International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
(A Peer Reviewed, Referred, Indexed and Open Access Journal)
DOI: 10.22192/ijarbs Coden: IJARQG (USA) Volume 10, Issue 4 -2023

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2023.10.04.014

Soil enhancer: A vital tool for plant stress management in heavy metal polluted environment

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Abstract

The damages observed in plant biomolecules caused by overproduction of reactive oxygen species (ROS) due to plants exposure to polluted environment is of serious concern. This research was to investigate the efficacy of soil enhancer as a vital tool in the management of plant stress pattern. Two (2) kilograms of homogenous heavy metal contaminated soil composite was weighed into 60 planting bags arranged in 4 blocks (A, B, C, D) alongside uncontaminated soil (block E) of 12 replications each. In a Randomized Complete Block Design the treatment were added as: NPK 15:15:15 was added to block A as A_1 :(40g/2kg), A_2: (80g/2kg), A_3: (120g/2kg), while block B and C contain orange and plantain peels respectively. Orange peels was added as B_1 :(100g/2kg), B_2 :(200g/2kg), B_3: (300g/2kg), and plantain peels as C_1: (100g/2kg), C_2: (200g/2kg), C_3:(300g/2kg) while no amendment was added to block D and E which stands as polluted and unpolluted control.The antioxidant enzymes: superoxide dismultase (SOD), glutathione (GSH), proline (P) and carotenoid (CA) were analyzed at 2 months intervals. Result showed that the highest in reactive oxygen species (ROS) production in root and shoot were recorded for *Echinochloa colona* grown in polluted control soil (0g amendment) and the least decrease in antioxidant response were recorded for *Echinochloa colona* grown in different concentrations of soil enhancer. The ecological impact of heavy metal exposure on plants can be ameliorated through the addition of soil enhancer such as NPK, orange and plantain peels (waste).These enhancers will also help mitigate the effects of heavy metals on plants when applying plant-based remediation techniques.

Keywords: Soil enhancer, *Echinochloa colona*, heavy metals, ROS production, Antioxidant.

1. Introduction

The benefits man derive from the environment cannot be enumerated, the environment play a crucial role in the survival of living things both as a reservoir of nutrient and dwelling place (Masindi *et al*., 2018). The environment is seen as a fundamental system of man's existence and productivity. The desire of man to gratify his insatiable wants and to become more industrialize and globalize in ranking especially the third world countries like Nigeria has led to an uncontrolled

exploitation of environmental resources (Ola *et al*., 2015). As result, these third world countries lacks data on daily, monthly and annual environmental pollution records hence environmental sustainability cannot be considerably accounted (Ola *et al*., 2015). Exploitation of the environment ought to be progressive in synergy with sustainability (conservation and preservation)in other to monitor the negative impact of the process on the environment for proper environmental management (Ola *et al*., 2015).Anthropogenic activities discharges numerous noxious pollutant into the environment some of these pollutant can be broken down into a smaller components when acted upon by natural processes. Pollutant such as heavy metals are recalcitrant in the environment and cannot be broken down rather they bio-accumulate and biomagnified along feeding process and increases in volume when present in food chain. Increase in the concentration of heavy metals influence all biotic organisms, these metals are predominant in the soil than other environmental components (Ali *et al*., 2013). The impacts of heavy metals in plants are observed directly on plant growth pattern, showing morphological symptoms such as stunted growth, root length inhibition, plant root elongation, and decrease in biomass production and reduction in root number (Ali *et al*., 2013).Heavy metals decrease optimal performance of plants through increasing production of Reactive Oxygen Species (ROS). Increase in ROS production lead in oxidative damage of the cell wall and other essential plant organs (Gill, 2010).Soil enhancer are organic biodegradable materials rich in essential nutrient wanted by plants for optimal growth, they can be regarded as natural inducing agents (chelators) which also help to mitigatethe oxidative stress effect of heavy metal pollution and soil nutrient depletion (Schmidt, 2003). Soil enhancers decrease the impact of heavy metals plants and thus help to stabilize the cellular and molecular responses of plant which is often regarded as an early pointer of environmental stress (Irato *et al*., 2003). Heavy metals generally cause an increase in peroxidative processes within the cells resulting in oxidative stress (Cheung *et al*., 2001). Excess production of ROS is reported in plants when they are subjected to extreme stressful condition. Plant stress is triggered when equilibrium between ROS production and antioxidant scavenger is distorted hence the radical produced in the electron transfer reaction chain which is a potential oxidants capable of damaging important cell component is released (Cheung *et al*., 2001). Increase in oxidative stress damage the antioxidative defense mechanisms of plants which is responsible for oxidative stress. This investigation is expected to review the benefit of soil enhancer in controlling plant stress pattern in heavy metal polluted soil.

2. Materials and Methods

2.1 Study area

This research was carried out at the Centre for Ecological Studies, University of Port Harcourt, located in the Niger Delta area of Nigeria on geographical coordinates of Latitude 4.90428°N and Longitude 6.92297°E. The area experiences two distinct seasons - dry and wet seasons. The dry season is from November to March and wet season is from April to October. The annual rainfall is usually at its peaks in July and September. The climate condition of the area is characterized by temperature range of 36°C and 45°C for daily and annual range respectively.

2.2 Sources of material and processing

Land race of orange was acquired from Otutu-Amaumara Ezinihitte Mbaise LGA Imo State. The ripe plantain obtained from Kaiama in Kolokuma/Opukuma L.G.A, Bayelsa State. 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: 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.

2.3 Experimental design

The experimental design used was a Randomized Complete Block Design (RCBD). Soil sample suspected of heavy metal polluted soil was obtained from an abandoned metal scrap site at Ikoku Rivers State Port Harcourt on geographical coordinate: Latitude 4.80083^{o}N and Longitude 6.991093^{o}E alongside with uncontaminated soil obtained from a fallow land with no know record of heavy metal pollution at University of Port Harcourt. The soils were collected at depth 0-20 cm using a spade which was then analyzed to ascertain heavy metal content and other soil chemical properties.The collected soils were 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 of height 18cm, diameter 14cm and surface area 0.095 m^2. The bags were arranged in 4 blocks (A, B, C, and D) alongside with uncontaminated soil designated as batch E. Batch A, B and C were subdivided into 3 sub plots designated as A_1, A_2, A_3, B_1, B_2, B_3, C_1, C_2 and $C_3$of 10 replications for each subplot.

Table 2. Physicochemical properties and heavy metal content of polluted and unpolluted soil

S/N	**Parameter**	**Unpolluted**	**Polluted soil**
1	Chloride (mg/kg)	213	3687
2	Sulphate (mg/kg)	28.4	269
3	Nitrate (mg/kg)	71.9	138
4	Phosphorus (mg/kg)	1.35	0.82
5	Sodium (mg/kg)	120	132
6	Calcium (mg/kg)	110	120
7	Magnesium (mg/kg)	258	280
8	Potassium (mg/kg)	43	68
9	pH	5.10	8.43
10	Conductivity (μsCm^{-1})	90	1193
11	Iron (mg/kg)	48.2	4410
12	Zinc (mg/kg)	0.94	107.5
13	Lead (mg/kg)	130	167.3
14	Cadmium (mg/kg)	0.80	15.3

Note: SOM = Soil Organic matter

2.4 Amendment treatments

Different concentration of NPK 15:15:15 was added to block A as A_1 :(40g/2kg), A_2: (80g/2kg), A_3: (120g/2kg), while block B and C contain orange and plantain peels respectively. Orange peels was added as B_1 :(100g/2kg), B_2 :(200g/2kg), B_3: (300g/2kg), and plantain peels as C_1: (100g/2kg), C_2: (200g/2kg), C_3:(300g/2kg) while no amendment was added to block D and E. That is 0g amendment which stand as polluted and unpolluted soil respectively. Acclimatization, two seedlings of test plant from the nursery after proper identification at the University of Port Harcourt Herbarium as *Echinochloa colona* Link was transplanted in block A,B, C,D and E. The planted seedlings were of the identical size and vigour. The experiment was monitored at 2 months interval. At the end of each interval, samples collected were properly characterised in line to treatments amendment and were taken to the laboratory immediately in an ice packed cooler for analysis.The parameters analysed were proline, carotenoid, glutathione and superoxidase dismutase.

2.5 Stress enzymes estimation

2.5.1 Superoxide Dismultase (SOD): The determination of SOD was based on the inhibition of NADI-phenazinemethosulpahte nitribleutetrazoliumformazon formation. The endcoloration obtained was extracted into butonal and measured at 560nm.The mixture containing 1.2ml of sodium pyrophosphate buffer, 0.1ml of phenazinemethosulphate(PMS), 0.3ml of nitroboluetrazolium (NBT), 0.2 ml of enzyme preparationand made up with water to a volume of 2.8 ml. The commencement of SOD determination was achieved by the addition of 2 ml of NADH and incubated for 90 seconds at 30^{o}C. After incubation, 0.1 ml of glacial acetic acid was added to bring the reaction to a stop. The reaction was shaked with 4.0 ml of n-butanol. This was read at 560nm in a spectrophometer (Genesys 10-S, USA). Enzyme activity (1 unit) was considered as the enzyme that gave 50% inhibition of nitroboluetrazolium reduction in 1 minute.

2.5.2 Proline: Proline was extracted using a cold extraction procedure by mixing 20-50mg fresh weight aliquots with 0.4 ml of ethanol water (40:60 v/v). The reaction mixture was left overnight on the pellet and supernatant pooled was used for the analyses. The solutions (store at 20^{0}C) Extract: 20 to 50 times diluted fresh weight (w/v), typically in a 70:30 ethanol: water mixture (v/v) Standard proline solution ranging from 0.04 to 1 mM, in the same medium as the one used for the extraction. Reaction mix: ninhydrin 1% (w/v) in acetic acid 60% (v/v), ethanol 20 % (v/v) protect from light.

1.5.3 Estimation of Total Carotenoids: Total caroteniod was determined using the method of Zakaria *et al.* (1979). As to avoid photolysis, the determination of carotenoid was carried out in dark. The homogenized sample 0.5g was saponified with 2.5ml of 12 % alcoholic potassium hydroxide in a water bath for 30 mins at 60^{o}C. The extract wastransferred into a separating funnel containing 15 ml of petroleum ether. The lower aqueous layer was poured into another separating funnel while the upper petroleum ether layer containing caroteniods was collected. This procedure was repeated continuously until colorless layer was observed. Sodium was added to petroleum ether as to remove excess mixture and final volume was noted. The yellow colour absorbance was read using a spectrometer (Gensys 10-S, USA) at 450nm and 503nm using petroleum ether as blank.

2.5.4 Estimate of Reduced Glutathione (GSH): Glutathione was determined by adopting the method of Moron *et al.* (1979). The mixture 2.5 ml of5 % TCA homogenized plant sample (0.5g) was collected and the precipitated protein was centrifuged at 1000rpm for10 mins. The supernatant (0.1 ml) was use to estimate GSH. The supernatant (0.1 ml) was made up to 1.0 ml with 0.2M sodium phosphate buffer (pH 8.0). Standard GSH corresponding to concentrations between 2 and 10 moles were also prepared, 2.0 ml of freshly prepared DTNB(5.5-dithiobis nitrobenzoieacid) solution was added as to intensify the yellow colour which was measured

using spectrophotometer (Gensys 10-S, USA) at 412 nm after 10 mins. The values were expressed as nmoles GSH/g sample.

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

3. Results and Discussion

Plant stress enzymes are biological indicators which shows or quantify the level of stress bioassay of plants. Plant stress enzymes are also known as biological markers. Excess production of reactive oxygen species are very dangerous and toxic to plant health and are capable of cause morphological and physiological damage to plants. The effects of amendment treatments was observe to improve plant health and performance. Amendment treatments also showed an improvement in shoot length, shoot fresh weight and dry weight and root fresh weight and dry weight. Plants grown in 300 g plantain showed an exceptional increase in shoot length. The application of amendment of various concentrations increased the height of *Echinochloa colona* grown in the treated soil, an increase in the height of plants was also recorded in plant grown in unpolluted with 0g amendments. It was found that there was significant difference in plant height between and within amendment treatments with time at ($p = 0.05$). Highest increase in plant height was observed in 300 g and 200 g plantain peels treated soil at 2 and 4 months respectively. This observation is possible since plantain peel amendment may have acted as a bio stimulant which enhanced soil depleted nutrienttherebystimulating microbial activitieswhich mayhave released growth hormone such as auxin (increased shoot length) and decreasedthe stress factor triggered in plants due to depletion of nutrient,. This assertion agrees with Bago (2000) who ascertain an increase in soil nutrients and microbial load in an enhanced soil.This findings also agrees with Oliver(2016) who observe an increase in plant grown in organic amendment treated soil. The decrease observed in growth performance of test plants grown in NPK amended soil as compared to plantain peel amendment may be credited to concentration of NPK added to soil. Furthermore, NPK application noxious to plant when added above recommended doses (Ibrahim, 2013). The test plant (*Echinochloa colona*) grown in NPK, powder orange and plantain peels of various concentration and unpolluted unamended soil showed an increase in root fresh weight yield and 300 g orange peels treated soil significant showed the highest increase in root fresh weight at 2 and 4 months, while least decrease was observed in polluted unamended soil. In figure 3, highest increase in root dry weight yield was also recorded for 300 g powder plantain peels soil amendment at 2 and 4 months. The result presented in Figure 4 and 5 also showed an increase in shoot fresh weight and dry weight of *Echinochloa colona*) grown in NPK, powder orange and plantain peels of various concentration showed an increase in shoot fresh weight and dry yield. Reduction in shoot fresh and dry weight yield was recorded in plant grown in polluted soil at 2 and 4 months. This increase in plant root and shoot fresh and dry weight may be attributed to the ready available soil mineral nutrients obtained from the added amendments might have influence shoot length and crop yield. The decrease in height of *Echinochloa* sp grown in polluted unamended soil may possibly be as a result of the negative toxicity effects of heavy metals which might have triggered the overproduction of reactive oxygen species which negatively influenced plant optimal performance. This findings corroborate with thereported by Joshi and Mohanty (2010) whodiscovers an effect of metals disrupting protein link and substitute itself with important plant nutrient hence influences plants negatively.

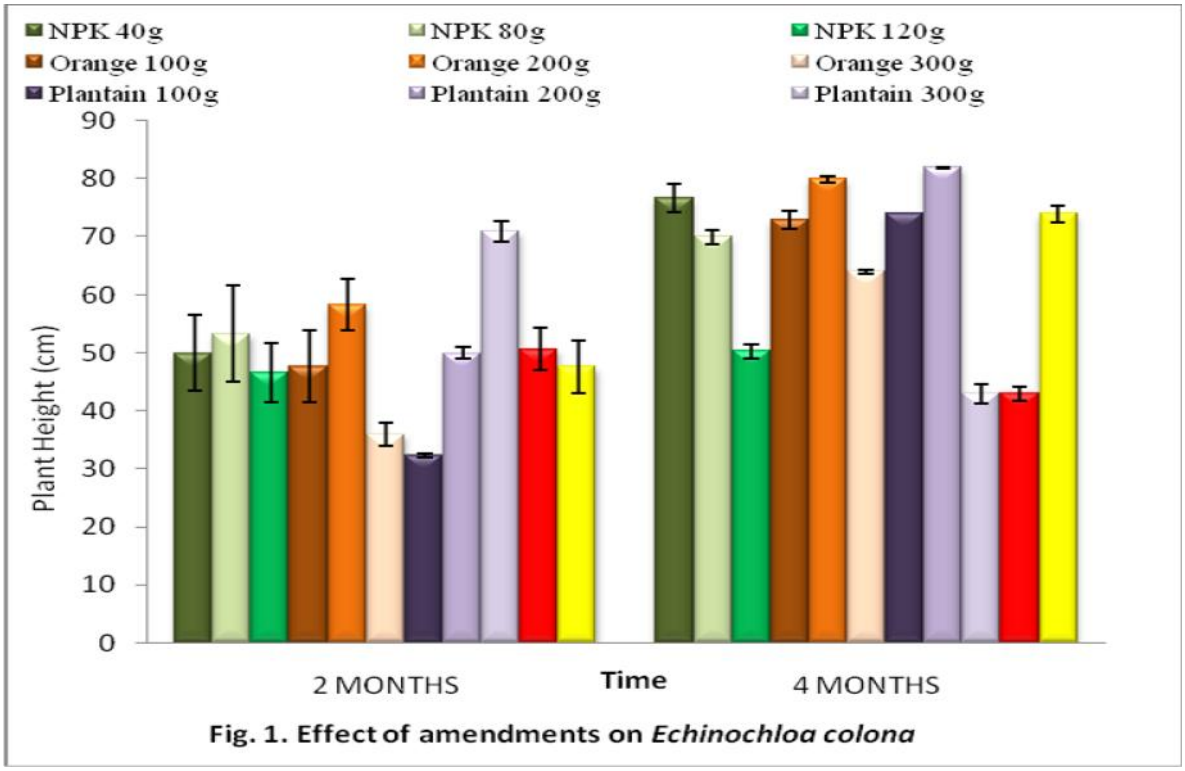

Fig. 1. Effect of amendments on *Echinochloa colona*

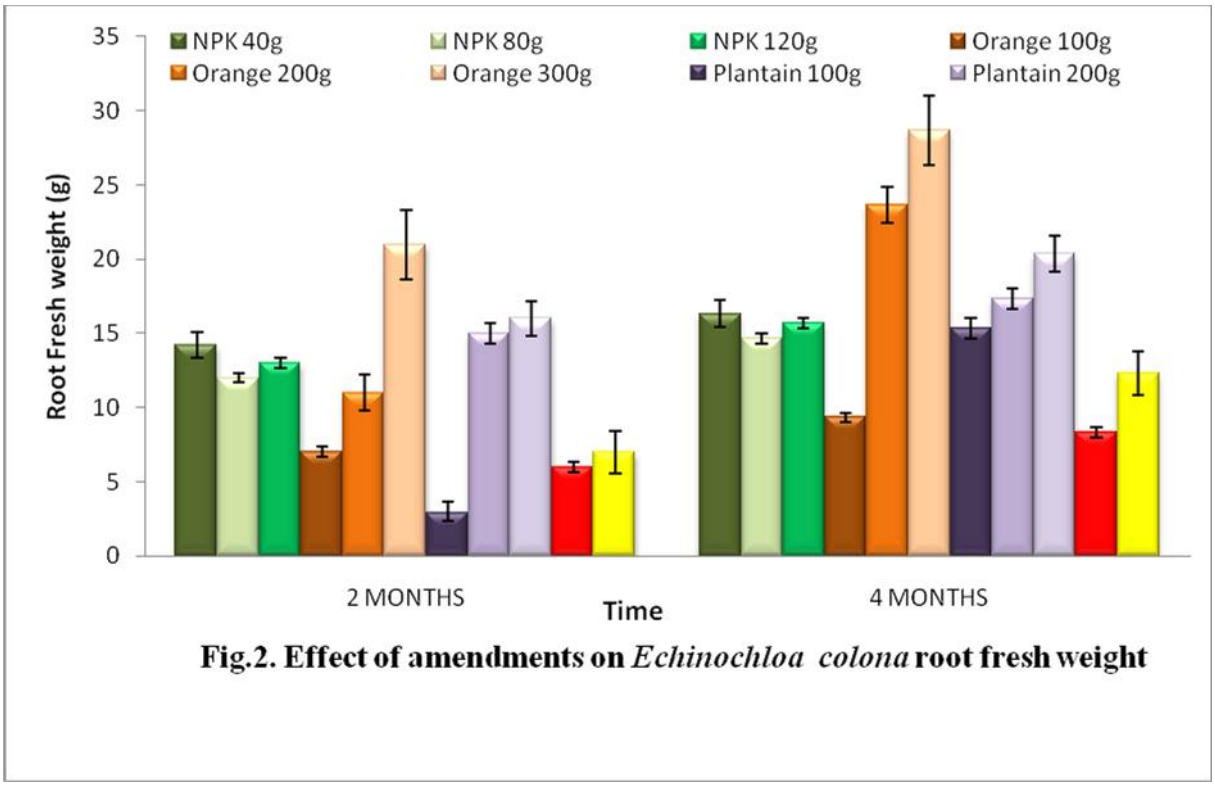

Fig.2. Effect of amendments on *Echinochloa colona* root fresh weight

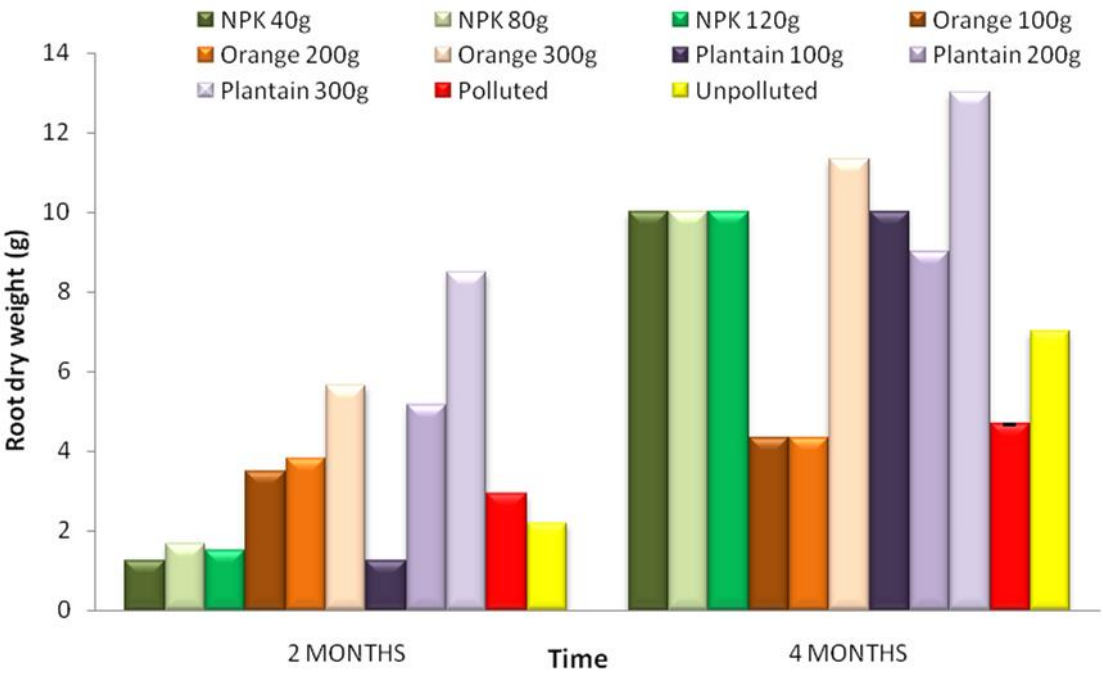

Fig. 3. Effect of amendments on *Echinochloa colona* root dry weight

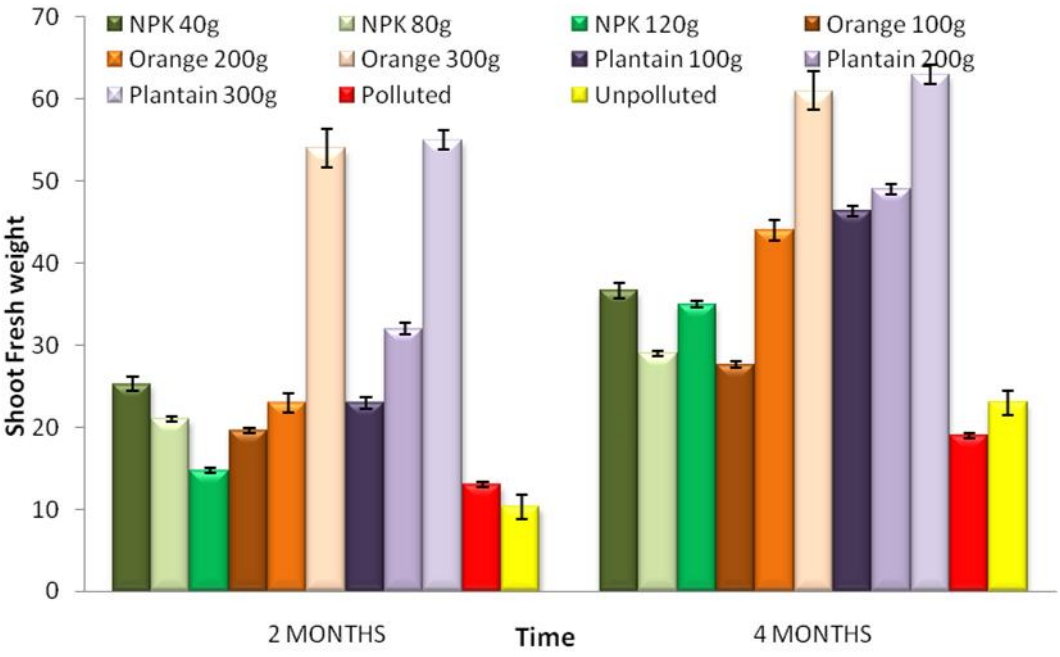

Fig. 4. Effect of amendments on *Echinochloa colona* shoot fresh weight

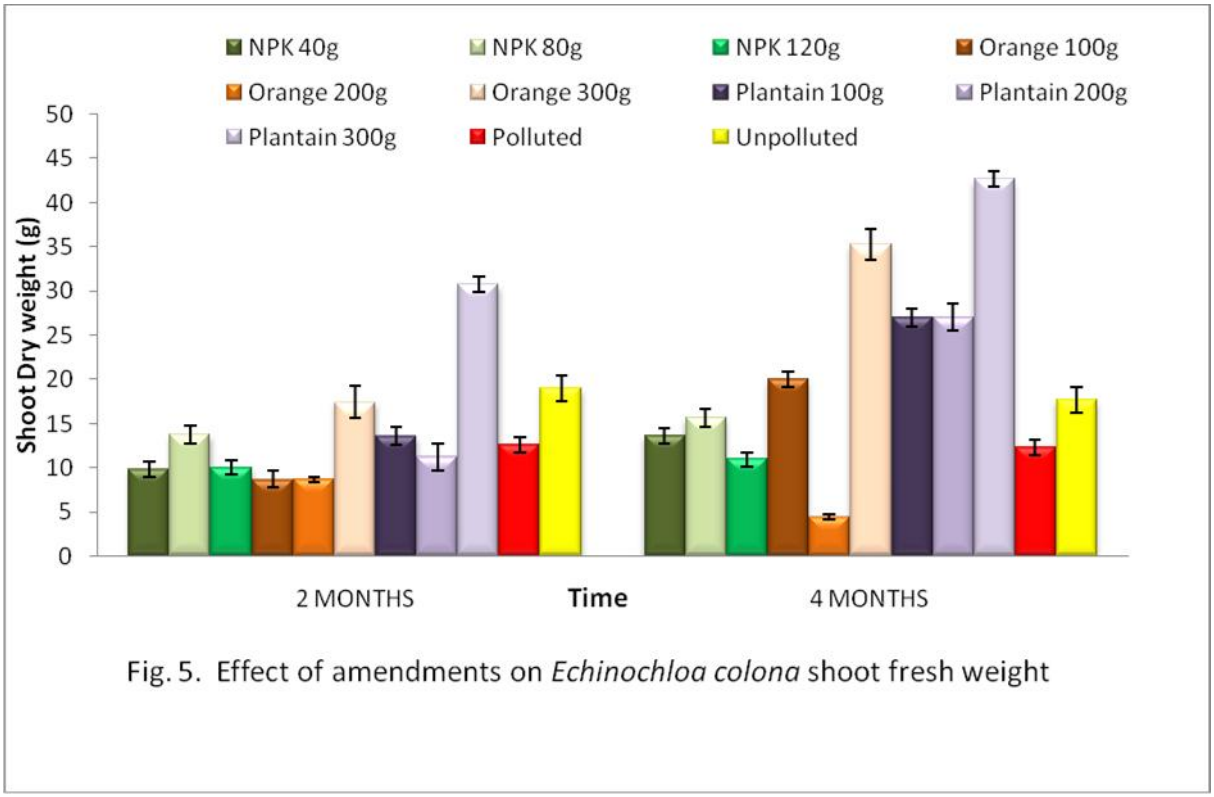

Fig. 5. Effect of amendments on *Echinochloa colona* shoot fresh weight

Proline Content

The added amendments of various concentrations showed a decrease on proline activity in roots and shoots of *Echinochloa colona is* presented in Figure. 6 and 7. Comparing the effects of amendment on proline at 2 and 4 months, result showed a reduction in proline activity in root of *Echinochloa colona* grown in powder orange peel (waste), powder plantain peel and NPK treated soil of various concentrations.The least in proline activity was recorded in plant grown in different concentrations of NPK plant while highest increase in proline activity was found in plants grown in polluted soil (0g amendments) at 2 and 4 months. There were significant statistical differences between and within amendment at (P = 0.05). The least decrease in proline content in root of *Echinochloa colona* was recorded for 120 g NPK and 200 g powder plantain peel soil treatment at 2 and 4 months. In Figure 7. Plant grown in unpolluted soil showed an increase in shoot proline content. In shoot of *Echinochloa colona*, amended soil showed a reduction in proline content.The activity of proline content in shoot of *Echinochloa colona* was lowest in unpolluted soil (0g amendment) at 2 month, while the highest increase at 4 months was recorded for plant grown in polluted soil. The increase in proline activity of plant *Echinochloa colona* grown in polluted un-amended soil could attributed to lack of available nutrients in polluted soil. This findings corroborated with the reported of Amadi *et al*., (2018) who reported that plant species growing in an enhanced soil will have improved growth performance and it will also show a stable morphological, anatomical and physiological process than plan growing in a polluted environment. This implies that, lack of available nutrients triggered the proline stress pathway of *Echinochloa colona*. The decrease in proline activity of *Echinochloa colona* grown amended soil could be attributed to amended amendment which might have stimulated microbial population capable of breaking down the amendment and converting them into 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 degradation of LMWOA's may be responsible for the decrease in pH (Zulfigar *et al*., 2012).

Additionally, soil pH also plays a major role in nutrient availability (Zulfigar *et al*., 2012; Yashin *et al*., 2014). The available nutrient decrease the toxic effects of heavy metals resulting to decrease in proline activity. Sharma, (2006) also reported similar findings on the phenomenon of proline accumulation is known to occur under water deficit, salinity, low temperature, heavy metal exposure and UV radiations contributes to stabilizing sub-cellular structures, scavenging free radicals and buffering cellular redox potential under stress conditions.

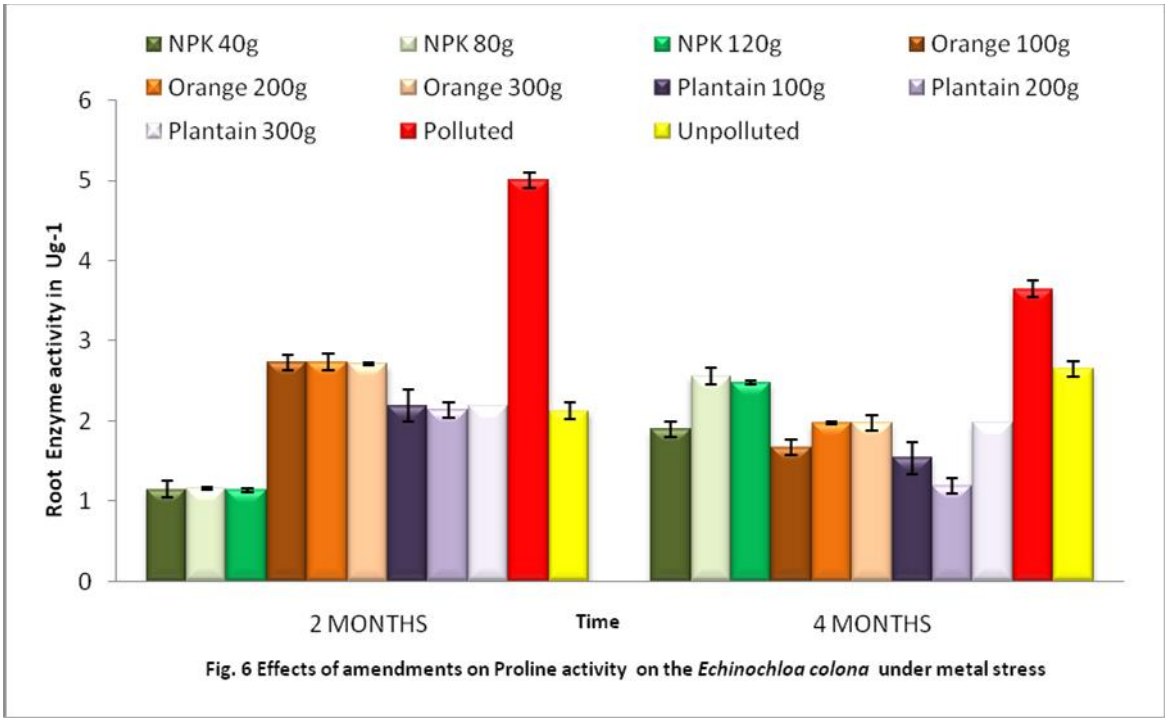

Fig. 6 Effects of amendments on Proline activity on the *Echinochloa colona* under metal stress

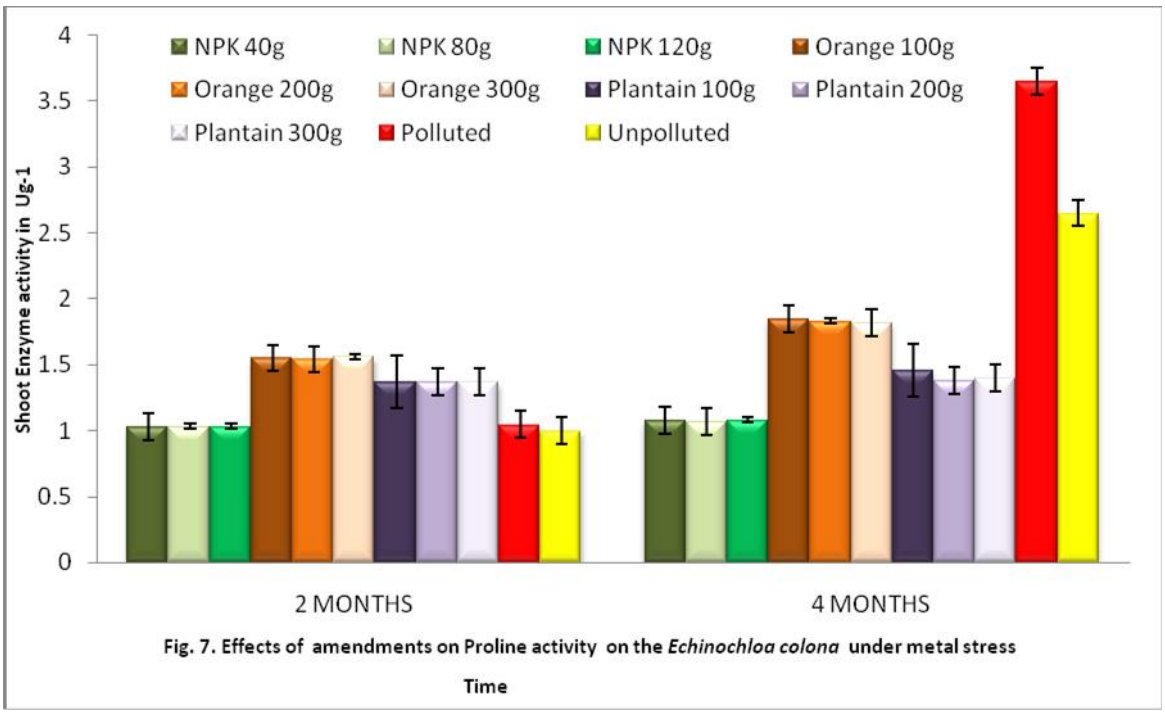

Fig. 7. Effects of amendments on Proline activity on the *Echinochloa colona* under metal stress

Super oxidase dismutase (SOD) Content.

Super oxidase dismutase (SOD) is an essential antioxidant which is very efficient in scavenging negative effects excess production of ROS (free oxygen radicals) when plant is expose to various environmental perturbation (Asada, 1994).The added amendments decreased SOD activity in roots and shoots of *Echinochloa colona* (Figure. 8). There were significant statistical differences between and within amendments at (P = 0.05). The least in SOD antioxidant biomarker in root of *Echinochloa* sp was observed in 300 g orange peel soil amendment at 2 and 4 months, while highest antioxidant activity was found for plant grown in polluted soil without amendment. The effect of amendment on SOD content in shoot of *Echinochloa* sp at 2 and 4 months was also observed. The amendment treatments also decreased SOD content of shoot *Echinochloa colona*. The least decrease in SOD shoot concentration was recorded for plant grown in 300g plantain peel amended soil at 2 and 4 months respectively. There were significant statistical differences between amendments at (P = 0.05).Highest SOD response in shoot of *Echinochloa* was found in polluted soil of 0g amendment at 2 and 4 months (Figure 9). The decrease in shoot and root SOD content of the test plant could be attributed to different type and concentrations of amendment added might have release the necessary nutrients needed by plant which may have help to ameliorate the nutrient depletion impact and also mitigate toxic effects of heavy metals. This findings corroborated with the report of Tewari *et al.* (2004) and Prochazkova *et al*. (2001) who observed greater SOD activity in nitrogen starved maize and also an increased SOD activity in maize after tasseling for 25 days respectively.

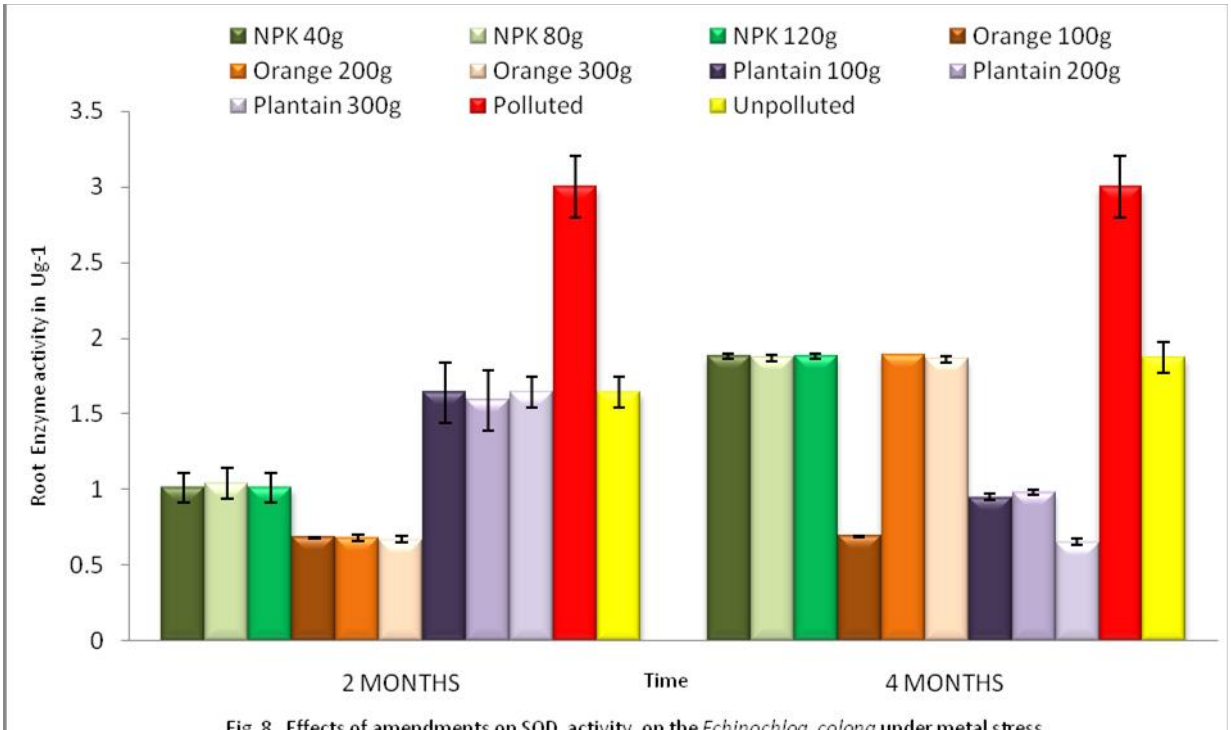

Fig. 8. Effects of amendments on SOD activity on the *Echinochloa colona* under metal stress

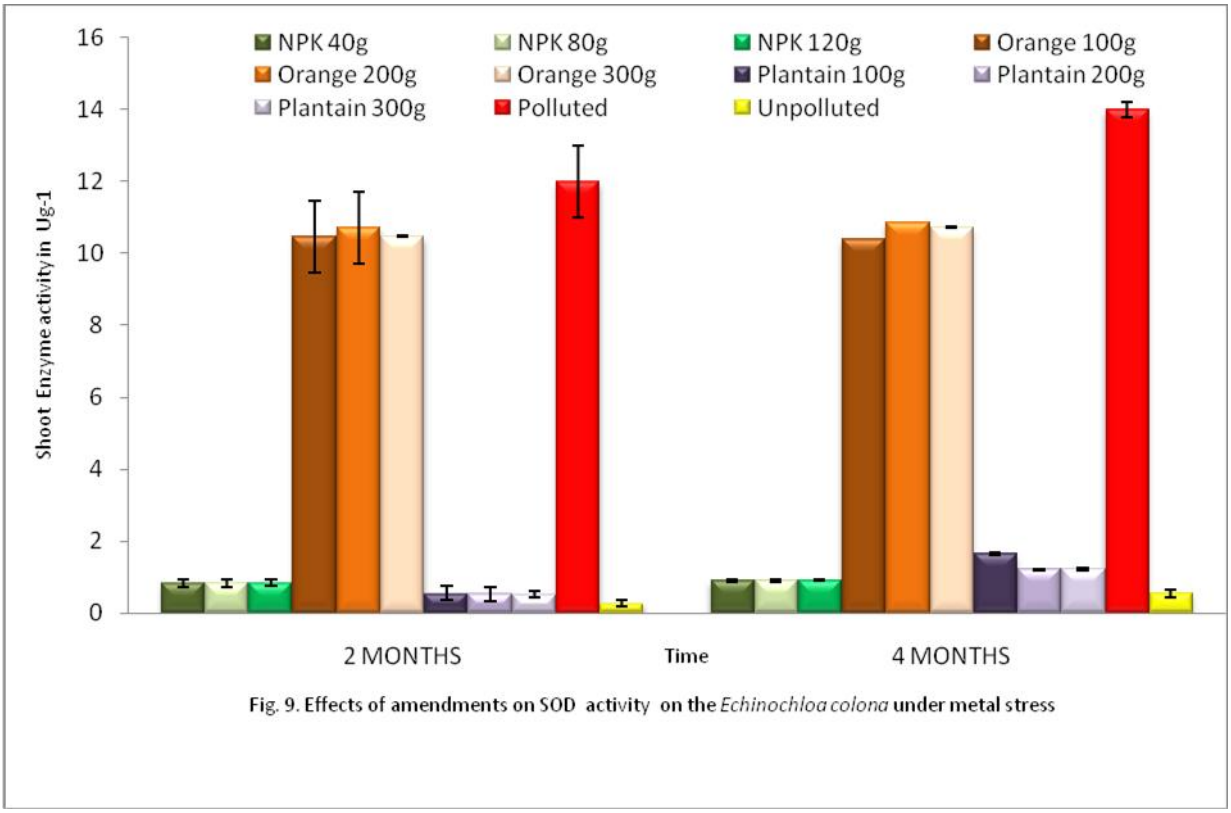

Fig. 9. Effects of amendments on SOD activity on the *Echinochloa colona* under metal stress

The various amendments showed a decrease on CARO activity in roots and shoots of *Echinchloa colona* plants is presented in Figure 10. Comparing the effects of amendment on CARO at 2 and 4 months the root of plant grown in the treated soil of different amendments and various concentrations showed a decrease in CARO. while plant grown in polluted unamended soil showed an increase in caroteniod antioxidant activity in root at 2 and 4 months. There were significant statistical differences between amendments at (P = 0.05).While least in antioxidant activity was observed in 300g and 100g orange peels soil amendment at 2 and 4 months respectively.

Conversely, there was significant difference as shown in (Figure 11). The effect of treatment on CARO content in shoot of *Echinochloa colona* at 2 and 4 months was compared. The shoot of plant grown in NPK, orange peels, plantain peels amendment, control and double control increased CARO content. There were significant statistical differences between amendments at (P = 0.05).The activity of caroteniod antioxidant in shoot of *Echinochloa colona* was lowest in 300 g orange peels soil amendment at 2 and 4 month. While, highest caroteniod activity was found polluted with 0g amendments at 2 and 4 months. The increase in carotenoid activity of plant grown in polluted soil could be attributed to the toxic nature of the heavy metals present in the soil might have impacted on the stress equilibrium. The alteration of the stability in equilibrium between ROS production and antioxidant enzymes can trigger increase in carotenoid content which is among the important pigment in plants and also responsible as biomarkers. This proposition is also response with the findings of Padmaja *et al*. (1990) who conducted an experiment to check the influence of heavy metals on plant pigment level and it was concluded that heavy metal impact influence negatively the pigment production levels in plants.

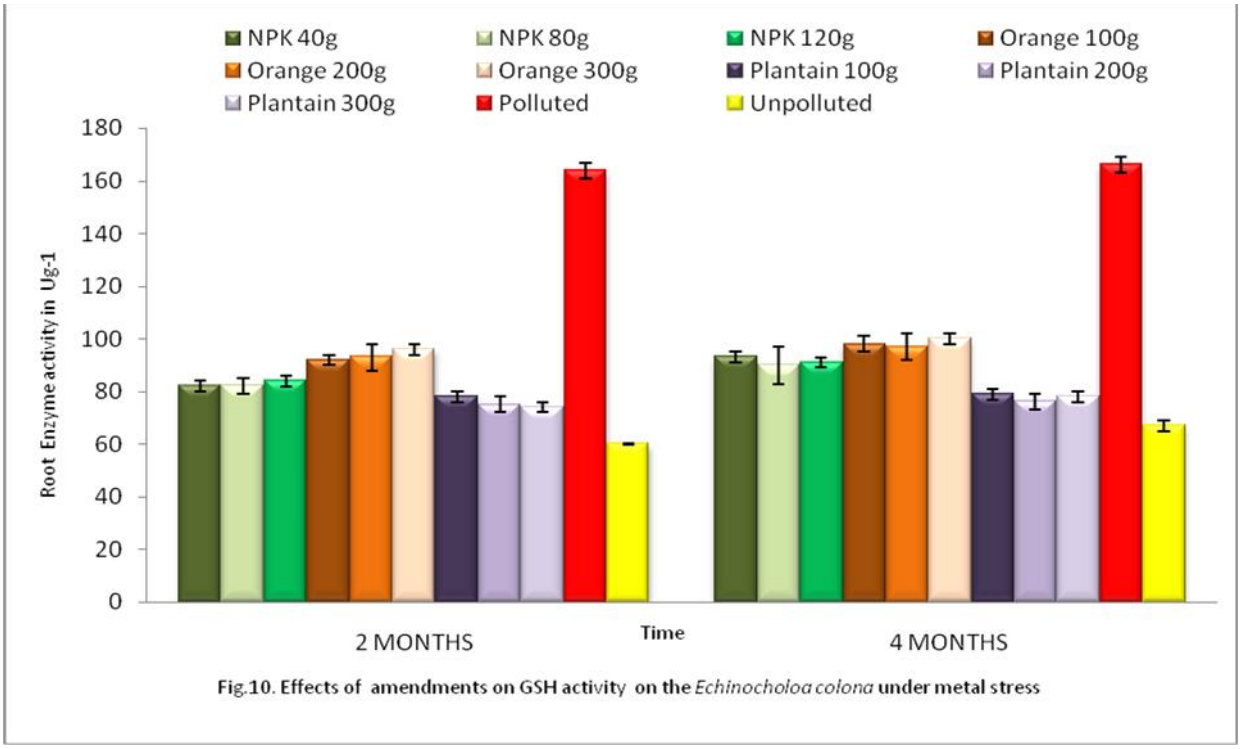

Fig.10. Effects of amendments on GSH activity on the *Echinocholoa colona* under metal stress

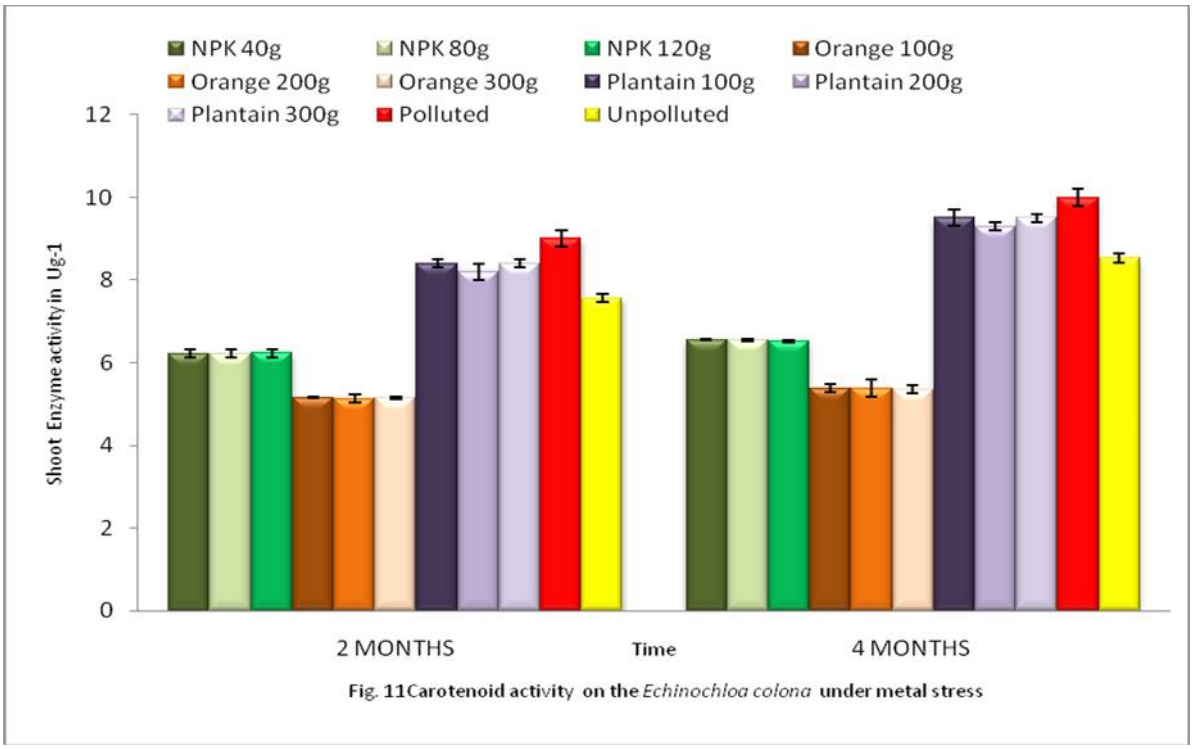

Fig. 11Carotenoid activity on the *Echinochloa colona* under metal stress

The various amendments showed a decrease on GSH activity in roots and shoots of *Echinchloa colona* plants is presented in Figure. 12. Comparing the effects of amendment on GSH at 2 and 4 months. The root of plant grown in NPK, orange peels, plantain peels amendment, polluted and unpolluted soil increased GSH content. There were significant statistical differences between and within amendments at (P = 0.05). Increase in GSH antioxidant activity in root of *Echinochloa colona* was found highest in polluted soil with 0g amendment at 2 and 4 months respectively. While lowest antioxidant activity was observed in unpolluted soil with 0g

amendments at 2 and 4 months. In Figure 13 showed the effect of amendment on GSH content in shoot of *Echinochloa colona* at 2 and 4 months. The shoot of plant grown in NPK, orange peels, plantain peels amendment, polluted and unpolluted soil shown an increase GSH content. There were significant statistical differences between amendments at P = 0.05.The activity of GSH antioxidant in shoot of *Echinochloa colona* was lowest 120 g NPK (20:20:20) orange peels soil amendment at 2 and 4 month while, highest GSH activity was found in polluted un-amended soil at 2 and 4 months respectively. The decrease in GSH of *Echinochloa colona* (root and shoot) grown in amended soil could be as the result of the amendment added may have enhance the availability of nutrient will help cushion the effect of ROS production. This findings agrees with the report of Sharma (2006) and Seth e*t al.*, (2012) who reported that nutrient depletion and toxicity level of a soil is capable of influencing ROS production. These factors are crucial determinate in the optimal growth of plant species.

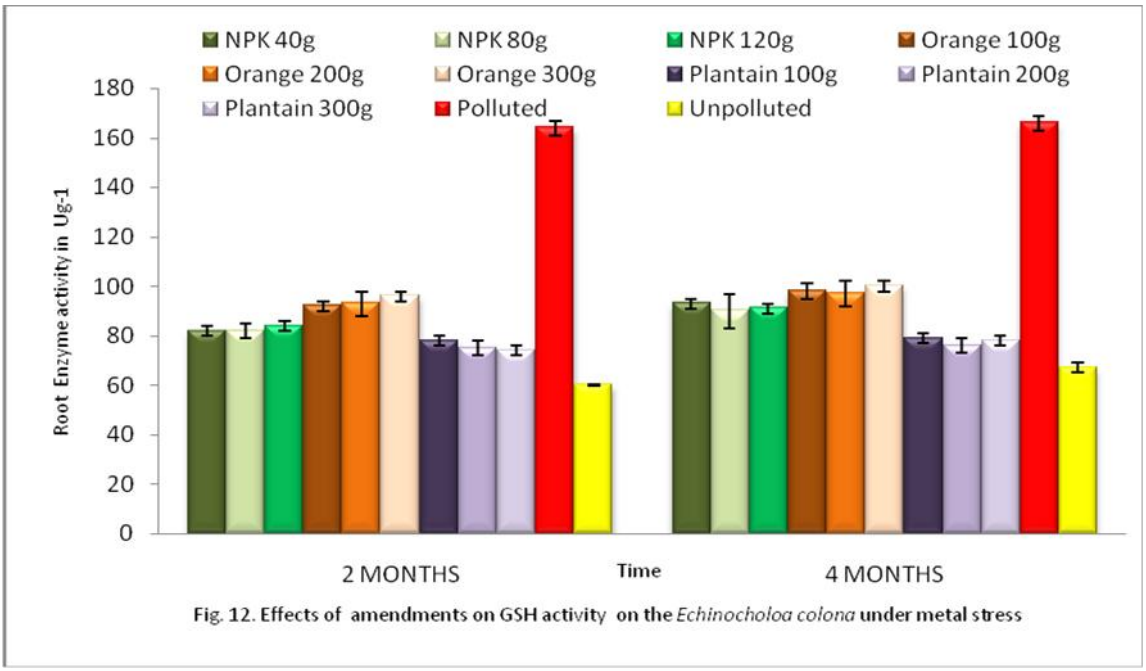

Fig. 12. Effects of amendments on GSH activity on the *Echinocholoa colona* under metal stress

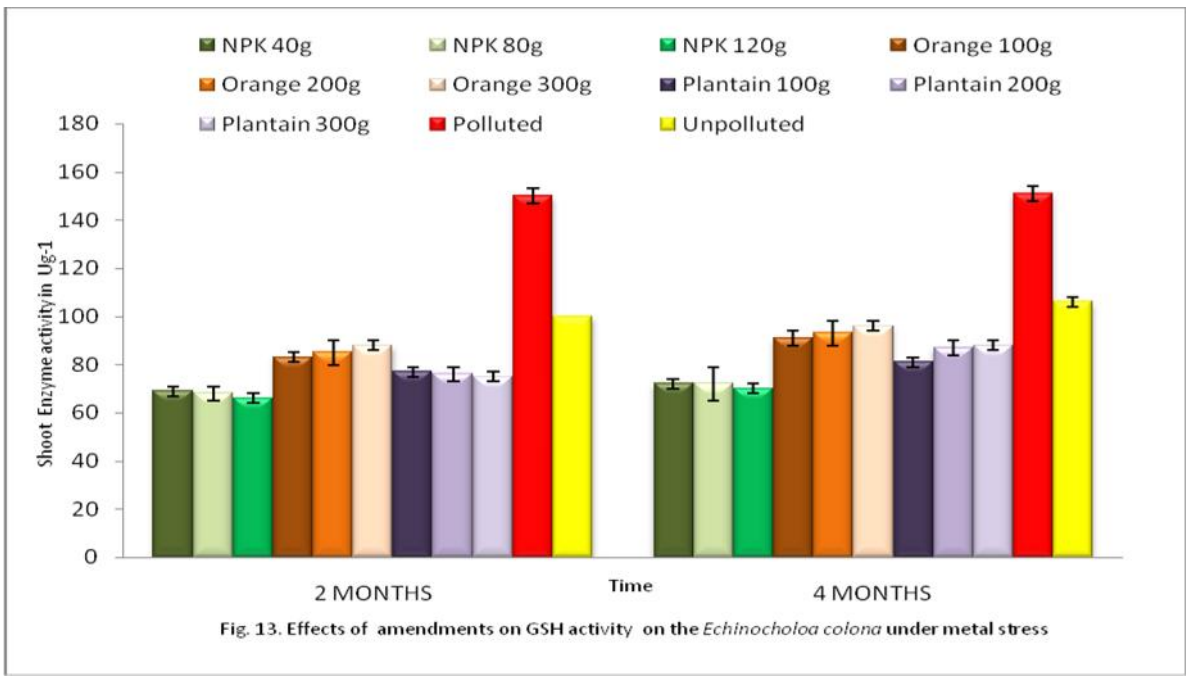

Fig. 13. Effects of amendments on GSH activity on the *Echinocholoa colona* under metal stress

4. Conclusion

It is well documented that anthropogenic activities is a major factor responsible for the increasing heavy metals concentration in the environment. The presence of these metals negatively influenced plant metabolic process due to its toxicity level and un-available nutrients. Results from the study showed the benefits of soil enhancer application significantly improved soil fertility and inhibited the toxicity impact of heavy metals on plants. These added amendments was effective in decreasing ROS production stress triggered by heavy metal toxicity exposure and soil nutrient depletion. The study therefore recommends that NPK fertilizer, orange and plantain peels (waste) could be an efficient amendment capable of enhancing optimal plant growth in a polluted environment and also useful in enhancing plants tolerance towards harsh environmental conditions which is envisage with recent trends of globalization, industrialization and increase in population.

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DOI:10.22192/ijarbs.2023.10.04.014	

How to cite this article:
Amadi. N, Okogbule, F.N.C and Chikere L.C. (2023). Soil enhancer: A vital tool for plant stress management in heavy metal polluted environment. Int. J. Adv. Res. Biol. Sci. 10(4): 168-182.
DOI: http://dx.doi.org/10.22192/ijarbs.2023.10.04.014