



Assessment of Nutritional Composition and Heavy Metal Levels in Okra and Tomato Crops Treated with Liquid Biofertilizer

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Abstract

The shift towards sustainable agriculture has intensified the use of liquid biofertilizers as alternative to synthetic fertilizers due to their role in improving soil health and plant nutrient uptake; but there are limited studies on their impact on the nutritional quality and toxicological safety of produce. This study is designed to assess the impacts of liquid biofertilizer on the nutritional and heavy metal concentrations of Okra (*Abelmoschus esculentus*) and Tomato (*Solanum lycopersicum*) crops. Okra and Tomato crops were treated with liquid biofertilizer containing microbial consortia (*Aspergillus niger*, *Penicillium chrysogenum*, *Bacillus cereus*, *Bacillus licheniformis*, *Pseudomonas fluorescens* and *Azotobacter chroococcum*). The proximate composition (%) for harvested Okra and tomato fruits treated with biofertilizer were; ash content (0.83 ± 0.04 and 1.00 ± 0.00); carbohydrate content (8.31 ± 0.04 and 9.93 ± 0.00); Fibre content (15.91 ± 1.35 and 8.55 ± 0.06); Lipid content (0.50 ± 0.01 and 0.43 ± 0.01); moisture content (98.60 ± 0.03 and 87.45 ± 0.02), protein content (6.52 ± 0.02 and 5.66 ± 0.04) and vitamin C content (Mg/Kg) (112.22 ± 1.57 and 94.46 ± 0.02) respectively. The heavy metal concentrations (mg/Kg) from harvested fruits ranged from 1.87 ± 0.028 - 38.025 ± 0.035 ; 0.00 ± 0.00 - 4.46 ± 0.269 ; 69.00 ± 0.14 - 183.67 ± 0.03 and 0.00 ± 0.00 for Zinc, Lead, Iron and copper, respectively. Statistically, the treatments were significant at $P < 0.000$ for concentrations of the heavy metals by the both plants. This study concludes that the use of the tested liquid biofertilizer is not only an effective strategy for boosting the productivity of okra and tomato but also a safe and valuable method for enhancing the nutritional value of the harvest, thereby recommending its integration into sustainable food production systems.

Keywords: Sustainable Agriculture, Liquid Biofertilizer, Nutritional Quality, Food Safety, Heavy Metals

Introduction

Contemporary agriculture stands at a critical juncture, challenged by the dual mandate of ensuring global food security for a growing population while mitigating the severe environmental impacts of conventional farming practices. The long-term and intensive use of synthetic chemical fertilizers has been linked to soil deterioration, water eutrophication, greenhouse gas emissions, and the disruption of soil microbial ecosystems (Kopittke et al., 2019). This has catalyzed a global shift towards sustainable agricultural inputs, with biofertilizers emerging as a cornerstone of this green revolution (Mahdi et al., 2020).

Biofertilizers are formulations containing live or latent strains of beneficial microorganisms that, upon application to seeds, plant surfaces, or soil, promote plant growth by improving the supply and availability of primary nutrients (Uzah et al., 2024; Bhardwaj et al., 2014). Unlike their chemical counterparts, biofertilizers do not directly provide nutrients but facilitate their mobilization from the non-available or fixed forms in the soil and atmosphere (Alori et al., 2017). Liquid biofertilizers, a specific formulation, offer distinct advantages such as ease of handling, uniform application, longer shelf life, and higher cell counts, making them more amenable for use in modern irrigation systems like fertigation (Mahdi et al., 2020). These microbial inoculants, which include nitrogen-fixing bacteria (e.g., *Azotobacter*, *Rhizobium*), phosphate-solubilizing bacteria (e.g., *Pseudomonas*, *Bacillus*), and plant growth-promoting rhizobacteria (PGPR), enhance soil fertility and plant

vigor through various mechanisms, including biological nitrogen fixation, phosphate solubilization, and the production of phytohormones (Backer et al., 2018).

Okra (*Abelmoschus esculentus*) and tomato (*Solanum lycopersicum*) are two of the most important and widely cultivated vegetable crops in tropical and subtropical regions, providing essential vitamins, minerals, and dietary fiber. They are high-value crops crucial for the income of smallholder farmers. However, both are typically cultivated under high-input systems, making them ideal candidates for studying the substitution potential of biofertilizers. Okra is known for its responsiveness to nutrient management, while tomato, a heavy feeder, requires substantial nitrogen and phosphorus for optimal fruit yield and quality (Altaf et al., 2021). The nutritional quality, particularly vitamin C content, lycopene in tomatoes, and mucilage content in okra, is highly influenced by the nutrient source and soil health.

While the growth-promoting and yield-enhancing effects of biofertilizers are well-documented for various crops (Ruzzi & Aroca, 2015), a critical, yet less explored, aspect is the comprehensive assessment of their impact on the nutritional and toxicological profile of the edible produce. PGPR can enhance the uptake of essential micronutrients like iron, zinc, and selenium, and increase the synthesis of secondary metabolites (antioxidants, vitamins), thereby improving the post-harvest nutritional quality of vegetables (Rosa-Palacios et al., 2022). Assessing parameters such as vitamin C, protein content, mineral composition, and antioxidant activity in biofertilizer-treated crops is therefore imperative. Moreso, the toxicological impacts, constitutes a significant knowledge gap. The source material for producing liquid biofertilizers (organic wastes, carrier materials) and the complex microbial consortia themselves could potentially introduce or mobilize hazardous substances. These may include heavy metals (like Cd, Pb, As), pathogenic microorganisms, or mycotoxins, which could bioaccumulate in plant tissues, posing a risk to human health (Kumar & Prasanna, 2021). A thorough toxicological screening is necessary to ensure the safety of biofertilizer-derived food products. This involves analyzing the heavy metal content in the soil, the biofertilizer itself, and the harvested fruits to ensure they are within safe limits prescribed by food safety authorities like the FAO/WHO Codex Alimentarius.

Previous studies have often focused singularly on the agronomic efficacy of biofertilizers. However, a holistic approach that concurrently evaluates the dual parameters of nutritional enhancement and toxicological safety is lacking, particularly for liquid formulations on high-consumption vegetables like okra and tomato (Kaushik et al., 2022; Sharma et al., 2023). Establishing that a liquid biofertilizer not only boosts yield but also improves the nutritional value of the produce without introducing health hazards is essential for gaining farmer confidence and ensuring consumer safety.

Therefore, this study is designed to fill this gap by providing a comprehensive assessment of the effects of a liquid biofertilizer on the growth, yield, nutritional composition, and toxicological profile (specifically heavy metal content) of okra and tomato crops. The findings will contribute critical data to the sustainable agriculture narrative, ensuring that the pursuit of productivity is aligned with the principles of food safety and nutritional security.

Methods and Materials

Experimental Site and Design

The experiment was conducted at the University of Port Harcourt research farmland, Rivers State Nigeria. A randomized complete block design (RCBD) for the field trial, with three replications per treatment. The treatments include; microbial consortia, negative control and commercial fertilizer as positive control. Both tomato (*Solanum lycopersicum*) and okra (*Abelmoschus esculentus*) were used as test crops. The harvested fruits were taken to the laboratory for proximate and toxicological analysis. The isolates used for the formulations were identified by Uzah et al., (2024a & 2024b) and assigned with their accession numbers as follows; *Bacillus cereus* (OP970172), *Bacillus licheniformis* (OP970169), *Pseudomonas fluorescens* (OP970170), *Azotobacter chroococcum* (OP970171), *Aspergillus niger* (OP970215) and *Penicillium chrysogenum* (OP970216).

Proximate Analysis of Harvested Fruits

Moisture Content

The method described by A.O.A.C (1995) was used to ascertain the moisture content of the harvested fruit samples. Five grams of the sample was weighed (W1) in duplicate into cans and then dried at a temperature of about 76 °C for 6 hours. Each sample was re-weighed every 2 hrs interval until a desiccator to avoid moisture uptake and the final weight (W2) recorded. The moisture content was calculated using the formula below:

$$\text{Moisture (\%)} = \frac{\text{weight of fresh sample (W1)} - \text{weight of dried sample (W2)}}{\text{weight of sample used (W1)}} \times 100 \quad \dots \text{Equation 1}$$

Ash Content

The total ash was ascertained using the method of Sluiter *et al.*, (2008). Five grams of the dried sample was weighed and placed in a previously weighed clean crucible and heated gradually until the substance was carbonized. The crucible was positioned inside a muffle furnace with the temperature kept at 600 °C and heated for 6 hours to burn off the fibre and nutrients present in order to obtain a white or grey ash. The ash content in percentage was calculated by the following formula.

$$\text{Ash (\%)} = \frac{\text{Ash weight}}{\text{Sample Weight}} \times 100 \quad \dots \text{Equation 2}$$

Crude Protein

Crude protein was determined using Micro Kjeldhal method (Sluiter *et al.*, 2011). One gram of dry milled sample (W) and a catalyst mixture containing HgSO₄ and K₂SO₄ (1:9) was digested using concentrated H₂SO₄. The digested sample was diluted with water to a volume of 250 ml and 10 ml aliquot of the diluted sample was mixed with 10 ml of NaOH solution (40%) to an excess alkaline reaction. Then, the mixture was distilled with steam in the presence of 50 mg zinc using a micro Kjeldhal distillation apparatus. Ammonia liberated during the process, was collected into 2% boric acid solution containing few drops of methyl red and methylene blue. The distillate thus obtained was titrated against 0.01N H₂SO₄. From the actual volume of 0.01N H₂SO₄ used, amount of % nitrogen was calculated by equating 1 ml of 0.01 (NH₃)₂SO₄ to 0.00014g of nitrogen. The percentage of crude protein was obtained by multiplying the % by 6.25.

$$\text{Nitrogen (\%)} = \frac{X \times 0.00014 \times 250 \times 100}{10 \times W} \times 100 \quad \dots \text{Equation 3}$$

$$\text{CP (\%)} = \text{Nitrogen (\%)} \times 6.25$$

$$X = \text{ml of 0.01 NH}_2\text{SO}_4 \text{ used}$$

$$1 \text{ ml of 0.01 NH}_2\text{SO}_4 = 0.00014 \text{ g of NH}_3 \text{ nitrogen}$$

$$W = \text{Weight of the sample in gram}$$

$$250 = \text{dilution factor; } 6.25 = \text{N to protein conversion factor}$$

Total Carbohydrate Content

Total carbohydrate content was determined using Clegg Anthrone Method (Sluiter *et al.*, 2011). 1ml of the sample was transferred into 250 ml flask. Precisely 62% of perchloric acid was added and shaken for 20 min for complete homogenization. The flask was filled with 25 ml of distilled water and the solution formed was allowed to sediment and then decanted. One millilitre of the solution was transferred into a test tube containing 5 ml of Anthrone reagent and mixed thoroughly. The mixture was read at 630 nm wavelengths and distilled water used as blank. A standard phase of 0.1 mg/mol was prepared and treated with Anthrone and the absorbance read. The total carbohydrate was calculated using the formula below;

$$\% \text{ Carbohydrate (glucose)} = \frac{25 \times \text{absorbance of sample}}{\text{absorbance of standard glucose} \times \text{volume of sample}} \times 100 \quad \dots \text{Equation 4}$$

Crude Fibre (CF)

The method by Sluiter *et al.* (2008) was adopted to determine crude fibre. Fat free sample was digested at simmering temperature (about 80 °C) with 200 ml of 1.25% H₂SO₄ solution for 30 min. The beaker was covered with a cooling device and the temperature of the boiling medium kept constant by frequent addition of water. Boiling was stopped after adding 500 ml cold water. The contents were filtered immediately under vacuum and the residue washed several times with 100 ml of hot water and digested with 200 ml 1.25% NaOH solution for half an hour. The sample was then filtered, and the residue washed with acetone and placed in an ash crucible. It was then dried to a constant weight at 105 °C in an oven and the weight of the residue recorded as (Wr). The crucible was placed on oxidizing flame to carbonize the substance and transferred into a muffle furnace with temperature at 600 °C for 4 hrs until complete combustion takes place. The weight of the ash (Wa) was recorded and crude fibre calculated with the following formula.

$$\text{CF (\%)} = \frac{W_r - W_a}{W} \times 100 \quad \dots \text{Equation 5}$$

Determination of Heavy Metals Concentration on Harvested Fruits

Heavy metals (Zinc, Lead, Iron, and Copper) concentrations of the harvested fruits were determined using atomic absorption spectroscopy technique as adopted by Asare *et al.* (2019). 1g of the fruit samples each were digested using a mixture of nitric acid and sulfuric acid the ratio of 1 : 10ml to break down organic substances .

The digested samples were made up to 25ml with distilled water, an aliquot was then analyzed using an atomic absorption spectrometer, which detects the concentrations of heavy metals in the samples.

Results

The proximate composition analysis of okra and tomato fruits as shown in Table 1 – 2, revealed that biofertilizer treatments significantly enhanced their nutritional profiles compared to commercial fertilizer and untreated controls. Biofertilizer-treated okra and tomato recorded the highest ash (0.83% and 1.00%), carbohydrate (8.31% and 9.93%), fibre (15.91% and 8.55%), and protein contents (6.52% and 5.66%) relative to other treatments. The vitamin C concentrations were also highest in the biofertilizer-treated fruits, with okra showing 112.22 mg/kg and tomato 94.46 mg/kg, indicating a marked improvement in micronutrient quality. In contrast, fruits from commercial fertilizer and untreated plots exhibited lower nutrient values, demonstrating that biofertilizer application supports enhanced nutrient accumulation and metabolic activity in crops.

The toxicological evaluation as indicated in Figures 1 – 4, revealed that all harvested fruits had zinc, iron, and copper concentrations below the World Health Organization (WHO) permissible limits, indicating they were safe for consumption. Zinc concentrations were notably low, with biofertilizer-treated okra and tomato recording 4.35 mg/kg and 1.87 mg/kg, respectively, far below the WHO limit of 99.40 mg/kg. Similarly, iron levels were within safe limits, ranging between 69.00 mg/kg and 183.67 mg/kg across treatments—well below the FAO/WHO maximum of 425.5 mg/kg. Copper was undetectable (0.00 mg/kg) across all treatments, further confirming the safety of the fruits concerning essential metal accumulation.

However, lead concentrations in most treatments exceeded the FAO/WHO safe threshold of 0.3 mg/kg, except for biofertilizer-treated okra, which showed no detectable lead (0.00 mg/kg). Biofertilizer-treated tomato contained 3.10 mg/kg, while commercial fertilizer-treated and untreated samples recorded even higher values, suggesting potential contamination from external environmental or soil sources. Statistically, the treatments were significant at $P < 0.000$ for concentrations of the heavy metals by the both plants. These findings imply that although biofertilizer use promotes superior nutritional quality and generally safe heavy metal levels, careful monitoring of soil and water sources is necessary to mitigate lead contamination risks and ensure the long-term safety of biofertilizer-grown crops.

Table 1: Proximate Composition of Okra Fruits Cultivated on Various Biofertilizer amended Soils

Samples	% Ash	% Carbohydrate	% Fibre	% Lipid	% Moisture	% Protein	mg/kg Vitamin C
Biofertilizer treated Okra	0.83±0.04	8.31±0.04	15.91±1.35	0.50±0.01	98.60±0.03	6.52±0.02	112.22±1.57
Commercial fertilizer treated Okra	0.64±0.05	6.26±0.03	13.92±2.55	0.40±0.01	79.45±4.54	4.34±0.06	58.89±4.71
Untreated Okra	2.65±0.21	4.65±0.25	0.56±0.35	0.39±0.00	87.92±0.04	3.94±0.00	46.67±3.15

Table 2: Proximate Composition of Tomato Fruits Cultivated on Various Biofertilizer amended Soils

Samples	% Ash	% Carbohydrate	% Fibre	% Lipid	% Moisture	% Protein	mg/kg Vitamin C
Biofertilizer treated Tomato	1.00±0.00	9.93±0.00	8.55±0.06	0.43±0.01	87.45±0.02	5.66±0.04	94.46±0.02
Commercial fertilizer treated Tomato	0.34±0.06	5.56±0.03	3.52±0.01	0.33±0.00	87.61±0.18	2.83±0.02	34.45±1.58
Untreated Tomato	0.72±0.03	5.38±0.06	1.45±0.01	0.33±0.00	87.76±0.04	4.37±0.02	64.44±3.14

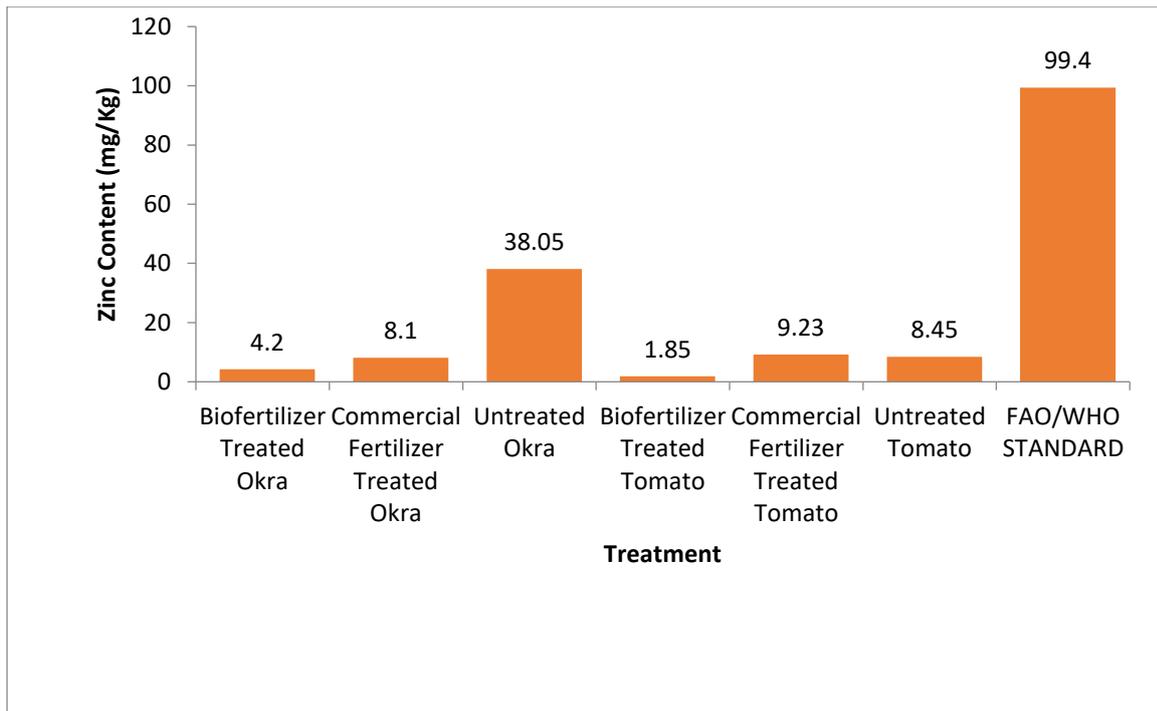


Fig. 1: Zinc Content of Fruits of Okra and Tomato Cultivated on Various Biofertilizer amended Soils

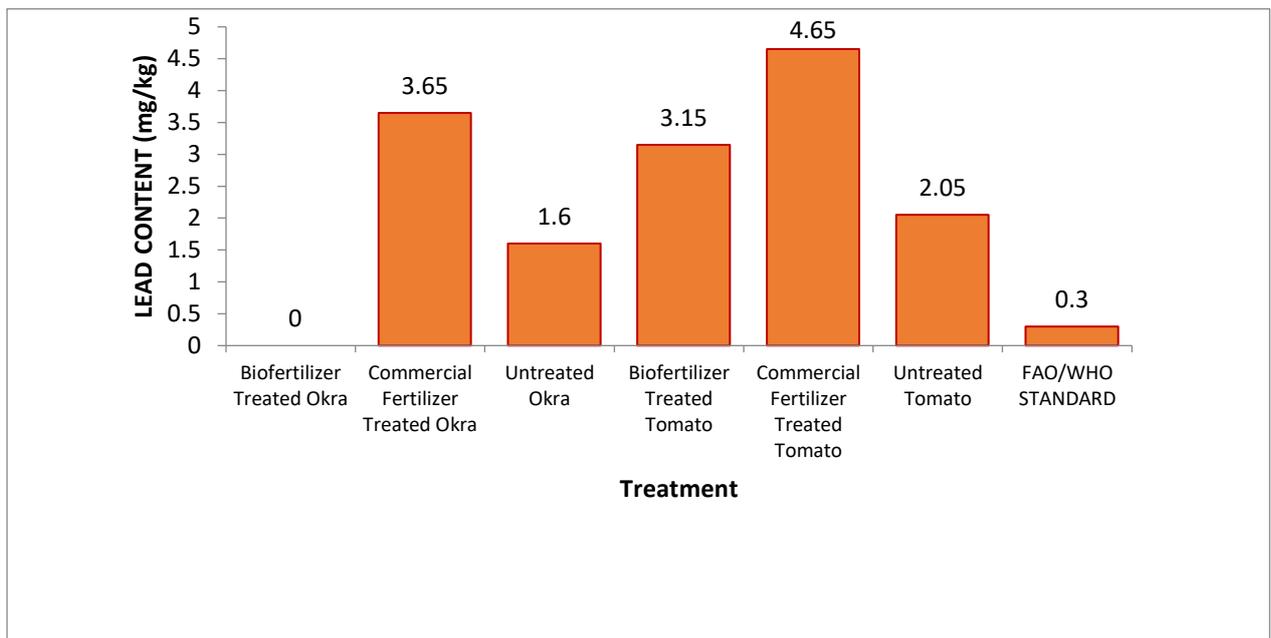


Fig. 2: Lead Content of Fruits of Okra and Tomato Cultivated on Various Biofertilizer amended Soils

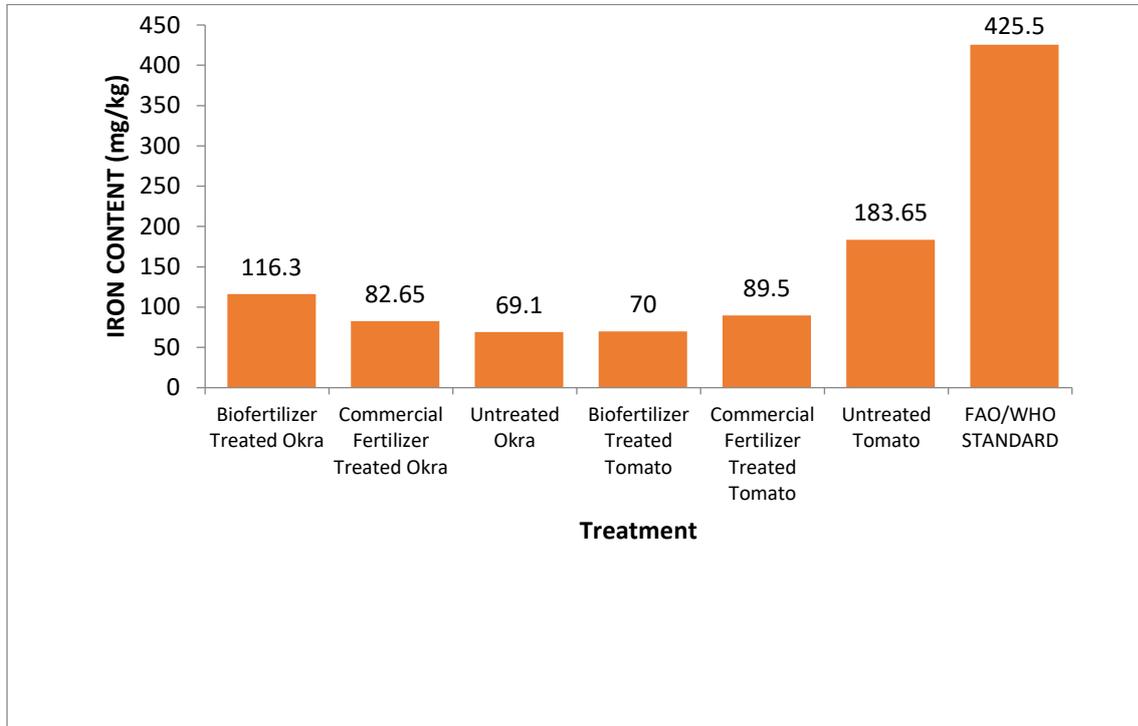


Fig. 3: Iron Content of Fruits of Okra and Tomato Cultivated on Various Biofertilizer amended Soils

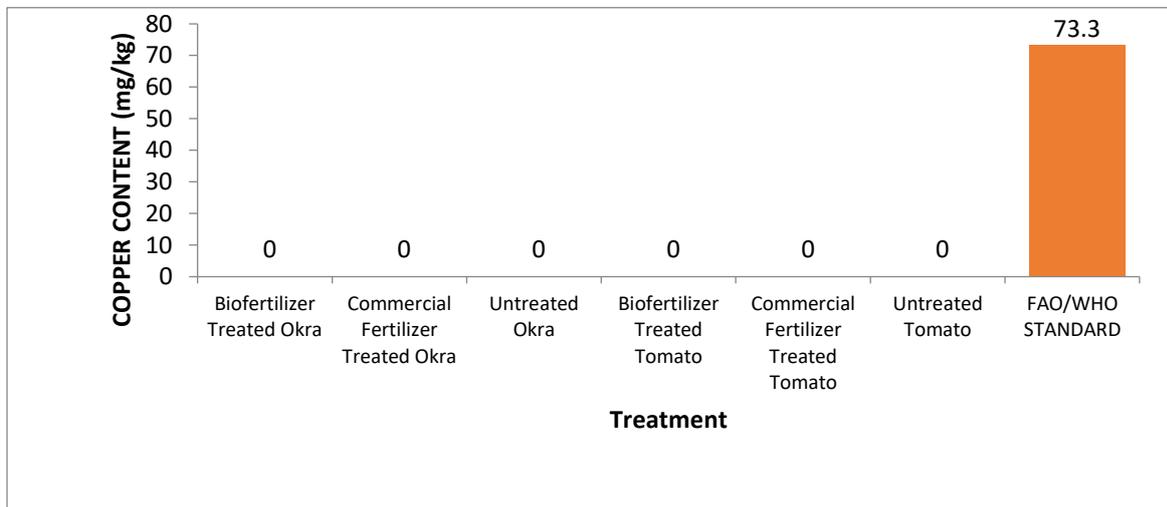


Fig. 4: Copper Content of Fruits of Okra and Tomato Cultivated on Various Biofertilizer amended Soils

Discussion

It is crucial to know how biofertilizer affects the nutritional value and heavy metal mitigation of crops. The ash level of the harvested Okra and tomato fruits varies based on the treatments used in this study which the result depicts the proximate composition of the fruits. As previously stated, the Okra and tomato plants treated with biofertilizer showed significantly higher ash content values (%) than the plants treated with conventional fertiliser. Ash levels were higher in the untreated Okra fruit than in the other plants treated with the same chemicals. Thus, beneficial microbes in the biofertilizer may be responsible for the difference in ash content between plants treated with and those not treated with the commercial biofertilizer. These microbes increase

nutrient absorption. After applying biofertilizer to Okra plants, Oluwagbenga and Solomon (2016) found that the plants' ash content rose. This observation is also in line with the reports of Kumar and Prasanna (2021), who stated that the ash content change reflects composting microorganisms' decomposition and mineralization of compost substrate. Also, compared to both commercially fertilised and untreated tomato and okra plants, plants treated with biofertilizer had increased amounts of vitamin C, carbohydrates, moisture, proteins, fibres, and lipids which further corroborate recent findings. For instance, a 2023 study by Rosa-Palacios et al. demonstrated that microbial inoculants enhance the synthesis of primary and secondary metabolites in vegetables, directly improving their nutritional composition and antioxidant capacity.

Biofertilizer improves crop yield and quality, according to many recent studies (Xu and Leskovar, 2015; Fan et al., 2013; Kim et al., 2018). Boosting the nutritional value and bioactive chemical concentration allows this to happen. Liquid biofertilizer increased the proximate composition of Okra plants, according to research by Oluwagbenga and Solomon (2016). This included bigger amounts of water, ash, crude fibre, protein, and fat. The mineral content and growth index were both enhanced in the treated plants. According to Maitlo et al. (2006), foliar fertilisation improves crop yields, nitrogen absorption, and plant growth traits. Crops also absorb nutrients more efficiently as a result (Fageria et al., 2009). The diverse range of minerals found in foliar fertilisers is likely responsible for their potential benefits. Plants that produce okra benefit from these nutrients, which boost the plant's growth traits and ultimately raise the yield of okra berries. Also, it allows nutrients that aren't getting to the plant system quickly via the leaves (Fageria et al., 2009). In their study, Alkaff and Hassan (2003) found that foliar fertilisation improved the growth and yield of okra. According to Mishra et al. (2003), micronutrients sprayed upon now mandarin leaves caused the fruit to grow earlier and mature more quickly.

In contrast to artificial fertilisers, microorganisms have a positive impact on heavy metal reduction, according to this study. It was observed in this study that the treatment plants' zinc, iron, and copper levels were lower than the FAO/WHO norm. However, the lead levels in all of the treatment centres were higher than the FAO/WHO guidelines. The reduction in heavy metals is as a result of the metabolic activity and mineralization of the microorganisms which agrees to the findings of Haroun et al. (2023); Ayangbenro and Babalola, (2017), who observed that biofertilizers facilitate both growth and metal uptake by plants and therefore act as promising biosorbents for metal remediation through a process called biosorption.

A high concentration of lead could be attributed to low mineralization ability of the microorganisms used for the biofertilizer production or high rate of pollutants in the soil through chemical fertilizer or other chemical substances. This observation is in line with the observation of Asfa et al. (2022), who stated that numerous bacterial communities with metal resistance abilities have been reported. Regardless of that report, microorganisms have been considered as the potential organisms for detoxification of pollutants and promoting the crop production under stressed soils (Khan et al., 2018) Also, the deposited metals in soils adversely affect the composition and functions of soil microbiome, soil health, and crop productivity and pose risks to human health through food chain (Kwunonwo et al., 2020; Haider et al., 2021). Because it is readily cultivatable, does not produce secondary pollution, and is more efficient at low metal concentrations, the soil microbiome mainly contributes considerably to metal bioremediation (Tarekegn et al., 2020; Bojórquez & Voltolina, 2016). Biofertilizers have an effect on the stressed environment directly, but they also impact the remediation process indirectly through changing the chelator molecules, the soil's hydrology, the nutrient pool, and the solubility and transport of metals (Ma et al., 2015). For example, siderophores, a low molecular weight (400–1500Da) iron-complexing compound, secreted by many biofertilizers like *Pseudomonas* (Kügler et al., 2020), *Azotobacter* (Ferreira et al., 2019), *Bacillus* (Esertas et al., 2020), rhizobia (Zhao et al., 2020), and *Azospirillum* (Vijayalakshmi et al., 2019) do play a significant role in mobilization and accumulation of metals.

Furthermore, without observing the post harvest shelf of the tested plants in this study, other researchers have reported the potentials of biofertilizers increasing shelf life of crops. This suggests that the fruits treated with this method had a longer storage life than fruits harvested from plants that were either untreated or treated with commercial fertiliser. Due to their susceptibility to spoiling and deterioration and spoilage, tomato fruits and okra pods have a limited post-harvest lifetime unless properly preserved (Singh et al., 1978). There are both outward and internal changes to the pods that lower their quality. Because of changes including dryness, colour degradation, and decay, the pods become mushy and unsuitable for immediate consumption. Costs go down or disappear altogether as a result (Huang & Jiang, 2012). After harvest, okra pods may get bruised and mechanically damaged, which can darken the pods on the ridges and calyx disc. Both value and price fall as a result of this. Additionally, browning, pigmentation, and the facilitation of microbial proliferation are among of the unfavourable outcomes of the several operations involved in processing okra fruit, which also includes trimming, peeling, coring, and cutting (Abdel-Haleem et al., 2022).

Tomatoes and okra have different shelf lives depending on factors like temperature and relative humidity. Fruits have a shorter shelf life and a faster rate of deterioration when temperatures are high. According to Abdel-Haleem et al. (2022), reducing the storage temperature of okra and tomato pods slows down the rate of respiration, evaporation, and ethylene production. Blackening, shrivelling, and decomposition characterise okra pods when left unattended at room temperature, leading to significant post-harvest loss in as little as two days. Okra pods reaped several benefits from a climate that was both cool (10-13 °C) and humid (93% to 100%). Because of this, they lost less weight, had a longer shelf life, and didn't go bad. Adetuyi et al. found this in their 2008 investigation. Research by Cheng et al. (2018) found that pod rigidity is strongly related to storage temperature.

Conclusion

In conclusion, the application of liquid biofertilizer demonstrably improved the nutritional profile of the crops, especially in boosting vitamin C, protein, and fibre content. While most heavy metals were within safe limits, the prevalent lead contamination underscores a potential risk in the agricultural system. The complete absence of lead in biofertilizer-treated okra suggests that this treatment may not only enhance nutrition but also mitigate the uptake of specific toxic heavy metals, presenting a compelling case for its use as a safer agricultural input.

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