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Growth and Nodulation of *Centrosema pubescens* Benth (Butterfly Pea) in Soils of High Copper Levels in the Humid Zone of Nigeria

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Abstract Some farming operations contribute to heavy metal toxicity of the soil which in turn affects above ground vegetation. Soils collected from cocoa and *Gmelina arborea* plantations were separately used for raising *Centrosema pubescens* in three pots (A, B and C) in Nigeria. The cocoa plantation soil was contaminated as result of use of Cu-based fungicide. Pot A had 5kg of soil with copper-contaminated soil while Pot B was same as Pot A above but amended with poultry manure using 2:1; representing media with moderate toxicity. Similarly, Pot C or the control contained soil obtained from a nearby *Gmelina* plantation 200m from the cocoa plantation. *C. pubescens* seedlings were raised in nursery for 4 weeks and planted out into the pots. Growth parameters (leaf number and total dry weight (g)) and chemical composition of biomass (N, P, K, Fe, Cu, Pb) were determined and compared using ANOVA at $_{@<0.05}$. Root nodulation was highest in *Centrosema* stands grown with control soil and was 260% higher than that of Cu contaminated soil (A). Both mean leaf number per plant and total dry weight (g) were highest in stands grown with Cu contaminated soil amended with poultry manure indicating possible suppression of Cu-toxicity by the manure. Cu- soil contamination affected the level of macronutrients (N, P, K) uptake especially in Pots A and B. N.P.K in Centrosema samples grown with control soils C were significantly higher than those of "A" by 48.3%, 61.4% and 66.5% respectively. Although toxicants have potential of being transmitted to man/livestock, *C. pubescens* showed relatively high absorption capacity for toxic elements which can enhance its choice for possible use in land clean-up and phytoremediation programme.

Keywords: *copper toxicity, legume, nodulation, biomass formation, mineral uptake, phytoremediation*

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1. Introduction

Heavy metals play critical roles in various metabolic and physiological processes in plants. Cu for example is known to be critical in respiration, photosynthesis, N and cell wall metabolism, carbohydrate distribution and seed production [1]. However, when the concentrations of even the biologically significant metals become higher, they cause toxicity. Heavy metal toxicity is becoming a common phenomenon in both developing and developed societies given the high human dependence on inorganics in food production and manufacturing sectors including the strong reliance on pesticide use in modern agriculture. This phenomenal dependence on pesticide use can be challenging especially in rain-fed agriculture and flood-prone regions of the world. Again, the evidence of widespread dispersal trend (mobility) of such toxicants into the agro-ecological system and food chain makes the situation even more disturbing [2,3].

On a global scale, available land is becoming insufficient to support growing demand for land uses (agriculture, housing, tourism, infrastructure etc.) caused by the rising human population [4]. Agricultural systems are introducing innovations such as the use of cover crops and N-fixing legumes (eg Centrosema) to support soil fertility and intensifying production systems. *Centrosema pubescens* Benth. is a leguminous dicot plant belonging to the family *Fabaceae*. It has a span (life cycle) of between 4-9 months but there is indication that it can complete its cycle within 8 months in humid regions of Nigeria [5]. Centrosema grows wild in fallow plots, forest gaps and under plantations such as cocoa. *Centrosema* is used for a variety of purposes and there are several researches involving the species in the field of agriculture, pharmaceuticals and manufacturing which signifies its potential value. Economically, *C. pubescens* is of immense use in the livestock sub-sector as forage whether managed under free-range or semi-intensive systems. The plant has antimicrobial properties against a wide range of microorganisms like *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and *Candida albicans*.

The symbiotic interaction between Rhizobia and legumes such as *Centrosema* is one of the dependable sources that provide nutrients to plants, increases soil fertility, facilitates plant growth and restores degraded/damaged ecosystem.

Environmental pollutants like heavy metals at lower concentrations are required for various metabolic activities of microbes including Rhizobia and legume crops. On the other hand, the excessive metal concentrations cause undeniable damage to Rhizobia, legumes and their symbiosis. As heavy metals build-up in the soil to abnormal levels, they cause dramatic changes in microbial composition which results in depletion nutrient pool and loss of soil fertility; a situation that adversely affects crop performance directly or indirectly through loss of soil fertility or by effect of the toxicants [6].

The strong linkage between toxicity of agricultural lands and human/animal health has been reported especially in the direct effects of toxicants in the food chain process [7]. *Centrosema pubescens* is one of the forage crops which have low empirically established reports of effect and/or growth response to toxicity in rural agro-based communities in developing countries [8]. Because there are a number of entry points through which Centrosema makes it into the food chain (either as forage or medicinal product) coupled with the high potential risk of being contaminated while growing in the wild under plantation crops such cocoa make Centrosema a plant to monitor for toxicity.

More so, there is limited information that is accessible to livestock managers and ecologists on the growth and performance of plants of *Fabaceae* family under metal-toxic lands [9 - 10]. This work provides information on the effect of Cu contamination on the nodulation and growth characteristics of *C. pubescens.*

2. Materials and Method

2.1. Study Area

The study was conducted at the Nursery Unit of the Department of Forestry and Environmental Management, Michael Okpara University of Agriculture, Umudike in South East Nigeria. It lies within latitude 5º 29´N and longitude 7º 33´E. Reference [11] reported that the region is typical of rainforest with relative humidity of about 60-80%. *Centrosema* grows across a variety of lands ranging from croplands, fringes, thicket, forested areas including mixed and monoculture plantation area [12]. The average annual rainfall and temperatures are 1245.33mm and 32°C respectively.

The soil in the area is largely acrisols with average pH of 4.8 [13]. Application of inorganic chemicals in agricultural production systems is common across the area particularly among plantation and commercial farmers [14]. Reference [15] reported the regular usage of Cu-based fungicides such as Bordeaux mixture by private cocoa plantation owners in the study area to control fungal infections in cocoa. Bordeaux mixture commonly used in the area is formulated locally by adding a mixture of slack lime and 125g of copper oxide to about 10 liters of water.

2.2. Method

Given the prevalence of the use of Bordeaux fungicide in disease control in agricultural lands, copper toxicity was of interest in this study given its high proportion and concentration in Bordeaux formulation. Seeds of *Centrosema* were sourced from the seed technology section in the Agronomy Department of the University of Agriculture Umudike. Seedlings of *Centrosema* were raised in nursery and transplanted into 3 sets of experimental polypots (A, B and C) each pot containing 5 kg weight of topsoil. The “A” set of pots contained soils collected from a cocoa plantation in the study area with history of receiving Bordeaux fungicide for controlling *Phytophthora palmivora* (Blackpod disease) since 1977. The cocoa plantation had been determined to have elevated levels of Cu in its soil through an earlier study [15]. The “B” pots contained soil mixtures from the cocoa plantation and organic manure (poultry droppings) in a respective ratio of 2:1. The B pots represent experimental treatment with moderate Cu toxicity. The reference pot “C” or control contained soils collected from 0-15 cm depth from a 15 years old *Gmelina* plantation located uphill at distance of 200m from cocoa plantation. The reference soil had no historical evidence of Cu toxicity. Each set of pot was replicated 10 times. Baseline soil chemical properties (pH, organic carbon%, N, P, K, Cu, Pb and Fe) of these soil treatments were determined prior to commencement of the experiment in line with standard methods [16].

Germination boxes were used in raising the seedlings of *Centrosema*. Two weeks after germination, C*entrosema* seedlings were transplanted into the experimental pots (A, B and C). The experiment was laid out in a Randomized Complete Block Design in three (3) replicates.

2.3. Data Collection and Analysis

Beginning from the 8 weeks after transplanting, monthly readings on the growth performance of the *Centrosema* plants were taken for 14 weeks. Growth parameters measured include counting of leaf number per plant monthly and random collection of Centrosema stands from each treatment which were gently uprooted to count the number of root nodules using the aid of a magnifying lens and determine total dry weight of plants. Also before oven-drying, aboveground plant samples were collected and washed, dried, digested and analyzed in a laboratory to determine levels of the Cu, Pb, Fe, N, P and K. Analysis of variance was used to determine variations in the parameters obtained from the treatments. Significant differences obtained in the result were separated using Fishers least significant difference (LSD) at 95 % confidence interval.

3. Results and Discussion

The results of the pre-trial soil analysis (baseline soil characteristics) are presented in Table 1. Heavy metals measured (Cu, Pb and Fe) in A and B sets of pots (representing areas with high and moderate doses of toxicity respectively) were more than twice the proportions obtained in Pot C (Control). Specifically the level of Cu concentration was lowest in the reference soil "C" (6.0mg/g) and more than double in Pots A and B (15.6mg/g and 15.2mg/g respectively).

Table 1. Background chemical properties of soil of the plots before the experiment

Parameters	Plot A (High toxicity)	Plot B (Moderate toxicity)	Plot C (Control)
Ph	6.70	7.20	6.30
Organic carbon (%)	3.33	2.19	7.03
N (%)	0.14	0.31	1.20
P (mg kg^{-1})	24.38	32.16	39.16
K (cmol kg^{-1})	0.76	0.78	1.74
Cu (mg g^{-1})	15.60	15.20	6.00
Pb (mg g^{-1})	3.00	3.60	1.30
Fe (mg g^{-1})	1386.34	1280.37	600.58

Conversely, macronutrient elements (N, P and K) in the soil were lowest in Plot A and improved progressively in the soils of Plots B and C; a pattern that expresses the substantial suppressive impact of the heavy metals. For instance, a comparison between the above values obtained for soil in Pot A and that in the control pot (C) indicates that Pot A had sharp decline in the levels of N and P below marginal permissible limits of 0.2% for N [17] and 26mg/kg for P [18]. Perennial low crop yield, land exhaustion and delayed land resuscitation for vegetation growth and metal accumulation are traceable effects of one form of pesticide use or the other [19]. Soil pH was more acidic in the reference soil C (6.3) than the pot A with high Cu toxicity (6.7). Amendment of Cu- contaminated soil with organic manure produced a moderation of soil pH towards neutrality (7.2). The pH range of these soils (6.7 – 7.2) which have evidence of Cu toxicity is reportedly not tolerable for optimal plant growth especially members of the *Fabaceae* family [20]. Comparing the soil pH level of 6.3 obtained from *Gmelina* plantation (Plot C) and the soil pH range of 4.91- 4.95 recorded from a cashew (*Anacardium*) plantation by Reference [21] in a location not very far from the study area showed that pH can be biologically regulated by the dominant species composition in a given site.

In Figure 1, formation of root nodules which are the structures that host the symbiotic interaction of the Rhizobia and the legume (*Centrosema*) was strongly influenced by the levels of Cu in the soil medium. The highest mean number of nodules (157.87 ± 12) was produced by the *Centrosema* stands grown with control soil (C) and it was significantly higher than the stands grown in Cu-contaminated soil (A) by 260%. The poultry manure amend soil (B) had 118.06 ± 8 mean number of nodules. Heavy metal soil toxicity has been reported in some other works to inhibit multiple metabolic processes in plants such as chlorophyll formation, inactivated protein synthesis, N-fixing ability of some plants and which consequently manifest as reduced plant growth and yield [6,22] and [23]. The inhibitory role of high levels of Cu in the development of root nodules has very serious implication on the ability of the *Centrosema* species to relate symbiotically with Rhizobia and ability to meet the host plant's N economy which consequently retards contribution to soil fertility. The capacity to form root nodules is directly connected with N-fixation because it is the Rhizobia species that carry symbiotic genes that codes for nodulation and N2 fixation and it is the same genes that confer in legumes the ability to nodulate and fix atmospheric N [6]. Heavy metals such as Cu, Hg, Cd and Cr have been shown to have adverse direct impact on the survival of N-fixing micro-organisms and the processes which they mediate [24,25].

The number of leaves produced by *C. pubescens* grown on the different soil media (experimental pots) varied significantly (Figure 1). The lowest number of leaves per plant (68.3 ± 3) was recorded from stands grown in "A" pots with Cu toxicity but the highest leaf number (138.9 ± 6) was from Centrosema stands in pot B whose toxicity level was amended with organic manure. For *Centrosema* plant, therefore, toxic soil amended with organic manure subdues the impact of toxicity and produces enhanced foliar formation. The mean number of leaves on Centrosema stand grown on control soil was 79.1 ± 4 which was not significantly different from the Cu toxic soil. Such variability in foliar growth performance was reported by Reference [26]. Leaves which form a delicacy for livestock in forage and pasture management therefore can as a result of this limitation (suppressive effect) impair photosynthetic rate and resultantly carbohydrate formation.

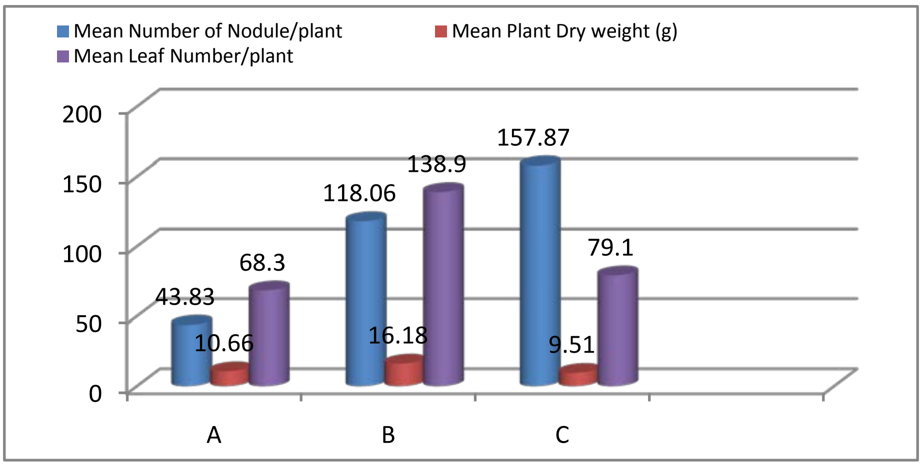

Figure 1. Mean number of root nodules per plant and mean dry weight of *Centrosema pubescens* after Fourteen (14) weeks of growth in Cu- contaminated soil (A), Cu-contaminated soil amended with poultry manure (B) and Control soil (C)

On mean total plant dry weight, the Cu-contaminated soil which was amended with poultry manure produced the highest total *Centrosema* dry weight of 16.18g ± 0.8/stand at 14 weeks of transplanting which was significantly higher than those grown in Cu-contaminated soil (A) and Control soils (C) that had 10.66g ± 0.5and 9.51g ± 0.3 respectively (Figure 1). Soil Cu-toxicity in this case appears to exert negative effect to *C. pubescens* total dry weight although not significantly different. Reference [27] similarly reported that field pea grown with soils loaded with heavy metals declined in aboveground biomass yield though not significant over the control. Also, it was reported that increased absorption of heavy metals by some plant species does not necessarily reflect as significant increase in plant biomass [23]. The higher biomass achieved by treatment B might be attributed to the contribution made by poultry manure amendment which enhanced aboveground growth of the plants and perhaps increased the availability of nutrients in the soil for plant uptake.

The lowest levels of essential macronutrients in plant samples (2.69% N; 268mg/g P and 11278.3kg/mg K) were recorded in *Centrosema* grown in Cu contaminated soil "A" (Table 2) The N:P:K values in plant samples grown in the control pots "C" were significantly higher than those of "A" by 48.3%; 61.4% and 66.5% respectively. It is possible that the higher heavy metal contents of the "A" soil inhibited the availability of the macronutrients for uptake by the plants. In addition, the Rhizobia and legume interaction which had been reported to enhance N fixation and uptake in leguminous plants was evidently greater in the control soil "C" which also had more nodulation in the *Centrosema* stands.

Furthermore, the low proportion of the mineral elements in the background soil analysis (Table 1) and its corresponding higher levels in plant biomass in Pot B which is an organic manure amended soil provides additional credence to possible remediation option for toxic/polluted soils through organic manure amendment as earlier demonstrated by [28]. This can enhance land productivity and vegetation growth. Given the high dependence of fungicides in arable and tree crop production especially Cu-containing compounds, the usefulness of organic manure which is often wasted or underutilized in land management deserves to be popularized among farmers and plantation managers. In Table 3, the level of heavy metals (Pb, Cu and Fe) in the stem of *Centrosema* raises serious concern especially for seedlings in Pot A. The capacity of young *Centrosema* seedlings to absorb and transport copper and other heavy metals from the soil unto the plant shoot is a source of worry to practitioners in the livestock industry. Potentially fatal diseases can develop through excessive accumulation of dietary heavy metals such as Cu, Cd, Pb and Cr in the human body [27,29]. The bioaccumulation and persistence of heavy metals have been observed in tissues of organisms (plants and animals) especially as it can be visibly transferred in the food chain process.

Significant proportions of the heavy metals absorbed by plant tissues are stored in the aboveground biomass [30]. The amendment of toxic soils with organic manure especially in regions where animal husbandry is predominant and the relatively high absorption capacity for toxic substances by *C. pubescens* should be highlighted for proposal in low-cost biological (phytoremediation) programmes and projects for polluted areas.

Table 2. Levels of macronutrients and heavy metals in the aboveground biomass of *Centrosema pubescens* after 14 weeks of growth in the study area

Pots	Macronutrients and heavy metals					
	Cu (mg kg^{-1})	Pb (mg kg^{-1})	Fe (mg kg^{-1})	N (%)	K (mg kg^{-1})	P (mg kg^{-1})
Plot A	5.03^{c}	2.59^{a}	344.7^{a}	2.69^{a}	$11,278.3^{a}$	268.3^{b}
Plot B	4.27^{b}	2.17^{b}	335.1^{b}	2.99^{ab}	$16,378.3^{b}$	298.3^{b}
Plot C	3.93^{a}	2.01^{ab}	325.1^{bc}	3.99^{c}	$18,782.3^{bc}$	433.3^{a}
LSD value	0.32	0.25	23.4	0.354	3328.3	51.7

Values within column with different superscript are significantly different at 0.05 level of significance.

4. Conclusion

There were very appreciable effects of soil toxicity on the growth of *C. pubescens* arising from build-up of Cu as a result of application of Bordeaux mixture. Elevated level of Cu toxicity in the soil had significant effect on nodulation, leaf formation and total dry weight. Amendment of toxic soil by poultry manure significantly minimized the impact of toxicity and in fact enhanced performance of leaf formation and total biomass. There was a perceived adaptation of *Centrosema* plant to sustain growth even in nutrient deficient soils (especially N) possibly through inherent capacity of similar leguminous plants to form N-fixing nodules. *Centrosema* plant grown on Cu toxic soil increased the levels of potassium and heavy metals (Pb, Cu, Fe) in the stem. *Centrosema* or other forage plants grown in such soils with elevated level of heavy metals in the soil should be avoided for livestock/human health and safety. In regions where the free range system is prevalent such as Nigeria, efforts need to be intensive to avoid land covers that regularly use Cu-based fungicides. However, where moderation of Cu soil toxicity and land management is the objective, using *Centrosema* as phyto-remediating plant would appear promising as a candidate plant species. Poultry manure also can be used where necessary in land restoration programmes.

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