereas in saline soil, residual zinc fraction was positively correlated with organic carbon. The negative correlation of different forms of zinc with pH may be due to the fact that at higher pH, insoluble calcium zincate or higher oxides of zinc are formed and zinc bound in these forms does not come into solution easily. This is in line with the findings of Kumar *et al*. (2010) [8]. The significant positive correlation of organic bound zinc with soil organic carbon (r= 0.41) suggests that the organic matter and clay provide exchange sites for the adsorption of zinc (Preetha and Stalin, 2014) [14]

Table 5: Correlation coefficients between different fractions of zinc and soil properties

At 0 DAI	WSEX-Zn	OC- Zn	CRYOX- Zn	AMOX- Zn	MnOX- Zn	Res- Zn	T- Zn
Normal soil							
pH	-0.1602	-0.2316*	-0.0702	-0.183	-0.0131	-0.2342	-0.2516
EC	-0.17181	-0.0456	-0.1735	-0.0802	-0.06584	-0.1859	-0.1429
Organic carbon	0.2252*	0.3156*	0.0839	0.0696	0.3412*	0.1179	0.1452
Saline soil							
pH	-0.0140	-0.1025	-0.1034	-0.0762	-0.0281	-0.0076	-0.047
EC	-0.0245	-0.1994	-0.225*	-0.080	-0.1608	-0.1839*	-0.1137
Organic carbon	0.01193	0.0818	0.1589	0.0438	0.1045	0.1692*	0.1247
At 90 DAI							
Normal soil							
pH	-0.0653	-0.2413	-0.0338	-0.0444	-0.1701	-0.0285	-0.2362
EC	-0.1386	-0.2056*	-0.0066*	-0.0040	-0.1716	-0.4369*	-0.4619*
Organic carbon	0.5409*	0.4108*	0.3893	0.1479	0.5519*	0.2049	0.1445
Saline soil							
pH	-0.0199	-0.05351	-0.0985	-0.0180	-0.0934	-0.0083	-0.0137
EC	-0.1398	-0.2115	-0.3197*	-0.1939	-0.1713	-0.0368	-0.0662
Organic carbon	0.1809	0.3593	0.0596	0.5072	0.0283	-0.4186	0.4387

Significant variations in all fractions were observed at all intervals of incubation study. The first study at 0 DAI produced the highest WSEX-Zn, which sharply fell down at 30 DAI and increased at 45 DAI (Fig 2). Thereafter, gradually decreased at 60 DAI and steeply increased at 90 DAI. Similarly, OM bound Zn decreased sharply at 15 DAI and increased up to 60 DAI, and gradually decreased at 90 DAI.

It is evident from the data presented in Fig 2a and 2b that the distribution of different fractions of Zn in soils was significantly affected by moisture and Zn and N application. In normal soil, the order was crystalline bound zinc < manganese oxide bound (Mn-OX) <WS and Ex<<organically bound zinc (OM)<amorphous sesquioxide bound (AMOX)<residual zinc (RES) whereas in saline soil, the fraction was followed the order: WSEX< amorphous sesquioxide bound<organically bound zinc (OM)< manganese oxide bound (MN-OX)<crystalline bound zinc<residual zinc (RES). The higher content of amorphous sesquioxide bound as compared to crystalline bound zinc in normal soil could be attributed to greater ability of latter to adsorb zinc because of their higher surface area (Devis and Leckie, 1978) [3].

In normal soil, WSEX- Zn attained a peak at 90 DAI and OM-Zn, at 30 DAI for OM- Zn. The per cent contribution of CRY-Zn (2.3), AM-Zn (3.2) and Mn-Zn (4.7) to the total Zn were highest at 60 DAI. WSEX-Zn fraction was the lowest among all the zinc fractions in saline soil. The value steeply decreased from 0.95 to 0.36 throughout the incubation period. High buffering capacity of these soils resulted in low amount of WSEX-Zn fractions. Similar findings were reported by Ghane (2011) [5]. The major portion of fraction in the soil is RES-Zn with values ranged from 56.1-90.5 mg kg^{-1} soil. The per cent contribution of this fraction to total Zn was higher than other fractions with 85.9 and 90.5% in normal and saline soil, respectively. It is considered as the primary form of the native Zn and associated with soil mineral fractions. Similar findings were also reported by Dhane and Shukla (1995) [4].

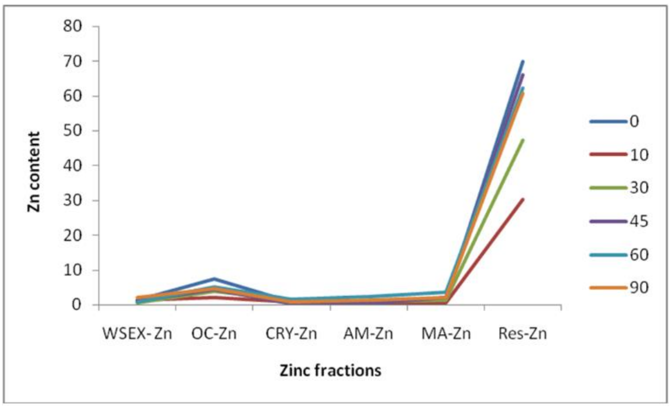

Fig 2a: Influence of moisture and nutrient levels on different fractions of zinc in normal soil

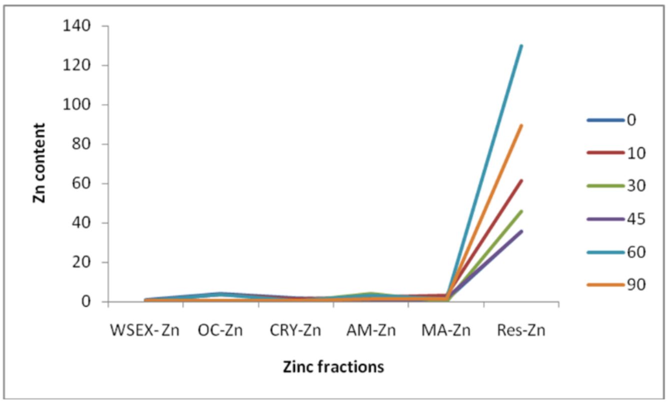

Fig 2b: Influence of moisture and nutrient levels on different fractions of zinc in saline soil

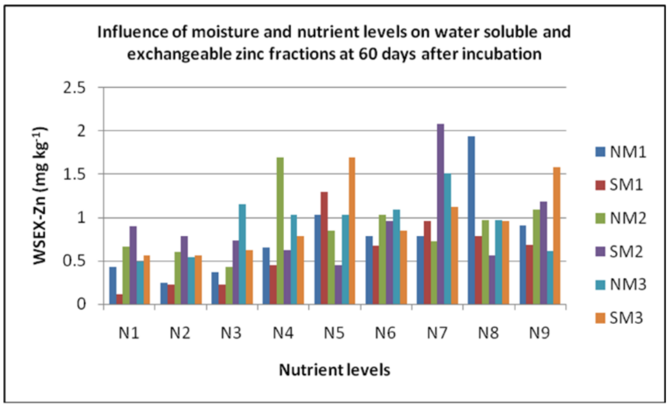

Fig 3: Influence of moisture and nutrient levels on water soluble and exchangeable zinc fractions at 60 days after incubation

Different treatments showed significant variation in WSEX-Zn fractions at 60 DAI. The highest WSEX-Zn (1.29 mg kg^{-1}) was recorded in N8 which was significantly higher as compared to other treatments (Fig 3) in normal soil. The treatment N7 recorded the highest value (1.39 mg kg^{-1}) of WSEX-Zn followed by N5 (1.15 mg kg^{-1}), that was at par with N9 (1.15 mg kg^{-1}). Among the different moisture levels, field capacity (M3) level significantly increased WSEX-Zn (0.94 and 0.97) as compared to M1 and M2 levels in both soils. These results indicated that added Zn and N play a very significant role in improving the WSEX- Zn fraction of soil, which is adversely affected due to longer period of anaerobic incubation. Anaerobic condition always causes a reduction in the Zn availability (Pavanasasivam and Axley, 1980 and Talukder*et al*. 2011) [13, 18].

Conclusions

Results of the study led us to conclude thatcontribution of plant available form of Zn fractions (WSEX-Zn and OM bound Zn) to the total Zn content was least and also reveals that moisture levels and nutrient (Zn and N) applications greatly influence the different zinc fractions in soils. Further, it confirms that integration of zinc and nitrogen influences readily available forms of zinc which is essential for the growth and development of crops grown in any soil. Mobility factor value clearly shows the difference in biologically available zinc in normal and saline soils at different incubation periods. Finally, the results suggest that fertilizer application; soil controlling factors on Zn fractionation and bioavailability are the areas that require more studies exclusively in heavy clay soils.

Acknowledgement

The authors acknowledge the advisory committee members and Sh.P.Mohan Kumar who provided their valuable guidance and technical support to conduct this experiment.

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