



Received: 10-11-2023  
Accepted: 20-12-2023

ISSN: 2583-049X

## **Effect of Cadmium on Chlorophyll Content, Lipid Peroxidation and some Antioxidative Enzymes in the Leaf of Maize (*Zea Mays*)**

<sup>1</sup> Obadoni A Isabel, <sup>2</sup> Omoremime E Dania, <sup>3</sup> Ugbeni O Cyril

<sup>1,2,3</sup> Department of Biochemistry, University of Benin, Benin, Edo State, Nigeria

Corresponding Author: **Obadoni A Isabel**

### **Abstract**

Cadmium is a heavy metal which at even low concentration is toxic to plants. We investigated the effects of varying cadmium concentrations on the anti-oxidative defence mechanism in the leaves of *Zea mays* seedlings. The soil sample used was divided into five groups containing four replicates each. Group one was uncontaminated. Groups two to five were contaminated with varying concentrations of cadmium chloride, ranging from 5mg, 10mg, 20mg and 30mg respectively. The leaf sample from each group was taken for weekly analysis starting from the third week after planting. The anti-oxidative defence enzymes determined were catalase, peroxidase, superoxide dismutase and thiobarbituric acid reactive species as indices for lipid peroxidation. Also determined were the leaf weight, leaf area and chlorophyll content of the leaves. Statistical analysis using SPSS showed that varying cadmium concentrations in the soil significantly ( $p < 0.05$ ) reduced the leaf area, chlorophyll content and weight of the leaves in *Zea mays*. An extremely significant ( $p < 0.05$ ) loss of

chlorophyll content and reduction in leaf area was observed in weeks 4, 5 and 6 under varying concentrations of cadmium exposure. With regards to the distribution of cadmium in leaves, the obtained data showed that there was no significant ( $p > 0.05$ ) difference between the cadmium concentrations of 20mg and 30mg in weeks 5 and 6. Furthermore, a significant ( $p < 0.05$ ) increase was observed in the anti-oxidative enzymes in response to varying concentrations of cadmium exposure when compared with the control. A significant ( $p < 0.05$ ) increase was observed in catalase and peroxidase activity of weeks 5 and 6 under varying cadmium exposure. A significant ( $p < 0.05$ ) increase was also observed in superoxide dismutase activity and malondialdehyde levels (in response to increased lipid peroxidation) of weeks 4, 5 and 6 under varying concentrations of cadmium exposure. The increased production of anti-oxidative enzymes observed in the leaf of *Zea mays* is to ameliorate the effects of oxidative stress caused by cadmium toxicity.

**Keywords:** Cadmium, Antioxidative Enzymes, Lipid Peroxidation, Maize Leaf

### **Introduction**

Heavy metals are conventionally defined as elements with metallic properties and atomic numbers greater than 20. The most common heavy metal contaminants are Cd, Cr, Cu, Hg, Pb, and Zn. Metals are natural components in soil (Tchounwou, Yedjou, Patlolla and Sutton, 2012) [36]. Some of these metals are micronutrients necessary for plant growth, such as Zn, Cu, Mn, and Co, while others have unknown biological function, such as Cd, Pb, and Hg (Saunders, Jastrzembski, Buckley, and Enriquez, 2013) [30]. Toxic heavy metals such as Pb, Co, Cd can be differentiated from other pollutants, since they cannot be biodegraded but can be accumulated in living organisms, thus causing various diseases and disorders even in relatively low concentrations (Roney, Osier and Paikoff, 2006) [28]. Heavy metals are known to have effect on plant growth, ground cover and have a negative impact on soil microflora (Rathnayake, Megharaj, Krishnamurti and Bolan, 2013) [26]. Phytoremediation is the use of plants to clean up a contamination from soils, sediments, and water. This technology is environmental friendly and potentially cost effective. Plants with exceptional metal accumulating capacity are known as hyper accumulator plants (Saunders *et al.*, 2013) [30]. The most common form of cadmium found in the environment exists in combination with other elements such as cadmium oxide, cadmium chloride, and cadmium sulphide (Sanz, Iamas and Ullrich, 2009) [29]. The largest source of cadmium release is from industrial sectors that burn fossil fuels like coal or oil, or that burn municipal waste (Lux *et al.*, 2011) [20]. Cd metal itself does not break down in the soil but it can change into different forms. This transformation and therefore the availability of Cd in soil, is influenced by factors such as pH, climate, agronomic practices, plant genotype, soil

temperature, soil organic matter, calcium concentration and chlorine salinity, oxidation-reduction reactions and the formation of complexes (Schwartz and Hu, 2007).

As cereals retain the majority of absorbed Cd in the roots variation in translocation of this pool can greatly affect Cd levels in shoot and grain (Koh, Andre, Edwards and Ehrhardt, 2005) [15]. Cd stress affects photosynthesis in various ways. It inhibits the synthesis of chlorophyll (Azevedo, Pinto, Fernandes and Loureiro, 2005; Shukla, Nurthy, and Kakkar, 2008) [3, 34] and their stable binding to proteins (Horváth *et al.* 1996) [14], thereby decreasing the accumulation of pigment– lipoprotein complexes, particularly photosystem (PS) I (Wang *et al.* 2009) [39]. Corn is perhaps the most completely domesticated of all field crops. It is cultivated worldwide and represents a staple food for a significant proportion of the world's population. No significant native toxins are reported to be associated with the genus *Zea* (*Zea mays* L.). Apart from satisfying the taste buds of its users, maize is also a good source of vitamins, minerals and dietary fibre (Adegoke and Adebayo, 1994) [1]. Toxicity of Cd has been related to an increase in lipid peroxidation and alterations in the antioxidant system (Romero-Puertas *et al.*, 2004) [27]. An unavoidable consequence of aerobic metabolism is production of reactive oxygen species (ROS). ROS include free radicals such as superoxide anion ( $O_2^{\bullet-}$ ), hydroxyl radical ( $\bullet OH$ ), as well as nonradical molecules like hydrogen peroxide ( $H_2O_2$ ), singlet oxygen ( $^1O_2$ ), and so forth. Environmental stresses such as drought, salinity, chilling, metal toxicity, and UV-B radiation as well as pathogens attack lead to enhanced generation of ROS in plants due to disruption of cellular homeostasis (Hassan and Aarts, 2011) [12]. When the level of ROS exceeds the defence mechanisms, a cell is said to be in a state of “oxidative stress” (Kudo, Anbo, Uemura and Kawai, 2011) [17]. Enhanced level of ROS can cause damage to biomolecules such as lipids, proteins and DNA. These reactions can alter intrinsic membrane properties like fluidity, ion transport, loss of enzyme activity, protein cross-linking, inhibition of protein synthesis, and so forth ultimately resulting in cell death (Hermans, Chen, Coppens, Inze and Verbruggen, 2011) [13]. To minimize the detrimental effects of heavy metal exposure and their accumulation, plants have evolved detoxification mechanisms. Such mechanisms are mainly based on chelation and subcellular compartmentalization (Dixit, Mukherjee, Ramachandran and Eapen, 2011) [10]. Those mechanisms can slow down or even stop the oxidation of biomolecules and block the process of oxidative chain reactions (Sgherri, Milone, Clijsters and Navari-Izzo, 2003) [32]. The most important antioxidant enzymes are: superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDAR), dehydroascorbate reductase (DHAR) and glutathione reductase (GR) (Yadav, 2010) [41]. Superoxide dismutase (SOD, 1.15.1.1) plays central role in defense against oxidative stress in all aerobic organisms (Scandalios, 1993) [31]. The enzyme SOD belongs to a group of metallo enzymes that catalyse the dismutation of  $O_2^{\bullet-}$  to  $O_2$  and  $H_2O_2$ . Catalase is unique among  $H_2O_2$  degrading enzymes in that it can degrade  $H_2O_2$  without consuming cellular reducing equivalents. When cells are stressed for energy and are rapidly generating  $H_2O_2$  through "emergency" catabolic processes,  $H_2O_2$  is degraded by catalase in an energy efficient manner. Peroxidase is any group of enzymes that

catalyses the oxidation of a compound by the decomposition of hydrogen peroxide or organic peroxide. They generally consist of a protein combined with haem.

Thiobarbituric acid reactive substances (TBARS) are formed as a by-product of the oxidation of fat cells that is lipid peroxidation and can be detected by the TBARS assay. The level of lipid peroxidation has been widely used as an indicator of ROS mediated damage to cell membranes under stressful conditions. Malondialdehyde (MDA) is one of the final products of peroxidation of unsaturated fatty acids in phospholipids and is responsible for cell membrane damage (Meriga, Reedy, Rao and Reedy, 2004) [21]. Two common sites of ROS attack on the phospholipid molecules are the unsaturated (double) bond between two carbon atoms and the ester linkage between glycerol and the fatty acid. One of the aims of this research is to determine the effect of varying concentrations of cadmium on some antioxidative enzymes such as catalase, peroxidase and superoxide dismutase in the leaf of *Zea mays*. Another aim is to determine the effect of varying concentrations of cadmium on the malondialdehyde levels (an index for lipid peroxidation), chlorophyll content, leaf area and leaf weight in the leaf of *Zea mays*.

## Materials and Method

### Soil Preparation

Loamy soil was obtained from an agricultural land opposite dentistry hostel, University of Benin. Root stumps, nails and other debris were removed from the soil. The soil was taken to the green house behind biochemistry laboratory, University of Benin. Water retention assay was determined as described by Piper, (1966) [24]. A soil of mass 5kg was weighed into 20 seedling bags of 45.8 cm high and 34.2 cm wide.

### Experimental Design

The seedling bags were then divided into 5 different groups. Group 1 was labelled normal with the aid of masking tape. This group was not treated with cadmium and therefore serves as the control. Groups 2-5 were treated with varying concentrations of cadmium and labelled with masking tapes. The maize seeds were obtained from an agro-based shop (Divine Agricultural Shop) along Oba Market Road Benin City. They were put in water to test their viability. Those that floated were thrown away but those that sank were deemed viable. The viable ones were then planted and watered. The seeds were planted 2 seeds per hole and 5 holes per bag, with a depth of 2cm into the soil. The planting was done in the evening at about 5pm. The seedling bags were watered continuously with clean water prior to germination and after germinating. After 3 weeks of planting, some of the plants were harvested and their leaves were cut out for analysis.

### Preparation of Plant Materials for Analysis

The leaves were homogenized with mortar and pestle, 8ml of phosphate buffer was added, to aid homogenization. The homogenates were centrifuged at 1500 rpm for 10 minutes and the supernatants were used for Malondialdehyde estimation, SOD, Catalase and Peroxidase assay.

### Leaf Area

Leaf area was determined by the use of graphical method. The leaf was placed on a graph sheet and traced after which the complete boxes were taken as  $1cm^2$  and the halves were

counted, divided by 2 and added to the number of the whole boxes.

### Leaf Weight

All the leaves on the maize plants were carefully detached based on the treatments applied and labelled separately. They were air dried in the laboratory at room temperature for seven days until constant weight was recorded. Dried leaves were carefully weighed using electronic weighing balance – Melter H80 Model, made in England.

### Determination of Catalase Activity

CAT activity was assayed by using the method of Cohen *et al.*, (1970) [9]. Each catalase unit specifies the relative logarithmic disappearance of hydrogen peroxide per minute and is expressed as  $K\text{min}^{-1}$ .

### Determination of Superoxide Dismutase Activity.

SOD activity was assayed by the method of Misra and Fridovich (1972) [22], the activity computed and expressed as described by Baum and Scandalios (1981) [5] in which one unit represents the amount of enzyme required for 50% inhibition of epinephrine during 1 min.

### Determination of Lipid Peroxidation

The amount of thiobarbituric reactive substances (TBARS) which are indicators of lipid peroxidation was assayed by the method of Buege and Aust (1978) [6]. Values for TBARS were quantified using a molar extinction coefficient of  $1.56 \times 10^5 \text{ M/cm}$  and expressed in terms of malondialdehyde (MDA) units per gram tissue. Each unit represents one micromole of MDA.

### Determination of Peroxidase Activity

Peroxidase activity was assayed by the method of Chance and Maehly (1955) [8]. Values for peroxidase were quantified using a molar extinction coefficient of 12 mg/ml of purpurogallin. Each unit represents 1.0 mg of purpurogallin from pyrogallol in 20sec.

### Chlorophyll Determination

Chlorophyll content was determined by the method described by Wintermans and De motts (1965) [40] with little modification. Twogrammes(2g) of leaf tissues was homogenised with 5ml of acetone in a mortar using pestle and the chloroplast suspension was used for chlorophyll assay.

## Result and Discussion

### Cadmium on the peroxidase activity of *Zea mays* leaf.

The activity of peroxidase in the leaves of *Zea mays* was significantly different ( $p < 0.05$ ) in soil contaminated with Cadmium. At concentrations of 20mg and 30mg of cadmium, there was an extremely significant ( $p < 0.05$ ) increase in peroxidase activity at 5 and 6 weeks when compared with the control (non contaminated soil), but for other weeks there were no significant ( $p > 0.05$ ) differences in the peroxidase activity. Table 1 shows that at high concentrations of cadmium, the activity of peroxidase is

increased due to an increase in the production of ROS by the plant.

**Table 1:** Effect of Cadmium on the peroxidase activity of *Zea mays* leaf

Group No	Treatment	Peroxidase Activity (Units/mg leaf)			
		Mean $\pm$ SEM $\times 10^{-1}$			
		Week 3	Week 4	Week 5	Week 6
1	Control	2.36 $\pm$ 0.02 <sup>a</sup>	2.62 $\pm$ 0.01 <sup>a</sup>	2.41 $\pm$ 0.22 <sup>a</sup>	2.69 $\pm$ 0.48 <sup>a</sup>
2	5mg Cd/kg	2.37 $\pm$ 0.05 <sup>a</sup>	2.64 $\pm$ 0.01 <sup>a</sup>	3.39 $\pm$ 0.28 <sup>c</sup>	3.96 $\pm$ 0.58 <sup>b</sup>
3	10mg Cd/kg	2.37 $\pm$ 0.05 <sup>a</sup>	2.65 $\pm$ 0.01 <sup>a</sup>	3.68 $\pm$ 0.89 <sup>b</sup>	4.74 $\pm$ 0.25 <sup>c</sup>
4	20mg Cd/kg	2.39 $\pm$ 0.05 <sup>a</sup>	2.68 $\pm$ 0.03 <sup>a</sup>	6.18 $\pm$ 0.71 <sup>d</sup>	5.22 $\pm$ 0.21 <sup>d</sup>
5	30mg Cd/kg	2.47 $\pm$ 0.07 <sup>a</sup>	3.22 $\pm$ 0.58 <sup>a</sup>	6.47 $\pm$ 0.69 <sup>e</sup>	10.15 $\pm$ 0.86 <sup>e</sup>

Values in the same column carrying different superscripts are significantly different ( $p < 0.05$ ) according to Duncan multiple range test.

### Cadmium on the superoxide dismutase activity of *Zea mays* leaf.

The activity of SOD in the leaves of *Zea mays* was significantly ( $p < 0.05$ ) different in soil contaminated with cadmium. At concentrations of 20mg, 30mg of cadmium, there weresignificant ( $p < 0.05$ ) increase in SOD activity in weeks 4, 5 and 6 compared with the activity in the control (Table 2). But for the other weeks, there were no significant ( $p > 0.05$ ) differences in the SOD activity. This increase in SOD activity is as a result of increased ROS production by the plant.

**Table 2:** Effect of Cadmium on the superoxide dismutase activity of *Zea mays* leaf

Group No	Treatment	Superoxide Dismutase Activity (Units/mg leaf)			
		Mean $\pm$ SEM $\times 10^{-1}$			
		Week 3	Week 4	Week 5	Week 6
1	Control	5.12 $\pm$ 0.07 <sup>a</sup>	5.58 $\pm$ 0.29 <sup>a</sup>	5.86 $\pm$ 0.65 <sup>a</sup>	10.96 $\pm$ 1.54 <sup>a</sup>
2	5mg Cd/kg	7.19 $\pm$ 0.05 <sup>a</sup>	7.78 $\pm$ 0.89 <sup>e</sup>	8.48 $\pm$ 1.80 <sup>e</sup>	11.43 $\pm$ 1.18 <sup>e</sup>
3	10mg Cd/kg	7.62 $\pm$ 0.68 <sup>a</sup>	8.55 $\pm$ 0.85 <sup>d</sup>	8.64 $\pm$ 2.47 <sup>d</sup>	11.76 $\pm$ 1.16 <sup>d</sup>
4	20mg Cd/kg	8.67 $\pm$ 0.99 <sup>a</sup>	9.32 $\pm$ 1.33 <sup>c</sup>	15.57 $\pm$ 2.31 <sup>c</sup>	20.30 $\pm$ 5.07 <sup>c</sup>
5	30mg Cd/kg	8.67 $\pm$ 0.78 <sup>a</sup>	10.34 $\pm$ 0.01 <sup>b</sup>	16.36 $\pm$ 0.88 <sup>b</sup>	36.96 $\pm$ 5.20 <sup>b</sup>

Values in the same column carrying different superscripts are significantly different ( $p < 0.05$ ) according to Duncan multiple range test.

### Cadmium on the catalase activity of *Zea mays* leaf.

The Catalase activity in the leaves of *Zea mays* was significantly different ( $p < 0.05$ ) in soils contaminated with cadmium (Table 3). At concentrations of 20mg and 30mg of cadmium, there were significant ( $p < 0.05$ ) increase in catalase activity at weeks 5 and 6 compared with the control. But for the other weeks, there were no significant ( $p > 0.05$ ) differences in catalase activity. The activity of catalase is increased due to an increase in the production of ROS by the plant.

**Table 3:** Effect of cadmium on the catalase activity of *Zea mays* leaf

Group No	Treatment	Catalase Activity (K/min)			
		Week 3	Week 4	Week 5	Week 6
1	Control	9.45±0.13 <sup>a</sup>	8.99±0.44 <sup>a</sup>	8.37±0.87 <sup>a</sup>	7.94±0.23 <sup>a</sup>
2	5mg Cd/kg	11.11±3.14 <sup>a</sup>	11.43±2.30 <sup>a</sup>	13.26±3.15 <sup>c</sup>	13.72±4.68 <sup>c</sup>
3	10mg Cd/kg	15.45±1.58 <sup>a</sup>	15.64±1.64 <sup>a</sup>	16.55±4.82 <sup>d</sup>	18.72±3.41 <sup>d</sup>
4	20mg Cd/kg	18.07±3.54 <sup>a</sup>	19.42±3.12 <sup>a</sup>	19.03±3.13 <sup>c</sup>	22.71±5.95 <sup>c</sup>
5	30mg Cd/kg	22.31±3.92 <sup>a</sup>	22.41±5.48 <sup>a</sup>	23.33±5.81 <sup>b</sup>	25.15±5.68 <sup>b</sup>

Values in the same column carrying different superscripts are significantly different ( $p < 0.05$ ) according to Duncan multiple range test.

#### Cadmium on the malondialdehyde level of *Zea mays* leaf

The malondialdehyde levels in the leaves of *Zea mays* was significantly ( $p < 0.05$ ) high in soil contaminated with cadmium. At concentrations of 20mg and 30mg of cadmium, there were significant ( $p < 0.05$ ) increase in the malondialdehyde levels in weeks 4, 5 and 6 when compared with the control (Table 4). There was no significant ( $p > 0.05$ ) difference in the malondialdehyde level in week 3 (Table 4). The increase in malondialdehyde level is as a result of increased lipid peroxidation caused by the attack of ROS on polyunsaturated fatty acid.

**Table 4:** Effect of cadmium on the malondialdehyde level of *Zea mays* leaf

Group No	Treatment	Malondialdehyde Level (MDA/g leaf) Mean ± SEM × 10 <sup>-5</sup>			
		Week 3	Week 4	Week 5	Week 6
1	Control	1.88±0.18 <sup>a</sup>	2.17±0.13 <sup>a</sup>	2.70±0.07 <sup>a</sup>	2.90±0.57 <sup>a</sup>
2	5mg Cd/kg	1.94±0.35 <sup>a</sup>	2.53±0.32 <sup>a</sup>	3.06±0.47 <sup>b</sup>	3.42±0.97 <sup>c</sup>
3	10mg Cd/kg	1.91±0.39 <sup>a</sup>	3.06±0.47 <sup>a</sup>	3.34±0.86 <sup>b</sup>	3.45±1.45 <sup>d</sup>
4	20mg Cd/kg	2.32±0.19 <sup>a</sup>	3.10±0.50 <sup>a</sup>	4.17±1.96 <sup>b</sup>	6.86±1.71 <sup>c</sup>
5	30mg Cd/kg	2.42±0.23 <sup>a</sup>	5.32±0.26 <sup>b</sup>	5.77±0.80 <sup>c</sup>	7.17±1.56 <sup>b</sup>

Values in the same column carrying different superscripts are significantly different ( $p < 0.05$ ) according to Duncan multiple range test.

#### Cadmium on the chlorophyll content of *Zea mays* leaf

**Table 5:** Effect of cadmium on the chlorophyll content of *Zea mays* leaf

Group No	Treatment	Chlorophyll Content (mg/g leaf) Mean ± SEM × 10 <sup>-1</sup>			
		Week 3	Week 4	Week 5	Week 6
1	Control	0.22±0.02 <sup>a</sup>	0.24±0.03 <sup>a</sup>	0.22±0.02 <sup>a</sup>	0.27±0.01 <sup>a</sup>
2	5mg Cd/kg	0.20±0.01 <sup>a</sup>	0.20±0.02 <sup>a</sup>	0.18±0.01 <sup>b</sup>	0.20±0.04 <sup>c</sup>
3	10mg Cd/kg	0.18±0.01 <sup>a</sup>	0.17±0.01 <sup>a</sup>	0.17±0.01 <sup>c</sup>	0.18±0.01 <sup>d</sup>
4	20mg Cd/kg	0.17±0.01 <sup>a</sup>	0.11±0.01 <sup>a</sup>	0.11±0.01 <sup>d</sup>	0.09±0.01 <sup>c</sup>
5	30mg Cd/kg	0.18±0.03 <sup>a</sup>	0.10±0.01 <sup>b</sup>	0.11±0.02 <sup>d</sup>	0.10±0.01 <sup>b</sup>

The chlorophyll content in the leaves of *Zea mays* was significantly low ( $p > 0.05$ ) in soils contaminated with cadmium. At concentrations of 5mg, 10mg, 20mg and 30mg of cadmium, there were significant ( $p < 0.05$ ) decrease of chlorophyll contents in weeks 4, 5 and 6 when compared with the control (Table 5). At week 3, there was no significant ( $p > 0.05$ ) difference in the chlorophyll

content (Table 5). The decrease in chlorophyll content observed in the leaves of contaminated soils could be as a result of chlorophyll modification by ROS or its reduced synthesis.

Values in the same column carrying different superscripts are significantly different ( $p < 0.05$ ) according to Duncan multiple range test.

#### Cadmium on the leaf area of *Zea mays*

The leaf area showed significant ( $p < 0.05$ ) differences in soils contaminated with cadmium (Table 6). At concentrations of 5mg, 10mg, 20mg and 30mg of cadmium, there was significant ( $p < 0.05$ ) decrease in leaf area at weeks 5 and 6 compared to the control. There was no significant ( $p > 0.05$ ) difference in the leaf area at weeks 3 and 4 (Table 6). The decrease in the rate of leaf enlargement in Table 6 is largely due to the inhibition by cadmium.

**Table 6:** Effect of Cadmium on the leaf area of *Zea mays*

Group No	Treatment	Leaf Area (cm <sup>2</sup> )			
		Week 3	Week 4	Week 5	Week 6
1	Control	79.80±2.67 <sup>a</sup>	80.87±4.58 <sup>a</sup>	83.24±6.16 <sup>b</sup>	87.99±3.56 <sup>a</sup>
2	5mg Cd/kg	73.74±1.01 <sup>a</sup>	72.73±2.31 <sup>a</sup>	56.72±6.49 <sup>c</sup>	50.02±0.94 <sup>c</sup>
3	10mg Cd/kg	76.46±3.82 <sup>a</sup>	72.10±2.09 <sup>a</sup>	56.67±2.31 <sup>c</sup>	45.08±7.09 <sup>b</sup>
4	20mg Cd/kg	69.70±0.87 <sup>a</sup>	69.13±9.67 <sup>a</sup>	51.94±0.56 <sup>c</sup>	48.84±9.04 <sup>c</sup>
5	30mg Cd/kg	64.22±7.20 <sup>a</sup>	59.67±2.39 <sup>a</sup>	48.38±1.93 <sup>b</sup>	46.79±0.43 <sup>c</sup>

Values in the same column carrying different superscripts are significantly different ( $p < 0.05$ ) according to Duncan multiple range test.

#### Cadmium on the leaf weight of *Zea mays*

The leaf weight of *Zea mays* was significantly ( $p < 0.05$ ) low in soils contaminated with Cadmium. At a concentrations of 20mg and 30mg of cadmium, there were significant ( $p < 0.05$ ) decrease in leaf weight at weeks 5 and 6 compared to the control (Table 7). There were no significant ( $p > 0.05$ ) differences in the leaf weight of weeks 3 and 4. The data in Table 7 shows that cadmium is capable of inhibiting cell division.

**Table 7:** Effect of cadmium on the leaf weight of *Zea mays*.

Group No	Treatment	Leaf Weight (g)			
		Week 3	Week 4	Week 5	Week 6
1	Control	2.02±0.84 <sup>a</sup>	2.43±0.14 <sup>a</sup>	4.03±0.19 <sup>a</sup>	3.90±0.24 <sup>a</sup>
2	5mg Cd/kg	1.83±0.25 <sup>a</sup>	2.07±0.38 <sup>a</sup>	3.14±0.19 <sup>b</sup>	3.87±0.34 <sup>c</sup>
3	10mg Cd/kg	1.76±0.63 <sup>a</sup>	1.96±0.42 <sup>a</sup>	3.02±0.51 <sup>c</sup>	3.18±0.20 <sup>d</sup>
4	20mg Cd/kg	1.76±0.36 <sup>a</sup>	2.63±0.43 <sup>a</sup>	1.42±0.18 <sup>d</sup>	0.91±0.16 <sup>c</sup>
5	30mg Cd/kg	1.89±0.10 <sup>a</sup>	2.26±0.26 <sup>a</sup>	0.89±0.01 <sup>e</sup>	1.74±0.57 <sup>b</sup>

Values in the same column carrying different superscripts are significantly different ( $p < 0.05$ ) according to Duncan multiple range test.

#### Discussion

In this study, we aimed to establish the effect of varying concentration of cadmium toxicity on some anti-oxidative enzymes, weight, chlorophyll content and area of *Zea mays* leaf. Studies have shown that heavy metals are widely recognised as highly toxic to plants. Plants can be affected directly by air pollutants or indirectly through the contamination of soil and water. To defend against oxidative stress and scavenge ROS plants possess a well organised anti-oxidative enzyme system and antioxidants (Apel and

Hurt, 2004) [2]. The increased activity of superoxide dismutase, catalase and peroxidase caused by cadmium has been observed in several plant species and is considered to be an adjustment response to stress (Kudo, *et al.*, 2011) [17]. This is in agreement with our findings in soils contaminated with cadmium which showed a significant ( $p < 0.05$ ) increase in anti-oxidative enzymes such as catalase, peroxidase and superoxide dismutase. The increase in these anti-oxidative activities is circumstantial evidence to support the hypothesis that cadmium treatment increases the formation of reactive oxygen species (Shehab, *et al.*, 2010) [33]. Rascio, *et al.*, (2002) [25] studied the effect of cadmium toxicity on maize plant and reported leaf bleaching, ultra structural alteration of chloroplast and lowering of photosynthetic activity. This was consistent with our findings which showed a significant ( $p < 0.05$ ) decrease in the chlorophyll content of cadmium contaminated soils leading to a decreased photosynthetic activity (Table 5). Yellowing of leaves was also observed as well as a reduction in leaf area. The decrease in chlorophyll content may be due to cadmium induced inhibition of delta aminolaevulinic acid hydratase (this is the enzyme that catalyzes the synthesis of porphobilinogen from delta aminolaevulinate in chlorophyll synthesis) or substitution of Mg atom in chlorophyll molecule leading to the breakdown in photosynthetic process (Kupper, *et al.*, 2007). Cadmium also inhibits the synthesis of chlorophyll stable binding proteins thereby decreasing the accumulation of pigment lipoprotein complexes particularly photosystem 1 (PS 1) (Wang *et al.*, 2009) [39]. The primary target of cadmium toxicity is photosystem 2 (PS II) and an enzymatic phase of photosynthesis particularly, ribulose 1, 5 bisphosphate carboxylase / oxygenase activity (Krantev, *et al.*, 2008) [16]. Vassilev, *et al.*, (2011) [38] claimed that on exposure of *Phaseolus vulgaris* to cadmium toxicity, there was a decrease in seed germination, total chlorophyll, leaf area and net electron photosynthetic transport. This was consistent with our results which showed a significant ( $p < 0.05$ ) decrease in the leaf area of *Zea mays* contaminated with cadmium (Table 6). The reduction of leaf area could be attributed to toxic cadmium levels which induce negative effects on some key metabolic processes coupled to growth in plants (VanAssche, *et al.*, 1984) [37]. Toxicity of cadmium has been related to an increase in lipid peroxidation and alteration in the anti-oxidative system in plants (Romero-Puertas, *et al.*, 2004) [27]. The extent of lipid peroxidation is assayed by the determination of the malondialdehyde concentration which is a product of this process. This work showed a significant ( $p < 0.05$ ) increase in malondialdehyde concentration in the presence of cadmium toxicity. Dixit *et al.*, (2001) [11] stated that cadmium enhances the level of lipid peroxidation and increases tissue concentration of  $H_2O_2$  in both roots and leaves.

In conclusion, we can say that cadmium is a heavy metal which causes oxidative stress as evident by the decrease in leaf area, chlorophyll content and leaf weight of *Zea mays* and by the increment in tissue concentration of  $H_2O_2$  and lipid peroxidation. These changes in turn led to the increased production of anti-oxidative enzymes to ameliorate the effect of cadmium toxicity.

## References

1. Adegoke GO, Adebayo CO. Production, nutritional profiles and storability of 'aadun' and 'kokoro', two

- corn products. *Plant Food Hum. Nutr.* 1994; 45:139-144.
2. Apel K, Hirt H. Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. *Annu. Rev. Plant Biol.* 2004; 55:373-399.
3. Azevedo H, Pinto CGG, Fernandes J, Loureiro S, Santos C. Cadmium effects on sunflower growth and photosynthesis. *Journal of Plant Nutrition.* 2005; 28:2211-2220.
4. Azmat R, Zill-e-Huma A, Hayat T, Khanumand R, Talat R. The inhibition of bean plant metabolism by cadmium metal: Effects of cadmium metal on physiological process of bean plant and *Rhizobium* species. *Pakistan Journal of Biological Sciences.* 2005; 8(3):401-404.
5. Baum JA, Scandalios JG. Isolation and characterization of cytosolic and mitochondria superoxide dismutase of maize. *Arch. Biochem. Biophys.* 1981; 206:249-264.
6. Buege JA, Aust SD. Microsomal lipid peroxidation. *Methods in enzymology.* 1978; 52:302-310.
7. Canfield RL, Henderson CR, Cory-Slechta DA, Cox C, Jusko TA, Lanphear BP. Intellectual impairment in children with blood lead concentrations below  $10\mu\text{g}/\text{dl}$ . *N. Engl. J. Med.* 2003; 348(16):1517-1526.
8. Chance B, Maehly AC. Assay of catalases and peroxidases. *Method in Enzymology.* 1955; 2:764-775.
9. Cohen G, Denbliec D, Marcus S. Measurement of catalase activity in tissue extracts. *Ann. Biochem.* 1970; 34:30-38.
10. Dixit P, Mukherjee PK, Ramachandran V, Eapen S. Glutathione transferase from *Trichoderma viridis* enhances cadmium tolerance without enhancing its accumulation in transgenic *Nicotiana glauca*. *PLOS ONE.* 2011; 6(1):1371.
11. Dixit V, Pandey V, Shyam R. Differential anti-oxidative responses to cadmium in roots and leaves of pea. *J. Exp. Bot.* 2001; 52(358):1101-1109.
12. Hassan Z, Aarts MGM. Opportunities and feasibilities for biotechnological improvement of Zn, Cd or Ni tolerance and accumulation in plants. *Environmental and Experimental Botany.* 2011; 72:53-63.
13. Hermans C, Chen J, Coppens F, Inze D, Verbruggen N. Low magnesium status in plants enhances tolerance to cadmium exposure. *New Phytologist.* 2011; 192:428-436.
14. Horváth G, Droppa M, Oravec Á, Raskin VI, Marder JB. Formation of the photosynthetic apparatus during greening of Cd poisoned barley leaves. *Planta.* 1996; 199:238-244.
15. Koh S, André A, Edwards H, Ehrhardt D, Somerville S. *Arabidopsis thaliana* subcellular response to compatible *Erysiphe cichoracearum* infections. *Plant J.* 2005; 44:516-529.
16. Krantev A, Yordanova R, Janda T, Szalai G, Popova L. Treatment with salicylic acid decreases the effect of cadmium on photosynthesis in maize plants. *Journal of Plant Physiology.* 2008; 165:920-931.
17. Kudo H, Kudo K, Ambo H, Uemura M, Kawai S. Cadmium absorption by plasma membrane isolated from barley roots. *Plant Science.* 2011; 180:300-305.
18. Kupper H, Kupper F, Spiller M. *In situ* detection of heavy metal substituted chlorophyll in water plants. *Phytosynth. Res.* 1998; 58:123-133.
19. Kupper H, Parameswaran A, Leitenmaier B, Trtilek M,

- Setlik I. Cadmium-induced inhibition of photosynthesis and long-term acclimation to cadmium stress in the hyperaccumulator. *Thlaspi caerulescens*. *New Phytologist*. 2008; 175:655-674.
20. Lux A, Martinka M, Vaculik M, White PJ. Root responses to cadmium in the rhizosphere: A review. *J. Exp. Bot.* 2011; 62:21-37.
21. Meriga B, Reddy BK, Rao KR, Reddy LA, Kishor PBK. Aluminium-induced production of oxygen radicals, lipid peroxidation and DNA damage in seedlings of rice (*Oryza sativa*). *Journal of Plant Physiology*. 2004; 16(1):63-68.
22. Misra HP, Fridovich I. The role of superoxide anion in the auto oxidation of epinephrine and a simple assay for superoxide dismutase. *J. Biol. Chem.* 1972; 247:3170-3175.
23. Musyimi DM, Netondo GW, Ouma G. Effects of salinity on growth and photosynthesis of avocado seedlings. *International Journal of Botany*. 2007; 3(1):78-84.
24. Piper CS. *Soil and Plant analyses: A laboratory manual of methods for the examination of soil and inorganic constituents of plant*. Hans Publishers, Bombay, 1966.
25. Rascio N, Vecchia FD, Ferretti M, Merlo L, Ghisi R. Some effects of cadmium on maize plants. *Arch. Environ. Contam. Toxicol.* 2002; 25:244-249.
26. Rathnayake IV, Megharaj M, Krishnamurti GS, Bolan NS, Naidu R. Heavy metal toxicity to bacteria - are the existing growth media accurate enough to determine heavy metal toxicity? *Chemosphere*. 2013; 90(3):1195-1200.
27. Romero-Puertas MC, Rodríguez-Serrano M, Corpas FJ, Gómez M, delRío LA, Sandalio LM. Cadmium-induced subcellular accumulation of O<sub>2</sub><sup>-</sup> and H<sub>2</sub>O<sub>2</sub> in pea leaves. *Plant Cell Environ.* 2004; 27:1122-1134.
28. Roney N, Osier M, Paikoff SJ. Evaluation of the health effects of zinc and relevance to public health. *Toxicol. Ind. Health*. 2006; 22(10):423-493.
29. Sanz A, Ilamas A, Ullrich CI. Distinctive phytotoxic effects of Cd and Ni on membrane functionality. *Plant Signaling and Behaviour*. 2009; 4:980-982.
30. Saunders JE, Jastrzebski BG, Buckey JC, Enriquez D, MacKenzie TA, Karagas MR. Hearing loss and heavy metal toxicity in a Nicaraguan mining community. *Audiol. Neurootol.* 2013; 18(2):101-113.
31. Scandalios JG. Oxygen stress and superoxide dismutases. *Plant Physiol.* 1993; 101:7-12.
32. Sgherri C, Milone MT, Clijsters H, Navari-Izzo F. Antioxidative responses of wheat treated with realistic concentration of cadmium. *Environmental and Experimental Botany*. 2003; 50:265-276.
33. Shehab GG, Ahmed OK, El-Beltagi HS. Effects of Various Chemical Agents for Alleviation of Drought Stress in Rice Plants (*Oryza sativa L.*). *Notulae Botanicae Horti Agrobotanici*. 2010; 38(1):139-148.
34. Shukla UC, Murthy RC, Kakkar P. Combined effects of ultraviolet β radiation and Cd contamination on nutrient uptake and photosynthetic pigments in *Brassica campestris L.* seedlings. *Environ. Toxicol.* 2008; 23:712-719.
35. Tausz M, Gullner G, Kömives T, Grill D. Role of thiols in plant adaptation to environmental stress. In: *Sulphur in plants*. Dordrecht, 2003, 221-244.
36. Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ. Heavy metal toxicity and the environment. *EXS*. 2012; 101:133-164.
37. Vanassche F, Cardinales C, Clijsters H. Premature leaf ageing induced by heavy metal toxicity. *Arch. Int. Physiol. Biochem.* 1984; 94:27-28.
38. Vassilev A, Nikolova A, Koleva L, Lidon F. Effect of excess zinc on growth and photosynthetic performance of young bean plants. *Journal of Physiology*. 2011; 3(6):58-62.
39. Wang H, Zhao SC, Liu RL, Zhou W, Jin JY. Changes in photosynthetic activities of maize (*Zea mays L.*) seedlings in response to cadmium stress. *Photosynthetica*. 2009; 47(2):277-283.
40. Winterman JF, De Mots A. Spectrophotometric characteristics of chlorophylls a and b and their pheophytin in ethanol. *Biochem. Biophys. Acta*. 1965; 109:448-453.
41. Yadav SK. Heavy metals toxicity in plants: An overview on the role of glutathione and phytochelatin in heavy metal stress tolerance of plants. *South African Journal of Botany*. 2010; 76:167-179.
42. Yang K, Ma W, Liang H, Ouyang Q, Tang C, Lai L. Dynamic Simulations on the Arachidonic Acid Metabolic Network. *PLOS Computational Biology*. 2007; 3(3):55.