GROWTH PARAMETERS AND TOXICITY OF COWPEA (*VIGNA UNGUICULATA*) GROWN IN COPPER POLLUTED SOIL

ABSTRACT

Heavy metals find their way into the soil and eventually the food chain via anthropogenic activities. The deleterious effects of some heavy metals depend on the concentration of the metal and the tolerant capacity of the plant. The present study investigated the morphological and toxicological effects of copper on cowpea. Exactly 2kg of soil was treated with 10mg/mL, 50mg/mL, 100mg/mL and 200mg/mL of copper sulphate (1g/L stock solution), and 5.20g/L of kocide (lower dose of kocide), 6.67g/L of kocide (positive control) and 10.70g/L of kocide (higher dose of kocide) and no treatment (negative control) to obtain eight groups. The experimental plant was cultivated for five weeks. Data were collected within and after the first week of cultivation and subsequent weeks until the fifth week. The data obtained from the various growth parameters considered were subjected to analysis of variance (ANOVA) using Statistical Product and Service Solutions (SPSS) software (version 16). The result obtained showed inconsistent increase with no significant difference in the percentage seed germination, leaf area and plant height as against the control between the weeks, while an inconsistent decrease within the weeks. There was reduction in the copper content in the leaf and steam, though, the content in the leaf exceeded in all the groups. Finally, the results of health risk factors/indices considered showed a decrease in the leaf in most of the treatments, and an increase in the stem when compared to United States Environmental Protection Agency (USEPA) permissible units. Data from the target hazard quotient and carcinogenic or health risk of the plant leaf and stem indicate that there is little or no risk associated in consuming the plant at certain level of copper contamination.

Key words: Copper, Cowpea, Growth and Tolerance.

ABBREVIATION

NC: Negative control (de-ionized water)

PC: Positive control (Normal kocide; that is applicable in field - 6.67g/L of kocide)

LD: Lower dose of kocide (5.20g/L)

HD: Higher dose of kocide (10.70g/L)

TA: Copper sulphate treatment (10mg/mL)

TB: Copper sulphate treatment (50mg/mL)

TC: Copper sulphate treatment (100mg/mL)

TD: Copper sulphate treatment (200mg/mL)

INTRODUCTION

The unconsolidated material on the surface of the earth that serves as a natural medium for the cultivation of plants is the soil (Thomas *et al*., 1999). Soil is the most indispensable part of our ecosystem which comprises mineral elements, organic matter of varying sizes and composition, chemicals, air and water that supports life (Thomas *et al*., 1999; Voroney and Heck, 2007; Shahid *et al*., 2013). A fertile soil is the most suitable for the cultivation of plants. The fertility of the soil is determined by its physicochemical and biochemical properties which vary from region to regions (Thomas *et al*., 1999; Nyle *et al*., 2009; Kumar *et al*., 2013). Research have shown that the soil biochemical properties such as dehydrogenase activities are influenced by some physical properties of the soil such as temperature, aeration, moisture content and pH, and even the application of pesticides (Kumar *et al*., 2013). Soil is a reservoir of minerals and nutrients. It serves as a harbor for living organisms and aids in curbing of flooding and drought (Ponge, 2003; De Deyn *et al*., 2005). Bending *et al*. (2002) reported that both crop residue and soil organic matter have effect on the fertility of the soil and the microbial community of the soil. It also serves as the foundation for various forms of construction projects and the essential component of industries such as mining, construction and landscaping development industries. In addition, soil is of great importance in the recycling of nutrient elements such as carbon and nitrogen, and curbing green house effect (Thomas *et al*., 1999). Harmful substances and even essential nutrient elements in a quantity beyond the required level, result in soil contamination or pollution.

Environmental pollution in recent times poses a tremendous health hazard to humans, animals and plants with local, regional and global implications (Anvar and Selvaraju, 2015). It also has adverse effects on land, water or air and the biotic and abiotic components of the ecosystem (Anvar and Selvaraju, 2015). Substances that contribute to this menace: environmental pollution, are not easily degradable to small harmless molecules and such toxic substances include: lead (Pb), mercury (Hg), cadmium (Cd), copper (Cu) and zinc (Zn). Other pollutants are sewage, chemicals, smoke and waste (Goyal and Chhibber, 2016). Most of these contaminants find their way into the soil through industrial activities and agricultural practices such as: application of fertilizers, liming of the soil and the use of pesticides, fungicides, and bactericides (Goyal and Chhibber, 2016; Asiamah *et al*., 2021). The accumulation of heavy metals in the soil could be as a result of the excessive use of these chemicals over a long period of time (Puschenreiter *et al*., 2005).

Metals such as copper, is often used in the production of some copper-based fungicides and bactericides (Dong, 2013). Prolong application of these copper-based chemicals, enhances the concentration of copper in the soil. This results in an increase in the copper load in the soil which has deleterious effects on plants.

Heavy metals contamination is of great concern due to its toxic effects on crop growth (Adrees *et al*., 2015). When plants bioaccumulate heavy metals beyond the (threshold) level or concentration that they can curb, the heavy metals hamper or thwart the morphological, physiological and biochemical processes of the plants (Tighe-Neira *et al*., 2022). This results in a drastic reduction in plant yield which brings about shortage of food supply.

Cowpea is a herbaceous legume that serves as food and feed crop cultivated in the semi-tropical regions of Africa, Asia, Europe, Central and South America (Nair *et al*., 2008). Cowpea (*Vigna unguiculata*) is of the order, *Fabales*; family, *Fabaceae*; tribe, *Phaseoleae* and genus, *Vigna* (Perrino *et al*., 1993; Ng, 1995; Singh *et al*., 1997). Unlike other crops, *V. unguiculata* thrives in poor dry conditioned soil of about 85% sand (Obatolu, 2003) with over 200million people consuming it daily (Langyintuo *et al*., 2003). Studies have shown that cowpea is a drought tolerant crop that has the capacity to fix atmospheric nitrogen through its nodules and thrive in sandy loam soil (Singh *et al*., 2003). However, when cultivated in a heavy metal contaminated soil, its morphology, physiology and yield deplete with regards to the concentration of the contaminant (Anvar and Selvaraju, 2015; Singh *et al*., 218). The yield of cowpea depletes due to the exogenous production of free radicals by the contaminant. Cowpea produces antioxidants which counteract the deleterious impact of free radicals by donating electron(s) to the free radicals or pulling unpaired valence electron(s) from them. These antioxidants could be enzymic such as superoxide dismutase which catalyses the conversion of superoxide anions to hydrogen peroxide and oxygen, catalase which catalyses the conversion of hydrogen peroxide to water and oxygen, and glutathione peroxidase which catalyses the conversion of hydrogen peroxide and organic (lipid) peroxides to water or alcohol; and the non-enzymic or low molecular weight antioxidants such as glutathione, ascorbate, carotenoids, tocopherol and phenolic compounds (Dontha, 2016; Ologundudu, 2021).

However, the production of free radicals often overwhelms the antioxidant defense mechanism of plants, resulting in oxidative stress which disrupts several cellular functions, physiological and biochemical processes (Goyal and Chhibber, 2016; Ologundudu, 2021).

The consequence of consuming plant that had bioaccumulated heavy metals could be the presence of the metals in the tissue of animals (secondary consumers). Human health risk assessment is a systemic process used to determine the health status and effect of an individual exposed to carcinogenic and non-carcinogenic chemicals (United State Environmental Protection Agency, 2001). The risk assessment consists of four steps: hazard identification, exposure assessment, toxicity (dose-response) assessment and risk characterization (United State Environmental Protection Agency, 2001). In the current study, the contaminant is copper. Thus, the toxicity study will be limited to three health risk factors: the estimated daily dietary intake of the heavy metal (copper) from the sample (Leo *et al*., 2002), the target hazard quotient (is the ratio of exposure of an individual and the reference oral dose which determines the health risk involved in the consumption of the sample) (Yu-Jun *et al*., 2011) and the carcinogenic risk which is determined by the slope factor (slope factor assesses risk which is used to evaluate an upper bound life time probability of an individual developing cancer as a result of exposure to a particular level of a potential carcinogen over a period of time) (United State Environmental Protection Agency, 2011). Here are some toxicity parameters:

Accumulation Factor (AF) or Transfer Factor (TF)

The accumulation factor or transfer factor is the index of the plants' ability to accumulate metals from the soil and is evaluated as:

$AF = C_{plant} / C_{soil}$

Where: C_{plant} and C_{soil} are the concentration of the heavy metal (mg/kg) in the plant part and soil respectively (Wilson and Pyatt, 2007).

Mobility Index or Translocation Factor

The mobility index (MI) or translocation factor is the index of heavy metals to move from the soils to the leaves of plants through the roots and stems. It is evaluated as:

$$MI = \frac{\text{Concentration of metal in the leaf}}{\text{Concentration of metal in the root}}$$

Biological Accumulation Coefficient (BAC)

Biological transfer coefficient is described as the ratio of heavy metal concentration in plant shoot to that in plant root.

Bioconcentration Factor

Bioconcentration factor is calculated as the ratio of concentration of heavy metal in the plant root to that of soil (Wilson and Pyatt, 2007).

Estimated Dietary Intake (EDI) of Heavy Metal from Sample

The estimated dietary intake of heavy metal depends on both the metal concentration in the sample and the consumption of the respective sample. The equation used for the determination of EDI of metals in sample is given below:

$$EDI = \frac{\text{Concentration of metal} \times \text{Daily sample intake}}{\text{Average body weight}}$$

Where: EDI = Estimated daily intake (kg/person/day).

Metal concentration in sample is expressed in mg/kg while the daily sample intake is expressed in kg/person/day and the average body weight of 60kg was used as reported by Leo *et al*. (2002). Average daily sample intake for adults was considered to be 0.09mg/person/day.

Target Hazard Quotient

Target hazard quotient (THQ) is defined as the ratio between exposure and reference oral dose (RfD). THQ is the used for the assessment of human health risks from the consumption of samples grown in contaminated sites. The ratio is used to express the risk of non-carcinogenic effects (Yu-Jun *et al*., 2011). The consumer is said to be faced with a health risk when the ratio is greater or equal to 1. The THQ value is evaluated using the equation below:

$$THQ = \frac{\text{Concentration of metals} \times \text{Daily sample (cowpea) intake}}{\text{RfD} \times \text{Average body weight}}$$

Where: THQ = Target Hazard Quotient

RfD = Oral Reference Dose (mg/kg/day)

Average body weight is 60kg.

Carcinogenic Risk

The carcinogenic risk is determined by the slope factor. The slope factor assesses risk which is used to evaluate an upper bound lifetime probability of an individual developing cancer as a result of exposure to a particular level of a potential carcinogen over a period of time (United State Environmental Protection Agency, 2011).

Lifetime probability of contracting cancer due to exposure to site related chemicals is evaluated as:

Lifetime Probability of cancer = EDI × CSF_{mg}

Where:EDI = Estimated daily intake of heavy metal (mg/kg/day).

CSF_{mg} = Cancer slope factor $(mg/kg/day)^{-1}$, CSF_{mg} for copper is $0.90(mg/kg/day)^{-1}$

Studies have shown that at minute concentrations, some heavy metals improve significantly the quality of crops (Anvar and Selvaraju, 2015; Nazir *et al*., 2016). Nazir *et al*. (2016) reported an improvement in the growth and yield of rice cultivated in a nickel contaminated soil (10-1000µM). Lower concentrations of zinc and copper had also been reported to enhance the growth and yield of cowpea

(Anvar and Selvaraju, 2015). However, a significant reduction in root elongation (a growth parameter) had also been reported of *Arabidopsis thaliana* exposed to a concentration range of copper oxide (0.5-100mg/L) (Nair *et al*., 2014). The aim of the current study is to investigate the growth parameter and toxicological effect of copper on cowpea grown in a copper polluted soil.

MATERIALS AND METHODS

Plant Material (Cowpea Seed)

Improved and healthy cowpea (*V. unguiculata*) seeds, Ife-brown species with specification: Ife brown cowpea IT 84E_124, were purchased from a renowned agrochemicals and seeds shop at Textile Mill road, Benin City, Nigeria, and taken to the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin for identification and authentication. A voucher specimen was deposited in the hebarium.

Soil

The uncontaminated sandy-loamy soil was collected from the Faculty of Agriculture demonstration farm land, University of Benin, Benin City, Nigeria.

Experimental Design

Physicochemical analyses were conducted on the soil sample to determine its fertility. Exactly 2kg of the suitable soil was treated with 1g/L of copper sulphate ($CuSO_4$) and kocide to obtain: 10, 50, 100 and 200mg/mL of $CuSO_4$ and 5.20 (lower dose), 6.67 (positive control) and 10.70g/L (higher dose) of kocide (each treatment and the soil treated with de-ionized water served as a group). The seeds of the plant were sown and cultivated for five weeks.

Growth Parameters

Within and after the first week, data were collected for percentage (%) germination, plant height, shoot width, leaf area and leaf number.

Sample Preparation

After the first two weeks of cultivation, part of the plant samples (the leaf and stem) were collected, rinsed, air-dried, pulverised and ashed. Thereafter, the ashed samples were subjected to metal analysis to determine the copper content. This procedure was repeated weekly till the fifth week.

Elemental Analysis (Ashing method)

The copper content of the samples was estimated using the method described by Agte *et al*. (1995).

Statistical Analysis

All data were statistically analyzed by analysis of variance (ANOVA). Statistical significance *(P=0.05)* of the means and standard error of the mean were determined by Tukey multiple range test using Statistical Product and Service Solutions (SPSS) software (version 16).

RESULTS

Table 1: Parameters for soil analysis

Parameter		**Value**
pH		5.60
Organic nitrogen content (g/kg)		0.80
Organic carbon content (g/kg)		18.58
Available phosphorus (mg/kg)		9.23
Particle size (g/kg)	Sand	837.30
	Silt	56.90
	Clay	105.80
Exchangeable acidity (cmol/kg)	Al^{3+}	0.58
	H^+	1.12
Exchangeable bases (cmol/kg)	K^+	0.25
	Na^+	0.15
	Ca^{2+}	0.72
	Mg^{2+}	0.26
Copper (mg/kg)	16.32	
Soil dehydrogenase activity (Concentration of TPF in μmol/mL× 10^{-2})	1.28	

Concentration was extrapolated from the Triphenylformazan (TPF) standard curve.

The results presented in table 1 from the soil analysis conducted showed that the soil used in this study met the prerequisite for a sandy-loam.

Table 2: **Growth parameters of cowpea treated with various concentrations of copper (Week 1)**

Group	**Number of germinated seeds per group**	**% germination per group**	**Leaf area (cm^2)**	**Plant height (cm)**
NC	6.67 ± 0.88	66.67 ± 8.82	25.50 ± 0.50	21.30 ± 0.50
PC	5.00 ± 0.58	50.00 ± 5.77	25.39 ± 0.50	20.87 ± 0.50
LD	7.00 ± 0.58	70.00 ± 5.77	24.44 ± 0.50	24.30 ± 0.50*
HD	5.33 ± 0.33	53.33 ± 3.33	23.87 ± 0.50	22.70 ± 0.50
TA	4.67 ± 1.45	46.67 ± 14.53	16.70 ± 0.50*	19.67 ± 0.50
TB	7.67 ± 1.20	76.67 ± 12.02	22.43 ± 0.50*	24.67 ± 0.50*
TC	6.67 ± 1.45	66.67 ± 14.53	21.79 ± 0.50*	24.30 ± 0.50*
TD	7.33 ± 0.67	73.33 ± 6.67	23.66 ± 0.50	23.23 ± 0.50*

Values are represented as mean ± SEM (Standard error of mean).

*Indicates value with significant difference relative to the negative control *(P=0.05)* in each column.

Few days after sowing, the third day precisely, the young shoot (plumule) sprouted out both in the controls (negative and positive) and the treatments with 66.67 ± 8.82% and 50.00 ± 5.77% as percentage germination rate of the negative and positive controls respectively (Table 2). The various treatments: lower dose of kocide, higher dose of kocide, 10mg/mL, 50mg/mL, 100mg/mL and 200mg/mL of copper; had the respective percentage germination values: 70.00 ± 5.77%, 53.33 ± 3.33%, 46.67 ± 14.53%, 76.67 ± 12.02%, 66.67 ± 14.53% and 73.33 ± 6.67%. The rate reduced slightly at 10mg/mL of copper treatment but the difference was not significant at *(P=0.05)*. After the first week of cultivation, there was significant reduction in the leaf area of the copper treatments as against the negative control, 25.50 ± 0.50cm^2 (Table 2). In contrast, the heights of the experimental plants of the copper treatments, 50mg/mL, 100mg/mL and 200mg/mL whose height are: 24.67 ± 0.50cm, 24.30 ± 0.50cm and 23.23 ± 0.50cm respectively, increased significantly *(P=0.05)* against the negative control, 21.30 ± 0.50cm. The plant heights of the lower and higher doses: 24.30 ± 0.50cm and 22.70 ± 0.50cm respectively also increased significantly when compared to the positive and negative controls.

Table 3: Growth parameters of cowpea treated with various concentrations of copper (Week 2)

Group	Leaf area (cm^2)	Total number of leaves	Plant height (cm)	Shoot width (cm)
NC	34.84 ± 1.50	8.00	25.83 ± 1.50	0.40 ± 0.01
PC	27.45 ± 1.50	7.00	23.67 ± 1.50	0.40 ± 0.01
LD	26.04 ± 1.50*	8.00	29.33 ± 1.50	0.40 ± 0.01
HD	28.53 ± 1.50	8.00	31.33 ± 1.50*	0.40 ± 0.01
TA	24.88 ± 1.50*	7.00	25.67 ± 1.50	0.30 ± 0.01*
TB	26.90 ± 1.50	7.00	29.67 ± 1.50	0.40 ± 0.01
TC	26.47 ± 1.50	8.00	30.33 ± 1.50	0.40 ± 0.01
TD	29.91 ± 1.50	8.00	29.67 ± 1.50	0.40 ± 0.01

Values are represented as mean ± SEM (Standard error of mean).

*Indicates value with significant difference relative to the negative control *(P=0.05)* in each column.

Cultivating for two weeks, the leaf area of the treatments tend to decrease insignificantly compared to the negative control (34.84 ± 1.50cm^2) *(P=0.05)*, but increased in the plant height (Table 3). There was little or no change in the total number of leaves and shoot width when comparing the treatments with the negative control at *(P=0.05)* (Table 3).

Table 4: Growth parameters of cowpea treated with various concentrations of copper (Week 3)

Group	**Leaf area (cm^2)**	**Total number of leaves**	**Plant height (cm)**	**Shoot width (cm)**
NC	28.40 ± 1.50	10.00	31.67 ± 1.50	0.40 ± 0.01
PC	41.16 ± 1.50*	9.00	29.67 ± 1.50	0.40 ± 0.01
LD	36.33 ± 1.50	11.00	33.00 ± 1.50	0.40 ± 0.01
HD	35.38 ± 1.50	11.00	31.67 ± 1.50	0.40 ± 0.01
TA	18.53 ± 1.50*	6.00	19.33 ± 1.50*	0.30 ± 0.01*
TB	29.26 ± 1.50	9.00	30.33 ± 1.50	0.40 ± 0.01
TC	27.92 ± 1.50	10.00	30.33 ± 1.50	0.40 ± 0.01
TD	30.19 ± 1.50	11.00	29.67 ± 1.50	0.40 ± 0.01

Values are represented as mean ± SEM (Standard error of mean).

*Indicates value with significant difference relative to the negative control *(P=0.05)* in each column.

After the third week of cultivation, a slight increase was observed with the treatments as against the negative control, but a significant increase with the positive control *(P=0.05)* (Table 4). In contrast, a slight decrease was recorded in the plant height of the treatments as against the negative control (31.67 ± 1.50cm) (Table 4). For the leaf number and shoot width, no significant differences were recorded.

Table 5: Growth parameters of cowpea treated with various concentrations of copper (Week 4)

Group	Leaf area (cm^2)	Total number of leaves	Plant height (cm)	Shoot width (cm)
NC	33.44 ± 1.30	16.00	45.17 ± 1.50	0.50 ± 0.02
PC	29.73 ± 1.30	10.00	32.33 ± 1.30*	0.30 ± 0.02*
LD	40.58 ± 1.30	13.00	32.00 ± 1.30*	0.50 ± 0.02
HD	40.56 ± 1.30	13.00	30.93 ± 1.30*	0.50 ± 0.02
TA	26.00 ± 1.30*	8.00	22.83 ± 1.30*	0.30 ± 0.02*
TB	35.94 ± 1.30	12.00	32.00 ± 1.30*	0.50 ± 0.02
TC	36.62 ± 1.30	15.00	39.00 ± 1.30*	0.50 ± 0.02
TD	42.69 ± 1.30*	13.00	35.33 ± 1.30*	0.50 ± 0.02

Values are represented as mean ± SEM (Standard error of mean).

*Indicates value with significant difference relative to the negative control *(P=0.05)* in each column.

At the fourth week, an increase in the leaf area was recorded with the treatments as against the negative control with that of 200mg/mL being significant at *P=0.05*. However, in plant height a tremendous significant decrease was observed in the treatments as against the negative control (45.17 ± 1.50cm) *(P=0.05)* (Table 5); while in the total leaf number, a decrease was recorded. In the shoot width, no significant differences were observed.

Table 6: Growth parameters of cowpea treated with various concentrations of copper (Week 5)

Group	Leaf area (cm^2)	Total number of leaves	Plant height (cm)	Shoot width (cm)
NC	37.12 ± 0.70	16.00	47.17 ± 0.70	0.60 ± 0.03
PC	31.39 ± 0.70*	10.00	33.67 ± 0.70*	0.40 ± 0.03*
LD	42.58 ± 0.70*	13.00	34.00 ± 0.70*	0.60 ± 0.03
HD	42.56 ± 0.70*	13.00	32.93 ± 0.70*	0.60 ± 0.03
TA	28.00 ± 0.70*	9.00	24.17 ± 0.70*	0.40 ± 0.03*
TB	38.94 ± 0.70	12.00	34.00 ± 0.70*	0.60 ± 0.03
TC	39.62 ± 0.70	15.00	41.00 ± 0.70*	0.60 ± 0.03
TD	45.69 ± 0.70*	13.00	37.33 ± 0.70*	0.60 ± 0.03

Values are represented as mean ± SEM (Standard error of mean).
*Indicates value with significant difference relative to the negative control *(P=0.05)* in each column.

At the fifth week, significant increase in the leaf area in the treatments as against the negative control (37.12 ± 70cm^2) was observed ($p<0.05$) excluding the positive control (Table 6), while also a corresponding significant decrease in their heights ($P=0.05$) (Table 6): both with the positive and negative controls. Results obtained for leaf number and shoot width showed a decrease and no significant increase respectively.

Table 7: Copper content in plant samples (leaf and stem)

Plant sample	NC	PC	LD	HD	TA	TB	TC	TD
Leaf copper content (mg/kg)	1.24	1.10	1.10	1.33	0.88	1.14	0.97	1.13
Stem copper content (mg/kg)	1.88	0.98	0.87	0.84	0.94	0.87	0.96	1.06

The plant parts in focus, the leaf and stem, were observed to have utilized copper molecules (ions) as presented in Table 7. In the leaf, an insignificant decrease was recorded in the treatments with mean value (1.09 ± 0.15mg/kg) as against the negative and positive controls (1.24 and 1.10mg/kg respectively): using the one-sample T-test (2-tailed) analysis (5% degree of freedom) (Table 6). For the stem, a significant decrease was observed in the treatments with mean value (0.92 ± 0.08mg/kg) when compared to the negative control (1.88mg/kg) *(P=0.05)*.

Table 8: Health risk assessment of the cowpea leaf

Group	Estimated daily intake (mg/Kg/day) $\times 10^{-4}$	Target hazard quotient $\times 10^{-1}$	Carcinogenic risk $\times 10^{-3}$	Lifetime probability of contracting cancer $\times 10^{-5}$
NC	5.17	0.13	0.07	3.62
PC	7.83	0.20	0.11	8.61
LD	4.58	0.11	0.07	3.21
HD	4.08	0.10	0.06	2.45
TA	4.58	0.11	0.06	2.75
TB	3.63	0.09	0.05	1.82
TC	5.54	0.14	0.08	4.43
TD	3.50	0.09	0.05	1.75

For the leaf target hazard quotient, the values for the treatments were significantly less than 1 (the reference value for health risk assessment according to the United States Environmental Protection Agency, 2011 and Yu-Jun *et al*., 2011). While the carcinogenic risk values for the treatments recorded were significantly lower than the United States Environmental Protection Agency (2011) cancer slope factor reference value, 0.90$(mg/kg/day)^{-1}$ (Table 8). However, values obtained for the three toxicity indices for health risk assessment; estimated daily intake, target hazard quotient and carcinogenic risk, showed a general decrease in the treatments as against the negative control. The lifetime probability of contracting cancer values recorded for most of the treatments were lower than the negative control.

Table 9: Health risk assessment of the cowpea stem

Group	Estimated daily intake (mg/Kg/day) $\times 10^{-4}$	Target hazard quotient $\times 10^{-1}$	Carcinogenic risk $\times 10^{-3}$	Lifetime probability of contracting cancer $\times 10^{-5}$
NC	3.67	0.09	0.05	1.82
PC	3.92	0.10	0.06	2.35
LD	4.75	0.12	0.07	3.33
HD	3.63	0.09	0.05	1.82
TA	4.04	0.10	0.06	2.42
TB	4.00	0.10	0.06	2.40
TC	4.71	0.12	0.07	3.30
TD	4.42	0.11	0.06	2.65

For the stem target hazard quotient, the values for the treatments were significantly less than 1 (the reference value for health risk assessment according to the United States Environmental Protection Agency, 2011 and Yu-Jun *et al*., 2011). While the carcinogenic risk values for the treatments recorded were significantly lower than the United States Environmental Protection Agency (2011) cancer slope factor reference value, $0.90(mg/kg/day)^{-1}$ (Table 9). However, the three toxicity indices for health risk assessment showed an increase in the treatment groups as against the negative control. The lifetime probability of contracting cancer values recorded for the treatments were higher than the negative control.

DISCUSSION

Anthroponegic and natural processes are responsible for the introduction of heavy metals in the soil which disrupt the growth of plant and cut down tremendously the yields of agricultural produce. The bioaccumulation of heavy metals by organisms especially plants beyond their threshold concentration results in toxicity. Several factors such as clay particle content, organic matter, available phosphorus and the presence of calcium, magnesium and aluminium, contribute immensely to the bioaccumulation of heavy metals (Schulte and Kelling, 1999). However, the latter factor depletes the bioavailability of heavy metals by chelating and eventually leaching the metals (Shahid *et al.*, 2014). Phytotoxicity is the consequence of the bioaccumulation and translocation of heavy metals in plant cells (Singh *et al.*, 2018). Phytotoxicity disrupts metabolic and physiological processes, and eventually growth retardation (Guala *et al*., 2010; Kamran *et al*., 2021). Plants often develop antioxidant defense mechanism, both enzymic and non-enzymic, and also utilize other special mechanisms for curbing stress. Plants with such adaptive features are good phytoextractors and phytoremediators (Wani *et al*., 2019; Moula *et al*., 2021).

In this study, the result of the soil analysis before cultivation showed that the soil is suitable for the experiment (Table 1) and met the criteria for a sandy-loam soil. The results obtained from the seed (%) germination showed an inconsistent increase with no significant mean difference for the treatments *(P=0.05)*. This indicates that cowpea is probably tolerant up to 200mg/mL of 1g/L concentration of copper treatment since over 50% of the seeds sown actually sprouted. Similar result was obtained for *Spenostylis stenocarpa* at concentration up to 10,000mg % NaCl (Tejovathi *et al*., 1988; Ikhajiagbe *et al*., 2007). However, Asiamah *et al*. (2021) reported no germination in cowpea grown in spilled engine oil polluted soil.

Growth parameters such as leaf area, total number of leaves, shoot width and plant height, are used to evaluate plants′ morphology and general performance. In this study, a significant reduction in the leaf area of the copper treated groups as against the negative control was observed, while a significant increase in the plant height for the first two weeks and the fifth week was also observed. However, in the subsequent weeks, 3 and 4, the reverse was observed: an increase in the leaf area and decrease in the plant height for the higher concentration of copper treatments.

Similar decrease was recorded in cowpea with increasing concentration of heavy metal (Sharma and Sharma, 1993; Vijayarengan, 2012; Anvar and Selvaraju, 2015; Bandopadhyay *et al*., 2021). Kamran *et al*. (2021) also recorded reduction in the leaf length, shoot length and root length of cowpea grown in zinc and biogas wastewater treated soil. But between the weeks, an inconsistent increase was observed in the leaf area and plant height of all groups. There was also an increase in shoot width and total number of leaves, when compared to the control. This result depicts that the plant was striving to develop a defense which enhances the tolerance capacity of the plant. The inconsistent increase in morphological parameters recorded between the weeks in the present study, indicates an improvement in the plant growth which might be as a result of the beneficial effect of copper treatment on the biochemical and physiological functions or activities of the plant (Vijayarengan and Mahalakshmi, 2013). Similar observation was reported by Sharma and Sharma (1993), Moustakas *et al*. (1994), and Vijayarengan (2012).

Cowpea tends to deplete the soil copper content by bioaccumulating it. This increases the level of copper in the plant tissues. From the results obtained in the present study, the copper content of the leaf exceeds that of the stem in almost all of the groups, because the tissues and cells in the leaf are more and contain more substances such as polyphenols, pigments and phytoalexins, which contribute effectively to the tolerant capacity of the plant (Sanginga *et al*., 2000; Carsky *et al*., 2001; Timko *et al*., 2007; Afutu *et al*., 2016). In addition, the tissues of the stem tend to translocate the nutrient elements (copper and others) to the leaves. This explains why the leaves are among the first organs in plants to be affected if a plant is exposed to any adverse condition.

Owing to the fact that plants bioaccumulate heavy metals, it is important to evaluate the probable health risk associated with the consumption of such plant (Chary *et al*., 2008; Jolly *et al*., 2013). Health risk assessment is a systematic process of surfing, collating and utilizing data for the enhancement or promotion of public health. In the current study, the health risk associated with the consumption of copper contaminated cowpea was assessed via estimated dietary intake (EDI) of copper, target hazard quotient (THQ) and carcinogenic risk (CR) (Food and Drug Administration (FDA), United States Environmental Protection Agency, 2011). A decrease in all three assessments were observed in the leaf of cowpea while an increase was recorded in the stem when compared to the negative control. Okeke

(2015) reported an increase in the dietary intake of heavy metal contaminated chicken. Similar, increase was reported by Khan *et al*. (2008). The target hazard quotient values of copper was observed to be less than 1 for the leaf and stem indicating that the consumption of the cowpea parts will leave the consumers with no health risk (Onuoha *et al*., 2016). Carcinogenic risk is a health risk assessment which estimates the probability of individuals consuming contaminated food to contract cancer over a lifetime of 70years (Onuoha *et al*., 2016). In the present study, the assessment of cancer risk from the bioaccumulation of copper as against the established guideline values did not indicate any possible carcinogenicity.

CONCLUSION

Heavy metals contamination has become a renowned global issue because of the detrimental impacts it has on the ecosystem. Natural processes such as volcanic eruption, earthquake, landslide and weathering of copper containing rocks, or anthropogenic activities which include; the use of waste water for irrigation, industrial activities, and the application of copper-based pesticides over a prolonged period of time are responsible for the introduction of heavy metals to the soil. The heavy bioaccumulation of copper by plants has adverse effects on their morphology, physiology and biochemistry and eventually distorts the food chain.

The consumption of such heavy metal contaminated plants poses a threat on the health of the individuals who consume such plants. In this study, the health risk associated with the consumption of copper contaminated cowpea seems to have an infinitesimal effect on the consumers and also no possibility of contracting cancer over a lifetime of 70years, at the concentrations considered in the study. Advance studies should include a chelating agent such as EDTA (ethylene diamine tetraacetic acid), higher concentration of copper and cultivating the plant to maturity.

CONTRIBUTION

The current study has contributed to knowledge in the following ways:

- The leaf of cowpea
- experiences much oxidative stress during metal contamination at (200mg/mL), but has little or no health risk when consumed.
- Copper contamination (due to the application of kocide pesticide) effects growth parameters in the leaf and stem of cowpea.

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