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# Changes In Morpho-Physiological And Antioxidants Enzymes In Fragrant Rice Seedlings Affected By Cadmium (CD) Stress

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***Abstract***
*Heavy metals like cadmium (Cd), found in agricultural soils are highly toxic to both plants and animals and pose threats to future crop production and food safety. This research investigates the impact of Cd stress on seedlings of five fragrant rice varieties: Meixiangzhan 2 (V1), Xiangyaxiangzhan (V2), Guixiangzhan (V3), Basmati (V4), and Nongxiang 18 (V5). The study assessed four Cd stress levels (0, 50, 100, and 150 mg/kg soil) conducted in a randomized complete block design. The results indicated that increased Cd toxicity disrupted both antioxidant and non-antioxidant enzyme activities, including Superoxide Dismutase (SOD) and Catalase (CAT). Elevated lipid peroxidation, measured by malondialdehyde (MDA) and hydrogen peroxide ($H_2O_2$), was also noted. Growth parameters such as plant height and total dry matter were adversely affected by Cd levels, with roots showing higher Cd uptake than shoots. Notably, Meixiangzhan 2 and Nongxiang 18 exhibited the highest accumulation of Cd, while Guixiangzhan (V3) showed better tolerance. The findings highlight the detrimental effects of soil Cd on rice seedling growth and the variation in cultivar responses to Cd stress at vegetative stage*

***Keywords:*** *Cadmium, Fragrant Rice Seedlings, Uptake, Antioxidants Enzymes, Vegetative Stage*

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## I. Introduction

Cadmium is among the most toxic heavy metals deposited in agricultural soils through natural and man-made activities, such as; the application of sewage sludge containing Cd contents, phosphate fertilizers application, and other waste disposal as well as metal smelting (Rizwan et al., 2012, Douay et al., 2009). Cd toxicity in agricultural soils is a serious threat to crop production worldwide (Rizwan et al., 2016). Even in low concentration, Cd becomes highly toxic to both growing plant as well as animals due to its nonessential form in living organisms, and thus affects rice seedlings morphologically, physiologically and biochemically during growth. (Song et al., 2015) reported that Cd can be taken up by rice roots and then translocated to shoots and grains of growing rice. This affects the growth and performance of plants, the most common visible symptoms of Cd toxicity in growing rice plants are; retardation of plant growth, chlorosis and eventually plant death.

High Cd accumulation in rice consumed by animals especially humans, has several health implications such as cardiac failure, anemia, cancer, hypertension, emphysema, proteinuria, cerebrovascular infarction, damage to the lungs, renal dysfunction in eyes, and osteoporosis (Ashraf et al., 2015, Sebastian et al., 2015, kanu et al., 2019). Cd

translocation from soil to shoot and finally to the edible parts of rice (grains) is the easiest pathway by which increasing Cd exposure opportunity to human beings is (Römkens et al., 2011). Research has shown that nearly sixteen percent (16%) of the agricultural soils in China have been polluted by varying heavy metals, of these, approximately 1.3×105 $hm^2$ of these soils have been polluted by varying degrees of Cd, and agricultural products polluted by Cd resulting to 1.46×108 kg, including 50 000 tons of rice (Xu et al., 2014).

Rice (Oryza sativa L.), is a major cereal crop cultivated and consumed worldwide, it's the second most important cereal crop after wheat in terms of area cultivated and consumption rate. Due to the level of importance attached to rice, it's therefore, becomes eminent to understand how different aromatic rice cultivars perform under varying levels of cadmium toxicity. Rice growth and development are most eminent during the vegetative growth stage where seedlings' development is initiated. Rice growth at tillering stage comprises roots development for nutrients uptake, hypocotyl elongation, and enzyme activation for mobilizing stored

energy and nutrients as well as photosynthetic processes. Cd stress has been proven to have some morpho-physiological and antioxidant effects in rice seedlings at the early growth stage (vegetative stage), especially in paddy polluted soils. Reports have shown a reduction in rice growth and biomass, which might possibly be a result of different Cd-mediated toxicity mechanisms in rice (Srivastava et al., 2014). Other studies have reported that the toxic effects of Cd increased rice seedlings' oxidative stress by releasing reactive oxygen species (ROS) like malondialdehyde (MDA) contents, hydrogen peroxide ($H_2O_2$) and electrolyte leakage which affects rice growth (Srivastava et al., 2014, Yu et al., 2013). Cadmium toxicity also altered leaf and root ultrastructure and caused structural damage to the photosynthetic apparatus of rice.

From several other studies conducted, marked differences in Cd uptake and translocation among plant species as well as among cultivars within the same species were observed (Kanu et al., 2017, Liu et al., 2007, Grant et al., 2008). However, considering the level of importance attached to fragrant rice, in terms of palatability and high market value compared to other rice varieties, several kinds of research conducted have not mainly focused on examining fragrant rice seedlings' performance at the vegetative stage when subjected to Cd stress conditions, little is known regarding different fragrance rice genotypes performances at tillering stage when grown under cadmium toxic soils. This study, therefore, took a close looked at the variation of Cd uptake in fragrance rice roots and translocation to stems and leaves as well as activities of antioxidant enzymes and growth parameters during the vegetative stage. The results will serve useful purposes for understanding the performance of different aromatic rice cultivars under varying cadmium stress.

## II. Research Design

**Soil Preparation And Pot Experiment**

A pot experiment was conducted in rain protected greenhouse under open-air conditions at the experimental research farm (College of Agriculture, South China Agricultural University (SCAU)), Guangzhou city (23°14′ N, 113°37′ E, 20 M altitude) during 2016/2017 cropping years. The pots were arranged in a two Factor Factorial Experiment with a Completely Randomized Design, each treatment replicated three times. Paddy soil from the research farm was collected to a depth of 20 cm and then air dried, after air-drying, the paddy soil was grinned and sieved through a 4 mm sieve, mixed thoroughly, and filled in the pots (25 cm diameter by 30 cm height). About 10 kilograms of soil was filled in each pot and Cd in the form of $CdCl_2.2\ 1/2\ H_2O$ was added to the soil to obtain the following Cd levels; a control with no added Cd (Cd 0), 50 mg Cd/kg of soil added (Cd 1), 100 mg Cd/kg of soil added (Cd 2) and150 mg Cd/kg of soil added (Cd 3). The pots once filled with soil were thoroughly mixed with the exact amount of Cd ($CdCl_2.2\ 1/2\ H_2O$) based on the treatment and kept for 15 days before the rice seedlings were transplanted in them. The soil used for the experiment was analyzed before use and was found to contain 4.96 mg/kg Cd content, 5.92 pH level, 18.73 g/kg organic matter contents, while total NPK was 0.81, 0.9 and 16.79 g/kg, and available NPK 69.15, 10.15 and 109.62 mg/kg respectively.

**Rice Seedlings Preparation And Transplantation**

Five aromatic rice varieties, Meixiangzhan 2 (V1), Xiangyaxiangzhan (V2), Guixiangzhan (V3), Basmati (V4), and Nongxiang 18 (V5), were secured from the Department of Crop Science and Technology, College of Agriculture, South China Agricultural University (SCAU)), Guangzhou. Rice seeds were first soaked in deionized water for about 48hrs. at room temperature (20-25°) and later nursed on March 3rd, 2016 in uncontaminated soil using parachute trays under moist conditions. After 15 days (29$^{th}$ March, 2016), the seedlings were transplanted into the pots (4 seedlings per pot). The pots were maintained under flooded conditions of about 2-3 cm water level above the soil surface during the whole vegetative growth period. Required rates of fertilizers were split and applied as basal. Entire rice was harvested and all sample parameters were collected after seven weeks of the vegetative growth period. The samples were separated into fresh samples stored in refrigerators at required temperatures for enzyme analysis, while the other set was oven dried at 80°C for analysis of plants biomass accumulation and Cd uptake in straw and root.

**Procedure for parameters Detection.**

Enzymes were extracted following Cho and Seo's (2005) method. In which rice straws were homogenized using a mortar and pestle with a 0.05 M sodium phosphate buffer of Ph 7.5 containing Mm ethylenediaminetetraacetic acid (EDTA) and 1% polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 12,000 g for 15 minutes and the supernatant was stored at $4^0$ c and later used to determine antioxidant enzymes.

Superoxide dismutase, (SOD) activity was determined following Zhou et al., (2015) with slight modification. The reaction mixture containing 81 mL of 14.5 mM methionine, 3 mL of 2.25 mM nitroblue tetrazolium chloride (NBT), 3 mL of 3 μM EDTA-$Na_2$ and 3 mL of 60 μM riboflavin, was prepared with a 0.05 M sodium phosphate buffer (pH 7.8) with the exception of riboflavin which was prepared using deionized water.

50 µL enzyme extract was added to the tubes containing 3 mL of the reaction mixtures. The mixtures in the tubes were then exposed to an illumination incubator for a duration of 20 min with one kept in the dark to serve a as control. The absorbance was recorded using a spectrophotometer at 560 nm. One unit of SOD enzyme activity was defined as the quantity of SOD required to produce a 50% reduction of NBT under experimental conditions and the specific enzyme activity expressed in units per g leaves fresh weight (FW).

Peroxidase (POD) activity was detected following the procedure of Xu et al., (2014), reaction mixtures containing 75 mL of 100 mM sodium phosphate buffer (pH 6.0), 28.5 µL $H_2O_2$ (30%), and 42 µL guaiacol, were prepared just before use. 1 mL enzyme extract then added to the 3 mL reaction mixtures. The increasing absorbance was measured at 470 nm at 1 min intervals up to 3 min using a spectrophotometer. One unit of POD enzyme activity was defined as absorbance changes at 470 nm per minute. Enzyme specific activity was expressed as units per g FW.

Catalase (CAT) activity was determined using a spectrophotometer following Xu et al., (2014), in which the reaction mixture containing 1.5 mL of 0.05 M sodium phosphate buffer (pH 7.8), 1 mL deionized water and 0.3 mL of 0.1 M $H_2O_2$ was prepared immediately before use, 0.2 mL enzyme extract was then added to the reaction solution. Catalase CAT activity was measured by monitoring the decrease in absorbance at 240 nm due to $H_2O_2$ consumption. One unit of CAT enzyme activity was defined as changes at 240 nm per minute. Enzyme specific activity was expressed as units per g FW.

Ascorbate Peroxidase (APX) was determined by observing the decreasing absorbance at 290 nM using a photo spectrometer machine. Glutathione Reductase (GR) was detected by observing the decreased absorbance at 340 nM using a photo spectrometer machine. Glutathione (GSH) and AsA were measured following Griffiths procedure based on enzymatic recycling.

Lipid peroxidation was expressed as malondialdehyde (MDA) content in µM per g FW following Zhang et al. (2015) with minor modifications. The fresh leaves were homogenized in 5 mL of 10% trichloroacetic acid (TCA) using a pestle and mortar. The homogenates were centrifuged at 4000 g for 10 min. To each 2 mL aliquot of the supernatant, 2 mL of 0.6% thiobarbituric acid (TBA) in 10% TCA was added. The mixture were heated at 100°C for 30 min and then cooled in an ice bath. The mixture was centrifuged at 10,000 g for 10 min and the absorbance of the supernatant recorded at 532 nm and 450 nm. Hydrogen peroxide ($H_2O_2$) content in the rice seedlings was measured by observing the absorbance at 410 nm using a photo spectrometer machine.

Growth parameters detection (shoot length and dry matter content), Plant heights and dry matter contents per plant were determined for every treatment. Seedling heights were measured using a ruler at harvest after 45 days growth period. Whereas, dry matter contents was determined by weighing 1hill of oven dried rice samples.

**Cd uptake detection**

After oven drying, plant samples were then ground to powdered form and digested using concentrated $HNO_3$ and $HClO_4$ in an 8:2, V/V as suggested by Allen (2001). After digestion, Cd concentrations in the roots and straw were analyzed using a flame atomic absorption spectrometer.

**Statistical analysis**

Data was analyzed using Microsoft excel 2010 and Analysis of variance was performed using Statistix 8 (Analytical, Tallahassee, Florida, USA). The data were analyzed by one-way analysis of variance to assess differences in parameters between treatments.

## III. Results

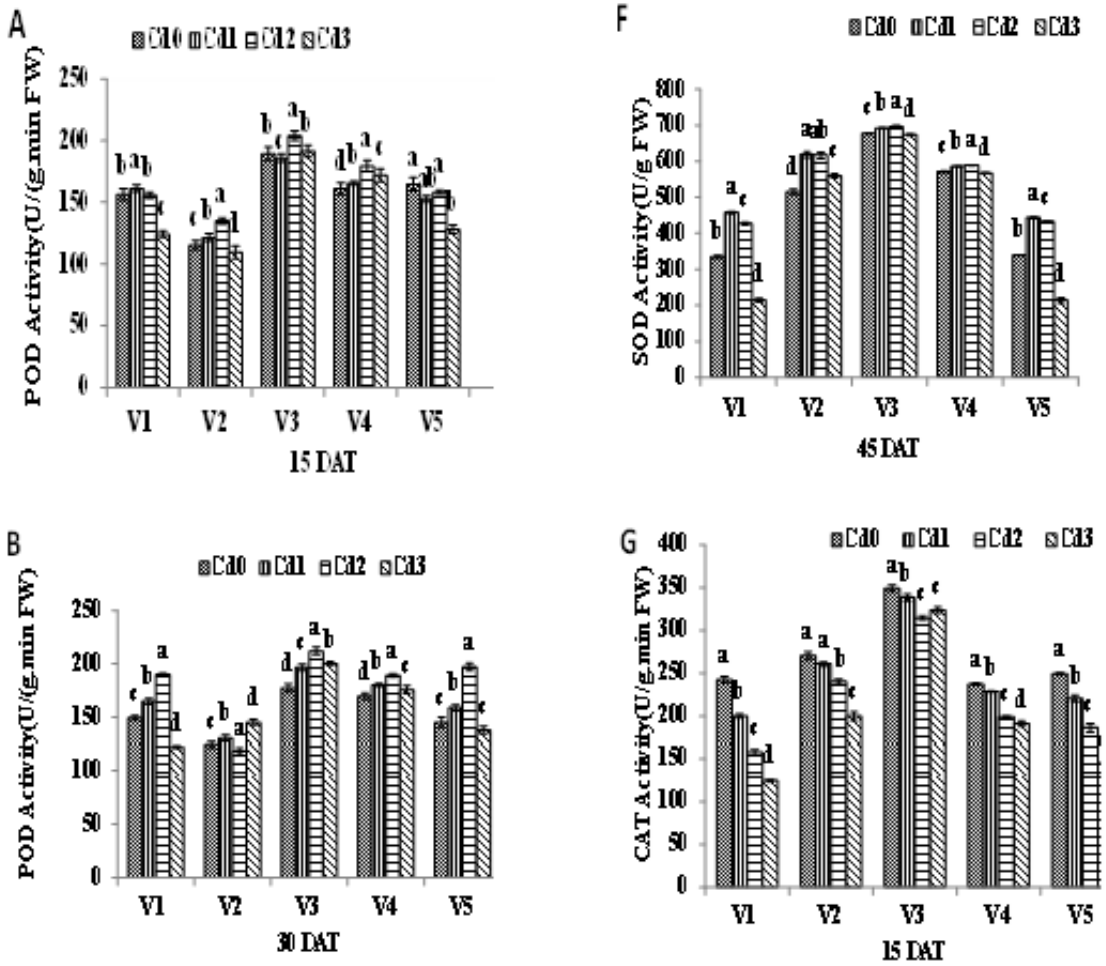
**Changes in rice seedlings antioxidant enzymes (POD, SOD, CAT) under induced cadmium stress at Different Sampling Time.**

Changes in POD activity for all treatments and cultivars were observed during the $15^{th}$, $30^{th}$ and $45^{th}$ days after transplant and the results presented in figures 1a, b and c. The results showed significant differences among treatments of the same cultivars. Initial increases in the activity of POD were observed with increased levels of Cd toxicity. When compared with control treatments (fig.1a), there was no regular increase or decrease in the activity of POD in all the cultivars under study except for cultivars V1, V2 and V5, which saw a 17.1, 4.96 and 4.97 % decrease in POD activity at the 150 mg/kg Cd toxicity respectively. From fig 1b, maximum initial increase in POD activity were observed in varieties V1 and V5 while varieties V2, V3 and V4 showed minimum initial increase when compared to their controlled. At maximum induced Cd concentrations (150 mg $Cd/kg^{-1}$), activity of POD were found decreased, such decrease was found higher in varieties 1 and 5 (V1 and V5) (fig 1b and c), for every increased Cd level, POD activity was found higher compared to control, except in the maximum induced Cd levels where POD activities decreased in response to stress conditions. From (fig. 1b), when rice seedlings were exposed to 150 mg/kg Cd toxicity, there was a 6, 5.2 and 4.9% decrease in POD

activities for cultivars V1, V2 and V5 respectively, and in fig 1c, there was a 10.5, 4.3 and 4.4% decrease in POD activities for cultivars V1, V2 and V5 respectively. The decrease in pod activity indicated toxic effects of Cd in rice seedlings.

Changes in the activities of SOD were also observed for all treatments in the different rice cultivars and results shown in figs. 1d, e and f. from fig. 1d, the results showed an increased activity of SOD with increased levels of Cd concentrations when compared with controls for some cultivars, whilst in other cultivars, there was not regular increase or decrease in SOD activity. Cultivars V2, V3 and V4 showed gradual increase at initial levels while higher decreases were observed in cultivars V1 and V5 at extreme toxic levels. In figures 1f, for every increased Cd level, SOD activity was higher compared to control except at maximum toxic levels. For V1 and V5 cultivars, when rice seedlings were subjected to 150 mg/kg Cd toxicity, there was a steadily decrease of 11.6 and 5.6% in SOD activities respectively.

Steady Changes in CAT activity for all treatments among the different cultivars were also recorded in fig1g, h and i) below. Significant decreases in CAT activities were observed with increased Cd toxicity, CAT activity in the treatments subjected to Cd toxicity saw a steady decrease in dose dependent manner. In all the three sampled time, when the decreasing CAT activities were compared with the Control, maximum CAT decreases were observed in cultivars 1 and 5(V1 and V5), while less decreases were observed in cultivars 2, 3 and 4(V2, V3 and V4). Similar decreased trends were observed in the three sampled periods (figs. 1g, h and i). From fig 1g, there was a 17.5, 18.6, 32.1 and 13.1, 16.1,29.9% decrease in CAT activity at 50, 100 and 150 mg/kg Cd toxicity for cultivars V1 and V5 respectively. While from fig. 1h, there was a 12.7, 16.7, 28.2 and 10.6, 16.9, 26.7% decrease in CAT activity at 50, 100 and 150 mg/kg Cd toxicity for cultivars V1 and V5 respectively. Results from fig 1i showed a 13.7, 16.8, 29.4 and 10.2, 14.7, 27.1% decrease in CAT activity at 50, 100 and 150 mg/kg Cd toxicity for cultivars V1 and V5 respectively. Such decrease with increased Cd concentrations shows the extent of damage in CAT as antioxidant enzymes and cultivars V1 and V5 were greatly affected while cultivars V2, V3 and V4 were less affected.



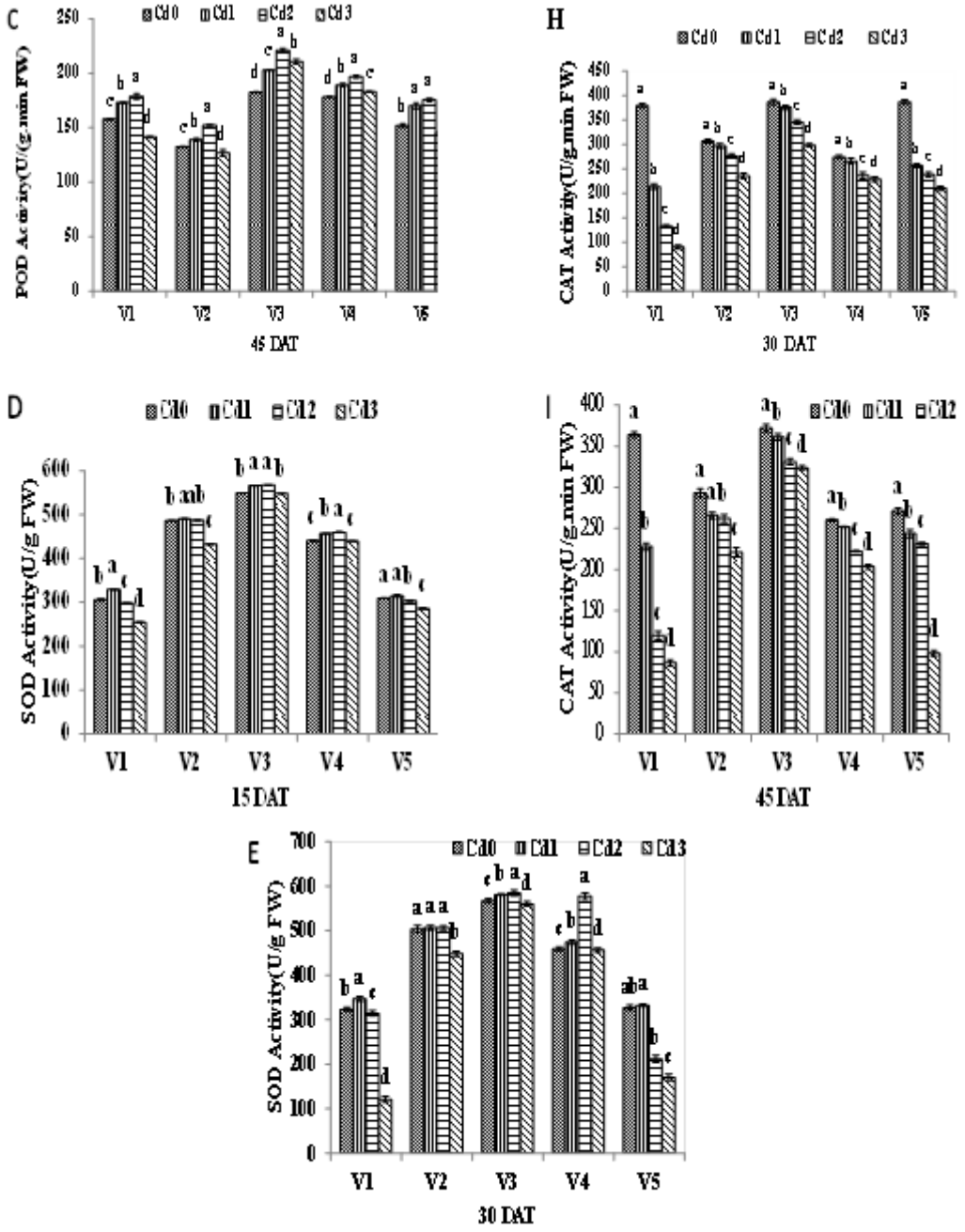
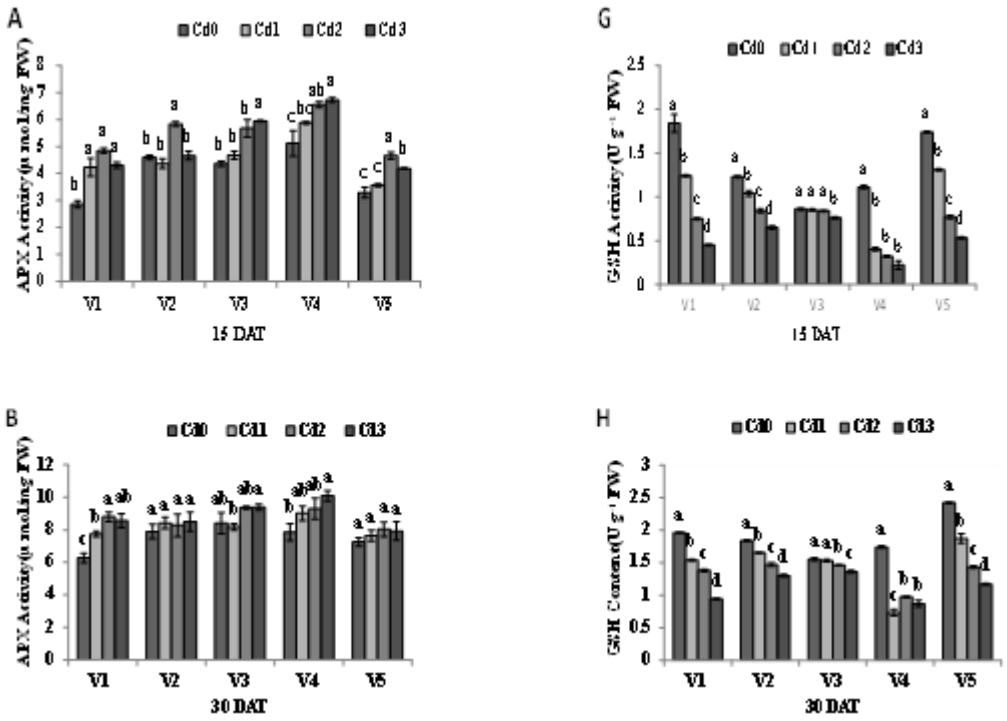



**Fig.1** a, b,c,d,e,f,g,h and I, show Changes in rice seedlings antioxidant enzymes (POD, SOD, CAT) under induced cadmium stress at different sampling time. Different letters indicate significant differences between treatments at P≤0.05, LSD.

**Changes in seedlings non-enzymatic antioxidants (APX, GR, GSH and AsA) under induced cadmium stress at Different Sampling Time.**

Changes in Ascorbate Peroxidase (APX) Activity were observed at Different Sampling period and the results shown in figures 2a, b and c. the results revealed no constant changes in some cultivars, while to some extent, other cultivars manifested consistent changes in all the three sampled stages. From figure 2a, cultivar V1 and V5 clearly showed consistent increase in APX activity with increased levels of induced Cd concentration, at every increased level, APX also increased when compared to control, whist at maximum induced Cd treatments, APX activities slightly declined. In cultivars V3 and V4, a steady increase was observed with increased Cd toxicity, whereas, V2 cultivar shown a fluctuating increase and decrease in APX activity. In figure 2b, no significant differences were observed in cultivars V2 and V5 while the other cultivars showed significant differences in treatment when compared to the control treatments. In figure 2c, cultivars V1 and V5 exhibited

similar increases in APX activity at initial Cd toxicity and decreases at maximum induced Cd toxicity. At the 50, 100 and 150 mg/kg Cd toxicity (fig. 2c), there were 8.2, 22.9 and 24.3% increases in APX activity in cultivars V1and V5 respectively. Activity of Glutathione Reductase (GR) for all treatments in the varying cultivars studied was observed and the results shown in figure 2d, e, and f. in figures 2d and e, there was a consistent increase in the GR activity for all treatments in dose dependent manner. GR activity was found increased as the levels of induced Cd toxicity increased. Maximum increase was observed in V1 cultivar and followed by V5 and V4 cultivars at the 150 mg/kg$^{-1}$ toxic level, while minimum increase was observed in cultivar V3. In comparison with the control treatments (fig. 2d), there were significant increases in GR activity of 29.7, 50.0, 78.8 and 8.5, 15.6, 28.1% for cultivars V1 and V5 respectively, while from fig. 2f, there were 31.2, 57.1, 57.4 and 10.7,24.6,29.2% increase for cultivars V1 and V5 respectively. GR activity was greatly affected in cultivar V1 when compared to the other cultivars. However, cultivar V3 was found less affected under Cd stress conditions. Changes in Glutathione (GSH) activity were observed and result recorded and presented in figure 2g, h and i. Like many other antioxidant enzymes, GSH is subsumed in the many known water-soluble non enzymatic antioxidants protecting organelles in plants. In our study, declining trends of GSH activity were observed in dose dependent manner, with increased levels of induced Cd toxicity in the soil, GSH activity was found decreased as shown in figure 2g, h and i below. When compared to the controlled treatments, maximum decrease was found in cultivars V1, V4 and V5, with minimal decreases observed in cultivars V3 and V2. In figure 2i, significant differences were observed in cultivars V1, V3 and V5, whilst no significant differences observed in cultivars V2 and V4. From fig. 2g, 48.4, 59.2, 75.5 and 32.8, 55.7 69.5% decrease in GSH activity were observed in cultivars V1 and V5 respectively. This shows the extent to which Cd affected GSH activity during seedling growth under Cd stress conditions. Changes in the contents of AsA were observed and results presented in fig 2j, k and l. significant differences were observed between treatments of the same cultivars. From figure2j, k and l, consistent increase in AsA contents were recorded in dose dependent manner, that is, at every level of induced Cd toxicity, AsA contents also increased. Maximum increase was observed in cultivars V1 and V5. In comparison with each cultivar's controlled treatments (fig. 2j), there were 34.5, 76.1, 91.5 and 24.2, 56.7 61.8% increased AsA contents in cultivars V1 and V5 respectively. Considering the significant role antioxidant enzymes play in plants defensive mechanism, any disruption caused through stress related factor do affect plants performance.



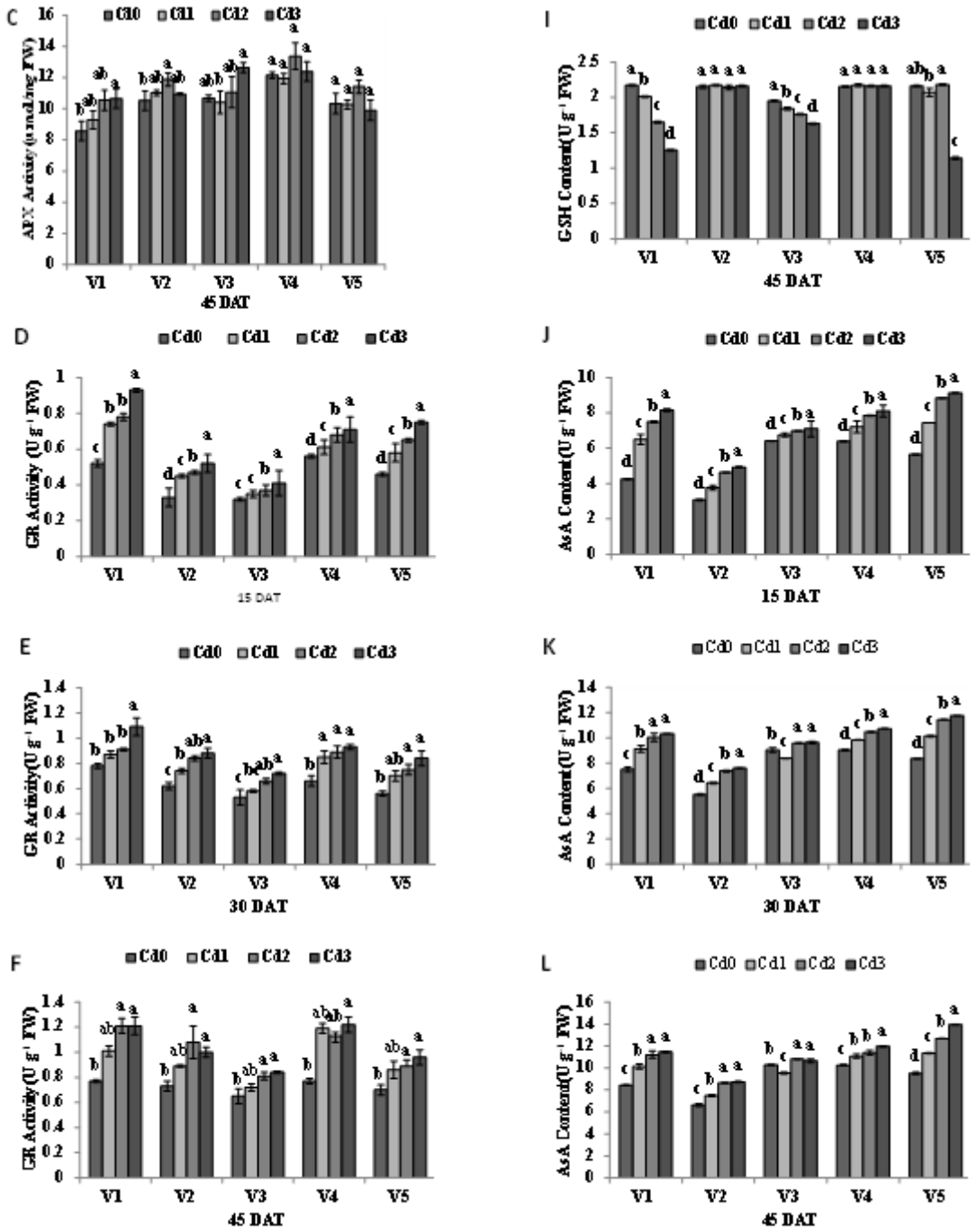



**Fig.2** a, b,c,d,e,f,g,h,I,j and k, show Changes in rice seedlings non- antioxidant enzymes (APX, GR, GSH and AsA) under induced cadmium stress at different sampling time. Different letters indicate significant differences between treatments at P≤0.05, LSD.

**Changes in seedlings MDA Content(μmol/g)**

Changes in MDA contents were observed for all treatments in the five cultivars and the results presented in table 1 below. Induced Cd toxicity showed significant increase in MDA contents for all cultivars. In cultivar V1and V5, higher MDA contents (10.34 and 6.45) at the 15 DAT, (12.4 and 6.9) at the 30 DAT and (12.99 and 8.43) at the 45 DAT were observed compared to other cultivars. Increased contents of lipid peroxides observed showed more production of toxic oxygen species. When rice grows under stressed conditions, free radicals generated in excess do accumulate in the cells, this leads to lipidperoxidation of biomembranes which forms MDA as end product. Hence, increase in MDA concentration is an indicator of physiological stresses and the aging process (Chen et al., 2003).

**Table 1: Changes in seedlings $H_2O_2$ Content(µmol/g)**

| Variety | Treatment | 15 DAT | 30 DAT | 45 DAT |
|---|---|---|---|---|
| V1 | Cd 0 | 3.48±0.04d | 5.20±0.06d | 6.18±0.09d |
| | Cd 1 | 4.36±0.03c | 7.52±0.04c | 8.47±0.03c |
| | Cd 2 | 6.76±0.01b | 10.42±0.09b | 11.09±0.09b |
| | Cd 3 | 10.34±0.01a | 12.40±0.03a | 12.99±3.33a |
| | | | | |
| V2 | Cd 0 | 2.34±0.01d | 3.14±1.00d | 4.14±2.90d |
| | Cd 1 | 2.95±2.18c | 3.77±0.01c | 4.34±1.15c |
| | Cd 2 | 3.13±2.96b | 3.96±0.01b | 4.77±2.30b |
| | Cd 3 | 3.32±0.02a | 4.17±0.01a | 4.98±3.71a |
| | | | | |
| V3 | Cd 0 | 2.15± 0.01d | 2.44±2.33d | 2.47±1.33d |
| | Cd 1 | 2.33±2.66c | 2.53±2.96c | 2.63±1.33c |
| | Cd 2 | 2.67±0.01b | 2.76±0.01b | 2.87±0.01b |
| | Cd 3 | 2.97±0.01a | 3.12±1.00a | 3.23±0.01a |
| | | | | |
| V4 | Cd 0 | 3.45±1.15d | 3.88±2.96d | 3.98±1.20d |
| | Cd 1 | 3.77±0.01c | 4.13±0.01c | 4.36±0.04c |
| | Cd 2 | 4.24±0.02b | 4.670±0.01b | 4.71±0.01b |
| | Cd 3 | 4.75±2.84a | 4.98±1.20a | 5.18±0.03a |
| | | | | |
| V5 | Cd 0 | 4.44±0.02d | 4.78±1.00d | 6.46±0.01d |
| | Cd 1 | 4.97±2.84c | 5.67±0.01c | 6.99±1.52c |
| | Cd 2 | 5.32±2.66b | 6.15±0.01b | 7.35±0.01b |
| | Cd 3 | 6.45±1.52a | 6.97±2.64a | 8.43±3.71a |

Three replicated means (±SE) were calculated for each treatment. Values with different letters are significantly different at P< 0.05. Vn=variety n, Cd0=0mg Cd/kg, Cd 1=50mg Cd/kg, Cd 2=100mg Cd/kg, Cd 3=150mg Cd/kg

**Changes in seedlings $H_2O_2$ Content(µmol/g)**

Changes in $H_2O_2$ contents in all treatments for all cultivars were observed at different time period and the results presented in table 2. $H_2O_2$ contents were found increased with increased levels of induced Cd toxicity. In all the three sampled periods, cultivar V1 recorded the highest $H_2O_2$ content at the highest induced Cd level and it's followed by cultivar V5. Highest content were recorded during the 45 DAT sampled period. However, cultivar V3 recorded the lowest $H_2O_2$ contents followed by cultivars V2 and V4 respectively.

**Table 2: Changes in seedlings $H_2O_2$ Content(µmol/g)**

| Variety | Treatment | 15 DAT | 30 DAT | 45 DAT |
|---|---|---|---|---|
| V1 | Cd 0 | 4.10±0.01d | 5.92±0.05d | 6.65±0.10d |
| | Cd 1 | 4.93±0.03c | 8.24±0.04c | 8.95±0.05c |
| | Cd 2 | 7.33±0.01b | 11.15±0.09b | 11.51±0.07b |
| | Cd 3 | 10.91±0.01a | 13.27±0.12a | 13.45±3.33a |
| | | | | |
| V2 | Cd 0 | 2.88±1.00d | 3.87±1.33d | 4.62±0.02d |
| | Cd 1 | 3.52±1.00c | 4.55±1.66c | 4.84±0.02c |
| | Cd 2 | 3.71±1.00b | 4.72±3.33b | 5.26±2.00b |
| | Cd 3 | 3.87±0.04a | 4.84± 0.02a | 5.45±1.33a |
| | | | | |
| V3 | Cd 0 | 2.67±0.01d | 3.15±0.01d | 2.94±2.66d |
| | Cd 1 | 2.93±0.01c | 3.26±2.00c | 3.16±1.00c |
| | Cd 2 | 3.22±1.00b | 3.43±0.02b | 3.36±1.00b |
| | Cd 3 | 3.56±1.00a | 3.82±0.01a | 3.68±1.33a |
| | | | | |
| V4 | Cd 0 | 4.03±2.00d | 4.63±0.01d | 4.45±2.88d |
| | Cd 1 | 4.34±0.01c | 4.82±0.02c | 4.80±0.04c |
| | Cd 2 | 4.81±1.66b | 5.42±1.20b | 5.17±0.01b |
| | Cd 3 | 5.35±0.01a | 5.72±1.33a | 5.64±0.03a |

| | | | | |
|---|---|---|---|---|
| V5 | Cd 0 | 5.01±2.33d | 5.43±0.09d | 6.92±2.66d |
| | Cd 1 | 5.55±1.33c | 6.41±2.00c | 7.45±2.00c |
| | Cd 2 | 5.87±0.01b | 6.84±0.02b | 7.75±0.02b |
| | Cd 3 | 7.03±2.00a | 7.71±1.66a | 8.89±1.33a |

Three replicated means (±SE) were calculated for each treatment. Values with different letters are significantly different at P< 0.05. Vn=variety n, Cd0=0mg Cd/kg, Cd 1=50mg Cd/kg, Cd 2=100mg Cd/kg, Cd 3=150mg Cd/kg

**Cadmium effects on Plants Heights**

Heights of rice seedlings for the different treatments in all cultivars were determined after the rice plants were all harvest (after 45 days vegetative growth period) and the results presented in figure 3. Heights of seedlings vary based on genotypic and stress conditions subjected to. Maximum heights were recorded at the control treatments for all cultivars and cultivar V3 showed the highest height, while the minimum seedlings height was recorded at 150 mg kg $^{-1}$ cadmium levels for all the cultivars and cultivar V1 was greatly affected and showed the most stunted seedlings height when treatments seedlings were compared to the control treatment. Maximum to minimum plants height for the different treatments followed the declining treatments order 150 mg/kg <100 mg/kg Cd < 50 mg/kg Cd < 0 mg/kg Cd respectively, while on cultivar bases V1<V5<V2<V4<V3.

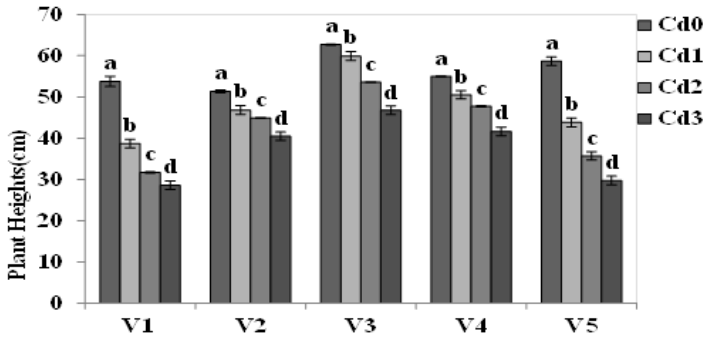



**Fig.3 Cadmium effects on Plants Heights. Different letters indicate significant differences between treatments at P≤0.05, LSD.**

**Cadmium effects on Dry Matter Contents**

Seedlings dry matter contents were determined after the rice the seedlings were all harvest (after 45 days vegetative growth period) dried to constant weights and the results showed in figure 4. Seedlings dry matter content varies based on genotypic and stress conditions subjected to. Maximum weights were recorded at the control treatments for all cultivars and cultivar V3 showed the highest dry matter content, while the minimum seedlings dry weight was recorded at 150 mg kg $^{-1}$cadmium levels for all the cultivars and cultivar V1 and V5 showed the least seedlings dry matter contents when compared to other cultivars. With increased levels of toxicity, seedlings dry weights reduce proportionately. Maximum to minimum seedlings dry matter content for the different treatments followed the declining treatments order 150 mg/kg <100 mg/kg Cd < 50 mg/kg Cd < 0 mg/kg Cd respectively, while on cultivar bases V1<V5<V2<V4<V3 respectively.

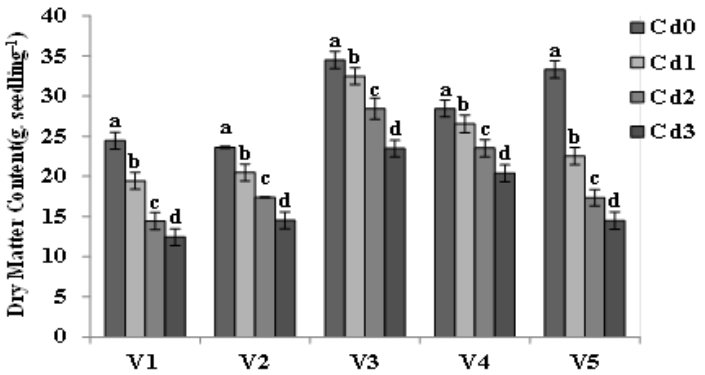



**Fig.4 Cadmium effects on Plants dry matter content. Different letters indicate significant differences between treatments at P≤0.05, LSD.**

**Cadmium Uptake and Distribution in Seedlings Parts(Roots, Stems and Leaves)**

Cadmium uptake, accumulation and distribution to the various plants parts (roots, stems and leaves) of the five rice cultivars used in the experiment were measured and results shown in figures 5a, b and c. In all cultivars, Cd accumulation in the various parts (roots, stems and leaves) was found elevated with increased concentrations of induced soil Cd contents. In average, Cd accumulation was in the descending order: root>stem>leaves for all the Cd treatments in all cultivars. The concentration of Cd in various rice organs (roots, stems and leaves) were significantly different among the five aromatic rice varieties as shown in fig. 5a, b and c. In terms of uptake, there were outstanding differences in Cd uptake between the different rice seedling organs especially from roots to stems, cultivar V3 showed the least uptake in both roots stems and leaves for all treatments followed by cultivar V4 and V2 while cultivar V1 and V5 showed the highest uptake respectively. Variation in cultivar uptake ability and accumulation in roots and transfer to stems and leaves were observed in the following descending order V3<V4<V2<V5<V1.

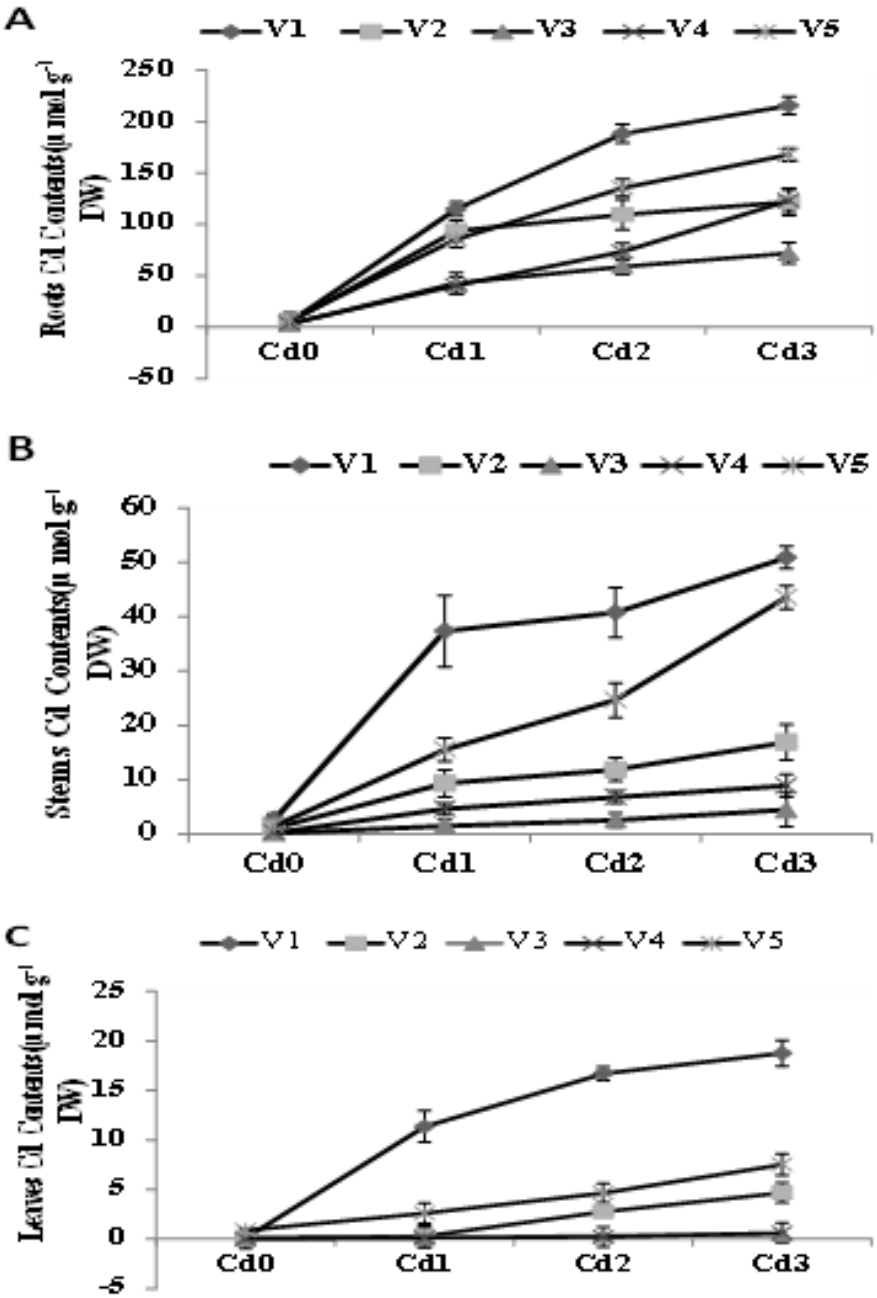



**Fig. 5 Cd accumulation in a) roots, b) stems, and c) leaves d) for the five different rice cultivars under induced Cd-stress at tillering stage. The Values are representative of three replicated means per treatment ±SE.**

**Cadmium Transfer Factor from Roots to Stems and from Stems to Leaves**

Variations in Cd transfer factor from roots to stems and from stems to leaves were recorded and result presented in table 3. Translocation factor from roots to stems and from stems to leaves were found higher in cultivars V1 and V5, while minimal translocation was observed in cultivars V3,V2 and V5. This may be associated to cultivars ability to uptake, accumulate and translocate Cd to the various plants parts.

**Table 3: Cadmium Transfer Factor from Roots to Stems and from Stems to Leaves**

| VARIETY | TREATMENTS | $TF_{Root\text{-}stem}$ | $TF_{stem\text{-}leaves}$ |
|---|---|---|---|
| V1 | Cd0 | 0.49 | 0.05 |
| | Cd1 | 0.32 | 0.30 |
| | Cd2 | 0.22 | 0.41 |
| | Cd3 | 0.24 | 0.37 |
| V2 | Cd0 | 0.21 | 0.13 |
| | Cd1 | 0.10 | 0.03 |
| | Cd2 | 0.11 | 0.23 |
| | Cd3 | 0.14 | 0.28 |
| V3 | Cd0 | 0.08 | 0.20 |
| | Cd1 | 0.11 | 0.03 |
| | Cd2 | 0.12 | 0.05 |
| | Cd3 | 0.12 | 0.05 |
| V4 | Cd0 | 0.04 | 0.29 |
| | Cd1 | 0.04 | 0.09 |
| | Cd2 | 0.03 | 0.09 |
| | Cd3 | 0.04 | 0.14 |
| V5 | Cd0 | 0.22 | 0.61 |
| | Cd1 | 0.18 | 0.17 |
| | Cd2 | 0.18 | 0.19 |
| | Cd3 | 0.26 | 0.17 |

## IV. Discussions

Antioxidant enzymes play very vital roles in plants defense mechanisms. Abiotic stresses like heavy metals especially Cd results to molecular damage in rice plant cells due to the generation of reactive oxygen species (ROS) (Hegedus et al., 2004). Even though Cd by itself does not directly generate ROS, but rather generates oxidative stress by interrupting essential antioxidant defense system. Antioxidant enzymes balance the production and destruction of ROS. In our experiment, enzymatic and non-enzymes antioxidants were measured and the results showed a significant difference in the means of the different treatments for all cultivars. SOD is often regarded as the first line of defense against abiotic stresses like heavy metals. Our study revealed that the activities of SOD for cultivars V1 and V5 decreased with the increased soil Cd toxicity (fig. 1d, e and f). The decrease in SOD activity can be attributed to Cd-induced phytotoxicity as concluded by Hou et al., (2007). This acts as an adaptive response means of plants exposed to heavy metals toxicity (Chaney et al., 1996). Our findings reveal that SOD activity were greatly affected in cultivars V1 and V5, decreased SOD activity in these two cultivars with increased soil Cd level, suggested that high soil Cd level might have damaged the antioxidant defense system by reducing SOD activity in the rice seedlings however, cultivars V3, V2 and V4 showed minimal disruption in their SOD activities, suggesting stronger defense mechanisms against Cd stress conditions similar findings were similar to those concluded by kanu et al., 2017. Initial increase in lower concentrations of induced Cd and decrease at highly Cd toxic levels in POD and a constant decrease in CAT activities with increased soil Cd levels was observed in all cultivars. It revealed that antioxidant enzymes like POD and CAT work in coordination to minimize oxidative stress in rice seedlings during growth. In summary, the activities of SOD, POD and CAT in all cultivars showed significant differences between rice seedling treatments as well as cultivars due to membrane damage after exposure to Cd toxicity. AsA and GSH (non-enzymatic antioxidants) while APX and GR(enzymatic antioxidants) are considered to be playing very significant roles in providing protection for biomolecules and cell organelles against oxidative damage through scavenging ROS directly. Under Cd stress condition, AsA and GSH forms critical components in maintaining cellular redox state in rice seedlings. AsA, APX and GR activities were all found increased at increased levels of Cd due to the increased oxidation during the detoxification process of ROS. Within the AsA-GSH cycle,

enzymes like APX and GR work in coordination with AsA and GSH. APX functions in converting $H_2O_2$ to $H_2O$ through oxidation of AsA to MDHA/DHAR using GSH. Oxidative stress in rice seedlings as a result of contamination by toxic metals can be manifested in the activity of MDA and $H_2O_2$ which are significant indicators of lipid peroxidation (Camp et al., 1996). Results from our study showed significant increases in MDA and $H_2O_2$ contents in all cultivars after exposure to Cd toxicity (Tables 1 and 2), this increase indicated Cd toxic effects in rice seedlings. Even though, Cd has not been proven to play an active role in the production of Reactive Oxygen Species (ROS), but rather, have been found to disrupt the mechanisms of essential elements in plants and or causes oxidative damage to plant by obstructing electron movement chain in plants. Most often, the indirect consequences of Cd results to the activation of NADPH oxidase found in the plasma membrane which increases $H_2O_2$ and $O_2^-$ production, hence, results to oxidative stress. Such increase in MDA and $H_2O_2$ were observed in all cultivars. Cultivars V1 and V5 exhibited higher ROS production whilst cultivars V3, V2 and V4 showed less ROS production. ROS were found increased in the various treatments on dose dependent manner, at every increased level of induced Cd, effects of lipid peroxidation were found higher in the production of MDA and $H_2O_2$. However, fewer increases in MDA and $H_2O_2$ contents were observed in cultivars V3, V2 and V4 when compared to the other two cultivars subjected under the same Cd stress conditions. This indicated that lipid peroxidation was higher in cultivars V1 and V5 than that in cultivars V3, V2 and V4, this is possibly due to the higher uptake ability of Cd in cultivars V1 and V5 when compared to cultivars V3, V2 and V4. In terms of seedling heights and dry matter accumulation in during the vegetative growth stage, seedlings were affected in proportion to accumulated Cd contents, at lower induced Cd levels, seedlings acquired higher heights and dry matter contents, with every increased level of Cd toxicity, seedling heights and dry matter also reduces due to the oxidative damage caused by metal toxicity which affected seedling growth parameters. Cd uptake and accumulation in roots, stems and leaves of all cultivars significantly increased with increased Cd levels (fig. 5). The significant differences observed in all treatments varied within cultivars as well as between treatment bases. Cadmium contents were found higher in roots than in stems and leaves for all the cultivars of the same cultivars. Cd contents in roots, stems and leaves were found higher in cultivar V1and V5 than that in cultivars V3, V2 and V4 for all corresponding treatments, this was probably as a result of the higher uptake and translocation ability of Cd from the soil medium to roots, from roots to stems and then stems to leaves. The differences observed in accumulation may be related to the genotypic tolerance nature of the cultivars to cadmium toxicity (Liu et al., 2005). Cadmium uptake and translocation from contaminated soil to plants edible parts were markedly different among plant species as well as cultivars within the same species (Liu et al., 2007). In our study, differences in Cd uptake and concentration between cultivars were observed, this might be as a result of seedling roots oxidation ability, roots organic acid secretions and roots acidification ability to uptake, accumulate and translocate Cd in the rice seedlings during growth (Liu et al., 2007). Result from this study has shown that, cadmium toxicity in growing rice seedlings impeded morpho-physiological and antioxidants activities in rice during early growth. Cd toxicity damages the antioxidant defense system resulting to oxidative stress in organs of growing rice seedlings. This resulted to alteration in antioxidant enzymes and growth parameters (plant height, and total dry matter content). At 150 mg/kg soil Cd toxicity, growing rice seedlings become critical and vulnerable to toxicity. The results suggested that soil Cd toxicity have negative consequences on rice seedling growth. Rice roots accumulated higher Cd than stems and leaves, and uptake varies amongst cultivars. Conclusively, Cd toxicity impaired early growth in rice by affecting physio-biochemical attributes, however, cultivar V3, V2 and V4 performed better than the other two cultivars V1 and V5.

## V. Conclusions

Results obtained from this study have shown that, cadmium toxicity in growing rice seedlings impeded morpho-physiological and activities of antioxidants enzymes in rice during early growth. Cd toxicity damages the antioxidant defence system resulting to oxidative stress in organs of growing rice seedlings, resulted to alteration in Antioxidant enzymes, rate of lipid peroxidation (MDA) level of Hydrogen Peroxide ($H_20_2$) and growth parameters (plant height, and total dry matter content). At 150 mg/kg soil Cd toxicity, growing rice seedlings become critical and vulnerable to toxicity. The results suggested that soil Cd toxicity have negative consequences on rice seedling growth. Rice roots accumulated higher Cd than shoots and uptake varied amongst cultivars. Conclusively, Cd toxicity impaired early growth in rice by affecting physio-biochemical attributes, however, cultivar V3 performed better than the other four cultivars.

**Conflicts of interest**

The authors have no existing competing interest.

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