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Antioxidant response of heavy metal stressed Cyperus Iria amended with organic and inorganic fertilizer

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Abstract
The effects of amendment on the growth of *Cyperus iria* exposed to heavy metal polluted soil was studied. Weighing balance (Setra 480S, USA) was used to weigh 2kg homogenized heavy metal polluted soil from an abandoned metal scrap dumpsite at Ikoku Rivers State Port Harcourt, Nigeria into planting bags which was arranged in 4 plots (A, B, C, and D) in addition to an uncontaminated soil designated as plot E adopting a factorial experimental design. The organic and inorganic amendments were added as: A_1: NPK (40g/2kg), A_2: NPK (80g/2kg), A_3: NPK (120g/2kg), B_1: orange peel (100g/2kg), B_2: orange peel (200g/2kg), B_3: orange peel (300g/2kg), and C_1: plantain peel (100g/2kg), C_2: plantain peel (200g/2kg), C_3: plantain peel (300g/2kg) plot D and E with no amendment stand as control and double control respectively and the test plant was introduced after harmonization. Results showed that organic amendment was very effective in controlling excess production of reactive oxygen species (ROS), while slight increase in ROS expression was shown *Cyperus iria* grown in NPK treated soil. Highest in oxidative stress production in *Cyperus iria* grown in polluted control soil (0g amendment). Reduction rate of ROS expression varies with different levels of amendment application, this is shown as: catalase < 300g plantain peel, GSH <100g and 300g orange peel, proline <120g NPK and superoxide dimultase <300g orange peel. Therefore, organic amendment is more efficient in controlling excess ROS production and hence enhance plant tolerability in polluted environment.

Keywords: Amendments, Heavy metals, Antioxidant biological marker, Reactive oxygen species

1. Introduction

Cadmium (Cd) and lead (Pb) are non-essential- elements to plants. These elements are predominately found in air, water and soil posing serious concern to human health. The effects of Pb on plants are observed directly on plant growth and metabolism showing visible symptoms like stunted growth resulting in membrane disorganization. Antioxidative defence mechanism protects the plants cells from oxidative damage caused by Reactive oxygen species (ROS) due to heavy metals. Biomarkers are biochemical, physiological or histological changes that measure effects of, or exposure to, toxic chemicals or environmental perturbation such as heavy metal pollution, depletion in nutrients, excess fertilizer application (Luebke *et al.*, 1997) [19], in general but not exclusively pertain to a response at a specific organ, cellular or subcellular level of organisation (O'Halloran *et al.*, 1998) [21], measuring biochemical endpoints (Bresler *et al.*, 1999) [8]. These cellular and molecular responses can be used as early warning pointer of environmental stress, before whole-organism effects become apparent (Regoli *et al.*, 1998) [26]. Exposure to high environmental levels of metals can induce synthesis of biomarker responses (Irato *et al.*, 2003) [18]. Biomarkers are being increasingly recognised as accurate and cost-effective methods for identifying the *in situ* toxic effects of pollutants on biota (Brown *et al.*, 2004) [9].

Naturally, molecular oxygen (O_2) is release into the environment on a regular bases by photosynthetic organisms. This regular release of molecular oxygen has increased the concentration of Reacting Oxygen Species (ROS). The production of Reactive Oxygen Species occurs when plants are subjected to stress conditions and production of O_2 molecule is frequently scavenged by plant biomarkers. This includes catalase, glutathione and super oxide dismutase (Foyer, 2005) [15]. The ROS alongside with antioxidant production are constantly in equilibrium which may be influenced by environmental stress. Environmental pollutants generally cause an increase in peroxidative processes within cells, causing oxidative stress (Cheung *et al.*, 2001; Nusetti *et al.*, 2001) [10, 20]. Hydroxyl radicals are produced in electron transfer reactions, and are potent oxidants capable of damaging important cell components, such as proteins and DNA (Doyotte *et al.*, 1997; Cheung *et al.*, 2001) [13, 10]. Lipid peroxidation (LPO) has often been used as a biomarker of environmental stress, reflecting damage to cell membranes from free radicals (Ringwood *et al.*, 1999) [27] and is an important feature in cellular injury (Reddy, 1997) [25]. The extent of damage caused by oxygen radical production is dependent on antioxidant defences, which include antioxidant enzymes and free radical scavengers, such as glutathione (Doyotte *et al.*, 1997) [13]. Therefore, antioxidant enzymes is a common biomarker used in environmental monitoring (Regoli *et al.*, 1998) [26]. The enzymes usually respond rapidly and sensitively to biologically active pollutants (Fitzpatrick *et al.*, 1997) [14]. Some of the most commonly used antioxidant enzyme biomarkers include catalase and glutathione-s-transferase. Catalase is induced by the production of hydrogen peroxide in the cells and catalyses the reaction, which reduces this compound to water and oxygen (Regoli *et al.*, 1998) [26]. Glutathione-s-transferase catalyses the conjugation of a large variety of xenobiotics containing electrophilic centres to reduced glutathione (Principato and Regoli 1995; Sharma *et al.*, 1997) [24]. Concentrations of this enzyme have been found to increase with exposure to contaminants (Fitzpatrick *et al.*, 1997) [14]. Glutathione (GSH) is often used in biomarker studies, as it is an overall modulator of cellular homeostasis (Ringwood *et al.*, 1999) [27]. The reduced form conjugates with electrophilic xenobiotics transforming them into water soluble products (Nusetti *et al.*, 2001) [20]. Many studies examine the responses of organisms to contamination, using the endpoints of reduced growth performance of plants or Antioxidant enzyme biomarkers in reproduction (Wright and Welbourne, 2002) [33]. Using a combination of biomarkers including antioxidant enzymes (CAT and GST) and free radical scavengers (GSH) in both a field and laboratory situations, ensure that all aspects of the biochemical effects of metal exposure are being assessed. Since antioxidative defense mechanisms protects the plant from Reactive Oxygen Species (ROS) damage, antioxidant enzymes like SOD, CAT, GSH are always on the increase when a plant is expose to pollution stress. It is expected that result obtained from the investigation will give a clearer view on the plant pollution stress interaction.

2. Materials and Methods

2.1. Study area /sources of material and processing

This research was carried out at the Center for Ecological Studies, University of Port Harcourt, located on geographical coordinates: Latitude 4.90428° N and Longitude 6.92297° E. The climate condition of the area is characterized by temperature range of 36 °C and 45 °C for daily and annual range respectively. Land race of sweet orange and plantain were acquired Otutu-Amaumara Ezinihitte Mbaise LGA., Imo State and Kolokuma/Opukuma L.G.A, Bayelsa State respectively. The plantain and orange peels were removed mechanically by hand peeling. The peels (waste) generated from mechanical process were dried and processed into powder form, which was then analyzed to make certain the nutritional value and heavy metals content of the peels (Table 1), while NPK (20:20:20) used was obtained from Rivers State Agricultural Development Program (ADP) Rumuodomaya, Port Harcourt.

2.2 Experimental design and treatment application

Factorial experiment fitted into Randomized Complete Block Design (RCBD) was adopted as a design for this experiment. A suspected heavy metal polluted soil was acquired from an abandoned metal scrap site at Ikoku Rivers State Port Harcourt on geographical coordinate: Latitude 4.80083° N and Longitude 6.991093° E alongside with uncontaminated soil obtained from a fallow land at University of Port Harcourt, at depth 0-20 cm using a spade. The soils collected were analysed to ascertain heavy metal content and other physicochemical properties. This was known as baseline analysis (Table 2). The soils were bulked together, homogenized and transported to the Centre for Ecological Studies University of Port Harcourt. The collected soil was mixed thoroughly, dried and sieve through 2 mm mesh to obtain a homogenous soil (fine fraction) composite. Weighing balance (Setra 480S, USA) calibrated in (kg) was used to weigh two kilograms (2 kg) of the homogenized soil into planting bags. The bags were arranged in 4 Plots (A, B, C, and D) alongside with uncontaminated soil designated as plot E. Block A was subdivided into 3 sub plots designated as A_1, A_2 and A_3. The same division process was adopted for block B and C as B_1, B_2 and B_3, C_1, C_2 and C_3 of 12 replications for each subplot. A weighing balance (Setra 480S, USA), was use to weigh various concentrations of amendment treatment into various plots. NPK was added to plot A, orange peel to plot B and plantain peels to plot C. Each plot was subdivided into 1,2, and 3 as A_1, A_2, A_3; B_1, B_2, B_3 and C_1, C_2, C_3. Amendment treatments were added as follows: Amendment treatment A_1: NPK (40g/2kg), A_2: NPK (80g/2kg), A_3: NPK (120g/2kg), Treatment B_1: orange peel (100g/2kg), B_2: orange peel (200g/2kg), B_3: orange peel (300g/2kg), and Treatment C_1: plantain peel (100g/2kg), C_2: plantain peel (200g/2kg), C_3: plantain peel (300g/2kg) while no amendment was added to plot D and E. That is 0g amendment which stand as control and double control respectively. The soil with the amendment treatment was thoroughly mixed to enhance harmonization of treatments with the soil. After two weeks, Two (2) seedlings of *Cyperus iria* Linn of identical sizes and vigour were transplanted into various plots. Direct rain water was controlled by shedding the experimental site and a standard watering technique was applied and weeding was done when needed. The experiment was monitored at 60 day interval. At 60th day of the experiment, the height of the plants was recorded and six replicates from each plot were harvested by uprooting, this was achieved by flooding the bags with water plants after an hour, the plants were carefully uprooted from the bags and dipped into a full bucket of water to help remove some

adhering soil particles attached. The shoots and roots plant samples were washed labelled according to plots and treatments and taken to the laboratory for the determination of proline, carotenoid, glutathione (GSH) and superoxidase dismutase. The amount of proline and GSH content in plant roots and shoot was estimated as described by Bates et al (1973), catalase activity in plant parts was determined following the method described by Aebi (1974), while superoxide dismutase activity was determined based on inhibition of the photochemical reduction of nitrobluetetrazolium (NBT), (Beauchamp and Fridovich, 1971) and carotenoid content in plants was estimated using the procedure described by Arnon (1949).

Statistical Analysis: The data generated (means and standard error of mean) was estimated using the Statistical Analysis System (SAS version 9.0).

Table 1: Nutrient and metal of the peels waste used

S/N	Parameter	Orange peels waste	Plantain peels waste
1	Phosphorus (mg/kg)	66.51	36.84
2	Sodium (mg/kg)	474.85	137.45
3	Potassium (mg/kg)	66,285	26,743
4	Magnesium (mg/kg)	1208	1614
5	Calcium (mg/kg)	278.70	4,400.10
6	Nitrogen %	0.119	0.196
7	Ash %	11.50	16.40
8	Fe (mg/kg)	767.7	483
9	Zn (mg/kg)	13.05	236.50
10	Pb (mg/kg)	ND	ND
11	Cd (mg/kg)	ND	ND
12	pH	5.56	9.08

ND = Not detected

Table 2: Soil physicochemical properties and heavy metal content

S/N	Parameter	Unpolluted	Polluted soil
1	Moisture (%)	45	43
2	Bulk density (%)	1.5	1.7
3	Particle density (%)	5.8	5.1
4	Porosity	0.35	0.3
5	SOM (%)	12	24
6	Sand (%)	95.6	93.6
7	Silt (%)	0.10	0.7
8	Clay (%)	4.3	5.7
9	Chloride (mg/kg)	213	3687
10	Sulphate (mg/kg)	28.4	269
11	Nitrate (mg/kg)	71.9	138
12	Phosphorus (mg/kg)	1.35	0.82
13	Sodium (mg/kg)	120	132
14	Calcium (mg/kg)	110	120
15	Magnesium (mg/kg)	258	280
16	Potassium (mg/kg)	43	68
17	pH	5.10	8.43
18	Conductivity (μsCm^{-1})	90	1193
19	Iron (mg/kg)	48.2	4410
20	Zinc (mg/kg)	0.94	107.5
21	Lead (mg/kg)	130	167.3
22	Cadmium (mg/kg)	0.80	15.3

Note: SOM = Soil Organic matter

3. Results and Discussion

Oxygen evolving photosynthetic organisms gave rise to the presences of molecular oxygen. The molecular oxygen now inseparable component of aerobic organisms. These biomarkers are biological components triggered in excess when a plant is perturbed. Plant biomarkers are well-known as plant stress enzymes. Increase metals accumulation influenced the equilibrium between scavengers and Reactive Oxygen Species (ROS). Increase Reactive Oxygen Species (ROS) is activated due to stress such as metal toxicity and this may have led to cellular damage of biomolecules (lipid, protein and nucleic acids). Gill (2010) [10] and Yang *et al*. (2011) [34] report that toxic nature of metals and nutrient depletion trigger excess production of ROS.

Superoxide dismutase

In aerobic organisms, SOD is the most active antioxidant which more abundant. The various amendments showed decrease in SOD activity in roots and shoots of *Cyperus iria* as presented in Figure 1. Comparing the effects of amendment on SOD at 60 and 120 day showed that the application of orange peels (waste) of various concentrations decrease the SOD content of the root of *Cyperus iria*. Plant grown in NPK and plantain peels amendments; control and double control showed increase in root SOD content. While the highest antioxidant activity was found in control at 60 and 120 day respectively (p= 0.05). The application of powder plantain peels (waste) of various concentrations decrease the SOD content in the shoot of *Cyperus iria* (Figure 2). *Cyperus iria* grown in NPK, orange peels, control and double control experienced increase in SOD content. Asada (1994) [4] observed that SOD is one of the most efficient antioxidants which is solely responsible in ameliorating and scavenge the negative effects from free oxygen radicals. This observation made here is justifiable because, the added amendments might have release the necessary plant nutrients which may have mitigated the toxicity effects of heavy metals on *Cyperus iria* thereby resulting in SOD reduction. This finding is in line with Tewari *et al*. (2004) who observed greater SOD activity in nitrogen starved maize. Additionally, Prochazkova

et al. (2001) also observed an increased SOD activity in maize after tasseling for 25 days. The increase in SOD observed in *Cyperus iria* grown in control could be attributed to decrease in essential plant nutrients in available form for plants uptake.

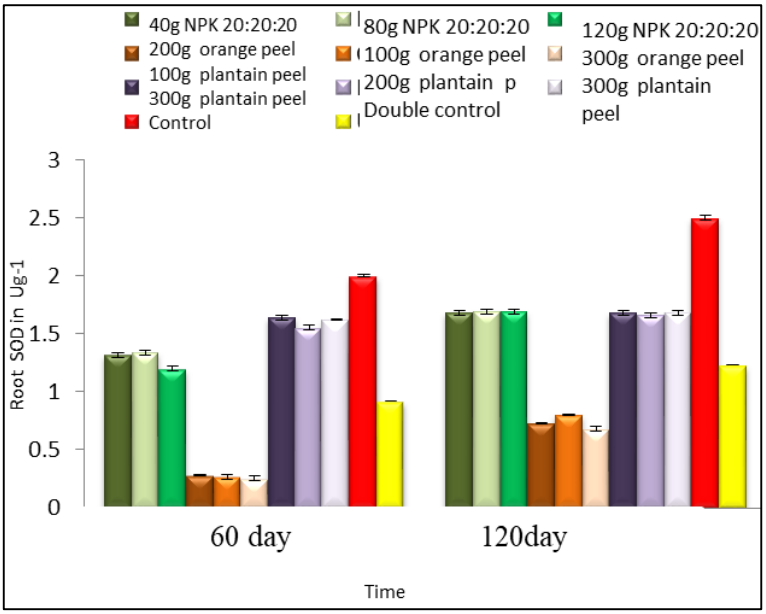

Fig 1: Effects of amendments on SOD activity in the root of *Cyperus iria* under metal stress

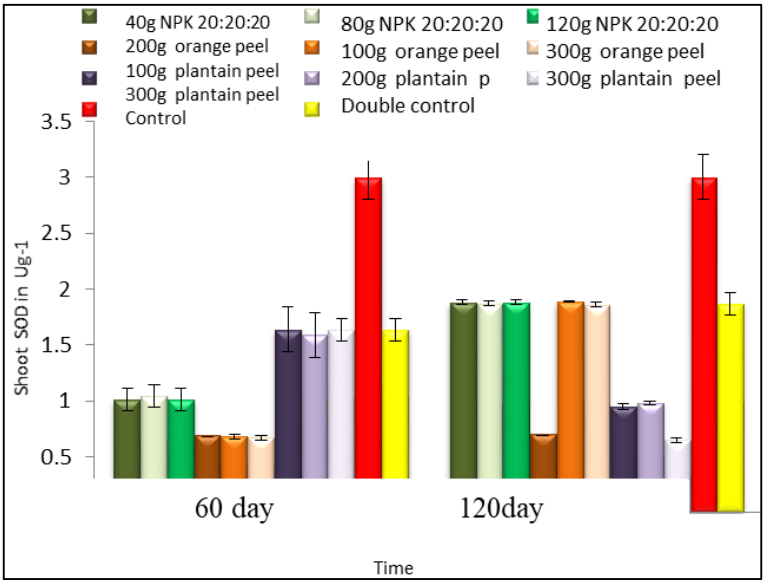

Fig 2: Effects of amendments on SOD activity in the shoot of *Cyperus iria* under metal stress

Proline

The various amendments showed a decrease on proline activity in roots and shoots of *Cyperus iria* as presented in Figure 3 and 4. Reduction was found in shoot of *Cyperus iria* grown in ameded polluted soil. The least decrease in root and shoot proline activity at 60 day was observed in 40 g NPK (20:20:20) and 200g plantain peel amendment respectively, while highest increase in shoot and root was in control (polluted soil without amendment). The decrease observed in proline activity of *Cyperus iria* grown in different concentrations of organic amendments is understandable since the amendments used are biodegradable by micro-organisms which make them a Low-Molecular Weight Organic Acids (LMWOA's)whose upon its addition might have acidified the soil. Furthermore, NH^{+}_{4}, CO_2 acid during microbial degredation of LMWOA's may be responsible for the decrease in pH (Albanell *et al*., 1988; Zulfigar *et al*., 2012) [2]. Additionally, soil pH also plays a major role in nutrient availability (Yashin *et al*., 2014). Due to nutrients availability, the depressing effects of heavy metals could be controlled hence leading to proline decrease. The phenomenon of proline accumulation is known to occur under water deficit, salinity, low temperature, heavy metal exposure and UV radiations (Sharma, 2006) [31]. Apart from acting as osmolyte for osmotic adjustment, proline contributes to stabilizing sub-cellular structures (e.g., membranes and proteins), scavenging free radicals and buffering cellular redox potential under stress conditions.

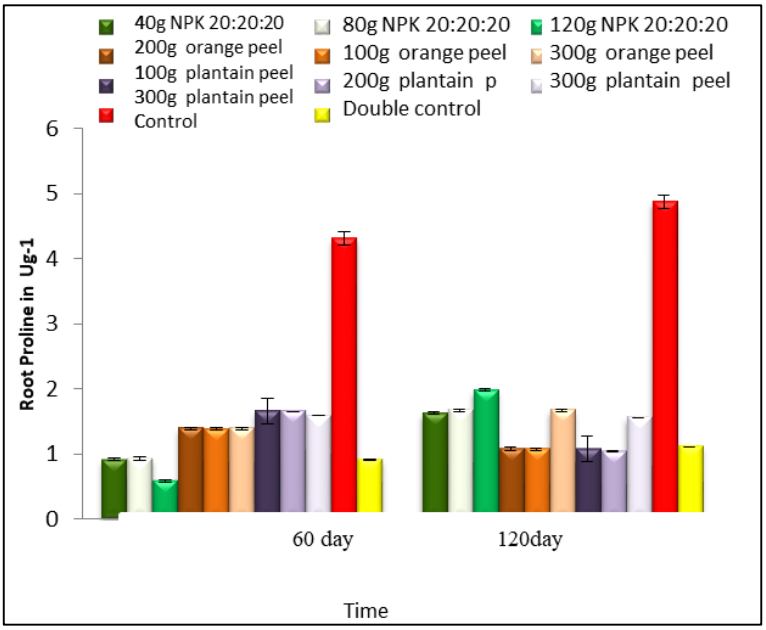

Fig 3: Effects of amendments on Proline activity in the root of *Cyperus iria* under metal stress

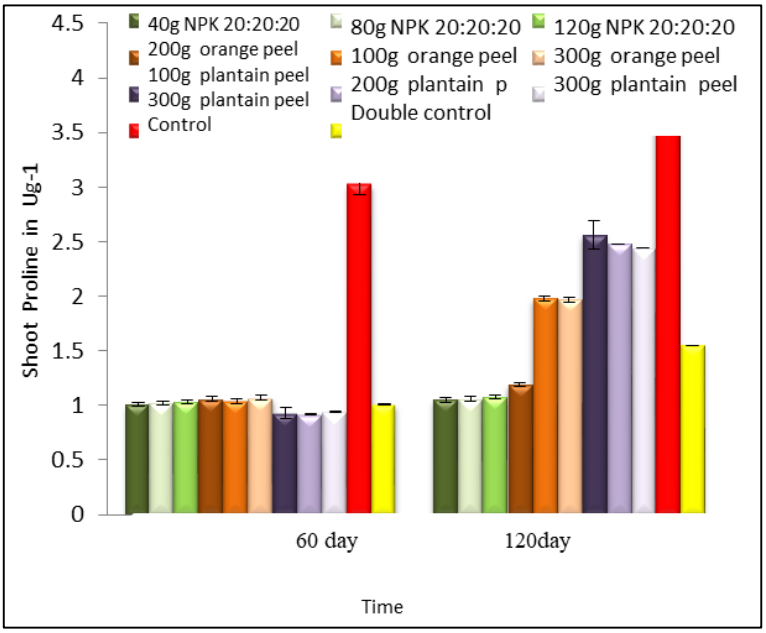

Fig 4: Effects of amendments on Proline activity in the shoot of *Cyperus iria* under metal stress

Caroteniod

The various amendments showed decrease in caroteniod activity in roots and shoots of *Cyperus* plants is presented in Figure 5and 6. The root of plant grown in NPK, orange peels, plantain peels amendment, control and double control accumulate high caroteniod. Least decrease in caroteniod antioxidant biomarker in root of *Cyperus iria* was observed in 300 g plantain peel soil amendment at 60 and 120 day and also in 100g and 300g orange peel amendment for shoot, while highest caroteniod in shoot and root antioxidant activity was found in control (polluted soil) at 60 and 120 day respectively. Several types of pigments are present in plants such as chlorophylls, xanthophylls and carotenoids. Among these, chlorophyll is the most abundant and important pigment in higher plants; responsible for photosynthesis as they capture light. In several cases, heavy metals are known to reduce the productivity by reducing the rate of photosynthesis. The increase in response of carotenoid content of *Cyperus iria* grown in control soil could be attributed to the toxicity nature of Pb and Cd. This corresponds to the results of Padmaja *et al.* (1990) [22] who observed a decline in the pigment composition of plants exposed to heavy metal stress.

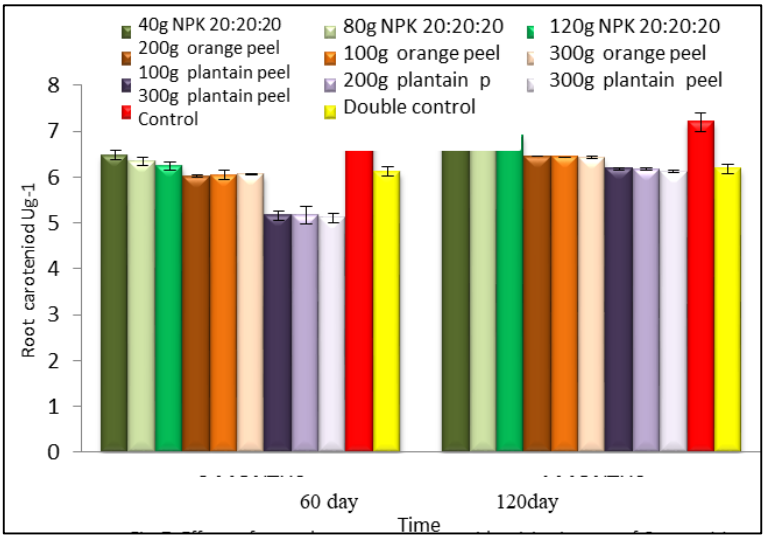

Fig 5: Effects of amendments on carotenoid activity in root of *Cyperus iria* under metal stress

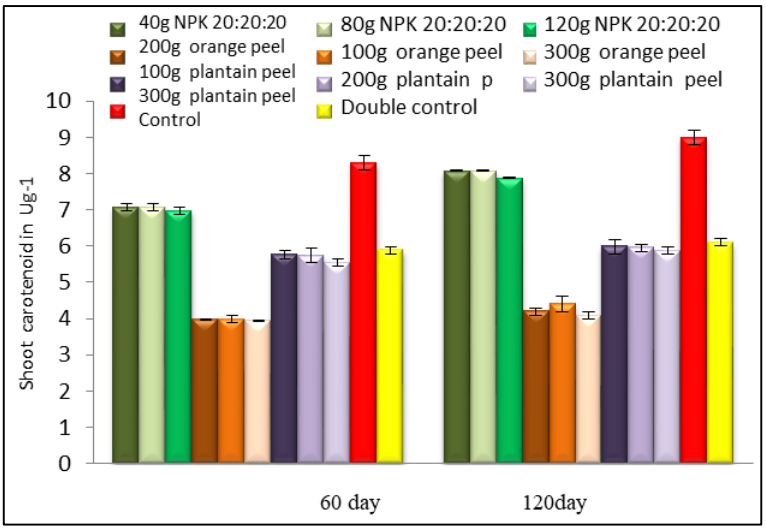

Fig 6: Effects of amendments on carotenoid activity of carotenoid activity in shoot of *Cyperus iria* under metal stress

Glutathione (GSH)

Glutathione (GSH) is a key component in metal scavenging due to the high affinity of metals to its thiol (-SH) group and as a precursor of phytochelatins (PCs). Besides metal homeostasis, plants possess a well-equipped antioxidative defense system to manage the metal-imposed oxidative challenge (Cuypers *et al*. 2010) [11]. The cysteine residue on GSH renders it an important antioxidant that, in addition to its primary antioxidant capacities, acts as a substrate for the regeneration of other essential antioxidants (Foyer *et al*. 2011) [16]. In this way, GSH performs in both metal homeostasis and the antioxidative defense, which influence the levels of free reduced GSH and its cellular redox state *i.e.*, oxidized glutathione disulfide (GSSG) *versus* reduced GSH]. Furthermore, the GSSG/GSH redox balance transmits specific information in order to fine tune cellular signaling pathways and responses under environmental stress conditions (Seth *et al*. 2012) [30]. The various amendments showed a decrease on GSH activity in roots and shoots of *Cyperus* plants is presented in Figure 7 and 8. The root of plant grown in NPK, orange peels, plantain peels amendment, control and double control showed increase in GSH content. Decrease in GSH antioxidant biomarker in root of *Cyperus iria* was observed in amended and double control at 60 and 120 day. The decrease in GSH content of *Cyperus iria* grown in amended soil may be attributed to the cushion effects due to nutrient availability. This findings is understandable since nutrients depletion have been report as a phenomenon leading to reactive oxygen production in plants (Sharma, 2006) [31]. The increase in GSH production found in *Cyperus iria* grown in control soil could be attributed to the toxicity nature of the studied metals. This result corroborated with Seth *et al*. (2012) [30] who reported that metal toxicity affect GSH at all levels.

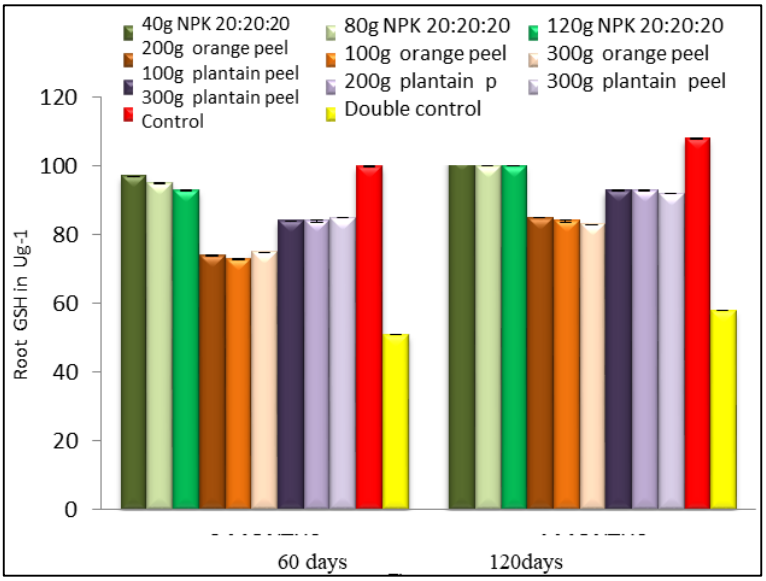

Fig 7: Effects of amendments on GSH activity in root of *Cyperus iria* under metal stress

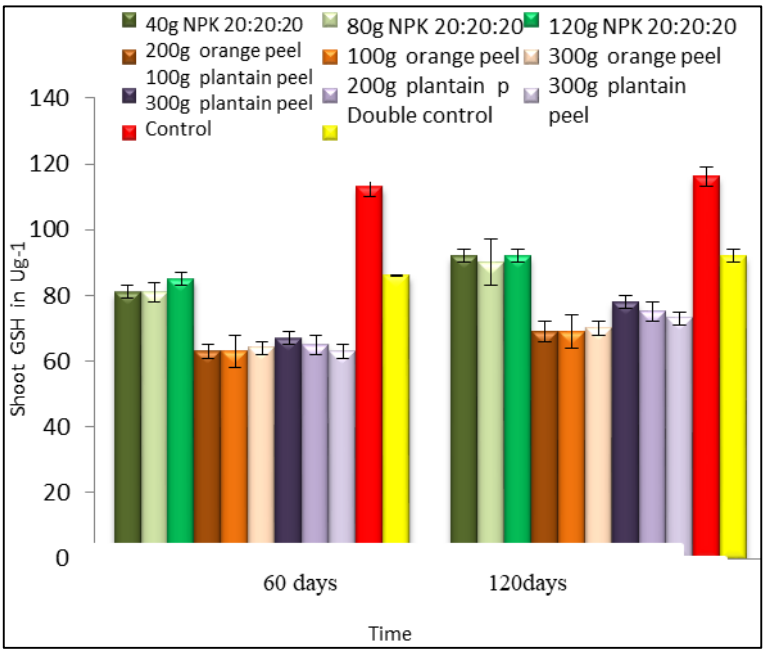

Fig 9: Effects of amendments on GSH activity in shoot of *Cyperus iria* under metal stress

Result in Figure 10. Showed the effects of amendments on plant height for *Cyperus iria*. The effect of amendments on the height of *Cyperus iria* is seen on different concentration of amendment treatment applied at 60 and 120 days. A decrease in plant height was found in plant grown in 40 g and 120 g NPK (20:20:20) treated soil. The test plant (*Cyperus iria*) grown in powder orange peel, plantain peels and double control showed an increase in height. It was found that there was significant difference in plant height between and within amendment treatments with time at ($p = 0.05$). The addition of 300 g plantain peels treated soil significantly increased the shoot growth of *Cyperus iria* at 60 and 120 days, while least decrease was observed in plant grown in 200 g powder orange peels and 40 g NPK amended soil at 60 day and 120 days respectively. The least decrease in plant height was recorded for plant grown in control soil (0g amended soil). The decrease observed in plants grown in NPK treated soil and the control soil could be attributed to the toxic nature of the heavy metal and the excess NPK fertilizer added to this soil. These components present in the growing metal triggered the stress equilibrium path in plant which may have turned out to decrease plant height. This findings was in line with the proposal made by Divya *et al*. (2016) [12] who reported a decrease in the growth of pigeon pea plants and the growth reduction correlated with different levels of Al amendment addition. This findings also corroborated with the report of Amadi et al (2023) who observed a decrease in the growth of *Echinochloa colona* expose to a heavy polluted environment.

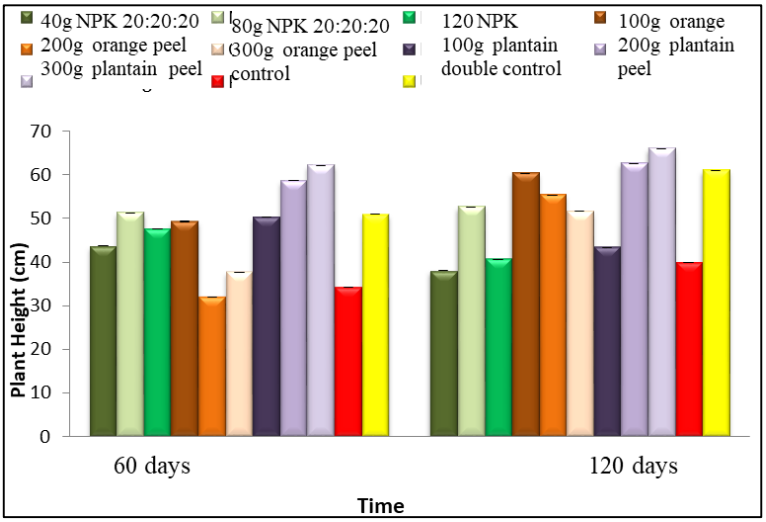

Fig 10: Effect of amendments on *Cyperus iria*

4. Conclusion

The present investigation lay credence to the fact that the addition of amendments such as NPK (20:20:20), orange and plantain peels can enhance the tolerance ability of plant species grown in contaminated soils to cope with heavy metal stress. The result demonstrated a significant decrease in antioxidant enzymes with addition of amendments. Conclusively, plant species grown in soil contaminated with heavy metal and the potential environmental perturbation ranging from heavy metal toxicity and nutrients depletion may be controlled by soil amendments addition as to enhance plant tolerance to pollution stress.

Competing Interests

Authors have declared that no competing interests exist.

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Abbreviations

GSH- Glutathione
CAR- Caroteniod
SOD- Superoxide dismutase
SOM- Soil organic matter.

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Not applicable.

Availability of data and material

The sets of data generated from this research are included in the article.

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