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Physiological Responses of Maize (*Zea mays* L.) Genotypes to Cadmium Stress

Enas Fahd Naji and Dhia S. Hassawi

Department of Biology, College of Sciences, University of Anbar, Al-Anbar, Iraq
E-mail: SC.enas-fahad@uoanbar.edu.iq

***Abstract*:** The research was performed to evaluate the influence of different levels of cadmium on various physiological parameters of maize (*Zea mays* L.). Three Iraqi varieties (5018, Baghdad-3, and Fajr-1) were exposed to four different concentrations of cadmium (0, 200, 300, 400 ppm). The results revealed different variations in the ability of these varieties to withstand cadmium stress, in terms of accumulating some components in their vegetative parts. The results also indicated that Baghdad-3 had the highest total chlorophyll and total carotenoids content which recorded 0.853 mg g^{1} and 3.537 mg g^{1}, respectively. The highest plant height (77.15 cm), shoot dry weight (11.35 g $plant^{1}$), content of proline (4.19 µmole g^{1}), total nitrogen (1.2018%), and total protein (7.499%) were recorded with Fajr-1. The addition of cadmium resulted in a decrease in plant height and shoot dry weight and an increase in all measured chemical contents. Higher levels of cadmium were accumulated in the shoot of variety 5018, while fajr-1 showed the least ability to absorb cadmium from the soil and accumulate it in its vegetative parts; the amount of cadmium accumulated by maize plant depends on its concentrations that added in the soil.

***Keywords*:** Maize, Cadmium, Physiological stress, Photosynthetic pigments, Proline

Pollution from heavy metals is a serious environmental issue and has become a global problem. Heavy metal production and emission increased along with increased industrial growth. This has led to increasing concern about food safety due to soil contamination with these toxic metals (Nagajyoti et al 2010**)**. Contamination of agricultural soils and crops with heavy metals has been considered a significant environmental concern due to their non-biodegradable existence and long biological half-life as well as their possible accumulation in human body (Muhammad et al 2011). Cadmium (Cd) has became one of the most dangerous and widespread contaminants in the atmosphere. In higher plants, cadmium is non-essential metal, and exposure to relatively low concentrations leads to severe toxicity (Fernandes et al 2008). The World Health Organization (WHO) has deemed an average intake of 0.83 µg kg-1 body weight or 58.1 µg Cd per person to be human toxic (WHO 2010). Cadmium is one of the heavy metals usually present in the soil at small concentrations and exposure to sources of pollution, crop rotation and management activities are the main causes of increased Cd content in soil (Siebers et al 2014). Cadmium is a non-necessary element easily absorbed by plant roots and can be loaded into the xylem for conveying into leaves. Many plants are susceptible to low concentrations of Cd, which inhibit plant growth as a result of changes in photosynthesis rate and macronutrient and micronutrient uptake and distribution (Benavides et al 2005). Maize (*Zea mays* L.) is the third important cereal crops after wheat and rice (Haddad et al 2016). Maize is one of the world's most important annual cereal crops, offering a staple diet and being used as an income source for many communities in developing countries. The way maize is processed and consumed differs greatly from country to country, with the most common products being maize meal and flour. It is a major source of carbohydrate in developing countries for human diets and in the developed world for animal feed (Ranum et al 2014). The aim of this study was to explore the physiological responses of maize genotypes grown in soil polluted with heavy metals, and to determine the capacity of uptake and performance of cadmium by maize.

MATERIAL AND METHODS

The experiment was conducted at University of Anbar during 2019 season. Seeds of three varieties of maize (5018, Baghdad-3, and Fajr-1) were obtained from the center of seeds certification at Abu Ghraib city. The seeds were surface sterilized with 10% sodium hypochlorite for 2 minutes and washed extensively with distilled water. The seeds were then germinated in plastic pots filled with16 Kg planting media, which were prepared from sandy loam soil and peat moss in 5:1 ratio. Some physical and chemical properties of soil are given in (Table 1).

About ten seeds were planted in each pot. After 10 days of planting, the germinated seedlings were thinned to five, as the most active and homogeneous plants were kept. The growing plants were irrigated with normal water, up to the field capacity, and then watering daily according to the needs of the plants. After 30 days of planting, cadmium was added to the soil in the form of cadmium nitrate tetra hydrates Cd $(NO_3)_2.4H_2O$, with concentrations of 0, 200 and 300, 400 mg kg^{-1}. It was applied after dissolving the specified weight of cadmium nitrate in the appropriate volume of distilled water in three batches with a period of 10 days interval between them. Physiological characteristics were measured 10 days after the last cadmium addition, as the plant was 60 days old after sowing. The maize plants were harvested after completing the experiment at an age of about 92 days. The aerial parts were taken and dried for the purpose of measuring plant height and taking dry weights of all plants in addition, estimating some chemical contents.

Total chlorophyll and carotenoid estimation: The pigments were extracted from fresh leaf that was collected from the three replicates for each treatment; about 0.5 g fresh weight of leaf samples were rapidly homogenized in 10 ml acetone 80%. The total chlorophyll and carotenoids concentrations were determined by a spectrophotometer according to the procedure describe by Lichtenthaler (1987) for total chlorophyll and total carotenoids. Absorption was measured at the wavelengths 470, 645 and 663 nm. Concentrations of photosynthetic pigments were quantified using the following equations:

C_a(mg /g tissue) = $12.7A_{663} - 2.69A_{645}$ *(V/1000*W)

C_b(mg/g tissue) = $22.9A_{645} - 4.68A_{663}$ *(V/1000*W)

Total chlorophyll = $C_a + C_b$

C_{x+c}(mg /g tissue) = $1000\,A_{470} - 1.82\,C_a - 85.02\,C_b/198$

Where C_a = chlorophyll a; C_b = chlorophyll b; $C_a + C_b$ = total chlorophyll;

C_{x+c} = carotenoid, $A\lambda$ = absorbance at λ (nm)

Proline analysis: Extraction and determination of free proline were achieved from fresh leaves according to the procedure of Bates et al. (1973). Sample of 0.5 g leaf material was homogenized in 10 ml of 3% 5-sulfosalysilic acid solution. The homogenate was filtered via Whatman No. 1 filter paper. Proline was evaluated by reacting 2 ml of the extract with 2 ml of glacial acetic acid and 2 ml of ninhydrin solution. The mixture was incubated in a boiling water bath for 1 h. After cooling, 4 ml of toluene was added and agitated. The absorbance of the toluene phase was determined at 520 nm in a Jenway (PD-6315). The proline content was determined using the following equation:

(µg proline ml^{-1} × ml toluene) / 115.5 µg $µ^{-1}$moles^{-1}]/ [(g sample)/5] = µmoles proline g^{-1} of fresh weight material.

Nitrogen and protein estimation: Nitrogen was estimated according to Kjeldahl method (Sawhney and Randhir 2000). From calculated nitrogen values, protein value was estimated by the following equation: Protein % = Nitrogen%*6.24.

Cadmium: Approximately 0.5 grams of dried and finely ground aerial parts were taken and digested by a mixture of concentrated acidsH_2SO_4, $HClO_4$, and HNO_3i n a ratio of 10:4:1, respectively. The samples were placed in Kjeldahl flasks with long neck and 10 ml of the above mixture was added. Then, they were left on the heaters until the color disappeared, after which it was cooled down and the volume was completed to 50 ml with distilled water. The cadmium element was measured using an atomic absorption spectrometer (Shimadzu 7000 Japan) and the concentrations of unknown samples were extracted by projecting the readings onto the standard curves which were drawn according to the standard curve fitting equation.

Statistical analysis: The experiment was conducted according to the complete randomize design (CRD) with three replicates for each treatment. The data were subjected to statistical analysis using the statistical software Gen Stat., (12^{th}) Edition.

Table 1. Chemical and physical properties of the soil used in this study

P (mg kg^{-1})	K^+ (mg kg^{-1})	Ca^{++} (mg kg^{-1})	Mg^{++} (mg kg^{-1})	Cd^{++} (mg kg^{-1})	Ecµs	TDS (ppm)	NaCl (%)
17.337	9.13	114.5	26.5	0.06642	2637	1318	5.2

Table 2. Plant height (cm) of maize varieties under different cadmium levels

Variety	Cd concentrations (ppm)				Average
	Control	200	300	400	
5018	81.400	63.300	73.650	69.450	71.950^b
Baghdad-3	71.500	69.100	60.800	54.500	63.975^c
Fajr -1-	98.500	78.800	70.500	60.800	77.150^a
LSD (p=0.05)	1.0354				0.5177
Average	83.800^a	70.400^b	68.317^c	61.583^d	Grand mean
LSD (p=0.05)	0.5978				71.025

RESULTS AND DISCUSSION

Plant height: There significant differences in the plant heights of the three maize varieties. Fajr-1- gave the maximum eight (77.150 cm), followed by 5018 variety and Baghdad-3. When maize plants were exposed to increased concentrations of cadmium, a significant and gradual decrease in plant height was observed 15.99, 18.47 and 25.51% in response to the concentrations 200, 300 and 400 ppm, respectively. The maximum plant height appeared in Fajr-1- variety with treatment 200 ppm, which decreased significantly from the control by 20%. Baghdad-3 variety with 400 ppm cadmium resulted in lowest value of plant height, which was significantly lower than the control, by 23.77%. Ling et al 2017) reported a decrease in the height of the maize plant under the influence of cadmium.

Shoot dry weight: The variety Fajr-1- recorded the highest dry weight value (11.35 g plant^{-1}) with a significant difference from the other two varieties, followed by 5018 (9.24 g plant^{-1}), and Baghdad-3 (8.63 g plant^{-1}(Table 3). The presence of cadmium in the soil caused a significant decrease in maize dry weight of shoots and when concentration of cadmium in the soil increased the decrease higher. The percentages of the decrease were 20.18% 22.01 and 32.69% in response to the concentrations 200, 300 and 400 ppm, respectively as compared with 11.99 g plant^{-1} for the control t. The maximum dry weight (11.06 g plant^{-1}) was in the Fajr-1- variety with 200 ppm, which decreased significantly from the control treatment (15.76 g plant^{-1}) by 29.82%. Baghdad-3 recorded the lowest dry weight (7.10 g plant^{-1}) when treated with 400 ppm, which was significantly lower than the control treatment of (9.26 g plant^{-1}) by 23.32% by (Ghani 2010) also reported that maize shoot dry weight declined under cadmium exposure. Wang et al (2001) explained that this decline as heavy metals alter the respiratory rate and thus reduce the rate of the energy complex ATP production which is necessary for the formation of new tissues and organs. Al-rumaih et al (2001) added that exposure to cadmium inhibited cell division and disturbed cell expansion and enlargement. In addition, a decrease in carbohydrate synthesis has also been shown to inhibit shoot growth due to the inhibitory effect of cadmium on carbohydrate metabolism.

Total chlorophyll content: The variety Baghdad-3 showed the highest total chlorophyll (0.853 mg g^{-1}) with significant differences with the lowest value of fajr-1 (0.722 mg g^{-1}). The total chlorophyll content of variety 5018 was 0.836 mg g^{-1} (Table 4). The results indicate that inclusion of cadmium at a concentration 200 ppm (0.623 mg g^{-1}) decreased the total chlorophyll content by 16.82%, with significant differences from the control (0.749 mg g^{-1}). The highest cadmium concentrations (400 ppm) caused an increase in the total chlorophyll content (1.051 mg g^{-1}) of the leaves by 40.32%. The variety 5018 at Cd concentration of 400 ppm caused 44.32% rise, with significant differences from the control (0.81 mg g^{-1}). In addition to significant differences from the lowest value in Baghdad-3 at Cd concentration of 200 ppm, the chlorophyll content decreased by 37.62%, with significant

Table 3. Shoot dry weight (g plant^{-1}) of maize varieties under different cadmium levels

Variety	Cd concentrations (ppm)				Average
	Control	200	300	400	
5018	10.94	8.21	9.36	8.47	9.24^{b}
Baghdad-3	9.26	9.43	8.74	7.10	8.63^{c}
Fajr -1-	15.76	11.06	9.95	8.64	11.35^{a}
LSD (p=0.05)		0.073			0.036
Average	11.99^{a}	9.57^{b}	9.35^{c}	8.07^{d}	Grand mean
LSD (p=0.05)		0.042			9.74

Table 4. Total chlorophyll content (mg g^{-1}) of maize varieties under different cadmium levels

Variety	Cd concentrations (ppm)				Average
	Control	200	300	400	
5018	0.810	0.616	0.749	1.169	0.836^{a}
Baghdad-3	0.885	0.552	0.844	1.132	0.853^{a}
Fajr -1-	0.551	0.700	0.785	0.853	0.722^{b}
LSD (p=0.05)		0.1957			0.0979
Average	0.749^{b}	0.623^{c}	0.793^{b}	1.051^{a}	Grand mean
LSD (p=0.05)		0.1130			0.804

differences from the control treatment (0.885 mg g^{-1}). Several studies mentioned the heavy metal inhibition of chlorophyll biosynthesis in higher plants. Regarding the heavy metal concentrations effect on the synthesis and aggregation of photosynthetic pigments, different reports showed decreased pigment content, while others found it is unchanged or even increased (Sfaxi-Bousbih et al 2010). Ling et al (2017) observed decrease in the total chlorophyll content with increasing external Cd levels. Cadmium exposure has been shown to increase stomatal closure and to inhibit photosynthesis in plants by chlorophyll degradation, With elevated external Cd supply levels, the concentrations of chlorophyll a, chlorophyll b, and chlorophyll a + b in corn leaves gradually decreased (Ling et al 2017). Photosynthetic machinery of plants is damaged by cadmium, particularly light harvesting complex-II and photosystem-I (PS-I) and PS-II. Inhibition of aminolaevulinic acid synthesis by chlorophyll reductase in chlorophyll biosynthesis is the most significant explanation for excessive doses of Cd to interrupt chlorophyll biosynthesis. The observed reduction in total chlorophyll content under Cd stress can be explained by reduced carbon metabolism ability or the low use of ATP and NADPH in the dark photosynthesis process. The reduction in chlorophyll content in plants exposed to Cd^{2+} stress is believed to be due to: (a) inhibition of important enzymes, such as δ-aminolevulinic acid dehydratase (AL-Adehydratase) and proto chlorophyllide reductase associated with chlorophyll biosynthesis; (b) impairment in the supply of Mg^{2+} and Fe^{2+} required for the synthesis of chlorophylls; (c) Zn^{2+}deficiencyresulting in inhibition of enzymes, such as carbonic anhydrase; (d) the replacement of Mg^{2+} ions associated with the tetrapyrrole ring of chlorophyll molecule (Subrahmanyam and Rathore 2000).

Total carotenoids content: The maximum carotenoid content was produced by Baghdad-3 variety (3.537 mg g^{-1}) with significant difference from the lowest value of 3.176 mg^{-1} in Fajr-1- variety (Table 5).The averages of cadmium concentrations revealed that the concentration 200 ppm reduced the carotenoids content (2.622mg g^{-1}) of corn leaves by 5.98% compared with the control (2.789 mg g^{-1}), but when the plant was exposed to high concentrations of cadmium (400 ppm), this has led to a significant increase in the content of carotenoids (4.443 mg g^{-1}). The treatment 400 ppm with Fajr-1 variety produced the highest carotenoid content, reaching 4.694 mg g^{-1}, with significant differences compared to control (2.130 mg g^{-1}). Treatment 200 ppm with Fajr-1 variety produced low carotenoid content (2.572 mg g^{-1}). Ling et al (2017) stated that quantity of pigment contents (including carotenoid) in corn leaves decreased slowly with increasing external Cd supply level. Aliu et al (2013) mentioned that the exposure of maize seedlings to Cd^{2+} resulted in a reduction of chlorophyll and carotene content in leaves compare to control treatment. Carotenoids serve as antioxidants against free radicals and photochemical damage, Carotenoids are known to quench the oxidizing species and triplet state of the chlorophyll and other excited molecules in the pigment bed, which are seriously involved in disrupting metabolism through oxidative damage to cellular components (Candan and Tarhan 2003).

Proline content: Fajr-1-variety showed the highest proline content (4.19 µmole g^{-1}) and the differences were significant comparing to the other two varieties, 5018 (2.53µmole g^{-1}) and Baghdad-3 (2.48 µmole g^{-1}) (Table 6). Irfan et al (2014) indicated that tolerant mustard cultivars had higher proline content than sensitive ones under Cd stress conditions. Cd-tolerant plants were reported to accumulate compatible osmolytes such as proline. The results of the concentration averages of cadmium revealed an increase in the content of proline corn leaves under the influence of cadmium stress. This increase was 19.58, 57.5 and 34.16%, in response to concentrations 200, 300 and 400 ppm respectively and the differences were significant compared to the control. The treatment 300 ppm Cd with Fajr-1 variety showed the highest proline content (5.27 µmole g^{-1}), which increased significantly by 56.84% as compared with 3.36 µmole/g control treatment. Yildirim et al (2019) confirmed that proline and sucrose

Table 5. Total carotienoids content (mg/g) of maize varieties under different cadmium levels

Variety name	Cd concentrations (ppm)				Average
	Control	200	300	400	
5018	2.859	2.656	3.632	4.513	3.415[a]
Baghdad-3	3.378	2.637	4.013	4.120	3.537[a]
Fajr -1-	2.130	2.572	3.307	4.694	3.176[b]
LSD (p=0.05)		0.3387			0.1694
Average	2.789[c]	2.622[c]	3.651[b]	4.443[a]	Grand mean
LSD (p=0.05)		0.1956			3.376

content increased with heavy metal treatments. Proline accumulation can play a role in the detoxification of heavy metal. Proline could be involved in metal chelation in the cytoplasm by increasing the biosynthesis of proteins and increasing the activity of the antioxidant enzymes (catalase and peroxidase) (Costa and Morel 1994). Proline thus seems to indirectly reduce the toxic impact of heavy metals. Proline increases the stress tolerance of plants through mechanisms as osmoregulation, protection of enzymes against denaturation, and stabilization of protein synthesis (Zengin and Munzuroglu 2005).

Shoot nitrogen content: Fajr-1- variety scored the highest nitrogen content of 1.2018%, which differed significantly from the Baghdad-3 variety (1.1270 %) and with the lower of 1.0441 %, was in 5018. The results also indicated that the accumulation of the cadmium element in the corn plant resulted in the accumulation of the nitrogen component in the shoot, recording at rates 24.85, 42.14 and 65.84 %, under the influence of concentrations 200, 300 and 400 ppm respectively and significant compared to the control treatment (0.8440 %). The 400 ppm Cd resulted in increased nitrogen levels in 5018 and Fajr-1 to reach 1.4230%. The lowest nitrogen content was at 200 ppm Cd in 5018 variety (0.9215%). These results do not comply with the results of Abdul Ghani (2010), who indicated that nitrogen content in shoots and roots decreased in maize plants treated with heavy. Kato et al (2020) recorded that cadmium-treated maize plants were with higher N concentrations than control plant. Dhir et al (2004) explained increase by the fact that heavy metals stimulate the accumulation of the amino acid proline in the wheat *Triticum aestivum* shoots, which includes nitrogen in its chemical structure.

Total proteins in shoot: The protein content of Fajr-1- variety was higher (7.499%) than Baghdad-3 (7.032%) and 5018 (6.515%) and the differences were statistically significant (Table 8). The cadmium concentrations caused aggregation of proteins in the shooting system of the plant, and the quantity of this aggregation increased as the concentration increased; 24.85, 42.13 and 65.82%, under the presence of 200, 300 and 400 ppm Cd concentrations, respectively. The highest protein content (8,880 %) in the shoots of maize plant were observed with the two varieties; 5018 and Fajr-1 at 4 00 ppm Cd, and the differences were significant. The lowest protein content was in 5018 variety with 200 ppm Cd, which increased significantly by 38.55 % over control treatment. The increase in the amount of proline, which plays a role in reducing osmotic stress, acts as a source of C and N, stabilizes protein synthesis, and functions as an antioxidant and pH regulator and is caused by many forms of stress. In maize, treatment with Cd resulted not only in a decrease in protease activity, but also in amino acid and proline accumulation. Siddhu and Khan (2012) revealed that the bean seed *Phaseolus mungo* had higher protein content at the low concentration of cadmium. Cuppers (2005)

Table 6. Proline content (μmole g^{-1}) of maize varieties under different cadmium levels

Variety	Cd concentrations (ppm)				Average
	Control	200	300	400	
5018	1.76	2.06	3.88	2.42	2.53^{b}
Baghdad-3	2.09	2.51	2.18	3.15	2.48^{b}
Fajr -1-	3.36	4.04	5.27	4.10	4.19^{a}
LSD (p=0.05)		1.630			0.815
Average	2.40^{b}	2.87^{ab}	3.78^{a}	3.22^{ab}	Grand mean
LSD (p=0.05)		0.941			3.07

Table 7. Nitrogen content (%) of maize varieties under different cadmium levels

Variety	Cd concentrations (ppm)				Average
	Control	200	300	400	
5018	0.6650	0.9215	1.1670	1.4230	1.0441^{c}
Baghdad-3	0.9800	1.0500	1.1250	1.3530	1.1270^{b}
Fajr -1-	0.8870	1.1900	1.3070	1.4230	1.2018^{a}
LSD (p=0.05)		0.06400			0.03200
Average	0.8440^{d}	1.0538^{c}	1.1997^{b}	1.3997^{a}	Grand mean
LSD (p=0.05)		0.03695			1.1243

Table 8. Protein content (%) of maize varieties under different cadmium levels

Variety	Cd concentrations (ppm)				Average
	Control	200	300	400	
5018	4.150	5.750	7.282	8.880	6.515[c]
Baghdad-3	6.115	6.552	7.020	8.443	7.032[b]
Fajr -1-	5.535	7.426	8.156	8.880	7.499[a]
LSD (p=0.05)	0.3993				0.1997
Average	5.267[d]	6.576[c]	7.486[b]	8.734[a]	Grand mean
LSD (p=0.05)	0.2306				7.016

Table 9. Cadmium content (ppm) of maize varieties under different cadmium levels

Variety	Cd concentrations (ppm)				Average
	Control	200	300	400	
5018	0.0	309.4	446.5	757.8	378.4[a]
Baghdad-3	0.1	250.4	379.9	565.4	298.9[b]
Fajr -1-	0.0	322.0	200.5	306.8	207.3[c]
LSD (p=0.05)	69.84				34.92
Average	0.0[d]	293.9[c]	342.3[b]	543.3[a]	Grand mean
LSD (p=0.05)	40.32				294.9

explained that plants build new proteins under stress, such as phytochelates and the metallicthionine also referred to the ability of heavy metals to induce plants to develop antioxidant enzymes that play an important role in reducing the toxicity of those minerals and/or preserving the stability of those minerals.

Cadmium content in shoot: The findings showed a variance in the ability of maize varieties to accumulate cadmium in their vegetative parts, as presented in (Table 9) and this depends on the ability of those varieties to transfer the element from the roots to the shoots. It is clearly infer that variety 5018 had highest ability (378.4ppm) to accumulate cadmium in the vegetative part, with a significant difference from the two other varieties, Baghdad-3 with 298.9 ppm and Fajr-1- with 207.3 ppm. The results also revealed that exposing corn plant to increased concentrations of cadmium led to a significant increase in the element's concentration in response to these concentrations, as the shoots content reached 293.9, 342.3 and 543.3 ppm under the influence of concentrations 200, 300 and 400 ppm, respectively compared to nil for the control. Kovačević (2019) mentioned both genotype and soil factors, especially soil pH, have considerable role in Cd uptake by plants, genotype affected the status of Cd in maize leaves (to an increased) relative to growing season and soil in the maturing process, The highest value of cadmium content (757.8 ppm) appeared at the concentration of 400 ppm for variety 5018, with a high significant difference compared to the control treatment. In contrast, the lowest cadmium content (200.5 ppm) appeared when treated Fajr-1- variety with 300 ppm Cd. Retamal-Salgado et al (2017) observed that Cd concentration was affected by environment and also by soil Cd application. Cannata et al (2013) revealed that plant Cd content elevated with increased doses of this element in root media. Plant uptake of Cd increased proportionally by soil Cd concentration.

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