

# CHARACTERIZATION OF MULBERRY STALK BIOCHAR AND ITS IMPACT ON SOIL NUTRIENTS RELEASE

## Abstract

An incubation study was conducted with graded levels of biochar to know the soil nutrients release till 120 days after incubation at Department of Soil Science and Agricultural chemistry, University of Agricultural Sciences, GKVK, Bengaluru during 2021. The experiment consisted of 4 treatments replicated five times and was laid out in a completely randomized design. The mulberry stalk biochar was characterized and recorded a pH of 8.53 and Electrical conductivity of  $0.39 \text{ dS m}^{-1}$ . The physical properties like bulk density ( $0.32 \text{ Mg m}^{-3}$ ) and maximum water holding capacity (93.14 %) were measured. Mulberry stalk biochar had 69.37 per cent of total carbon, 0.89 per cent Nitrogen, 0.22 per cent Phosphorus, 0.65 per cent Potassium and secondary nutrients like Calcium of 0.96 per cent, 0.48 per cent Magnesium and 0.18 per cent sulphur and micronutrients like Iron ( $493 \text{ mg kg}^{-1}$ ), Zinc ( $34.59 \text{ mg kg}^{-1}$ ), Manganese ( $94.1 \text{ mg kg}^{-1}$ ), copper ( $20.55 \text{ mg kg}^{-1}$ ) and boron ( $33.5 \text{ mg kg}^{-1}$ ) were recorded. It was observed that with increased levels of biochar application to soil from 5 to  $10 \text{ t ha}^{-1}$  positively influenced the nutrient release pattern in the soil. Higher primary, secondary and micronutrients were recorded with application of biochar @  $10 \text{ t ha}^{-1}$  ( $T_3$ ) at 60, 90 and 120 days after incubation and lower values were recorded in control treatment ( $T_4$ ).

**Keywords:** Biochar, nutrient release, mulberry stalk

## Introduction

Efficient use of biomass like crop residues and other farm wastes by converting into a useful organic source of nutrients (*i.e.*, biochar) is one way to manage soil health and soil fertility otherwise they are being dumped as waste material. Different crop residues like wood, mulberry stalk, cob rind and stover of maize, grasses, animal manure, areca husk, *etc.*, could be used for biochar production. Maize biomass waste is the largest agricultural waste after timber and rice waste, Mulberry (*Morus alba* L.) a perennial, deep rooted, fast growing and high biomass producing foliage plant and is the only source of food for the silkworm (*Bombyx mori*) and is grown under varied climatic conditions ranging from temperate to tropics. The sustainable production of mulberry leaf is entirely dependent on the maintenance of the soil fertility of mulberry garden through the periodical application of organic sources and inorganic fertilizers in required quantities. Though lot of mulberry stalk has been generated throughout the year and therefore conversion of this underutilized mulberry stalks into biochar helps to increase

potential biomass use and to conserve the nutrients apart from effective crop residue management and this could have a dramatic impact on the society and on agriculture worldwide.

Biochar is a carbon rich organic solid material derived from biomass pyrolysis *i.e.*, thermal decomposition of biomass in the absence or limited oxygen condition. During the pyrolysis process biomass is heated to 250 to 700°C temperature results in the production of volatile compounds, which are condensed to give bio-oil. The other products produced from pyrolysis include a gaseous material called "syngas" and carbon (C) rich charcoal material known as bio-char (Lehmann, 2007).

The left over organic solid biochar in the reactor contain C, O, H, N and ash (Ca and K). Biochar produced at low temperatures (250 to 400°C) contain C=O and CH functional groups that can act as nutrient exchange sites after slow oxidation. These may be better substrates for mineralization by bacteria and fungi, which have an integral role in nutrient turnover processes and aggregate formation compared to biochar produced at high temperature (400 to 700°C) which contain less ion-exchange functional groups due to dehydration and decarboxylation, potentially limiting its usefulness in retaining soil nutrients.

The importance of biochar for soil improvement is mainly due to its high surface area and a greater number of micro pores which helps to retain nutrients also provide habitat for beneficial soil microorganisms. It promotes storage of organic carbon (OC) in soil and it has characteristic ability to endure in soil with very little biological decay (Lehmann *et al.*, 2006). It's incorporation in the soil had been suggested to increase water holding capacity, soil fertility and enhance agricultural productivity (Ali *et al.*, 2018). It improves soil cation exchange capacity (Chintala *et al.*, 2014), soil physical properties and as a soil additive along with organic and inorganic fertilizers. With this backdrop, incubation study was conducted to know the composition of biochar and the soil nutrient release with application of graded levels of biochar.

## **Material and methods**

### **Characterization of biochar**

The biochar was characterised by various standardized analytical procedures for its specific physico-chemical properties such as bulk density, water holding capacity, pH, EC and total elementary composition (Table 1).

## Incubation study

To know the dissolution and release of nutrients from biochar, an incubation study was conducted. One hundred gram of soil was filled in a small plastic cup with graded levels of biochar and incubated at field capacity for 120 days. The experiment consisted of 4 treatments replicated five times and was laid out in a completely randomized design. Moisture content was maintained at field capacity by weighing the cups periodically and weight loss was made by adding distilled water.

Destructive sampling of soil was done at 30, 60, 90 and 120 days after incubation and samples were analysed for primary nutrients (nitrogen, phosphorus and potassium), secondary nutrients (calcium, magnesium and sulphur) and micronutrients (iron, zinc, manganese and copper) by adopting standard procedures and the results obtained from the analysis are presented in Table 3. The treatment details of the experiment are given below.

### Experimental details

Design	: Completely Randomized Design
No. of treatments	: 04
No. of replications	: 05
Biochar levels	: 03 (5, 7.5 and 10 t ha <sup>-1</sup> )

### Treatment details

- T<sub>1</sub>: Biochar @ 5 t ha<sup>-1</sup>
- T<sub>2</sub>: Biochar @ 7.5 t ha<sup>-1</sup>
- T<sub>3</sub>: Biochar @ 10 t ha<sup>-1</sup>
- T<sub>4</sub>: Control (without biochar)

The physical and chemical characteristics of the soil was given in Table 1.

**Table 1: Initial chemical properties of the soil**

---

Particulars	content
-------------	---------

---

Available nitrogen (kg ha <sup>-1</sup> )	261.37
Available phosphorus (P <sub>2</sub> O <sub>5</sub> kg ha <sup>-1</sup> )	35.84
Available potassium (K <sub>2</sub> O kg ha <sup>-1</sup> )	210.26
Available sulphur (ppm)	15.82
Exchangeable calcium [cmol(p <sup>+</sup> ) kg <sup>-1</sup> ]	4.52
Exchangeable magnesium [cmol(p <sup>+</sup> ) kg <sup>-1</sup> ]	1.85
DTPA extractable iron (mg kg <sup>-1</sup> )	12.66
DTPA extractable copper (mg kg <sup>-1</sup> )	1.56
DTPA extractable manganese (mg kg <sup>-1</sup> )	4.91
DTPA extractable zinc (mg kg <sup>-1</sup> )	0.83

### Biochar used for the study

Biochar is the C-rich solid product resulting from the heating of biomass in an oxygen-limited environment. Due to its highly aromatic structure, biochar is chemically and biologically more stable compared with the organic matter from which it is made. Generally, the properties of biochar vary widely, depending on the source of biomass used and the conditions of production of biochar. The biochar used for the study was obtained from mulberry stalk and after the production, the biochar was ground and sieved through a 2mm sieve. The physico-chemical characteristics of the mulberry stalk biochar used in this study are presented in the Table 2.

**Table2: Physico-chemical characteristics of mulberry stalk biochar**

Parameters	Value
Bulk density (Mg m <sup>-3</sup> )	0.32
WHC (%)	93.14
pH (1: 2.5)	8.53
EC (dS m <sup>-1</sup> ) (1: 2.5)	0.39
Total carbon (%)	69.37

Nitrogen (%)	0.89
Phosphorus (%)	0.22
Potassium (%)	0.65
Calcium (%)	0.96
Magnesium (%)	0.48
Sulphur (%)	0.18
Iron (ppm)	493
Manganese (ppm)	94.1
Zinc (ppm)	34.59
Copper (ppm)	20.55
Boron (ppm)	33.5

## Results and discussion

### Physico-Chemical Characterization of mulberry stalk biochar

The locally produced mulberry stalk biochar has been used in the present investigation and characterised by various standardized analytical procedures for its specific physico-chemical properties such as bulk density, water holding capacity, pH, EC and total elementary composition. Representative samples were taken from mulberry stalk biochar. The mulberry stalk biochar was ground to move through a sieve of 2 mm and tested for various chemical parameters and findings are shown in Table 2.

The properties of the mulberry stalk biochar used in the present study are greatly varied. The variation in mulberry stalk biochar's physico-chemical properties may be due to influence of the type of feedstock and the temperature of pyrolysis. Similar results were also reported by Gaskin *et al.* (2008) and Singh *et al.* (2010).

The mulberry stalk biochar had shown the lower bulk density of 0.32 g cm<sup>-3</sup> and higher maximum water holding capacity of 93.14 per cent (Table 1). Rodriguez *et al.* (2009) reported that such lower BD values and higher maximum water holding capacity was recorded in the biochar produced from woody materials. Brewer *et al.* (2014) in a study estimated the BD values of biochar produced from different feedstocks and it ranged from 0.25 to 0.6 g cm<sup>-3</sup> collected from natural fire site.

The biochar of mulberry stalk has a comparatively high pH (8.53) and is organically alkaline (Table 2). Such higher pH values of biochar were also reported by Gaskin *et al.* (2008) and Singh *et al.* (2010). The high pH of biochar can be attributed to hydrolysis of salts of Ca, Mg and K. The soluble salt in mulberry stalk biochar was 0.39 dS m<sup>-1</sup>. The carbon content (69.37 %) was higher in mulberry stalk biochar (Table2). In the coconut shell biochar, Total nitrogen, phosphorus and potassium amounts were respectively 0.89, 0.22 and 0.65 per cent. Mulberry stalk biochar contains a total of 0.96, 0.48 and 0.18 per cent respectively of calcium, magnesium, sulphur respectively. The iron, manganese, zinc, copper and boron concentrations were 493, 94.1, 34.59, 20.55 and 33.5 mg kg<sup>-1</sup>, respectively. This is in accordance with the report of [Rondon *et al.* (2007) and Emmanuel *et al.* (2010)].

### **Incubation study**

The results of the incubation study conducted to know the releasing pattern of nutrients from mulberry stalk biochar in the soil is presented below

#### **Effect of different levels of mulberry stalk biochar on available N release during incubation period (30, 60, 90 and 120 DAI)**

Addition of different levels of biochar to the soil increased the available N at 120 DAI when compared to 30 DAI. Available N content of soil as influenced by different levels of biochar was found to be significant at all intervals of incubation except at 30 DAI, while application levels was found to significant at 60, 90 and 120 DAI. Although, there was a significant difference in the available N content with different levels of biochar application when compared to control, the highest N content (281.19 kg ha<sup>-1</sup>) was recorded with application of biochar @ 10 t ha<sup>-1</sup> and the lowest (273.88 kg ha<sup>-1</sup>) with biochar @ 5 t ha<sup>-1</sup> at 120 DAI (Table 3 and Fig. 1.).

The available nitrogen content in the soil has been increased with incubation period because organic matter present in the native soil might have maintained more microbes with days of incubation and accounted for the increase in available nitrogen content (Singh *et al.* 2018). The increased mineralization rate of native soil organic matter and increase in soil carbon content might have contributed for the higher available N content in the soil (Luca *et al.* 2009).

#### **Effect of different levels of mulberry stalk biochar on available P<sub>2</sub>O<sub>5</sub> release during incubation period (30, 60, 90 and 120 DAI)**

Application of different levels of biochar was found to have a significant influence on available P<sub>2</sub>O<sub>5</sub> content at all intervals of incubation (Table 3 and Fig. 1). However, application of biochar at different levels was found to be non-significant only at 30 DAI. In general, there was a significant increase in the available P<sub>2</sub>O<sub>5</sub> content from 60 DAI to 120 DAI with all the levels of biochar including control and ranged from 35.36 to 47.75 kg ha<sup>-1</sup>. The highest available P<sub>2</sub>O<sub>5</sub> (47.75 kg ha<sup>-1</sup>) was recorded with the application of biochar @ 10 t ha<sup>-1</sup> at 120 DAI and the lowest (36.08 kg ha<sup>-1</sup>) with biochar @ 5 t ha<sup>-1</sup> at 30 DAI.

Biochar application increased available P<sub>2</sub>O<sub>5</sub> content in soil over the incubation period from 60 to 120 DAI due to increase of mineralization rate in the soil. The nutrient content of biochars is largely influenced by the type of feedstock and pyrolysis conditions (Singh *et al.* 2010), whereas the availability of nutrients in biochar is related to the type of bonds associated with the element involved (Luca *et al.* 2009 and Yao *et al.* 2010).

Phosphorus is mainly found in the ash fraction, with pH-dependent reactions and presence of chelating substances controlling its solubilisation (Luca *et al.* 2009). The increase in P availability due to presence of soluble and exchangeable phosphate in biochar and could be due to the release of soluble P directly from biochar and high ion exchange capacity of biochar which may alter P availability by providing anion exchange capacity or by influencing the activity of cations (Ca, Mg, Al, Fe) that interact with P (Venkatesh *et al.* 2018).

Biochar may also influence the bioavailability of P through several other mechanisms associated with P precipitation, such as biochar-induced surface sorption of chelating organic molecules which was also reported by Parvage *et al.* (2013) and Hass *et al.* (2012).

#### **Effect of different levels of mulberry stalk biochar on available K<sub>2</sub>O release during incubation period (30, 60, 90 and 120 DAI)**

A significant increase in available potassium content was observed with application of different levels of biochar at all intervals (Table 3 and Fig. 1).

Available potassium content significantly differed with the application of different levels of biochar at all intervals except at 30 DAI in the soil. Increase in available potassium content was observed with increased incubation time recording higher at 120 DAI. Among the treatment combination, biochar @ 10 t ha<sup>-1</sup> recorded higher available potassium content of 230.44 kg ha<sup>-1</sup> followed by application of biochar @ 7.5 (230.44 kg ha<sup>-1</sup>) and 5 t ha<sup>-1</sup> (230.44 kg ha<sup>-1</sup>) and

lowest potassium content ( $220.24 \text{ kg ha}^{-1}$ ) was recorded in the control treatment which is devoid of biochar at 120 DAI.

The available potassium content of biochar amended soil was significantly increased with increasing period of incubation time. This might be due to increase in the biochar induced K transformation in the soil. The rate of biochar had a significant effect on the available potassium content at all periods of incubation. Increase in the rate of biochar application noticeably enhanced the potassium availability. This might be due to the fact that biochar contained at least 0.3 per cent of ash and their application to soils registered an increase in availability of potassium. The supply of available potassium in biochar is generally higher and increased potassium availability as a result of biochar application has been reported by Lehmann *et al.* (2003).

#### **Effect of different levels of mulberry stalk biochar on exchangeable calcium release during incubation period (30, 60, 90 and 120 DAI)**

The data on effect of different levels of biochar application on exchangeable Ca content in the soil is presented in Table 4 and Fig. 2. Application of graded levels of biochar showed a significant effect on exchangeable Ca at 60, 90 and 120 DAI. A continuous increasing trend of exchangeable Ca content was observed from 30 to 120 DAI and with a maximum at 120 DAI.

The exchangeable Ca content varied with the application of different levels of biochar over an incubation time recording highest ( $5.89 \text{ cmol (p}^+) \text{ kg}^{-1}$ ) with the application of biochar @  $10 \text{ t ha}^{-1}$  at 120 DAI and lowest ( $4.58 \text{ cmol (p}^+) \text{ kg}^{-1}$ ) with biochar @  $5 \text{ t ha}^{-1}$  at 30 DAI

#### **Effect of different levels of mulberry stalk biochar on exchangeable Mg release during incubation period (30, 60, 90 and 120 DAI)**

The data on influence of different levels of biochar on exchangeable Mg content in the soil is presented in Table 4 and Fig. 2. Exchangeable Mg content in the soil differed significantly as a result of the application of levels of biochar at all intervals. In general, exchangeable Mg content showed an increasing trend from 30 DAI to 120 DAI. Changes in exchangeable Mg content as a result of the application of different levels of biochar over period of incubation which was ranged from  $1.91$  to  $2.95 \text{ cmol (p}^+) \text{ kg}^{-1}$ .

The highest exchangeable Mg content was recorded in the treatment which was received biochar @ 10 t ha<sup>-1</sup> which was followed by biochar @ 7.5 and 5 t ha<sup>-1</sup>.

Exchangeable bases of Ca and Mg content in soil varied significantly with the application of biochar at different levels. Higher exchangeable Ca content in soil was observed with the application of biochar @ 10 t ha<sup>-1</sup> than other two levels of biochar application at 5 and 7.5 t ha<sup>-1</sup>. Irrespective of levels, application of biochar significantly increased the exchangeable Ca, Mg and available S content as compared to control. Increase in exchangeable bases in soil can be attributed to release of basic cations from biochar. This might be due to high porosity and surface/volume ratio and improved the Ca and Mg availability (Yamato *et al.* 2006 and Chan *et al.* 2007).

UNDER PEER REVIEW

**Table 3: Effect of different levels of mulberry stalk biochar on available N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O release during incubation period (30, 60, 90 and 120 DAI)**

Treatments	Available N (kg ha <sup>-1</sup> )				Available P <sub>2</sub> O <sub>5</sub> (kg ha <sup>-1</sup> )				Available K <sub>2</sub> O (kg ha <sup>-1</sup> )			
	Days after incubation				Days after incubation				Days after incubation			
	30	60	90	120	30	60	90	120	30	60	90	120
<b>T<sub>1</sub>:Biochar @ 5 t ha<sup>-1</sup></b>	259.24	262.87	267.15	273.88	36.08	37.43	40.65	41.59	211.32	218.00	221.72	224.92
<b>T<sub>2</sub>:Biochar @ 7.5 t ha<sup>-1</sup></b>	261.33	266.56	270.74	276.17	35.71	38.15	42.48	43.01	212.64	220.04	223.44	227.52
<b>T<sub>3</sub>:Biochar @ 10 t ha<sup>-1</sup></b>	260.29	267.61	272.83	281.19	36.18	38.78	44.78	47.75	212.36	223.96	226.48	230.44
<b>T<sub>4</sub>:Control</b>	261.33	262.33	264.29	267.33	36.42	37.28	39.35	40.36	211.96	212.52	217.04	220.24
<b>S.Em ±</b>	<b>1.28</b>	<b>1.28</b>	<b>1.73</b>	<b>1.56</b>	<b>0.34</b>	<b>0.32</b>	<b>1.25</b>	<b>1.87</b>	<b>1.15</b>	<b>1.52</b>	<b>1.15</b>	<b>1.46</b>
<b>CD @ 1%</b>	<b>NS</b>	<b>4.17</b>	<b>5.65</b>	<b>5.11</b>	<b>NS</b>	<b>1.07</b>	<b>4.07</b>	<b>6.11</b>	<b>NS</b>	<b>4.96</b>	<b>3.76</b>	<b>4.76</b>

### **Effect of different levels of mulberry stalk biochar on available sulphur release during incubation period (30, 60, 90 and 120 DAI)**

Significant influence of different levels of biochar on available S content was observed in the soil at different intervals (Table 4 and Fig. 2). Application of biochar @ 10 t ha<sup>-1</sup> recorded higher available S content at 60, 90 and 120 DAI. Available S was found to increase with increased period of incubation time from 30 DAI to 120 DAI. Of all the treatments, application of biochar @ 10 t ha<sup>-1</sup> recorded highest available S content in soil (22.03 mg kg<sup>-1</sup>) at 120 DAI and lowest (17.32 mg kg<sup>-1</sup>) with biochar @ 5 t ha<sup>-1</sup> at 30 DAI.

Sulphur content in the soil varied significantly with application of different levels of biochar. This may be due the contribution of available sulphur to soil after the mineralization of organic sulphur in biochar. The results suggest that biochar also improves the bioavailability of sulphur; which mainly depends on mineralization of organic forms of sulphur to inorganic form. (Luca *et al.* 2009).

### **Effect of different levels of mulberry stalk biochar on DTPA extractable Fe release during incubation period (30, 60, 90 and 120 DAI)**

DTPA extractable Fe content differed significantly with the application of different rates of biochar at all intervals (Table 5 and Fig. 3). Application of biochar at different rates differed Fe content significantly at all intervals except 30 DAI. The Fe content showed a decreasing trend with increased level of biochar application at all intervals. Over a period of incubation, application of biochar @ 5 t ha<sup>-1</sup> recorded the highest (13.92 mg kg<sup>-1</sup>) Fe content at 120 DAI and biochar @ 10 t ha<sup>-1</sup> recorded lowest Fe content (12.56 mg kg<sup>-1</sup>) at 30 DAI

**Table 4: Effect of different levels of mulberry stalk biochar on exchangeable calcium, magnesium and available sulphur release during incubation period (30, 60, 90 and 120 DAI)**

Treatments	Exchangeable Ca (cmol (p <sup>+</sup> ) kg <sup>-1</sup> )				Exchangeable Mg (cmol (p <sup>+</sup> ) kg <sup>-1</sup> )				Available S (mg kg <sup>-1</sup> )			
	Days after incubation				Days after incubation				Days after incubation			
	30	60	90	120	30	60	90	120	30	60	90	120
<b>T<sub>1</sub> :Biochar @ 5 t ha<sup>-1</sup></b>	4.58	4.94	5.13	5.20	1.95	2.06	2.22	2.37	17.32	18.69	19.65	20.17
<b>T<sub>2</sub> :Biochar @ 7.5 t ha<sup>-1</sup></b>	4.62	5.06	5.37	5.61	1.91	2.16	2.45	2.72	17.82	18.91	20.07	21.11
<b>T<sub>3</sub> :Biochar @ 10 t ha<sup>-1</sup></b>	4.61	5.23	5.64	5.89	1.96	2.23	2.56	2.95	17.75	19.78	21.14	22.03
<b>T<sub>4</sub> :Control</b>	4.65	4.73	4.93	5.15	1.94	1.96	2.04	2.19	17.63	18.12	18.96	19.74
<b>S.Em ±</b>	<b>0.03</b>	<b>0.08</b>	<b>0.14</b>	<b>0.21</b>	<b>0.01</b>	<b>0.04</b>	<b>0.09</b>	<b>0.17</b>	<b>0.11</b>	<b>0.32</b>	<b>0.43</b>	<b>0.54</b>
<b>CD @ 1%</b>	<b>NS</b>	<b>0.28</b>	<b>0.45</b>	<b>0.68</b>	<b>NS</b>	<b>0.12</b>	<b>0.30</b>	<b>0.56</b>	<b>NS</b>	<b>1.06</b>	<b>1.41</b>	<b>1.78</b>

### **Effect of different levels of mulberry stalk biochar on DTPA extractable Mn release during incubation period (30, 60, 90 and 120 DAI)**

Among the biochar levels, application of biochar @ 10 t ha<sup>-1</sup> recorded lower DTPA extractable Mn content (4.57 to 4.85 mg kg<sup>-1</sup>) at all intervals (Table 5 and Fig. 3). Application of biochar increased Mn content from 30 to 120 DAI. Higher Mn content of 4.75, 4.89, 4.98 and 5.12 mg kg<sup>-1</sup> was recorded with the application of biochar @ 10 t ha<sup>-1</sup> at 30, 60, 90 and 120 DAI. Application of biochar @ 10 t ha<sup>-1</sup> recorded significantly lower Mn content of 4.57, 4.79, 4.88 and 4.85 mg kg<sup>-1</sup> at 30, 60, 90 and 120 DAI.

### **Effect of different levels of mulberry stalk biochar on DTPA extractable Zn release during incubation period (30, 60, 90 and 120 DAI)**

Application of biochar at different rates differed Zn content significantly at all intervals except 30 DAI. Higher Zn content of soil was observed at 120 DAI (0.81 to 0.98 mg kg<sup>-1</sup>) and lower at 30 DAI (0.72 to 0.78 mg kg<sup>-1</sup>) over a period of incubation (Table 5 and Fig. 3). Among different treatment combinations, highest Zn content (0.98 mg kg<sup>-1</sup>) was noticed with application of biochar @ 5 t ha<sup>-1</sup> and the lowest (0.70 mg kg<sup>-1</sup>) with biochar @ 10 t ha<sup>-1</sup> at 30 DAI.

**Table 5: Effect of different levels of mulberry stalk biochar on DTPA extractable iron, manganese, zinc and copper (mg kg<sup>-1</sup>) release during incubation period (30, 60, 90 and 120 DAI)**

Treatments	Fe (mg kg <sup>-1</sup> )				Mn (mg kg <sup>-1</sup> )				Zn (mg kg <sup>-1</sup> )				Cu (mg kg <sup>-1</sup> )			
	Days after incubation				Days after incubation				Days after incubation				Days after incubation			
	30	60	90	120	30	60	90	120	30	60	90	120	30	60	90	120
<b>T<sub>1</sub>:Biochar @ 5 t ha<sup>-1</sup></b>	12.68	13.25	13.63	13.92	4.75	4.89	4.98	5.12	0.78	0.86	0.95	0.98	1.54	1.72	1.76	1.87
<b>T<sub>2</sub>:Biochar @ 7.5 t ha<sup>-1</sup></b>	12.60	13.06	13.32	13.59	4.60	4.87	4.90	4.97	0.77	0.84	0.89	0.92	1.53	1.69	1.70	1.76
<b>T<sub>3</sub>:Biochar @ 10 t ha<sup>-1</sup></b>	12.56	12.92	13.14	13.26	4.57	4.79	4.88	4.85	0.70	0.76	0.83	0.87	1.51	1.63	1.65	1.72
<b>T<sub>4</sub>:Control</b>	12.62	12.78	12.92	13.08	4.63	4.69	4.72	4.78	0.72	0.75	0.79	0.81	1.52	1.54	1.58	1.64
<b>S.Em ±</b>	<b>0.09</b>	<b>0.09</b>	<b>0.14</b>	<b>0.18</b>	<b>0.10</b>	<b>0.02</b>	<b>0.03</b>	<b>0.05</b>	<b>0.007</b>	<b>0.02</b>	<b>0.03</b>	<b>0.03</b>	<b>0.01</b>	<b>0.02</b>	<b>0.03</b>	<b>0.03</b>
<b>CD @ 1%</b>	NS	<b>0.32</b>	<b>0.45</b>	<b>0.60</b>	NS	<b>0.09</b>	<b>0.12</b>	<b>0.17</b>	NS	<b>0.08</b>	<b>0.11</b>	<b>0.10</b>	NS	<b>0.07</b>	<b>0.10</b>	<b>0.12</b>

## **Effect of different levels of mulberry stalk biochar on DTPA extractable Cu release during incubation period (30, 60, 90 and 120 DAI)**

Application of different levels of biochar recorded a significant effect on DTPA extractable Cu content at all intervals except 30 DAI. During study period, higher Cu content was observed at 120 DAI and lower at 30 DAI (Table 5 and Fig. 3). Over a period of incubation, significantly higher Cu content ( $1.87 \text{ mg kg}^{-1}$ ) was recorded with application of biochar @  $5 \text{ t ha}^{-1}$  at 120 DAI and lower ( $1.51 \text{ mg kg}^{-1}$ ) with biochar @  $10 \text{ t ha}^{-1}$  at 30 DAI.

The variation in micronutrients content in soil supplied with the biochar over control can be attributed to their physical and chemical properties. Application of biochar at different levels recorded significant effect on micronutrient content at all intervals.

Higher micronutrients content was observed at 120 DAI and lower micronutrient status at 30 DAI. With increased level of biochar application, there is a decreasing trend in micronutrients content with incubation time over control (devoid of biochar). The organic materials form chelates and increase the availability of zinc. The available manganese status might be increased because of enhanced solubilisation of manganese due to reduction potential of manganese and non-complexation by organic ligands. Lentz and Ippolito, (2011) supported the significant increment in available manganese on biochar addition as biochar acts as a source of manganese. Hass *et al.* (2012) reported increase in extractable micronutrients (Fe, Zn, Mn and Cu) as a result of biochar application.

With increased level of biochar application recorded lower status of micronutrients in the soil which might be due to the adsorption, possible immobilization and precipitation. As pH of soil and micronutrients availability was negatively correlated, immobilization and precipitation of micronutrient occurs in soil and there by recorded lower micronutrient content in the soil with increased level of biochar application.

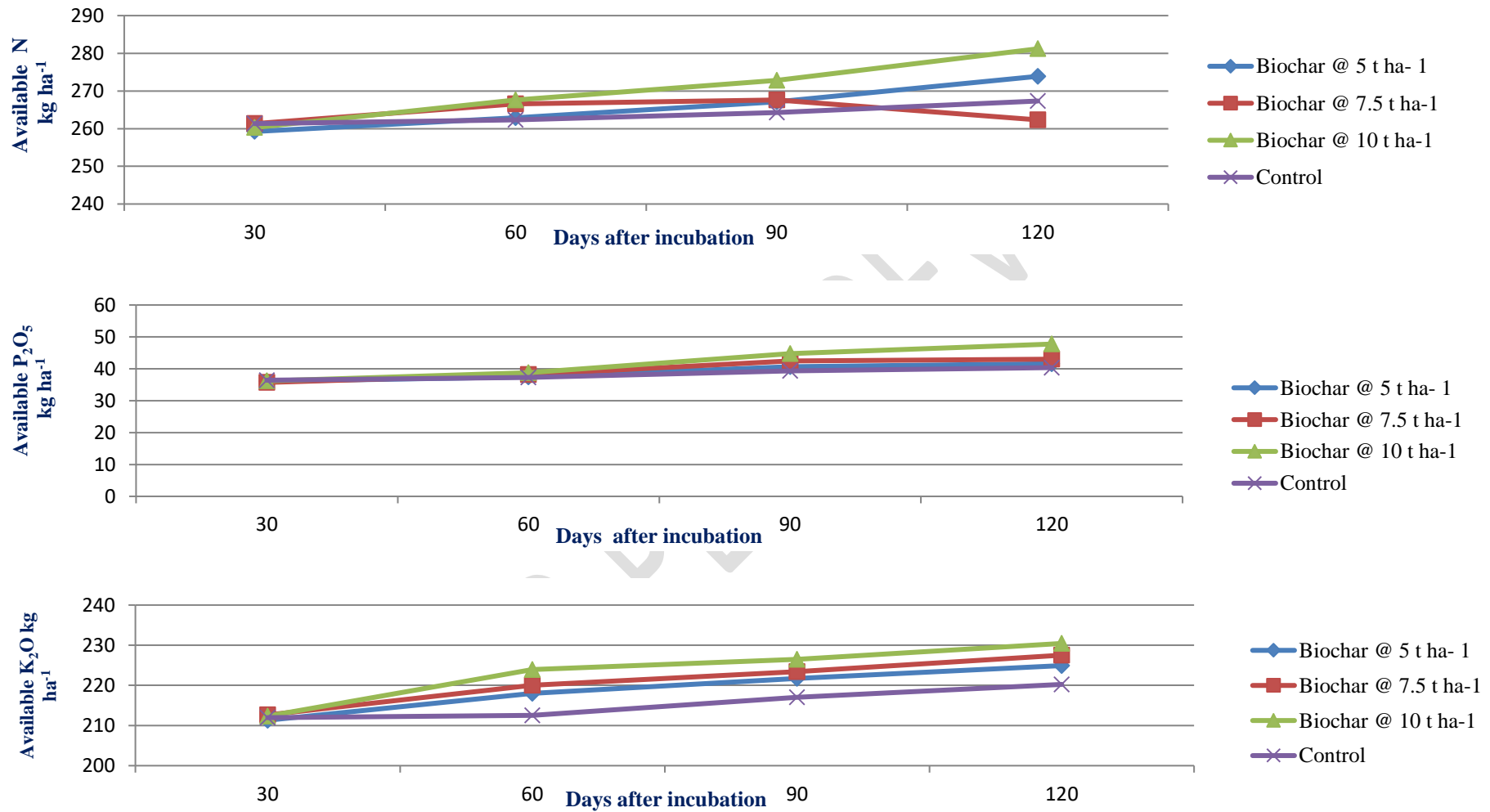
### **Conclusion**

Under incubation study, the nutrient status showed a significant increase with increased level of biochar application in soil. The better results were recorded in the treatment with biochar @  $10 \text{ t ha}^{-1}$  at 120 days after incubation.

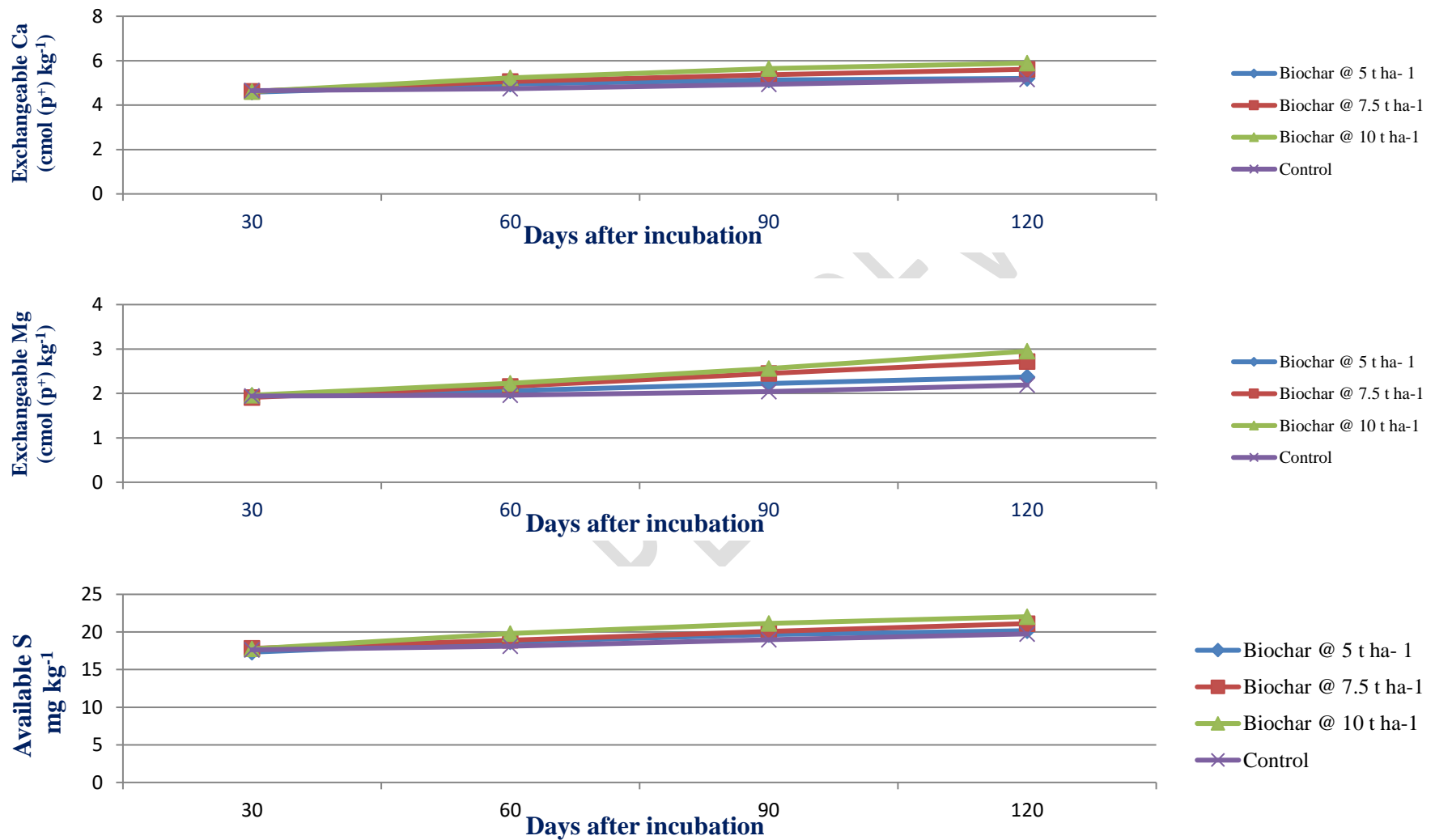
## References

- Ali K, Arif M, Badshah I, Zafarhayat, Ali A, Naveed K, Shah F. Formulation of biochar based fertilizer for improving maize productivity and soil fertility. *Pak. J. Bot.* 2018; 50(1): 135-141.
- Brewer C E, Schmidt K, Satrio J A, Brown R C. Characterization of biochar from fast pyrolysis and gasification systems. *Soil Sci.* 2014; 88: 164-167.
- Chan K Y, Van Zwieten L, Meszaros I, Downie A, Joseph S. Agronomic values of green waste biochar as a soil amendment. *Aust. J. Soil Res.*, 2007; **45**: 629-634.
- Chintala R, Javier M, Thomas E, Schumacher, Douglas D, Malo, James L. Effect of biochar on chemical properties of acidic soil. *Arch. Agron. Soil Sci.* 2014; **60**(3): 393-404.
- Emmanuel D, Anne V, Steve R, Sarana S. Biochar from saw dust, maize stover and charcoal: Impact on water holding capacity of three soil from Ghana. In: *19<sup>th</sup> world congress of soil science, soil solutions for a changing world 1-6 August 2010*, Brisbane, Australia.
- Gaskin J W, Steiner C, Harris K, Das K C, Bibens B. Effect of low temperature pyrolysis conditions on biochars for agricultural use. *American Soc. Agric. Biol. Eng.*, 2008; 51 (6): 2061-2069.
- Hass A, Gonzalez J M, Lima I M, Godwin H W, Halvorson J J, Boyer D G. Chicken manure biochar as liming and nutrient source for acid Appalachian soil. *J. Environ. Qual.*, 2021; 41(4): 1096-1106.
- Lehmann J. A handful of carbon. *Nature*, 2007; 447:143-144.
- Lehmann J, Da Silva J R J P, Steiner C, Nehls T, Zech W, Glaser B. Nutrient availability and leaching in an archaeological Anthrosol and a Ferralsol of the Central Amazon basin: fertilizer, manure and charcoal amendments. *Plant Soil*, 2003; 249: 343-357.
- Lehmann J, Gaunt J, Rondon M. Biochar sequestration in terrestrial ecosystems: A review. *Mitig. Adapt. Strategies Global Change*, 2006; 11(2): 395-419
- Lentz R D, Ippolito J A. Biochar and manure affect calcareous soil and corn silage nutrient concentrations and uptake. *J. Environ. Qual.*, 2011; 41: 1033-1043.

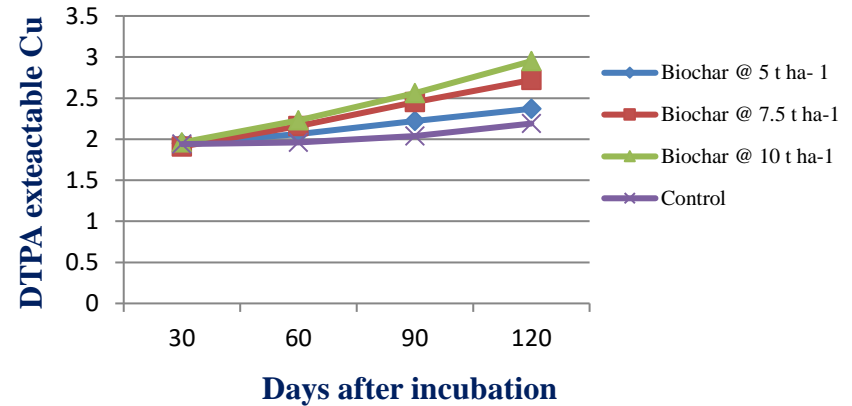
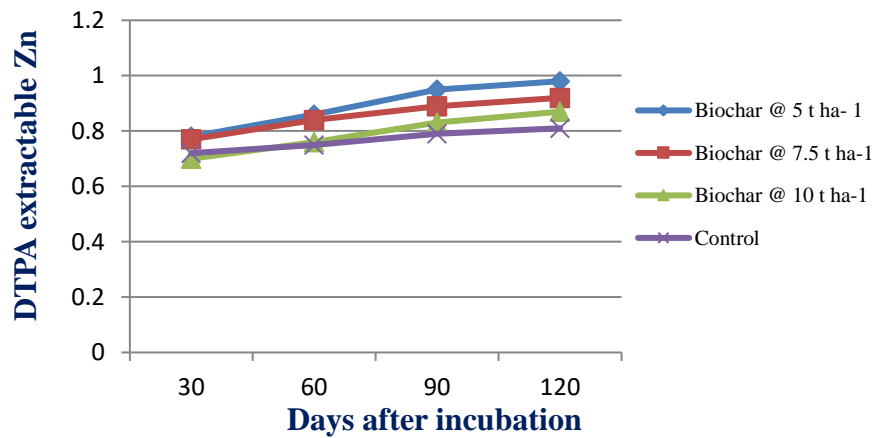
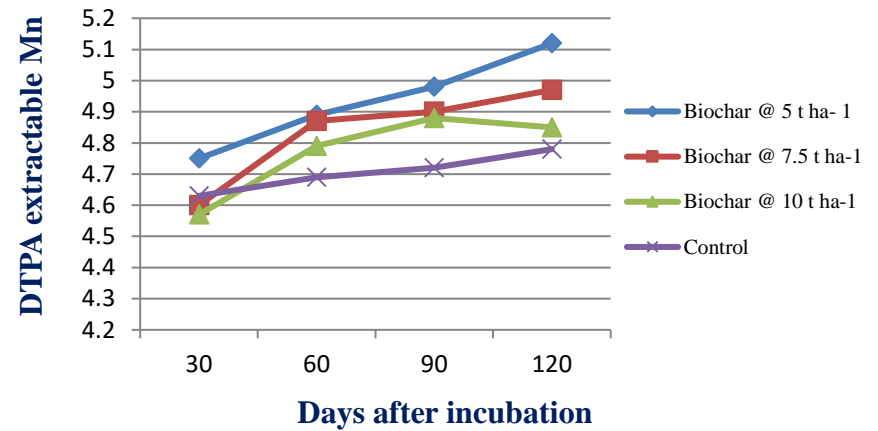
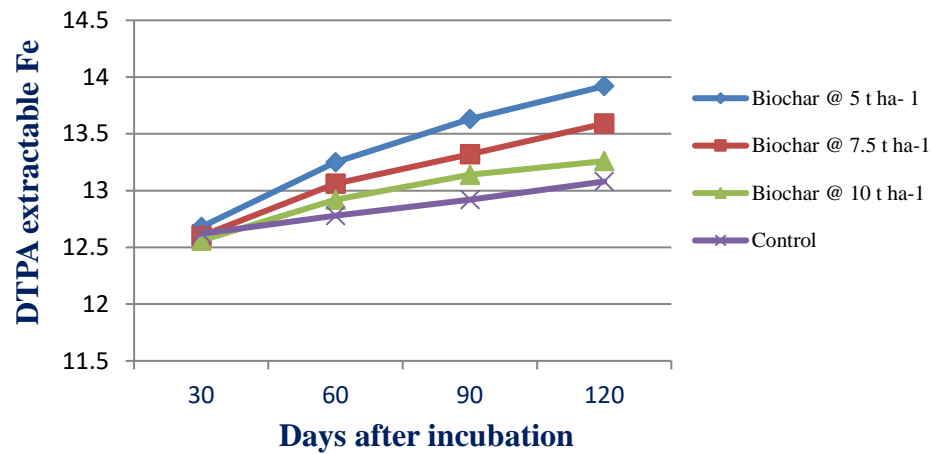
- Luca T H D, Mackenzie M D, Gundale M J. Biochar effects on soil nutrient transformations. *Biochar for environmental management science and technology. Earthscan, London.* 2009; pp:72-87.
- Parvage M M, Barbro U, Eriksson J, Jeffery S, Holger K. Phosphorus availability in soils amended with wheat residue char. *Biol. Fert. Soils*, 2013; 49(2): 245-250.
- Rodriguez L, Salazar P, Preston T R. Effect of biochar and bio digester effluent on growth of maize in acid soils. *Livestock Res. Rural Develp.*, 2009; 21(7): 110.
- Rondon M A, Lehmann J, Ramírez J, Hurtadoe M. Biological nitrogen fixation by common beans (*Phaseolus vulgaris* L.) increases with bio-char additions. *Bio. Fert. Soils.* 2007; 43(6): 699-708.
- Singh A, Singh A P, Purakayastha T J. Characterization of biochar and their influence on microbial activities and potassium availability in an acid soil. *Arch. Argon. Soil. Sci.*, 2010; 3 (6): 1 – 14.
- Singh C, Tiwari S, Gupta V K, Singh J S. The effect of rice husk biochar on soil nutrient status, microbial biomass and paddy productivity of nutrient poor agriculture soils. *Catena*, 2018; 171: 485-493.
- Venkatesh G, Sammireddy K, Gopinath K A, Visha kumari V, Chary R G. Biochar for enhancing the soil, water and crop productivity in rainfed areas. *Rainwater Management for Climate Resilient Agriculture in Drylands*. 2018; 496-523.
- Yamato M, Okimori Y, Wibowo I F, Anshiori S, Ogawa M. Effects of the application of charred bark of acacia mangium on the yield of maize, cowpea and peanut, and soil chemical properties in south Sumatra, Indonesia. *Soil. Sci. Plant Nutr.*, 2006; 52: 489-495.
- Yao F X, Camps A M, Virgel S, Blanco F, Arostegui J, Maciaagullo J A, Macias F. Simulated geochemical weathering of a mineral ash rich biochar in a modified Soxhlet reactor. *Chemosphere*. 2010; **80**: 724-732.



**Fig 1. Effect of different levels of mulberry stalk biochar on available N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O release during incubation period (30, 60, 90 and 120 DAI)**



**Fig 2. Effect of different levels of mulberry stalk biochar on exchangeable calcium, magnesium and available sulphur release during incubation period (30, 60, 90 and 120 DAI)**



**Fig 3. Effect of different levels of mulberry stalk biochar on DTPA extractable iron, manganese, zinc and copper (mg kg<sup>-1</sup>) release during incubation period (30, 60, 90 and 120 DAI)**

UNDER PEER REVIEW