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Pathogen and heavy metal contamination in urban agroecosystems of northern Ghana: Influence of biochar application and wastewater irrigation

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Abstract

The benefit of biochar as a soil fertility enhancer is well known and has been broadly investigated. Equally, many tropical and subtropical countries use wastewater for irrigation in urban agriculture. To assess the related health risks, we determined pathogen and heavy metal fate associated with biochar application and wastewater irrigation in the urban agriculture of northern Ghana. Rice (*Oryza L.*) husk biochar (20 t ha⁻¹), N–P–K 15–15–15 fertilizer (212.5 kg ha⁻¹), and their combinations were evaluated in a field-based experiment. Untreated wastewater and tap water served as irrigation water. Red amaranth (*Amaranthus cruentus L.*) was used as a test crop and was grown in wet (WS) and dry (DS) cropping seasons. Irrigation water, soil, and vegetables were analyzed for heavy metals, *Escherichia coli*, fecal coliform, helminth eggs, and *Salmonella* spp. Unlike the pathogens, analyzed heavy metals from irrigation water and soil were below the FAO/WHO permissible standard for agricultural activities. Wastewater irrigation caused *E. coli* concentrations ranging from 0.5 to 0.6 (WS) and from 0.7 to 0.8 (DS) log₁₀ colony forming units per gram fresh weight (CFU g_{FW}⁻¹) on vegetables and from 1.7 to 2.1 (WS) and from 0.6 to 1.0 (DS) log₁₀ CFU per gram dry weight (g_{DW}⁻¹) in soil. Average log₁₀ CFU g_{FW}⁻¹ rates of 6.19 and 3.44 fecal coliform were found on vegetables, whereas in soil, 4.26 and 4.58 log₁₀ CFU g_{DW}⁻¹ were observed in WS and DS, respectively. Helminth egg populations were high in wastewater and were transferred to the crops and soil. Biochar did not affect bacteria contamination. Pathogen contamination on vegetables and in soil were directly linked to the irrigation water, with minimal or no difference observed from biochar application.

Abbreviations: CFU, colony forming units; DS, dry season; DW, dry weight; FC, fecal coliform; FW, fresh weight; STEC, Shiga toxin-producing *Escherichia coli*; WS, wet season.

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1 | INTRODUCTION

Urban and peri-urban agriculture plays an integral role in food security and supports the health and economy of urban dwellers (Artmann & Sartison, 2018). However, urban and peri-urban agriculture in developing countries is challenged by limited space and resources, making it more intensive with a high rate of nutrient extortion (Drechsel & Keraita, 2014). Biochar, a carbon (C)-rich organic material, is an option to improve soil fertility in urban agriculture (Häring et al., 2017), which in turn could result in an improved crop yield potential of up to 25% in tropical acidic soils (Jeffery et al., 2017; Manka'abusi et al., 2019).

Besides being a dependable water source for irrigation, wastewater contains essential plant nutrients such as nitrogen (N), phosphorus (P), and potassium (K) (Barreto et al., 2013). It could serve as a substitute for commercial fertilizer, such as mineral NPK fertilizer. Hence, up to 20-fold increases in crop yields have been reported for wastewater application on unfertilized soils in the West African urban production system (Akoto-Danso et al., 2019). Wastewater, therefore, becomes a vital resource, more especially for poorly resourced smallholder farmers in developing countries (Mateo-Sagasta & Burke, 2011).

Despite the potential benefits of crop yield improvement, it is essential to be mindful of the health hazards that may result from the reuse of untreated wastewater in irrigated farming. Enteric pathogens are the prime biological pollutants of untreated wastewater and may lead to the contamination of irrigated soils and their crop produce (Kaetzl et al., 2019). Fecal coliforms, specifically *E. coli* and *Salmonella* spp., are among the top human enteric bacteria present in untreated urban wastewater (Kaetzl et al., 2020; Tchobanoglous et al., 2003) and are linked to several hygiene-related diseases (Iwu & Okoh, 2019). More so, the addition of biochar favors soil microbial survivability and abundance by providing refuge against predators (Palansooriya et al., 2019) and a niche for colonization (Hardy et al., 2019) and buffers conditions such as pH and organic matter for microbial nutrition and proliferation (Rutigliano et al., 2014). Such soil conditions may as well be favorable for helminth eggs (Paller & Babia-Abion, 2019) delivered to the soil by wastewater.

Heavy metals have remained a persistent chemical pollutant of wastewater with the potential to contaminate the food chain, leading to human health impacts (Tytła, 2019). High cation exchange capacity, electronegativity, and functional groups on biochar surfaces are among other properties of biochar that facilitate heavy metal immobilization in the soil (He et al., 2019). Several studies have reported reduced mobility and bioavailability of heavy metals in wastewater irrigated soil following biochar application (Ahmad et al., 2014; Medyńska-Juraszek & Ćwieląg-Piasecka, 2020; van Dang et al., 2019). For example, in a study by Nzediegwu et al. (2019) using synthetic wastewater, plantain (*Musa* spp. L.) peel biochar signif-

Core Ideas

- Soil and vegetable contamination were linked to irrigation water source.
- Rice husk-derived biochar did not affect bacteria contamination on the vegetables.
- Wastewater and biochar application did not affect heavy metal contamination in the soil.
- Helminth eggs and bacteria pathogens in soil were not significantly affected by biochar.

icantly adsorbed Cd and Zn at the soil surface, thereby reducing their uptake into the flesh of potatoes (*Solanum tuberosum* L.) by 69 and 33%, respectively. According to Chen et al. (2018), factors including biochar type, application rate, metal contaminant, and soil properties are critical for effective heavy metal remediation in contaminated soil.

This study used a field-based experiment to evaluate the effects of biochar on pathogen and heavy metal concentrations in irrigated urban agriculture with the hypotheses that biochar application results in (a) reduced contamination of plant-available heavy metals in the soil, (b) higher pathogen concentration in soils and on vegetables under cultivation, and (c) prolonged survival of pathogen in the wet season without irrigation.

2 | MATERIALS AND METHODS

2.1 | Field setup

The field experiment was conducted in Tamale, northern region of Ghana (9°28'28.75" N, 0°50'53.48" W). This area lies in the Guinea savannah zone and experiences semi-arid climatic conditions with a monomodal rainfall pattern. The daily mean temperature is 28.9 °C, and annual mean precipitation is 1,090 mm. Following the World Reference Base (WRB, 2015) classification system, the soil was classified as Petroplinthic Cambisol, with 5.90% clay, 48.40% silt, and 45.70% sand. At a depth of 20 cm, the soil initially contained organic C of 4.1 g kg⁻¹, 0.4 g kg⁻¹ total N, and an effective cation exchange capacity of 36.1 mmol_c kg⁻¹ (Häring et al., 2017).

The soil amendments on the experimental field included rice (*Oryza* L.) husk biochar (20 t ha⁻¹), commercial NPK 15–15–15 fertilizer (212.5 kg ha⁻¹) according to the normal agricultural practice by farmers, combined biochar (20 t ha⁻¹) and NPK fertilizer (212.5 kg ha⁻¹), and an unamended control. Each treatment was replicated four times and irrigated either with untreated wastewater (domestic sewage) or tap water (domestic tap water supply). Rice husk biochar was locally produced in a kiln at 550 °C under limited oxygen conditions.

TABLE 1 Initial soil and biochar characterization of the field experiment (adapted from Häring et al., 2017)

| Parameter | Soil | Biochar |
|---|-------|---------|
| Sand, % | 45.7 | – |
| Silt, % | 47 | – |
| Clay, % | 5.9 | – |
| CEC, mmol _c kg ⁻¹ | 36.1 | ND |
| pH (CaCl ₂) | 5.1 | ND |
| pH (deionized H ₂ O) | ND | 9.1 |
| SOC, % | 0.41 | – |
| Bulk density, g cm ⁻³ | 1.42 | ND |
| C, % | 0.4 | 42.4 |
| N, % | 0.04 | 0.6 |
| Available P, mg kg ⁻¹ | 7.7 | ND |
| Total P, mg kg ⁻¹ | 110.9 | 861.3 |
| K, mg kg ⁻¹ | 38.9 | 977.1 |
| Specific surface area (BET), m ² g ⁻¹ | – | 62.9 |
| Volatile matter, % | ND | 23.2 |
| Ash content, % | ND | 45.2 |
| H/C (molar ratio) | ND | 0.05 |
| O/C (molar ratio) | ND | 0.27 |

Note. BET, Brunauer–Emmett–Teller; CEC, cation exchange capacity; ND, not determined; SOC, soil organic C.

TABLE 2 Mean total aboveground biomass of amaranth grown in the wet and dry season

| Irrigation water | Soil treatment | Mg _{FW} ha ⁻¹ | |
|------------------|----------------|-----------------------------------|---------------|
| | | Wet season | Dry season |
| Tap water | control | 0.97 ± 0.95a | 2.11 ± 1.23a |
| | biochar | 0.59 ± 0.32a | 2.15 ± 0.48a |
| | NPK | 8.70 ± 2.02b | 14.22 ± 3.35b |
| | NPK + biochar | 6.06 ± 1.45b | 13.12 ± 0.97b |
| Wastewater | control | 5.24 ± 1.11b | 22.93 ± 1.35c |
| | biochar | 6.53 ± 0.86b | 21.87 ± 0.93c |
| | NPK | 14.77 ± 1.45c | 32.38 ± 2.25d |
| | NPK + biochar | 15.01 ± 0.96c | 33.90 ± 1.03d |

Note. Variation ranges are given as SD. Letters indicate a significant difference of the mean (ANOVA, $p < .05$) within each cropping season after post hoc analysis (adopted from Akoto-Danso et al., 2018). FW, fresh weight.

The obtained rice husk biochar had an average C content of 42.4 and 0.6% N (Table 1).

2.2 | Crop growth cycles and agronomic practices

Prior to this study, multiple crops had been grown on the field consecutively in the dry cropping season (DS) and wet

cropping season (WS) for 18 mo. To allow for a comparison between DS and WS, amaranth was grown in the peak periods of each cropping season for 4 wk. Detailed agronomic activities conducted on the experimental field are described in Akoto-Danso et al. (2019) and Häring et al. (2017). They also analyzed biochar effects on yields and soil properties on the same experimental site within a common cooperative study to enhance nutrient use efficiency in urban agriculture of West African cities. Biochar (20 t ha⁻¹) was manually incorporated and thoroughly tilled to a depth of 20 cm. Amaranth seeds were sown by broadcasting, and NPK 15–15–15 fertilizer (212.5 kg ha⁻¹) applied 7 d after seed emergence. Irrigation water types were kept in an open storage system (as practiced by farmers) and applied by means of manual irrigation using a watering can. The quantity of irrigation water input was 850 mm in the DS and 416 mm in the WS. Total aboveground biomass of amaranth was measured at harvest to determine treatment effect on crop yield (Table 2).

2.3 | Microbiological analyses

Pathogen analysis of irrigation water types was performed twice every week throughout the experimental period, whereas soil and vegetables (amaranth) were analyzed at the end of each cropping cycle. A uniform mix of amaranth leaf was carefully taken from each plot and hygienically transferred to sterile sample bags. Also, a composite soil sample (0–10 cm) was obtained from six randomly distributed points within each plot. All samples were transported on ice and immediately analyzed for *E. coli*, fecal coliform (FC), *Salmonella*, and helminth eggs.

CHROMagar Coliform (ECC) and *E. coli*, as described by Alonso et al. (1999), were adopted for the isolation of *E. coli* and FC bacteria. Briefly, 25 g of carefully chopped amaranth leaf and 10 g soil were homogenized using a paddle blender (STO-80, Tekmar Co.) with 225 and 90 ml phosphate-buffered saline, respectively. Samples were serially diluted, isolated, and identified with CHROMagar *E. coli* and Coliform ECC (CHROMagar). Results were log-transformed and reported as colony forming units (CFU) per 100 ml (water), per gram fresh weight (FW, vegetables), or per gram dry weight (DW, soil).

Shiga toxin-producing *E. coli* (STEC) was isolated following the method in Hirvonen et al. (2012) with CHROMagar STEC (CHROMagar) bacteria culture media. A CHROMagar STEC interpretation chart was used to detect STEC-positive plates based on the colony appearance.

Semi-solid Rappaport-Vassiliadis, Xylose-Lysine Deoxycholate agar, and buffered peptone water produced by Merck were used to isolate and identify *Salmonella* as elaborated by the International Organization for Standardization (ISO, 2004). The samples were precultured with buffered

peptone water and transferred to solidified semi-solid Rappaport-Vassiliadis for motile *Salmonella*. Presumptive *Salmonella* colonies were transferred to a Xylose Lysine Deoxycholate agar plate for confirmation.

2.4 | Helminth eggs

The flotation and sedimentation method by Schwartzbrod (1998) was used for the helminth egg determination. Two liters of the irrigation water samples and washing extracts from soil and vegetables were allowed to settle overnight. The supernatant was removed, leaving about 50 ml, and then the samples were centrifuged for 3 min at 1,450 rpm. The resultant was decanted, resuspended in 150 ml ZnSO₄ (1.2 sp. gr), and again centrifuged for 3 min at 1,450 rpm. The ZnSO₄ supernatant was poured into a 2-L flask, diluted with 1 L distilled water, and allowed to settle for 24 h. The supernatant was sucked up, and the deposit was centrifuged at 1,600 rpm for 3 min. The supernatant was again removed, and the precipitate was resuspended with 5 ml acid-alcohol (0.1 NH₂SO₄ + 35% C₂H₅OH) buffer solution and 2 ml concentrated ethyl ether. The mixture was shaken with the occasional opening of the tube and centrifuged again at 2,200 rpm for 3 min. With a micropipette, as much of the supernatant as possible (~6 ml) was removed, leaving about 1 ml of deposit. The deposit was observed under a microscope (×100), and the eggs were manually counted. A chart for the diagnosis of intestinal parasites developed by Guy (1995) was used to identify the helminths captured by a digital camera microscope (3.0 MP, OMAX).

2.5 | Heavy metal and nutrients analysis of water and soil

Irrigation waters were periodically sampled and immediately measured for PO₄-P (Ohno & Zibilske, 1991), NO₃-N (Cataldo et al., 1975), and NH₄-N (Grasshoff, 1976) photometrically using UV/VIS spectrophotometer (Pharo 300 Spectroquant, Merck GmbH). A conductivity meter (Basic 20, Crison Instruments S.A.) and a pH meter (basic 20, Crison Instruments S.A.) were used for electrical conductivity and pH measurement, respectively. Subsamples were acidified and transported to Germany for heavy metal analysis with inductively coupled plasma–optical emission spectrometry (Ciros CCD, SPECTRO Analytical Instruments GmbH).

Heavy metals in soil samples were measured after microwave digestion with concentrated nitric acid (65% HNO₃) in Teflon tubes. Briefly, 0.25 g of ground soil was digested in 10 ml nitric acid in a MARS express (CEM, Kamp-Lintford) microwave at 120 °C for 15 min. The digested sample was allowed to cool to room temperature, and 10 ml of deionized water added to the vessel. The mixture was fil-

tered with 0.2-μm cellulose membrane filter paper. The resultant filtrate was analyzed for heavy metals with inductively coupled plasma–optical emission spectrometry (Ciros CCD, SPECTRO Analytical Instruments GmbH). Calibration for precision and quality control of heavy metal measurement were ensured using ICP Multi-Element Standard Solution XVI (Merck).

2.6 | Data analysis

Fecal coliform and *E. coli* counts per plate were converted to CFU and log transformed to obtain normalized data for statistical analysis. We performed a two-way ANOVA to test the effect of irrigation water qualities and soil amendments on the pathogen and heavy metal contaminations separately for the WS and DS. A post hoc test was performed with Fisher's LSD at $p < .05$. Additionally, we ran Pearson moment correlations between irrigation water qualities, pathogen, and heavy metals levels. The statistical analyses were carried out using R Language and Environment for Statistical Computing (R Core Team, 2017), and the figure was created with OriginPro 2020 software (Origin Lab Corporation).

3 | RESULTS

3.1 | Pathogens and nutrient loads of irrigation water

Wastewater was highly contaminated with human enteric FC (7.01 log₁₀CFU 100 ml⁻¹) and *E. coli* (4.35 log₁₀CFU 100 ml⁻¹). *Salmonella* spp. was detected in both water sources, whereas STEC was found only in the wastewater. Helminth eggs were abundant in the wastewater compared with tap water. Plant essential nutrients, such as ammonium (NH₄-N) and phosphate (PO₄-P), were at much higher levels in wastewater when compared to tap and rainwater (Table 3). Heavy metals in wastewater were negligible, with relatively lower concentrations in the wet season (Supplemental Table S2).

3.2 | Fecal coliform and *E. coli* contamination of vegetable and soil

Fecal coliform and *E. coli* contamination on vegetables and in soil was significantly higher on wastewater irrigated plot in both cropping seasons. However, the contamination of vegetables was reduced to about half in the WS (Figure 1). On the other hand, amaranthus grown in the DS had a significant level of contamination ranging, from 5.8 to 7.1 log₁₀CFU g_{FW}⁻¹ for fecal coliform and from 0.75 to 0.84 log₁₀ CFU g_{FW}⁻¹ for *E.*

TABLE 3 Average concentration of pathogens and nutrients in irrigation water in dry and wet season growing periods

| Parameter | Unit | Dry season | | Wet season | |
|-------------------------|--------------------------------------|--------------|---------------|--------------|---------------|
| | | Tap water | Wastewater | Tap water | Wastewater |
| Faecal coliform | \log_{10} CFU 100 ml ⁻¹ | 3.07 ± 0.20b | 7.46 ± 0.30a | 3.59 ± 0.50b | 6.54 ± 0.40a |
| <i>Escherichia coli</i> | \log_{10} CFU 100 ml ⁻¹ | 1.08 ± 0.14b | 4.34 ± 0.08a | 1.1 ± 0.10b | 4.37 ± 0.19a |
| <i>Salmonella</i> spp. | | + | + | + | + |
| STEC | | - | - | - | + |
| Helminth | Eggs L ⁻¹ | 2.66 ± 1.00b | 13.00 ± 4.00a | 2.33 ± 1.00b | 16.66 ± 3.00a |
| NO ₃ -N | mg L ⁻¹ | 0.34 ± 0.09b | 0.15 ± 0.05a | 0.2 ± 0.06ab | 0.17 ± 0.06ab |
| NH ₄ -N | mg L ⁻¹ | 0.03 ± 0.01b | 30.74 ± 6.45a | 0.05 ± 0.01b | 40.34 ± 8.76a |
| PO ₄ -P | mg L ⁻¹ | 0.05 ± 0.10a | 11.34 ± 3.98c | 0.04 ± 0.02a | 4.92 ± 1.00b |
| K | mg L ⁻¹ | 1.44 ± 0.50a | 4.55 ± 1.18b | 0.87 ± 0.19a | 4.37 ± 0.63b |
| pH | | 7.05 ± 0.38a | 7.31 ± 0.23a | 7.16 ± 0.46a | 7.43 ± 0.81a |
| EC | μS cm ⁻¹ | 108 ± 9a | 590 ± 87b | 87 ± 19b | 503 ± 79b |

Note. The SD is presented as a variation range of 20 samples. Positive and negative signs express the presence or the absence of an organism in the irrigation water. Groups that do not share the same letter are significantly different from each other (ANOVA, $p < .05$ and Fisher's post hoc test). CFU, colony forming units; EC, electrical conductivity; STEC, Shiga toxin-producing *E. coli*.

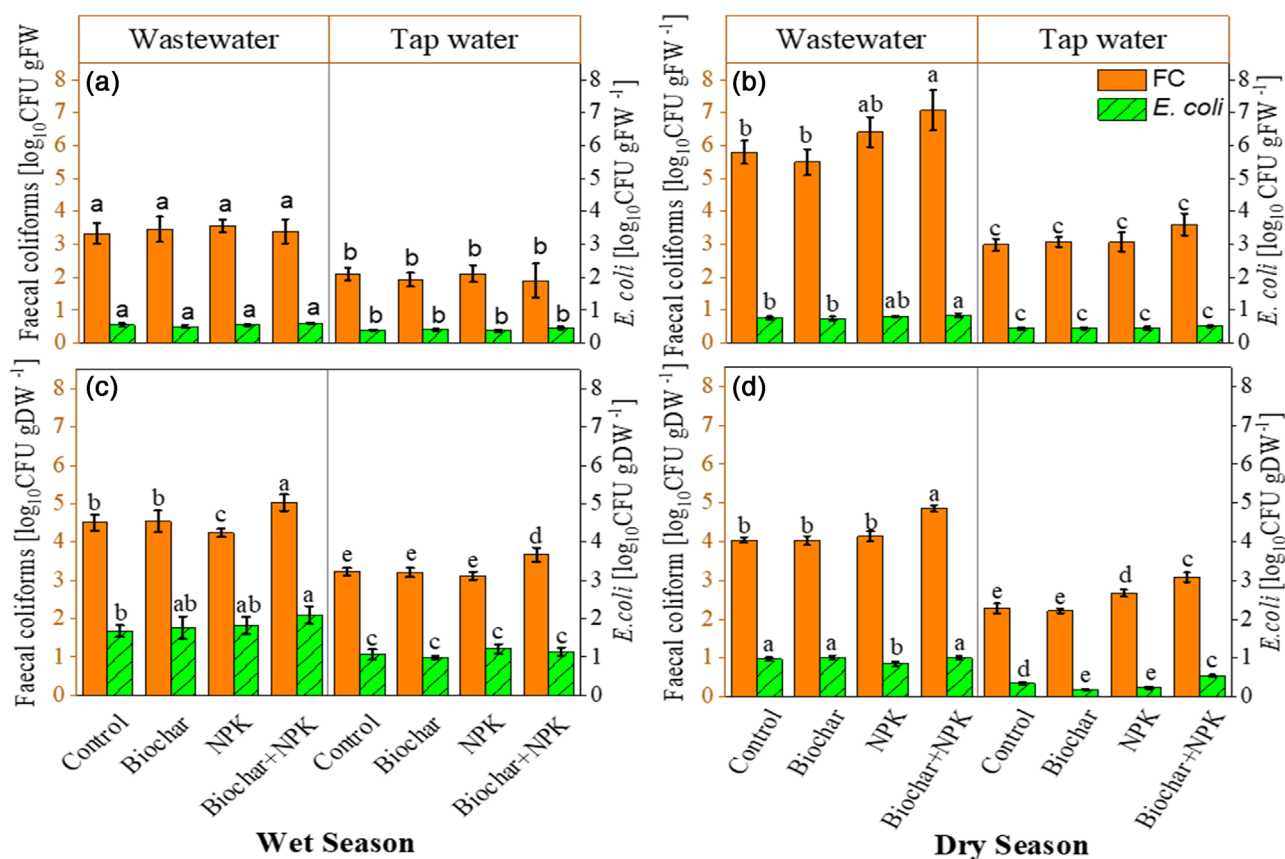


FIGURE 1 Fecal coliform (FC) and *Escherichia coli* counts on *Amaranthus cruentus* L. (A and B) and soil (C and D) in wet (A and C) and dry season (B and D). Error bars represent SDs of means of four ($n = 4$) replicates. Letters indicate a significant difference in the mean (ANOVA, $p < .05$) between treatments following a Fisher's post hoc test. CFU, colony forming units; DW, dry weight, FW, fresh weight.

coli on wastewater-irrigated plots. Compared with the control, the addition of biochar had no influence on bacterial contamination in soil. However, co-amendment of biochar and NPK resulted in a significant FC contamination of 5.03 ± 0.2 and $4.86 \pm 0.08 \log_{10}$ CFU g_{DM}^{-1} soil⁻¹ in the DS and WS, respectively, and *E. coli* in the WS at $2.1 \pm 0.2 \log_{10}$ CFU g_{DM}^{-1} soil⁻¹ compared with the unamended control.

3.3 | Helminth egg counts of soil and vegetables

Helminth egg count was significantly higher in soil than on the vegetables. No helminth eggs were detected on vegetables grown during the WS. A count of 2 eggs $100 g_{FW}^{-1}$ was the only observed egg on vegetable recorded in the DS and was irrigated with wastewater. Biochar+NPK-amended soil with wastewater irrigation showed the highest count of helminth eggs (23 ± 7 egg $100 g_{DW}^{-1}$ in the WS and 17 ± 2 eggs $10 g_{DW}^{-1}$ in the DS). However, biochar additions did not result in a statistically significant difference when compared to the control soil.

3.4 | Heavy metal contamination of soil

In general, the amount of heavy metals in soil was low regardless of the irrigated water quality and amendment applied. A significant effect of wastewater on heavy metal in soil was only observed for Cd, Ti, and Zn, with mean values of 0.27, 16.07, and 7.27 mg kg^{-1} compared with tap water, with mean values of 0.21, 13.03, and 5.33 mg kg^{-1} , respectively. The addition of rice husk biochar (20 t ha^{-1}) did not significantly affect the heavy metal concentration in soil (Table 4).

4 | DISCUSSION

4.1 | Pathogenic properties of irrigation water

The water quality assessment in this study focused on enteric bacteria, including FC concentration, *E. coli* loads, presence of *Salmonella* spp., STEC, and helminth eggs. The levels of enteric bacteria revealed in the wastewater were high (Table 3) above WHO (2006) recommended value of $3 \log_{10}$ CFU $100 ml^{-1}$ for FC and *E. coli*, zero counts for *Salmonella* spp., Shiga toxin-producing *E. coli*, and helminths eggs. The dissolution of human and animal excreta from the surrounding communities is believed to contribute to contamination levels in the wastewater. These results correspond to an earlier finding by Amoah (2008) with similar reports from Tamale and other major cities in Ghana. Furthermore, smallholder farm-

ers' common means of irrigation is by using a watering can, which requires manually fetching water from an open storage source (Arimiyaw et al., 2020). Such an open water storage system practiced by farmers might have exposed the tap water to fecal contamination because FC was above WHO acceptable standards. In addition, tap water was stored in an open 10-m³ plastic container. Therefore, recontamination of tap water during storage could not be excluded.

The influence of seasonal variation (dry and wet) caused about 15% reduction in FC, confirming the study by McLain and Williams (2008). This can be explained by the potentials of rainfall input to dilute surface water and decrease the concentration of indicator bacteria. *Escherichia coli* strain O157:H7 (STEC) is virulent and can cause bloody diarrhea and hemolytic uremic syndrome, leading to kidney failure (Jelacic et al., 2003). The positive STEC detected in the wastewater of our study supports the findings of Saba et al. (2015), who reported about 44 and 42% of STEC-positive incidents in Tamale teaching hospital and cattle feces in the study area. Gyles (2007) stated that the primary hosts of STEC are the intestinal guts of cattle and humans, which are an essential source of STEC contamination to the wastewater. It seems possible that this result is due to the free-range animal production system in the study area, which is the largest producer of cattle and related ruminants in the country (Rahman et al., 2019). The helminth population in the irrigation water exceeded the WHO (2006) recommended standard of <1 egg L^{-1} for unrestricted irrigation. *Ascaris lumbricoides*, *Schistosoma* spp., and *Strongyloides stercoralis* were the main eggs detected in the irrigation water. About 75% of identified eggs were *Ascaris lumbricoides*, confirming the study of Abagale et al. (2013), which investigated the types and seasonal diversity of helminth eggs in wastewater used for peri-urban vegetable crop production in Tamale metropolis and also recorded a predominant number of *Ascaris lumbricoides*.

4.2 | Microbial contamination of vegetables (*Amaranthus cruentus* L.)

The health risk posed by pathogens is among the main disadvantages of using untreated wastewater, especially for irrigation on vegetable farms. Fecal coliform contamination on vegetables was high and had a positive correlation ($\rho = .92$) with the pathogenic quality of irrigation water. The primary source of coliform contamination to irrigated vegetable is probably the irrigation water (Okafo et al., 2003). Hence, there is a need to treat wastewater before irrigation to remove pathogens (Kaetzl et al., 2019). High pathogen load on the test vegetable (amaranthus) has been reported by Cobbina et al. (2013), who recorded high levels of pathogen above the level recommended by the International Commission on Microbiological Specifications for Foods recommended level.

TABLE 4 Mean concentrations of heavy metals in the irrigated soil

| Irrigation | Be | Cd | Co | Cr | Cu | Hg | Ni | Pb | Se | Ti | V | Zn |
|---------------------------------|------------|----------------|--------------|--------------|--------------|--------------|-------------|--------------|---------------|----------------|--------------|---------------|
| mg kg ⁻¹ of dry soil | | | | | | | | | | | | |
| Tap water | | | | | | | | | | | | |
| Control | 2.30 ± 0.3 | 0.30 ± 0.01abc | 2.91 ± 0.4a | 5.36 ± 0.4a | 0.91 ± 0.3b | 2.18 ± 0.02a | 1.29 ± 0.1a | 3.46 ± 0.50a | 4.91 ± 0.30a | 16.86 ± 0.8ab | 8.49 ± 1.10a | 4.55 ± 0.5def |
| Biochar | 2.20 ± 0.2 | 0.24 ± 0.03abc | 2.03 ± 0.6b | 4.79 ± 0.3ab | 1.45 ± 0.1a | 2.19 ± 0.01a | 1.24 ± 0.4a | 3.86 ± 0.08a | 4.57 ± 0.7ab | 12.13 ± 1.02cd | 7.53 ± 1.10a | 7.84 ± 1.03b |
| NPK | 2.20 ± 0.4 | 0.19 ± 0.04c | 2.02 ± 0.2b | 4.84 ± 0.4ab | 0.66 ± 0.1c | 2.21 ± 0.10a | 1.09 ± 0.1a | 3.23 ± 0.60a | 4.91 ± 0.80a | 12.02 ± 0.9cd | 8.29 ± 1.30a | 4.59 ± 0.7def |
| NPK + biochar | 2.0 ± 0.2 | 0.21 ± 0.02bc | 2.04 ± 0.1b | 4.53 ± 0.1b | 0.76 ± 0.3b | 2.20 ± 0.10a | 1.16 ± 0.1a | 3.32 ± 0.30a | 3.77 ± 0.10c | 11.12 ± 0.02d | 7.10 ± 0.70a | 4.33 ± 0.30f |
| Wastewater | | | | | | | | | | | | |
| Control | 1.90 ± 0.2 | 0.25 ± 0.03abc | 1.79 ± 0.2b | 5.1 ± 0.3ab | 0.80 ± 0.2bc | 2.19 ± 0.1a | 1.15 ± 0.1a | 3.31 ± 0.1a | 3.89 ± 0.3bc | 16.45 ± 0.6b | 7.12 ± 0.2a | 5.93 ± 1.4cde |
| Biochar | 2.30 ± 0.2 | 0.23 ± 0.02abc | 1.95 ± 0.2b | 5.4 ± 0.4a | 0.97 ± 0.1b | 2.16 ± 0.02a | 1.27 ± 0.2a | 3.62 ± 0.04a | 4.65 ± 0.4abc | 18.19 ± 1.01a | 8.12 ± 0.02a | 6.49 ± 0.3bc |
| NPK | 2.20 ± 0.3 | 0.28 ± 0.06ab | 2.32 ± 0.4b | 4.9 ± 0.3ab | 1.29 ± 0.3ab | 2.11 ± 0.2a | 1.43 ± 0.2a | 3.69 ± 0.3a | 4.20 ± 0.7ab | 12.92 ± 1.5c | 7.7 ± 0.8a | 10.59 ± 1.2a |
| NPK+biochar | 1.90 ± 0.2 | 0.31 ± 0.08a | 1.99 ± 0.2ab | 5.01 ± 0.2ab | 0.94 ± 0.2b | 2.19 ± 0.02a | 1.37 ± 0.1a | 3.08 ± 0.5a | 4.05 ± 0.6abc | 16.70 ± 1.3ab | 7.4 ± 0.8a | 6.057 ± 1cd |
| F value | | | | | | | | | | | | |
| Treatment | .483 | .747 | .284 | .223 | .001 | .879 | .963 | .285 | .101 | <.001 | .593 | <.0001 |
| Water quality | .443 | .045 | .119 | .168 | .339 | .305 | .174 | .816 | .13 | <.001 | .512 | <.0001 |
| Treatment × water quality | .572 | .105 | .012 | .218 | <.0001 | .566 | .170 | .528 | .12 | <.001 | .324 | <.0001 |
| WHO/FAO (MAC) | ND | 1–5 | 20–50 | 50–500 | 60–150 | 0.5–5 | 20–60 | 20–300 | ND | ND | 150 | 100–300 |

Note. Groups that do not share the same letter are significantly different from each other ($p < .05$; ANOVA and Fisher's post hoc test). Values after ± sign represent the SD of means.

The study revealed that the microbial contamination of irrigated vegetables is dependent on the season. The significant reduction of about 80% in FC and 44% in *E. coli* concentration as well as zero records of *Salmonella* spp. during the WS can be attributed to natural rainfall that may have washed the pathogens off the surfaces of the vegetables, thereby reducing the prevalence of enteric bacteria. Vegetables with larger leaf surfaces are more prone to microbial contamination from irrigation water, especially when overhead irrigation techniques are used (Amoah et al., 2005). This phenomenon could have contributed to the elevated loads of pathogens and the presence of *Salmonella* spp. on the amaranth grown on Biochar+NPK and NPK soil-amended plots, which had higher aboveground biomass compared with other treatments as a result of improved soil nutrients (Akoto-Danso et al., 2019). The WHO has recognized STEC as one of the leading causes of human foodborne diseases worldwide (WHO, 2006). Contaminated vegetables can be an essential vector for STEC transmission to humans (Khatib et al., 2015). Although *E. coli* was detected on the vegetables in this study, none of the vegetable samples from all the treatments was positive for *E. coli* strain O157 in the two seasons (see Supplemental Material). *Escherichia coli* strain O157 has a high proportion of population die-off in most environments and on plant surfaces due to its short survival time (Erickson et al., 2010; Moyne et al., 2020). Our study was conducted in a savanna climate, characterized by high temperatures and solar radiation. These conditions might have rendered the *E. coli* strain O157 on amaranth inactive or nonculturable in the DS, as similarly reported in a field study by Bezanson et al. (2012). During the peak periods of the WS, amaranth crop was subjected to limited wastewater irrigation (prime source of pathogens) due to the intensity of rainfall. The reduced irrigation regime might have lowered *E. coli* strain O157 on the amaranth surfaces, or the strain may have further been washed away by the natural rainfall.

4.3 | Prevalence of pathogens in soil

The potentials of pathogenic contamination of soil resulting from wastewater application are of concern due to their risk of causing enteric disease outbreak (Mara et al., 2007). The presence of bacteria contaminant in wastewater was directly transferred and significantly increased pathogens in soil, with a higher occurrence in the DS (Figure 1). The intense irrigation regime in the DS compared with the limited wastewater irrigation in the WS (due to rainwater) could explain the increased pathogen load in wastewater-irrigated soil in the DS. The survivability of pathogens in wastewater-irrigated soil may have been improved by dissolved organic matter in wastewater (Table 3), enhancing the properties of soil organic components to stimulate microbial respiration and activities

(Asirifi et al., 2021). The improved organic matter content might have also accounted for the higher pathogen loads in Biochar+NPK-treated plot under both water qualities and seasons. The forward adjustment of soil C, N, and pH and the increase in soil volumetric moisture content (Akoto-Danso et al., 2019) by biochar amendment under the same conditions may have accounted for the elevated pathogens in the biochar-amended soils. This result supports earlier findings that the soil microbial activity increases with biochar additions (Steiner et al., 2008). Higher soil volumetric moisture content between 22 and 47% detected in the WS, compared with 10–18% volumetric moisture content in the DS, might have catalyzed the growth and proliferation of pathogens during the WS. These findings agree with Chu et al. (2010), who reported that soil moisture strongly correlated with bacterial composition and abundance. *Salmonella* spp. was absent in tap water but was detected in all wastewater-irrigated soil in both seasons. The result is related to the irrigation water because the microbial quality of irrigated soil depends on the source and type of the irrigation water (Armon et al., 2002).

4.4 | Helminth eggs load in the soil and on vegetables

All soils were contaminated with helminth eggs irrespective of the soil amendments applied or irrigation water quality. However, the prevalence was significantly higher in soil irrigated with wastewater than in soil irrigated with tap water (Figure 2). This clearly originates from wastewater-borne fecal matter in the soil. Amoah et al. (2016) reported similar findings of helminth contamination in urban vegetable production in Kumasi, Ghana. The improved organic matter (biochar application) and high moisture content (WS) may have enhanced the loads of helminth eggs up to 57% (WS) and 23% (DS). These findings are consistent with those of Eteya et al. (2016), who reported a positive correlation between the prevalence of helminth distribution and soil properties, including organic matter and moisture. *Ascaris lumbricoides*, *S. stercoralis*, *Trichuris trichura*, and *Schistosoma mansoni* were the identified helminths in the soil, confirming the importance of the parasite as a health hazard in wastewater used for irrigation. *Ascaris lumbricoides* was the most predominant species, with about 94% of the total population, probably due to their high persistence and survival time in the soil. Abagale et al. (2013) and Amoah et al. (2006) reported a similar prevalence of *Ascaris* spp. in irrigation water and vegetables in urban agriculture in Ghana. According to Silva et al. (2003), *Ascaris* spp. occur most frequently in tropical and subtropical regions where inadequate sanitation prevails, especially in the case of using wastewater for agricultural activities. Unlike the soil, amaranthus vegetable showed fewer helminth eggs. For instance, an average of 2 eggs $100\text{ g}_{\text{FW}}^{-1}$

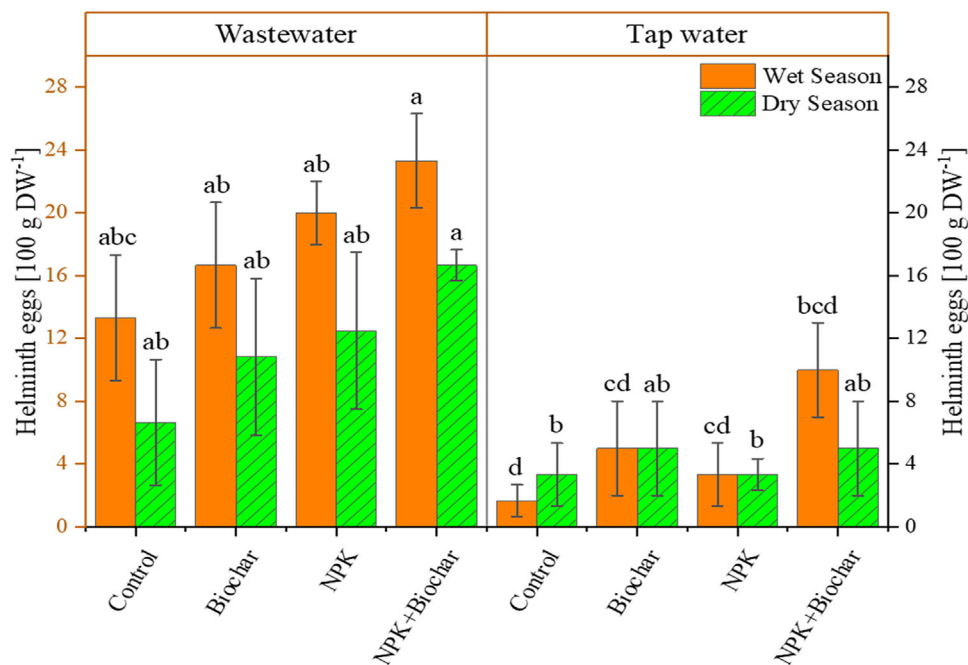


FIGURE 2 Helminth eggs counts of soil in wet and dry season. Error bars represent SDs of means of four ($n = 4$) replicates. Letters indicate a significant difference in the mean (ANOVA, $p < .05$) between treatments following a Fisher's post hoc test. DW, dry weight.

was counted on amaranth, which is slightly above the WHO standard of <1 egg $100 \text{ g}_{\text{FW}}^{-1}$ of irrigated vegetables. The leaf morphology of the amaranth plant and its height above the ground may explain the relatively low population of helminth eggs. Luna-Guevara et al. (2019) indicated that the morphology of irrigated crops plays a role in their exposure to biological contaminant. Similarly, Amoah et al. (2006) found a range of 3–6 eggs 100 g^{-1} on lettuce and related the findings to the broader leaf and higher exposure of lettuce to soil splashes due to its proximity to the ground.

4.5 | Nutrient and heavy metal concentration irrigation water

Deductions from the lower concentration of heavy metals found in wastewater, even below WHO acceptable standards, confirm the wastewater source as domestic, with minimal or no interference from industrial waste. Other studies that reported higher concentrations of heavy metal in wastewater linked its source to effluent from textile (Jaishree & Khan, 2014), mining (Muhammad et al., 2013), and electroplating industries (Venkateswaran et al., 2007). The higher concentration of Fe in tap water than in wastewater can be related to the average amount of Fe in the tap water supply within the Tamale municipality as 3.5 mg L^{-1} (Ewusi et al., 2015). The wastewater contained more essential plant nutrients than the tap water and increased crop yield comparable to inorganic NPK fertilization (Akoto-Danso et al., 2019). This confirms

the report that the major plant nutrients available in wastewater are N, P, and K due to dissolved ions and organic materials (Barreto et al., 2013).

4.6 | Trace element accumulations in soil

All heavy metals in the soil were below the WHO (2006) and FAO (2003) permissible concentrations for agriculture activities. This relatively lower heavy metal concentration is due to the low levels of heavy metal in the domestic wastewater used for irrigation. Our finding agrees with Kim et al. (2015) in a study that assessed heavy metal pollution in domestic irrigated soil. An appreciable higher concentration of metals in the wastewater used in the study explains the greater concentrations of metal in wastewater-irrigated plots. The addition of biochar to the soil did not affect heavy metal concentration, which confirms the report by Trakal et al. (2011). In this case, the rate of biochar (20 t ha^{-1}) applied was mainly for agronomic purpose and may not have been sufficient to influence the heavy metal content in the soil. For instance, biochar application rates up to 72 t ha^{-1} (Xing et al., 2019), 60 t ha^{-1} (Gonzaga et al., 2020), and 40 t ha^{-1} (Bian et al., 2014) have been reported for the efficient remediation of heavy metal-contaminated soil. Moreover, the heavy metal concentration of the wastewater was low and therefore could not contaminate the soil within the study period to justify the biochar effect. Studies on biochar's remediation effect on soil heavy metal contamination usually occurred in severely

contaminated soil (Bian et al., 2014), and the influence differs with the type and concentration of heavy metals in question (Rees et al., 2014).

5 | CONCLUSION

This study showed higher pollution of fecal coliforms, *E. coli*, *Salmonella*, and helminth eggs in the wastewater above WHO (2006) standards. These pathogenic pollutants were transferred to the soil and vegetables under irrigation, although reduced contaminations were observed during the WS. Contrarily, heavy metals found in irrigation water and soil were below FAO/WHO permissible standard for agricultural activities. There was no significant interference of biochar application on the contamination levels. Indications from the results show that, due to its fertilization (plant nutrient) potential and low heavy metal concentration, domestic wastewater from the study area can serve as an alternative source of water for irrigation. To reduce health risks from the pathogens, we recommend the use of simple on-site water filtration systems (Kaetzl et al., 2019) to treat the wastewater for pathogen removal prior to use.

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AUTHOR CONTRIBUTIONS

Isaac Asirifi: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Visualization; Writing-original draft; Writing-review & editing. Steffen Werner: Conceptualization; Methodology; Writing-original draft; Writing-review & editing. Korbinian Kaetzl: Formal analysis; Validation; Visualization; Writing-original draft; Writing-review & editing. Courage K. S. Saba: Conceptualization; Investigation; Methodology. Felix K. Abagale: Conceptualization; Formal analysis; Investigation; Methodology. Philip Amoah: Conceptualization; Methodology. Bernd Marschner: Conceptualization; Funding acquisition; Project administration; Resources; Supervision; Writing-original draft; Writing-review & editing.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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