Interactive Effects of Biochar and Sewage Sludge on Bioavailability and Plant Uptake of Cu, Fe, and Zn, and Spinach (Spinacia oleracea L.) Yields under Wastewater Irrigation

Ugele Majaule 1,* Oagile Dikinya 1 and Bruno Glaser 2

1 Department of Environmental Science, University of Botswana, Private Bag, Gaborone UB 0704, Botswana; dikinyao@mopipi.ub.bw
2 Soil Biogeochemistry, Institute of Agronomy and Nutritional Sciences, Martin Luther University Halle-Wittenberg, von-Seeckendorf-Platz 3, 06120 Halle (Saale), Germany; bruno.glaser@landw.uni-halle.de

* Correspondence: umajaule@gmail.com

Received: 23 October 2020; Accepted: 26 November 2020; Published: 2 December 2020

Abstract: Biochar can influence bioavailability of micronutrients and crop yields in sewage sludge-treated soils, but the mechanisms of its effects remain poorly understood. Therefore, this field experiment was conducted on a Luvisol and Cambisol to investigate the bioavailability and uptake of some micronutrients and spinach (Spinacia oleracea L.) yields grown in soil amended with biochar and sewage sludge. Ten treatments arranged in randomized complete block design with three levels of biochar (0, 2.5, 5 t/ha) and sewage sludge (0, 6, 12 t/ha) and combinations thereof were applied. High rate of sole sewage sludge, and its combination with biochar significantly (p < 0.05) increased yield on the Luvisol. On the Cambisol, only marginal yield increase resulted from high rates of sole organic amendments and chemical fertilizer, while co-applications decreased yields. Co-amendments generally increased bioavailability of micronutrients relative to sole amendments in the order Fe > Cu = Zn, with greater increase on the Cambisol, but uptake of micronutrients decreased with co-application rates of amendments. Contents of micronutrients in plant leaves were within the normal range, except for a combination of highest dosage of co-amendments on the Cambisol (Fe; 560 mg/kg), which resulted in leaf necrosis and 7% yield depression. The results showed greater yield response of spinach to co-application of amendments on the Luvisol.

Keywords: co-application; organic amendments; micronutrients; Spinacia oleracea L.; wastewater

1. Introduction

Recently, large quantities of sewage sludge are generated from wastewater treatment plants in Botswana as more wastewater treatment facilities are being developed. Application of the sludge and other organic wastes to croplands for production of important vegetable crops (e.g., spinach (Spinacia oleracea L.), cabbage (Brassica oleracea var. capitata), tomato (Solanum lycopersicum L.), etc.) is gaining popularity as a waste disposal option, and low-cost fertilizer [1]. However, there are concerns about the risks of soil pollution and elevated concentrations of heavy metals in plants grown on sewage sludge-treated soils [2–4].

Although micronutrients such as Cu, Fe, and Zn are essential for plant physiological processes and plant productivity, they become toxic at high contents in soil [5]. The frequency, level of contamination and rates of sewage sludge applications to agricultural soils determines the bioavailability and uptake of micronutrients [3]. Important soil factors affecting the bioavailability of these elements and their leaching potential include soil pH, organic matter content, soil texture, redox potential, nutrient interactions, and Al, Fe, and Mn hydroxides contents and temperature [6–8]. Moreover,
plant roots’ association with rhizosphere microbes can regulate the bioavailability and plant uptake of heavy metals [5,9,10]. The strong influence of soil pH on bioavailability of heavy metals is illustrated by the fact that the permissible limits for total heavy metals in sewage sludge are also based on the prevailing soil pH [11].

The uncertainty in bioavailability of heavy metals under fluctuating soil pH and diminishing soil organic matter levels over long time-scale in the field remains a concern by scientists [12]. This concern is due to rapid decomposition of sewage sludge in the soil, which can reduce the retention sites for heavy metals over time, ultimately increasing risk of toxicity and mobility of heavy metals [13,14]. The retention of heavy metals in the soil profile can be enhanced by co-application of sewage sludge with biochar, maintaining adequate levels of bioavailable micronutrients in the soil, without inducing plant deficiency and crop yield losses. Negative priming effects of biochar on sludge organic matter may also moderate the rate of micronutrients release, thus maintaining a steady supply for plant growth [15,16].

Biochar application as a blending agent during sewage sludge composting [17–19], or mixed into matured composts [20,21] significantly increased plant growth and yields, and had contrasting effects on bioavailability of heavy metals in different soils [22–24]. Enhanced oxidation of biochar surfaces and cation exchange capacity (CEC) in the presence of sewage sludge increases retention sites for heavy metals to levels greater than other organic matter in soils [25–27], while the biochar liming effects promote precipitation of heavy metals [28,29]. However, the majority of these studies were conducted under controlled greenhouse and laboratory conditions, or were based on application of biochar to polluted soils [17]. Thus, there is limited research on the potential effects of direct co-application of biochar and sewage sludge on reducing phytoavailability and uptake of heavy metals by vegetable crops in agricultural soils [30].

The hypothesis was that co-application of biochar and sewage sludge would significantly improve the bioavailability and plant uptake of selected micronutrients (Cu, Fe and Zn) and improve spinach yields. The objective of this study was to determine whether the interactive effects of co-application of the biochar and sewage sludge on bioavailability and uptake of trace metals and spinach yields was more favorable than sole amendments in texturally different soils.

2. Materials and Methods

2.1. Experimental Design and Trial Management

The experiment comprised 10 different treatments (Table 1) with three replicates and so each site comprised 30 plots (1.8 × 1.5 m²) arranged in a randomized complete block design (RCBD). Mineral fertilizer (CHEM) treatments received a pre-sowing basal dressing of 18.9 kg ha⁻¹ N, 28 kg/ha P, and 18.9 kg/ha K, which was applied as 2:3:2 (NPK, 22%) at 300 kg/ha. Furthermore, ammonium sulfate (46% N) was top-dressed on CHEM plots at 92 kg N/ha after 2 weeks of transplanting and after the first harvest, hence these plots received a total of 200 kg N/ha/season.

Both sites were disk plowed to about 30 cm depth before the study. Sewage sludge and biochar (in suitable doses) were manually incorporated into the soil using hand-hoes. Spinach (variety; Fordhook Giant) (Sakata, Morgan Hill, CA, USA) seedlings were propagated on garden compost for 3–4 weeks before transplanting after 1 day of irrigation. The trials were drip-irrigated based on soil moisture conditions for about 2 h per irrigation.

The spinach crop in the first season was planted on the 5 March and first harvested on 29 April 2018. The second harvest was done on 5 June 2018. In the second season, the plots were cleared of crop residues, but the soil was not plowed. The planting lines and treatment plots were maintained as during the first season. Similar amendments were applied before the plots were re—planted in the second season with spinach seedlings on the 5 July 2018. The first and second harvests were done on 9 September and 29 October 2018, respectively. The mean temperature and total rainfall were 24.8 and 120 mm, and 19.5 and 24 mm in the first and second season, respectively.
Table 1. Treatments, their application amounts per season and abbreviations.

| Treatment          | Sewage Sludge (t/ha) | Biochar (t/ha) | Chemical Fertilizer (kg/ha) |
|--------------------|-----------------------|---------------|-----------------------------|
| CONT (Control) *   | 0                     | 0             | 0                           |
| 2.5BC              | 0                     | 2.5           | 0                           |
| 5BC                | 0                     | 5             | 0                           |
| CHEM (NPK)         | 0                     | 0             | N—200, P—28, K—18.9        |
| 6SS                | 6                     | 0             | 0                           |
| 6SS + 2.5BC        | 6                     | 2.5           | 0                           |
| 6SS + 5BC          | 6                     | 5             | 0                           |
| 12SS               | 12                    | 0             | 0                           |
| 12SS + 2.5BC       | 12                    | 2.5           | 0                           |
| 12SS + 5BC         | 12                    | 5             | 0                           |

* Symbols represent additions of; CONT—no amendment (control); 2.5BC—2.5 t/ha biochar; 5BC—5 t/ha biochar; CHEM—NPK mineral fertilizer at 300 kg/ha; 6SS—6 t/ha sewage sludge; 6SS + 2.5BC—6 t/ha sewage sludge and 2.5 t/ha biochar; 6SS + 5BC—6 t/ha sewage sludge and 5 t/ha biochar; 12SS—12 t/ha sewage sludge; 12SS + 2.5BC—12 t/ha sewage sludge and 2.5 t/ha biochar; 12SS + 5BC—12 t/ha sewage sludge and 5 t/ha biochar.

2.2. Soil, Plant, Sewage Sludge and Biochar Analysis

Biochar was produced from chipped mixed-wood debris through a home-made pyrolysis unit with two drums. The smaller drum with the feedstock had a lid to ensure low oxygen concentration, while the space between the drum was filled with the chipped wood for heating the pyrolysis tank. After pyrolysis biochar samples were ground and sieved to a particle size of <2 mm. Air-dried soil samples (0–15 cm) were sieved (<2 mm). Random samples of sewage sludge and wood biochar (535 °C, 6 h) were obtained by scoping and stored for laboratory analysis or soil application. Bioavailable trace metals (Cu, Fe, Zn) were determined following the Mehlich-3 extraction procedure as described by [31] (pp. 81–88). Briefly, 3 g of soil samples (2 mm) were extracted in 30 mL of M-3 extracting solution (0.2 M CH₃COOH + 0.015 M NH₄F + 0.013 M HNO₃ + 0.001 M EDTA + 0.25 M H₄NO₃). Extractions were done in an orbital shaker (120 oscillations/min) for 5 min and in triplicate for each soil. Extracts were filtered through a Whatman No. 42 filter paper into plastic vials and then stored at 4 °C until analysis. Exchangeable cations and soil CEC were determined using the ammonium acetate method (pH 7), using a mechanical extractor on a 2.5 g sample [32]. Exchangeable cations were quantified using a 4210 MP-AES (Agilent Technologies, Santa Clara, CA, USA). Soil pH was potentiometrically determined in a 1:5 distilled water and 0.01 mol/L CaCl₂ solution. The pH of sewage sludge samples was determined in a 1:5 of 0.01 mol/L CaCl₂ solution. Biochar pH was measured on a 1:20 (w/v) biochar-to-water ratio via an Orion benchtop pH meter after shaking the samples on an end-to-end shaker for 1 h and settling the mixtures for another hour [33].

Total nitrogen (TN) of soil, sewage sludge, plant and biochar samples was analyzed according to the micro—Kjeldahl procedure [34]. Plant-available phosphorus was determined following the Mehlich III protocol as described by Ziadi and Tran [31] (pp. 81–88). For total content of P and bases in sludge and plant, 1.25 g of sample was wet digested in 2.5 mL of sulphuric acid—selenium mixture [35]. Determination of the total content of P, K, S, Mg and Ca in biochar was done after dry combustion by heating the biochar samples for 2 h at 500 °C as described by Enders and Lehmann [36]. Basic cations were determined in the diluted digests via 4210 MP-AES. The samples were maintained at 500 °C for 8 h, before adding 5 mL of concentrated HNO₃ to each sample vessel and further digesting the residues at 120 °C until dry. After cooling, 1.0 mL HNO₃ and 4.0 mL H₂O₂ were added to the vessels. Furthermore, the samples were heated at 120 °C until dryness. Subsequently, the mixture was dissolved in concentrated HNO₃ (1.4 mL) before the volume was adjusted with deionized water (18.57 mL), then filtered (Whatman No. 42 (Sigma-Aldrich, St. Louis, MO, USA)). Soil organic carbon (SOC) was determined in 1 g samples digested with 0.167 mol/L K₂Cr₂O₇ (10 mL) in presence of 98% concentrated H₂SO₄ (20 mL) according to the Walkley-Black method [37]. The organic C concentrations were determined spectrophotometrically at 570 nm after calibration with sucrose standards solutions.
Total carbon (TC) of the biochar and sewage sludge was characterized by ashing in muffle furnace at 500 °C for 48 h.

Trace metals (Cu, Fe and Zn) in plant leaf tissues were quantified after digestion of samples in concentrated nitric acid (HNO₃). To 1 g of each dry plant sample, 10 mL of trace metal grade nitric acid (65% w/v) (Merck, Darmstadt, Germany) was added and the mixture was allowed to stand overnight, then heated for 1 h at 100 °C in a microwave digester. The mixture was then diluted to 100 mL in borosilicate volumetric flasks with distilled water and filtered through Whatman No. 42 filter papers. Concentrations of Cu, Fe and Zn in the diluted samples were determined by using a 4210 MP-AES.

2.3. Biomass Yields

The spinach plants were grown for about 60 days from the date of transplanting. Harvesting was done on plot basis, disregarding the outer rows of each plot. At physiological maturity, randomly selected plants were cut at about 5 cm above the soil surface on each plot. Biomass yields were determined by taking fresh weight of leaves each plot at each harvest stage.

2.4. Statistical Analysis

Data were subjected to 2-way ANOVA using SAS version 9.4 (SAS Institute Inc, Cary, NC, USA) after employing the Shapiro-Wilk test for normal distribution of the data for each variable. Duncan’s multiple range test was performed to test the significance of the difference between the treatments.

3. Results

3.1. Soil, Sewage Sludge and Biochar Properties

The physicochemical properties of the surface soils (0–15 cm), sewage sludge and biochar used in this study are presented in Table 2. The sewage sludge used in this study was characterized by low heavy metal contents (115 mg Cu/kg and 314 mg Zn/kg) which were within the maximum permissible limits for the EU Council Directive for Cu (1000–1750 mg/kg) and Zn (2500–4000 mg/kg, dry basis) in sludge for agricultural use [11]. Fe content in sewage sludge was the highest among the other heavy metals, and greater than in biochar.

3.2. Effects of Amendments on Mehlich III Extractable Micronutrients

The ANOVA model was significant \(p = 0.04; r^2 = 0.52\) and soil type had significant effects on Mehlich-extractable Cu \(p = 0.0027\). Treatments with sewage sludge slightly increased extractable Cu on the Cambisol relative to the control (CONT) (Figure 1a), but there was marginal decrease in Cu contents in the control compared to the baseline Cu concentrations, possibly due to plant uptake. Treatments had no significant \(p > 0.05\) effects on extractable Cu on the Luvisol. Significant \(p < 0.05\) increase in extractable Cu on the Cambisol was observed under sole and combined applications of organic amendments, the largest values of 6.6 mg/kg resulting from high rates of both amendments (12SS + 5BC) (Figure 1a).

Furthermore, combined application of amendments at high rates (12SS + 5BC) on the Luvisol significantly \(p < 0.05\) increased extractable Cu compared to application of sole amendments at the same rates. Mineral fertilizer marginally increased Cu content compared to the un-amended control on both soils. Cu bioavailability had significant correlations with soil pH \(r^2 = -0.33; p = 0.0099\), CEC \(r^2 = 0.44; p = 0.0004\) and soil organic carbon \(r^2 = 0.53; p < 0.0001\) (Table 3).
Table 2. Pre-crop planting soil properties and basic characteristics of sludge and biochar used in the study.

| Properties | Luvisol | Cambisol | Biochar | Sewage Sludge |
|------------|---------|----------|---------|---------------|
| pH (CaCl₂) | 7.5 ± 1.5 | 6.8 ± 1.3 | 7.7 ± 1.1 | 6.3 ± 0.3 |
| CEC (cmolc/kg) | 8.4 ± 1.1 | 26.2 ± 3.2 | 12± 2.5 | 38 ± 6 |
| Organic Carbon (%) | 1.0 ± 0.2 | 1.8 ± 0.3 | nd * | nd * |
| Total P (mg/kg) | 103 ± 27 | 91.3 ± 14.3 | 824 ± 123 | 5753 ± 525 |
| Available P (mg/kg) | 42.3 ± 5.1 | 24.0 ± 4.1 | 51 ± 8 | 272 ± 38 |
| Total N (%) | 0.08 ± 0.01 | 0.04 ± 0.01 | 1.1 ± 0.6 | 4.5 ± 1.2 |

| Properties | Luvisol | Cambisol | Biochar | Sewage Sludge |
|------------|---------|----------|---------|---------------|
| pH (CaCl₂) | 7.5 ± 1.5 | 6.8 ± 1.3 | 7.7 ± 1.1 | 6.3 ± 0.3 |
| CEC (cmolc/kg) | 8.4 ± 1.1 | 26.2 ± 3.2 | 12± 2.5 | 38 ± 6 |
| Organic Carbon (%) | 1.0 ± 0.2 | 1.8 ± 0.3 | nd * | nd * |
| Total P (mg/kg) | 103 ± 27 | 91.3 ± 14.3 | 824 ± 123 | 5753 ± 525 |
| Available P (mg/kg) | 42.3 ± 5.1 | 24.0 ± 4.1 | 51 ± 8 | 272 ± 38 |
| Total N (%) | 0.08 ± 0.01 | 0.04 ± 0.01 | 1.1 ± 0.6 | 4.5 ± 1.2 |

Table 3. Pearson’s correlation matrix of heavy metals concentrations, crop yields and soil properties.

|          | pH       | Yield    | CEC      | OC       | % M3Cu    | M3Fe    | M3Zn    | Leaf Cu | Leaf Fe  | Leaf Zn |
|----------|----------|----------|----------|----------|-----------|---------|---------|---------|----------|---------|
| pH       | 0.4409 * | −0.8347 * | −0.6553 * | −0.3305 * | −0.7286 * | 0.0494  | −0.0804 | −0.4358 * | −0.3238 * |
| (0.0015) |          | (0.0001) | (0.0099) | (<0.0001) | (0.7079)  | (0.5451) | (0.0006) | (0.0124) |
|          | −0.4738 * | −0.3135 * | −0.1037 | −0.2963 * | 0.2156  | 0.1271  | −0.3615 * | −0.0796 |
| (0.0006) |          | (0.0283) | (0.4784) | (0.0387) | (0.1369)  | (0.3841) | (0.0107) | (0.5866) |
|          | 0.8148 * | 0.4427 * | 0.8729 * | −0.15752 | −0.08492 | 0.33504 | 0.2706 * |          |
| (0.0001) |          | (0.0004) | (<0.0001) | (0.2294) | (0.3252)  | (0.0095) | (0.0382) |          |
|          | 0.5305 * | 0.8250 * | 0.2391 | −0.0793 | 0.2323  | 0.2428  |          |          |
| (0.0001) |          | (<0.0001) | (0.0658) | (0.3506) | (0.0767)  | (0.0638) |          |          |
|          | 0.6471 * | 0.3995 * | 0.2391 | −0.0793 | 0.2323  | 0.2428  |          |          |
| (0.0001) |          | (<0.0001) | (0.0658) | (0.3506) | (0.0767)  | (0.0638) |          |          |
|          | 0.7358 * | 0.5367 | 0.2931 | −0.0441 | 0.1156  | 0.1818  |          |          |
| (0.0016) |          | (0.3834) | (0.1681) |          |          |          |          |          |
|          | 0.1016 | −0.0626 | 0.1529 | 0.2083 |          |          |          |          |
| (0.4399) |          | (0.374)  | (0.2476) | (0.1134) |          |          |          |          |
|          | 0.0706 | 0.0114 | −0.0153 |          |          |          |          |          |
| (0.3953) |          | (0.932)  | (0.9086) |          |          |          |          |          |
|          | 0.3109 * | 0.3479 * |          |          |          |          |          |          |
| (0.0166) |          | (0.0069) |          |          |          |          |          |          |
|          | 0.3764 * | (0.0033) |          |          |          |          |          |          |

* nd—not determined, (±, standard deviation, n = 3).

Table 3. Pearson’s correlation matrix of heavy metals concentrations, crop yields and soil properties.

** M3Cu, M3Fe, M3Zn represent three Mehlich-extractable heavy metals. * Correlation significant at 5% level of significance Values in parentheses are p-values at 5% level of significance.
Cambisol e e e e

2020 rates of both amendments (12SS + available Fe above the control on both soil types, but the differences on the Luvisol were statistically (mean = 92 mg/kg). Results of ANOVA showed that treatments (p < 0.001) had significant effects on the levels of Mehlich-extractable Fe. All amendments increased Mehlich-extractable Cu (12SS + 5BC) (Figure 1a). Furthermore, combined application of amendments at high rates (12SS + 5BC) on the Luvisol p < 0.001) had significant effects on the levels of Mehlich-extractable Fe. All amendments increased pH Yield CEC OC ¶ M3Cu M3Fe M3Zn Leaf Cu Leaf Fe Leaf Zn

| Treatment | MeHlich Cu (mg/kg) | MeHlich Fe (mg/kg) | MeHlich Zn (mg/kg) |
|-----------|--------------------|--------------------|--------------------|
| CONT      | 1.2 ± 0.2          | 20 ± 2             | 5 ± 1              |
| 2.5BC     | 2 ± 0.3            | 25 ± 3             | 6 ± 2              |
| 5BC       | 2.5 ± 0.5          | 30 ± 4             | 9 ± 3              |
| CHEM      | 6 ± 0.8            | 50 ± 5             | 15 ± 4             |
| 6SS       | 12 ± 1.5           | 80 ± 6             | 30 ± 5             |
| 6SS+2.5BC | 15 ± 1.8           | 120 ± 8            | 60 ± 7             |
| 6SS+5BC   | 20 ± 2.0           | 170 ± 10           | 100 ± 9            |
| 12SS      | 30 ± 3.0           | 220 ± 12           | 150 ± 10           |
| 12SS+2.5BC| 40 ± 4.0           | 280 ± 14           | 200 ± 11           |
| 12SS+5BC  | 50 ± 5.0           | 340 ± 18           | 300 ± 15           |

Figure 1. Mehlich-extractable contents of (a) Mehlich Cu, (b) Mehlich Fe, (c) Mehlich Zn at the end of the season. For each variable, columns for both soil types with different letters are significantly different (p < 0.05) according to Duncan’s multiple range test. Comparison of treatment means for both soil types indicate the statistics were done simultaneously. Error bars denote standard error of the mean (SEM) (n = 3).

Available Fe was significantly (p < 0.05) higher on the Cambisol (mean = 159.7 mg/kg) relative to the Luvisol (mean = 92 mg/kg). Results of ANOVA showed that treatments (p = 0.006) and soil type (p < 0.001) had significant effects on the levels of Mehlich-extractable Fe. All amendments increased available Fe above the control on both soil types, but the differences on the Luvisol were statistically insignificant (Figure 1b). Contrastingly, on the Cambisol, application of mineral fertilizer, low rate of sewage sludge (6SS), combination of 6 t/ha of sewage sludge and 5 t/ha biochar (6SS + 5BC), and high rates of both amendments (12SS + 5BC) caused significant increases in available Fe above the control.
The highest Fe content was caused by co-applications of 6SS + 5BC and 12SS + 5BC for the Luvisol and Cambisol, respectively (Figure 1b).

Treatments resulted in higher mean extractable Zn on the Luvisol (5.9 mg/kg) compared to the Cambisol (4.3 mg/kg). Generally, co-applications had greater effects on increasing plant-available Zn than sole applications, and this effect was consistent on the Cambisol (Figure 1c). Co-application of 6 t/ha sewage sludge and 5 t/ha biochar significantly increased Mehlich-extractable Zn on the Luvisol to 11.7 mg/kg relative to the control (2.5 mg/kg). Combined application of high rates of amendments led to the highest available Zn on the Cambisol (8.8 mg/kg) compared to the control (2.3 mg/kg).

3.3. Effects of Amendments on Micronutrients in Leaf Tissues

Cu and Zn contents in the spinach tissues were in the normal range and did not reach the phytotoxic levels (mg/kg): Cu (20–100) and Zn (100–400) [38] (pp. 304–363), mainly due to the low heavy metal contents in the sewage sludge used in this study. Similarly, except for co-application of 6SS+5BC (Fe; 559 mg/kg), leaf Fe was within the normal range (<500 mg/kg). Furthermore, the contents of heavy metals in leaf tissues were generally higher on the Cambisol than on the Luvisol, for the respective treatments (Figure 2a–c).
In general, there was a greater yield response to treatments on the Luvisol compared to the Cambisol (Figure 3). Applications of sole amendments and co-amendments consistently produced significantly ($p < 0.05$) greater yields on the Luvisol compared to the same treatments on the Cambisol.

A slightly insignificant ($p = 0.056$) linear regression between yields and biochar was determined. Increasing the application rate of sole sewage sludge increased crop yields on both soil types, with significant effects determined at high rate (12 t/ha) on the Cambisol only. Interestingly, except for 12SS + 2.5BC, other co-applications decreased yields on the Cambisol. Yields had significant but weak linear correlation to pH ($r^2 = 0.44; p = 0.0015$), CEC ($r^2 = 0.47; p = 0.0006$), soil organic matter content ($r^2 = 0.36; p = 0.0283$), Mehlich-3-extractable Fe ($r^2 = 0.30; p = 0.0387$), and leaf Fe content ($r^2 = 0.36; p = 0.01$) (Table 3).
4. Discussion

4.1. Effects of Amendments on Bioavailability of Micronutrients

Higher bioavailability and uptake of micronutrients on the Cambisol, which had a relatively lower soil pH, confirm findings from previous studies that the solubility and plant uptake of heavy metals are enhanced under acidic pH conditions [39,40]. Furthermore, these findings show that soil acidity was more significant than soil texture in regulating bioavailability of Cu and Fe. Our results support the results from previous studies which showed that soil acidity and coarse soil texture increased bioavailability and uptake of heavy metals by food plants [41,42]. This also corroborates the findings from a long-term study on soils previously amended with sewage sludge by Sauerbeck [43]. The author reported that soil pH was more important than soil texture in regulating the availability of Cd, Cu, Ni and Zn to several plant species. It is well known that Cu bioavailability is inversely related to soil pH [44], hence the significant ($p < 0.05$) negative correlation between available Cu and soil pH (Table 3). This was confirmed in this study as available Cu was higher for each of the corresponding treatments on the Cambisol.

Except for extractable Zn, the bioavailable micronutrients contents in soil and leaf tissues had significantly ($p < 0.05$) negative correlations with soil pH, CEC, and crop yield (Table 3). The treatments on the Cambisol had greater CEC values than the corresponding treatments on the Luvisol (Table 4). Therefore, higher clay and SOC contents of the Cambisol provided more sorption sites, which explains negative correlation between available micronutrients and the other soil parameters (CEC, SOC) [45]. Thus, the relatively lower soil pH of the Cambisol plots, increased bioavailability and uptake of micronutrients. Furthermore, the comparatively higher crop yields on the Luvisol probably resulted in higher uptake of Cu, Fe and Zn, but a dilution effect may explain the negative correlations between yield, and concentrations of micronutrients in the soil and leaf tissues (Table 3).
At the end of the season, extractable Cu and Zn in the control plots on both soil types decreased as compared to the baseline values presumably due to plant uptake [2]. In contrast, available Fe in the control plots significantly increased over 5-fold on both soil types (Figure 1b). These results suggest high Fe concentrations in the irrigation water; therefore Fe toxicity could be a serious problem for sensitive crops over the long term. Application of more alkaline biochar might be a sustainable strategy to keep solution Fe concentrations below toxic levels. Further research is needed to explore optimum pyrolysis conditions to optimize biochar moderation of Fe bioavailability in the study sites.

Significant ($p < 0.05$) positive correlation between SOC and available Cu (Table 3) confirms previous observations indicating that SOC has a strong positive influence on Cu bioavailability. Beesley et al. [46] reported that Cu availability in an acidic soil obtained from a contaminated site increased with the concentrations of dissolved organic carbon (OC) increased in the pore water. However, contrasting results have been reported by Gomez-Eyles et al. [47] who reported that Cu mobility and water soluble carbon simultaneously decreased after application of wood biochar to soil samples obtained from a contaminated site. It should, therefore, be presumed that increasing concentrations of SOC does not always reduce Cu bioavailability.

The values for extractable Zn ranged from 1.99 to 11.7 mg/kg at the end of the season (Figure 1c). Combined applications of 6SS + 5BC and 12SS + 5BC on the Luvisol and Cambisol, respectively resulted in significant ($p < 0.05$) increase of available Zn above the control. Notably, while both rates of sole sewage sludge did not result in substantial changes in available Zn, co-applications of amendments generally increased Zn, regardless of the rate on both soil types. Simultaneously, on the Cambisol, co-application of amendments significantly ($p < 0.05$) increased SOC relative to sole amendments. These results suggest greater soil microbial activities and decomposition rates resulting from co-amendments, leading to release of higher amounts of Zn and other nutrients from the sewage sludge. This is plausible because previous studies have reported that biochar retention of pollutants in soil can promote microbial activities [48–50].

On both soil types, bioavailable Fe increased due to organic amendments (Figure 1b). Corresponding treatments had higher Fe on the Cambisol relative to the Luvisol, mainly due to the lower soil pH in the former soil type. Since the Luvisol was under long-term effluent water irrigation, accumulation of carbonates from the wastewater possibly precipitated Fe and reduced its solubility compared to the Cambisol which was recently brought under cultivation.

Bioavailable Fe was also most increased by combined applications of high rates of sewage sludge and biochar on both soils. Greater production of organic acids as shown by higher SOC under co-applications on the Cambisol may have accentuated available Fe via chelation [38] (pp. 304–363).
This theory is supported by the observation of significant correlation between SOC and available Fe (Table 3). Greater Fe uptake on the Luvisol due to higher biomass production may have decreased bioavailable Fe hence the significant ($p < 0.05$) negative correlation between yield and available Fe.

The observed general trend of increasing extractable Cu, Fe and Zn levels under high rates of co-applications of sewage sludge and biochar shows indicates synergistic effects of amendments, and that the quantity of micronutrients in the organic amendments was important in increasing their bioavailability. In addition, extractable Cu and Fe were inversely related with soil pH suggesting that soil pH was an important determining factor in controlling bioavailability of these metals. This is further illustrated by the higher extractable metal contents (Cu, Fe, Zn) on the Cambisol which had comparably lower pH (Tables 2 and 3), possibly due to less exchange sites for metal retention caused by higher contents of exchangeable cations.

### 4.2. Effects of Amendments on Leaf Micronutrients’ Contents

The mean Cu and Fe contents in the dry matter of spinach leaves were higher on the Cambisol (Figure 2a,b), following the same trend as Mehlich-extractable concentrations. Values for leaf Cu and Zn across all the treatments were below the maximum permissible limits; 20 and 50 mg/kg, respectively. Leaf contents of Cu, Fe and Zn were not significantly ($p < 0.05$) correlated with bioavailable contents (Table 3). The disconnect between bioavailable and plant uptake of micronutrients is possibly due to accumulation of heavy metals in the roots while suppressing translocation to the leaves which has been reported for spinach [4].

The consistent trend in elevated leaf Fe contents due to combined applications of medium rates of amendments, followed by very low concentrations at high rates of combined applications on both soils is noteworthy (Figure 2b). These changes are attributed to the increased soil pH due to co-applications of higher rates of amendments (Table 4), leading to precipitation of Fe on hydroxides of Fe, Mn, and Al. As can be seen in Figure 2b, 12SS + 5BC had a significantly ($p < 0.05$) lower leaf Fe compared to 6SS + 2.5BC, for both soil types. This phenomenon is paramount to mitigate leachability of both macro- and micronutrients due to over-irrigation to leach out excess salts from the root zone. Thus, further field-based research is required to optimize sorption properties of biochar for nutrients and heavy metals from both the wastewater and fertilizers [51,52].

### 4.3. Effects of Amendments on Spinach Yields

Significant ($p < 0.05$) yield improvements due to co-application of biochar and sewage sludge, particularly on the Luvisol (i.e., 6SS + 5BC and 12SS + 2.5BC) is probably linked to enhanced microbial decomposition of sewage sludge and consequent release of plant nutrients [49,53]. Our findings corroborate results from a pot study by Jatav et al. [23] which showed that complimentary effects of biochar and sewage sludge on rice (Oryza sativa L.) yields grown on an Inceptisol (pH = 7.4). The greater spinach yield response to co-amendments on the Luvisol also support results from previous studies regarding greater biochar effects on crop productivity on infertile soils with low organic matter contents [54]. Inconsistent biochar effects on crop biomass yields has been highlighted in many reports [55–57].

As sole sewage sludge dose increased from 6 to 12 t/ha, spinach yields increased significantly ($p < 0.05$) from 19.4 to 24.4 t/ha on the Cambisol, while the increase on the Luvisol was marginal (Figure 3). This trend is linked to increased nutrient availability at higher sludge application rates. Increase in spinach yields with the application of sewage sludge has also been reported [38]. Application of sole biochar significantly increased yields over the control (CONT), but the yield decreased with biochar dose on both soils (Figure 3) possibly due to nutrient deficiency caused by biochar retention of some nutrients.

Treatments on both soils showed sufficiency levels of leaf Fe contents (50–250 mg/kg); [38] (pp. 304–363), whereas on the Cambisol, Fe toxicity (560 mg/kg) (Eid et al. 2017) was caused by co-application of 6 t/ha sewage sludge and 2.5 t/ha biochar (6SS + 2.5BC). Brown spots on young
leaves (necrosis) of plants grown in 6SS + 2.5BC treatments on the Cambisol were observed, and this treatment also had the lowest yields (16.5 t/ha, Figure 3). Thus, Fe toxicity possibly contributed to depressed yields under this treatment. The tendency of higher leaf Fe on the Cambisol is consistent with trend for greater extractable Fe on this soil type, mainly due to lower soil pH.

5. Conclusions

Results of this study shows that co-applications of sewage sludge and biochar generally increased bioavailability of heavy metals, with greater increase determined for the Cambisol which had a lower soil pH. Mehlich III extractable Fe was high, followed by similar levels of Cu and Zn. Leaf tissue nutrient contents were in the order of Fe > Zn > Cu. The contents of micronutrients were more critical in regulating their bioavailability as the solution concentrations tended to increase with sewage application rates.

Biochar—sewage sludge complimentary effects on yields were significant on the Luvisol, with 6SS + 5BC resulting in the highest yields in this study. This study recommends that combined application of 6 t/ha sewage sludge and 5 t/ha biochar (6SS + 5BC) on the Luvisol and sole application of 12 t/ha of sewage sludge on the Cambisol.

Author Contributions: Conceptualization, U.M., O.D. and B.G.; Data curation, U.M.; Formal analysis, U.M.; Investigation, U.M.; Methodology, U.M., O.D. and B.G.; Supervision, O.D.; Validation, U.M.; Writing—original draft, U.M.; Writing—review & editing, U.M., O.D. and B.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research did not receive any specific funding.

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

References

1. Eid, E.M.; El-Bebany, A.F.; Alrumman, S.A.; Hesham, A.E.-L.; Taher, M.A.; Fawy, K.F. Effects of different sewage sludge applications on heavy metal accumulation, growth and yield of spinach (Spinacia oleracea L.). Int. J. Phytoremediation 2017, 19, 340–347. [CrossRef] [PubMed]

2. Dikinya, O.; Areola, O. Comparative analysis of heavy metal concentration in secondary treated wastewater irrigated soils cultivated by different crops. Int. J. Environ. Sci. Technol. 2010, 7, 337–346. [CrossRef]

3. Antonious, G.F.; Kochhar, T.S.; Coolong, T. Yield, quality, and concentration of seven heavy metals in cabbage and broccoli grown in sewage sludge and chicken manure amended soil. J. Environ. Sci. Health 2012, 47, 1955–1965. [CrossRef] [PubMed]

4. Kumar, V.; Chopra, A.; Srivastava, S. Assessment of heavy metals in spinach (Spinacia oleracea L.) grown in sewage sludge–amended soil. Commun. Soil Sci. Plant Anal. 2016, 47, 221–236. [CrossRef]

5. Mishra, J.; Singh, R.; Arora, N.K. Alleviation of heavy metal stress in plants and remediation of soil by rhizosphere microorganisms. Front. Microbiol. 2017, 8, 1706. [CrossRef]

6. Wang, Q.; Huang, Q.; Guo, G.; Qin, J.; Luo, J.; Zhu, Z.; Hong, Y.; Xu, Y.; Hu, S.; Hu, W.; et al. Reducing bioavailability of heavy metals in contaminated soil and uptake by maize using organic-inorganic mixed fertilizer. Chemosphere 2020, 261, 128122. [CrossRef]

7. Yang, G.; Zhu, G.; Li, H.; Han, X.; Li, J.; Ma, Y. Accumulation and bioavailability of heavy metals in a soil–wheat/maize system with long-term sewage sludge amendments. J. Integr. Agric. 2018, 17, 1861–1870. [CrossRef]

8. Karwowska, B.; Dbowska, L. Bioavailability of heavy metals in the municipal sewage sludge. Ecol. Chem. Eng. A 2017, 24, 75–86.

9. Janeeshma, E.; Puthur, J.T. Direct and indirect influence of arbuscular mycorrhizae on enhancing metal tolerance of plants. Arch. Microbiol. 2020, 202, 1–16. [CrossRef]

10. Singh, S.; Parihar, P.; Singh, R.; Singh, V.P.; Prasad, S.M. Heavy Metal Tolerance in Plants: Role of Transcriptomics, Proteomics, Metabolomics, and Ionomics. Front. Plant. Sci. 2016, 6, 1143. [CrossRef]

11. Hudcová, H.; Vymazal, J.; Rozkošný, M. Present restrictions of sewage sludge application in agriculture within the European Union. Soil Water Res. 2019, 14, 104–120. [CrossRef]
12. Bogusz, A.; Oleszczuk, P. Effect of biochar addition to sewage sludge on cadmium, copper and lead speciation in sewage sludge-amended soil. *Chemosphere* 2019, 239, 1–9. [CrossRef] [PubMed]
13. Antoniadis, V.; Robinson, J.; Alloway, B. Effects of short-term pH fluctuations on cadmium, nickel, lead, and zinc availability to ryegrass in a sewage sludge-amended field. *Chemosphere* 2008, 71, 759–764. [CrossRef] [PubMed]
14. Głąb, T.; Gondek, K.; Mierzwia-Hersztel, M.; Szewczyk, W. Effects of Straw and Biochar Amendments on Grassland Productivity and Root Morphology. *Agronomy* 2020, 10, 1794. [CrossRef]
15. Brodowski, S.; John, B.; Flessa, H.; Amelung, W. Aggregate-occluded black carbon in soil. *Sci. Total Environ.* 2019, 680, 181–189. [CrossRef]
16. Greenberg, I.; Kaiser, M.; Gunina, A.; Ledesma, P.; Wiedner, K.; Mueller, C.W.; Glaser, B.; Ludwig, B. Substitution of mineral fertilizers with biogas digestate plus biochar increases physically stabilized soil carbon but not crop biomass in a field trial. *Sci. Total Environ.* 2019, 680, 181–189. [CrossRef]
17. Zhang, J.; Lü, F.; Shao, L.; He, P. The use of biochar-amended composting to improve the humification and degradation of sewage sludge. *Bioresour. Technol.* 2014, 168, 252–258. [CrossRef]
18. Awasthi, M.K.; Wang, Q.; Huang, H.; Li, R.; Shen, F.; Lahori, A.H.; Wang, P.; Guo, D.; Guo, Z.; Jiang, S. Effect of biochar amendment on greenhouse gas emission and bio-availability of heavy metals during sewage sludge co-composting. *J. Clean. Prod.* 2016, 135, 829–835. [CrossRef]
19. Maliniska, K.; Golarska, M.; Caceres, R.; Rotar, A.; Weisser, P.; Slezak, E. Biochar amendment for integrated composting and vermicomposting of sewage sludge—the effect of biochar on the activity of Eisenia fetida and the obtained vermicompost. *Bioresour. Technol.* 2017, 225, 206–214. [CrossRef]
20. Glaser, B.; Wiedner, K.; Seelig, S.; Schmidt, H.P.; Gerber, H. Biochar organic fertilizers from natural resources as substitute for mineral fertilizers. *Agron. Sustain. Dev.* 2015, 35, 667–678. [CrossRef]
21. Waqas, M.; Li, G.; Khan, S.; Shamshad, I.; Reid, B.J.; Qamar, Z.; Chao, C. Application of sewage sludge and sewage sludge biochar to reduce polycyclic aromatic hydrocarbons (PAH) and potentially toxic elements (PTE) accumulation in tomato. *Environ. Sci. Pollut. Res.* 2015, 22, 12114–12123. [CrossRef] [PubMed]
22. Gwengi, W.; Muzava, M.; Mapanda, F.; Tauro, T.P. Comparative short-term effects of sewage sludge and its biochar on soil properties, maize growth and uptake of nutrients on a tropical clay soil in Zimbabwe. *J. Integr. Agric.* 2016, 15, 1395–1406. [CrossRef]
23. Jatav, H.S.; Singh, S.K.; Singh, Y.; Kumar, O. Biochar and sewage sludge application increases yield and micronutrient uptake in rice (*Oryza sativa* L.). *Commun. Soil Sci. Plant Anal.* 2018, 49, 1617–1628. [CrossRef]
24. Penido, E.S.; Martins, G.C.; Mendes, T.B.M.; Melo, L.C.A.; Do Rosário Guimarães, I.; Guilherme, L.R.G. Combining biochar and sewage sludge for immobilization of heavy metals in mining soils. *Ecotoxicol. Environ. Saf.* 2019, 172, 326–333. [CrossRef] [PubMed]
25. Liang, B.; Lehmann, J.; Solomon, D.; Kinyangi, J.; Grossman, J.; O’neill, B.; Skjemstad, J.; Thies, J.; Luizao, F.; Petersen, J. Black carbon increases cation exchange capacity in soils. *Soil Sci. Soc. Am. J.* 2006, 70, 1719–1730. [CrossRef]
26. Luo, Y.; Durenkamp, M.; De Nobili, M.; Lin, Q.; Brookes, P. Short term soil priming effects and the mineralisation of biochar following its incorporation to soils of different pH. *Soil Biol. Biochem.* 2011, 43, 2304–2314. [CrossRef]
27. Wiedner, K.; Fischer, D.; Walther, S.; Criscuoli, I.; Favilli, F.; Nelle, O.; Glaser, B. Acceleration of biochar surface oxidation during composting? *J. Agric. Food Chem.* 2015, 63, 3830–3837. [CrossRef]
28. Park, J.H.; Choppala, G.K.; Bolan, N.S.; Chung, J.W.; Chauasavath, T. Biochar reduces the bioavailability and phytotoxicity of heavy metals. *Plant Soil* 2011, 348, 439. [CrossRef]
29. Xiao, R.; Awasthi, M.K.; Li, R.; Park, J.; Pensky, S.M.; Wang, Q.; Wang, J.J.; Zhang, Z. Recent developments in biochar utilization as an additive in organic solid waste composting: A Review. *Bioresour. Technol.* 2017, 246, 203–213. [CrossRef]
30. Koriczak, M.; Oleszczuk, P. Application of biochar to sewage sludge reduces toxicity and improve organisms growth in sewage sludge-amended soil in long term field experiment. *Sci. Total Environ.* 2018, 625, 8–15. [CrossRef]
31. Ziadi, N.; Tran, T.S. Mehlich 3-Extractable Elements. In *Soil Sampling and Methods of Analysis*, 2nd ed.; Carter, M.R., Gregorich, E.G., Eds.; CRC Press: Boca Raton, FL, USA, 2008; p. 81.
32. Gumbara, R.H.; Darmawan; Sumawinata, B. A comparison of cation exchange capacity of organic soils determined by ammonium acetate solutions buffered at some pHs ranging between around field pH and 7.0. *Earth Environ. Sci.* 2019, 393, 012015. [CrossRef]

33. Wang, S.; Gao, B.; Zimmerman, A.R.; Li, Y.; Ma, L.; Harris, W.G.; Migliaccio, K.W. Physicochemical and sorptive properties of biochars derived from woody and herbaceous biomass. *Chemosphere* 2015, 134, 257–262. [CrossRef] [PubMed]

34. Dempster, D.N.; Jones, D.L.; Murphy, D.V. Clay and biochar amendments decreased inorganic but not dissolved organic nitrogen leaching in soil. *Soil Res.* 2012, 50, 216–221. [CrossRef]

35. Van Reeuwijk, L. Particle Size Analysis. In *Technical Paper 9: Procedures for Soil Analysis*; International Soil Reference and Information Centre: Wageningen, The Netherlands, 2002; Available online: www.isric.org (accessed on 14 March 2018).

36. Enders, A.; Lehmann, J. Comparison of wet-digestion and dry-ashing methods for total elemental analysis of biochar. *Commun. Soil Sci. Plant Anal.* 2012, 43, 1042–1052. [CrossRef]

37. Aregahegn, Z. Optimization of the analytical method for the determination of organic matter. *J. Soil Sci. Environ. Manag.* 2020, 11, 1–5.

38. Tisdale, S.L.; Nelson, W.L.; Beaton, J.D.; Havlin, J.L. *Soil Fertility and Fertilizers*; Macmillan: New York, NY, USA, 1993; pp. 304–363. ISBN 0-02-420835-3.

39. Hou, S.; Zheng, N.; Tang, L.; Ji, X.; Li, Y. Effect of soil pH and organic matter content on heavy metals availability in maize (*Zea mays* L.) rhizospheric soil of non-ferrous metals smelting area. *Environ. Monit. Assess.* 2019, 191, 634. [CrossRef] [PubMed]

40. Kaninga, B.K.; Chishala, B.H.; Maseka, K.K.; Sakala, G.M.; Lark, M.R.; Tye, A.; Watts, M.J. Review: Mine tailings in an African tropical environment-mechanisms for the bioavailability of heavy metals in soils. *Environ. Geochem Health* 2020, 42, 1069–1094. [CrossRef] [PubMed]

41. Wan, Y.; Huang, Q.; Wang, Q.; Yu, Y.; Su, D.; Qiao, Y.; Li, H. Accumulation and bioavailability of heavy metals in an acid soil and their uptake by paddy rice under continuous application of chicken and swine manure. *J. Hazard. Mater.* 2020, 384, 1–10. [CrossRef] [PubMed]

42. Xu, Y.; Liang, X.; Xu, Y.; Qin, X.; Huang, Q.; Wang, L.; Sun, Y. Remediation of Heavy Metal-Polluted Agricultural Soils Using Clay Minerals: A Review. *Pedosphere* 2017, 27, 193–204. [CrossRef]

43. Sauerbeck, D. Plant element and soil properties governing uptake and availability of heavy metals derived from sewage sludge. *Water Air Soil Pollut.* 1991, 57, 227–237. [CrossRef]

44. Khan, A.Z.; Ding, X.; Khan, S.; Ayaz, T.; Fidel, R.; Khan, M.A. Biochar efficacy for reducing heavy metals uptake by Cilantro (*Coriandrum sativum*) and spinach (*Spinacia oleracea*) to minimize human health risk. *Chemosphere* 2020, 244, 125543. [CrossRef] [PubMed]

45. Navarro-Pedreño, J.; Almendro-Candel, M.B.; Gómez Lucas, I.; Jordán Vidal, M.M.; Bech Borras, J.; Zorpas, A.A. Trace Metal Content and Availability of Essential Metals in Agricultural Soils of Alicante (Spain). *Sustainability* 2018, 10, 4534. [CrossRef]

46. Beesley, L.; Moreno-Jiménez, E.; Gomez-Eyles, J.L. Effects of biochar and greenwaste compost amendments on mobility, bioavailability and toxicity of inorganic and organic contaminants in a multi-element polluted soil. *Environ. Pollut.* 2010, 158, 2282–2287. [CrossRef] [PubMed]

47. Gomez-Eyles, J.L.; Sizmur, T.; Collins, C.D.; Hodson, M.E. Effects of biochar and the earthworm Eisenia fetida on the bioavailability of polycyclic aromatic hydrocarbons and potentially toxic elements. *Environ. Pollut.* 2011, 159, 616–622. [CrossRef] [PubMed]

48. Deenik, J.L.; Mcellean, T.; Uehara, G.; Antal, M.J.; Campbell, S. Charcoal volatile matter content influences plant growth and soil nitrogen transformations. *Soil Sci. Soc. Am. J.* 2010, 74, 1259–1270. [CrossRef]

49. Anderson, C.R.; Condron, L.M.; Clough, T.J.; Fiers, M.; Stewart, A.; Hill, R.A.; Sherlock, R.R. Biochar induced soil microbial community change: Implications for biogeochemical cycling of carbon, nitrogen and phosphorus. *Pedobiologia* 2011, 54, 309–320. [CrossRef]

50. Palansooriya, K.N.; Wong, J.T.F.; Hashimoto, Y.; Huang, L.; Rinklebe, J.; Chang, S.X.; Bolan, N.; Wang, H.; Ok, Y.S. Response of microbial communities to biochar-amended soils: A critical review. *Biochar* 2019, 1, 3–22. [CrossRef]

51. Yao, Y.; Gao, B.; Chen, J.J.; Zhang, M.; Inyang, M.; Li, Y.C.; Alva, A.; Yang, L.Y. Engineered carbon (biochar) prepared by direct pyrolysis of Mg-accumulated tomato tissues: Characterization and phosphate removal potential. *Bioresour. Technol.* 2013, 138, 8–13. [CrossRef]
52. Thomas, E.; Borchard, N.; Sarmiento, C.; Atkinson, R.; Ladd, B. Key factors determining biochar sorption capacity for metal contaminants: A Literature Synthesis. *Biochar* 2020, 2, 151–163. [CrossRef]
53. Rogovska, N.; Laird, D.; Cruse, R.; Fleming, P.; Parkin, T.; Meek, D. Impact of biochar on manure carbon stabilization and greenhouse gas emissions. *Soil Sci. Soc. Am. J.* 2011, 75, 871–879. [CrossRef]
54. Glaser, B.; Lehr, V.I. Biochar effects on phosphorus availability in agricultural soils: A meta-analysis. *Sci. Rep.* 2019, 9. [CrossRef] [PubMed]
55. Mukherjee, A.; Lal, R. The biochar dilemma. *Soil Res.* 2014, 52, 217–230. [CrossRef]
56. Novak, J.M.; Ippolito, J.A.; Lentz, R.D.; Spokas, K.A.; Bolster, C.H.; Sistani, K.; Trippe, K.M.; Phillips, C.L.; Johnson, M.G. Soil health, crop productivity, microbial transport, and mine spoil response to biochars. *BioEnergy Res.* 2016, 9, 454–464. [CrossRef]
57. Jeffery, S.; Abalos, D.; Prodana, M.; Bastos, A.C.; Van Groenigen, J.W.; Hungate, B.A.; Verheijen, F. Biochar boosts tropical but not temperate crop yields. *Environ. Res. Lett.* 2017, 12, 053001. [CrossRef]
58. Ngole, V.M. Variations in sludge effects on selected properties of four soil types and vegetable yield. *Afr. J. Agric. Res.* 2010, 5, 3279–3290.

**Publisher’s Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.