

Study of the Physiological Behavior of Both Varieties of Bread Wheat (*Triticum aestivum* L) Subjected to a Metallic Stress of the Cadmium

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Abstract: The cadmium is one of the metallic element traces very toxic for vegetables, animals and human. Its presence in the soil is aggravated by the various cultural practices. This study is to test the effect of cadmium on the physiological parameters and its accumulation in two varieties of bread wheat (*Triticum aestivum* L). The relative growth of seedlings, the rate of chlorophyll a and b, the proline, soluble sugars and rate of accumulation of Cadmium are measured under four concentrations of Cd staggered from 0 to 150mg/l and tested on the varieties Arz and Hiddab. Results show an important reduction of the relative growth of the seedlings of both varieties but in a different way. A disturbance of the various physiological processes is observed a decrease of the photosynthesis gradually with the increase of the dose in cadmium on the chlorophyll a and b compared with the control, increase of the rates of the proline, the soluble sugars and the rate of cadmium in seedlings following the increase in concentration of the metal study. The Arz variety is more accumulating than Hiddab. The presence of the cadmium in the environment causes a physiological disturbance of the processes and affects dramatically the production of the biomass.

Keywords: Cadmium, Bread Wheat, the Photosynthesis, Process Physiological, Proline

1. Introduction

Metals and metalloids are, presents naturally in soil. The development of the industrial activity (metallurgy and chemical industries) and the agricultural activity (Pesticides, fertilizers) for the last century pulled sometimes intensive contributions of these elements which become pollutant for the environment. Even in low concentrations, they can establish a severe public health problem because of their toxicity and their organic character – accumulative [1]. Plants are exposed by two ways to heavy metals on one hand by the top parties and on the other hand by the roots. Heavy metals can be deposited on the surface of leaves and roots or penetrate into the plant. They can penetrate by the air parts there (leaves, stalks and fruits), from airborne particles, from gaseous compounds or from compounds dissolved in the water of rain or irrigation and by roots from the ground. Once taken by the plant, heavy metals can be trapped and not

circulate in the plant, or then transported from the site of the absorption towards another organ.

Many studies showed that the presence of metallic elements traces and particularly the presence of Cd in a crop can be translated, beyond a certain limit, by the appearance of symptoms of poisoning, accompanied with an inhibition of the growth of plants, with a reduction of the photosynthetic activity and with a decrease of the absorption of nutriment [2, 3]. Wheat is one the crop the most cultivated in Algeria and the most consumed by the population. The objective is not applied on the surface of the soil to increase the production, however with, the intensive use of fertilizers especially those of the bottom and particularly the phosphated fertilizer creates another problem which is the soil pollution by heavy metals particularly Cd; According to Szolnoki & al, (2013) [4] The contents of cadmium in fertilizers of phosphate extend between 2 and 200 mg / kg. It increases the exhibition of the plant to the stress and therefore endangers the human being

health [5]. The most common effect of cadmium consists of a reduction of the growth of the various parts of the plant. Depending on the importance of the stress, leaves can present a chlorose due a photosynthetic reduction in pigments therefore, such as the chlorophyll a and the chlorophyll b and the carotenoids, and leads to a physiological disturbance which is expressed by an oxidative stress. According to Poghosyan & al (2014) and Tauqeer & al (2016) [6, 7]. The present study is to investigate the effect of this toxic element on the physiological parameters and the accumulation of Cd in both varieties of bread wheat.

2. Materials and Methods

The experiment is done in the polycarbonate greenhouse in the laboratory of the biotechnology department of Blida 1 University, during year 2016. Two varieties of common wheat (*Triticumaestivum L*) ARZ and Hiddab widely used by our farmers are the object of this study. Seeds supplied by the ITGC (technical Institute of the field crops) are disinfected with 2% of sodium hydrochlorite during 10 minutes then rinsed with some distilled water to eliminate the fungal contaminations. They were seeded in some compost with alveoli and irrigated by solutions charged with cadmium; 10 repetitions for each treatment of chloride of cadmium 0, 50, 100, 150 mg / l were used. After one month of growth, we made the following measures:

2.1. Relative Dry Biomass of Roots and Leaves

The dry mass of the seedlings is measured with a precision balance after dehydration in a steam room at 70°C, during 72 hours, using the following formula:

$$\text{The relative growth} = (\text{Mass of the treated plants} / \text{Mass of controlled plants}) \times 100$$

2.2. Rate of the Photosynthesis

Chlorophyll a and b are determined by using the method of [8]. Which consists in making a maceration of 0.1g of fresh material of leaves in 10ml of a mixture of 75% of acetone and 25% of ethanol with a 80 and 40% concentration successively; 48 hours later we proceed to a reading at 645, 663 and 470 nm of the optical density by spectrophotometer.

The rate of chlorophyll is determined by the following formula:

$$\text{Chl a } (\mu\text{g/g MF}) = 12,7 \times \text{DO (663)} - 2,59 \times \text{DO (645)} \times V / (1000 \times W)$$

$$\text{Chl b } (\mu\text{g/g MF}) = 22,9 \times \text{DO (645)} - 4,68 \times \text{DO (663)} \times V / (1000 \times W)$$

V: Volume of the extracted solution and W the weight of the fresh material of the sample

2.3. Dosage of the Proline

The proline is measured according to the technique used by Monneveux & Nemmar, (1986) [9]. The principle is the quantification of the reaction proline-ninhydrine by

spectrophotometric measure. The proline is coupled with the ninhydrine by forming a colored complex. The intensity of the coloring is proportional to the quantity of proline in the sample. We proceed to the reading of the optical density of the samples with the spectrophotometer in the wavelength of 528 nm. The determination of the content of the proline is done according to the formula:

$$\text{Proline } (\mu\text{g/g MF}) = \text{DO528} \times 0.62$$

2.4. Dosage of the Soluble Sugars

Total soluble sugars (saccharose, glucose, fructose, divert them methyl and polysaccharides) are dosed by the method of Dubois and Gillet, (1965) [10]. It consists in taking a 100mg of plant material leaves in essay tubes; we added 3ml of ethanol at 80% in order to extractie sugars at a room temperature during 48 hours. At the time of dosage tubes are placed in the steam room at 80°C with a purpose to evaporate the alcohol. In cleaned glass tubes, we put 2ml of the solution to be analyzed; we added 1ml of phenol at 5% and 5 ml sulphuric acid concentrate at 96% while avoiding pouring some acid. We obtain an orange yellow solution at the top, and then with a weshaped whirlpool the solution is shacked in order to homogenize the color, then placed in the bath marry for 10 to 20mn at a temperature of 30°C. The determination of the content of soluble sugars is done according to the formula: Soluble Sugars ($\mu\text{g} / \text{g MF}$) = $\text{DO490} \times 1.657$.

2.5. Extraction and Dosage of the Cadmium

The mineralization took place under an extractor fan in a jar made with teflon, masses of 0.25g of every sample of dry material were weighed, by adding a volume of 7ml of nitric acid (HNO₃) at 69% and 1ml of hydrogen peroxide (H₂O₂) at 30%, Jars are hermetically closed with crowbars and left at the room temperature during one hour, afterward jars were placed in a steam room at temperature of 110°C during 120min. After cooling, samples were filtered in 50 ml phials, affermards, jars are rinsed several times with some distilled water. Then the phials are completed with distilled water up to their capacity mark and kept in a refrigerator at 4°C to avoid any contamination until we make the analysis by spectrophotometer of atomic absorption.

2.6. Statistical Analysis

The statistical analysis of the obtained results was calculated by the software SPSS © version 2000 for Windows™. The comparison between the averages of the various treatments also has been, established by an ANOVA followed by the test of Tukey for the comparison of the averages. This to be able to select the concentrations having a significant impact on the germination. In addition the correlation of Pearson between the parameters in the various concentrations in cadmium was also studied.

3. Results

The results of our study are represented in the table 1 which contains the averages obtained from the parameters studied under the influence of the concentration of the

cadmium, by quoting: the relative dry biomass, the chlorophyll a, b, the proline, the soluble sugars and the dosage of the cadmium which are illustrated by graphs and represented in figures 1-6.

Table 1. Averages of the biomass dry seedlings (PS of seedlings, relative growth (CR %), chlorophyll a and b, the proline, soluble sugars and rate of Cd.

Variety	Cd dose mg/L	PS des plantules (g)	CR (%)	Ch a (µg/g MF)	Ch b (µg/g MF)	Proline (µg/g MF)	Sucessolubles (µg/g MF)	Taux Cd (mg)
Arz	Témoin	0.201 ^b	100	18,960 ^a	13,700 ^a	0.050	0.985	0.024 ^j
	50	0.119 ^c	59,2	17,840 ^b	13,260 ^b	0.127 ^b	1.520 ^a	0.154 ^e
	100	0.056 ^c	27,86	14,940 ^d	10,570 ^d	0.111 ^c	1.570 ^a	0.231 ^b
	150	0.036 ^f	17,91	12,203 ^f	8,883	0.194 ^a	1.510 ^a	0.333 ^a
Hiddab	Témoin	0.271 ^a	100	18.433 ^a	12.970 ^b	0.034	0.921 ^d	0.025 ^j
	50	0.115 ^c	46.70	14.000 ^e	9.493 ^c	0.081 ^d	1.108 ^b	0.120 ^f
	100	0.106 ^d	39.11	16.240 ^c	11.710 ^c	0.079 ^e	1.009 ^c	0.170 ^d
	150	0.069 ^e	25.46	16.140 ^c	11.390 ^c	0.067 ^f	1.024 ^c	0.192 ^c

3.1. Relative Growth

The exposure of both varieties Arz and Hiddab to the various concentrations of Cd causes a decrease of the dry biomass of the seedlings; however the decrease is different for the two varieties. Hiddab was less affected when exposed to the lowest concentration of the environment in Cd (50 mg/l) (Figure 1a) and spreads out gradually with to the increase of the concentration in Cd until a weight of 0.036 g under the concentration of 150 mg/l of Cd.

On the other hand, the variety Hiddab registered an important reduction under the concentration 50mg / l of Cd then the decrease will be slow with the increase of the concentration of Cd in the environment (Figure 1b).

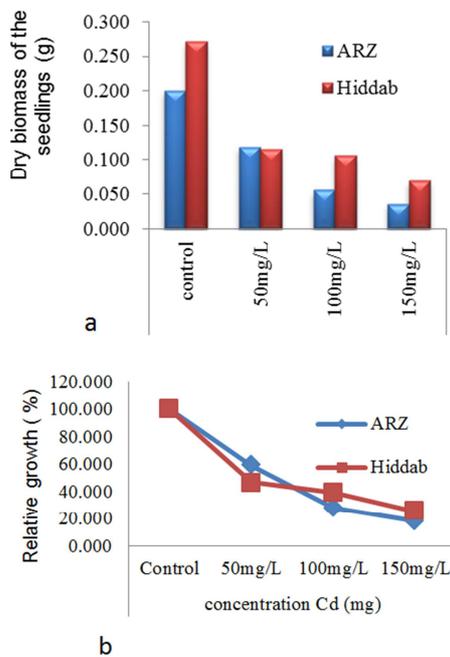


Figure 1. Effect of various concentrations of cadmium on the variation of the dry biomass of the seedlings of the studied varieties of bread wheat. Old seedlings 30 days were exposed to various concentrations of CdCl₂ for 30 days. The samples were taken from each plant. The values are averages from 10 repetitions. The bars of error correspond to the standard errors.

3.2. Chlorophyll a and b

The treatment by the cadmium causes a disturbance of the photosynthesis for both types of chlorophyll a and b (Figure 2) in both studied varieties Arz and Hiddab. The analysis of variance revealed a very highly significant difference of this rate under the influence of the increasing doses of cadmium.

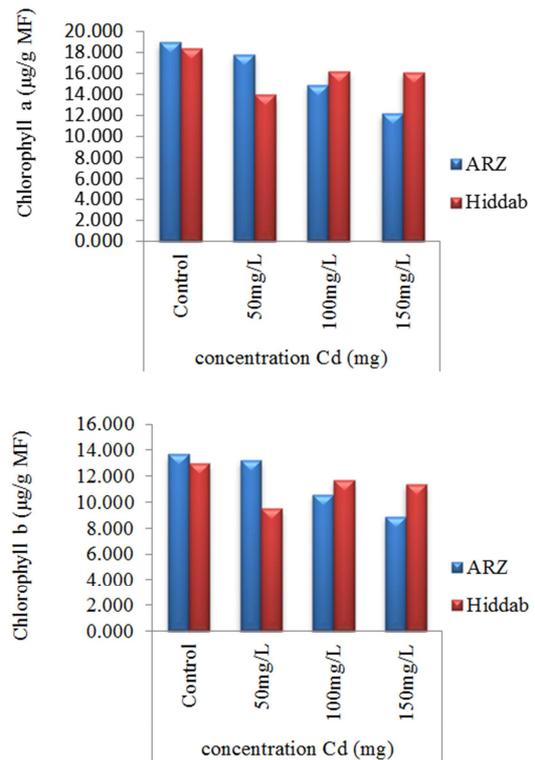


Figure 2. Effect of different concentrations of Cadmium on the assimilation of the chlorophyll a and b. The bars of error correspond to the standard errors.

However, the rate of the chlorophyll whether it is or b decrease, gradually according to the increase of the concentration of Cd in the medium for the variety Arz. In this case we registered a percentage of reduction more than 60%. On the other hand, the variety Hiddab behaves differently, we noted a decrease of the photosynthesis when we added Cd in

the middle then, the variety took back its photosynthetic activity and kept this stability by increasing the concentration of Cd in the medium with rates of (16,24; 11,71) and (16,14; 11,39) ($\mu\text{g} / \text{g MF}$) of chlorophyll a and b under the concentrations of 100 and 150 mg / l successively (Figure 2).

3.3. Proline

The proline is an amino acid often considered as a bio marker of stress, indeed; its dosage is to estimate the state of stress of the plant. The analysis of the variance reveals a very highly significant difference of the effect of Cd on the content of the proline (Figure 3).

The proline content increases when we added Cd in the environment with low concentration (50 mg/g) for varieties, however the variety Arz shows a disturbance following the increase of Cd in the environment (0.127, 0.111 and 0.194 $\mu\text{g/g MF}$) under the concentrations 50,100 and 150 mg/g successively. Contrary Hiddab shows a certain stability of synthesis of the proline (0.081, 0.079 and 0.067 $\mu\text{g} / \text{g MF}$) under the concentrations 50, 100 and 150 mg/l of Cd.

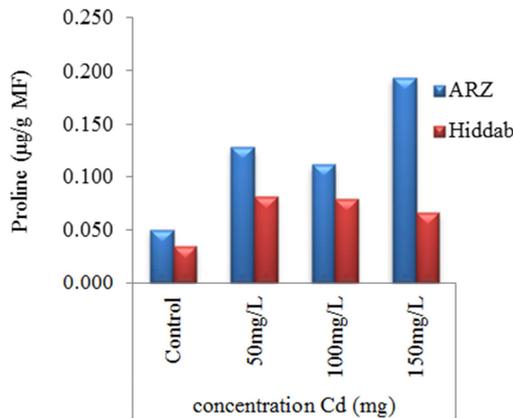


Figure 3. Effect of various concentrations of cadmium on the proline of the studied varieties of common wheat. The bars of error correspond to the standard errors.

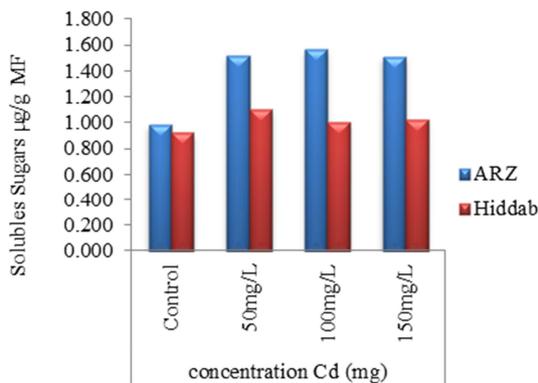


Figure 4. Effect of various concentrations of cadmium on soluble sugars of the studied varieties of bread wheat. The bars of error correspond to the standard errors.

3.4. Soluble Sugars

The effect of the cadmium on the contents of soluble

sugars is illustrated in the figure. A very highly significant difference is recorded for the effect of Cd on soluble sugars, the content of the latter increases in the presence of Cd in the environment compared to the control where Cd was not added. Both subdued varieties represent the same shape for the contents in soluble sugars; however the contents registered by the variety Arz are higher than report those recorded by Hiddab (Figure 4).

3.5. Rate of Cd

A considerable variation of the concentration of Cd is registered for both varieties subjected to the various concentrations of the medium in Cd, indeed, the analysis of the variance shows a very highly significant difference ($p=0.000$).

The concentrations of Cd in the parts of the plants above the gown of the studied varieties increase according to the increase of the concentration of Cd in the middle, however the varieties do not accumulate Cd at the same degree. Arz seems more accumulative than Hiddab with concentrations of 50, 100 and 150 mg / l successively.

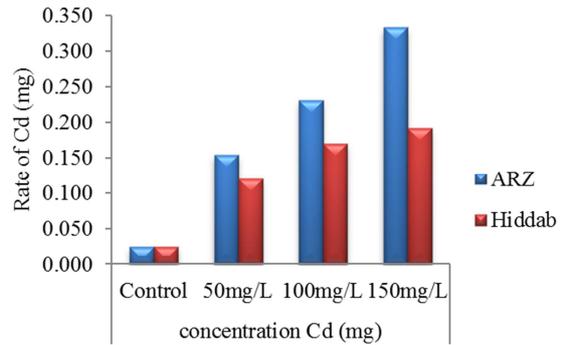


Figure 5. Effect of various concentrations of cadmium on its accumulation by the studied varieties of common wheat. The bars of error correspond to the standard errors.

3.6. Correlation

The test of correlation of the relative growth according to the rate of Cd is inversely proportional with one $r=0.973$ and $R^2=0.846$ (Figure 6). Indeed the growth of the seedlings of both varieties subjected to Cd stress decreases strongly with the accumulation of Cd in seedling

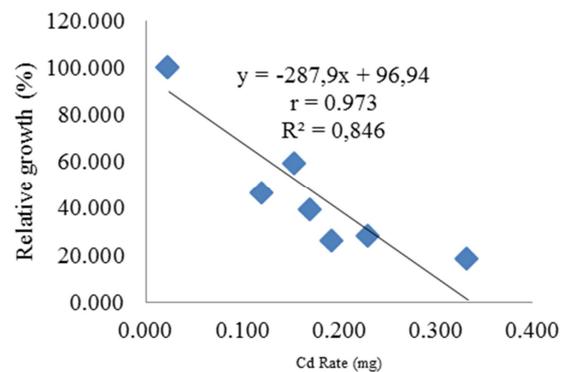


Figure 6. Correlation of the relative growth and the rate of Cd in the air parts of varied studied.

4. Discussion

Disturbance of the photosynthetic process, the reduction of the production of the dry vegetative biomass, the induction of the synthesis of bios markers and the accumulation of the agent causing stress in the plant are the results of the presence of the cadmium in the medium of culture.

An important reduction of the vegetative biomass of both studied varieties following the increase of Cd in the medium is registered, the results found are similar to those several in studies on the effect of heavy metals on plants generally [11] and the effect of Cd on the wheat in particular [12-14]. However, the decrease is different in both varieties, the growth of the variety of which hiddab is more affected than the Arz variety due to the trek genetically explain [15].

We note a reduction of the photosynthesis expressed by the decrease of rate of chlorophyll following the increase of the concentration of cadmium as it shows several studies such as those of Moustakas and al, (1997), Singh and al, (2004) [16, 17]. Other more thorough studies were led to know the targets of actions of heavy metals in the biosynthetic way. According to Oncel and al (2000)[18], the enzymes which return in set in the presence of cadmium could prevent the formation of the complex photoasstphotochlorophyllide-NADPH-enzyme by inhibiting the protochlorophyllidéréductase enzyme or still-aminolévaliniquedés-hydrogénase and it further to the interaction of the Cadmium to the thiols groupings (SH) of enzymes.

Our results are in agreement with those found by Mobin and Khan, (2007), Ebbs and Uchil, (2008) and Ekmekci and al, (2008) [19-21], whose showed that the reduction of the photosynthesis leads automatically to a reduction in the growth of the plant.

As reported by studies done by Mujahid and al, (2013), Deyala (2016) [22, 23]; the presence of Cd in the environment leads to the synthesis of molecules anti oxidizing. In our research we were interested to study the variation of the rates of proline and the soluble sugars in both varieties subjected to various concentrations of Cd in the medium. A considerable disturbance was registered expressed by the gradual accumulation of these two molecules in tissues of seedlings [24], this accumulation results from a change of the enzymatic activities, in particular the acid invertase, saccharose synthase and the starch phosphorylase. However the rates were different in both varieties.

The assimilation of cadmium in the wheat varies with the type of soil, the atmospheric pollution and cultivated varieties of wheat [25, 26].

Our study was based on the factor variety. A remarkable difference of the accumulation of Cd is registered between both studied varieties; these results are similar to those found by Arduini and al, (2014), Ehab and al, (2013) [27, 28]. According to Adeniji and al (2010), Ci and al, (2010a) [29, 12] a higher concentration of Cd is accumulated in roots and less is transferred to stalks according to the wheat varieties grown.

5. Conclusion

In the present work, we studied the physiological behavior

of two varieties of bread wheat Arz and Hiddab widely cultivated in Algeria under a range of concentration of cadmium on the physiological parameters and its accumulation in two varieties of bread wheat (*Triticum aestivum* L). This heavy metal is very toxic for the human being and known very to be common on these.

Our results shows that it presence in the environment leads to considerable disturbance of the physiology of both subdued varieties expressed by the decrease of the rates of chlorophyll a and b and therefore influences the production of the dry biomass of seedlings, and so active the synthesis of molecules occurring at the state of stress. However, the severity of this stress is different between both varieties; however Hiddab is shows to be more tolerant than Arz.

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