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# Effect of Mg,Cu,Cd and Mg/Cd, Cu/Cd on stress biomarkers in durum wheat.

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## Abstract

This work aims to evaluate the effects of few trace metals (Cd,Cu) and a major element (Mg) and their interactions (Cd/Cu and Cd/Mg) on the growth rate of durum wheat roots (Var. *Simeto*) and stress biomarkers: glutathione and catalase activity (CAT) in the roots and leaves of *durum* wheat. A treatment with concentrations (5,20,50µM) of Cu and Mg combined or not to (100 µM) of Cd is applied. The results show an increase growth rate for all Mg concentrations whereas copper has stimulating at low concentrations and inhibiting at high concentrations. As for combinations Mg/Cd and Cu/Cd, inhibit root growth except at [100 µM] of Cd stimulating it during the first 96 hours. However GSH is stimulated at low [Mg/Cd] and increasing concentrations of Cu except the lowest dose where no significant increase is observed. GSH levels are stimulated at low concentrations of Cu/Cd and 100µM of Cd in the roots compared to controls. In addition, increasing concentrations of Mg increase the production of GSH, So that an inhibition of GSH for combination treatment Mg/Cd. should be noted that Cu has no effect on GSH except at (50 /100µM). Meanwhile, the catalase activity is stimulated to the different concentrations of Mg and Mg/Cd in the plants. Unlike increasing Cu concentrations where there is a significant decrease in this activity. Similarly, the combined treatment (Cu/Cd) inhibits catalase activity [ 50/100 µM ] in roots and leaves. In the end, Cd induces catalase activity in leaves and inhibits in the roots.

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## Introduction

Since the beginning of the industrial revolution, pollution of the environment, including soil, accelerated dramatically (Yanai *et al.,* 2006). The unregulated industrial waste represent an important contribution in trace elements in the environment (air, water or land) that contribute to anthropogenic pollution of the biosphere. The enrichment xenobiotic compounds is accentuated by various transformation process including non-ferrous metal production (As, Cd, Cu, Zn), the combustion of coal (Ni, Pb), agricultural practices (As, Cd,Pb) and road transport (Pb, Mn) ( Kabata - Pendias, 2011; Grant, 2008). The major elements (O, Si, Al, Fe, Ca, Na, K, Mg, Ti and P) alone represent 99 % of the composition of the Earth's crust. Some of these trace elements are essential micronutrients for many living organisms (plants, animals and microorganisms), among which are copper (Cu), nickel (Ni) and zinc (Zn). Others occupy no physiological function in living organisms and are considered toxic non-essential elements such as arsenic (As), mercury (Hg), lead (Pb), cadmium (Cd) and antimony (Sb) (Chiffoleau *et al.,* 2001; Kabata - Pendias and Pendias 2001).

In plants, if the metals are often essential to the conduct of biological processes (trace elements), many of them may be contaminants for various forms of life, when their concentration exceeds a threshold, itself a function of ' physicochemical state (speciation) of the element. This is the case of iron (Fe), copper (Cu) , zinc ( Zn), nickel (Ni), cobalt (Co), vanadium (V), selenium (Se), molybdenum (Mo), manganese (Mn), chromium (Cr), arsenic (As) and titanium (Ti) ( Miquel, 2001).Some of these metals are also involved in the molecular processes such as control of gene expression, protein biosynthesis, nucleic acids, growth substances, chlorophyll, secondary metabolites, lipid metabolism or tolerance stress (Rengel, 1999). For example, copper is used in the reactions of photosynthesis and during breathing (Yruela, 2009; Hopkins, 2003).

Cadmium (Cd) is a relatively rare heavy metal in the ecosystem (McBride,1995) and particularly toxic to humans (Moulis *et al.,* 2014).

Several studies have shown that the presence of trace metal elements, and more particularly Cd in the culture medium can result, beyond a certain limit by the appearance of signs of toxicity, accompanied by an inhibition of weight growth of plants, reduction of photosynthetic activity and a decrease in the absorption of nutrients (Zhou and Qiu, 2005; Clemens, 2006; Verbruggen *et al.,* 2009 and DalCorso *et al.,* 2013). Using approaches ' omics ' also allowed to reveal or confirm the cellular and molecular mechanisms disturbed by trace metals and particularly Cd (Herbette *et al.,* 2006; Le Lay *et al.,* 2006; Sarry *et al.,* 2006; Villiers *et al.,* 2011; Doustaly *et al.,* 2014 Ovecka and Takac, 2014).

The objective of our work is to study the impact of cadmium associated or not with magnesium and / or copper on the growth rate of wheat roots. In addition, we analyzed the impact of this pollution on the defense system "antioxidant" of the plant measuring some enzyme activities which play a major role in this defense.

## Materials and methods

*Culture Conditions*

Durum wheat seeds (*Triticum durum* Desf. Var. *Simeto*) were sterilized with 10% (v/v) hydrogen peroxide ($H_2O_2$) at 10% (v/v) for 15min, and thoroughly washed with distilled water. Seeds germinated during 96H in darkness at 23 ± 1°C. A solution of $MgSO_4$, $CuSO_4$ (5,20,50µM) combinate or not at 100µM of Cd was added at culture medium. After, the seeds are planted in 6cm in diameter plastic pots and 9cm in height, containing disinfected soil and gravel with the following proportions: 3: 1/3 then seeded number 15 seeds per pot with three repetitions by treatment. The seedling depth is 1 cm. Watering is done two (02) times a week with the nutrient solution Hoshang added or not to the two types of treatment.

*Analytical Techniques*

*Determining the root growth rate*

The average root length is measured during 96H (cm/d).

*Determination of Biomarkers*

*Determination of Glutathione*

(GSH) The enzyme extract is homogenised in a solution of 0.02M E.D.T.A and undergoes deproteinization by the sulfo - salicylic acid 0.25%. After centrifugation at 2000 g for 10 minutes the supernatant is used for the spectrophotometric assay with DTNB reagent to 0.01M to 412 nm. Glutathione levels were measured with the method of (Weckbecker and Cory, 1988) and expressed in µM/mg protein.

*Determination of catalase activity (CAT)*

*Preparation of the enzyme extract*

The enzyme extract of the roots of wheat treated with different metals used Loggini *et al.,* (1999) method. After 07 days of treatment, the fresh root (1g) are ground cold with a mortar in 5 ml of phosphate buffer (50 mM phosphate, pH = 7.5). The homogenate is then filtered using an adequate before centrifugation at 12000g for 20min in cold (Sigma centrifuge 3-16K). The supernatant obtained is used as the extract for the determination of different enzyme activities.

*The spectrophotometric assay of catalase activity (CAT)*

It is performed according to the method of Cakmak and Horst, 1991.The decrease of absorbance is recorded for three minutes (spectrophotometer Jenway 6300) for a 240 nm wavelength and an extinction coefficient ε = molar linear 39400 M- 1.cm - 1.To the final volume of 3ml, the reaction mixture contains: 100 µl of the enzyme extract, 50µl of hydrogen peroxide $H_2O_2$ 0.3% and 2850µl of phosphate buffer (50mM, pH = 7.2). Device Calibration is performed in the absence of the enzymatic extract. The reaction is initiated by the addition of hydrogen peroxide. Catalase activity was expressed in nmol/min/ mg protein.

*Statistical analysis*

All results are given as mean values ± SD. To assess the significance of differences observed in metal accumulation or in biomarker responses, we used a one-way analysis of variance analysis (ANOVA).

**Results**

*Effect of Mg,Cu,Cd and Mg/Cd, Cu/Cd on growth rate of durum wheat roots*

The measurement of the root length of wheat allowed us to calculate during 4 days. The results for different treatments (Mg,Cu,Cd, Mg/Cd and Cu/Cd) are reported in Table 1.

**Table 1.** Effect of Mg,Cu,Cd and Mg/Cd, Cu/Cd on growth rate of durum wheat roots (cm/d).

| Metals [µM] | Time (H) | Growth rate (cm/d) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Control | Cd | Mg | Mg/Cd | Cu | Cu/Cd |
| 0 | 48 | 0,00 | | | | | |
| | 72 | 1,05 | | | | | |
| | 96 | 1,22 | | | | | |
| | V($d_3$-$d_2$) | 0,17 | | | | | |
| 5 | 48 | | | 0,84 | 0,5 | 0,6 | 0,7 |
| | 72 | | | 1,22 | 0,79 | 0,81 | 1,05 |
| | 96 | | | 1,31 | 0,92 | 1,15 | 1,34 |
| | V($d_3$-$d_2$) | | | 0,09 | 0,13 | 0,34 | 0,29 |
| 20 | 48 | | | 0,6 | 0,5 | 0,63 | 0,73 |
| | 72 | | | 1,1 | 1,21 | 0,81 | 0,97 |
| | 96 | | | 1,16 | 0,88 | 0,63 | 1,32 |
| | V($d_3$-$d_2$) | | | 0,06 | -0,33 | -0,18 | 0,35 |
| 50 | 48 | | | 0,6 | 0,56 | 0,57 | 0,33 |
| | 72 | | | 1,43 | 1,1 | 0,77 | 1,19 |
| | 96 | | | 0,98 | 0,79 | 0,84 | 0,91 |
| | V($d_3$-$d_2$) | | | -0,45 | -0,31 | 0,07 | -0,28 |
| 100 | 48 | | 0,00 | | | | |
| | 72 | | 0,69 | | | | |
| | 96 | | 0,77 | | | | |
| | V($d_3$-$d_2$) | | 0,08 | | | | |

($d_3$= third day, $d_2$=second day).V= Growth rate.

Table 1 shows an increase in average root length as a function of time, this increase is very significant for (5,20µM) of Mg which is reached average growth rate (0.09; 0.06 cm/d) simultaneously, the high concentration of Mg (50 µM) slightly slows growth rate which is estimated to 0,45cm/d. Copper treatment stimulates the growth at low concentration (5 µM) as function of time, however a concentrations of 20 and 50µM Cu slow the speed of root growth which is equivalent to 0.18 and 0,77cm/d respectively. In parallel, the combined treatment Cu/Cd, slows the growth rate of an average of 0,28 cm/d at 50/100µM. The same is observed for combination treatment (Mg/Cd) at 20, 50 µM of Mg equivalent to 0.31 and 0,33 cm/d respectively. Finally treatment with (100 µM) Cd stimulates the root growth rate on average of 0,08 cm/d during the first 96h.

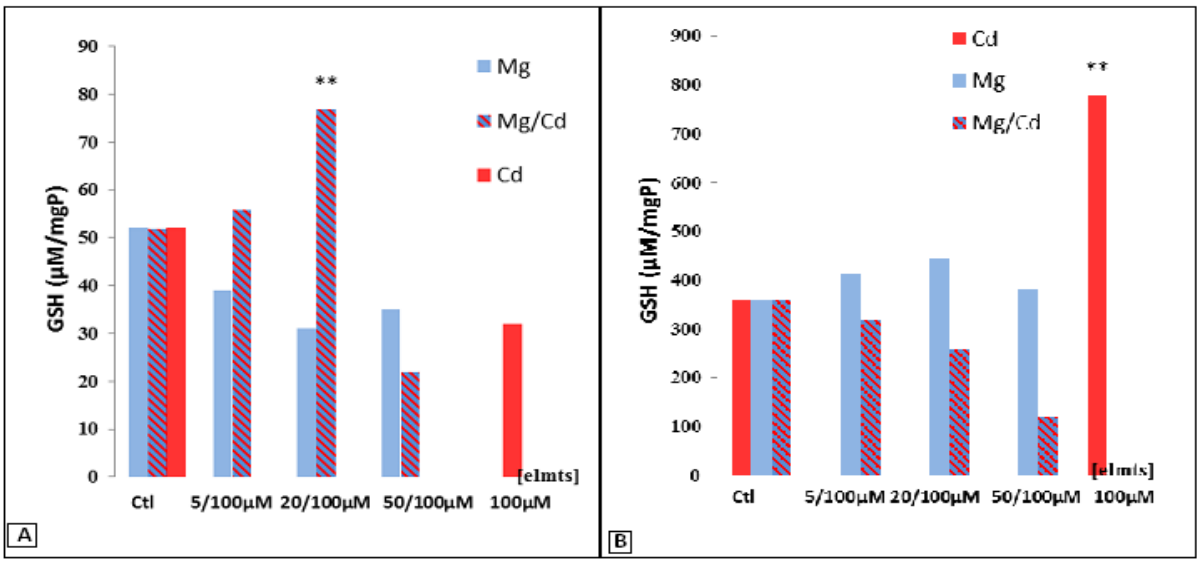


**Fig. 1.** Effect of Cd, Mg and Mg/Cd on the GSH in roots (A) and leaves (B) of durum wheat. * P< 0.05; *** P < 0.001 vs control Elmts: elements, Ctl: Control.

*Effect of Mg,Cu,Cd and Mg/Cd, Cu/Cd on stress biomarkers in the leaves and roots of durum wheat.*

*Glutathione*

*Effect of Mg and Mg/Cd on the GSH*

The results obtained are Fig1 which represent the variation of the GSH levels according to different concentrations of Cd, Mg and Mg/Cd in the roots (A) and leaves (B) of wheat after 7days of treatment. It shows that GSH not affected when increasing concentrations of Mg added in the culture medium (< to control) in wheat roots. However the combined treatment (Mg/Cd) stimulates the synthesis of GSH at (5/100, 20/100µM) for successively reach values of 56 and 77µg/mgp compared to control while the concentration (50/100µg/ mgP) inhibits production of GHS (<control).Similarly, treatment with (100µM) of $CdCl_2$ decreases the production of GSH (32µM/mgp) remains lower than the control (32µM/mgP).

We show that a synthesis of GSH in leaves with the different concentrations of Magnesium. However the combined treatment Mg/Cd inhibits its production (fig.1B) witch the greatest value is (465µM/mgP) reached at 50µM of Mg. Finally treatment with 100µM of Cd stimulates significantly the GSH levels and reaches the maximum value of 779µM/ mgP.

*Effect of Cu and Cu/Cd on GSH levels*

The Fig. 2A represents a GSH levels in wheat roots treated with concentrations of Cd, Cu and Cu/Cd. It is show that at (20, 50µM) of Cu a GSH levels decrease (< control) except for 5µM of $CuSO_4$, where it reached 61µg/mgP. The combined treatment Cu/Cd stimulates significantly the synthesis of GSH in wheat roots at 5/100µM, 20/ 100µM concentrations which reached 106µM and 288µM/mgP compared to the control.

In the fig. 2B, the leaves wheat GSH levels is not affected by different concentrations of Cu and Cu/Cd (<control), except at (50/100µM) where is significantly increase the GSH which is reached (524µg/mgP) compared to control (361µg/mgP). However, the treatment of 100µM of Cd increases significantly the GSH levels which are superior of Cu and Cu/Cd. However, a treatment with 100µM of Cd inhibits the production of wheat roots GSH.

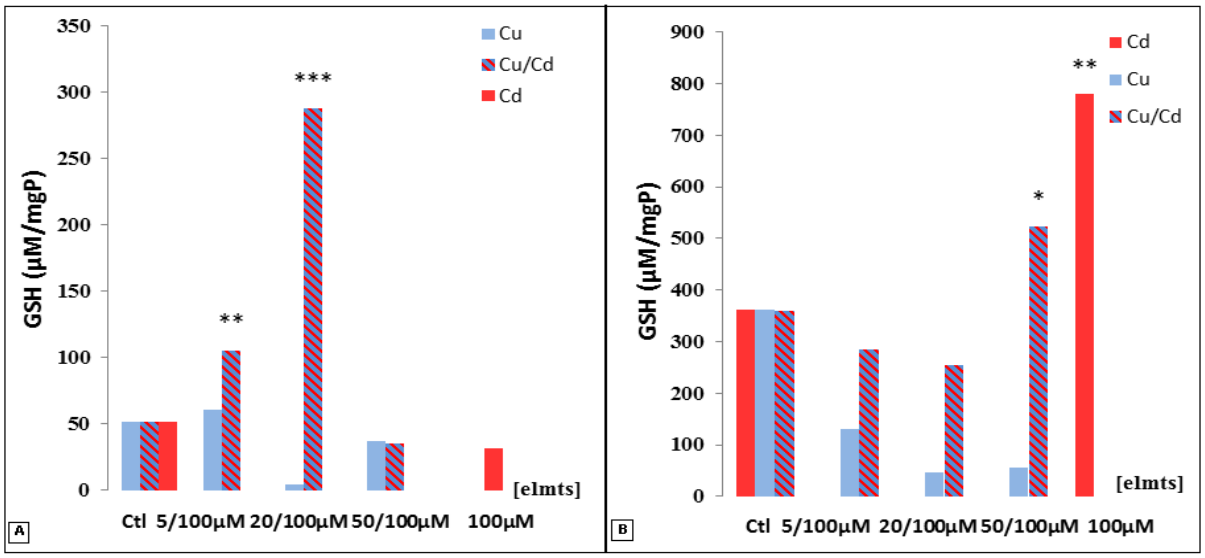


**Fig. 2.** Effect of Cd,Cu and Cu/Cd on GSH in the roots (A) and leaves (B) of durum wheat. * (P< 0.05); *** (P < 0.001) vs control. Ctl: control, elmts: elements.

*Effect of Cd on the content of GSH*

The addition of 100µM of Cd in the culture medium decreases not significantly wheat roots GSH levels from 52 to 32 µM/mgP. However, it is increases significantly in leaves from 361 to 779µM/mgP (fig.3).

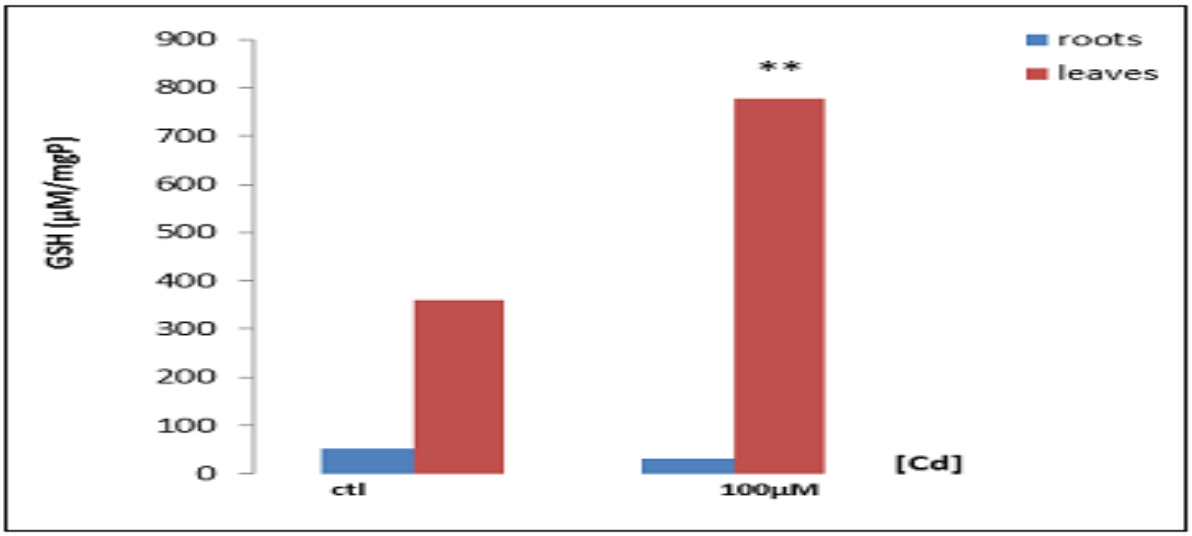


**Fig. 3.** Effect of Cd on GSH in durum wheat roots and leaves. Ctl: Control, * (P< 0.05); *** (P < 0.001) vs control.

*Catalase activity*

*Effect of Mg and Mg/Cd on CAT activity*

We observed in fig.4A which represents the effect of Cd, Mg and Mg/Cd on wheat roots catalase activity. A slightly stimulation of this activity at different concentrations of Mg, Mg/Cd is observed which reached $59.10^{-5}$nmole/mgP at 50µM of Mg: $51.10^{-5}$nmole/µgP at 50/100µM of Mg/Cd.

The treatment with 100µM of Cd shows not significantly decrease catalase activity which reaches 2. $10^{-4}$nmole/mn /mgP compared to control (245.$10^{-6}$nmole/mn/mgP).

A leaves wheat CAT activity is represented in fig. 4 B. We show that a not significant decrease of catalase activity with different concentrations of Mg; except at 5µM (> control). However, the combined treatment Mg/Cd stimulates very significantly catalase activity at 20/100µM: (17.$10^{-4}$ nmol/mgP).

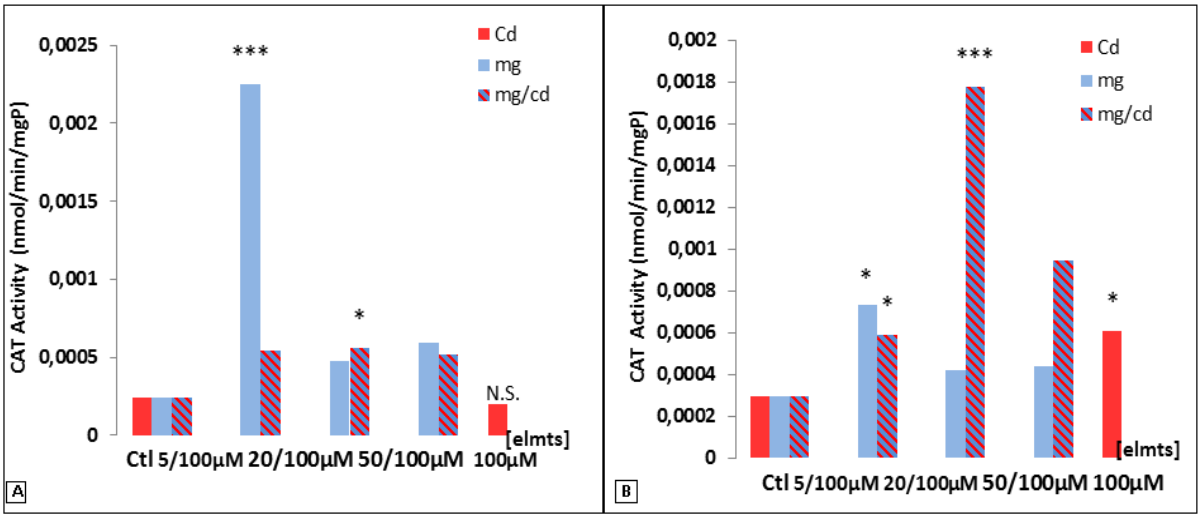


**Fig. 4.** Effect of Cd,Mg and Mg/Cd on the durum wheat roots ( A) and leaves ( B) of CAT activity.
Ctl: Control, Elmts: elements, N.S.: not significant. * (P< 0.05); *** (P < 0.001) vs control.

*Effect of Cu and Cu/Cd on CAT activity*

A significantly decrease of root wheat catalase activity showed with different concentration of Cu (fig.5 A). However, the combined treatment (Cu/Cd) shows an increase of catalase activity (77.$10^{-5}$ nmol/mn/mgP) at 5/100µM: (11.$10^{-4}$ nmol/mn/mgP) and at 20/100µM except of highest concentrations of Cu/Cd where is inhibits them (≤ control)

By catalase activity against the leaves wheat (Fig.5B) treated with different concentrations of Cd, Cu and Cu/Cd significantly decreases but remains higher than the controls. The highest activities were observed at lower concentrations (5µM : 9.$10^{-4}$nmole/nm/mgP and at 5/100µM: 13.$10^{-4}$ nmole/mn/mgp) and for both treatments (Cu and Cu/Cd).

*Effect of Cd on CAT activity*

The effect of 100µM of cadmium on the wheat roots catalase activity (Fig.6) shows a decrease of catalase activity (2.$10^{-4}$nmol/mn/mgP) compared to the control (24.$10^{-5}$nmol/mn/mgP).

Catalase activity in wheat leaves treated with 100µM of Cd is significantly stimulated twice (6.$10^{-4}$ nmol/mn/mgP) more than the control (29.$10^{-5}$ nmol/mn/mgP).

**Discussion**

We are interested, in this work at the biological response of our plant model durum wheat (*Triticum durum* Desf.) against a heavy metal "cadmium ". Firstly, known for its influence on morpho metric parameters, physiological and biochemical, and certain nutrients other, essential to the functioning of certain life cycles of the plant, associated or not to the presence of cadmium chloride in the culture medium.

The first parameter measured in our work is the average root growth which is calculated from the average root length. The results obtained after applying the treatment of "Cd" show an increase in average root length and therefore their growth rate. Several studies have shown that the presence of cadmium in the culture medium can result, beyond a certain limit by the appearance of symptoms of intoxication accompanied by an inhibition of growth in weight of plants (Ouariti *et al.,* 1997; Djebali *et al.,* 2002; Ghnaya *et al.,* 2005; Zorrig *et al.,* 2010).

In our study the presence of Cd improves slightly the root length as a function of time. This could be explained by the fact that the treatment at an early stage does not affect negatively on the fitness of the plant. It is important to note that the treatment (100µM Cd) in wheat seedlings does not reflect those in contact with the roots due to their soil dissipation.

Wheat seedlings employ the compounds available at their roots, the dissipation concentration of cadmium in soil that a small amount is brought into contact with plant roots. This small amount will slightly affect seedling growth (Sbartai *et al.,* 2008). Similarly, concentrations of 5 and 20 µM of Mg and 5 µM of Cu stimulate root growth since these Mg and Cu nutrients are essential to the growth and development of the plant.

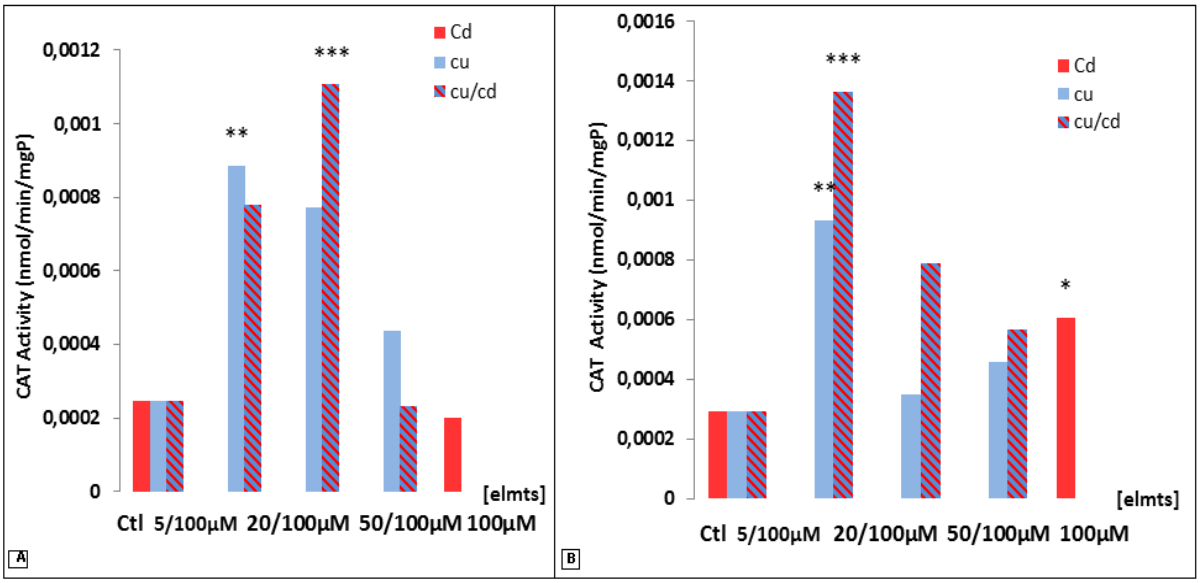


**Fig. 5.** Effect of Cd,Cu and Cu/Cd on *Durum* wheat roots (A) and leaves (B) of CAT activity.
Ctl: Control, elmts: elements, * (P< 0.05); *** (P < 0.001) vs control.

Beyond a certain level these elements become toxic and inhibit the root growth. Indeed, Cu concentrations (20 and 50µM) and Mg (50µM) inhibit root growth. Our results are similar to those obtained by Cuypers (2000) which shows that plants initially focus Cu in the roots and it is the organs that undergo first impacts of Cu and which are the most affected (Paschke and Redente 2002).

Decreased root growth has the consequence that the sampling surface of the plant and therefore a reduction of the sample in water and nutrients (Brown *et al.,* 2003). When the copper is in excess in the root cells the concentration of $H_2O_2$ and the quantities of peroxidases involved in the synthesis of lignin increases (Lin *et al.,* 2005) this increase may cause a decrease in accessibility of cells to Cu (Cuypers , 2000, Dos Santos *et al.,* 2004).

In parallel, the combined treatment inhibits root growth. Knowing that the Mg distributes metabolites (exp : Acid amine and carbohydrates), the formation of certain metabolites is limited by the presence of several items at once that comes into competition such as carbohydrates and causing a decrease in root growth and increases the risk of deficiency of nutrients and environmental stress (Cakmak and Kirkby 2008).

Regarding the effects of Cd, Mg and Mg/Cd combination on stress biomarkers in leaves and roots of durum wheat, the results show that simple treatments (Mg and Cd) inhibit GSH levels in wheat roots then this same rate is stimulated in leaves. In contrast, the combined treatment Mg/Cd stimulates the GSH levels in roots and inhibits in the leaves. In general,

the presence of cadmium stimulates the GSH in the roots treated with cadmium suggesting the involvement of this element in the detoxification of reactive oxygen species generated by the Cd (Asada and Takahashi, 1987). Our results are in agreement with those of Zhu *et al .,* 1999; Cao *et al .,* 2004 and Freeman *et al .,* 2004 and Sbartai *et al.,* 2012 observed that the level of GSH is enhanced with increasing the tolerance to the accumulation of Cd for low concentrations, along with those observed by Gallego *et al.,* 1996; Nagalakshmi and Prasad,2001; Ducruix *et al.,* 2006 Sbartai *et al.,* 2012 where the level of GSH decreases in response to stress induced by high concentrations of Cd.

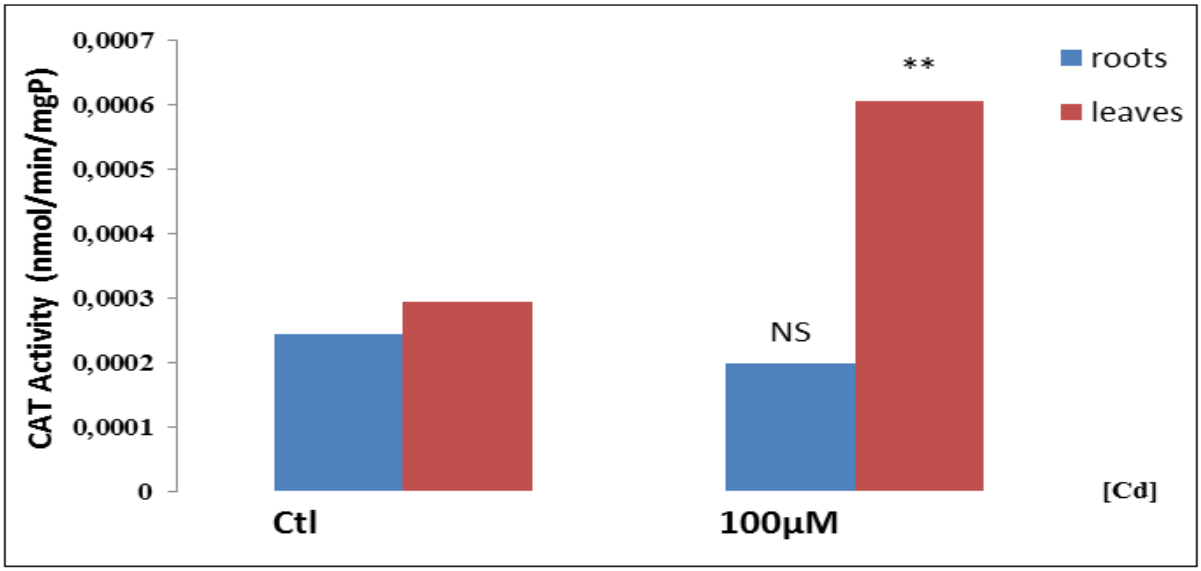


**Fig. 6.** Effect of Cd on the CAT activity in the roots and leaves of durum wheat. * (P< 0.05); *** (P < 0.001) vs control. Ctl : control. N.S.: Not significant.

The presence of magnesium in the roots is not seeking the intervention of GSH that Mg is an essential element for the growth and development of the plant and its mobility in the phloem ( White and Broadley, 2008) depends on several factors (Mikkelsen, 2010). In the plant, except 20% of total Mg is used for the synthesis of chlorophylls while 80% is in various mobile platforms (Marschner, 2012).

However its massive translocation to the leaves is explained by Gransee and Fuhrs (2013), knowing that Mg is the central atom of the chlorophyll molecule and thus the photosynthetic catalyst, induces the production of GSH which increases in response to increasing concentrations of Mg thus proving the existence of oxidative stress knowing that GSH is a prominent and highly reactive thiol cell non-enzymatic scavenging systems belonging to the first antioxidant defense line which maintains the intracellular redox balance and the non-enzymatic antioxidants in their biologically active form reduced. In contrast, the combination treatment stimulates the production of GSH for the first two levels in roots and inhibits the highest concentration while it is inhibited that some or the applied concentration in leaves thus proving the drastic effect of Cd associate Mg.

The single treatment with Cu completely inhibits glutathione in roots and leaves proving that the system in question is outdated and replaced by a more powerful antioxidant defense system (enzyme) in response to oxidative stress caused by increasing concentrations of Cu. The same is observed for combination treatment in leaves. However, in the roots combined treatment stimulates the production of GSH. Indeed the presence of excess copper in the cells actually increased the concentration of $H_2O_2$ (Lin *et al.,* 2005) which can induce oxidative stress.

In addition, the Cu disturbs indirectly the homeostasis of other elements by changing the permeability and membrane integrity and directly modifying the plant requirements or competes with other elements (Cook *et al.,* 1997, Cuypers 2000).

Indeed, the evaluation of oxidative stress is generally performed by changes in the levels of enzyme activity (CAT, SOD, etc ...) involved in the antioxidant defense system (Torres *et al.,* 2008). CAT is the first antioxidant defense line; it catalyzes the dismutation of hydrogen peroxide into water and oxygen. In our work, the measure of the CAT enzymatic activity involved in detoxification shows that treatment with cadmium inhibits this activity in the roots while an induction was observed in the leaves.

This could be explained by the fact that in these conditions of stress there is outbreak of detoxification systems which mostly consist of enzymes catalase. The induction of this activity following the installation of oxidative stress cause by the presence of Cd shows his role in the removal of oxygenated water ($H_2O_2$) formed following Cd accumulation in roots wheat. These observations highlight a phenomenon of tolerance which would be responsible for the adaptation of plants to xenobiotics (Cho *et al.,* 2000; Souguir, 2009, Sbartai *et al.,* 2012).

In parallel treatment with Mg and the combination Mg/Cd stimulates dose-dependent manner catalase activity in roots proving triggering the plant defense system think back to oxidative stress induced by increasing concentrations of Mg. However treatment with copper inhibits the activity in the roots and leaves of wheat proving its high toxicity to the cell that is raised by the presence of the Cd that comes into competition thus preventing penetration and translocation of Cd to leaves.

**Conclusion**

In conclusion, all the results obtained in our work compared to those reported in the literature allowed us assumed some hypotheses explaining the phenomenon of pollution.

We noticed that when the wheat seedlings were submitted to chemical stress in the presence of essential elements, the rate growth, GSH and CAT activity appear to be sensitive and rapidly respond to the presence of the pollutant.

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