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Effect of EDTA and Na_2EDTA along with Mussoorie rock phosphate and Tata basic slag on N,P,K,Ca,Mg,Na and Fe uptake by Paddy Field

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***Abstract**: Chelates such as EDTA and DTPA are often used to increase micronutrient solubility, but micronutrients complexed by these chelates are not readily taken up by plants. A field experiment was conducted to assess the effect of the application of EDTA coupled with Tata basic slag and Mussorie Rock Phosphate on* Rice . *The aim of this study was to identify the interaction of Na,Ca,Mg,Fe,Cu and Zn when the rice was planted on the test soil and to evaluate the effect of EDTA ,Na_2EDTA with MRP and TBS of these metals. It was found from the study that by applying increased the organic carbon, organic matter, micronutrient i.e. Fe,Cu and Zn in the soil. Further it was noticed that TBS, MRP increases the N,P and K content in soil. From the results of this experiment, it could be concluded that combined application of MRP and TBS improved micronutrients in rice.*

***Key Words:** EDTA,Interaction,Fe,Cu,Zn,Micronutrients,Mussorie Rock Phosphate,Tata Basic Slag*

I. Introduction:

"More than 2 billion people in the world today are estimated to be deficient in key vitamins and minerals, particularly Vitamin A, iodine, iron, and zinc. Most of these people live in low-income countries and are typically deficient in more than one micronutrient" (WHO, World Food Programme, & UNICEF, 2007). Essential nutrients are taken up by plant roots and translocated to different plants parts. The availability of these essential elements varies from place to place may be due to the soil, environment and microbial activities. The amount of nutrient taken up by plants is a function of the amount of available nutrients in the soil. Micronutrients are essential for the normal growth of plants. Deficiencies of micronutrient drastically affect the growth, metabolism and reproductive phase in plants, animal and human beings. Wide spread deficiencies of micronutrients has been found in Indian soils(Singh,2009)

This article is part of an overall study in which the effects of EDTA (with Tata Basic Slag and Mussorie Rock Phosphate),on the extractability and accumulation of micronutrients and Fe,Cu and Zn by Rice crop. The Mussorie Rock Phosphate is a sedimentary type of phosphate rock. Tata basic slag is a phosphate bearing byproduct of the steel industries .Studies showed that MRP and TBS were as efficient as superphosphate(Verma et al,1993,Kaleeswari et al,2001)For this purpose an artificially contaminated soil sample was used. EDTA has been utilized in micronutrient fertilizers since the1950 s (Wallace et al., 1992; Bucheli-Witschel and Egli, 2001) It was (Wallace et al. 1974) who first reportedthat metal–EDTA complexes could increase solubility and phytoavailabilityof metals in soils.

For more than 40 years, synthetic chelates have been used to supply plants with micronutrients in both soil and hydroponics. Some evidence suggests that the Fe-chelate EDTA can be absorbed by plants and translocated to shoots (Weinstein et al.; 1954, Hill-Cottingham and Liyod-Jones 1961,1965.) High organic matter (O.M.)content and cation exchange capacity (CEC) are some of the most important soil factors that determine the bioavailability of metals to plants (Liphadzi and Kirkham, 2005). Several studies showed that chemical amendments such as chelating agentsincrease metal availability and uptake by plants (Blaylock et al., 1997; Huang et al., 1997; Wu et al., 1999; Kirkham, 2000;Madrid et al., 2002). EDTA effectively increased the mobility of total water-soluble macronutrients (Ca, K, Mg, P) and micronutrients (Fe, Mn) in the soil solution. Its application may increase the fertility of the soil and also in maintaining the soil pH. Mussoorie rock phosphate was 44-52% as efficient as concentrated superphosphate (Kaleeswari et al,2001).

Low dietary intake of Fe and Zn appears to be the major reason for the widespread prevalence of Fe and Zn deficiencies in human populations. In countries with a high incidence of micronutrient deficiencies, cereal-based foods represent the largest proportion of the daily diet (Cakmak 2008).

II. Materials and Method:

i)Soil Sampling: Soil was taken from the surface layer(0-10cm) of a paddy field from the area situated on the right bank of river Yamuna and the left side of Agra-Delhi National Highway about 5km away from Agra city. The pH value of the soil was measured by using glass electrodes in the solution of soil in water in 1:1 ratio.[Smith et al ,2004)]The soil is sandy loam with pH 7.34.The soil samples were collected 5cm diameter sampler in zigzag along different transect of area until the whole area 3mx3m was covered. About 2 kg composite samples from 10-20 sub samples were taken in plastic bags of 4kg capacities by quartering technique(EPA,1992). Composite samples of soil were rapidly air dried and then dried in oven at 105-110^{0}C.Later it was ground to pass 60 mesh sieve. The total concentration of Zn,Cu and Fe in the soil samples were determined by using AASas described by Suruchi (2013),and cation exchange capacity by reported method(Skinner et al,2001).

The samples were analyzed for total nitrogen by Kjeldhal method(Sahrawat,1983),Ca and Mg content by EDTA titration method(IITA,1982),Na and K by Flame photometer(Aleksandraet al,1975) and P by colorimeter method(Gupta et al ,1993).(Table-1)

TABLE I:**Physico chemical properties of the studysoil**

pH	Oma	OCb	CECc	Total concentration of heavy metals(mg kg-1)d					
	(%)	(%)	(cmol(+)kg-1soil)	ZnT	ZNA	CuT	CuA	FeT	FeA
7.34	8.5	4.8	12.9	58.8	1.8	52	1.6	22190	33.3

[a]OM-Organic Matter
[b]OC-Organic Carbon
[c]CEC-Cation Exchange Capacity
[d] heavy metals.,subscript T indicates the total and A refer to available values

The field consisted of a factorial experiment(3x2x2)with a randomized complete block design(three replications) The purpose of the experiment is to determine the effect of TBS and MRP coupled with EDTA on rice crop .

.Plot dimensions were 3x3m.The factors were four levels of EDTA(0,5,10,15ppm),four levels of TBS (0,5,10,15ppm),three levels of Na_2EDTA(0,5,10ppm) and three levels of MRP(0,5,10ppm). Table: 2.

TABLE II:Treatment Description-

Treatment	Doses(ppm)	Symbol
EDTA	0	E_0
	5	E_5
	10	E_{10}
	15	E_{15}
TBS	0	T_0
	5	T_5
	10	T_{10}
	15	T_{15}
Na2 EDTA	0	NaE_0
	5	NaE_5
	10	NaE_{10}
MRP	0	M_0
	5	M_5
	10	M_{10}

TBS-Tata Basic Slag,MRP-Mussorie Rock Phosphate

Soil was thoroughly mixed to achieve uniformity in metal spiking and then was placed in large containers lined with polyethylene sheets. The soil was kept wet to near saturation and was allowed to equilibrate with periodic mixing for 3 weeks. The recommended dose was applied to per pot. Distilled water was used to avoid any contamination.
Afterharvesting, the rice plant and grains were dried in oven at 60^0C, weighed and ground to powder.

III. Statistical Analysis-

The observed quantitative data were tabulated to statistical analysis with the help of ANOVA.The F-test was used to determine whether any real difference between the treatments exists or there were only error of sampling. The composition of testing the significance was made at 5% level.

IV. Result and Discussion:

The interaction of N,P,K,Na and Mg-

It is revealed from the data pertaining to N content given in table 3and 4 clearly indicated that the N content in rice plant and seed increased with the rise in EDTA and Na_2EDTA along with MRP and TBS.The maximum N concentration in plant at $E_{10}T_{10}NaE_5M_5$ level,i.e.42.57 mg pot^{-1}and for seed was observed 34.56 mg / pot^{-1}.It is revealed that fertilization of EDTA and Na_2EDTA along with MRP and TBS found significant superior over control. The increased N content with chelating agent application may be ascribed to increased root system and higher absorbing capacity.

Phosphorus is an essential element in plants, and deficiency can significantly limit plant growth. The highest phosphorus concentration in plant and seed was obtained at $E_{15}T_{15}NaE_5M_5$ level i.e. 41.49mg^{-1} and 12.68 mg^{-1} respectively. It is further revealed that the TBS and MRP application also found significant in both plant and seed.(Table 3 and 4) It is obvious that due to interactive effect of EDTA and Na EDTA along with MRP and TBS,phosphrous content went high.

TABLE:3 Interaction Effect of chelates with MRP and TBS levels in plants

elements	EDT+TBS (PPM/mg pot^{-1})	Na_2EDTA+MRP(ppp/mg pot^{-1})		
	Treatment	NaE_0M_0	NaE_5M_5	$NaE_{10}M_{10}$
N	MeanSEm ±1.03	*30.89*	31.63	34.22
P	mean SEm=±8.59	9.58	17.53	10.33
K	mean SEm=±1.54	56.17	46.67	51.25
Na	mean SEm=±0.65	34.25	37.33	40.83
Ca	mean SEm=±0.64	7.25	6.67	8.25
Mg	mean SEm =±0.41	3.67	3.25	4.58
Fe	mean SEm =±0.22	1.61	1.51	1.46
Cu	mean SEm =±0.01	0.09	0.1	0.1
Zn	mean SEm =±1.11	22.31	26.65	31.04

TABLE:4 Interaction Effect of chelates with MRP and TBS levels in seeds

S.No.	EDT+TBS (PPM/mg pot^{-1})	Na_2EDTA+MRP(ppp/mg pot^{-1})		
	Treatment	NaE_0M_0	NaE_5M_5	$NaE_{10}M_{10}$
N	meanSEm ±0.19	*25.4*	26.38	27.95
P	mean SEm=±0.09	11.25	11.4	11.72
K	mean SEm=±0.57	36.17	33.83	33.33
Na	mean SEm=±0.54	15.33	17.42	20.17
Ca	mean SEm=±0.42	2.5	3.17	2.83
Mg	mean SEm =±0.01	0.06	0.06	0.06
Fe	mean SEm =±0.06	0.33	0.3	0.29
Cu	mean SEm =±0.07	0.06	0.06	0.07
Zn	mean SEm =±0.25	26.67	25.42	30.42

It is further noted that Potassium content increased significantly to a greater extent at lower concentration of EDTA and Na EDTA along with MRP and TBS,while it reduced tremendously reduced with increasing levels of chelating agents. The maximum K concentration in plant was observed in both the cases of plant and seed. The maximum K concentration was observed at control i.e. 61.67ppm while minimum was recorded at $E_{10}T_{10}NaE_9M_0$ level i.e. 50.00ppm.The potassium concentration in the seed also follow the same trend. The maximum K concentration recorded at $E_{10}T_{10}NaE_{10}M_{10}$ i.e. 41.67ppm while minimum was recorded at $E_0T_0NaE_5M_5$ i.e. 21.67ppm.

The $E_0T_0NaE_0M_0$ did not increase the sodium concentration in comparison to the other levels. The minimum values of Na content in the plant and seed was recorded in control. The increase in Na content due to Na_2 EDTA level may be due to higher concentration of Na in soil solution. The maximum value of sodium concentration of plant and seed was found at $E_{10}T_{10}NaE_{10}M_{10}$ level at 51.67 and 28ppm respectively. From the data it is further revealed that interaction effect of compounds is found to be significant in both plant and seed.

It is obvious from the data pertaining to uptake in plant and seed that higher concentration of EDTA and Na_2EDTA along with MRP and TBS did not significantly increase the Magnesium content in appreciable manner in both plant and seeds. The highest Magnesium uptake in plant got increased at $E_{15}T_{15}NaE_0M_0$ level i.e.8.3ppm.Similar trend was also observed in seed where interaction increased Mg uptake maximum at 0.08ppm.It is found that these results were significant at 5% level of significance.

The interaction of Fe,Cu and Zinc-

From the examined data, it was found that there was significant difference within the levels of MRP and TBS as well as chemical ameliorants which slightly decreased the Fe uptake over control. It was further revealed from the data that higher concentration of EDTA decreased the Fe content in plant and seeds whereas better results were obtained at medium concentration (table-5).

These results are also favours the results given by Smith et al .The treatment does not show any significant difference ($p= <0.05$)It is also clear from the data (Table-5)that in all treatments copper utilization pattern in plant and seed was very similar to Luo et al also reported that EDTA is an effective chelate for mobilizing the metal from the contaminated soils and for enhancing the uptake of metal by the hyperaccumulater like thlaspi and Brassica junceae.

From the results it was found that the zinc content increased in appreciable proportion in all treatments positively correlated with increasing concentrations of Zn in the sample ($p< 0.05$). It is evident from the data that due to interaction effect of EDTA and Na_2EDTA with MRP and TBS ,zinc concentration went high(table-5). It is further revealed that these results are found to be more encouraging as compared from the other treatments. These results are in consonance with the findings of Collins et al.Gangloff et al also suggested that $ZnSO_4$ and Zn-EDTA were always the most effective material in supplying the plant's need.

Statistical Analysis-To investigate the relationship between plant and seed, linear regression were performed on the seed versus plant concentration of nutrient of crop. The value of R^2 clearly showed the positive effect of chelates on the nutrient concentration of rice crop. It is important to note that the sample size used for unvaried regressive curves in this study were relatively small (n=12) and there is possibility that the correlation coefficient were significantly affected by one or two extremely high levels of data. For these reasons, interpretation of correlation results should be taken as suggestive rather than definitive. Data pertaining to the correlation coefficient studies in rice crop is shown in Table: 5It is obvious from the results that 'r' value of chemical nutrients of plant vs. seed is found positively correlated to each other.

The observed quantitative data tabulated to statistical analysis with the help of variance(ANOVA) technique. The F- test was used to determine whether any real difference between the treatments existed or there were only error of sampling, the composition for treating the significance was made at 5% level. The results were analyzed and found significant at 5% level(Table-6).

TABLE;5

TABLE;6

Results of ANOVA for micronutrients and heavy metal concentration in rice crop

	Micro nutrients and Heavy metals in Plants									
Sources/variables	df	N	P	K	Na	Ca	Mg	Fe	Cu	Zn
factor-1	3	22.07**	1.43*	17.05**	628.85**	47.82**	42.69**	25**	4.64**	2011.33**
factor-2	2	52.86**		38.13**	101.28**	6.21**	11**	0.5*	1.66*	447.09**
interaction	6	11.48**	1.04*	12.85**	7.87**	8.86**	22.82**	0.11*	0.31*	1618.96**
	Micro nutrients and Heavy metals in Seeds									
Sources/variables	df	N	P	K	Na	Ca	Mg	Fe	Cu	Zn
factor-1	3	659.27**	139.55**	344**	578.91*8	47.82**	7.25**	12.93**	2.92*	162.87**
factor-2	2	1955.94**	28.12**	27.94**	80.48**	6.21**	0.21*	0.33*	0.66*	61.8**
interaction	6	176.68**	12.4**	54.82**	8.25**	8.86**	2.79**	0.07*	2.04*	8.02**

df=Degree of freedom
**significant $p<0.05$
* non significant

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