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Improvement in Beetroot's (*Beta vulgaris*) Growth and Yield Parameters in Soils Fertilized with Maize (*Zea mays*) Biochar Under Drip Irrigation with Treated Wastewater

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Abstract

Global water scarcity due to climate change coupled with environmental pollution from artificial fertilizers threatens sustainable agricultural productivity. To address these challenges, innovative circular and sustainable agricultural practices are being sought after globally. Two promising sustainable approaches are the utilization of biochar coupled treated wastewater (TWW). In our experiment, maize biochar (MB) was locally produced by pyrolysis of maize stover waste in a Top-Lit-Up-Draft (TLUD) biochar kiln and characterized using FTIR, SEM, AAS, UV-vis and adsorption isotherms. Then, the performance of beetroots (*Beta vulgaris*) in terms of the germination rate, growth rate and yield in the cultivation soils fertilized with MB under drip irrigation with TWW was investigated. Post-hoc analysis (Bonferroni correction, α = 0.01667) revealed that cultivation soils fertilized with MB produced beetroots with significantly longer leaves and larger-heavier bulbs than the control. However, doubling MB fertilization dosage from 10 g to 20 g in the cultivation soils did not yield statistically significant improvement in the average leave height, mass and circumference of beetroot bulbs. Therefore, the findings demonstrated that combining MB with TWW enhanced beetroot growth and yield, highlighting a sustainable and circular agricultural system.

Keywords: biochar; fertilization; pyrolysis; treated wastewater; cultivation soils; germination rate; growth rate; drip irrigation; sustainability; circular economy

1. Introduction

Some of the contemporary threats to the existence of mankind are the interconnected climate change and unsustainable agricultural activities because of global population explosion [1]. Extreme weather patterns due to climate change have already impacted agricultural production negatively in every corner of the globe and developing countries have been badly affected because of limited resources [2]. Extreme weather patterns such extended droughts and high temperatures significantly reduced agricultural productivity and threaten food security particularly for developing countries [3]. Furthermore, drought leads to water scarcity and negatively impacting household's food-baskets leading to children malnutrition particularly in developing countries. To meet food demands and avert famine, countries globally have resorted to the use of synthetic fertilizers and pesticides. While the use of synthetic fertilizers and pesticides has managed to resolve food shortage and ensured surplus, they leave behind a trail of severely polluted surface waters, ground waters and soils [4–6]. Furthermore, some health issues have been linked to pesticides and fertilizers in the food chain [7,8] and people are now conscious of what they eat and now demand organic foods cultivated under green and sustainable methods without the use of synthetic chemicals [9,10]. On the other hand, commercial fertilizers are less accessible to poor communities because of high prices.

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Pollution from agriculture does not only come from synthetic fertilizers and pesticides, intensive crop production to sustain rapid population growth also generates large volumes of agricultural crop residue such as maize stover, rice husks, sorghum stalks etc which remain underutilized after harvest and their improper disposal leads to environmental pollution [11]. The current and common means of agricultural crop residue disposal involves a burning process which produces green-house gases (GHGs) such as carbon dioxide and therefore directly contributes to climate change [12]. However, climate change mitigation strategies recommend practicing climate smart agriculture (CSA) to reduce greenhouse gas emission [13]. Hence to reduce agricultural contributions to environmental pollution and climate change, scientists adopted an ingenious concept of Circular Economy (CE) which is a form of sustainable agricultural waste management. CE concept is a recycling idea based on 3R principles: Reduce, Reuse and Recycle [14]. Agricultural crop residue is readily available and presents itself as a valuable biomass for extraction of high value chemicals and conversion into many high value products. Applying CE concept on agricultural residues not only support CSA, but it also empowers small scale farmers to diversify their revenues through selling their agricultural residues after harvest thus promoting rural development [15].

To this end, conversion of agricultural crop residue into biochar has been identified as a smartest and most sustainable use of CE concept to reduce agricultural residue pollution in the environment and a robust approach to mitigate climate change [16]. Biochar production and utilization have been described as an innovative solution to achieve Sustainable Development Goals (SDGs) on zero to hunger and poverty by supporting food security [17]. Biochar is a mineral rich black and stable porous material that is produced by pyrolyzing biomass in limited or absence of oxygen [18]. It is highly rated for its soil amendment properties in agriculture and regarded as an alternative low cost and sustainable fertilizer [19]. Other desirable and beneficial properties of biochar in agriculture include stabilization of soil pH, increased soil microbial activity, and immobilization of toxic substances such as heavy metals [20]. The high porosity in biochar renders it the propensity to trap and deliver moisture and essential nutrients to plants while its carbonous nature imparts rugged physical stability which allows it to remain buried but active in the soil for several years, thus eliminating the need for frequent replenishment [21]. It helps farmers achieve high crop yield while at the same time drastically reducing the proliferation of environmental pollution compared to synthetic fertilizers [22]. In addition, biochar production can be carried out in simple constructed biochar kilns, making it ideal for small scall-scale farmers in developing countries [23]. Synthetic fertilizers, by contrast, are often highly priced and largely inaccessible to many small-scale farmers in developing countries. Biochar offers a sustainable, locally producible alternative that enables these farmers to increase agricultural output while protecting the environment. The use of biochar for soil amendment is not a modern innovation; it is a rediscovery of techniques practiced by indigenous Amazonian populations thousands of years ago. By fertilizing their fields with charcoal, they created the remarkably fertile Amazonian Dark Earths (Terra Preta) long before the advent of industrial fertilizers [24].

Several studies have reported an improved germination rate and growth rate upon application of biochar for different crops such as soybeans [25], maize [26], sorghum [27], cowpeas [28]. In vegetables, benefits have been shown in lettuce (Lactuca sativa) [29], beetroot (Beta vulgaris) [30], spinach (Spinacia oleracea) [31].

It is well known that biochar affords farmers sustainable means to improve soil fertility and crop yield. However, water scarcity in arid and semi-arid regions remains a hurdle in achieving SDS 2 on Zero Hunger as outlined in United Nations Sustainable Development Goals [32–34]. To address water scarcity, several countries with arid or semi-arid conditions have implemented the use of TWW for agricultural irrigation purposes [35–37]. Many studies have reported that crops irrigated with TWW outperforms crops irrigated with freshwater [38]. Already, some farmers in semi-arid countries like South Africa, Iraq, Algeria Israel and many others such as Botswana utilize TWW for vegetable irrigation[39]. However, the drawback is the limited awareness on biochar's capacity to enhance seed germination, growth rates, and overall yield. Given the high cost of commercial fertilizers, biochar

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offers a sustainable, affordable solution to maximize horticultural productivity. Furthermore, heightened global energy prices exacerbated by the 2026 Israel-USA-Iran conflict have severely increased fertilizer costs, threatening food security and creating economic challenges for the horticultural sector. Therefore, combining biochar and TWW in agricultural production is an attractive and yet a cheap circular and sustainable solution in achieving food security and accessibility in arid and semi-arid regions.

To provide farmers with cost-effective, sustainable management strategies, this study evaluated the effects of MB on the germination, growth, and yield of beetroot (*Beta vulgaris*) irrigated with TWW from the municipal treatment plant, thus supporting food security and UN SDGs.

Beta vulgaris is commonly referred to as beetroot and belongs to the *Chenopodiaceae* family [40]. It is cultivated for its valuable nutrition and various health beneficial properties, such as antioxidants, antibacterial, antiviral, anti-inflammatory, anti-cancer, and anti-diabetic activity, as well as hepatoprotective, hypotensive, and healing effects [41,42]. The results are necessary to highlight circular economic approach to farmers, showcasing biochar as a sustainable, low-cost fertilizer in irrigation with TWW.

2. Materials and Methods

2.1. Materials and Reagents

Distilled water was obtained locally from MilliporeSigma™ Milli-Q™ Direct Water Purification System from MilliporeSigma™, Germany, methylene blue >82% was purchased from Sigma/Aldrich-Merck, South Africa, calibration curves for elemental analysis were performed using Reagecon Atomic Absorption (AAS) Multi Element Standard (13 Elements) 100 µg/mL (100 ppm) in 2-5% Nitric Acid (HNO_3) purchased form Merck/Sigma-Aldrich, South Africa and diluted to different concentrations. Nitric acid, ACS reagent, 70%, Hydrochloric acid, 37%, ACS reagent and Hydrogen peroxide solution, 50 wt. % were all purchased from Merck/Sigma-Aldrich from South Africa.

2.2. Location and Planting Material

Field experiment was carried out at Glen Valley farm, located at 24°36'21.6"S 25°58'18.1"E in Gaborone, Botswana. The Starke Ayres Detriot Dark Red beetroots seeds were purchased from local supermarket. Three maize biochar levels 0, 10 g and 20 g/hole constituted the treatment variables.

2.3. Experimental Design

The experiment was a completely randomized design, with each experimental unit being an irrigation lateral and its associated plants. The lateral was a single conventional dripline (CD). Emitter spacings were the same on all the laterals, so that plant populations were equal. The emitter spacing for the CD was 60 cm, giving 60 cm between plants. There were seven (7) CD lines/ plots in the experiment. Three treatments were completely randomized across the seven lines/plots. Two beetroot seeds were planted adjacent to each emitter. The plot size was 25 m * 7.70 m. The treatment consisted of three levels of maize biochar on the 7 lines: 0 g/hole, 10 g/hole, 20 g/hole. Before applying maize biochar to the soil, water was allowed to wet the soils to locate the drip emitters and where to place the biochar. Shallow holes were made under drip emitters and then maize biochar was placed there. No maize biochar was applied for lines 1, 6, 7 and served as control. Lines 2 and 3 were treated with 10 g/hole while lines 4 and 5 were treated with 20 g/hole. The plots were drip-irrigated for 1 hour daily with treated wastewater from Gaborone city municipal Glen Valley treatment plant. Weeding was carried out whenever necessary.

2.4. Maize Biochar Production

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Dry maize stover harvested at Glen valley farm from previous DIVAGRI experimental project was cut into smaller pieces by matchet before grinding in JF 2D HAMMER MILL manufactured in Gaborone, Botswana. 200 g of dried maize ground biomass was then placed in a locally fabricated DIVAGRI TLUD biochar kiln designed by Namibian University of Science and Technology (NUST). The contents of the kiln were heated by fire for a duration of about 3 hours at a temperature of roughly 300 °C. Biochar was cooled overnight before collecting and storing in plastic bags for weighing. Since the temperature of TLUD kiln was not controlled, maize biochar was also produced by pyrolyzing 5 g of ground maize stover at three (250 °C, 350 °C and 500 °C) in a temperature-controlled microwave oven. This was carried out in an Ethos UP High-Performance Microwave oven from Milestone set at 800 w for 45 minutes at those specified temperatures. This was carried out to assess the effect of temperature on the functional groups of biochar.

2.5. Measurement of pH and Electrical Conductivity (EC) of Biochar

pH and EC of MB were measured for three different masses. Sample masses (1.25 g, 2.50 g and 5.02 g) of dry MB were weighed into separate 100 mL Duran sample bottle then 100 mL of distilled water was added. The mixtures were then mechanically shaken on Innova 40 incubator Shaker Series at 200 rpm for 1 hour at 25°C. Suspensions were then allowed to stand for 45 minutes before measuring pH and electrical conductivity (EC). The pH of the suspension was measured in triplicate with Orion Star A111 pH meter from Thermo Scientific (USA). Before pH measurement, the pH meter was calibrated based on three points calibration using pH 4, pH 7, pH 10 buffer solutions. The EC of the suspension was measured in triplicate with DDS-12DW conductivity meter from Bante Instruments (China). Before EC measurement, the conductivity meter was calibrated based on three points calibration with reference calibration standards solutions of 84.0 mS/cm, 12.88 mS/cm and 1413 mS/cm conductivity. The pH and EC for distilled were measured as 7.25 and 0.552 mS/cm respectively.

2.6. Surface Area Determination of MB Based on Methylene Adsorption

Methylene blue adsorption method was used to estimate the surface area of MB using Langmuir adsorption models (Linear and Non-linear models) and Freundlich adsorption models (Linear and Non-linear models). To achieve this, a stock solution of methylene blue dye was prepared by dissolving 1 g of dye in 1L of distilled water to give the concentration of 1000 mg/L. Then experimental dye solutions of desired concentrations were obtained by appropriate dilution of the stock solution. Equilibrium studies were carried out by batch adsorption techniques at 35 °C with fixed biochar dosage of 0.2 g into different 100 mL of Duran bottles containing 100 mL of different initial concentrations (10, 15, 20, 25, 30 and 35 mg/L) of dye solution. The mixtures were then shaken in an Innova 40 Incubator series form New Brunswick Scientific at 150 rpm for 5 hrs to equilibrate, then filtered with a Whatman filter paper. The filtrates were subsequently scanned between 200 nm - 800 nm for UV-vis absorbance measurements with Genesys 10S UV-vis spectrometer from Thermo Scientific. Then the maximum absorbance at 668 nm in the spectra was used to calculate the differences in initial concentrations of methylene blue solution and residual methylene blue concentrations after methylene blue adsorbed per unit mass of MB (q_e) using equation 1. In all measurements, distilled water was used as blank, and results were reported as a mean 3 measurements. The calibration curve was obtained using the maximum absorbance at λ_{max} 668 nm by using MB standard series in the range of 5.0 - 40 mg L^{-1}.

$$q_e = \left(\frac{C_o - C_e}{m}\right) * V \qquad (1)$$

Where;

q_e is mass of methylene blue adsorbed on MB in mg/g at equilibrium

C_o is the initial concentration of Methylene blue before adsorption (mg/L)

C_e is the equilibrium concentration of Methylene blue in solution after adsorption (mg/L)

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V is the volume of methylene blue solution used in adsorption experiments in L

m is the mass of the MB used for adsorption experiment in g

The experimentally derived q_e values were then plotted against equilibrium methylene blue concentrations subsequently fitted with both non-linear and linear Langmuir models (equations 2 and 3 respectively) using K_L and q_m as fitting parameters in Excel solver. K_L and q_m values were obtained by minimizing sum of squares of residuals.

non-linear Langmuir adsorption model

$$q_e = \frac{q_m K_L C_e}{1+K_L C_e} \quad (2)$$

linear Langmuir adsorption model

$$\frac{C_e}{q_e} = \frac{C_e}{q_m} + \frac{1}{K_L q_m} \quad (3)$$

Where;

q_e is mass of methylene blue adsorbed on MB in mg/g at equilibrium

q_m is maximum capacity of methylene adsorbed on MB in mg/g

K_L is the Langmuir equilibrium constant L/g

C_e is the concentration of methylene blue remaining in solution at equilibrium (i.e. unabsorbed) in mg/L

The surface area of MB was estimated using;

$$SA = \frac{(q_m a_{mb} N_A 10^{-20})}{M} \quad (4)$$

Where;

SA is the specific surface area of MB (m^2/g)

q_m is maximum capacity of methylene adsorbed on MB in mg/g

a_{mb} is area per methylene blue in $Å^2$ (197.2 $Å^2$)

N_A is the Avogadro's number ($6.02*10^{23}$ mol^{-1})

M is the molar mass of methylene blue (373.8 $gmol^{-1}$)

Data was also fitted to both linear and non-linear Freundlich models.

2.7. Characterization of Surface Morphology of MB

The surface morphology of MB was determined by scanning electron microscopy (SEM) using a Carl Zeiss FEGSEM Gemini 500 instrument fitted with energy dispersive spectroscopy (EDS) to allow elemental nutrients analysis. Samples were ground into a powder, placed on a piece of microscope slide (5x5 cm) then coated with carbon to reduce charging. The slide was then mounted on a SEM sample holder and then subjected to high vacuum conditions subsequently signals were detected based on secondary electron (SE) and backscattered electron (BSE) detectors. SEM imaging and EDS analysis were conducted under high vacuum conditions and images were obtained using a beam energy of 3 kV for SEM imaging and EDX analysis, working distance 3.9 - 4.3 mm and aperture of 20 µ. Back scatter imaging and EDS analysis were conducted under high vacuum conditions and images were obtained using a beam energy of 20 kV at working distance between 10.3 – 10.4 mm and aperture of 60 µ.

2.8. Determination of Surface Functional Groups on MB

Different surface functional groups on MB were identified using a PerkinElmer Spectrum Two Fourier Transform Infrared (FTIR) spectrometer fitted with an attenuated total reflection (ATR) diamond crystal. Samples were directly placed on the ATR diamond crystal subsequently subjected to a force of 80 N to improve sample contact and signal. FTIR spectra were collected between the range from 550 cm^{-1} to 4000 cm^{-1} running 4 scans per sample at medium scan rate and 1 cm^{-1} resolution in the absorbance mode.

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2.9. Trace Metal Analysis

2.9.1. Digestion of MB for Trace Elements (Fe, Cu, Mn & Zn) Analysis

0.3 g of ground fine powder of MB was weighed into Teflon microwave digestion tube. Digestion was carried out using 7.5 mL of Nitric acid, 2.5 mL of HCl and 2 mL of Hydrogen peroxide in a digestion tube. MB was then digested in Ethos UP High-Performance Microwave oven from Milestone set at 800 w and 80 °C for 2 hrs. The solution was removed when it was colourless. The solution was filtered into a 50 mL volumetric flask and made up to the mark with distilled water. The solution was then analysed for (Fe, Cu, Mn, Zn) micronutrients using atomic absorption spectrophotometer (Agilent Technologies 200 Series AA, Germany). Flame atomization was achieved using air and acetylene at 13.50 L/min and 2.00 L/min flow rate respectively. The calibration curves were made up of Reagecon Atomic Absorption (AAS) Multi Element Standard. Analytical parameter settings for AAS instrument for each element are shown in Table 1.

Table 1. Atomic absorption spectroscopy (AAS) setting for sample analysis for 4 trace metal analysis.

Element	Wavelength/nm	Flame
Copper (Cu)	324.8	Air-acetylene
Manganese (Mn)	279.5	Air-acetylene
Iron (Fe)	248.3	Air-acetylene
Zinc (Zn)	213.9	Air-acetylene

2.9.2. Digestion of Leaves and Beetroot Tubers for Trace Elements Analy Sis

Samples of leaves and beetroot tubers were treated in the same way. Leaves and tubers were first washed with tap water and finally rinsed with distilled water to remove dirt. Tubers harvested from different treatments (No biochar added, 10 g of biochar treatment, 20 g of biochar treatment) were then sun dried for 3 days before complete drying in an oven at 90 °C for 24 hrs. The samples were then ground to fine powder with mortar and pestle. Then 0.3 g of ground biochar was weighed into Teflon microwave digestion tube. Digestion was carried out using 7.5 mL of Nitric acid, 2.5 mL of HCl and 2 mL of Hydrogen peroxide in a digestion tube. Samples were then digested in Ethos UP High-Performance Microwave oven from Milestone set at 800 w and 80 °C for 2 hrs. The solution was removed when it was colourless. The solutions were filtered into separate 50 mL volumetric flasks and made up to the mark with distilled water. The solutions were analysed for (Fe, Cu, Mn, Zn) micronutrients using atomic absorption spectrophotometer (Agilent Technologies 200 Series AA, Germany). Flame atomization was achieved using air and acetylene 13.50 L/min and 2.00 L/min flow rate respectively.

2.9.3. Digestion of Cultivation Soils for Trace Elements Analysis

Soil samples were collected in separate labelled beakers according to MB treatment lines from Glen Valley farm. Each soil sample (no biochar added, 10 g of MB, 20 g of MB) was dried in an oven at 90 °C for 3 days. 0.3 g of each soil sample was weighed into Teflon microwave digestion tube. Digestion was carried out using 7.5 mL of Nitric acid, 2.5 mL of HCl in a digestion tube. Samples were then digested in Ethos UP High-Performance Microwave oven from Milestone set at 800 w and 80 °C for 2 hrs. The solution was removed when it was colourless. Each solution was filtered into a different 50 mL volumetric flask and made up to the mark with distilled water. Solutions were the used for analysis of (Fe, Cu, Mn, Zn) micronutrients using atomic absorption spectrophotometer (Agilent Technologies 200 Series AA, Germany). Flame atomization was achieved using air and acetylene 13.50 L/min and 2.00 L/min at flow rate respectively.

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2.9.4. Digestion of TWW for Trace Element Analysis

Water was collected in an acidified water sample bottle. 10 mL of water sample was placed into Teflon microwave digestion tube. Digestion was carried out using 7.5 mL of Nitric acid, 2.5 mL of HCl in a digestion tube. Samples were then digested in Ethos UP High-Performance Microwave oven from Milestone set at 800 w and 80 °C for 2 hrs. The solution was removed when it was colourless. The solution was filtered into a 50 mL volumetric flask and made up to the mark with distilled water. The aqueous solution was then analysed for (Fe, Cu, Mn, Zn) micronutrients using atomic absorption spectrophotometer (Agilent Technologies 200 Series AA, Germany). Atomization was achieved by flame using air and acetylene at 13.50 L/min and 2.00 L/min flow rate respectively.

2.9.5. Effects of MB on Beetroot Germination Rate, Beetroot Growth Rate and Yield

Germination rate was carried out by counting visible shoot above ground after 2 weeks of sowing and converted to percentage using the number of planted holes in each line. Growth rate was assessed based on leaf heights measured with a measuring tape after 1st month and 3rd month of sowing the seeds. The yield of beetroots was assessed by weighing masses of harvested tubers on a MOHAK Sf-400 7 KG Stainless Steel Electronic LCD Weighing balance

(India) and measuring the circumferences of the harvested beetroot tubers with a measuring tape.

2.9.6. Statistical Analysis

Data was analysed using MS Excel statistical package (Version 2601). The means were analysed at significance levels of p value < 0.05 in a one-way analysis of variance (ANOVA) followed by post-hoc test (Bonferroni Correction method, $\alpha = 0.01667$) to test where the group differences lie. This was carried out by performing separate T-tests and control the multiple comparisons with Bonferroni correction method, α=0.016667. Microsoft excel was also used to draw graphs.

3. Results and Discussion

3.1. pH and Electrical Conductivity (EC) of MB

The results for the pH and EC are shown in Table 2. The results show that pH was not affected much by increasing the mass of MB. However, increasing mass of MB increased EC. This was consistent with results from [43] who reported that EC of soil increased with increasing biochar application rate. High pH biochar has been reported to neutralized acidic soils leading to improved crop yield [44].

Table 2. Effects of mass of MB on pH and EC.

Mass of MB/g	pH	Electrical Conductivity/mS/cm
1.25	8.41	73
2.50	8.54	1018
5.02	8.41	1275

3.2. Surface Morphology of Biochar (SEM) and EDS

The surface area of biochar is among the most important features affecting the adsorption and retention properties of biochar [45,46]. The morphology of MB was observed by SEM analysis coupled with an energy-dispersive X-ray spectroscopy (EDS). The SEM images of the MB are shown in Figure 1. As shown in this Figure 1a, the cross-sectional surface area of MB was characterized by high porosity with honeycomb like polyhedral shaped pores. The SEM also revealed MB interior with long parallel vascular bundle structures connected to each other through micro pores in Figure 1b. These vascular bundle structures were attributed to xylems which are water and mineral channels

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from the roots to other parts of maize plant. The small pores interconnecting xylems cells were attributed to pits which facilitates nutrients transfer across adjacent xylem cells. This characteristic of high porosity is constant with reported literature [47–49]. The release of volatile matter, particularly celluloses, and hemicelluloses, during pyrolysis has been reported to lead to the formation of a porous structure in biochar [50]. The average of major diameter of MB surface pores as measured by SEM was around 10 μm while the average of the minor pore was around 4 μm.

EDS spectrum in Figure 1c revealed the presence of the following elements in MB; 1.5 % w/w Si, 2.5 % w/w P, 1.0 % w/w Cl, 6.4 % w/w K, with large amount of C at 88.1 %w/w and very small quantities of Mg at 0.5 %w/w. The macro elements are known to influence growth and development in plants [51–53]. Porous structure of biochar is reported to be a habitable zone for beneficial to plants [54]. As seen in Figure 1c, MB was characterized by high C content which has been reported to be beneficial to microorganisms in the soil leading to increased plant development [55].

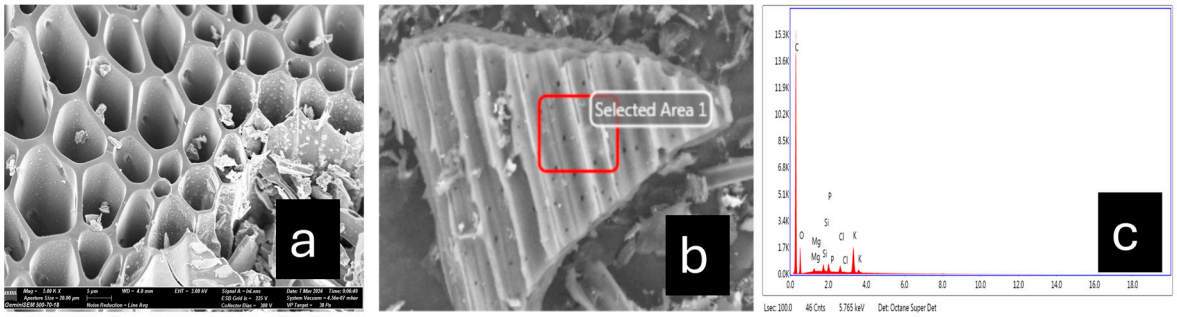

Figure 1. Scanning electron microscope micrographs a) micropore on cross section maize biochar, b) side view of maize biochar revealing micro channels and nano pore connecting micro channels, c) EDS for surface elemental analysis in selected area 1 depicted in 1b.

3.3. MB Specific Surface Area by Methylene Blue Absorption

The specific surface area (SSA) of MB was estimated through methylene adsorption based on both linear and non-linear Langmuir models and Freundlich models. The SSA from Langmuir models was found to be 51.93 m^2/g for the linear model and 51.38 m^2/g for the non-linear model shown in adsorption summary Table 3. Both values of SSA were different from literature values because the physical and chemical properties of biochar have been reported to be dependent on many factors such as heating rate, kiln pressure, gaseous atmosphere, and type of pre- or post-treatment of biochar and pre-treatment [56]. Pyrolysis temperature was identified as a predominant parameter influencing biochar surface area [57]. Ali et al reported that increasing pyrolysis temperature (300 °C -700 °C) of sheep manure increased Brunaeur Emmet Teller (BET) surface area significantly from 17.072 $m^2\ g^{-1}$- 89.885 $m^2\ g^{-1}$ [58]. In another study, Septiana *et al* studied characteristics of biochar from different plant biomass at low temperature. The authors observed increasing BET surface area upon increasing temperature from 250 °C to 350 °C for all plant biomass under study [59]. Apart from BET method, methylene blue adsorption experiments have been employed to determine SSA [60] of different materials such as Iron dioxide (FeS_2) [61] and Betulin particles [62]. The maximum adsorption capacity (q_{max}) fitted by Langmuir model was 16.18 mg/g from non-linear model and 16.36 mg/g from linear model as shown in Table 3. This value was close to 13.4 mg/g which was obtained by Chem *et al* [63]**.** The micropores seen in Figure 1a were responsible for this surface area and the high adsorptive capacity of methylene blue by MB. It can be seen from Figure 2 that the Langmuir model (R^2 were 0.9969 non-linear and 0.9971 linear) was more suitable than the Freundlich model (R^2 were 0.9677 for the non-linear and 0.9571 linear), indicating that the adsorption sites of biochar were evenly distributed, and adsorption was mainly monolayer [63]. The Langmuir model was based on the uniform and monolayer adsorption hypothesis, whereas the Freundlich model was established

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on multilayer heterogeneous surface sorption [64]. The presence of high surface area provided biochar with the ability to hold nutrients necessary to support beetroot growth.

Table 3. Langmuir and Freundlich adsorption isotherm parameters for MB on Zea mays at 35 °C.

Model	Fitting Parameters
Langmuir linear model	q_{max} = 16.36 mg/g, K_L = 0.70 L/mg, SSA = 51.93 m^2/g, R^2 = 0.9971
Langmuir non-Linear	q_{max} = 16.18 mg/g, K_L = 0.73 L/mg, SSA = 51.38 m^2/g, R^2 = 0.9969
Freundlich linear model	K_F = 6.60 mg/g n = 2.45 R^2 = 0.9571
Freundlich non-linear model	K_F = 6.92 mg/g n = 2.74 R^2 = 0.9677

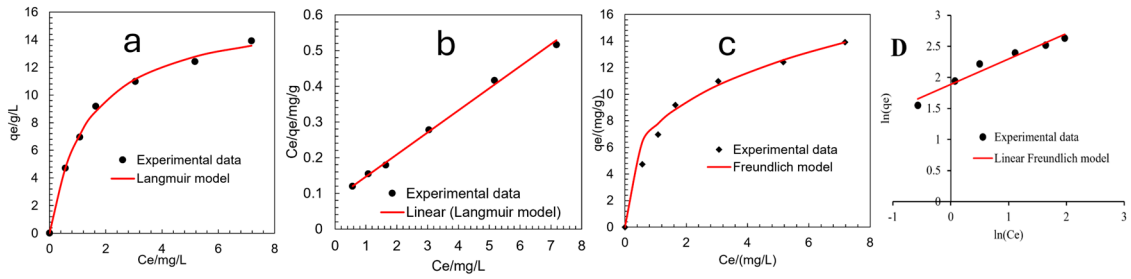

Figure 2. Adsorption Isotherms of adsorption of methylene blue by maize biochar at 35 °C a) non-linear Langmuir adsorption isotherm, b) linear Langmuir adsorption isotherm c) non-linear Freundlich adsorption isotherm d) linear Freundlich adsorption isotherm for maize biochar.

3.4. Surface Functional Groups (FTIR-ATR)

Assessing biochar surface functional groups with FTIR was a critical analysis because adsorption of certain adsorbate and adsorption rate are known to be governed by biochar surface chemistry [65]. Furthermore, FTIR analysis was necessary because biochar functional groups are reported to be influenced by temperature and the biomass feedstock [66,67]. Therefore, MB was analysed with FTIR and its spectrum was compared to raw maize biomass and biochar made under controlled temperature using microwave energy at two different temperatures. Figure 3a shows the MB FTIR spectra under different pyrolytic conditions. The spectrum of raw maize showed a medium vibrational absorption peak between 3037-3635 cm^{-1} associated with -O-H stretch arising from cellulose, hemicellulose and lignin [68]. A weak vibrational peak at 2879 cm^{-1} in the spectrum was assigned to -C-H from sp^3 vibrational stretch. An observed peak at 1729 cm^{-1} was assigned to a -C=O vibrational absorbance band. Two peaks at 1603 and 1515 cm^{-1} were assigned to -C=C- vibrational stretch from aromatic rings [69,70]. Two vibration bands at 1334 and1425 cm^{-1} were assigned to -C-H bending and a very intense vibrational peak at 1066 cm^{-1} was assigned to -C-O stretch from the cellulose and hemicellulose backbone [71–74]. The spectrum of raw maize biomass differs from the MB spectra in terms of peak intensities. The spectra of MB pyrolyzed in DIVAGRI TLUD kiln and MB prepared under controlled temperature using microwave energy exhibited similar vibrational characteristics. However, all biochar spectra exhibit reduced vibrational band intensities between 3037-3635 cm^{-1} and a vibrational peak at 2879 cm^{-1} associated with -O-H stretch and -C-H stretch respectively [75]. Other researchers also reported the disappearance/weakening of absorption bands for aliphatic surface functional groups upon increasing pyrolysis temperature to 500 °C [76]. At the same time, an increase in the intensities of vibrational peaks at 1711 cm^{-1} and 1582 cm^{-1} associated with -C=O stretch and -C=C- stretch respectively were observed. Pyrolysis of maize biomass led to decomposition of cellulose, hemicellulose and lignin which vaporized -O-H and -C-H hence the observed reduction in the intensities associated with these two functional groups [77,78]. The spectra also revealed a significant decrease in vibrational bands at 1066 cm^{-1} associated with -C-O confirms the decomposition of cellulose, hemicellulose and lignin [79,80]. However, no obvious differences were observed between the spectra of MB from TLUD kiln and MB produced under controlled

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temperature using microwave energy. Furthermore, no change in the FTIR spectra was observed upon increasing temperature of the microwave from 250 °C to 350 °C. This is consistent with other researchers, Hu et al. Hu et al investigated the effect of pyrolysis temperature on removal efficiency and mechanisms of Hg(II), Cd(II), and Pb (II) by maize straw biochar at 3 different temperatures (300 °C, 400 °C, 500 °C). They reported that types of biochar functional groups were similar at all three pyrolysis temperatures [81]. However, increasing temperature of microwave energy to 500 °C led to increase in ash content of the sample as seen in Figure 3b. This in contrast to conventional muffle furnace which can produce biochar above 650 °C as reported by other researchers [82]. The reason could be that microwave energy has more heating power than conventional furnaces.

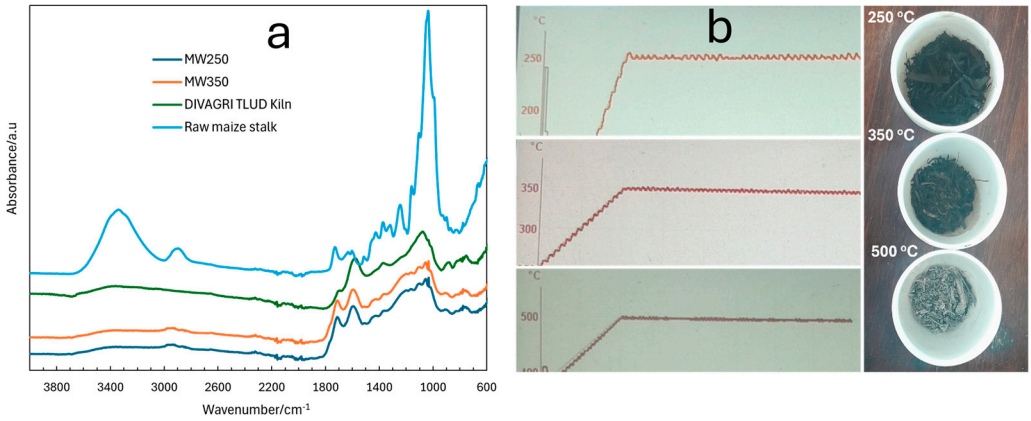

Figure 3. a) FTIR spectrum of raw maize stalk, MB, MW250- biochar microwave pyrolyed at 250 °C and M350 – biochar microwave pyrolyed at 350 °C. b) Microwave pyrolysis of MB at different temperatures; 250 °C, 350 °C. and 500 °C.

3.5. Trace Elements Analysis in MB

The content of four trace elements copper (Cu), manganese (Mn), iron (Fe) and zinc (Zn) were analysed in beetroot leaves, beetroot tubers, cultivation soils, irrigating treated wastewater using atomic absorption spectroscopy (AAS). The results are presented in Table 4 and Figure 4- Figure 5. Samples were harvested from different treatments (10 g biochar treated soils, 20 g biochar treated soils, soils without biochar as control). The highest Cu content was recorded from MB at 54.97±1.3 mg/L while the lowest Cu amount was observed in treated wastewater at 0.001±13.3 mg/L. On the other hand, the highest Mn content was recorded in the leaves of samples harvested from cultivation soils treated with 20 g of MB at 189.08±1.5 mg/L while the lowest Mn amount was observed in treated wastewater at 0.031±24.6 mg/L. As for Fe, the highest content was recorded in the cultivation soils treated with 20 g of MB at 7300.78±2.1 mg/L while the lowest Fe amount was observed in treated wastewater at 0.003±100 mg/L. However, Fe was the highest detected trace element in the cultivation soil samples followed by Mn. Finally, the highest Zn content was detected in MB at 371.74±2.0 mg/L while the lowest Mn amount was observed in treated wastewater at 0.024±6.6 mg/L. Moreover, for each trace element evaluated, the lowest level was found in treated wastewater sample.

Table 4. Average elemental content of Copper (Cu), Iron (Fe), Manganese (Mn) and Zinc (Zn) in mg/L in beetroot leaves (L), TWW, beetroot tubers (R), soils (S).

Sample	[Cu]/mg/L	[Mn]/mg/L	[Fe]/mg/L	[Zn]/mg/L
L0	32.10 ±1.6	118.00 ± 2.0	134.33 ± 3.2	22.95± 3.0
L1	9.356 ± 2.5	131.98 ± 1.9	147.25 ± 1.2	9.22 ± 5.5
L2	18.90 ± 6.7	189.08 ± 1.5	180.77± 2.0	27.14 ± 1.8
R0	7.39 ± 1.3	7.86 ± 9.0	6.62 ± 16.6	29.19 ± 1.1

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R1	18.99 ± 1.2	116.97 ± 2.7	128.33 ± 1.6	28.20 ± 1.0
R2	21.16± 1.5	15.44 ±14.2	300.93 ± 1.5	4.20 ± 6.0
S0	28.64 ± 1.5	109.99 ± 4.4	6500.99 ± 4.4	34.78 ± 2.1
S1	11.38 ± 2.6	120.41 ± 3.4	7000.00 ± 2.6	36.49 ± 1.9
S2	9.35 ± 2.5	136.70 ± 3.0	7300.78 ± 2.1	31.38 ± 0.2
MB	54.97 ± 1.3	123.63 ± 3.2	6135.82 ± 0.7	371.74 ± 2.0
TWW	0.001± 13.3	0.031 ± 24.6	0.003 ± 100	0.024 ± 6.6

Values expressed as mean ± relative standard deviation, n=3, NB: L= beetroot leaves, R= beetroot roots, S= soils, 0 means no biochar added (control), 1 means treatment with 10 g of biochar, 2 means treatment with 20 of biochar.

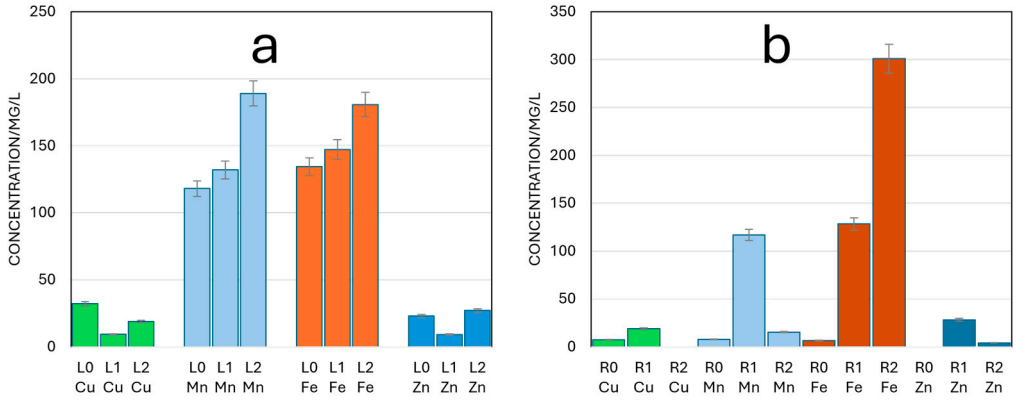

Figure 4. a) Trends of measured trace elements in beetroot leaves and b) Trends of measured trace elements in beetroot tubers, L ≡ beetroot leaves, R ≡ beetroot tuber, 0≡no biochar added to soil, 1≡10 g of biochar added to soil and 2≡20 g of biochar added to soil. Error bars at 5% standard deviation. .

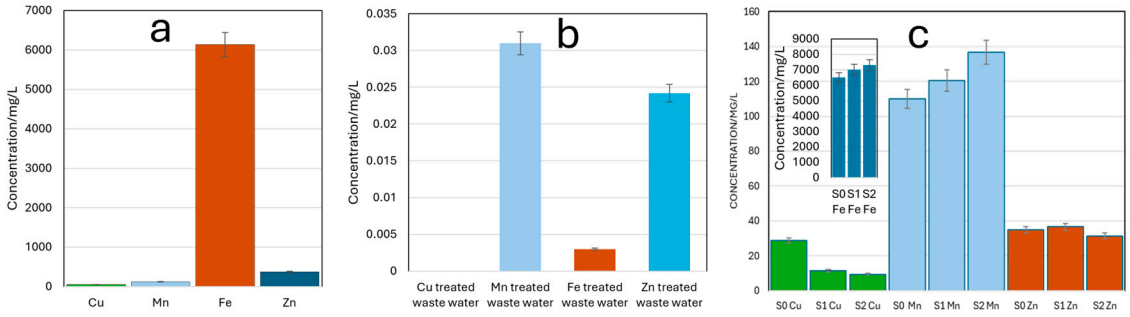

Figure 5. a) Trends of trace elements measured in maize biochar (MB), b) Trends of measured trace elements in treated wastewater (TWW), c) Trends of measured trace elements in cultivation soil, insert for high Fe content in the soil. Error bars at 5% standard deviation. s ≡ soil, 0≡no biochar added to soil, 1≡10 g of biochar added to soil and 2≡20 g of biochar added to soil.

3.5.1. Copper

Copper plays a pivotal role in hormonal balance and plant growth. Cu amount in the samples varied from leaves (32.10 ±1.6 - 18.90 ± 6.7 mg/L), roots (7.39 ± 1.3 - 21.16± 1.5 mg/L), soil (28.64 ± 1.5 - 9.35 ± 2.5 mg/L). As shown in Table 4 and Figure 4-Figure 5, the Cu amount in the tested samples was relatively lower than the other test trace elements. It was observed that the controls for the soil and leaves had more Cu content than biochar treated leaves and soils whereas biochar treated roots showed higher content of Cu than their control. These differences might be due to the translocation rate of Cu from the soil to other parts of the beetroot which is dictated by metabolic needs of plants and environmental factors such as pH and temperature [83]. High content of Fe has been reported to

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compete with Cu for uptake by suppressing its bioavailability and translocation leading to lower concentration of Cu in plant tissues. As seen in Table 4 the results of the trace elements analysis were characterized by highly elevated Fe concentration in the samples and consistent low concentration of Cu in the leaves, roots and soil, the Cu suppression by Fe could be at play.

3.5.2. Manganese

Manganese (Mn) is an essential micronutrient for plants, playing a crucial role in soil–plant–microbial interactions [84]. It is necessary for plant growth and development and sustains metabolic process in the cells [85]. Although needed for plant sustainability, it has been reported that too much Mn can prevent the uptake and translocation of other essential elements such as Ca, Mg, Fe, and P [86]. High Fe content could lead to competition with Mn for adsorption sites on soil particles and oxidize Mn^{2+} to Mn^{3+} and Mn^{4+}, which are less available for plant uptake. Mn was the second highest abundant trace element analysed in beetroot tuber (7.86 ± 9.0 - 189.08 ± 1.5 mg/L), and soil samples (109.99 ± 4.4 -136.7 ± 3.0 mg/L) after Fe. It was the highest element in the beetroot leaves (118.00 ± 2.0 - 189.08 ± 1.5 mg/L) and its lowest amount was found in treated wastewater (0.031 ± 24.6 mg/L) as shown in Figure 4a and Figure 4b respectively. The tuber and soil contents for Mn varied from (7.86 ± 9.0- 15.44 ±14.2 mg/L) and (109.99 ± 4.4 -136.7 ± 3.0 mg/L) respectively. Samples cultivated in biochar treated soils exhibited higher Mn content. The content order of Mn was as follows; leaves > roots > soil > controls. Increasing the amount of biochar from 10 g to 20 g increased Mn concentration in the leaves, roots and biochar treated soils. On applying 20 g of biochar, Mn content in MB observed to be less than Mn content detected in leaves and soil. This was attributed to easy uptake of Mn by beetroots because it has been reported that Mn can be easily absorbed and accumulated by beetroots. Literature reported that increasing in Mn in the presence of Fe increased Mn content in leaves and stems of rice below a certain threshold [87]. Therefore, this could also be a plausible explanation of the observed increase in content of Mn in the leaves and soil upon increasing biochar in the soil with high content of Fe. Although Mn content in the roots (7.86 ± 9.0 -15.44 ±14.2 mg/L) did not follow the increasing pattern observed for the leaves and soil, the content in their respective control was less than the content in biochar treated samples. The lowest Mn concentration was found in treated wastewater (0.031 ± 24.6 mg/L). Deficiency of Mn leads to stunted root growth and decrease in biomass therefore this could explain lower beetroot germination rate and shorter beetroot leaves in control throughout.

3.5.3. Iron

Its deficiency in plants can be solved by applying iron bearing fertilizers [88]. Fe is key trace element needed for plant photosynthesis to produce plant sugars for energy production from light and carbon-dioxide [89]. Fe was the most abundant trace element in the roots, soil and biochar with the highest content recorded in the soil (6500.99 ± 4.4 - 7300.78 ± 2.1 mg/L) as shown in Figure 5c insert and the lowest Fe amount was recorded in treated wastewater (0.003 ± 100 mg/L) as shown in Figure 5b. It was observed that the concentration of Fe in the leaves, roots and biochar treated soils increased with increase in amount of biochar applied to the soil. Although application of biochar increased Fe concentration in the leaves and roots, Fe content in biochar treated soil samples and biochar remained higher, shown in Figure 4. Plants have several enzymatic and non-enzymatic mechanisms to protect against oxidative stress caused by excess of Fe [90]. This could explain the reason for observed lower concentrations in the leaves and roots. Considering the extremely low Fe concentration in irrigation treated wastewater, it was concluded that its Fe contribution was negligible. Therefore, the application of maize biochar served as external Fe enrichment source for the cultivation soils. The other reason for the observed high Fe amount could be that the test soils have naturally high Fe.

3.5.4. Zinc

Zinc is an essential and important nutrient for the normal growth and reproduction of crop plants, a small amount of Zn is required for the proper physiological, enzymatic and metabolic

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processes of plants [91]. It is an effective nutrient that enhances germination, cell membrane stability, stomatal movement, photosynthesis, and respiration regulation. It interacts with enzymes for protein synthesis and its scarcity reduces chlorophyll content and protein synthesis [92]. It is needed in small amount because literature reported that excessive Zn has the potential to affect growth and development, including seed germination and root and stem elongation, and even lead to death [93]. On the contrary, deficiency affects plant growth and development, decreasing yields and nutritional quality [94,95]. Zn application has proven beneficial in improving crop yield and quality [96]. The highest amount of Zn was detected in the biochar (371.74 ± 2.0 mg/L) shown in Figure 5a and the lowest Zn content was found in the treated wastewater (0.024 ± 6.6 mg/L) shown in Figure 5b. Zn content in the samples varied as follows; leaves (9.22 ± 5.5 - 27.14 ± 1.8 mg/L), tubers (4.2 ± 6.0 - 29.19.19 ±1.1 mg/L) and soils (31.38 ± 0.2 -36.49 ± 1.9 mg/L) as shown in Table 4. It was observed that samples from biochar treated soils possessed higher Zn content in the leaves, roots and soil compared to their respective controls. It was further noted that the content of Zn in the leaves, tubers and biochar treated soils increased with increase in amount of biochar applied to the soil. Comparing beetroot samples, the highest amount was detected in the leaves than the tuber. In addition to the presence of micro elements; Cu, Mn, Fe and Cu, EDS revealed the presence of the following macro elements; Mg, K, P, C, Cl, Si and S shown in Figure 1c. Mg is major component of chlorophyll pigments involved in the light-capturing complex of chloroplasts [97]. Mg, K, P and S are known for their crucial roles in plant growth and development [97–99]. The presence of these beneficial micro and macro elements was the reason for improved germination rate and growth rate in biochar treated experimental lines compared to the control. The differences in relative abundance of trace elements in leaves and tubers of beetroots implied that these parts have different accumulation capacity for different metals.

3.6. Effects of MB on Beetroot (Beta vulgaris) Germination Rate

In our experiment, our Null hypothesis (H_o) was that adding MB to the cultivation soils increased germination rate while our alternative hypothesis (H_a) was that adding MB to the soils has no effect on the germination rate. As shown in Figure 6 addition of MB to cultivation soils improved germination rate compared to the control therefore retaining our H_o. The germination rate of the untreated soil after 14 days was observed at 76%, lower than for biochar treated soils which were 96%. When 10 g of biochar was applied, the germination rate was at 93% but when 20 g of biochar was applied to the soil, a lower germination rate was observed at 84%. Several researchers also reported significant increase in germination rate in biochar treated soils compared to controls [100–102]. For example, Shamim et al reported that biochar treated soils exhibited positive germination rate and seedling growth of rice (*Oryza sativa*) seeds compared to control [103]. Other studies revealed that biochar application improved the early growth of seedlings and crop growth [104]. Several reasons have been advanced for observed improved germination rates upon application biochar to cultivation soils. For example, some researchers reasoned that its high porosity has adsorption capacity which enhances soil-water holding capacity in turn maintaining the soil moisture level necessary to promote germination rate of seeds [100]. In agreement with this concept, our SEM analysis of maize biochar revealed surface pores and cross-sectional channels in Figure 1a with the ability to trap water molecules and increase soil moisture leading to observed increased germination rate. Low soil moisture in untreated soils resulted in decreased germination rate in accordance with the above concept. Yang et al suggested that the observed increased seed germination of maize plants in their experiment was due to the mineral nutrients present in biochar which were released slowly to maintain the fertility level of the soil necessary for germination [105]. In our experiment, we detected beneficial trace elements (Cu, Mn, Fe and Zn) and macro elements (K, Mg, P, Si, C and Cl) in the biochar and soil which could also explain the increased germination rate in biochar treated soils. Keital et al reported that plant growth and development depend heavily on micronutrients like iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu) [106]. These elements were detected in the soil, treated wastewater and biochar. Therefore, like Yang et al we suggested that the improved germination of beetroots in biochar treated soils compared to controls was because of mineral soil

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enrichment from biochar. It was observed that all biochar treated soils had more mineral nutrient content than the control except for Zn. The contribution from treated wastewater was negligible based on the extremely low elemental concentrations recorded as shown in Figure 5b and Table 4. The detection of Si macro element by EDS as shown in Figure 1c in the biochar could also explain the increased germination rate in biochar treated soils compared to the control. Shi et al reported that Si significantly increases germination rate and shoot length under both normal and salt conditions [107].

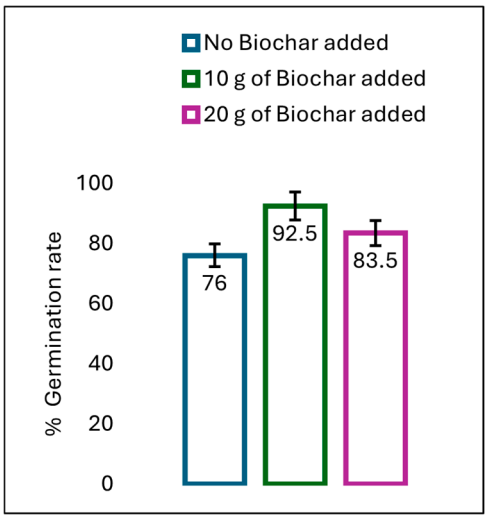

Figure 6. Effects of maize biochar on germination rate as measured at 14 days.

3.7. Effects of MB on Beetroot (Beta vulgaris) Growth Rate

The growth rate of beetroot was monitored by measuring leaf heights after 1 month and after 3 months. We hypothesized (H_o) that adding biochar to the cultivation soils will improve the growth rate than the control. The results agreed with our H_a. As shown in Figure 7a, applying MB improved growth rate at 1 month and continued to enhance growth rate until harvest time in 3 months as shown in Figure 7b. The growth rates were measured by leaf heights. This is consistent with data from literature reports. Shaaban et al reported that biochar as an amendment agent for soil, boosts crop growth and yield by improving the physico-chemical and biological properties of the soil [108]. Manka'abusi et al reported that biochar significantly improved total fresh matter yields of two amaranth cycles by 39% and 17%, lettuce by 7% and carrot by 11% [109]. The effect of rice-husk biochar application on the growth of transplanted lettuce (*Lactuca sativa*) and Chinese cabbage (*Brassica chinensis*) was found to increase the final biomass, root biomass, plant height and number of leaves in all the cropping cycles in comparison to the control [110]. Bu attributed the improved growth due to biochar porosity and presence of soluble nutrients, which help in water-holding capacity and nutrient availability respectively [111]. In our experiment, the germination rate was measured on day 14 and it can be seen in Figure 6 that adding biochar to the soil improved germination rate compared to the control. However, it was observed that doubling amount of biochar from 10 g to 20 g slightly slowed germination rate although it was still higher than for the control. In addition, soils treated with 10 g of biochar exhibited longer leaves of beetroots at one month than soils treated with 20 g of biochar as shown in Figure 6. One Factor ANAOVA analysis (α=0.05) revealed that there was a significant difference in the average leave lengths of beetroot from the 3 treatment $F(2, 232) = 8.85$), p value = 0.0002 measured at 1 month. Post-hoc analysis (Bonferroni correction, $\alpha = 0.01667$) showed that the average leave heights of beetroot samples measured in cultivation soils with no biochar treatment (43.26 ± 16.90 mm) were significantly smaller than average leave heights of beetroot samples measured in cultivation soils treated with 10 g of MB (56.40 ± 20.75 mm, p value < 0.00001). However, there was no significant difference between the leave heights from beetroot samples measured in the control and sample leaves measured in cultivation soils treated with 20 g of MB (49.83 ± 25.43 mm, p value > 0.05).

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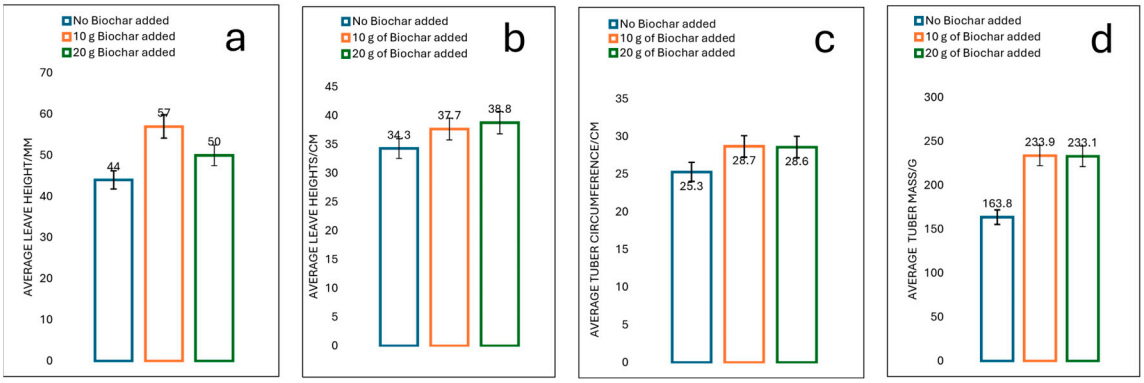

Figure 7. a) Effects of MB on beetroot growth rate based on leave height as measured after one month b) b) Effects of MB on beetroot growth rate based on leave height as measured after three months c) Effects of MB on beetroot yield based on circumference of beetroot tubers d) Effects of MB on beetroot yield based on mass of beetroot tubers and effects of maize biochar of beetroot yield.

We had hypothesized that doubling the amount of MB in the cultivation soils will have a significant effect on the leave heights (H_o) taking H_a as doubling MB will not have any effect. When comparing leave samples measured in cultivation soils treated with 10 g and 20 g of MB, the results revealed that there was no significant difference between the beetroot leaves samples measured in cultivation soils treated with 10 g of MB and 20 g of MB, p value > 0.01. Liao et al also reported an improvement in the initial growth of shoot in soil treated with biochar at a lower rate [112]. Other researchers reported a decreased growth rate at high biochar concentration. The authors evaluated the effect of rice husk and woodchip biochar at varying application rates (1%, 2% and 5% by weight) on seed germination and seedling growth of *Robinia pseudoacacia L.* in calcareous soils by a 240-day glasshouse pot experiment. They reported an increased germination rate for *Robinia pseudoacacia L.* seeds in biochar-treated soils compared to the control. They reported a significant increase of shoot and root biomass of seedlings when 1% and 2% biochar was added. However, they noted that increasing the levels of biochar led to a sharp decrease in root to shoot ratio which they attributed to the increased soil capillary water and nutrient supply (i.e., P and K) [113]. At harvest time (3 months), one Factor ANAOVA ($\alpha = 0.05$) analysis revealed that there was a significant difference in the average leave lengths of beetroots from the 3 treatment $F(2, 133) = 6.66$), $p < 0.05$. Post-hoc analysis (Bonferroni correction, $\alpha = 0.01667$) showed that the average height of beetroot leaves harvested from the control (34.26 ± 6.38 cm) were significantly smaller than average leave heights of beetroot harvested from cultivation soils treated with 10 g of MB (37.65 ± 5.18 cm, p value < 0.05) as well as significantly different from cultivation soils treated with 20 g of MB (38.84 ± 6.73 cm, p value < 0.05). However, there was no significant difference observed between the beetroot leaves harvested from cultivation soils treated 10 g of MB and 20 g of MB at 3 months, p value > 0.1. Solaiman et al study showed that biochar at a lower rate enhanced the early growth of maize seedlings [114] and improved plant macro- (N, P, K) and micronutrients (Zn, Mn, Fe, B, Cu, etc.) than at higher rate. In addition to micro elements, macro elements are known to influence plant growth and development [115]. This could be another reason for observing longer leaves in biochar treated soils compared to controls.

3.8. Effects of MB on Beetroot (Beta vulgaris) Yield

The beetroot yield was measured based on the masses and circumferences of the beetroot tubers. We had hypothesized that adding MB to the cultivation soils will have a significant effect on the size/diameter and weight of the beetroot tubers (H_o). In agreement with H_o, the results revealed that MB treated cultivation soils produced heavier beetroot tubers shown in Figure 7d with average larger diameter compared to beetroot tubers harvested from the control as shown in Figure 7c. This was contrasting to what was reported by Kwizera et al who reported that biochar did not have a significant effect on beetroot diameter, mineral content [116]. Rodgers et al reported that biochar

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increased coffee and maize yields from 1 t ha^{-1} to 3 t ha^{-1} [117]. Therefore, biochar applied in treated wastewater irrigation increased the yield and biomass of beetroot. One Factor ANAOVA (α = 0.05) analysis revealed that there was a significant difference in the average masses of beetroot tubers from the 3 treatment F (2, 133) = 6.46), p < 0.0002. Post-hoc analysis (Bonferroni correction, α = 0.01667) showed that the average mass of beetroot tubers harvested from soils with no biochar treatment (163.79 ± 88.20 g) were significantly smaller than masses of beetroot tubers harvested from cultivation soils treated with 10 g of MB (231.92 ± 105.48 g, p value < 0.0015) as well as cultivation soils treated with 20 g of MB (230.28 ± 109.06 g, p value < 0.0025). We also hypothesized that doubling the amount of MB in the cultivation soil will cause an increase in the average mass of the tubers (H_o). However, there was no significant difference observed between the masses of beetroot tubers harvested from cultivation soils treated with 10 g of MB and 20 g of MB, p value > 0.5.

We postulated that cultivation soils treated with MB will produce tubers with larger diameter (H_o). In agreement with H_o, One Factor ANAOVA (α = 0.05) analysis revealed that there was a significant difference in the average length of circumference of beetroot tubers from the 3 treatment F (2, 133) = 7.69), p < 0.001. Post-hoc analysis (Bonferroni correction, α = 0.01667) showed that the average circumference of beetroot tubers harvested from cultivation soils with no biochar treatment (25.35 ± 4.37 cm) were significantly smaller than length of circumferences of beetroots harvested from cultivations soils treated with 10 g of MB (28.69 ± 4.44 cm, p value < 0.001) and soils treated with 20 g of MB (28.56 ± 4.76 cm, p value < 0.005). We also postulated that doubling MB in the cultivation soils will increase the diameter of the harvested beetroot tubers. However, there was no significant difference observed between the circumferences of biochar tubers harvested from cultivation soils treated 10 g of MB and 20 g of MB, p value > 0.5.

4. Conclusions

The findings revealed that MB fertilization improved key beetroot parameters such as germination rate, leave height, and yield in terms of biomass compared to the control. Post-hoc analysis (Bonferroni correction, α = 0.01667) revealed that cultivation soils fertilized with both 10 g and 20 g of MB produced significantly longer leaves, larger and heavier bulbs than the control. However, doubling MB dosage from 10 g to 20 g in the cultivation soils did not produce statistically significant additional improvements in the average leave heights, masses and circumferences. Therefore, the findings conclude that combining MB with TWW in vegetable production serves as an effective and sustainable circular approach in addressing environmental pollution and mitigating climate change while achieving food security in arid/semi-arid regions.

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Conflicts of Interest: The authors declare no conflicts of interest.

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Abbreviations

The following abbreviations are used in this manuscript:

MB	Maize Biochar
TWW	Treated Wastewater
AAS	Atomic Absorption Spectroscopy
FTIR-ATR	Fourier Transform Infrared Spectroscopy-Attenuated Total Reflectance
SEM	Scanning Electron Microscopy
EDS	Energy Dispersive X-ray Spectroscopy
BET	Brunauer Emmentt Teller

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