



***In vitro* Availability of Macro Nutrients in Various North Indian Soils by Application of Biochar**

**Akansha Singh ^{a*}, B. P. Dhyani ^a, U. P. Shahi ^a, Vipin Kumar ^a,
Satendra Kumar ^a, N. S. Rana ^a, Akash Tomer ^a
and Anand Singh ^a**

^a *Department of Soil Science and Agricultural Chemistry, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut - 250110, Uttar Pradesh, India.*

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The use of organic amendments is popular these days therefore we conducted a study on biochar in various North Indian region soils under pot culture to evaluate the changes in soil characteristics. Four types of soils taken for 75 days during *Kharif* and *Rabi* season were studied out of which Soil-1 is slightly acidic while Soil-2 to Soil-4 are alkaline. The soil samples were collected thrice at the interval of 25 days using standard procedures and analyzed for various macronutrients (pH, EC, OC, N, P, and K) essential for plants in the laboratory. The change in status of available NPK in soils and other soil properties like pH, electrical conductivity (EC), and organic carbon (OC) content due to inoculation of biochar was assessed. Results reveal that all treatments having biochar leads to an increase in the content of available N, P, and K together with OC. There recorded a slight elevation in EC initially at 25th DAI due to its inoculation. The pH during the initial period had reduced but later on rise as the number of DAI increased.

Keywords: *Electrical Conductivity (EC); Organic Carbon (OC); biochar; Days After Inoculation (DAI).*

1. INTRODUCTION

Recent agricultural advent needs various organic products which can enhance productivity and

provide sustainability to the farming system while yet cost-effective. Considering all these grounds in mind we had explored the historical products used by our ancestors for the betterment of

*Corresponding author: E-mail: dr.akanshasingh16@gmail.com;

agricultural production. Biochar is one of those products which for a long time had been used as a major component of the fertile *Terra Preta* i.e. dark earth, of the Amazon Basin. It can sequester large quantities of carbon in the soil for a long time thereby sustaining the productivity of ancient agroforestry of the Amazon vegetation.

Now the day's farmers are taking interest in using it as a source of long-term carbon which can sustain in crop fields. Biochar can be defined as a carbon enriched, fine-grained, porous by-product of slow pyrolysis, it is formed when organic material (feedstock) is thermally decomposed at low to moderate temperatures during a long heating period under a limited supply of oxygen [1]. The feedstock for its preparation may have crop residues of any type, organic wastes, dairy manure, sewage, sludge, etc. First time in 2013 in Europe, the Swiss Federal Ministry of Agriculture approved the use of certified biochar for agriculture.

Generally, biochar can increase soil electrical conductivity by 124.6 % [2]. It can also increase the soil pH and organic carbon content of soils. The elemental like K, Ca, Na, and Mg together with carbon, nitrogen, and hydrogen are generally found in biochar [3]. The amounts of the extractable nutrient elements in soil (e.g., Na, K, Ca, and Mg) could be increased after the application of biochar. These available nutrients can enhance soil chemical properties thereby increasing soil fertility.

The application of inorganic fertilizers in the soil, air and water pollution gets enhanced. These chemicals also possess a threat to human or livestock health and contaminate groundwater and the environment. Indian farmers mostly depend on these fertilizers to inculcate available nutrients (N, P, and K) into soils. The overuse of these chemical imbalances the organic carbon in agricultural land, destruct soil's physical characteristics, and accumulate toxic chemicals in water bodies. Therefore, there is a need among farmers, for organic source to

supply available nutrients into the soil. Hence, biochar can act as a promising approach for this.

2. MATERIALS AND METHODS

2.1 Site Description and Soil Sampling

The *in vitro* pot analysis was done on four different soil samples in Department of Soil Science and Agricultural Chemistry, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut. The soil samples collected from various locations for analysis having their description in Table 1. Each pot was filled with half kilogram soil. The biochar @ 1.25 g pot⁻¹ were then incorporated by manual mixing into the soils. At the interval of 5 to 7 days according to the requirement of the pots watering was done up to 60% of maximum water holding capacity. During the experiment the soil samples were collected thrice in the amount of 100 g pot⁻¹ from each experimental pot at the interval of 25 days (25, 50 and 75) during both the seasons (*Kharif* and *Rabi*) for year 2019-2020. The samples taken were cleaned by removing gravels and stones and then processed. The samples were air dried and grinded with wooden roller, separately. Each sample was then passed through 2mm sieve. All the samples of the soil were stored in labeled polythene bags for conducting laboratory analysis. The new soil samples were taken in pot for each *Kharif* and *Rabi* seasons.

2.2 Materials

The treatment material biochar used in this study is made up of sugarcane bagasse and rice residue at the temperature around 300° to 350°C, having pH 9.2, Electrical Conductivity (EC) 12.5 dSm⁻¹, Total Organic Carbon (TOC) 54.17%, Total Nitrogen 9234 ppm, Total Phosphorus 3190 ppm and Total Potassium 6542 ppm.

Table 1. Description of various soil samples taken for analysis

Sr. No.	Sample No.	Place of Collection of Soil sample	Rhizospheric soil
1.	Soil-1	Krishi Vigyan Kendra (KVK), Distt. Champawat (Uttarakhand)	Field
2.	Soil-2	Horticultural Research Centre, SVPUAT, Meerut (U.P.)	Field
3.	Soil-3	College of Agriculture, SVPUAT, Meerut (U.P.)	Field
4.	Soil-4	Krishi Vigyan Kendra (KVK), Bulandshar (U.P.)	Field

2.3 Chemical Analysis and Experimental Design

The analytical work was carried out in the laboratory of Department of Soil Science, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, (U.P) India for soil pH, EC, Organic Carbon and available macronutrients (N, P and K) by using standard protocols. The pH was determined with combined glass electrode pH meter by soil water suspension method [4]. Electrical conductivity was with the help of EC meter soil water suspension method [4]. Organic carbon determination was done by wet oxidation method by Walkely and Black, [5]. Available nitrogen analysis was done using oxidative hydrolysis through alkaline permanganate (KMnO_4) as outlined by Subbiah and Asija, [6]. The available phosphorus estimation for soils was done using Olsen's Reagent [7] available P in the aliquot was determined by developing blue color through Ascorbic acid method using Spectrophotometer. Finally, available K soil determination was done by neutral ammonium acetate extractant [8], using Flame photometer.

Experimental design used for this study is Completely Randomized Block Design (CRD) because it is a pot culture experiment. Statistical Analysis was worked out using OPSTAT software.

3. RESULTS AND DISCUSSION

The analysis about the effect of biochar was done on four different location soils. The description about the initial soil properties can be observed in Table 2.

The initial parameter to be analyzed for any soil must be its pH because it tells about the type of reaction going on in the soil. The change in soil pH values from the control T_1 , due to the addition of biochar in T_2 during *Kharif* and *Rabi* season can be observed in Table 3.1a and 3.1b. In every season for each soil, there recorded an increase in pH value due to biochar addition. Also, there is an increase in pH value recorded as the number of Days After Inoculation (DAI) increases. The highest increase in pH from T_1 to T_2 during *Kharif* season for Soil-1 to Soil-4 is 36.98%, 9.74%, 7.25% and 9.79%. During *Rabi* season percentage increase in pH for Soil-1 to Soil-4 is 36.23%, 10.81%, 8.22% and 13.33%. The decrease in exchangeable Al^{3+} and exchangeable acidity while increasing exchangeable base content due to addition of

biochar leads to an increase in pH of soil samples [9]. Chan et al. [10], Laird et al. [11], Abbruzzini et al. [12] and Bista et al. [13] reported a considerable increase in pH values (around 1 pH unit) by biochar inclusion. There recorded a decrease in pH of the soil at 25th DAI in T_2 samples is might be due to the production of organic acids [14] during both seasons (*Kharif* and *Rabi*). The highest increase in pH during both seasons was recorded for Soil-1 which is acidic because of liming effect produced by biochar incorporation in that acid soil [15].

Soil EC represents the amount of salts present in the soil. The change in EC during both crop seasons can be observed in Table 3.2a and 3.2b. The increase in EC was observed at 75th DAI for T_2 due to higher EC value of biochar. EC values increases significantly with the addition of biochar in all soils [16,17], it is due to the release of weakly bound nutrients (cations and anions) into the soil solution, which are available for plant uptake [18-20].

Organic Carbon (OC) is the most important parameter pertaining to soil health. It is the carbon component of Soil Organic Matter (SOM). The immobilization of OC till 25th DAI for biochar treatment T_2 was recorded then a decrease in OC content for each soil from 25th to 75th DAI takes place in each season. The maximum percentage increase in value of T_2 from control during *Kharif* for Soil-1 is 14 g kg^{-1} , Soil-2 and Soil-3 is 8 g kg^{-1} , while for Soil-4 is 8.1 g kg^{-1} respectively. In *Rabi* season the maximum increase in T_2 from control in Soil-1 is 13.2 g kg^{-1} , Soil-2 is 7.8 g kg^{-1} , Soil-3 is 7.9 g kg^{-1} and Soil-4 is 8 g kg^{-1} respectively. The changes occur due to biochar inoculation on OC can be seen in Table 3.3a and 3.3b. Laird et al. [21] elucidate that biochar amended soils significantly increased soil organic C. Liu et al. [22] reported that the pot studies showed the greatest increases in SOC response to biochar amendment. Most of the studies confirmed that the biochar carbon recalcitrance enhances SOC storage in soils [3].

The out-turn of T_2 on soil available nitrogen (N) content during both the seasons is presented in Table 3.4a and 3.4b. The addition of biochar in T_2 leads to increase in value of available N in *Kharif* and *Rabi* from control up to at 75th DAI. The incremental values for *Kharif* from control in Soil-1 to Soil-4 is 14.80%, 15.44%, 12.56% and 7.60% respectively. The increased values during *Rabi* for N at 75th DAI for Soil-1 to Soil-4 is

Table 2. Initial soil properties

Sr. No.	Soil Parameters	Soil-1	Soil-2	Soil-3	Soil-4
1.	pH	5.60	7.13	7.45	7.23
2.	EC (dS m ⁻¹)	0.45	0.32	0.34	0.30
3.	Organic Carbon (g kg ⁻¹)	8.6	2.7	3.0	2.6
4.	Available N (kg ha ⁻¹)	224.35	197.01	190.65	195.14
5.	Available P (ppm)	9.5	9.15	8.24	8.94
6.	Available K (kg ha ⁻¹)	181.84	170.67	175.89	220.34

Table 3.1a. Soil pH (Kharif)

Treatment Name	Trt. No.	Soil-1			Soil-2			Soil-3			Soil-4		
		25 days	50 days	75 days	25 days	50 days	75 days	25 days	50 days	75 days	25 days	50 days	75 days
Control	T1	5.65	5.6	5.57	7.18	7.20	7.19	7.4	7.42	7.45	7.23	7.21	7.25
Soil + Biochar	T2	7.41	7.57	7.63	7.70	7.79	7.89	7.84	7.90	7.99	7.78	7.89	7.96
CD		0.68	0.68	0.69	0.19	0.14	0.02	0.22	0.10	0.27	0.38	0.27	0.26
SEm±		0.17	0.17	0.17	0.05	0.04	0.01	0.06	0.03	0.07	0.09	0.07	0.06

Table 3.1b. Soil pH (Rabi)

Treatment Name	Trt. No.	Soil-1			Soil-2			Soil-3			Soil-4		
		25 days	50 days	75 days	25 days	50 days	75 days	25 days	50 days	75 days	25 days	50 days	75 days
Control	T ₁	5.68	5.61	5.63	7.22	7.25	7.21	7.43	7.41	7.42	7.23	7.21	7.2
Soil + Biochar	T ₂	7.44	7.59	7.67	7.82	7.93	7.99	7.89	7.99	8.03	7.99	8.02	8.16
CD		0.68	0.68	0.69	0.27	0.33	0.23	0.22	0.48	0.48	0.30	0.54	0.44
SEm±		0.17	0.17	0.17	0.07	0.08	0.06	0.05	0.12	0.12	0.07	0.13	0.11

Table 3.2a. Soil EC [dSm⁻¹] (Kharif)

Treatment Name	Trt. No.	Soil-1			Soil-2			Soil-3			Soil-4		
		25 days	50 days	75 days	25 days	50 days	75 days	25 days	50 days	75 days	25 days	50 days	75 days
Control	T ₁	0.21	0.22	0.23	0.30	0.28	0.31	0.3	0.29	0.31	0.28	0.30	0.31
Soil + Biochar	T ₂	0.29	0.30	0.31	0.38	0.43	0.44	0.37	0.43	0.45	0.42	0.43	0.44
CD		0.02	0.02	0.02	0.04	0.04	0.05	0.03	0.04	0.05	0.04	0.04	0.05
SEm±		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

Table 3.2b. Soil EC [dSm⁻¹] (Rabi)

Treatment Name	Trt. No.	Soil-1			Soil-2			Soil-3			Soil-4		
		25 days	50 days	75 days	25 days	50 days	75 days	25 days	50 days	75 days	25 days	50 days	75 days
Control	T ₁	0.21	0.22	0.23	0.32	0.31	0.3	0.31	0.28	0.33	0.29	0.31	0.30
Soil + Biochar	T ₂	0.30	0.31	0.31	0.37	0.43	0.45	0.36	0.42	0.45	0.42	0.43	0.43
CD		0.02	0.02	0.02	0.04	0.05	0.04	0.04	0.04	0.05	0.04	0.05	0.04
SEm±		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

Table 3.3a. Soil organic carbon [g kg⁻¹] (Kharif)

Treatment Name	Trt. No.	Soil-1			Soil-2			Soil-3			Soil-4		
		25 days	50 days	75 days	25 days	50 days	75 days	25 days	50 days	75 days	25 days	50 days	75 days
Control	T ₁	8.9	9	8.9	3.4	3.3	3.1	3.3	3.1	3.3	3.4	3.4	3.5
Soil + Biochar	T ₂	14	13.8	13.5	8	7.4	6.7	8	7.5	7	8.1	7.6	6.8
CD		1.1	1.1	1.1	0.5	0.5	0.6	0.5	0.6	0.6	0.5	0.6	0.6
SEm±		0.3	0.3	0.3	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

Table 3.3b. Soil organic carbon [g kg⁻¹] (Rabi)

Treatment Name	Trt. No.	Soil-1			Soil-2			Soil-3			Soil-4		
		25 days	50 days	75 days	25 days	50 days	75 days	25 days	50 days	75 days	25 days	50 days	75 days
Control	T ₁	8.5	8.6	8.7	3.2	3	3.1	3.2	3	3	3.3	3.2	3.4
Soil + Biochar	T ₂	13.2	12.9	12	7.8	7.2	6.6	7.9	7.4	6.9	8	7.5	8
CD		1.2	1.2	1.2	0.5	0.6	0.6	0.5	0.6	0.6	0.5	0.6	0.6
SEm±		0.3	0.3	0.3	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

Table 3.4a. Soil available Nitrogen [kg ha⁻¹] (Kharif)

Treatment Name	Trt. No.	Soil-1			Soil-2			Soil-3			Soil-4		
		25 days	50 days	75 days	25 days	50 days	75 days	25 days	50 days	75 days	25 days	50 days	75 days
Control	T ₁	224.68	226.48	225.65	195.73	196.70	199.63	192.27	190.03	191.11	193.06	195.36	196.08
Soil + Biochar	T ₂	214.81	246.14	259.05	179.41	220.24	230.45	177.28	198.16	215.11	178.39	206.68	210.99
CD		8.46	10.69	25.21	8.12	21.73	22.36	7.44	3.29	21.16	11.54	10.54	6.38
SEm±		2.10	2.65	6.25	2.01	5.39	5.55	1.84	0.82	5.25	2.86	2.61	1.58

Table 3.4b. Soil available Nitrogen [kg ha⁻¹] (Rabi)

Treatment Name	Trt. No.	Soil-1			Soil-2			Soil-3			Soil-4		
		25 days	50 days	75 days	25 days	50 days	75 days	25 days	50 days	75 days	25 days	50 days	75 days
Control	T ₁	224.68	223.05	224.17	198.72	196.71	198.68	191.47	190.13	192.09	193.02	194.06	193.53
Soil + Biochar	T ₂	215.89	242.44	245.56	180.34	215.48	222.17	178.85	195.17	212.92	179.57	199.75	202.93
CD		8.79	1.95	7.22	9.15	9.89	21.94	5.99	1.57	21.13	9.27	3.98	8.31
SEm±		2.18	0.48	1.79	2.27	2.45	5.44	1.49	0.39	5.24	2.30	0.99	2.06

Table 3.5a. Available P in Kharif season (ppm)

Treatment Name	Trt. No.	Soil-1			Soil-2			Soil-3			Soil-4		
		25 days	50 days	75 days	25 days	50 days	75 days	25 days	50 days	75 days	25 days	50 days	75 days
Control	T ₁	9.18	9.16	9.13	9.18	9.16	9.13	8.40	8.45	8.43	9.35	9.31	9.36
Soil + Biochar	T ₂	32.25	32.76	32.83	32.25	32.76	32.83	32.30	34.13	33.94	36.18	36.65	37
CD		2.19	2.34	2.27	2.19	2.34	2.27	2.23	2.34	2.34	2.51	2.54	2.56
SEm±		0.54	0.58	0.56	0.54	0.58	0.56	0.55	0.58	0.58	0.62	0.63	0.63

Table 3.5b. Available P in Rabi season (ppm)

Treatment Name	Trt. No.	Soil-1			Soil-2			Soil-3			Soil-4		
		25 days	50 days	75 days	25 days	50 days	75 days	25 days	50 days	75 days	25 days	50 days	75 days
Control	T ₁	9.24	9.23	9.22	8.90	8.95	8.93	7.44	8.43	8.57	8.99	9.10	9.23
Soil + Biochar	T ₂	21.99	22.74	22.98	31.84	31.99	32.05	28.44	32.22	32.75	34.79	35.22	35.72
CD		1.63	1.68	1.69	2.22	2.23	2.23	1.97	2.23	2.27	2.41	2.44	2.47
SEm±		0.41	0.42	0.42	0.55	0.55	0.55	0.49	0.55	0.56	0.60	0.61	0.61

Table 3.6a. Soil available Potassium [kg ha⁻¹] (Kharif)

Treatment Name	Trt. No.	Soil-1			Soil-2			Soil-3			Soil-4		
		25 days	50 days	75 days	25 days	50 days	75 days	25 days	50 days	75 days	25 days	50 days	75 days
Control	T ₁	180.29	182.07	182.31	172.43	170.26	171.61	174.77	175.27	175.57	220.24	221.08	222.36
Soil + Biochar	T ₂	275.05	280.33	283.21	249.21	250.8	253.6	248.13	251.2	254.92	250.65	253.85	254.67
CD		23.47	23.76	23.94	21.69	21.65	21.86	21.77	21.94	22.15	24.51	24.70	24.83
SEm±		5.82	5.90	5.94	5.38	5.37	5.42	5.40	5.44	5.49	6.08	6.13	6.16

Table 3.6b. Soil available Potassium [kg ha⁻¹] (Rabi)

Treatment Name	Trt. No.	Soil-1			Soil-2			Soil-3			Soil-4		
		25 days	50 days	75 days	25 days	50 days	75 days	25 days	50 days	75 days	25 days	50 days	75 days
Control	T ₁	180.4	181.21	182.07	170.61	168.76	169.32	174.27	173.38	172.66	219.08	220.42	219.51
Soil + Biochar	T ₂	275.43	280.01	282.88	248.67	251.2	252.17	249.47	252.61	253.19	249.17	251.23	252.76
CD		23.44	23.71	23.71	21.57	21.60	21.68	21.80	21.91	21.90	24.37	24.55	24.57
SEm±		5.81	5.88	5.88	5.35	5.36	5.38	5.41	5.43	5.43	6.05	6.09	6.09

9.54%, 11.82%, 10.84% and 4.86%. The rise in the content of N is because of the higher mineralization rate of biochar that happens due to readily decomposable organic carbon present into it [23-25] and also the priming effect on biochar within 90 days of its application into the soil that leads to higher N availability into soil [23,24]. The initial decrease in the value of N upto 25th DAI happens due to immobilization of N as it contains acid hydrolysable N (e.g. amino sugars, amino acids) which can be readily used by soil microbes [26,27]. Khan et al., 2019 reported the rise in content of N by the application of biochar in onion crop. The increased in nitrate nitrogen content is mainly due to increased transformation of NH_4^+ to NO_3^- [28] or due to enhanced activity of nitrifying bacteria in the soil [29].

Available P content of the soils clearly represents the plant available form of phosphorus in soil. The significant increase in content of available P in T₂ for both the seasons at 75th DAI can be seen in Table 3.5a and 3.5b. The utmost increase in value for T₂ during *Kharif* season for soils (Soil-1 to Soil-4) is 24.12 ppm, 32.84 ppm, 34.13 ppm and 9.36 ppm. While for *Rabi* the maximum rise for soils (Soil-1 to Soil-4) is 22.98 ppm, 32.05 ppm, 32.75 ppm and 35.72 ppm.

The increase in P due to soil biochar inoculation is also reported by Zhu et al., 2014. Diversity of soils and variable environmental state promotes the available P content due to biochar application [30]. The increase in P availability also happens in acid soils due to application of high pH biochar which is rich in neutral metal oxides (Ca and Mg oxides) it is due to its liming effect in soils [31, 32]. Various P species are found in biochar, out of which the soluble P form can be released into the soils that leads to increase in soil available P pool [33-36]. Biochar is slow P discharging source which replenishes the soil reliably and continuously with liable P [27,37,38] and also it has been proved that biochar also activates soil endogenous P.

The seasonal effect on biochar treatment T₂ on soil available potassium (K) content during *Kharif* and *Rabi* season is presented in Table 3.6a and 3.6b. In *Kharif*, addition of biochar in T₂ leads to increase in value of available K from control up to 75th DAI in Soil-1 to Soil-4 is 55.34%, 47.78%, 45.16% and 14.53%. In *Rabi* due to the addition of biochar in T₂ leads to increase in value of available K from control up to 75th DAI in Soil-1 to Soil-4 is 55.37%, 49.02%, 46.64% and 15.15%.

There are various reasons for increase in content of K in soils due to biochar addition. The biochar contain various carbonates and oxides of potassium which can be solubilized into soil solution therefore increases content of available K in soil [39-42]. At the time of preparation of biochar the potassium ions don't volatilize in its burning process hence remains as free nutrient cation [42]. Another reason may be increase in number of K solubilizing microbes and relative abundance of other bacteria, this also promotes solubilized K in soils [43]. The Potassium (K) in various organic residues like biochar exists in inorganic forms and after its application to soil it sets free the easily soluble and exchangeable fractions of K.

4. CONCLUSION

In modern agriculture, nutrient management is the most crucial factors affecting plant growth, yield, and quality performances. The results of this study represents that the application of organic fertilizer like biochar enhanced basic soil physicochemical properties as well as improve soil health. Additionally, the inoculation of this factor into acid soils is helpful in bringing pH to its neutral range that promotes the availability of major nutrients especially available P. The increase in organic carbon under all soils upgrades soil health and increases soil microbial reserve, is therefore causes overall soil development. The other macro nutrients also display the positive growth in all soils and availability of all these nutrients got enhanced by inoculation of biochar factor into the soil. Overall biochar is useful because of its long residence time in soil which makes it a slow and constant supply source for soil nutrients. The research must be undertaken to discover more positive effects of biochar on soil nutrient properties. Exploring more about this component can help us to slowly integrate organic more into our farming system and can prevent environmental problems pose due to inorganic fertilization.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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