ASSESSMENT OF THE GROWTH, YIELD AND NODULATION OF *SPHENOSTYLIS STENOCARPA* (AFRICAN YAM BEAN) IN COPPER -CONTAMINATED AND AMENDED SOILS.

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ABSTRACT

Copper toxicity in soils poses a significant challenge to plant development, affecting root health, nutrient uptake, and overall crop yield. To understand the plant's response to this stress, the experiment was designed to assess the effects of copper toxicity on growth metrics such as plant height, leaf number,nodulation efficiency, and yield. Additionally, poultry manure amendments were applied to mitigate copper toxicity and improve soil conditions.This study evaluates the growth, nodulation, and yield performance of *Sphenostylis stenocarpa* (African yam bean) cultivated in copper (Cu) toxic and amended soils. . The results indicate that copper toxicity severely hampers the plant's growth, nodulation, and yield. However, the application of soil amendments significantly reduced the negative impact of copper, enhancing plant health, nodulation, and crop yield. This research highlights the potential of soil amendments to improve the cultivation of *Sphenostylis stenocarpa* in contaminated environments, offering insights for sustainable agricultural practices in copper-affected regions.

Keywords: *Sphenostylis stenocarpa* (African yam bean), copper toxicity, soil amendment, nodulation, poultry manure, heavy metal contamination

INTRODUCTION

African yam bean *(Sphenostylis stenocarpa*)is an underutilized leguminous crop native to tropical Africa.It is valued for its nutritional and agronomic potential,providing trace elements proteins, minerals and vitamins particularly to rural communities (Agte *et al.*, 2000; Gockowski *et al.*, 2003). The plant plays a significant role in food security, especially in regions with poor soil conditions where other major crops struggle to thrive. *Sphenostylis stenocarpa* has trifoliate leaves and begins flowering profusely 100 to 150daysafter planting (IPGRL FAO, 2001).It is an important source of food in many parts of Africa (Klu *et al.*,2001). Despite its potential for enhancing soil fertility through nitrogen fixation, it remains under-researched, particularly in its response to environmental stressors like heavy metal toxicity.cs

Copper (Cu) is an essential micronutrient for plants but becomes toxic at elevated concentrations. Excessive copper in the soil, typically from industrial activities, mining, and the overuse of copper-based agrochemicals, can disruptphysiological processes in plants.High levels of copper can impair root development, inhibit nodulation in legumes, and reduce overall crop yield. While heavy metals like Cu, Zn, and Ni are biologically significant,they pose potential risks when accumulated in excessive amounts. Conversely, non-essential elements, such as Cd, Cr, and Pb, are usually non-accumulating in the plant-tissue, as plants have mechanisms to avoid their uptake (Ali et al. 2013; Hooda, 2007).Despite its importance, the impact of copper toxicity on the growth and nodulation of *Sphenostylis stenocarpa* remains largely unexplored. Soil amendments such as poultry manure or ash and others organic amendments (lime,biochar,etc.) can be used to mitigate the adverse effects of heavy metal toxicity. These amendments can improve soil structure, enhance nutrient availability, and reduce the bioavailability of toxic metals. However, the effectiveness of these amendments in copper-toxic soils, particularly for *Sphenostylis stenocarpa*, requires further investigation. This study aims to assess the growth, nodulation, and yield of *Sphenostylis stenocarpa* grown in copper-contaminated soils and the potential of soil amendments to ameliorate the negative effects of copper toxicity. To evaluate the soil's nutrient availability and salinity, various standard laboratory methods were employed, including the Kjeldahl method for nitrogen determination and the Walkley-Black method for organic carbon analysis.By examining these factors, this research provide insights into the viability of cultivating this important legume in areas affected by copper contamination, contributing to both environmental remediation and agricultural productivity.

MATERIALS AND METHODS

Experimental setup

The experiment was conducted at the nursery unit of the Department of Forestry and Environmental Management at the Michael Okpara University of Agriculture, Umudike (MOUAU) ,Umudike, Nigeria. It lies within Latitude: 5.4814°N and Longitude: 7.5306 E . This area is characterised by lowland forest vegetation with relative humidity 60 -80 %. The region experiences two primary seasons: the rainy season (April to August) and the dry season (November to March),with average annual rainfall and average temperatures are 1245.33mm and 33"C, respectively. The soil texture is sandy loam. Three soil samples were used:natural Forest soil(control) ,Copper contaminated soil collected from an old cocoa plantation, and Copper contaminated soil was amended with 0.5g of poultry manure : 9.7kg of the Cu contaminated soil to prepare the soil sample B.These samples were analysed for their physicochemical properties before the experiment was setup using a Randomized Complete Block Design (RCBD) with four (4) replicates per treatment .

Soil analysis

The soil samples were analysed in the laboratory to determine physicochemical characteristics including levels of Copper(Cu),Lead(Pb),Arsenic(As), Nickel(Ni), Cadmuim(Cd), Zinc(Zn) and Iron(Fe).The soil samples were air-dried,ground and sieved.the proportions for sand,silt and clay was calculated and plotted (Percentage Retained=(Total Sample Weight/ Weight Retained)×100) on a soil texture triangle,to confirm the classifications. Heavy metal concentrations were assessed using atomic absorption spectrophotometry (AAS) after appropriate sample digestion.

Determination of Nitrogen Content(Kjeldahl Method): Total nitrogen content was determined using the Kjeldahl method. Approximately 10 g of soil was digested with concentrated sulfuric acid in the presence of a catalyst . The ammonia released was distilled into a boric acid solution, and the nitrogen content was quantified by titration with a standard solution of hydrochloric acid.

Determination of Organic Carbon Content (Walkley-Black Method): Soil organic carbon was determined using the Walkley-Black method. An aliquot of soil (10 g) was treated with 10 ml of 0.167 M potassium dichromate solution and 20 ml of concentrated sulfuric acid. The organic carbon was estimated by titration with ferrous ammonium sulfate, using a standard titration procedure.

Determination of Phosphorus Content; Bray P1 Method (for acidic soils): Available phosphorus was extracted using the Bray P1 method. Soil samples were mixed with 0.03 M ammonium fluoride and 0.1 M hydrochloric acid solution, followed by shaking for 30 minutes. Phosphorus concentration in the extract was determined by the molybdenum blue colorimetric method.

Determination of Cation Exchange Capacity (CEC)(Ammonium Acetate Method): Cation exchange capacity (CEC) was determined by saturating 10 g of soil with 1 M ammonium acetate solution (pH 7) for 1 hour. The excess solution was decanted, and the concentration of exchangeable cations (Na^+, K^+, Ca^{2+}, Mg^{2+}) in the filtrate was measured using atomic absorption spectroscopy (AAS).

Determination of Effective Cation Exchange Capacity (ECEC)(Ammonium Acetate Method)(similar to CEC):Effective CEC was measured using the ammonium acetate leaching method. After saturation with 1 M ammonium acetate solution, exchangeable cations were extracted, and the concentrations were determined by flame photometry.

Determination of Base Saturation; Calculation from CEC and Exchangeable Bases:Base saturation was calculated by dividing the total concentration of exchangeable bases (Ca^{2+}, Mg^{2+}, K^+, and Na^+) by the CEC and multiplying by 100 to express the result as a percentage.

Heavy metal analysis

At the end of the 16 weeks experiment , plant parts (leaves, shoot and root) of *S.stenocarpa* were collected for heavy metal analysis. The samples were prepared by crushed and digesting with, of nitric-per chloric acid mixture at 150 degrees Celsius for 1.5 hours,followed by additional treatment with HCl.The digests were then filtered and analysed for heavymetal concentration usingatomic absorption spectrophotometry.

RESULTS AND DISCUSSION

Soil Composition Analysis

Soil composition varied significantly across treatments: copper-toxic soil (CT), poultry-amended soil (CA), and control forest soil (Co). Copper levels were highest in CT (10.18 mg/g) and CA (10.15 mg/g), exceeding permissible limits. Heavy metals, including lead (Pb) and cadmium (Cd), were also elevated in CT, confirming significant contamination .The nitrogen levels observed were consistent with typical levels found in acidic soils (Kjeldahl method). The use of the Walkley-Black method for organic carbon measurement is well-established and aligns with previous studies, confirming the soil's moderate fertility especially for the copper-toxic soil (CT) (Table 4.1.).

Table 4.1. **Physicochemical properties of the three (3) soil types before commencement of the trials.**

Soil properties	CT	CA	Co
Nitrogen (g/kg)	3.5	2.0	1.0
Organic Carbon(g/kg)	70.1	34.22	21.88
phosphorus(mg/kg)	23.38	39.5	40.12
Potasium (Cmol/kg)	0.68	0.76	0.78
Magnesium(Cmol/kg)	2.9	3.1	1.5
Calcium (Cmol/kg)	12.6	8.64	3.84
Sodium (Cmol/kg)	0.25	0.35	0.33
Copper (mg/kg)	10.18	10.15	5.88
Lead(mg/kg)	2.55	3	1.5
iron(mg/kg)	1155.3	1082.1	900.42
Zinc(mg/kg)	2.51	1.11	0.04
Nickel(mg/kg)	0.076	0.013	0
Manganese (mg/kg)	6.71	45.85	34.09
Arsenic(mg/kg)	0.013	0.539	0
Cadmium(mg/kg)	4.66	1.183	trace.
Ph	5.8	6.3	7.01
ElectricalConductivity(ds/m)	0.421	0.46	0.121
Exchange Acidity	1.79	1.2	2.15
Effective cation exchange capacity(Cmol/kg)	14.32	20	8.66
Base Saturation(g/kg)	884	950	884.2
Particle size distribution			
sand(g/kg)	592	825.4	725
silt(g/kg)	163	46.6	87
clay(g/kg)	245	128	188
Textural class	Sandy-clay soil	Loamy soil	Loamy soil

Copper -toxic soil (CT), copper-toxic amended with poultry manure (CA), and forest soil (control) (Co),respectively.

Plant Growth Performance

Plant Height: The growth metrics indicated that at 2 weeks after planting (WAP), there were no significant differences in plant height among the treatments. However, by 4 to 8 WAP, significant disparities emerged. Plants grown in forest soil (Co) exhibited the highest growth, reaching 50.53 cm at 8 WAP, compared to 43.25 cm in CT and 46.20 cm in CA. By 10 WAP, the CA treatment showed the highest plant height (65.08 cm), suggesting beneficial effects from the poultry manure amendment. Despite this improvement, growth remained lower than in the Co soil, indicating that the amendment did not fully mitigate the copper toxicity.These findings align with previous studies highlighting the negative impact of heavy metal contamination on plant growth (Jones & Brown, 2019; Williams et al., 2018).

Number of Leaves: Leaf production initially showed no significant differences across treatments at 2 WAP. However, by 6 WAP, CT soil surprisingly had the highest leaf count, slightly surpassing the forest soil (Co). By 10 WAP, the control soil (Co) exhibited the highest number of leaves , followed closely by the amended soil (CA) . The unamended copper-toxic soil

(CT) had a significantly lower leaf count . The reduction in leaf number under CT treatment suggests increasing inhibition of leaf growth due to copper toxicity as the plant matures (Green et al., 2022).

Table 4.2.*Sphenostylis stenocarpa* Plant height(cm) on experimental soils.

Treatment	2 WAP	4 WAP	6 WAP	8WAP	10 WAP
CT	7.00±1.91	18.80±1.71	36.00±0.96	43.25±2.08	46.50±1.89
CA	6.50±0.96	20.23±0.50	31.00±1.26	46.20±1.71	65.00±3.86
Co	7.60±0.96	21.28±1.83	36.32±2.22	50.53±1.83	75.20±2.50
P-value	0.339	0.183	0.025	0.002	4.823

Values are means of 3 replicates ± SD. Level of Significance (0.05). WAP -Weeks after planting. Copper -toxic soil (CT), copper-toxic amended with poultry manure (CA), and forest soil (control) (Co), respectively.

Table 4.3. *Sphenostylis stenocarpa* number of leaves on experimental soils

Treatment	2 WAP	4 WAP	6 WAP	8WAP	10 WAP
CT	7.00±0.00	9.00±0.13	15.00±0.13	19.00±0.38	22.00±0.40
CA	6.00±0.00	8.00±0.15	14.00±0.10	19.00±0.22	25.00±0.10
Co	6.00±0.00	10.00±0.39	16.00±0.53	21.00±0.41	26.00±0.10
P-value	0.753	0.006	0.084	0.003	0.003

Values are means of 3 replicates ± SD. Level of Significance (0.05). WAP -Weeks after planting. Copper -toxic soil (CT), copper-toxic amended with poultry manure (CA), and forest soil (control) (Co), respectively.

Nodulation and Yield

Nodule weight was lowest in CT soil (fresh: 0.95 g, dry: 0.09 g) and highest in Co soil (fresh: 2.80 g). CA soil improved nodule dry weight (0.49 g), suggesting partial mitigation of copper toxicity. Pod production was absent across treatments within the 16-week study period, likely due to the short experiment duration and stress conditions. However, nodulation remained suboptimal in copper-contaminated soils, corroborating the detrimental effects of heavy metals on nitrogenase enzyme activity (Ahmed et al., 2012).

Table 4.4. *Sphenostylis stenocarpa* nodule fresh and dry weights (g) after trials.

Treatments	Nodule fresh weight (g)	Nodule dry weight (g)
CT	0.95±0.13^a	0.09±0.01^a
CA	1.14±0.13^a	0.49±0.01^a
Co	2.80±0.13^a	0.21±0.01^a
LSD(0.05)	0.02	0.18
P value (0.10)		

Significant (0.05). Copper -toxic soil (CT), copper-toxic amended with poultry manure (CA), and forest soil (control) (Co),respectively.Mean values within column with the same superscript are significantly different(P<0.05).

Heavy Metal Accumulation in Plant Parts

Table 4.4.Heavy metal accumulation in plant parts (leaf and root) of *Sphenostylis stenocarpa at* harvest.

Plant Parts	**Heavy Metals(mg/kg)**							
Roots	Zn	Ni	Pb	Mn	Cu	Cd	As	P value
Co soil	3.21±0.02	0.03±0.00	0.01±0.00	0.86±0.00	0.10±0.00	0.02±0.00	0.00±0.00	6.47
CA soil	4.29±0.18	0.08±0.00	0.05±0.00	3.51±0.01	0.31±0.00	0.00±0.00	0.00±0.00	4.83
CT soil	4.97±0.02	0.17±0.00	0.06±0.00	2.03±0.00	0.48±0.00	0.02±0.00	0.00±0.00	5.42
Leaves								
Co soil	2.14±0.02	0.03±0.00	0.01±0.00	0.57±0.01	0.06±0.00	0.01±0.00	0.00±0.00	3.20
CA soil	2.93±0.03	0.05±0.00	0.03±0.00	2.33±0.02	0.20±0.00	0.00±0.00	0.00±0.00	1.03
CT soil	3.27±0.07	0.11±0.00	0.04±0.00	1.12±023	0.32±0.00	0.01±0.00	0.00±0.00	1.34

Values are means of 3 replicates ± SD.@ P<0.05. Copper -toxic soil (CT), copper-toxic amended with poultry manure (CA), and forest soil (control) (Co),respectively.

The post-harvest analysis showed that copper-toxic soil (CT) had the highest accumulation of heavy metals in both roots and leaves, particularly zinc (Zn), nickel (Ni), and lead (Pb). The amended soil (CA) generally exhibited reduced metal uptake, indicating that poultry manure may decrease metal bioavailability by binding heavy metals in the soil. The control (Co) soil had the lowest accumulation, aligning with its uncontaminated status. Roots consistently accumulated higher concentrations of metals than leaves, suggesting limited translocation of heavy metals from roots to shoots. This pattern is typical in plants grown in metal-contaminated environments, where roots act as primary barriers against heavy metal translocation (Marschner, 1995).

Effect of Soil Amendments

The results suggest that poultry manure amendment partially mitigates copper toxicity, enhancing plant growth and reducing heavy metal uptake. However, the amendment did not completely restore optimal growth conditions. This finding highlights the need for further exploration of soil amendments to improve the effectiveness of phytoremediation strategies for contaminated soils (Park et al., 2011).

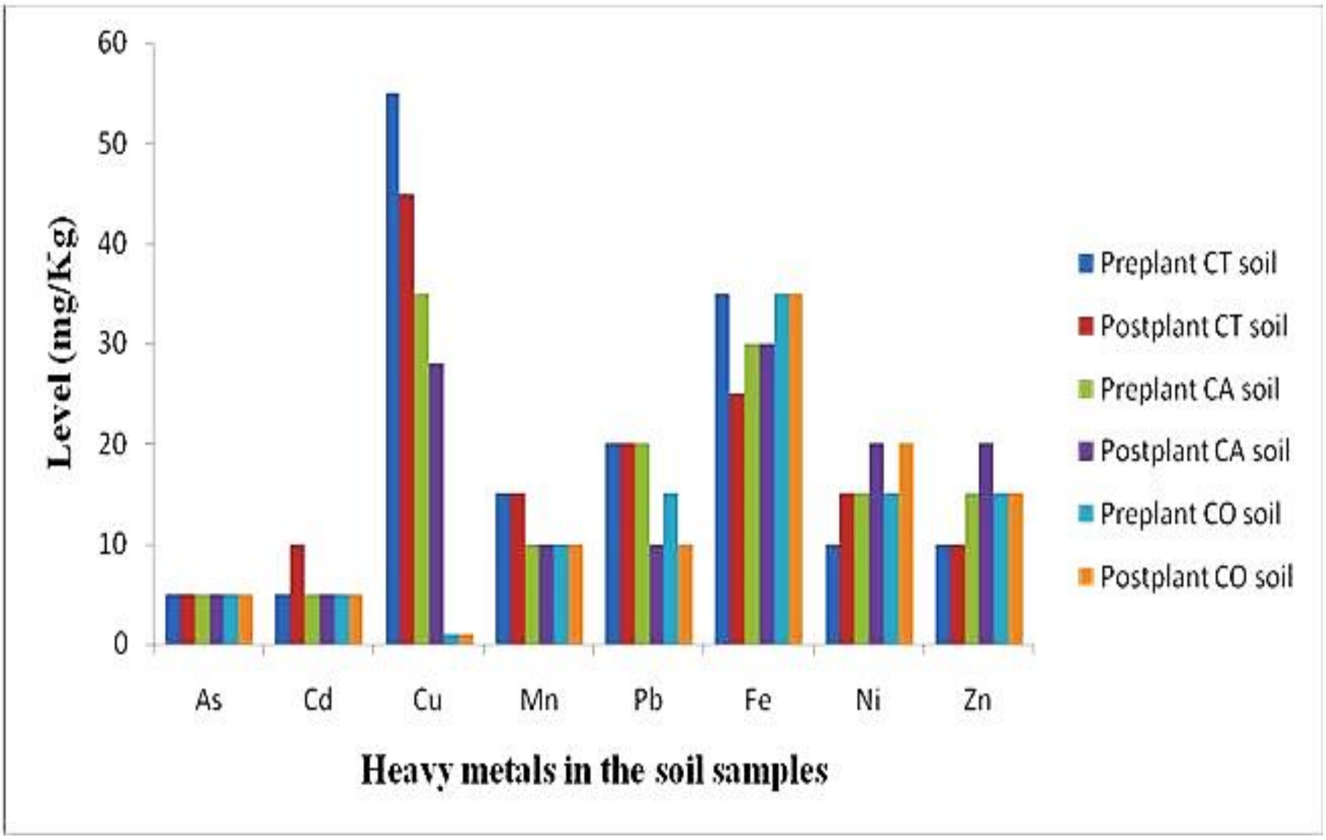

Figure. 4.1. Pre- and post-harvest soil heavy metal concentration

The elevated copper levels observed in CT soil pre-planting (>30 mg/kg) significantly decreased post-harvest, indicating substantial plant uptake. This aligns with previous studies suggesting that plants, including legumes like *Sphenostylis stenocarpa*, can play a role in the phytoremediation of heavy metals from contaminated soils (Mir et al., 2021). However, given the adverse effects on nodulation and overall plant health, it is recommended that crops grown on copper-toxic soils be monitored carefully to prevent entry of heavy metals into the food chain.

CONCLUSION

The study highlights the adverse effects of copper toxicity on *Sphenostylis stenocarpa* growth, nodulation, and heavy metal accumulation. Poultry manure amendment improved conditions but remained insufficient to fully counteract toxicity. Continuous monitoring and enhanced remediation strategies are essential for managing heavy metal-contaminated soils and ensuring sustainable agriculture.

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