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Effect of Mixed Wood Biochar on Physico-chemical Properties of Sandy Soil, Haryana Region, India

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Abstract

Biochar is a carbon-rich source which significantly affects the soil properties. In this study, we investigated the effect of different concentrations of mixed wood biochar synthesized from Sal, Cheed and Teak with poly-aromatic hydrocarbons (50 mg/kg naphthalene and 100 mg/kg phenanthrene) and observed its effect on various physico-chemical parameters of sandy soil. The significant results were found with increasing biochar application rate. Soil amended with1%, 5% and 10% biochar effectively help in the reduction of bulk density 8.18%, 12.58% and17.61% respectively, as compared to control. Soil porosity (40 to 50%) and water holding capacity (113.2 to 219.6%) was also increased with biochar concentration. The non-significant ($P \leq 0.05$) results were observed in case of increase in pH and EC values in biochar amended and non-biochar amended soil samples. CEC and SOC value were significantly increased from 24.13 to 32.94% and 37.68 to 50.82% respectively, in 10% biochar-amended soil as compared to biochar non-amended soil samples. However, biochar helps in conditioning of soil by increasing the surface negative charge, nutrient retention capacity, soil porosity, organic carbon content and cation exchange capacity. It could be safely applied in agriculture for improvement of soil health and crop yield.

1. Introduction

Soil is a life supportive component of the environment that contains several minerals and biological forms (Singh *et al.,* 2020). A good quality of soil is a strong determinative for agronomic production. Drastic changes in loss of soil fertility and declining agricultural yield is mainly due to the reduction of soil organic matter (SOM). Moreover, soil organic carbon (SOC) is necessary for sustainable crop production as it is able to retain water and nutrients. It provides a habitat for soil biota and improves soil structure (Lal, 2009; Lorenz *et al*., 2007). But technological and industrialization advances have led to the enhancement of organic and inorganic contaminants in soil. Similarly, various anthropogenic activities such as incomplete combustion of fossil fuels and biomass, smelting, motor vehicle emissions and natural exposure sources include volcanoes, forest fire and hydrothermal processes contaminate the soil and sediments in many places throughout the world (Singh *et al.,* 2020; Wang *et al*., 2020). Generally, these pollutants are non-degradable and accumulate in the environment. The well-known categories of organic pollutants are fuel hydrocarbons, polychlorinated biphenyls (PCBs), poly-aromatic hydrocarbons (PAHs), chlorinated compounds and detergents and pesticides (Pandey *et al*., 2018). The present study based on PAHs which are organic pollutants containing one or more fused aromatic rings (mostly benzene rings) in linear and angular arrangements. PAHs also differ in their transport, distribution and fate in the environment and their effects on the biological system (Seo *et al*., 2009). A list of 17 PAH components have been identified which are in greatest concern with their adverse effects on human health and on environment. These PAH compounds are considered as a mutagen, teratogens and carcinogens (Abdel-Shafy and Mansour, 2016). Naphthalene and phenanthrene are the simplest form of PAHs formed by a fusion of benzene rings. These PAHs are difficult to remove from polluted soil and are toxic in nature. Remediation of PAHs through biochar is an important aspect to reducing the toxic effect of different contaminants that present in soil because of relatively low costs and environmentally friendly approach (Singh *et al*., 2020). Now, different environmentally acceptable techniques have been found and reported for the sustainable management dealing with this problem.

To overcome this problem biochar has received great interest since last few years, due to its wider agricultural and remediation applications (Kaur and Sharma, 2017; Dume *et al*., 2016; Major *et al*., 2010). The amendment of biochar in polluted soil effectively binds with soil contaminants that help to provide suitable conditions to promote plant growth (Singh *et al*., 2020). Biochar is more recalcitrant form of carbon, it is observed that biochar persists in soils for hundreds of years (Schmidt *et al*., 2014; William and Qureshi, 2015; Wang *et al*., 2015; Verheijen *et al*., 2017). Biochar makes the soil cleaner and healthier, which enhances the growth of agricultural productivity and microbial population. Farmers can cheaply convert agricultural raw materials into a biochar this will meet at least the primary needs to improve soil quality and crop yield. This is a sustainable approach to use the agricultural byproducts (Schouten, 2010).

"Biochar is carbon-rich source obtained from the pyrolysis of various organic materials (crop residues, manures, wood chips, sewage waste) and industrial wastes (paper sludge's and biosolids) when heated in a closed container or pyrolysed at low oxygen conditions" (Lu *et al*., 2020; Huang *et al*., 2017; Zhang *et al*., 2018; Lehmann and Joseph, 2009). The effect of biochar application on environment depends on the type of different raw materials used during pyrolysis as well as on optimized temperature conditions. The pyrolysis condition shows that, how much labile carbon in the form of volatile matter (VM). The VM content is lost during pyrolysis and thus the final fixed organic carbon content roughly equals to the ash content (Xie *et al*., 2015). Biochar contains the carbonized and non-carbonized organic matter such as wood fiber, cellulose and ligno-cellulose (Chen *et al*., 2008; Chen and Yuan, 2011). The concentration of carbon content and volatile matter in the biochar synthesized from plant biomass are higher than the agricultural waste, this is due to the presence of higher lingo-cellulose content in the plant wood as compared to other species. Biochar is a potentially valuable and sustainable tool to improve soil quality and crop yield. Various research applications of biochar in the field of environment, energy and agriculture have been increased dramatically in the form of environmental pollution, contaminant remediation (from soil and water bodies), energy shortage and food security (Lyu *et al*., 2020; Chen *et al*., 2019). The proximate and ultimate analysis of biochar consists: fixed carbon content, moisture content, ash content and volatile matter with different elemental composition (Kaur and Sharma, 2019b). Out of these, carbon is an important component of life, of human society and of biological energy system (Schouten, 2010).

Primary properties of biochar are directly influenced by feedstock type and production conditions. These properties are: crystalline carbon, amorphous carbon, porous structure, surface functional groups, volatile matter, resident matter, ash content and elemental composition (like C, H, N, O, S, Ca, Mg, P, Si). On the other hand, secondary properties of biochar are factors of its primary properties which include: pH, EC, CEC, surface area, bulk density, particle density, stability and surface charge. Both properties facilitate the different applications (Kaur and Sharma, 2019c; Igalavithana *et al*., 2017; Nigussie *et al*., 2012). Biochar have several potential benefits, which includes bioenergy production (Mia *et al*., 2014; Laird, 2008; Lehmann *et al*., 2009), carbon sequestration (Mia *et al*., 2014; Streubel *et al*., 2011; Kookana *et al*., 2011; Laird, 2008), adsorption of organic and inorganic contaminants due to high surface area (Latawiec *et al*., 2017; Hale *et al*., 2011; Jiang *et al*., 2012; Mia *et al*., 2014), increased nutrient cycling (Adeyemi and Idowu 2017; Shenbagavall and Mahimairaja, 2012), soil health (Atkinson *et al*., 2010; Sohi *et al*., 2010; Lehmann *et al*., 2009) and other environmental benefits (Spokas *et al*., 2012). Due to its porous structure, it serves as a soil conditioning agent that can reduce the bulk density, improves soil texture, pore size distribution and rate of soil water percolation (Rogovska *et al*., 2014; Kookana *et al*., 2011). High soil pH helps to enhance the metal sorption rate from contaminated sites because of the de-protonation of pH-dependent cation exchange sites on soil surface (Sizmur *et al*., 2016). Electrical conductivity (EC) of biochar depends on its surface porosity, surface area and crystalline structure. EC indirectly affects the soil nutrient cycling (Gul *et al*., 2015; Wang *et al*., 2015). WHC of biochar are affected by the average pore diameter and the total pore volume. The presence of large pores in biochar can not only hold the maximum water in it, but also acts as the passage for small pores. The rate of SOC increases with increased biochar application rate (Shenbagavall and Mahimairaja, 2012). Cation exchange capacity (CEC) and exchangeable bases are one of the important parameters of soil. They relate information on a soils ability to sustain plant growth, a measure of fertility, retain nutrients, buffer acid deposition or sequester toxic metals.

Furthermore, mixing of biochar in the soil helps to increase nutrient retention by increasing CEC and also helps to increase exchangeable bases. Cation exchange occurs due to the negative charges carried by soil particles, in particular, clay minerals, sesquioxides and organic matter. These negative charges are cancelled out by the sorption of cations from solution. The CEC can be estimated by summation of exchangeable bases (Ca^+, Mg^+, Na^+ and K^+) and exchangeable Al^+. CEC changes with soil type clay have a great capacity to attract and hold cations because of its chemical structure. Sand has no capacity to exchange cations because it has no electrical charge. This means sandy soils such as podzolic top soils have very low CEC (Liang *et al*., 2006). Sandy soils are more prone to high bulk density. High bulk density is an indicator of low soil porosity and soil compaction. It may cause restrictions to root growth and poor circulation of air and water through the soil (Rogovska *et al*., 2014; Carter *et al*., 2013). Hence, it helps to enhance the soil physico-chemical properties or restore degraded soils (Conte *et al*., 2016; Latawiec *et al*., 2017; Kaur and Sharma, 2019a). This problem overcomes by adding biochar in sandy soil and increases its quality. Amendment of biochar in soil has therefore been proposed as a potential mechanism to not only increase soil fertility but also to lock up biogenic carbon by offsetting carbon emissions associated with the burning of fossil fuels (Streubel *et al*., 2011; Kookana *et al*., 2011). Biochar is also referred as an effective tool in agricultural waste management practices and in energy production.

The present research addressed mainly three hypothesis: (1) berseem grown in sandy soil having different concentrations of mixed wood biochar, showed higher influence on soil physico-chemical properties as compared to non-biochar-amended soil samples; (2) biochar application rate effectively enhances the soil organic carbon content as well as cation exchange capacity and (3) Amendment of biochar in sandy soil helps to increase soil water holding capacity and surface porosity. Keeping view in effectivity of biochar to improve soil quality or acts as a soil conditioning agent, the main objectives of present study was: (i) to determine the effect of biochar and their application rates on physico-chemical properties of sandy soil and (ii) to evaluate the effect of biochar applications at different sampling times,

2. 2. Material and Methods

2.1. Sample & material collection

The mixed wood biochar (MWBC) was prepared from Sal, Cheed and Teak wood biomass, procured from Chorahi village, Bilaspur, District Yamunanagar, Haryana, India. Biochar was synthesized by traditional earth mound kiln method at approximately 450ºC, 48h. It was crushed to homogenize before pot filling. Farm soil (sandy soil) used in this study was collected from the University Energy Park, Guru Jambheshwar University of Science & Technology, Hisar, India. The air-dried soil and biochar were subsequently sieved to pass through a 2mm sieve and stored at room temperature prior to the experimental study. The physico-chemical properties of soil and biochar sample are represented in table1. The soil was mixed homogeneously with different concentration of biochar (0%, 1%, 5% and 10%) by developing soil beds. Naphthalene (50 mg/kg) and phenanthrene (100 mg/kg) separately dissolved in hexane and stirred continuously for 30 min without any disturbance. Finally, spiked the measured concentration of 50 mg/kg naphthalene and 100 mg/kg phenanthrene on soil beds and mixed homogeneously.

2.2. Pot experiment setup

The pot experiments were designed under natural conditions from October, 2016 to April, 2017. The experiments designed in replicates. The sixteen treatment combinations were arranged in a randomized complete block design with three replicates for a total 144 pots. Seeds of *Trifolium alexandrinum* (berseem), variety Mescavi were procured from Chaudhary Charan Singh Haryana Agriculture University (CCSHAU), Hisar, India and sterilized by using mercuric chloride solution. Approximately, 100 seeds/ pot were sown during the experimental study. Rhizospheric soil samples from pots having different concentration of biochar (0%, 1%, 5% and 10%) were taken on an interval of approximately 60 days. The different set of treatments was shown in table 2. A soil: biochar (w/w) mixture of the suitable ratio for each application was used for filling the different pots 19.6cm diameter × 20.6cm height. In order to manage the same final volume of pot for different treatments, the soil volume was adjusted as needed in order to retain the total volume of the soil: biochar mixture. Hence, the potential rooting depth of plants was same for all treatments. Soil and plant samples were collected at different time intervals, oven dried, sieved by 2mm sieve and stored at room temperature in plastic containers for further characterization.

Table: 1. Physico-chemical properties of the soil and biochar used in the experimental study.

Sr. No.	**Characteristics**	**Soil**	**Biochar**
1.	Soil Type	Sandy soil	Alkaline
2.	Soil Texture	•Sand-85.09% •Silt-10.71% •Clay-4.20%	ND
3.	Soil & biochar colour	-	Black
4.	Bulk density (g/cm^3)	1.59±0.01	0.49±0.03
5.	Soil structure	Granular	-
6.	Water holding capacity (%)	25.7±0.12	107±0.11
7.	Biochar Type	-	Mixed wood
8.	pH(1:10 solid water suspension)	8.09±1.01	8.59±1.01
9.	Electrical conductivity (EC) dsm^{-1} (1:10 soil water extract)	1.31±0.28	1.41±1.01
10.	Cation exchange capacity (cmol/kg)	9.24±0.31	19.5±1.25
11.	Total Organic Carbon (TOC) (%)	5.27±0.00	183.6±2.01
12.	Total carbon (TC) (%)	8.73±0.29	183.6±1.41
13.	Inorganic carbon (IC) (%)	3.46±0.41	ND
14.	Exchangeable Na (g Kg^{-1})	0.21±0.01	0.71±0.01
15.	Exchangeable K (g Kg^{-1})	0.80±0.00	0.81±0.00
16.	Exchangeable Mg (g Kg^{-1})	0.04±0.00	0.07±0.00
17.	Exchangeable Ca (g Kg^{-1})	3.09±0.21	11.9±1.01
18.	Cu (mg/Kg)	10.0±0.00	ND
19.	Mn (mg/Kg)	190.0±0.00	70.0±0.00
20.	Ni (mg/Kg)	10.0±0.00	ND
21.	Zn (mg/Kg)	30.0±0.01	90.0±0.00
22.	Fe (mg/Kg)	50.0±0.00	17.0±0.01
23.	Naphthalene (mg/kg)	ND	0.011±0.00
24.	Phenanthrene (mg/kg)	ND	0.043±0.01

Values are mean±standard error (S.E) of triplicate samples.

Table: 2. Different set of treatments under pot experiment.

Set. 1	**A0** Control	**A1** Soil+1% biochar	**A2** Soil+5% biochar	**A3** Soil+10% biochar
Set. 2	**B0** Soil+50 mg/kg Naph.	**B1** Soil+50 mg/kg Naph.+ 1% biochar	**B2** Soil+50 mg/kg Naph.+ 5% biochar	**B3** Soil+50 mg/kg Naph.+ 10% biochar
Set. 3	**C0** Soil+100 mg/kg Phen.,	**C1** Soil+100 mg/kg Phen.+ 1% biochar	**C2** Soil+100 mg/kg Phen.+ 5% biochar	**C3** Soil+100 mg/kg Phen.+ 10% biochar
Set. 4	**D0** Soil+50 mg/kg Naph.+ 100 mg/kg Phen.	**D1** Soil+50 mg/kg Naph.+ 100 mg/kg Phen.+ 1% biochar	**D2** Soil+50 mg/kg Naph.+ 100 mg/kgPhen.+ 5%biochar	**D3** Soil+50 mg/kg Naph.+ 100mg/kgPhen.+ 10%biochar

2.3. Different methods used for the analysis of soil and biochar characteristics

The different materials used in present study were soil, biochar, berseem seeds, naphthalene and phenanthrene. Physico-chemical parameters of soil were studied, before spiking contaminants in the soil. pH was determined by using a pH meter (1:10 m/v), electrical conductivity (EC) was measured by using conductivity meter (1:10 m/v) (ISO 11265:1994), TOC, TC and IC were analysed with the help of Total organic carbon analyzer (Shimadzu, Japan), soil organic carbon (SOC) was determined by Walkley-Black method or wet digestion method and CEC was determined by using Flame Photometer (ELICO CL-378). The amount of micro-nutrients (Mn, Cu, Ni, Zn and Fe) was analysed by Atomic Absorption Spectrophotometer (AAS) (AA 6300- SHIMADZU). Available Na and K concentrations were determined by using Flame Photometer. Ca and Mg were measured by using EDTA-titration method. Soil texture was determined by sieve test method, using 0.09mm –1mm standard test sieves (ISO 11277:2009). Soil Water holding capacity (%) was analysed with the help of brass box (Devi and Singh, 2015). Soil Volume (cm^3), percentage solid space (%), porosity (%), particle density (g/cm^3) and bulk density (g/cm^3) was calculated by using Core Method (ISO 9001:2000) and soil particle density (g/cm^3) by using (ISO 11508:2017) method. Volatile matter, ash and moisture content were analysed by using the American Society for Testing and Materials ASTM (D1762-84) method (Kaur and Sharma, 2020). Fixed-carbon content was calculated by subtracting volatile matter, ash content and moisture content from the total dry weight of biochar (Kaur and Sharma, 2019b; Sun *et al*., 2017; Zhao *et al*., 2017). Energy Dispersive X-Ray analysis (EDX) is an X-Ray technique which was used to identify the elemental composition of MWBC. Moreover, a solid phase extraction procedure was used for the determination of naphthalene and phenanthrene in dried soil and biochar sample using Gas-chromatography coupled with Flame Ionization Detector (GC-FID) (Varian 450-GC). Moreover, the standard peaks as well as GC-FID chromatogram of phenanthrene & naphthalene are represented in Fig 5 and 6. The extraction efficiencies of PAHs analysed are shown in Table 6 (Supplementary file).

2.4. Statistical analysis

All experiments were performed in triplicates. The data was homogeneous and normal distributed. For comparison between all set of treatments at different durations Two-way ANOVA and Duncan test was performed by using SPSS V.18 and presented as mean of three replicates ± S.E. level of significance between treatments was checked at ($P \leq 0.05$).

3. Results

3.1. Proximate and ultimate analysis of biochar

The proximate and ultimate/elemental compositions of biochar sample prepared at 450ºC are shown in Table 5 (Supplementary file) (as already published in Kaur and Sharma, 2019b).

3.2. Bulk density, Particle density, Percent solid space, Soil porosity and Water holding

The bulk density, particle density, percent solid space, water holding capacity and soil porosity are the important parameters of soil which indicate the soil quality and its productivity. The analysis of variance showed that the significant results were observed in case of soil bulk density, percent solid space, soil porosity and water holding capacity at ($P \leq 0.05$) significance level. On the other hand, non-significant effect was recorded for particle density in biochar amended and non-biochar amended soil samples (as represented in Table 3). Soil amended with 1%, 5% and 10% biochar concentration significantly helps to reduce the soil bulk density 8.18%, 12.58% and 17.61% respectively, as compared to control. Similarly, percent solid space effectively decreased with increased biochar application rate from 1% to 10% in soil. The reduction percentage calculated as 8 to 17% respectively, as compared to control.

Moreover, water holding capacity and soil porosity was significantly enhanced with biochar application rate. Soil having different concentrations of biochar i.e. 0%<1%<5%<10% showed an increase in the water holding capacity from 34.4%, 41.04% and 48.4% respectively, as compared to control.

Table: 3. Physical characterization of sandy soil.

Sr. No.	Treatments	Bulk density (g/cm^3)	Particle density (g/cm^3)	Percent solid space (%)	Porosity (%)	Water holding capacity (%)
1.	A0	1.59±0.01 a	2.65±0.06 a	60.01±1.01 a	40±0.01 d	113.22±0.96 d
2.	A1	1.46±0.03 b	2.65±0.09 a	55.20±0.80 b	45±0.01 c	172.80±0.90 c
3.	A2	1.39±0.02 c	2.65±0.11 a	52.49±1.68 c	48±0.03 b	192.06±1.41 b
4.	A3	1.31±0.09 d	2.64±0.12 b	49.76±0.46 d	50±0.01 a	219.63±0.94 a

Means±S.E (standard error) with the different letters denote significant differences in soil properties and mean value with same letter are not significantly different at ($P \leq 0.05$) level of significance. Here, A0= control, A1= soil+1% biochar, A2= soil+5% biochar, A3= soil+10% biochar application rate.

3.3. pH and Electrical conductivity (EC)

The statistical analysis revealed that non-significant ($P \leq 0.05$) results were observed in case of increase in pH and EC values in biochar amended and non-biochar amended soil samples (as shown in Fig 1 & 2).

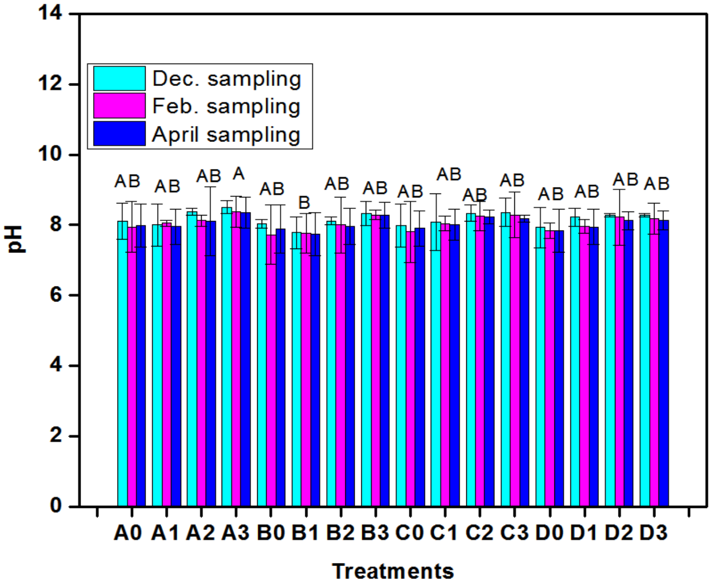

Fig: 1. Soil pH (Means±S.E) with the different letters denote significant differences in soil pH value and mean value with same letter are not significantly differ at ($P \leq 0.05$) level of significance using Duncan multiple comparison test.

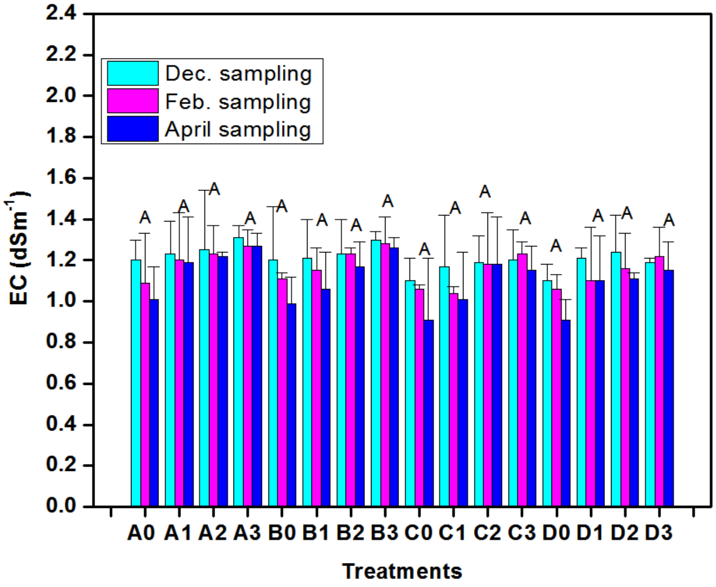

Fig: 2. EC value (Means±S.E) with the same letter denote that all treatment sets at different time are not significantly differ from each other. Comparisons using a Duncan multiple comparison test at ($P \leq 0.05$).

During the study period, initially (Dec. 2016) the pH of control soil sample was observed to be 8.10 and EC value has been found to be 1.20 dsm^{-1}. Whereas, the non-significant percent increase was observed in 10% biochar amended soil sample i.e. pH (8.51) and EC value was found to be 1.31 dsm^{1}. Similarly, at the end of approximately 121days (April 2016), there has been no effective difference was noticed in pH (7.99) and EC values (1.01 dsm^{-1}). Furthermore, it was reported that with time period (Dec. to April), soil pH and EC values was non-significantly reduced from 8.51 to 8.36 and 1.32 to 1.28 dsm^{-1} respectively, in 10% biochar amended soil sample.

3.4. Cation exchange capacity (CEC) and exchangeable bases

The CEC and exchangeable bases are the important characteristics of soil which determines the nutrients sorption and their availability in the soil. The statistical analysis revealed that a significant ($P \leq 0.05$) increase in CEC value as well as in exchangeable bases was noticed in biochar amended sandy soil (0%<1%<5%<10%) samples as compared to control. Similarly, CEC and exchangeable bases was effectively increased with time period (from Dec. to April) in biochar-amended soil samples as compared to biochar non-amended soil sample (as represented in Fig 3 and exchangeable bases in Table 4).

Initially in control soil (Dec. 2016), the CEC was observed to be 9.43 cmol/kg which was significantly reduced to 9.18 cmol/kg (April 2017). In biochar non-amended soil samples, the CEC significantly decreased at the rate of 2.54% (Dec. to Feb.), 2.65% (Dec. to April) and 0.10% (Feb. to April). The cation exchange capacity of sandy soil increases with biochar application rate (0%<1%<5%<10%). Moreover, the soil having different concentration of biochar, the CEC value was increased from Dec. to April as compared to control. The percent increase was observed to be 24.13-32.94% in 10% biochar-amended soil (A3) as compared with control (A0). If we compared B3 and B0 soil sample, the percent increase was recorded to be 24.45-30.08%. Similarly, the comparison study between C3 and

C0 soil sample, the percent increase was observed to be 21.50-27.38%. Furthermore, in mixed contaminated soil sample (D3 and D0) the percent increase was reported to be 22.45-28.44%.

Table: 4. Comparison study of exchangeable bases with respect to biochar amended and non-biochar amended soil samples.

		Exchangeable bases (mg/kg)											
Sr.No.	Treatments	Na^+			K^+			Ca^+			Mg^+		
		I	II	III	I	II	III	I	II	III	I	II	III
1.	A0	125dB	127dB	139dA	570dC	595dB	675dA	3015dB	3062dB	3110dA	35.41dC	36.71dB	38.10dA
2.	A1	170cC	220cB	515cA	595cC	605cB	1105cA	3690cC	3745cB	4310cA	36.07cC	46.92cB	69.17cA
3.	A2	235bC	335bB	524bA	695bC	715bB	1335bA	3735bC	3875bB	4670bA	38.78bC	49.12bB	73.13bA
4.	A3	265aC	380aB	573aA	705aC	800aB	1560aA	3820aC	4025aB	4870aA	39.40aC	51.21aB	75.53aA

Here, the small letters shows the significance difference with different biochar dose effect (1% to 10%) and capital letters indicates the significance difference at different time intervals (Dec. to April).Mean values within column followed by the same letters are not significantly different and mean values with different superscripts denote significant difference between all treatments at different sampling intervals using Duncan multiple comparison test ($P \leq 0.05$).

[Here, I=Dec. sampling, II= Feb. sampling, III= April sampling and A0= control, A1= soil+1% biochar, A2= soil+5% biochar, A3= soil+10% biochar dose effect. These results showed that, the soil amended with 10% biochar concentration significantly increased the exchangeable base level in sandy soil as compared with other treatments or control (10%>5%>1%>0%).

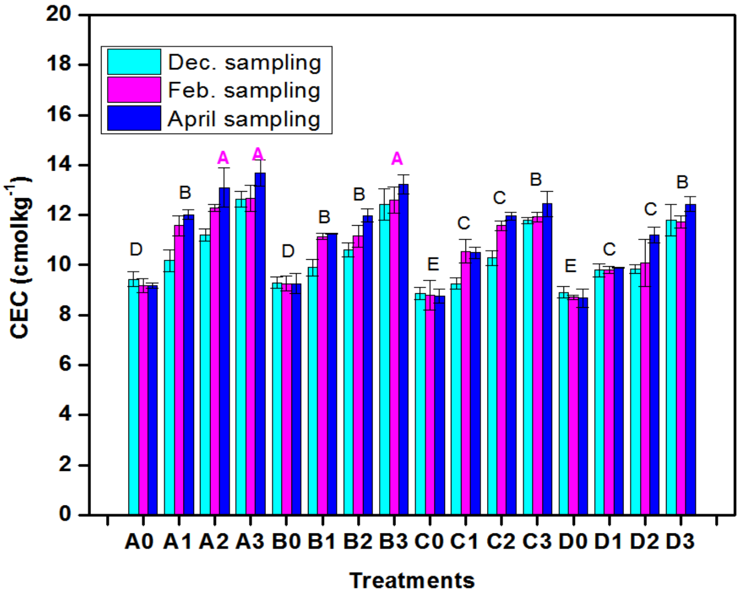

Fig: 3**.** Cation exchange capacity (Means±S.E) with the different letters denote significant differences in soil CEC value and mean value with same letter are not significantly differ at ($P \leq 0.05$) level of significance using Duncan multiple comparison test.

3.5. Soil organic carbon content (SOC)

The statistical analysis revealed that a significant ($P \leq 0.05$) increase in SOC content in biochar amended soil as compared to control and other set of treatments (as represented in Fig 4).

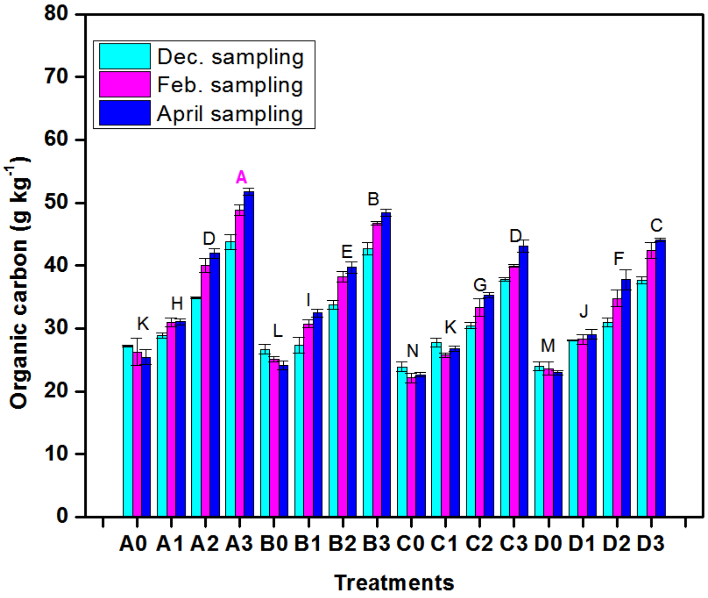

Fig: 4. Organic carbon (Means±S.E) with the different letters denote significant differences in SOC values and mean value with same letter are not significantly differ at ($P \leq 0.05$) level of significance using Duncan multiple comparison test.

In Dec. 2016, the SOC was recorded to be 27.30 g Kg^{-1} which was reduced to 25.47 g Kg^{-1} (April 2017) in control soil. Hence, in non-biochar amended soil samples the SOC level significantly reduced at the rate of 3.66% (Dec. to Feb.), 6.60% (Dec. to April) and 3.04% (Feb. to April). The organic carbon content of soil increases with biochar application rate (0%<1%<5%<10%). Furthermore, in biochar amended soil samples the SOC content was significantly increased with the time period (from Dec. to April). The percent increase was observed to be 37.68-50.82% in 10% biochar-amended soil sample (A3) as compared with control (A0). The comparison between B3 and B0 soil samples, the percent increase was found to be 36.60-49.73%. Similarly, if we compared the C3 and C0 soil sample, the percent increase was observed to be 33.88-45.63%. Furthermore, in mixed contaminated soil sample (D3 and D0) the percent increase was reported to be 35.19-47.84%.

4. Discussion

In this study, water holding capacity and soil porosity was significantly enhanced with biochar application rate. These results are in strong agreement with (Yu *et al*., 2013). Moreover, soil having 10% biochar application rate results in 20% increase in soil porosity as compared to the non-biochar amended soil sample. These results were found to be in close proximity to the results of (Rogovska *et al*., 2014; Carter *et al*., 2013). The non-significant results were recorded for pH and EC value in biochar amended and non-biochar amended soil. This might be due to the release of protons (H^+) from the exchangeable sites of biochar and due to the proliferation of acid producing soil microbes. Lucchini *et al*., (2014) revealed that, non-significant increase in pH and EC value as a result of the addition of biochar as compared to control. The lack of significant changes observed in pH and EC at different biochar doses may be due to the displacement of exchangeable acidity, high buffering capacity and biochar production conditions (Dume *et al*., 2015). Moreover, it may be due to the production of organic acid during the decomposition of organic matter and leaching of cations present in the soil. Similar results were also observed by Shenbagavalli and Mahimairaja, 2012. The soil CEC increased with an increase in soil organic matter. The presence of organic matter in sandy soil helps to increase the negatively charged sites on their surface, which results in electrostatic attraction by holding and adsorbing the positively charged ions. The direct supply of positively charged nutrients or cations (e.g. Na^+, Ca^+, Mg^+ and K^+) to plants was a critical step. This may have happened due to the presence of a large amount of negatively charged sites that causes the strong electrostatic attraction and results to improve soil fertility because they hold maximum cations (Mckenzie *et al*., 2004). Biochar has high surface area, porosity, surface negative charge, surface adsorption capacity, base saturation and possesses a variable charge organic material that has potential to increase soil CEC and exchangeable bases (Na^+, Ca^+, Mg^+ and K^+) when mixed in soil (Carter *et al*., 2013; Dume *et al*., 2016; Nartey and Zhao, 2014; Abdul and Abdul, 2017). CEC represents the capacity of soil for the exchange of positively charged ions between the soil and the soil solution. The exchangeable base level significantly increased with biochar application rate (0%< 1%< 5%<10%). The maximum increase was observed in soil having 10% biochar concentration as compared to control or other treatments. As biochar ages, the more negatively charged exchange sites develop on biochar surface and positive exchange sites decreases, this might be due to presence of oxidized functional groups (Cheng *et al*., 2006; Shenbagavalli and Mahimairaja, 2012). The presence of oxidized functional groups indicated the high oxygen and carbon ratio on biochar surface (Liang *et al*., 2006; Shenbagavalli and Mahimairaja, 2012).

In present research, the maximum percent increase in CEC value was found in biochar-amended soil as compared to another set of treatments. Soil spiked with naphthalene dose and amended with different concentrations of biochar (2nd set of treatment) didn't show any significant difference as compared with 1st set of treatment (having no PAH concentration). Significant results were found if we compared the 3rd set of treatment in which different concentrations of biochar was mixed with soil and spiked with 100 mg/kg phenanthrene dose as well as in 4th set of treatment in which soil is spiked with mixed dose of both naphthalene and phenanthrene at various sampling intervals. Similarly, the maximum percent increase in SOC level was noticed in biochar-amended soil samples as compared to control. The increase in SOC with an increase in time period was due to the microbial decomposition. Same results were also represented by Dume *et al*., 2015; Rogovska *et al*., 2014; Liang *et al*., 2006. Similar results were observed by Dume *et al*., (2015). In this study, biochar was synthesized from corn cob and coffee husk at 350 and 500ºC. The physico-chemical parameters were determined and they found that with increase in the biochar application rate from 1% to 10%, the SOC content was increased significantly. The increase in SOC with biochar concentration might be due to enrichment of soil with organic carbon content and a large amount of carbon was sequestered in soil amended with biochar and recalcitrance of OC in biochar (Nigussie *et al*., 2012; Lehmann, 2007; Solomon *et al*., 2007; Liang *et al*., 2006; Oleszczuk *et al*., 2014). About 38.5 to 51.4% increase in SOC was observed in the biochar-amended soil as compared to control with the time period (as discussed above). These results are in close agreement with Shenbagavalli and Mahimairaja, 2012; Domene *et al*., 2014; Oleszczuk *et al*., 2014.

5. Supplementary file

Table: 5. Proximate and Ultimate analysis of biochar derived from mixed wood plant biomass. (Data set are already published) (Kaur and Sharma, 2019b)

Sr. No.	**Constituents**	**MWBC**
Proximate Analysis		
1	Moisture (%)	0.97
2	Volatile Matter (%)	14.86
3	Ash content (%)	7.98
4	Fixed carbon (%)	76.19
Ultimate Analysis		
5	Hydrogen (%)	1.71
6	Sulfur (%)	0.03
7	Oxygen (%)	20.26
8	Nitrogen (%)	1.67

➢ Optimization of extraction procedure and choice of extraction solvent

Extraction efficiency of PAHs was significantly depending on the affinity of extraction solvent. To achieve better extraction efficiency of PAHs from biochar sample were first analysed with 2 solvents having wide polarity range like n-hexane and toluene. The extraction efficiency of PAHs expressed as a percentage determined from both extraction solvent is given in table. 6.

Table: 6. The extraction efficiencies of PAHs analysed.

PAHs	Extraction efficiencies ± RSD (%)	
	n-hexane	Toluene
Naphthalene	ND	66.39±0.26
Phenanthrene	52 ±2	74.36 ±0.39

➢ Sample preparation for PAHs analysis

The 2g sample was transferred into a glass beaker and 20-30 ml extraction solvent (toluene) was added to it. Samples were sonicated by using probe sonicator for 30 min at 30-40 duty cycles. The same procedure was followed 2-3 times by adding toluene for all samples. After that high-speed centrifuge MPW-350R was used at 4000 rpm for 15 min, the sample was filtered by using Whatman filter-paper No. 1, pore size 11 µm. Silica gel for chromatography 60-120 mm mesh size glass column was used to filter-out the colored samples to make it colorless. Collect the whole extract in amber glass GC-vials. Finally, extract volume were reduced to 1-2 ml by allowing solvent evaporation with the help of rotator distillation unit at 100°C for approximately 45 min followed by GC-FID for PAHs analysis.

➢ Reagents and Standards for PAH analysis

The Poly-aromatic hydrocarbons analyzed in the present study were naphthalene and phenanthrene. An intermediate standard set of the solution was developed by transferring the equal dose of both naphthalene and phenanthrene (0.1 g to 25 ml) volumetric flask and dissolved in toluene. The final concentration prepared was 4000 ppm. A set of calibration standard solution of 1, 0.5 and 0.1 ppm was prepared from the standard working solution and used to fortify soil and plant samples. Toluene was HPLC grade and silica gel for chromatography 60-120 mm mesh size was purchased from Loba chemicals. The ultrapure Milli-Q water was used in the present study. Naphthalene and phenanthrene standard chemicals were purchased from Sigma-Aldrich.

➢ GC-FID conditions

The analysis of PAHs was carried out by GC (Varian-450, 2700 Mitchell drive, Walnut Creek, CA 94598-1675/USA) equipped with a flame ionised detector (FID) and an automatic split-splitless injector model. The column used for separation was 30 m × 0.53 mm, Id BPX 5, 1.5 (Serial no. 741901).

GC-FID operating conditions were as follows:
Injector port temperature= 280°C,
Nitrogen as carrier gas at a flow-rate = 1.2 ml/min.
Pulsed splitless mode (pulsed pressure 45 psi= 310 kPa for 1.5 min).
Column temperature = 140°C for 3 min, then programmed at 6°C/min to 250°C; increased to 300°C at a rate of 10°C/min and held for 5 min.
The total analysis time was 42 min and the equilibration time was 2 min. A 1µl volume was injected to the injector. Make-up (Nitrogen) flow rate 28 ml/min; combustion (Hydrogen) flow rate 30ml/min and combustion (Air) flow rate 30 ml/min were programmed.

➢ Analytical characteristics of standards by gas chromatography

Linear calibration curve was obtained by using four-point standards ranged from 0.1, 0.5 and 1 ppm. The linear graph plotted std. conc. v/s chromatographic area whose, R^2=0.999 for phenanthrene and R^2 = 0.997 for naphthalene. 2g of dried, sieved soil (2 mm sieve) and 3g of plant sample was dissolved in 20 ml and 30 ml toluene respectively, used for the extraction of PAHs. PAHs were analysed by GC-FID. Representative GC-FID chromatogram of naphthalene and phenantharene analysed in a mixed wood biochar sample Fig 5. Both naphthalene and phenanthrene were satisfactory separated with adequate sensitivity.

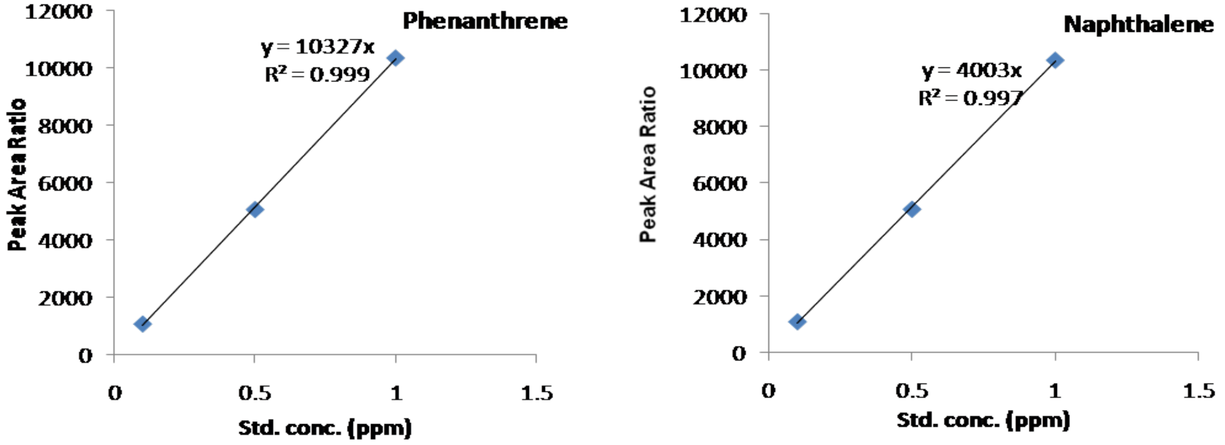

Fig: 5. Represents the standard peaks of Phenanthrene & Naphthalene (Conc. v/s peak area).

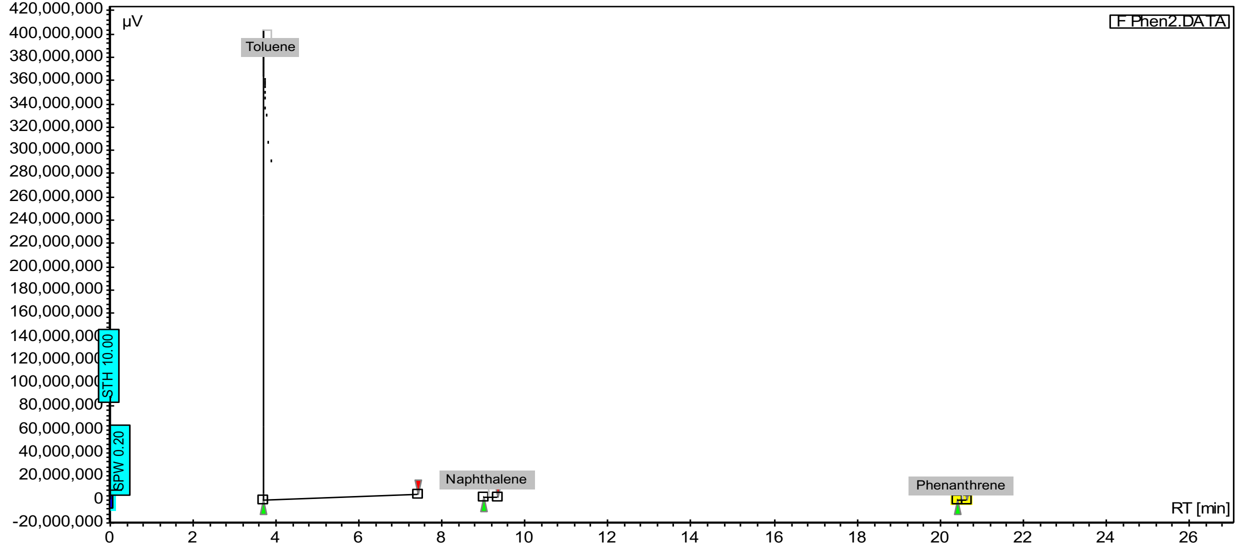

Fig: 6. Representative GC-FID chromatogram of naphthalene and phenantharene analysed in a mixed wood biochar sample**.**

6. Conclusions

The incorporation of biochar into agricultural land helps to improve soil physico-chemical characteristics and reduces environmental consequences if farmers are safely and willingly implement this amendment. Biochar application dramatically improves the soil health. It helps to reduce the increasing fertilizer demand and their negative effects on soil physical, chemical and biological properties. Proximate analysis of biochar samples provides the percentage of the material that burns in the solid state (fixed carbon), in a gaseous state (volatile matter) and the percentage of inorganic waste material (ash). Moreover, ultimate analysis of biochar was performed, for the determination of elemental composition of the biomass used during pyrolysis. The proposed study demonstrated that, biochar application in the soil helps to enhance its quality and reduces the negative effect of contaminants due to its high surface area, surface adsorption capacity, electrostatic attraction and highly porous structure. Biochar amended soil helps to reduce soil bulk density from 1.59 to 1.31 g/cm^{3}and increases soil porosity and water holding capacity from 40 to 50% and 114.05 to 230.52, respectively. In this study, CEC level increased from 9.43 to 13.69 cmol/kg and SOC from 27.27 to 51.79 g Kg^{-1}. Further work is needed to understand the reasons behind bacterial accumulation, PAH mineralization, contaminants surface attraction with biochar and different methodologies for the production of high-quality biochar for soil quality improvement.

Authors' contributions: Varinder Kaur performed all experiments, prepared the samples, calculations and wrote the manuscript. Prof Praveen Sharma (Professor and Dean) has done final editing and corresponding author.

Conflicts of interest: The authors declare that there is no conflict of interests regarding the publication of this paper.

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