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Impact of EDTA application on chromium contaminated soil on yield and nutrient content in soil under maize (*Zea mays* L.) cultivation

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Abstract

A pot experiment was conducted to assess the effect of EDTA addition on the phytoremediation efficiency of chromium contaminated soil by *Zea mays* L. Treatments consist of four level of Ethylene diamine tetra acetic acid (EDTA) and four level of chromium. Results showed that the different level significantly increased plant height and straw yield at different stages *i.e.*, at 30 and 60 days after sowing respectively of maize crop. Maximum plant height at 30 DAS maize was recorded in (95.17cm) treatment in $C_0 E_0 i.e.$, control.

Keywords: Chromium, EDTA, maize, plant height, nutrient content

Introduction

Land and water are precious natural resources on which rely the sustainability of agriculture and the civilization of mankind. Unfortunately, they have been subjected to maximum exploitation and severely degraded or polluted due to anthropogenic activities. Increased population, industrialization and urbanization are responsible for environmental contamination Environmental decontamination is a big problem these days. Contaminated land could be described as surface environments which have been affected by both natural and anthropogenic sources. The pollution includes point sources such as emission, effluents and solid discharge from industries, vehicle exhaustion and metals from smelting and mining and nonpoint sources such as soluble salts (natural and artificial), use of insecticides or. Pesticides, disposal of industrial and municipal wastes in agriculture and excessive use of fertilizers.

Each source of contamination has its own damaging effects to plants, animals and ultimately to human health, but those that add heavy metals to soils and waters are of serious concern due to their persistence in the environment and carcinogenicity to human beings. Heavy metals are natural elements that are found at various high background levels at different places throughout the world, due to various concentrations in the bedrock. Thus, for example, Ni, Cr and Co are abundant in serpentine soils, whereas Zn, Pb and Cd are high in calamine soils. Heavy metals are persistent and cannot be deleted from the environment. Thus, a problem arises when their availability is high due to high background levels or to human activity. Since the beginning of the industrial revolution, heavy metal contamination of the biosphere has increased considerably and became a serious environmental concern. Contamination by heavy metals can be considered as one of the most critical threats to soil and water resources as well as to human health (Yoon *et al.*, 2006) ^[1]. Soil contamination has increased noticeably within the past few years (Ahmadpour *et al.*, 2006) ^[2].

Plant species have different responses to heavy metal pollution of soils. Although it may exist a relationship between heavy metal accumulation and plants tolerance, many plant species grow on contaminated soils and yet do not accumulate metals (Gabberielli *et al.*, 1990)^[3]. Plants that possess the ability to tolerate, uptake and accumulate high levels of metals in their biomass are termed as hyper accumulators (Brown *et al.*, 1995)^[4].

There are, two strategies of phyto extraction: (i) continuous phyto extraction, which depends on the natural ability of some plants to accumulate, translocate and resist high amounts of metals over the complete growth cycle (e.g., hyper accumulators), and (ii) chemically/chelateenhanced, assisted, or induced phyto extraction, based on the application of chelating agents to the soil to enhance metal uptake by plants (Garbisu and Alkorta, 2001) ^[5]. Phyto extraction refers to the use of metal-accumulating plants that translocate and concentrate metals from the soil in roots and aboveground shoots or leaves (Schnoor, 1997) ^[6]. Chromium (Cr) was first discovered in the Siberian red lead ore (crocoite) in 1798 by the French chemist Vauquelin. Chromium is essential to human and animal life but nonessential for the vegetable kingdom. In the soil environment, Cr can be found in two main oxidation forms-Cr (III) and Cr (VI), which show contrasting properties. The trivalent Cr is apparently useful or harmless to living organisms at reasonable concentrations, while Cr (VI) is extremely toxic. In addition, Cr (III) is not mobile in soil, therefore risks of its leaching are negligible. On the other hand, Cr (VI) is mainly present in the forms of chromates (CrO₄²⁻) and dichromates $(Cr_2O_7^{-2})$ and is generally mobile and often a part of crystalline minerals (ophiolites and serpentines). Chromium rich soils (serpentine soils) exist in many countries and Cr content in these soils may exceed 10,000 mg kg⁻¹. Chromium primarily exists in two stable oxidation states: hexavalent [Cr(VI)] and trivalent [Cr(III)] chromium (Mishra et al., 1997) [7]. There has been much interest in Cr since the discovery that it participates in mammalian glucose metabolism and appears to be essential to man and animals. As yet, however, there is no evidence of an essential role in plant metabolism (Huffman and Allaway, 1973)^[8]. EDTA (Ethylene diamine tetra acetic acid), is an amino poly carboxylic acid and a colorless, water-soluble solid. Its conjugate base is named ethylene di amine tetra acetate. Its usefulness arises because of its role as a hexa dentate ("sixtoothed") ligand and chelating agent, i.e., its ability to sequester metal ions. Chelating agents such as ethylene diamine tetra acetic acid, EDTA (Maxted et al, 2007 and Saifullah, 2009) [9, 10], [S,S]-ethylene diamine di succinate, EDDS (Komárek et al, 2010) [11]; low molecular weight organic acids (Nascimento et al, 2006)^[12] have been tested as mobilizing amendments for heavy metals in soils. Application of EDTA to soil contaminated with heavy metals increases their uptake from the soil to more than 1 percent of shoot dry biomass (Huang et al., 1997) ^[13]. A chelate is a chemical compound composed of a metal ion and a chelating agent. A chelating agent is a substance whose molecules can form several bonds to a single metal ion. In other words, a chelating agent is a ligand that can form a chelate with a metal atom and also known as 'polydentate-ligand. A true chelating compound must consist of at least two sites capable of donating electrons (coordinate covalent bond) to the metal it chelates. For true chelation to occur the donating atom must also be in a position within the chelating molecules so that a formation of a ring with ion can occur. Several forms of chelating compounds are commonly used in agriculture as foliar and soil application such as synthetic chelates, lingo organic acids, protein (amino sulfonates. acids). biodegradable chelating agents and humic or fulvic acids. Chelating agents have been used for the dissolution of metalcontaining materials in a variety of applications. Maize or corn (Zea mays L.) is a plant belonging to the family of grasses (Poaceae). It is an important cereal crop in world after wheat and rice. The importance of maize lies in its wide industrial applications besides serving as human food and animal Maize is called 'queen of cereal' as it is grown throughout the year due to its photo-thermo insensitive character and highest genetic yield potential among the cereals. Maize is a versatile crop grown over a range of agro climatic zones. In fact the suitability of maize to diverse environments is unmatched by any other crop. It is grown from 58°N to 40°S, from below sea level to altitudes higher than 3000 m, and in areas with 250 mm to more than 5000 mm of rainfall per year (Shaw, 1988 and Dowswell et. al., 1996) ^[14, 15] and with a growing cycle ranging from 3 to 13 months (CIMMYT 2000) [16].

Material and Methods

Experimental site and soil properties

A pot experiment was conducted with maize (Zea mays L.) variety DHM-117during kharif season of 2012-2013 in the net house of the Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India. The Varanasi is Situated at an altitude of 80.71 meters above mean sea level and located between 25° 14 and 25°23 N latitude and 82° 56 and 83°30E longitude has a semi-arid to sub humid climate with moisture deficit index between 20-40. The average annual rainfall of this region is about 1100 mm. Generally, the maximum and the minimum temperature ranged between $20^{\circ}-42$ °C and $9^{\circ}-$ 28 ^oC, respectively. May and June are the hottest months with the maximum temperature ranging from 39° to 42 °C. The cold period lies between November and January with the minimum temperature varying between 9º-10 °C. The mean relative humidity is about 68% which rise to 82% during wet season and goes down to 30% during dry season. The bulk soil (0-15 cm depth) sample was collected from BHU's agricultural research farm had pH 7.1 (Sparks, 1996) [17]; electrically conductivity 0.21 dS m⁻¹ (Sparks, 1996) ^[17]; Organic carbon 0.34%, (Walkley and Black, 1934) ^[18]: available N 126.44 kg ha⁻¹ (Subbiah and Asija 1956) ^[19]; available P 18.15 kg ha⁻¹ (Olsen 1954) ^[20]; and available K 141.30 kg ha⁻¹ (Hanway and Heidal, 1952)^[21]. The DTPA extractable zinc (Zn), copper (Cu), iron (Fe) manganese (Mn) and chromium (Cr) contents in soil were 1.74, 2.33, 35.62 12.44 and 0.69 mg kg⁻¹ respectively (Lindsay and Norwell 1978) ^[22] analyzed by using atomic absorption spectrophotometer (UNICAM 969).

Crop Management

The recommended dose of fertilizer (RDF) for maize is 120:60:50 N, P_2O_5 and K_2O . Required quantities of fertilizers for 5 kg soil was calculated and applied in liquid form using Urea [CO (NH₂)₂], Potassium dihydrogen phosphate (KH₂PO₄) and muriate of potash (KCl) as source of N, P_2O_5 and K_2O , respectively. Full dose of P_2O_5 and K_2O and half dose of N were applied before sowing and remaining nitrogen was added in two equal splits at 30 and 60 days after sowing in each pot. The growth and yield attribute recorded at different stage after sowing of maize crop. The treatment details are given in Table 1.

Table 1: Treatment details

Chelating compound	Symbol
EDTA (0 ppm)	E ₀
EDTA (10 ppm)	E 10
EDTA (30 ppm)	E ₃₀
EDTA (50 ppm)	E ₅₀
Heavy Metal	Symbol
Chromium (0 ppm)	C_0
Chromium (10 ppm)	C10
Chromium (30 ppm)	C30
Chromium (50 ppm)	C50

Analysis of plant and soil

The height of plants was measured from the surface of soil to the tip of plant at 30 and 60 days after sowing with the help of a meter scale. The plants were harvested at pre-tasselling stage with the help of seizer from the above 3 to 4 cm ground. Nitrogen content in plant samples was determined by modified kjeldahl method as per procedure outlined by Jackson (1973) ^[23] The plant samples were digested in a di-

acid mixture of nitric acid and perchloric acid (HNO₃:HClO, 9:4 v/v) and analyzed for P and K.

The soil samples were collected after the harvest of maize crop and analyzed for pH and electrical conductivity in 1:2.5 soil: water suspension (Sparks, 1996) ^[17]; organic carbon by methods of Walkley and Black (1934) ^[18]; available N by alkaline potassium permanganate (Subbiah and Asija, 1956) ^[19]; sodium bicarbonate (NaHCO₃) extractable-P (Olsen *et al.*, 1954) ^[20] by spectrophotometer; ammonium acetate-extractable K (Hanway and Heidal, 1952) ^[21] by flame photometer.

Result and discussion Plant height

At 30 days, plant height significantly varied from 77.17 to 95.17 cm (Table 2). It was found maximum (95.17 cm) in combination C_0E_0 and minimum (77.17cm) in $C_{50}E_{50}$. Treatments $C_{50}E_0$ and $C_{30}E_0$ decreased plant height by 18% and 10.5%, respectively over C_0E_0 . Interaction effect of application was found to be significant. Almost similar trend was noticed with the plant height recorded at 60 DAS except a slight increase in plant height as compared to observations recorded at 30 DAS and the interaction effect showed significant increase in plant height.

Plant height (cm) at 30 DAS							
Treatments	C_0	C10	C ₃₀	C50	Mean		
E_0	95.17	93.00	85.17	77.83	87.79		
E10	94.83	93.17	86.00	78.33	88.08		
E ₃₀	94.17	93.67	86.83	78.67	64.79		
E50	92.67	94.17	87.33	77.17	87.84		
Mean	70.67	93.50	86.33	78.00	82.13		
]	Plant height (cm) at	60 DAS				
Treatments	C_0	C ₁₀	C ₃₀	C ₅₀	Mean		
Eo	119.5	112.87	102.77	92.18	106.83		
E ₁₀	115.49	113.5	104.58	93.5	106.77		
E30	116.34	114.69	107.67	96.8	108.88		
E50	117.65	116.32	108.94	103.17	111.52		
Mean	117.25	114.35	105.99	96.41	108.5		

		30 DAS	60 DAS		
	SEm±	CD (P=0.05)	SEm±	CD (P=0.05)	
Е	0.13	0.44	0.02	0.07	
С	0.11	0.41	0.03	0.06	
ExC	0.24	0.85	0.03	0.13	

The increase in plant height may be attributed to the adequate supply of nutrients by the nitrogenous fertilizer application. A significant decrease in the shoot length of *Zea mays* L. at 9 μ mL⁻¹ Cr (VI) after 7 days. In the hydroponically grown rice, wheat and green gram seedlings where Cr⁺⁶ induced toxic effect as observed by the noticeable decrease in root length, shoot length, biomass content, stimulation in the seedling growth when supplemented with chelating agents such as EDTA, DTPA, and EDDHA as reported by Mohanty *et al.* (2012) ^[24].

Biomass yield

The fresh weight of maize (biomass yield) obtained after harvesting has been presented in Table 3. It is evident that fresh weight of plant ranged from 80.35 to 130.85 g pot⁻¹. The lowest oven dry weight (80.35 g pot⁻¹) was recorded with $C_{50}E_0$ and maximum (130.85 g pot⁻¹) was recorded with C_0E_0 which have shown about 63% increase. The treatment $C_{50}E_{50}$ has shown an increase of 6 and 2% over treatments $C_{50}E_{10}$ and $C_{50}E_{30}$, respectively.

Treatments	Treatments C ₀		C ₃₀	C50	Mean	
E ₀	130.85	111.56	96.52	80.35	104.82	
E10	130.62	115.39	98.41	85.03	107.36	
E ₃₀	126.55	118.37	110.4	88.05	110.84	
E50	124.51	122.62	111.83	90.05	112.25	
Mean	128.13	116.99	104.29	85.87	108.82	
	SEm±		CD(P=0.05)			
Е	0.21		0.75			
C	0.22		0.78			
E×C	0.42		1.56			

Table 3: Effect of EDTA application on biomass yield of maize

Effect of EDTA compound on concentration of nutrients in maize

Nitrogen

The critical perusal of data presented in (Table 4) showed that nitrogen content in maize increased significantly with application of EDTA compound. The interaction effect of EDTA and chromium was found to be non- significant. The nitrogen content in maize ranged from 0.65% to 1.86%. The maximum content (1.86%) was recorded in treatment C_0E_{50} which showed 2.8 times increase over minimum value (0.65%) observed in control. Treatments C_0E_{50} increased nitrogen content by 87% over control.

Phosphorus

A critical perusal of data pertaining to phosphorus content in maize (Table 4) showed a significant increase with application of EDTA compound. Interaction effect of EDTA and chromium was also found to be significant. The phosphorus content in maize ranged between 0.28% to 0.79%. Maximum phosphorus content (0.79%) was observed in treatment C_0E_{50} .

maize (Table 4) with the application of EDTA compound. The interaction effect of EDTA & chromium was also significant. The potassium content in maize ranged between 3.19% to 7.8%. Maximum (7.8%) K content was recorded in treatment C_0E_{50} which have shown about 65% increase over control. Minimum (3.19%) was recorded in $C_{50}E_{0}$.

Potassium

There was a significant increase in potassium content in

Ν						
Treatments	C ₀	C10	C30	C50	Mean	
E ₀	0.99	0.85	0.71	0.65	0.80	
E_{10}	1.13	1.1	1.04	1.01	1.07	
E_{30}	1.23	1.19	1.14	1.07	1.16	
E_{50}	1.86	1.64	1.35	1.01	1.47	
Mean	1.30	1.20	1.06	0.94	1.13	
		Р				
Treatments	C ₀	C10	C30	C50	Mean	
E ₀	0.37	0.31	0.29	0.28	0.31	
E_{10}	0.6	0.56	0.5	0.41	0.52	
E ₃₀	0.72	0.6	0.54	0.46	0.58	
E_{50}	0.79	0.73	0.65	0.53	0.68	
Mean	0.62	0.55	0.50	0.42	0.52	
		K				
Treatments	C ₀	C10	C ₃₀	C50	Mean	
E ₀	4.73	4.2	3.5	3.19	3.91	
E_{10}	6.28	5.82	5.38	4.8	5.57	
E_{30}	6.99	6.66	6.27	5.98	6.48	
E_{50}	7.8	7.12	6.85	6.39	7.04	
Mean	6.45	5.95	5.50	5.09	5.75	

	Ν		Р		K	
	SEm±	CD (P=0.05)	SEm±	CD (P=0.05)	SEm±	CD (P=0.05)
E	0.06	NS	0.014	NS	0.19	NS
С	0.09	0. 22	0.014	0.041	0.24	0.72
ExC	0.13	NS	0.026	0.076	0.44	1.25

Effect of EDTA compound on chemical properties of postharvest soil

Soil reaction (pH)

The data pertaining to pH of soil (Table 5) showed that the pH varied from 7.28 to 7.5. Maximum pH (7.5) was observed in treatments $C_{50}E_{30}$ and minimum pH (7.28) was observed in

 C_0E_0 , EDTA compound showed no effect on soil reaction and their interaction effect was also non-significant. The non-significant effect on pH of soil with the application of chelating compounds was also reported by Ghestem and Bermond (1998) ^[25] and Luo *et al.* (2000) ^[26].

Table 5: Effect of EDTA application on pH, EC and organic carbon content in post-harvest soil

pH						
Treatments	C ₀	C ₁₀	C ₃₀	C ₅₀	Mean	
E ₀	7.28	7.31	7.33	7.38	7.33	
E10	7.35	7.41	7.43	7.45	7.41	
E ₃₀	7.33	7.35	7.45	7.5	7.41	
E50	7.33	7.35	7.36	7.43	7.37	
Mean	7.32	7.36	7.39	7.44	7.38	
	EC (d Sm ⁻¹)					
Treatments	C ₀	C ₁₀	C ₃₀	C ₅₀	Mean	
E ₀	0.18	0.19	0.19	0.18	0.19	
E10	0.21	0.19	0.18	0.2	0.20	
E ₃₀	0.21	0.21	0.2	0.2	0.21	
E50	0.21	0.21	0.2	0.2	0.21	
Mean	0.20	0.20	0.19	0.20	0.20	
	Organic carbon (%)					
Treatments	C ₀	C ₁₀	C ₃₀	C ₅₀	Mean	

	E ₀	0.38	0.41	0.32	0.41	0.38
	E10	0.41	0.43	0.39	0.36	0.40
	E ₃₀	0.39	0.42	0.42	0.41	0.41
	E50	0.47	0.39	0.49	0.38	0.43
N	Mean	0.41	0.41	0.41	0.39	0.41
		pН	EC		Org. C	
	SEm±	CD (P=0.05)	SEm±	CD (P=0.05)	SEm±	CD (P=0.05)
Е	0.02	NS	0.02	NS	0.02	0.05
С	0.02	NS	0.04	0.07	0.03	0.07
ExC	0.04	NS	0.05	NS	0.04	NS

Electrical conductivity (EC)

Organic carbon

Data pertaining to EC of soil has been presented in (Table 5). The EC of soil didn't increased significantly with the application of chelating compounds. The interaction effect of EDTA and chromium was non-significant. It is evident that the EC of soil ranged between 0.18 to 0.21 dS m⁻¹. The minimum value of EC (0.18 dS m⁻¹) was recorded in C_0E_0 and maximum (0.21 dS m⁻¹) was recorded in treatment C_0E_{10} , C_0E_{30} , C_0E_{50} , $C_{10}E_{30}$, $C_{10}E_{50}$.

It is evident from (Table 5) that organic carbon content in soil

significantly varied with the application of chelating

compound. However, interaction effect of EDTA and

chromium was non-significant. The minimum organic carbon

content was recorded in $C_{30}E_0$ (0.32%) and maximum organic carbon content in $C_{30}E_{50}$ (0.49%). Increase in organic carbon content of soil due to application of EDTA of low organic acid also reported by Knight *et al.* (1998) ^[27] and Wu *et al.* (2004) ^[28].

Available N

A perusal of (Table 6) indicated that the available nitrogen content of soil was in lower range and it increased non-significantly with application of chelating compound. Interaction effect of EDTA and chromium was also found to be non-significant. The available nitrogen content of soil ranged between 117.05 to 129.05 kg ha⁻¹, minimum being in C_0E_0 and maximum in treatments $C_{10}E_0$ and $C_{10}E_{50}$.

Table 6: Effect of EDTA application on available N, P, K (Kg ha⁻¹) in post-harvest soil

Available N						
Treatments	C ₀	C10	C ₃₀	C50	Mean	
Eo	117.05	129.05	125.89	122.89	123.72	
E10	123.42	126.08	126.48	123.84	124.96	
E30	126.74	126.96	128.72	125.6	127.01	
E50	127.87	129.05	126.08	128.72	127.93	
Mean	123.77	127.79	126.79	125.26	125.90	
		Available P				
Treatments	C ₀	C10	C30	C50	Mean	
Eo	17.03	18.63	19.68	18.47	18.45	
E_{10}	17.84	18.93	19.59	19.05	18.85	
E30	20.51	20.51	21	21.67	20.92	
E ₅₀	21.93	22.02	19.05	21	21.00	
Mean	19.33	20.02	19.83	20.05	19.80	
		Available K				
Treatments	C ₀	C10	C30	C50	Mean	
Eo	141.79	141.59	141.67	142.09	141.79	
E ₁₀	141.61	142.52	142.09	142.42	142.16	
E30	142.65	143.03	143.89	143.83	143.35	
E50	143.89	144.89	145.67	145.89	145.09	
Mean	142.49	143.01	143.33	143.56	143.09	

	Available N		Available P		Available K	
	SEm±	CD (P=0.05)	SEm±	CD (P=0.05)	SEm±	CD (P=0.05)
Е	0.06	NS	0.012	NS	0.20	NS
С	0.09	0. 21	0.014	0.043	0.24	0.76
ExC	0.15	NS	0.027	0.072	0.46	1.30

Available P

A critical perusal of data presented under (Table 6) showed that the available P in soil was in medium range. A significant increase was recorded with the application of EDTA compound. The available P in soil ranged between 17.03 to 22.02 kg ha⁻¹ minimum being in control C_0E_0 and maximum available P content in treatment $C_{10}E_{50}$.

Hovsepyan and Greipsson (2005) ^[29] reported that phosphorus occurs as a negatively charged ion and therefore is not complexed by EDTA which is present as an anion itself. Inceasing concentrations of P in the extract by respectively

increasing doses of EDTA and may resulted from the dissolution of metal phosphates by EDTA. Stunzi (2006) ^[30] reported that metals are complexed by EDTA and thereby the P concentrations in the extracts increased.

Available K

The result obtained from (Table 6) showed that the available K content of soil was found to be increased with application of EDTA compound. Interaction effect of EDTA and chromium was also found to be significant. The available K content in soil ranged between 141.59 to 145.89 kg ha⁻¹.

Lowest K content in soil (141.59 kg ha⁻¹) was reported in $C_{10}E_0$ while maximum K content (145.89 kg ha⁻¹) was found in treatment $C_{50}E_{50}$ which was increased by 3%.

Conclusion

Plant height decreased with application of increased doses of chromium (30 and 60 DAS). Interaction effect of was significant 30 DAS as well as 60 DAS. Combined effect of 50 ppm EDTA with 50 ppm chromium has shown minimum plant height at 30 DAS and that of EDTA 0 ppm combined with 50 ppm chromium at 60 DAS. With increasing level of chromium plant growth was found to be decreased but increased with increased EDTA levels. Biomass of maize with the application of EDTA compound was positively increased. Maximum (130.85 g pot-1) was recorded with treatment C₀E₀ which have shown about 63% increase over $C_{50}E_0.$ Treatment $C_{50}E_{50}$ has shown an increase of 6% and 2% over treatment $C_{50}E_{10}$, $C_{50}E_{30}$ respectively. The pH of soil varied from 7.28 to 7.5. EDTA compound showed no effect on soil reaction. Maximum pH (7.5) was observed in treatments C₅₀E₃₀ where, EDTA (50 ppm) was applied. The percent organic carbon content in soil ranged from 0.32 to 0.49. Maximum organic carbon (0.49%) was found in $C_{30}E_{50}$, which was 29% increase over control. There was a nonsignificant effect on organic carbon content in soil due to EDTA application. Nitrogen content of soil was found in lower range. Application of EDTA compound, increased nitrogen content in soil from 117.05 to 129.05 kg ha⁻¹. The treatment $C_{10}E_0$ and $C_{10}E_{50}$ have shown highest available nitrogen content in soil (129.05 kg ha⁻¹) with increased N build up in soil by 10.25% over control. The available P content in soil significantly increased from 17.03 to 22.02 kg ha⁻¹. Treatment $C_{10}E_{50}$ showed maximum (22.02 Kg ha⁻¹) available P content which was increased by 29.30% over control (C_0E_0). The available K content of soil significantly varied between 141.59 to 145.89 kg ha⁻¹. Maximum K content (145.89 kg ha⁻¹) was found in treatment C₅₀E₅₀ which was increased by 2.9% over control.

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