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Ascorbic Acid, Sugars, Phenols, and Nitrates Concentrations in Tomato Grown in Animal Manure Amended Soil

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Abstract: We studied the impact of animal manure that was mixed with biochar (a product of wood pyrolysis) on the nitrates (NO^{-}_3), vitamin C, total phenols, and soluble sugars concentrations in tomato fruits (*Solanum lycopersicum* var. Marglobe) of plants that were grown in raised plastic-mulch of freshly tilled soils. Sewage sludge (SS), horse manure (HM), chicken manure (CM), vermicompost (worm castings), commercial inorganic fertilizer, commercial organic fertilizer, and bare soil used for comparison purposes were the soil amendments. Each of the seven treatments was mixed with 10% (*w*/*w*) biochar to make a total of 42 treatments. Chemical analysis of mature tomato fruits revealed that the fruits of plants grown in SS amended soil contained the greatest concentration of NO^{-}_3 (17.2 μg g^{-1} fresh fruits), whereas those that were grown in SS biochar amended soils contained the lowest concentrations of nitrate (5.6 μg g^{-1} fresh fruits) compared to other soil treatments. SS that was amended with biochar increased vitamin C and total phenols in tomato (22 and 27 μg g^{-1} fresh fruits, respectively) when compared to SS alone (11μg g^{-1} fresh fruits). Growers and scientists are seeking strategies to increase antioxidants and reduce anti-nutritional compounds, like nitrates in food, while recycling animal waste. The results of this investigation revealed the role of biochar in reducing nitrates and optimizing the nutritional composition of tomato.

Keywords: vermicompost; chicken manure; horse manure; sewage sludge; vitamin C; biochar

1. Introduction

Tomato has been a significant part of culinary culture worldwide after the exploration of the New World in the 1400's, and it has since become ubiquitous worldwide as an important foodstuff [1]. Tomato is the second most popular vegetable that is consumed in the United States [2] and it has long been recognized as a dominant crop of the worldwide economy [3]. The last 30 years have seen a greater public and research interest regarding the antioxidant properties of tomatoes and human health benefits. On the other hand, tomato accumulates large amounts of nitrate like most vegetables. Ingested nitrate is converted in saliva and gastrointestinal tract to the more toxic nitrite [4].

Nitrate ions in food are due to the addition of nitrogen fertilizers, decaying plants, and animal manure application to agricultural soils and other organic residues or food additives in canned vegetables. Human and animal exposure to nitrate is exogenous (originating from outside an organism), which mainly occurs from intake of vegetables and other foods. Accumulation of large amounts of nitrate in edible plants may also be due to plants low in Nitrate Reductase Activity (NRA) [5]. Due to the increased use of nitrogen fertilizers and livestock manure in intensive agriculture systems, vegetables, and drinking water may contain high concentrations of nitrate [6]. A reduction in nitrate content represents a beneficial value for vegetable products that are rich in carotenoids, vitamins C and E, selenium, dietary fiber, and plant phenols, etc.). It is important to mention here that, some components of vegetables, such as ascorbic acid and phenols, have been reported to mitigate the toxic effects of nitrates [6].

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the European Communities' Scientific Committee for Food [7] set an Acceptable Daily Intake (ADI) for nitrate at 0 to 3.65 mg kg^{-1} body weight in evaluating nitrate and nitrite. Accordingly, the use of nitrogen fertilizers in vegetables should be monitored to avoid the accumulation of nitrate over the allowable ADI limits. Growers and scientists are seeking strategies to reduce anti⁻nutritional compounds, like nitrates in food, while recycling animal wastes. Nitrate and nitrite are two ions that we should monitor in vegetables and fruits. Nitrate ($NO^-{}_3$) and nitrite ($NO^-{}_2$) are a naturally vital part of the nitrogen cycle in the environment. Nitrate is formed from animal manure, plants decomposition, and fertilizers that are used in agriculture. $NO^-{}_3$ interacts with secondary, tertiary, and amides in the stomach and it forms N-nitroso compounds, which have a carcinogenic effect on human and animal cells [8]. Current trends in agricultural practices focus on recycling animal wastes for use as organic fertilizers due to the presence of organic matter, nitrogen, phosphorus, and potassium (NPK), which can be used as alternative organic supplements to inorganic fertilizers.

Phenolic compounds in tomato fruits are important for human health. Studies have consistently linked tomato based phenolic compounds with lower rates of cancer and serum cholesterol [9]. 2015). Studies have examined the metabolic significance and the beneficial effects of various phenolic compounds, such as caffeic acid, ferulic acid, cinnamic acids, coumaric acids, chromogenic acids, quercetin-3-β-O-glycoside, and quercetin on human health and their impact on the harmful effects of herbivore insects and some fungi. Increasing the total phenolic contents of tomatoes through manipulations of the agricultural environment using animal manure as fertilizer is a unique way of improving plant nutritional composition and recycle wastes [1]. Plant phenols and polyphenols have the ability to scavenge radicals, such as hydroxyl, superoxide, and peroxyl, preventing their damage to the DNA molecules. Physiological antioxidant protection is one of the principal mechanisms to combat the side effects of free radicals.

Ascorbic acid (vitamin C) is a major antioxidant in plant cells. Its derivatives, which are tested on cancer cells, revealed anticancer activity [10]. In addition, ascorbic acid, as found in most fruits and vegetables, protects against heart disease, high cholesterol, high blood pressure, and cancer [11]. Accordingly, the increasing awareness of the value of vegetables and fruits in the human diet requires the monitoring of antioxidant content and the undesirable $NO^-{}_3$ content in plants that are grown under new soil management practices, such as recycling animal manure for land farming.

Biochar is the carbon-rich product that is obtained when biomass, such as wood, manure, or leaves, is heated in a closed container with little or no air. The conversion of biomass into biochar cannot only result in renewable energy (synthetic gas and bio oil), but it also decreases the content of carbon dioxide (CO_2) in the atmosphere. The extremely hygroscopic and porous nature of biochar is very effective in retaining both water and water-soluble nutrients, thus making biochar a habitat for many beneficial soil microorganisms [12]. The addition of biochar to soil also decreases trace elements availability due to the alkalinity of biochar. The three main mechanisms by which biochar reduces soil the availability of trace metals to plants are: its oxygen functional groups, aromatic compounds, and high pH [13]. In general, crops that are grown on acidic soils accumulate higher concentrations of most trace elements in their tissues [14]. Accordingly, an increase in soil pH can cause strong adsorption on soil particles and/or metal precipitations, which, in turn, allows for lower mobility of trace elements into edible plant tissues.

The objectives of this investigation were to: (1) assess the effect of biochar and animal manures on tomato yield and (2) test the impact of soil amendments on tomato fruit composition (nitrates, vitamin C, total phenols, and soluble sugars).

2. Materials and Methods

Tomato (*Solanum lycopersicum* var. Marglobe) seedlings of 70 days old were planted in raised, plastic-mulched, freshly tilled soil of 42 plots (3 replicates × 14 treatments) in a randomized complete block design (RCBD). The soil treatments were: (1) no-mulch (NM) unamended soil; (2) municipal sewage sludge (SS); (3) horse manure (HM); (4) chicken manure (CM); (5) vermicompost (worm castings); (6) inorganic fertilizer (NPK 19-19-19); and (7) organic fertilizer (Nature Safe NPK 10-2-8). Each soil amendment was added at 5% nitrogen (N) and roto-tilled with the top 15 cm of native soil in amounts that correspond to the N composition of each soil amendment (Table 1). Of the 14 treatments, seven soil treatments were mixed with 10% (*w*/*w*) biochar (a product of wood pyrolysis), while no biochar was added to the other seven soil treatments.

The sewage sludge (SS) was obtained from the Metropolitan Sewer District, Louisville, KY; CM was obtained from the Department of Animal and Food Sciences, University of Kentucky, Lexington, Kentucky; HM was obtained from the Kentucky Horse Park, College of Agriculture, University of Kentucky, Lexington, KY; vermicompost was obtained from Worm Power Company (Montpelier, Vermont); and, inorganic (NPK 19:19:19) and organic fertilizers (Nature Safe NPK 10:2:8) were obtained from Southern State Co., KY. The native soil was used as a no-mulch (NM) control treatment (rototilled bare soil) for comparison purposes. The soil was transplanted with tomato seedlings in raised black plastic mulch (Figure 1) and was irrigated by a uniform drip irrigation system. All of the agricultural practices were implemented according to the University of Kentucky Growers Guide [15]. Mature tomato fruits were harvested six times during the growing season on August 15, 22, 30, September 7, 18, and 27, 2018. The fruits were weighted and counted for each of the fourteen soil treatments. Samples of tomato fruits (ten fruits), which were collected at the fifth harvest (September 7, 2018) from each soil treatment, were used to determine nitrates, vitamin C, total phenols, and soluble sugars concentrations.

Table 1. Nitrogen, phosphorus, and potassium in soil amendments and amounts of nitrogen used for growing tomato plants in Fayette County, Kentucky, USA.

Soil Amendment	Nitrogen (% N)	Phosphorus (% P)	Potassium (% K)
Sewage Sludge	5.00	3.00	0.00
Chicken Manure	1.10	0.80	0.50
Horse Manure	0.70	0.30	0.60
Vermicompost	1.50	0.75	1.50
Organic Fertilizer	10.00	2.00	8.00
Inorganic Fertilizer	20.00	20.00	20.00
Amounts of Soil Amendments Added to in lbs. Acre $^{-1}$			
Soil Amendment	**Nitrogen (N)**	**Phosphorus (P)**	**Potassium (K)**
Sewage Sludge	2000.00	1200.00	0.00
Chicken Manure	5882.00	4277.80	2673.60
Horse Manure	14,285.00	6122.00	12,244.29
Vermicompost	8333.00	4166.50	8333.00
Organic Fertilizer	1000.00	200.00	800.00
Inorganic Fertilizer	500.00	500.00	500.00

Figure 1. Tomato, *Solanum lycopersicum* var. marglobe grown in raised black plastic mulch.at Fayette County, Kentucky, USA.

2.1. Tomato Yield

At each six harvests (June, 26; July, 21; August 9; August 29; and Sept. 7 and 26, 2017), ripe tomato weight and number of fruits were recorded and combined for each of the fourteen soil treatments for statistical analysis.

2.2. Chemical Analysis

Nitrate concentrations in tomato fruits that were collected at random from plants grown in soils amended with biochar and plants grown in soils without biochar amendments was determined while using the electrode method [16]. At the fifth harvest, ten mature fruits were randomly collected from each soil treatment. Fruits were homogenized and 50 g were taken from the homogenate and blended for one minute using a household blender with 150 mL of 80% ethanol to extract phenols. The homogenates were filtered through Whatman No. 1 filter paper and one mL aliquots of filtrates used for determining total phenols [17] while using a standard calibration curve (1–10 μg g^{-1} of chlorogenic acid). Vitamin C (ascorbic acid) was extracted by blending 20 g of chopped fruits with 100 mL of 0.4% (*w*/*v*) oxalic acid solution and quantified using the potassium ferricyanide method [18]. To determine the degree of fruit sweetness, soluble sugars in mature tomato fruits were extracted with 80% ethanol and then quantified using the methods that were used by Antonious et al. [19].

2.3. Statistical Analysis

Data containing the fruit weights, numbers, and compositions of vitamin C, total phenols, soluble sugars, and nitrates were statistically analyzed while using analysis of variance (ANOVA) and the means were compared using Duncan's multiple range test [20].

3. Results and Discussion

The results revealed no significant differences ($p > 0.05$) among the biochar and no-biochar treatments of the tomato yields that were obtained from manures amended soils. However, the total fruit weights that were obtained from vermicompost-amended soils were significantly ($p < 0.05$) greater when compared to plants grown in soils amended with organic fertilizers (Figure 2A). Regardless of biochar treatments, the total number of fruits obtained from the vermicompost treatments were significantly greater when compared to CM and biochar mixed with vermicompost treatments (Figure 2B). This response may be due to the increase in nutrient availability and water holding capacities in soils that were amended with vermicompost. Some investigators [21] reported that vermicompost has important properties that can be explored as a new technology for converting organic wastes into a product rich in plant nutrients. In our investigation, we applied each soil amendment at 5% N, which indicated that the N rate of application is not a limiting factor in tomato yield, but more important is the availability of N to the growing plants in such soluble forms, as ammonium (NH^{+}_{4}) and nitrate (NO^{-}_{3}) ions.

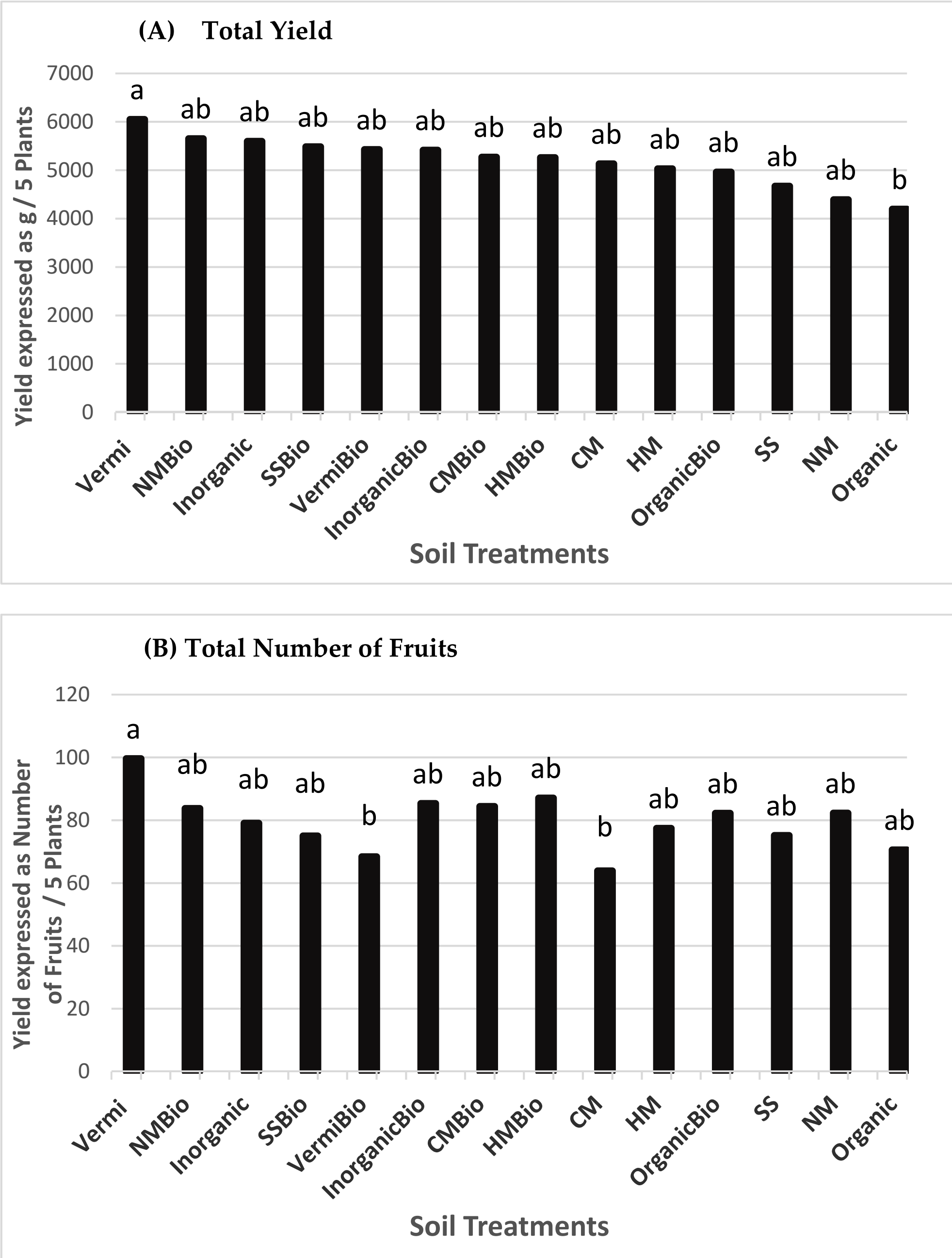

Figure 2. Yield of tomato (**A**) and fruits numbers (**B**) of plants grown in soils mixed with amendments: Vermicompost (Vermi), no-mulch soil mixed with biochar (NMBio), inorganic fertilizer (Inorganic), sewage sludge mixed with biochar (SSBio), vermicompost mixed with biochar (VermiBio), inorganic fertilizer mixed with biochar (InorganicBio), chicken manure mixed with biochar (CMBio). horse manure mixed with biochar (HMBio), chicken manure (CM), horse manure (HM), organic fertilizer mixed with biochar (OrganicBio), sewage sludge (SS), no-mulch bare soil (NM), and organic fertilizer (Organic). Values accompanied by the same letter(s) are not significantly different ($p > 0.05$).

Vitamin C was greatest (23 µg g^{-1} fresh fruits) in fruits of plants grown in SS amended with biochar (SS Bio) compared to all other amendments (Figure 3).

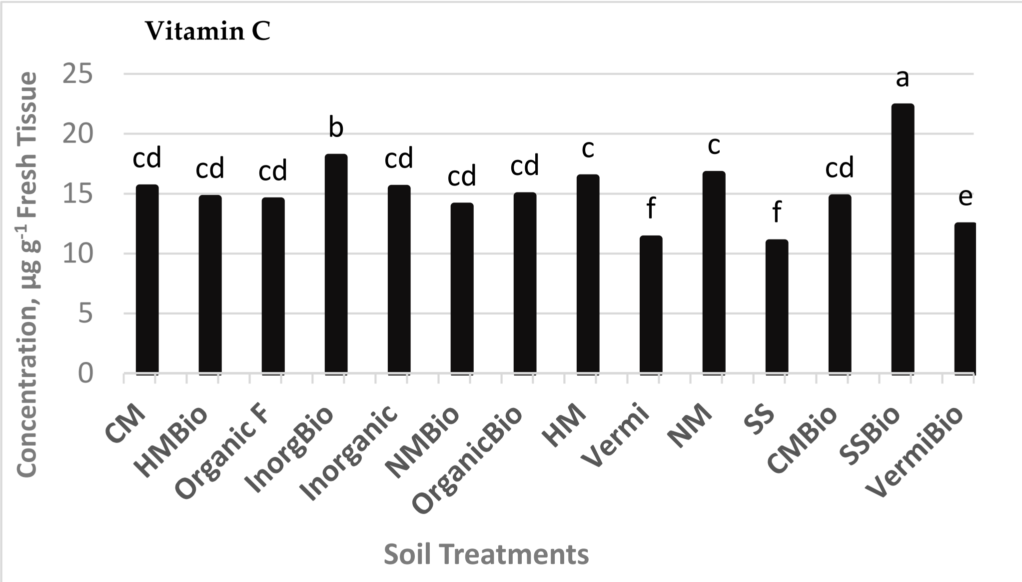

Figure 3. Concentrations of vitamin C in tomato grown in soil mixed with amendments: Vermicompost (Vermi), no-mulch soil mixed with biochar (NMBio), inorganic fertilizer (Inorganic), sewage sludge mixed with biochar (SSBio), vermicompost mixed with biochar (VermiBio), inorganic fertilizer mixed with biochar (InorganicBio), chicken manure mixed with biochar (CMBio), horse manure mixed with biochar (HMBio), chicken manure (CM), horse manure (HM), organic fertilizer mixed with biochar (OrganicBio), sewage sludge (SS), no-mulch bare soil (NM), and organic fertilizer (Organic). Values accompanied by the same letter(s) are not significantly different ($p > 0.05$).

The total phenol concentrations varied among the various soil treatments and they were greatest (27 µg g^{-1} fresh fruits) in the fruits of plants grown in SS Bio and HM amended soil when compared to no-mulch biochar amended soil (NMBio), whereas no significant differences were found among other soil treatments (Figure 4). Tomato plants that were grown in soil amended with SS mixed with biochar (SS Biochar) or horse manure (HM) mixed soil displayed a significant increase in total phenolic content relative to SS, CM Biochar, Inorganic fertilizer, vermicompost mixed with biochar, or no-mulch (NM soil) that was mixed with biochar treatments. Investigators reported that the levels of total phenolic compounds in tomato vary due to the ripening and location of the fruits within the plant [22,23]. We made every effort to only collect ripe fruits for chemical analysis.

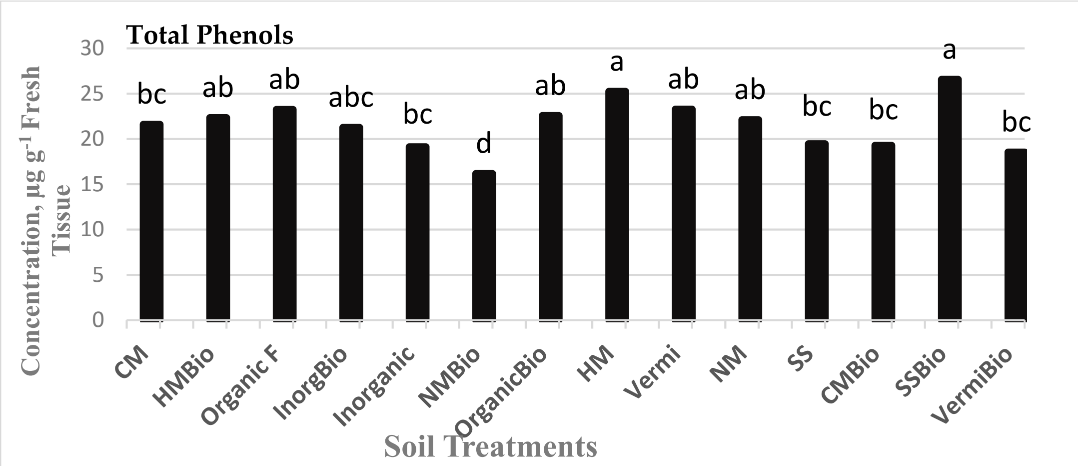

Figure 4. Concentrations of total phenols in tomato grown in soils amended with: Vermicompost (Vermi), no-mulch soil mixed with biochar (NMBio), inorganic fertilizer (Inorganic), sewage sludge mixed with biochar (SSBio), vermicompost mixed with biochar (VermiBio), inorganic fertilizer mixed with biochar (InorganicBio), chicken manure mixed with biochar (CMBio), horse manure mixed with biochar (HMBio), chicken manure (CM), horse manure (HM), organic fertilizer mixed with biochar (OrganicBio), sewage sludge (SS), no-mulch bare soil (NM), and organic fertilizer (Organic). Values accompanied by the same letter(s) are not significantly different ($p > 0.05$).

Soluble sugars concentration was greatest in fruits that were collected from plants grown in SS amended soil as compared to all other amendments (Figure 5). Chemical analysis of mature tomato fruits revealed that fruits of plants grown in SS amended soil contained the greatest concentration of $NO^{-}{}_{3}$ (17.2 µg g^{-1} fresh fruits), whereas the fruits of plants grown in SS biochar amended soil contained the lowest concentrations of nitrate (5.6 µg g^{-1} fresh fruits), as shown in Figure 6, indicating the role of biochar in reducing $NO^{-}{}_{3}$ in SS amended soils. Overall, the $NO^{-}{}_{3}$ concentrations in tomato fruits were highly significant ($p < 0.001$) among the seven amendments used in this investigation (Table 2).

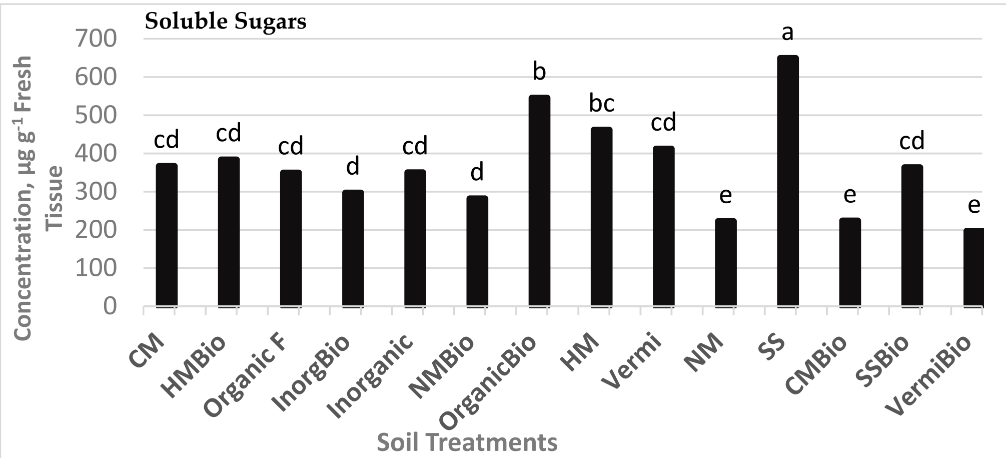

Figure 5. Sugars concentrations in tomato grown in soils amended with: Vermicompost (Vermi), no-mulch soil mixed with biochar (NMBio), inorganic fertilizer (Inorganic), sewage sludge mixed with biochar (SSBio), vermicompost mixed with biochar (VermiBio), inorganic fertilizer mixed with biochar (InorganicBio), chicken manure mixed with biochar (CMBio), horse manure mixed with biochar (HMBio), chicken manure (CM), horse manure (HM), organic fertilizer mixed with biochar (OrganicBio), sewage sludge (SS), no-mulch bare soil (NM), and organic fertilizer (Organic). Values accompanied by the same letter(s) are not significantly different ($p > 0.05$).

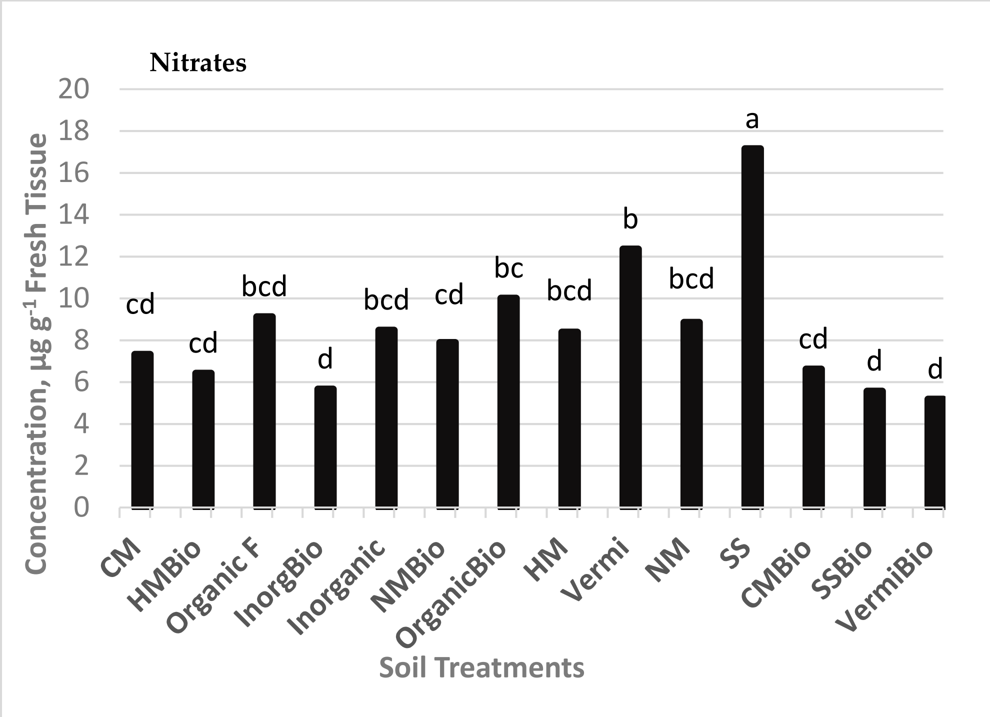

Figure 6. Nitrates concentrations in tomato grown in soils amended with amendments: Vermicompost (Vermi), no-mulch soil mixed with biochar (NMBio), inorganic fertilizer (Inorganic), sewage sludge mixed with biochar (SSBio), vermicompost mixed with biochar (VermiBio), inorganic fertilizer mixed with biochar (InorganicBio), chicken manure mixed with biochar (CMBio), horse manure mixed with biochar (HMBio), chicken manure (CM), horse manure (HM), organic fertilizer mixed with biochar (OrganicBio), sewage sludge (SS), no-mulch bare soil (NM), and organic fertilizer (Organic). Values accompanied by the same letter(s) are not significantly different ($p > 0.05$).

Table 2. Analysis of Variance (ANOVA) of the effect of soil amendments on soluble sugars, nitrates, vitamin C, phenols, total number of fruits, and total yield.

Variable	Dependent Variable	Sum of Squares	df	Mean Square	F-Test	*p*-Value
Soil Type	Soluble sugars	883,573.38	13	67,967.18	0.491	0.910 ^{ns}
	Nitrates	389.53	13	29.96	6.337	<0.001 ***
	Vitamin C	319.10	13	24.55	2.86	0.034 **
	Phenols	900	13	69.3	2.17	0.015 **
	Total no. of fruits	15,056	13	1158	2.11	0.016 **
	Total yield	5.95×10^7	13	4.58×10^6	0.986	0.467 ^{ns}

, * indicate statistical significance at $p \leq 0.05$ and 0.01, respectively. ns indicates statistically no significant.

4. Conclusions

We investigated the impact of animal manure and animal manure mixed with biochar (a product of wood pyrolysis) on the total tomato weight and the numbers at harvest. No impact of biochar application on tomato yield was recorded. However, the results revealed variability among the amendments that were tested for vitamin C, total phenols, nitrates, and soluble sugars contents. Soil amended with vermicompost (worm castings) alone significantly ($p < 0.05$) increased the tomato total yield as compared to the commercial organic fertilizer that was used in this investigation (Nature Safe NPK 10-2-8). Vermicompost also increased the total number of fruits as compared to chicken manure (CM) and vermicompost amended with biochar (VermiBio). Plants that were grown in SS amended soil contained the greatest concentration of $NO^-{}_3$ (17.2 µg g^{-1} fresh fruits), whereas the fruits of plants grown in SS biochar amended soil contained the lowest concentrations of nitrate (5.6 μg g^{-1} fresh fruits) as compared to other soil treatments.

Author Contributions: G.A. designed the study, conducted the laboratory analysis, and wrote the paper. E.T. and M.D. designed the field study and project administration.

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Conflicts of Interest: The authors declare that there are no conflict of interest.

References

1. Antonious, G.F. The impact of Animal Manure on Phytochemicals in Hot Pepper Fruits. In *Solanaceae: Cultivation, Nutrition and Health*; Rocka, I.S., Ed.; Plant Science Health and Practices; Nova Science Publishers: New York, NY, USA, 2018; Chapter 1; pp. 1–42.
2. Luthria, D.L.; Mukhopadhyay, S.; Krizek, D.T. Content of total phenolics and phenolic acids in tomato (*Lycopersicon esculentum* Mill.) fruits as influenced by cultivar and solar UV radiation. *J. Food Compos. Anal.* **2006**, *19*, 771–777. [CrossRef]
3. Peralta, I.E.; Spooner, D.M. History, Origin and Early Cultivation of Tomato (Solanaceae). In *Genetic Improvement of Solanaceous Crops*; Razdan, M.K., Mattoo, A.K., Eds.; Chapter 1 (Solanaceae); Science Publishers: New Hampshire, NH, USA, 2006; Volume 2, pp. 1–24.
4. Pannala, A.S.; Mani, A.R.; Spencer, J.P.E.; Skinner, V.; Bruckdorfer, K.R.; Moore, K.P.; Rice-Evans, C.A. The effect of dietary nitrate on salivary, plasma, and urinary nitrate metabolism in humans. *Free Radic. Biol. Med.* **2003**, *34*, 576–584. [CrossRef]
5. Jana, J.C.; Moktan, P. Nitrate concentration of leafy vegetables: A survey of nitrite concentrations in retail fresh leafy vegetables from daily markets of different locations. *J. Food Agric. Sci.* **2013**, *3*, 1–5.
6. Santamaria, P. Nitrate in vegetables: Toxicity, content, intake and EC regulation. *J. Sci. Food Agric.* **2006**, *86*, 10–17. [CrossRef]
7. Scientific Committee on Food. *Assessment of Dietary Intake of Nitrates by the Population in the European Union, as a Consequence of the Consumption of Vegetables, in Reports on Tasks for Scientific Cooperation: Report of Experts Participating in Task 3.2.3*; European Commission: Brussels, Belgium, 1997; p. 34.
8. Speijers, G.; van den Brandt, P.A. Nitrite and potential endogenous formation of N-nitroso compounds; Safety evaluation of Food Additives; JECFA. *WHO Food Addit. Ser.* **2003**, *50*, 49–74.
9. Giudice, R.D.; Raiola, A.; Tenore, G.C.; Frusciante, L. Antioxidant bioactive compounds in tomato fruits at different ripening stages and their effects on normal and cancer cells. *J. Funct. Foods* **2015**, *18*, 83–94. [CrossRef]
10. Naidu, K.A. Vitamin C in human health and disease is still a mystery? An overview. *Nutr. J.* **2003**, *2*, 2–7. [CrossRef] [PubMed]
11. Ruxton, C.H.; Gardner, E.J.; Walker, D. Can pure fruit and vegetable juices protect against cancer and cardiovascular disease too? A review of the evidence. *Int. J. Food Sci. Nutr.* **2006**, *57*, 249–272. [CrossRef] [PubMed]
12. Antonious, G.F. Biochar and animal manure impact on soil, crop yield and quality. In *Agricultural Waste*; Aladjadjiyan, A., National Biomass Association, Bulgaria, Eds.; Intec-Open Science Books: Rijeka, Croatia, 2018; Book Chapter.
13. Beesley, L.; Moerno-Jiminez, E.; Fellet, G.; Carrijo, L.; Sizmur, T. Biochar and heavy metals. In *Biochar for Environmental Management: Science and Technology*; Lehmann, J., Joseph, S., Eds.; Earthscan from Routledge: London, UK, 2015; Chapter 20; pp. 563–594.
14. Antonious, G.F.; Silitonga, M.R.; Tsegaye, T.; Unrine, J.M.; Coolong, T.; Snyder, J.C. Elevated concentrations of trace elements in soil do not necessarily reflect metals available to plants. *J. Environ. Sci. Health B* **2013**, *48*, 219–225. [CrossRef] [PubMed]
15. *University of Kentucky Vegetable Production Guide for Commercial Growers, College of Agriculture, Food and Environment*; Kentucky Cooperative Extension Service: Lexington, KY, USA, 2018; pp. 95–108.

16. American Public Health Association (APHA). *Standard Methods of Examination of Water and Wastewater*, 21st ed.; Eaton, A.D., Clesceri, L.S., Rice, E.W., Greenberg, A.E., Eds.; Port City: Baltimore, MD, USA, 2005.
17. McGrath, R.M.; Kaluza, W.Z.; Daiber, K.H.; Yan der Riel, W.R.; Glennie, C.W. Polyphenols of sorghum grain, their changes during malting and their inhibitory nature. *J. Agric. Food Chem.* **1982**, *30*, 450–456. [CrossRef]
18. Hashmi, M.H. Spectrophotometric determination with potassium ferricyanide. In *Assay of Vitamins in Pharmaceutical Preparations*; John Wiley and Sons: London, UK, 1973.
19. Antonious, G.F.; Dennis, S.O.; Unrine, J.M.; Snyder, J.C. Ascorbic acid, β-carotene, sugars, phenols, and heavy metals in sweet potato grown in soil fertilized with municipal sewage sludge. *J. Environ. Sci. Health B* **2011**, *46*, 112–121. [CrossRef] [PubMed]
20. SAS Institute Inc. *SAS/STAT Guide 2016*; Version 6.4; SAS Inc.: Cary, NC, USA, 2016; p. 27513.
21. Yadav, A.; Gupta, R.; Garg, V.K. Organic manure production from cow dung and biogas plant slurry by vermicomposting under field conditions. *Int. J. Recycl. Org. Waste Agric.* **2013**, *2*, 3–7. [CrossRef]
22. Slimestad, R.; Verheul, M.J. Content of chalconaringenin andchlorogenic acid in cherry tomatoes is strongly reduced duringpostharvest ripening. *J. Agric. Food Chem.* **2005**, *53*, 7251–7256. [CrossRef] [PubMed]
23. Slimestad, R.; Verheul, M.J. Seasonal variations in the level of plantconstituents in greenhouse production of cherry tomatoes. *J. Agric. Food Chem.* **2005**, *53*, 3114–3119. [CrossRef] [PubMed]