IN VITRO AND *IN VIVO* ANTIOXIDANT CAPACITY OF COWPEA (*VIGNA UNGUICULATA*) GROWN IN COPPER POLLUTED SOIL

ABSTRACT

Vigna unguiculata is a drought tolerant legume which serves as a staple in sub-Sahara West Africa region especially in Nigeria. Studies have shown that cowpea possesses adaptive features which enables it to strive in dry regions. The present study investigates the antioxiodant capacity of the leaf and stem of cowpea (*Vigna unguiculata*) grown in copper polluted soil. Exactly 2kg of soil was treated with 10mg/mL, 50mg/mL, 100mg/mL and 200mg/mL of copper sulphate stock solution (1g/L) 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 in one group (negative control) to obtain eight groups. The experimental plant, cowpea, was cultivated for five weeks. Data were collected after two weeks of cultivation and subsequent weeks until the fifth week, for the assays both on the homogenate and methanolic extract of the plant leaf and stem. The data obtained from the various assays conducted were subjected to analysis of variance (ANOVA) using Statistical Product and Service Solutions (SPSS) software (version 16). The results obtained showed that the malondialdehyde (MDA) level for both the leaf and stem showed an inconsistent increase (in lower dose of kocide, higher dose of kocide, 100mg/mL and 200mg/mL with 63.00 ± 8.82, 50.00 ± 5.77, 163.60 ± 3.33 and 157.00 ± 3.33 unit in mol/g wet weight × 10^{-4} respectively as against the negative control 73.00 ± 3.33MDA unit in mol/g wet weight × 10^{-4}) and decrease (in lower dose of kocide, higher dose of kocide, 100mg/mL and 200mg/mL with 17.00 ± 3.33, 23.00 ± 3.33, 17.00 ± 3.33 and 10.00 ± 0.00 unit in mol/g wet weight × 10^{-4} respectively as against the negative control 20.00 ± 5.77MDA unit in mol/g wet weight × 10^{-4}) both within and between weeks: depicting a stress condition in the plant. In the case of catalase, superoxide dismutase activities (SOD), total antioxidant capacity (TAC), ferric reducing antioxidant potential (FRAP), reducing power and nitric oxide (NO) free radical scavenging potentials; a consistent and significant increases within and between the weeks for both the leaf and stem were observed. The results obtained from the present study depict that *V. unguiculata* plant could tolerate a specific level of copper pollution (200mg/mL) by enhancing the activities of enzymic antioxidants and other features.

Key words: Copper, Cowpea, Antioxidant capacity 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)

Formatted: Numbering: Continuous

Formatted: Font color: Red

Formatted: Font color: Red

TA: Copper sulphate treatment (10mg/mL)

TB: Copper sulphate treatment (50mg/mL)

TC: Copper sulphate treatment (100mg/mL)

TD: Copper sulphate treatment (200mg/mL)

SEM: Standard error of mean.

INTRODUCTION

Soil is the basic and most essential part of the ecological system that serves as a natural medium where plants are cultivated (Thomas *et al*., 1999; Shahid *et al*., 2013). The ability of the soil to provide the essential nutrients, organic matters and water required for the production or cultivation of plant is known as soil fertility. The physicochemical and biochemical properties of the soil which are used to determine the fertility of the soil vary from region to regions (Thomas *et al*., 1999; Nyle *et al*., 2009; Kumar *et al*., 2013). Some physical properties of the soil such as temperature, aeration, moisture content and pH, and even the application of pesticides have been reported to influence the soil biochemical properties such as dehydrogenase activities (Kumar *et al*., 2013). Soil serves as the foundation for various forms of construction projects and the essential component of industries such as mining, construction and landscaping development industries. It is a reservoir of minerals and nutrients. It also serves as a harbor for living organisms and aids in curbing of flooding and drought (Ponge, 2003; De Deyn *et al*., 2005). Furthermore, soil is important in the recycling of nutrient elements such as carbon and nitrogen, and curbing green house effect (Thomas *et al*., 1999). The presence of harmful substances, pollutants, or even essential nutrient elements in a quantity beyond the required level, results in soil contamination or pollution.

Anvar and Selvaraju (2015) reported that environmental pollution in recent times poses a detrimental health hazard to plants, animals and human with local, regional and global implications. Pollution has negative impact on land, water or air and the biotic and abiotic components of the ecosystem (Anvar and Selvaraju, 2015). Heavy metals are the major substances that contribute to this menace and are not easily degradable. Such toxic substances include; lead (Pb), mercury (Hg), cadmium (Cd), copper (Cu) and zinc (Zn) (Bhatt *et al*., 2021). Other contaminants such as sewage, chemicals, smoke and waste also contribute to environmental pollution (Goyal and Chhibber, 2016). Heavy metals gain access into the soil via

anthropogenic activities such as industrial activities and agricultural practices which include; fertilizer application, liming of the soil and the use of pesticides, fungicides, and bactericides (Goyal and Chhibber, 2016). Heavy metals accumulation 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).

Copper-based fungicides and bactericides could introduce copper to the soil (Dong, 2013). The consequence of the prolonged application of these copper-based chemicals is an increase in copper concentration in the soil. The increase in the copper load in the soil has deleterious effects on plants cultivated in such soil.

Free radicals are chemical species, capable of independent existence and possessing one or more unpaired electrons (Dontha, 2016). Some chemical compounds in the cells or tissues of plants bind to these free radicals preventing them from imposing their debilitating effect on healthy cells or tissues (Dontha, 2016). These chemical compounds (substances) are called antioxidants: they oppose oxidation and break the free radical chain of reactions by sacrificing their own electrons to feed the free radicals, without becoming free radicals themselves (Dontha, 2016). In Plants, the antioxidant properties are provided by vitamins and phenolic compounds. Both substances possess this capacity because of their ability to donate electrons to free radicals thus, stabilizing or neutralizing the free radicals which are generated from metabolic process such as the electron transport chain and photosynthetic process or from severe environmental conditions. Thus, antioxidants create a defense system for plants against free radicals (Yuan, 2012; Upadhyay *et al*., 2020). However, further production of free radicals overwhelms the antioxidant defense system; resulting to an imbalance in the redox state of the plant system (Yadav, 2010; Rascio and Navari, 2011; Durackova, 2010). The above condition is called oxidative stress. Oxidative stress is induced in plants grown in a heavy metal polluted soil (Shahid *et al*., 2014). This imbalance or oxidative stress leads to the damage of important biomolecules (such as lipid, proteins and DNA) and cells, with potential impact on the whole organism (Durackova, 2010; Adrees *et al*., 2015; Wang *et al*., 2019; Tighe-Neira *et al*., 2022).

The drought tolerant and herbaceous legume, *Vigna unguiculata*, serves as a staple and feed crop cultivated in the tropical regions (Obatolu, 2003; Nair *et al*., 2008). The genus of *V. unguiculata* is *Vigna*, and it is of the order, family and tribe; *Fabales*, *Fabaceae* and *Phaseoleae* respectively (Perrino *et al*., 1993; Ng, 1995; Singh *et al*., 1997). Cowpea is consumed on a daily bases by over 200million people (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*., 2013). However, when cowpea is

Formatted: Font color: Red

cultivated in a heavy metal contaminated soil, its morphology, physiology and biochemistry change adversely with regards to the concentration of the contaminant (Anvar and Selvaraju, 2015).

Studies have shown that at minute concentrations, some heavy metals improve significantly the quality of crops (Anvar and Selvaraju, 2015; Singh *et al*., 2018). *V. unguiculata*, a drought tolerant crop that is consumed because of its high nutrient content, has been reported to show reduction in growth and biochemical parameters when grown in a high (toxic) copper contaminated soil (Rangel *et al*., 2003; Singh *et al*., 2003; Muhammad *et al*., 2015). The purpose of this study is to investigate the antioxidant capacity of the leaf and stem of cowpea (*Vigna unguiculata*) grown in copper polluted soil.

Formatted: Font color: Red

Formatted: Font color: Red

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.

Formatted: Font color: Red

Soil

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

Formatted: Font color: Red

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.

Sample Preparation

After the first two weeks of cultivation, parts (1g) of the plant (the leaf and stem) were collected, rinsed with distilled water and homogenized (using 10mL of chilled 0.1M sodium phosphate buffer, pH 8.0) to give the crude homogenate, while about 1g of some samples were air-dried, macerated and the methanolic extract was obtained, using about 10mL of methanol (Atawordi *et al*., 2003). Some biochemical studies were conducted on the methanolic extract and homogenate. These preparatory steps of the plant sample were repeated after the third, fourth and fifth weeks of cultivation.

Formatted: Font color: Red

Antioxidant Status

The methanolic extract and homogenate of the plant were subjected to antioxidant analysis.

In vivo Antioxidant Assays

The *in vivo* antioxidant assays were conducted on the homogenate of the plant leaf and stem.

The catalase activity was estimated using the method described by Cohen *et al*. (1970); superoxide dismutase activity was estimated as described by Misra and

Fridovich (1972) and the total antioxidant capacity was determined according to the method of Prieto *et al*. (1999).

In vitro Antioxidant Assays

The *in vitro* antioxidant assays were conducted on the methanolic extract of the plant leaf and stem.

A modified method of Benzie and Strain (1999) was adopted for Ferric ion reducing antioxidant potential assay; reducing power was determined according to the method described by Lai *et al*. (2001) and nitric oxide free radical scavenging activity was determined according to the method described by Marcocci *et al*. (1994).

Malondialdehyde (MDA) Determination for Lipid Peroxidation

Malondialdehyde level was determined according to the method of Gutteridge and Wilkins (1982).

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

LIPID PEROXIDATION DETERMINATION

Table 1: MDA assay values for leaf (MDA unit in mol/g wet weight)

Groups	Week 2 (Unit × 10^{-4})	Week 3 (Unit × 10^{-4})	Week 4 (Unit × 10^{-4})	Week 5 (Unit × 10^{-4})
NC	116.00 ± 0.33^{a}	150.00 ± 12.61^{b}	73.00 ± 3.33^{c}	393.00 ± 0.33^{d}
PC	53.00 ± 0.67*^{a}	101.00 ± 0.26^{a}	145.00 ± 48.20^{a}	257.00 ± 85.67*^{a}
LD	59.00 ± 1.53*^{a}	126.00 ± 2.49^{b}	83.00 ± 8.82^{c}	8.00 ± 0.00*^{d}
HD	163.00 ± 6.94*^{a}	99.00 ± 0.69^{b}	150.00 ± 5.77^{a}	199.00 ± 0.00*^{c}
TA	81.00 ± 1.16*^{a}	291.00 ± 96.91^{a}	175.00 ± 6.01^{a}	98.00 ± 32.62*^{a}
TB	124.00 ± 2.31^{a}	174.00 ± 1.08^{b}	77.00 ± 3.33^{c}	164.00 ± 0.39*^{d}
TC	55.00 ± 2.19*^{a}	221.00 ± 1.08^{b}	163.00 ± 3.33*^{c}	265.00 ± 0.15*^{d}
TD	154.00 ± 3.76*^{a}	161.00 ± 0.26^{a}	157.00 ±3.33* ^{a}	155.00 ± 0.15*^{a}

Values represent mean ± SEM of triplicate determinations.
*Indicates value with significant difference relative to the negative control *(P=0.05)* in each week.
Means with similar superscript letters between weeks do not differ significantly *(P=0.05)*.

Table 1 shows the results of MDA concentration for the leaf homogenate. Results obtained at the second week, show a significant increase *(P=0.05)* in the higher dose of kocide 50mg/mL and 200mg/mL copper treatments as against the negative control 116 ± 0.33MDA unit in mol/g wet weight. A tremendous increase was observed in the lower dose and copper treatments for week 3. At week 4, MDA concentration was increased in all the treatments as against the negative control: 73.00 ± 3.30MDA unit in mol/g wet weight, while at the fifth week, a decrease was observed (Table 1). For all groups (treatment and control), a significant *(P=0.05)* but inconsistent increase was observed; between the weeks excluding the positive control, 50mg/mL and 200mg/mL copper treatments.

Table 2: MDA assay values for stem (MDA unit in mol/g wet weight)

Groups	Week 2 (Unit × 10^{-4})	Week 3 (Unit × 10^{-4})	Week 4 (Unit × 10^{-4})	Week 5 (Unit × 10^{-4})
NC	50.00 ± 1.57^{a}	54.00 ± 4.33^{a}	20.00 ± 5.77^{b}	16.00 ± 0.15^{b}
PC	$47.00 \pm 0.67^{*a}$	$32.00 \pm 0.58^{*b}$	$10.00 \pm 3.33^{*c}$	$325.00 \pm 108.29^{*d}$
LD	$29.00 \pm 0.33^{*a}$	$23.00 \pm 0.33^{*b}$	17.00 ± 3.33^{c}	14.00 ± 0.15^{c}
HD	$64.00 \pm 0.33^{*a}$	$25.00 \pm 0.58^{*b}$	23.00 ± 3.33^{b}	14.00 ± 0.15^{c}
TA	$42.00 \pm 2.65^{*a}$	$51.00 \pm 17.16^{*b}$	$10.00 \pm 3.33^{*c}$	16.00 ± 5.17^{d}
TB	$42.00 \pm 2.65^{*a}$	$9.00 \pm 0.65^{*b}$	17.00 ± 3.33^{c}	21.00 ± 0.54^{c}
TC	$43.00 \pm 1.20^{*a}$	$34.00 \pm 0.39^{*b}$	17.00 ± 3.33^{c}	34.00 ± 0.26^{b}
TD	$29.00 \pm 1.33^{*a}$	37.00 ± 0.29^{b}	10.00 ± 0.00^{c}	16.00 ± 0.26^{d}

Values represent mean ± SEM of triplicate determinations.
*Indicates value with significant difference relative to the negative control *(P=0.05)* in each week.
Means with similar superscript letters between weeks do not differ significantly *(P=0.05)*.

Table 2 shows the level of MDA in the stem (homogenate) of the treated groups. MDA level tend to decrease in the treatments as against the negative control, with few exceptions in some treatments within each week. For weeks two and three, the decrease was significant *(P=0.05)* excluding the higher dose of kocide for week two only: 64.00 ± 0.3 and control 50.00 ± 1.57MDA units in mol/g wet weight. Furthermore, an increase in MDA concentration was observed in higher dose treatment only as against the negative control 20.00 ± 5.77MDA unit in mol/g wet weight for week 4. The 50mg/mL and 100mg/mL copper treatments: 21.00 ± 0.54 and 34.00 ± 0.26MDA units respectively had higher values as against the negative control: 16.00 ± 0.15MDA unit for week 5. Between the weeks, a quite significant decrease was observed in the negative control and treatments *(P=0.05)*. A remarkable decrease in MDA concentration was recorded comparing the stem to the leaf.

IN VIVO ANTIOXIDANT ASSAYS (ON THE HOMOGENATE)

Table 3: CATALASE assay values for leaf (Catalase unit at 30seconds in mol/g wet weight)

Groups	Week 2 (Unit × 10^{-2})	Week 3 (Unit × 10^{-2})	Week 4 (Unit × 10^{-2})	Week 5 (Unit × 10^{-2})
NC	9.58 ± 0.11^{a}	82.37 ± 1.56^{b}	50.00 ± 2.03^{c}	218.76 ± 1.43^{d}
PC	$81.08 \pm 39.82^{*a}$	$198.57 \pm 5.03^{*b}$	56.40 ± 18.90^{b}	216.37 ± 72.12^{a}
LD	$56.47 \pm 0.54^{*a}$	$204.43 \pm 1.70^{*b}$	46.73 ± 1.45^{c}	205.88 ± 1.02^{b}
HD	36.66 ± 17.22^{a}	113.77 ± 5.03^{b}	69.63 ± 1.76^{c}	206.99 ± 1.27^{d}
TA	31.50 ± 0.73^{a}	97.37 ± 48.91^{a}	$132.90 \pm 66.59^{*a}$	142.55 ± 71.28^{a}
TB	41.57 ± 0.44^{a}	108.97 ± 0.58^{b}	54.60 ± 2.26^{c}	181.98 ± 1.05^{d}
TC	35.25 ± 0.58^{a}	$197.80 \pm 2.57^{*a}$	49.40 ± 0.90^{a}	129.51 ± 4.27^{a}
TD	$211.12 \pm 10.42^{*a}$	$198.50 \pm 38.01^{*b}$	72.60 ± 1.50^{c}	197.00 ± 1.05^{d}

Values represent mean ± SEM of triplicate determinations.
*Indicates value with significant difference relative to the negative control *(P=0.05)* in each week.
Means with similar superscript letters between weeks do not differ significantly *(P=0.05)*.

Catalase activity for the leaf (homogenate) increased quite significantly for the first three weeks in the treatments as against the negative control, 9.58 ± 0.11, 82.37 ± 1.56 and 50.00 ± 2.03 catalase unit at 30seconds in mol/g wet weight for respective weeks (*P=0.05*, Table 3). However, at the fifth week, the activity of catalase reduced. Between weeks, a significant but inconsistent increase in catalase activity was observed (P=*0.05*).

Table 4: CATALASE assay values for stem (Catalase unit at 30seconds in mol/g wet weight)

Groups	Week 2 (Unit × 10^{-2})	Week 3 (Unit × 10^{-2})	Week 4 (Unit × 10^{-2})	Week 5 (Unit × 10^{-2})
NC	8.88 ± 0.39^{a}	75.63 ± 1.39^{b}	3.03 ± 0.28^{c}	165.63 ± 2.51^{d}
PC	$8.80 \pm 0.55^{*a}$	86.00 ± 1.21^{b}	$40.60 \pm 12.9^{*b}$	210.41 ± 69.83^{a}
LD	$42.23 \pm 0.33^{*a}$	94.03 ± 1.65^{a}	$37.17 \pm 1.45^{*b}$	202.70 ± 2.51^{b}
HD	$42.32 \pm 0.27^{*a}$	92.87 ± 2.52^{b}	$57.67 \pm 0.79^{*c}$	197.58 ± 0.83^{d}
TA	$35.67 \pm 0.33^{*a}$	91.75 ± 30.99^{a}	$37.15 \pm 12.38^{*a}$	212.53 ± 70.84^{a}
TB	$43.51 \pm 1.39^{*a}$	$39.47 \pm 4.01^{*b}$	$43.07 \pm 1.92^{*b}$	126.37 ± 3.07^{c}
TC	$43.89 \pm 0.62^{*a}$	54.60 ± 1.64^{b}	$44.70 \pm 0.21^{*a}$	182.89 ± 0.86^{c}
TD	$18.66 \pm 0.95^{*a}$	46.13 ± 0.48^{b}	$37.17 \pm 0.68^{*c}$	157.54 ± 0.96^{d}

Values represent mean ± SEM of triplicate determinations.
*Indicates value with significant difference relative to the negative control *(P=0.05)* in each week.
Means with similar superscript letters between weeks do not differ significantly *(P=0.05)*.

In the stem homogenate, catalase activity followed a similar trend as in the leaf, but a higher significant increase was observed between the first alternating weeks at *P=0.05* (Table 4).

Table 5: SOD assay values for leaf (SOD Activity at 60seconds in units/g)

Groups	Week 2 (Unit × 10^{-2})	Week 3 (Unit × 10^{-2})	Week 4 (Unit × 10^{-2})	Week 5 (Unit × 10^{-2})
NC	198.63 ± 0.02^{a}	199.22 ± 0.04^{b}	198.63 ± 0.05^{a}	199.63 ± 0.00^{c}
PC	$199.16 \pm 0.00^{*a}$	199.07 ± 0.04^{a}	199.28 ± 66.43^{a}	199.15 ± 66.38^{a}
LD	198.40 ± 0.00^{a}	198.66 ± 0.10^{b}	199.26 ± 0.01^{c}	198.98 ± 0.28^{d}
HD	$198.07 \pm 0.02^{*a}$	199.42 ± 0.03^{b}	198.82 ± 0.03^{c}	199.12 ± 0.01^{d}
TA	198.62 ± 0.32^{a}	198.71 ± 66.24^{a}	198.93 ± 6.63^{a}	199.12 ± 66.37^{a}
TB	$199.17 \pm 0.01^{*a}$	199.07 ± 0.02^{a}	198.64 ± 0.07^{b}	198.90 ± 0.26^{c}
TC	198.89 ± 0.00^{a}	199.57 ± 0.01^{a}	195.48 ± 0.58^{b}	199.76 ± 0.00^{c}
TD	$199.07 \pm 0.00^{*a}$	199.36 ± 0.02^{b}	198.46 ± 0.07^{c}	199.37 ± 0.01^{c}

Values represent mean ± SEM of triplicate determinations.
*Indicates value with significant difference relative to the negative control *(P=0.05)* in each week.
Means with similar superscript letters between weeks do not differ significantly *(P=0.05)*.

In the negative control and the treatments within the weeks, a significant but inconsistent increase was observed for the leaf homogenate as presented in Table 5. A significant increase was also recorded between the weeks when compared to both the negative control and treatments at *P=0.05*.

Table 6: SOD assay values for stem (SOD Activity at 60seconds in units/g)

Groups	Week 2 (Unit × 10^{-2})	Week 3 (Unit × 10^{-2})	Week 4 (Unit × 10^{-2})	Week 5 (Unit × 10^{-2})
NC	197.65 ± 0.01^{a}	158.89 ± 4.84^{b}	198.39 ± 0.03^{a}	195.84 ± 0.23^{a}
PC	198.30 ± 0.02^{a}	$194.58 \pm 0.53^{*a}$	197.83 ± 65.94^{a}	196.24 ± 65.41^{a}
LD	197.94 ± 0.01^{a}	$191.52 \pm 2.13^{*b}$	194.92 ± 0.29^{c}	197.57 ± 0.15^{d}
HD	196.48 ± 0.19^{a}	$159.19 \pm 29.62^{*a}$	196.66 ± 0.29^{a}	197.05 ± 0.10^{a}
TA	197.95 ± 0.01^{a}	$191.89 \pm 63.96^{*a}$	197.66 ± 65.89^{a}	197.16 ± 65.72^{a}
TB	198.29 ± 0.01^{a}	$193.91 \pm 0.53^{*b}$	197.37 ± 0.31^{c}	197.36 ± 0.25^{d}
TC	197.89 ± 0.07^{a}	$193.06 \pm 2.80^{*a}$	194.55 ± 0.58^{b}	197.21 ± 0.13^{b}
TD	198.49 ± 0.01^{a}	$197.37 \pm 0.17^{*b}$	196.49 ± 0.88^{a}	197.45 ± 0.09^{b}

Values represent mean ± SEM of triplicate determinations.
*Indicates value with significant difference relative to the negative control *(P=0.05)* in each week.
Means with similar superscript letters between weeks do not differ significantly *(P=0.05)*.

Table 6 shows SOD activity of the stem homogenate and the results obtained showed an increase within the weeks with a significant increase *(P=0.05)* in week 3.

Table 7: TAC values for leaf (Concentration in µg/mL)

Groups	Week 2 (Unit × 10^{-1})	Week 3 (Unit × 10^{-1})	Week 4 (Unit × 10^{-1})	Week 5 (Unit × 10^{-1})
NC	6.30 ± 0.91^{a}	7.18 ± 0.46^{a}	51.10 ± 1.23^{b}	28.00 ± 4.63^{c}
PC	8.05 ± 0.93^{a}	5.43 ± 0.76^{a}	$7.61 \pm 2.58^{*a}$	37.28 ± 12.44^{a}
LD	4.90 ± 0.46^{a}	9.80 ± 0.63^{b}	$16.63 \pm 0.76^{*c}$	27.65 ± 0.63^{d}
HD	$12.25 \pm 0.76^{*a}$	$12.60 \pm 2.19^{*b}$	$16.80 \pm 0.30^{*c}$	18.90 ± 0.61^{d}
TA	$17.15 \pm 0.46^{*a}$	$1.05 \pm 0.46^{*a}$	$46.20 \pm 1.54^{*a}$	34.13 ± 11.39^{a}
TB	$13.30 \pm 0.46^{*a}$	$3.15 \pm 0.61^{*b}$	$15.40 \pm 0.46^{*c}$	24.33 ± 0.76^{d}
TC	8.05 ± 0.46^{a}	7.18 ± 1.53^{a}	$10.33 \pm 0.63^{*b}$	40.08 ± 0.76^{c}
TD	$8.75 \pm 0.18^{*a}$	$18.90 \pm 1.82^{*b}$	$15.93 \pm 0.46^{*a}$	$48.83 \pm 1.32^{*c}$

Values represent mean ± SEM of triplicate determinations.
*Indicates value with significant difference relative to the negative control *(P=0.05)* in each week.
Means with similar superscript letters between weeks do not differ significantly *(P=0.05)*.

The results for the total antioxidant capacity for the leaf homogenate show a significant increase in the treatments as against the negative control: 6.30 ± 0.91 µg/mL (Table 7, *P=0.05*). For the weeks 3 and 5, the increase in TAC was inconsistent as compared to the negative control. Week 4 had a significant decrease in the treatments as against negative control, 51.10 ± 1.23 µg/mL at *P=0.05* (Table 7). Between the weeks, a quite significant increase was observed (*P=0.05)*.

Table 8: TAC values for stem (Concentration in µg/mL)

Groups	Week 2 (Unit × 10^{-1})	Week 3 (Unit × 10^{-1})	Week 4 (Unit × 10^{-1})	Week 5 (Unit × 10^{-1})
NC	1.75 ± 0.17^{a}	2.45 ± 0.93^{a}	37.28 ± 0.61^{b}	15.23 ± 0.91^{c}
PC	$5.78 \pm 0.30^{*a}$	$5.95 \pm 0.46^{*a}$	$4.99 \pm 1.67^{*a}$	19.95 ± 6.66^{a}
LD	2.28 ± 0.46^{a}	3.68 ± 0.80^{a}	$5.60 \pm 0.63^{*b}$	24.33 ± 0.35^{c}
HD	1.05 ± 0.30^{a}	3.50 ± 0.46^{b}	$7.18 \pm 0.46^{*c}$	$26.43 \pm 0.18^{*d}$
TA	$7.53 \pm 0.80^{*a}$	3.15 ± 1.39^{a}	$11.56 \pm 3.90^{*a}$	19.43 ± 6.54^{a}
TB	0.88 ± 0.30^{a}	$9.80 \pm 1.23^{*b}$	$1.05 \pm 0.30^{*b}$	18.33 ± 0.80^{c}
TC	$3.33 \pm 0.80^{*a}$	2.63 ± 0.91^{a}	$5.78 \pm 0.30^{*b}$	23.80 ± 0.93^{c}
TD	$28.18 \pm 0.30^{*a}$	$5.78 \pm 0.30^{*b}$	$9.98 \pm 0.61^{*c}$	24.15 ± 0.30^{d}

Values represent mean ± SEM of triplicate determinations.
*Indicates value with significant difference relative to the negative control *(P=0.05)* in each week.
Means with similar superscript letters between weeks do not differ significantly *(P=0.05)*.

In the stem homogenate, an increase in TAC of the treatments as against the negative control was observed within the weeks excluding week 4, where a significant decrease was observed at *P=0.05* (Table 8). Similar trend of a significant increase was recorded between the week (*P=0.05*). The TAC value for the stem is quite lower than the leaf both within and between the weeks (Table 8).

IN VITRO ANTIOXIDANT ASSAYS (ON THE METHANOLIC EXTRACT)

Table 9: FRAP assay (Concentration in µmol/g of leaf extract)

Groups	Week 2	Week 3	Week 4	Week 5
NC	446.35 ± 4.11^{a}	620.98 ± 5.09^{b}	854.48 ± 1.17^{c}	706.07 ± 7.08^{d}
PC	611.08 ± 1.47^{a}	466.15 ± 5.38*^{b}	693.09 ± 8.66*^{c}	831.32 ± 1.68*^{d}
LD	674.26 ± 7.31*^{a}	406.18 ± 1.44*^{b}	507.58 ± 8.55*^{c}	745.96 ± 1.22^{d}
HD	613.87 ± 6.80^{a}	612.90 ± 8.65^{a}	859.08 ± 1.10^{b}	998.28 ± 4.66*^{c}
TA	551.24 ± 4.89^{a}	444.53 ± 1.89*^{a}	705.93 ± 7.79*^{a}	633.81 ± 6.41*^{a}
TB	451.23 ± 9.38^{a}	545.80 ± 6.03*^{b}	741.49 ± 6.73*^{c}	798.26 ± 6.80*^{d}
TC	612.05 ± 2.32^{a}	632.00 ± 4.62^{a}	728.38 ± 3.75*^{b}	679.14 ± 3.02^{b}
TD	624.96 ± 2.08^{a}	829.09 ± 1.33*^{b}	786.55 ± 0.74*^{c}	592.39 ± 1.16*^{d}

Values represent mean ± SEM of triplicate determinations.
*Indicates value with significant difference relative to the negative control *(P=0.05)* in each week.
Means with similar superscript letters between weeks do not differ significantly *(P=0.05)*.

In the leaf extract, the activity for FRAP increased at week 2 with the treatments as against the negative control (446.35 ± 4.11µmol/g) and also a significant increase was observed in the fifth week with the treatments as compared to the negative control (706.07 ± 7.08µmol/g) (*P=0.05*; Table 9). A significant decrease was observed within weeks 3 and 4. Between the weeks, there was significant but inconsistent increase at *P=0.05*.

Table 10: FRAP assay (Concentration in µmol/g of stem extract)

Groups	Week 2	Week 3	Week 4	Week 5
NC	444.67 ± 4.30^{a}	345.64 ± 9.32^{b}	603.54 ± 5.71^{c}	775.53 ± 1.69^{d}
PC	$561.84 \pm 6.03^{*a}$	358.61 ± 5.18^{b}	616.94 ± 4.80^{a}	$947.23 \pm 4.66^{*c}$
LD	$227.22 \pm 9.63^{*a}$	367.68 ± 7.74^{b}	$514.00 \pm 14.87^{*c}$	776.36 ± 4.69^{d}
HD	$521.95 \pm 1.02^{*a}$	$468.39 \pm 5.82^{*b}$	627.12 ± 1.83^{c}	$952.39 \pm 2.61^{*d}$
TA	$310.63 \pm 7.83^{*a}$	$303.38 \pm 1.01^{*b}$	$430.45 \pm 4.27^{*c}$	$543.29 \pm 1.47^{*d}$
TB	$472.01 \pm 1.67^{*a}$	326.81 ± 5.44^{b}	604.94 ± 6.93^{c}	$551.24 \pm 1.54^{*d}$
TC	$543.71 \pm 0.85^{*a}$	353.45 ± 8.28^{b}	$649.43 \pm 1.60^{*c}$	$551.66 \pm 1.40^{*d}$
TD	$732.99 \pm 1.15^{*a}$	$520.83 \pm 2.79^{*b}$	$703.42 \pm 3.61^{*c}$	756.56 ± 2.93^{d}

Values represent mean ± SEM of triplicate determinations.
*Indicates value with significant difference relative to the negative control *(P=0.05)* in each week.
Means with similar superscript letters between weeks do not differ significantly *(P=0.05)*.

In the stem extract, the FRAP activity for weeks 2, 3 and 4 had significant but inconsistent increase in the treatment as against the negative control at *P=0.05* (Table 10). For the fifth week, the FRAP values increase in positive control, lower and higher doses of kocide with values: 947.23 ± 4.66µmol/g, 952.39 ± 2.61µmol/g and 776.36 ± 4.69µmol/g of stem extract respectively as against 755.53 ± 1.69µmol/g of negative control (Table 10). An inconsistent increase was observed in each group between weeks; while the value of leaf tends to be quite higher than the stem *(P=0.05)*.

Table 11: Reducing power assay (Concentration in µmol/g of leaf extract)

Groups	Week 2	Week 3	Week 4	Week 5
NC	17.59 ± 0.68^{a}	39.99 ± 0.74^{b}	43.41 ± 0.84^{b}	269.72 ± 4.08^{c}
PC	15.43 ± 1.35^{a}	$159.01 \pm 3.97^{*b}$	$215.92 \pm 13.53^{*c}$	264.15 ± 4.39^{d}
LD	20.33 ± 0.45^{a}	37.77 ± 1.45^{b}	$97.05 \pm 5.16^{*c}$	269.42 ± 1.36^{d}
HD	21.37 ± 0.46^{a}	47.19 ± 0.59^{b}	$118.72 \pm 1.00^{*c}$	$359.65 \pm 4.31^{*d}$
TA	19.22 ± 1.68^{a}	33.91 ± 1.97^{a}	$26.04 \pm 1.61^{*a}$	$154.26 \pm 2.66^{*b}$
TB	19.37 ± 1.05^{a}	37.77 ± 0.32^{b}	$96.83 \pm 1.14^{*c}$	$52.53 \pm 0.84^{*d}$
TC	27.90 ± 0.97^{a}	44.09 ± 6.96^{b}	$120.80 \pm 0.93^{*c}$	$58.25 \pm 0.86^{*b}$
TD	34.28 ± 11.45^{a}	40.88 ± 1.05^{b}	36.51 ± 0.68^{b}	$31.24 \pm 2.09^{*c}$

Values represent mean ± SEM of triplicate determinations.
*Indicates value with significant difference relative to the negative control *(P=0.05)* in each week.
Means with similar superscript letters between weeks do not differ significantly *(P=0.05)*.

The results in the leaf extract for weeks 2, 3 and 4 had an inconsistent increase in most of the treatments with values: 17.59 ± 0.68µmol/g, 39.99 ± 0.74µmol/g and 43.41 ± 0.84µmol/g of extract for the negative control of the respective weeks (Table 11) with significant difference at *P=0.05* for week 4. For the fifth week, a reduction was observed in the values compared, most of which were significant at *P=0.05*: while between the weeks (columns), there was significant increase in most of the values at *P=0.05*.

Table 12: Reducing power assay (Concentration in µmol/g of stem extract)

Groups	Week 2	Week 3	Week 4	Week 5
NC	16.62 ± 1.39^{a}	37.55 ± 0.96^{b}	24.26 ± 0.84^{c}	149.51 ± 0.20^{d}
PC	18.33 ± 1.34^{a}	34.50 ± 2.12^{b}	$92.75 \pm 1.53^{*c}$	151.81 ± 1.14^{d}
LD	18.03 ± 1.94^{a}	34.80 ± 0.52^{b}	25.23 ± 0.78^{c}	147.06 ± 0.37^{d}
HD	19.14 ± 0.77^{a}	43.93 ± 0.78^{b}	$28.20 \pm 0.32^{*c}$	$158.05 \pm 0.34^{*d}$
TA	$30.35 \pm 0.63^{*a}$	$25.82 \pm 8.61^{*b}$	$33.69 \pm 0.27^{*b}$	$97.87 \pm 2.21^{*c}$
TB	20.18 ± 1.75^{a}	35.48 ± 6.84^{a}	25.23 ± 0.27^{b}	$26.04 \pm 0.26^{*b}$
TC	15.81 ± 1.02^{a}	38.36 ± 2.16^{b}	$27.97 \pm 0.75^{*c}$	$31.02 \pm 0.90^{*c}$
TD	85.40 ± 0.54^{a}	$50.38 \pm 3.57^{*b}$	$34.65 \pm 0.97^{*c}$	$31.09 \pm 0.83^{*c}$

Values represent mean ± SEM of triplicate determinations.
*Indicates value with significant difference relative to the negative control *(P=0.05)* in each week.
Means with similar superscript letters between weeks do not differ significantly *(P=0.05)*.

In the stem extract, the reducing potential of the extract increased in the treatments as against the negative control for weeks 2 and 4 (Table 12). In contrast, a significant decrease was seen at week 5 in the treatments as against the negative control *P=0.05*. A variation in the trend was observed except for the 200mg/mL treatment (*P=0.05*), while between the leaf and stem only a slight difference was observed.

Table 13: NO radical scavenging assay (Concentration in µmol/g of leaf extract)

Groups	Week 2	Week 3	Week 4	Week 5
NC	13.82 ± 0.12^{a}	27.46 ± 0.49^{b}	25.49 ± 0.08^{c}	24.56 ± 0.32^{c}
PC	$16.69 \pm 0.27^{*a}$	$36.26 \pm 0.31^{*b}$	$42.03 \pm 0.30^{*c}$	$25.67 \pm 0.15^{*d}$
LD	13.95 ± 0.28^{a}	$48.40 \pm 0.23^{*b}$	$46.52 \pm 0.22^{*c}$	$36.44 \pm 0.17^{*d}$
HD	14.32 ± 0.34^{a}	$32.30 \pm 0.07^{*b}$	$39.69 \pm 0.23^{*c}$	$50.78 \pm 0.04^{*d}$
TA	$25.09 \pm 0.55^{*a}$	$36.95 \pm 0.10^{*b}$	$39.24 \pm 0.82^{*c}$	$21.13 \pm 0.04^{*d}$
TB	$27.19 \pm 0.33^{*a}$	$37.73 \pm 0.12^{*b}$	$44.13 \pm 0.28^{*c}$	$33.51 \pm 0.19^{*d}$
TC	13.52 ± 0.31^{a}	$43.28 \pm 0.04^{*b}$	$27.17 \pm 0.04^{*c}$	$25.71 \pm 0.11^{*d}$
TD	$34.26 \pm 0.45^{*a}$	$35.55 \pm 0.12^{*b}$	$22.40 \pm 0.26^{*c}$	$10.00 \pm 0.12^{*d}$

Values represent mean ± SEM of triplicate determinations.
*Indicates value with significant difference relative to the negative control *(P=0.05)* in each week.
Means with similar superscript letters between weeks do not differ significantly *(P=0.05)*.

A significant increase was observed in the nitric oxide (NO) scavenging activity for the leaf extract within each week in the treatment as against the negative control at *P=0.05* (Table 13), while between the weeks a tremendous but inconsistent increase was observed (*P=0.05*).

Table 14: NO radical scavenging assay (Concentration in µmol/g of stem extract)

Groups	Week 2	Week 3	Week 4	Week 5
NC	12.54 ± 0.11^{a}	59.31 ± 3.53^{b}	54.50 ± 0.17^{b}	28.39 ± 0.13^{c}
PC	$23.20 \pm 0.46^{*a}$	$22.15 \pm 0.22^{*b}$	$33.05 \pm 0.20^{*c}$	$43.53 \pm 0.20^{*d}$
LD	$17.88 \pm 0.34^{*a}$	$66.31 \pm 0.31^{*b}$	$63.20 \pm 0.07^{*c}$	$51.79 \pm 0.03^{*d}$
HD	$21.75 \pm 0.52^{*a}$	$33.25 \pm 0.16^{*b}$	$68.20 \pm 0.19^{*c}$	$32.86 \pm 0.02^{*d}$
TA	$25.13 \pm 0.36^{*a}$	$37.82 \pm 0.20^{*b}$	$27.91 \pm 0.13^{*c}$	$47.45 \pm 0.11^{*d}$
TB	$25.68 \pm 0.26^{*a}$	$36.10 \pm 0.20^{*b}$	$41.11 \pm 0.15^{*c}$	$30.61 \pm 0.19^{*d}$
TC	$33.17 \pm 2.16^{*a}$	$31.17 \pm 0.19^{*a}$	$44.66 \pm 0.18^{*b}$	$45.78 \pm 0.18^{*b}$
TD	$25.65 \pm 0.26^{*a}$	$66.96 \pm 0.33^{*b}$	$52.20 \pm 0.38^{*c}$	$48.27 \pm 0.05^{*d}$

Values represent mean ± SEM of triplicate determinations.
*Indicates value with significant difference relative to the negative control *(P=0.05)* in each week.
Means with similar superscript letters between weeks do not differ significantly *(P=0.05)*.

In the stem extract, a significant increase was observed in the treatments as against the negative control (*P=0.05*) for weeks 2 and 5, whereas there was a significant but inconsistent decrease in the treatments as compared to the negative control (*P=0.05*) for weeks 3 and 4 (Table 14). A significant but inconsistent increase was observed between the weeks at *P=0.05*, while the leaf extract values differed from the stem slightly (Table 14).

Table 15: 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.

Formatted: Font color: Red

Formatted: Font color: Red

The results presented in Table 15 from the soil analysis conducted showed that the soil used in this study met the prerequisite for a sandy-loam.

DISCUSSION

Heavy metals find their way into the soil and eventually the food chain via anthropogenic activities. Heavy metals have detrimental effects on plant growth, food supply and economics. Bioavailability of heavy metals could result to toxic substances depending on their concentration in living organisms such as plants, animals and microorganisms which tend to bioaccumulate them (Shahid *et al*., 2014). However, the bioaccumulation of heavy metals depends on several factors which include organic matter content, available phosphorus content and clay particles in the soil (Ferraz and Lourençlo, 2004; Aigberua, 2018). Also, the presence of other elements such as calcium, magnesium and aluminium, influences the uptake of heavy metals (Schulte and Kelling, 1999) through chelating heavy metals, thus leaching or reducing their bioavailability (Shahid *et al*., 2014). Plants bioaccumulate and translocate heavy metals to various organs, tissues and cells (Singh *et al*., 2018). Bioaccumulation of high concentration of heavy metals results in phytotoxicity (Guala *et al*., 2010). At toxic level, the heavy metals disrupt metabolic and physiological processes, and eventually growth retardation (Guala *et al*., 2010; Wani *et al*., 2019).

Plants often develop an antioxidant defense mechanism which alleviates the oxidative stress imposed on the plant system by these metals. However, some plants synthesize both enzymic and non-enzymic antioxidants and utilize other special mechanisms for curbing the stress. Such tolerant plants are used as 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. The values for the pH, particulate nature, copper content and organic matter content of the soil met the requirements for a sandy-loam (Thomas *et al*., 1999).

Formatted: Font color: Red

Heavy metals are potentially toxic and their bioaccumulation in plant system results to various physiological and biochemical dysfunctions such as oxidative stress, yield depression, depletion of nutrient uptake, metabolic disruption (disorder) and disruption in the symbiotic relationship between cowpea and nitrogen fixing microbes (Guala *et al*., 2010; Adesina and Adelasoye, 2014; Asiamah *et al*., 2021). Plants cultivated in a heavy metal polluted soil tend to undergo oxidative stress because of the profuse free radicals produced.

Consequently, lipid peroxidation occurs whose hallmark is an elevated level of malondialdehyde (MDA). In the present study, the level/concentration of MDA recorded in the leaf quite consistently increased both within the weeks and between the weeks; and the increase was significant (*P=0.05*). In contrast, a significant decrease was observed in the stem both within and between weeks. An elevated level of MDA was recorded for the leaf as against the stem. The increase/elevated level of MDA in the leaf was supported by the earlier report of Goyal and Chhibber (2016) which showed an increase in MDA content in mung beans treated with manganese (Mn) as against control. The increase in the MDA level of the leaf shows that the plant tissues in the leaf were undergoing lipid peroxidation, which indicates that the experimental plant was stressed up in the course of the cultivation. However, there was a reduction in the MDA concentration between the weeks. This could probably be as a result of the translocation of the toxicant after it has been taken up from the stem to other tissues. In addition, the chelation of the heavy metals by some soluble species in the stem facilitates their translocation which could reduce the MDA level in the stem (Barbadier *et al*., 2012; Apodaca *et al*., 2017).

Plants cultivated in a heavy metal (copper) contaminated site are subjected to oxidative stress condition where excess free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), are generated (Anjum *et al*., 2015; Goyal and Chhibber, 2016; Apodaca *et al*., 2017). The consequences of the profuse amount of ROS is deleted directly or indirectly by detoxification or scavenging the numerous free radicals (Anjum *et al*., 2015). The system responsible for the alleviation of ROS mediated oxidative stress condition is referred to as the antioxidant defense system. It consists of the enzymic antioxidants such as, catalase (CAT), superoxide dismutase (SOD), guaiacol peroxidase (GPX), glutathione sulfotransferase (GST) and glutathione reductase (GR); and the non-enzymic antioxidant often called the lower molecular weight scavengers such as carotenoids, tocopherols, phenolics, glutathione and ascorbate, which could be estimated via ferric ion reducing antioxidant power assay, reducing power assay, total antioxidant capacity assay and nitric oxide radical scavenging assay (Anjum *et al*., 2015; Goyal and Chhibber *et al*., 2016; Apodaca *et al*., 2017).

Catalase, a heavy metal toxicity bioindicator is an enzyme that scavenges for hydrogen peroxide (H_2O_2) in order to deplete its toxic level in cells, and eventually

alleviates oxidative damage in tissues. In the present study, a significant increase in CAT activity was observed both within and between the weeks in the leaf and stem. Much significant increase was observed in the stem as against the leaf. This increase in activity consequently would lead to the depletion of the causative agencies of oxidative stress. This was indicated by the reduced levels of MDA as presented in Tables 1 and 2. Similar decrease in MDA levels of plant cultivated in a heavy metal contaminated soil due to the increased activity of antioxidant enzymes was reported by Anjum *et al*., 2015; Goyal and Chhibber *et al*., 2016; Apodaca *et al*., 2017. Another vital antioxidant enzyme SOD assists in the curbing of oxidative stress condition. The increase in the activity of SOD in the leaf and stem homogenate both within and between the weeks; is a reflection of the adaptive nature of the cowpea at the concentration of copper considered. Furthermore, the significant difference recorded between the weeks and also between the leaf and stem buttresses the response of cowpea to salvage oxidative stress. In the study by Olubodun and Eriyamremu (2013), a reduction in activities of SOD in maize radical grown in a crude oil contaminated soil was observed. In contrast, other studies recorded an increase in the activities of SOD (Anjum *et al*., 2015; Goyal and Chhibber *et al*., 2016; Apodaca *et al*., 2017). The latter finding supports the claim of the present study.

The total antioxidant capacity of the cowpea parts considered in this study was observed to have increased both within and between the weeks: suggesting a probable adaptive nature of the plant. In general, the ferric ion reducing antioxidant potential (FRAP) and the reducing power parameters conducted on the methanolic extract, evaluate the scavenging capacity of a plant. A quite consistent increase in FRAP and reducing power was recorded both within and between the weeks, while between the parts considered, there was quite a significant increase. This shows that as the cultivation period increases, the plant tends to develop adaptive features to withstand the adverse condition induced by the heavy metal, copper.

Another scavenging potential of plant determinant is nitric oxide radical scavenging assay. Nitric oxide is a signaling molecule involved in the defense system developed to ameliorate oxidative stress in plants (Sadeghipour, 2017). In the current study, an inconsistent increase in nitric oxide radical scavenging properties of the methanolic extract within and between weeks for both the leaf and

stem were observed. In addition, the radical scavenging capacity of the leaf quite exceeds that of the stem. The result obtained in the current study is in agreement with that recorded by previous studies (Sadeghipour, 2017; Sadeghipour, 2018).

Lisa and Ching (2003) observed that non-enzmic antioxidants such as tocopherol and ascorbic acid, play a vital role in alleviating the impacts of copper-induced oxidative stress by depleting the concentration of free radicals generated in the process. Previous studies had presented similar report of an increase in nitric oxide level in response to lead-induced oxidative stress in cowpea (Sadeghipour, 2016; Bücker-Neto *et al*., 2017; Sadeghipour, 2017). The increase in the level of *in vitro* antioxidant defense potentials evaluated via various assays demonstrates the adaptive features developed by the plant to check oxidative damage, that is, increase in heavy metal tolerance in cowpea (Xiaong *et al*., 2010; Sadeghipour, 2017).

CONTRIBUTION

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

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

REFERENCES

Adesina, G.O. and Adelasoye, K.A. (2014). Effects of crude oil pollution on heavy metal contents, microbial population in soil; and maize and cowpea growth. *Agricultural Science.* **5**:43-50.

Adrees, M., Ali, S., Rizwan, M., Ibrahim, M., Abbas, F. and Farid, M. (2015). The effect of excess copper: A review on growth and physiology of important food crops. *Environmental Science and Pollution Research*. **22**:8148–8162.

Aigberua, A. and Tarawou, T. (2018). Speciation and mobility of selected heavy metals in sediments of the Nun-river system, Bayelsa State, Nigeria. *Environmental Toxicological Studies Journal*. **2**(1):1-9.

Anjum, N.A., Adam, V., Kizek, R., Duarte, A.C., Pereira, E., Igbal, M., Lukatin, A.S. and Ahmad, I. (2015). Nanoscale copper in the soil-plant system-toxicity and underlying potential mechanisms. *Elsevier, Environmental Research*. **138**(13): 306-325.

Anvar, A.S. and Selvaraju, M. (2015). Growth and biochemical contents of cowpea (*Vigna unguiculata*) on the application of zinc. *World Scientific News.* **10**:73-83.

Apodaca, S., Wenjuan, T., Osvatdo, E.D.M., Jose, A.H., Jose, R.P. and Jorge, L.G. (2017). Physiological and biochemical effects of nanoparticulate copper, bulk copper, copper chloride and kinetin in kidney bean plants. *Science of the Total Environment*. **599**(10):2085-2094.

Asiamah, J.Y., Otwe, P.E., Danquah, A., Apori, S.O. and Hanyabui, E. (2021). Effect of spilled engine oil on soil quality indicators and physiological performance of maize, cowpea and sorghum. *Afr. J. Environ. Sci. Technol.* **15**(7):262-269.

Atawordi, S.E., Bulus, T., Ibrahim, S., Ameh, D.A., Nok, A.J., Mamman, M. and Galadima, M. (2003). *In vitro* trypanocidal effect of methanolic extract of some Nigerian savannah plants. *African Journal of Biotechnology*. **2**(9):317-321.

Barbadieri, M., Peralta-Videa, J.R. Pedron, F. and Gardea-torresdey, J.I. (2012). Plant growth regulators and improvements in phytoremediation process efficiency. In: Naser, A.A., Pereira, M.E., Ahmad, I., Duarte, A.C., Umar, S. and Khan, N.A. (eds). *Phytotechnologies: Remediation of Environmental Contaminants*. Pp 377-390.

Bhatt, S.C., Rawat, D. Pachauri, S.P. and Srivastava, C.P. (2021). Effect of different methods of Zn application on Zn, Fe, Cu and Mn concentration and uptake in some cowpea genotypes grown in mollisols of India. Journal of Pharmacognosy and Phytochemistry. **10**(1):380-388.

Benzie, I. F. F. and Strain, J.J. (1999). Ferric reducing antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods of Enzymology.* **299**: 15-27.

Bücker-Netol., Paiva, A.L.S., Machado, R.D., Arenhart, R.A. and Margis-Pinheiro, M. (2017). Interactions between plant hormones and heavy metal responses. *Genetics and Molecular Biology*. **40**(1):373-386.

Cohen, G., Dembiec D. and Marcus J. (1970). Measurement of catalase activity in tissue extracts. *Analytical Biochemistry.* **34**: 30-38.

De Deyn, G.B., vander Putten and Wim. H. (2005). Linking aboveground and belowground diversity. *Trends in Ecology and Evolution*. **20**(11):625-633.

Dong, Y. (2013). Plasma membrane NADPH oxidase and apoplastic POD can be responsible for accumulation of H_2O_2 in mesophyll cells of *Elsholtzia haichowensis* under copper stress. *African Journal of Agricultural Research*. **8**(16): 1466-1471.

Dontha, S. (2016). A review of antioxidant methods. *Asian Journal Pharmaceutical and Clinical Research*. **9**:14-32.

Durackova, Z. (2010). Some current insights into oxidative stress. *Physiological Research*. **59**(4):495-469.

Ferraz, M.A. and Lourençlo, J.C.N. (2004). Influence of organic matter content of contaminated soils on the leaching rate of heavy metals. *Environmental Progress*. **19**(1):53-58.

Goyal, A. and Chhibber, S. (2016). Effect of manganese in relation to antioxidative metabolism in germinating mungbeans. *International Journal of Biology, Pharmacy and Allied Sciences.* **5**(6):1167-1175.

Guala, S.D., Vega, F.A. and Covelo, E.F. (2010). The dynamics of heavy metals in plant soil interactions. *Ecological Modelling*. **221**:1148-1152.

Gutteridge, J. M. and Wilkins, S. (1982). Copper dependent hydroxyl radical damage to ascorbic acid: Formation of a thiobarbituric acid-reactive product. *Federation of European Biochemical Societies Letters.* **137**: 327-330.

Kumar, S., Chaudhuri, S. and Maiti, S.K. (2013). A review of soil dehydrogenase enzyme activity in natural and mine soil. *Middle-East Journal of Scientific Research*. **13**:898-906.

Lai, L. S., Chou, S. T. and Chao, W. W. (2001). Studies on the antioxidant activities of Hsian-tsao (*Mesona procumbens* Hemsl) leaf gum. *Journal of Agriculture and Food Chemistry.* **49**: 963-968.

Langyintuo, A.S., Lowenberg-Deboer,J., Faye, M., Lambert, D., Ibro, G., Moussa, B., Kergna,A., Kushwaha, S., Musa,s. and Ntoukam, G.(2003). Cowpea supply and demand in West and Central Africa. *Field Crops Research.* **82**:215-231.

Lisa, M.G. and Ching, K.C. (2003). Copper toxicity, oxidative stress, and antioxidant nutrients. *Science Direct.* **189**:147-163.

Marcocci, L., Packer, L., Droy-Lefaix, M. T., Sekaki, A. and Gardes-Albert, M. (1994). Antioxidant action of *Ginkgo biloba* extract EGb 761. *Methods in Enzymology.* **234**: 462-475.

Misra, H. P. and Fridovich, I. (1972). The role of superoxide anion in the autooxidation of epinephrine and simple assay for superoxide dismutase. *Journal Biology and Chemistry.* **247**: 3170-3175.

Muhammad, S., Sana, K., Ghulam, A., Naeem, S., Muhammad, N., Muhammad, S., Muhammad, A. and Camille, D. (2015). Heavy metal stress and crop productivity. *Crop Productivity and Global Environmental Issues*. **319**:1-25.

Moula, A., Ellafi, A., Borgi, M.A., Rhimi, S., Akermi, N., Namsi, A., Chaieb, M. and Mekki, A. (2021). Enhanced bioremediation of heavy metals from phosphate processing wastewater using the indigenous bacterium *Serratia rubidaea* NCTC12971. *Geomicrobiology Journal*. DOI: 10.1080/01490451.2021.1979696

Nair, P.M.G. and Chung, I.M. (2008). Impact of copper oxide nanoparticles exposure on Arabidopsis thaliana growth, root system development, root lignification and molecular level changes. *Environmental Science and Pollution Rasearch*. **21**(22):12709-12722.

Ng, N.Q. (1995). Cowpea, Vigna unguiculata (Leguminous-Papilionoideae): Smart, J. and Simmonds, N.W. (Eds). Evolution of crop plants. *Longman, Harlow, United Kingdom.* Pp 326-332.

Nyle, C., Brady, R. and Ray, R.W. (2009). Elements of the nature and Properties of soils. *Prentice Hall*. (3rd Ed.). Pp 208-278.

Obatolu, V.A. (2003). Growth pattern of infants fed with a mixture of extruded malted maize and cowpea. *Nutrition*. **19**:174-178.

Olubodun, S.O. and Eriyamremu, G.E. (2013). Effect of different crude oil fraction on growth and oxidative stress parameters of maize radical. *International Journal of Plant and Soil Science*. **2**(1):144-154.

Perrino, P., Laghetti, G., Spagnoletti-Zeuli, P.L. and Monti, L.M. (1993). Diversificatiom of cowpea in the Mediterrean and other centres of cultivation . *Genetic resources and crop evolution*. **40**:121-132.

Ponge, J. (2003). Humus forms in terrestrial ecosystems: a framework to biodiversity. *Soil Biology and Biochemistry*. **35**(7):935-945.

Prieto, P., Pineda, M. and Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analysis of Biochemistry.* **269**: 337–341.

Puschenreiter, M., Schnepf, A., Molina-Millan, I., Fitz, W.J., Horak, O., Klepp, J., Schreft, T., Lombi, E. and Wenzel, W.W. (2005). Changes of Ni Biogeochemistry in the Rhizosphere of the Hyperaccumulator *Thlaspi goesingense*. *Plant and Soil*. **271**:205-218.

Rangel, A., Domont, G.B., Pedrosa, C. and Ferreira, S.T. (2003). Functional properties of purified Vicilins from cowpea and pea and cowpea protein isolate. *Journal of Agricultural and Food Chemistry*. **51**:5792-5797.

Rascio, N. and Navari-Izzo, F. (2011). Heavy metal hyperaccumulating plants: how and why do they do it? And what makes them so interesting? *Plant Science (Shannon Ireland).* **180**:169–181.

Sadeghipour, O. (2016). Pretreatment with nitric oxide reduces lead toxicity in cowpea. *Archaeology and Biological Science, Belgrade.* **68**(1):165-175.

Sadeghipour, O. (2017). Nitric oxide increases Pb tolerance by lowering Pb uptake and translocation as well as phytohormonal changes in cowpea (*Vigna unguiculata*). *Sains Malayisiana*. **46**(2):189-195.

Sadeghipour, O. (2018). Drought tolerance of cowpea enhanced by exogenous application of methyl jasmonate. *International Journal of Modem Agriculture*. **7**(4):51-57.

Schulte, E.E. and Kelling, K.A. (1999). Soil and applied copper. *Understanding Plant Nutrients*. **7**:1-2

Shahid, M., Xiong, T., Castrec-Rouell, Leveque, T. and Dumat, C. (2013). Water extraction kinetics of metals, arsenic and dissolved organic carbon from industrial contaminated poplar leaves. *Journal of Environmental Science*. **25**:2451–2459.

Shahid, M., Xiong, T., Masood, N., Leveque, T., Quenea, K., Austruy, A., Foucault, Y., Dumat, C. (2014). Influence of plant species and phosphorus amendments on metal speciation and bioavailability in a smelter impacted soil: a case study of food-chain contamination. *Journal Soils Sediments.* **14**:655–665.

Singh, B.B., Mohan, D.R., Dashiell, K.E. and Jackal, L.E.N. (1997). Advances in Cowpea Research, IITA, Ibadan, Nigeria, and Japan International Institute of Tropical Agriculture, Sayce, Devon, UK. Pp.313-325.

Singh, B., Ajeigbe, H.A., Tarawali, S.A., Fernandez-Rivera, S. and Abubakar, M. (2003). Improving the production and utilization of cowpea as food and fodder. *Fields Crops Research*. **84**:169-180.

Singh, R., Ahirwar, N.K., Tiwari, J. and Pathak, J. (2018). Review on sources and effect of heavy metal in soil: its bioremediation. *Impact Journals.* 1-22.

Tighe-Neira, R., Gonzalez-Villagra, Nunes-Nesi, A. and Inostroza-Blancheteau, C. (2022). Impact of nanoparticles and their ionic counterparts derived from heavy metals on the physiology of food crops. *Plant Physiology and Biochemistry*. **172**:14-23.

Thomas, E.L., Kirk, W.B., Terence, H.C. and Murray, H.M. (1999). Sustaining our soils and society. *American Geological Institute: Environmental Awareness series.* **2**:36-68.

Upadhyay, S.K., Ahmad, M., Srivastava, A.K., Abhilash, P.C. and Sharma, B. (2020). Optimization of eco-friendly novel amendments for sustainable utilization of Fly ash based on growth performance, hormones, antioxidant. and heavy metal translocation in chickpea (*Cicerarietinum L*.) plant. *Chemosphere*. **268**:1-8.

Wang, N., Han, J., Wei, Y., Gang, L. and Sun, Y. (2019). Potential ecological risk and health risk assessment of heavy metals and metalloid in soil around Xunyang mining areas. *Sustainability*. **11**: 4828.

Wani, P.A., Garbak, S.H. and Mashood (2019). Prevention of oxidative damage and phytoremediation of Cr(VI) by Chromium(VI) reducing *Bacillus subtilus* PAW3 in cowpea plants. *Bulletin of Environmental Contamination and Toxicology*. **103**:476-483.

Xiong, J., Fu, G., Tao, L. and Zhu, C. (2010). Roles of nitric oxide in alleviating heavy metal toxicity in plants. *Archaeology Biochemistry and Biophysics*. **497**:13-20.

Yadav, S.K. (2010). Heavy metals toxicity in plants: an overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. *South African Journal Mar Science*. **76**:167–179.

Yuan, D. (2012). Plasma membrane NADPH oxidase and apoplastic POD can be responsible for accumulation of hydrogen peroxide in mesophyll cells of Elshltzia haichowensis under copper stress. *African Journal of Agricultural Resource*. **8**:1466-1471.