



Morpho-physiological Characteristics of *Brassica napus* L. Under Cadmium Stress

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Received: 4 August 2015

Accepted: 19 January 2016

Abstract

Cadmium as a heavy metal is also considered as a serious threat for the environment. This element is accumulated in soil through industrial processes as well as phosphate fertilizers and aggregates into the food chain. This study was conducted in order to investigate the different levels of cadmium effects on growth features of rapeseed (*Brassica napus* L.) plants. Root and shoot weight and height, contents of chlorophyll a and b, total chlorophyll, carotenoid and peroxidase enzyme activity were measured after one month of planting. Generally, an increase in cadmium concentration showed a significant effect on morphological characteristics of root and shoot but revealed no significant effect on chlorophyll a and b as well as on carotenoid contents, though it caused a significant decline in peroxidase activity level specially for concentrations above 90 μM of cadmium treatment.

Key words: *Brassica napus* L.; cadmium; chlorophyll; heavy metals; peroxidase activity

Introduction

Human hygienic status depends directly upon foods on which he lies and the environment in which he lives. Plants stay on the focus of general attentions because of their richness in nutritional elements and vitamins (Omale and Emmanuel, 2011). *Brassica napus* L. (canola or rapeseed) is regarded as an annual herb belonging to *Brassicaceae* family (Ghahraman, 1993).

Currently, canola as one of the major oil seeds is classified into protein rich plants growing in moderate cold and moist cold region, in which most of other oil seed plants cannot tolerate. Canola is also capable of producing substantial protein and oil content per area unit even in semi-temperate regions (Shahidi and Sepehr, 2002).

One of the major causes that put heavy metals among dangerous chemical materials is their ability to biologically over-accumulate into live organism's body and to be increased continuously. Plant pollution by heavy metals is a result of soil and water pollution on which the plant grows (Maleki and Zarasvand, 2008). Over-accumulation of cadmium can occur as a consequence of its distribution in rivers and sewages through cadmium containing deposits.

There is less amount of cadmium found in sewage produced in residential regions compared to industrial ones. Cadmium is found in ionic forms (Cd^{++} , CdCl^+ , CdOH^+) in a mixture of colloids in soil. Cadmium binds to absorbable soil colloids in a nonspecific manner, via not very strong bounds. The aforementioned bounds became strengthen, so that the Cd absorption will be reduced at high pH. The nutrients available to plants are controlled through soil physicochemical features, climatic conditions, different vegetative geotypes, irrigation water resources and agronomic management modes (Golia *et al.*, 2008). Heavy metals ionic solubility and biological accessibility are functions of causes affecting their concentrations in soil including soil pH, soil clay content and soil organic matter (Tokalioglu and Kartal, 2006). Different responses to cadmiumic reactions are probably dependent on its concentration in soil and thiol groups concentrations in plants. Thiols show strong antioxidant characteristics of which they are capable of neutralizing oxidative stresses (Mejare and Bulow, 2001).

Compared to other heavy metals, Cd is not heavily absorbed through organic matters by plant roots. Indeed, organic matters strongly absorb Cd ions in acidic soils causing low

absorbability of these ions by plants. Cd is also introduced to soil resources through phosphate fertilizers even though in very low levels (Chen *et al.*, 2011).

Cadmium is also found with zinc compounds because of their physiochemical similarities. These are readily transported to stems (Chen *et al.*, 2011). Cd shows cytotoxicity only in high concentrations causing metabolic disturbances. For example, for transgenic cotton, it provoked seed germination at low concentrations (10 and 100 μM) but inhibited plant growth at high levels (1000 μM) due to changes in root cellular ultrastructure (Daud *et al.*, 2009). For most plant species, Cd is accumulated mostly in roots. In fact it is rarely transported to leaves (Karimi and Nojavan, 2007).

Some of the symptoms of cadmium toxicity in plants include: aboveground growth prohibition (either in height or weight), decline in root growth, young leaves chlorosis resulting in decreased photosynthesis, disruption of CO_2 fixation and transpiration, substantial changes in cells membrane permeability as well as disturbances in nutritional absorptions in various forms (Ghani, 2010). Activation of Cd ions results in a significant reduction in the activity of superoxide dismutase, catalase, glutathione transferase and glutathione peroxidase.

Regarding the extensive reports introducing cadmium as an accumulating element in soil superficial levels and its inevitability to be absorbed by plants we studied its effects on the growth features of *Brassica napus* L. in order to determine its tolerance thresholds against this heavy metal.

Materials and Methods

Experimental design

The experiment was conducted as completely randomized design in 10 treatments (0, 10, 30, 50, 70, 90, 130, 110, 150 and 200 μM of Cd in the form of CdSO_4), with four replica. A one-liter pot is considered as an experimental unit. 10 seeds of *Brassica napus* L., (cultivar RGS 003) were planted in each pot added with 100 ml of Cd-containing solution in different concentrations. Pots were kept at $25 \pm 2^\circ\text{C}$ and 35% RH in greenhouse environment under 16/8 hours of photoperiodic arrangements for 30 days. Pots were regularly irrigated by distilled water in 47-72 hours to preserve farm capacity (FC).

Measuring of chlorophyll meter number

After four weeks and before any harvesting activity, the chlorophyll contents were measured using SPAD -502 set (MINO LTACO- LTD JAPAN) for each replica.

Harvesting

After a month of treatment, plants were harvested precisely and roots and shoots were separated and used for recording the parameters. The total length of root and shoot was measured and expressed in centimeter. Their fresh weights were measured and then were dried for two days at 80°C in an oven and dry weight was taken and expressed in grams.

Determination of photosynthetic pigments

In order to measure the photosynthetic pigment contents, a specific weight of leaves in each sample was rubbed in the 80% acetone till the containing chlorophyll was resealed. The resulting solution was centrifuged at 400 rpm for 10 min.

After attenuation, the volume of the solution was increased to 25 ml. using a spectrophotometer (model UV/1100), the light absorption level were measured at 440, 646 and 663 nm. The following formula were used to calculate total chlorophyll, chlorophyll a, chlorophyll b and carotenoid contents (Arnon, 1956).

$$\begin{aligned} \text{Total chlorophyll (mg/g leaf)} &= (A_{625}) / (34/5 \times V/W) \\ \text{Chlorophyll a (mg/ g leaf)} &= (12/7(A_{663}) - 2/69(A_{645})) \times \\ &V/W/1000 \\ \text{Chlorophyll b (mg/ g leaf)} &= (22/9(A_{645}) - \\ &4/68(A_{663})) \times V/W/1000 \\ \text{Carotenoid (mg/gfw)} &= [4.69 (A_{440} - 0.267 (ch1 \\ &a+b)) / (FW \times 1000)] \times V \end{aligned}$$

where A=light absorption, W=leaf weight (g), V= solution volume (ml) and FW=leaf fresh weight.

Determination of guaiacol peroxidase activity

Firstly, 0.1 g of leaf washed by distilled water a rubbed in 1 ml of 0.8M potassium chloride in a mortar. The resulting solution was centrifuged at 7000 rpm for 10 min at 4°C (Mae- Adam and Nelson, 1992). Then 3 ml of 0.1M phosphate buffer, 50 μl of guaiacol and 50 μl of 3% hydrogen peroxidase were added orderly. The absorbance was measured at 436

nm in 15 seconds intervals for 3 minutes (Mae-Adam and Nelson, 1992).

Statistical analysis

Statistical analyses were done using analysis of variance (ANOVA) and Tukey mean difference comparison test in Minitab statistical package v.16 environment. Graphs were plotted using Microsoft Excel 2013.

Results and Discussion

Results obtained from analysis of variance and Tukey test showed that Cd has a significant effect on rapeseed shoot and root length in treated plants compared to control group. Indeed, both root and shoot represented a decrease in length except for 150 μM of CdSO_4 in which caused a non-significant increase compared to control group (Figs. 1, 2).

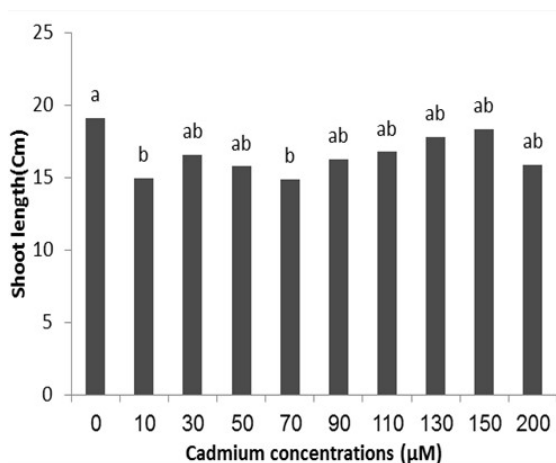


Fig. 1. Effects of cadmium on shoot length of *Brassica napus* L.

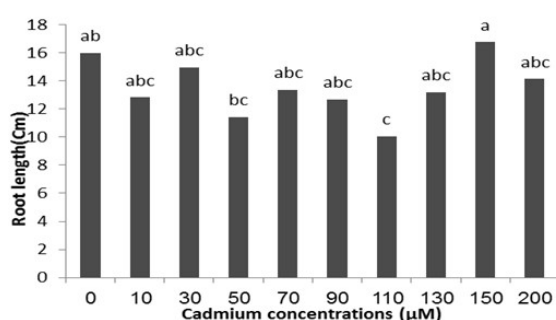


Fig. 2. Effects of cadmium on root length of *Brassica napus* L.

Results showed a non-significant decrease in root and shoot fresh weights of *Brassica napus* L. However, the least shoot fresh weight was observed at 10 and 50 μM of CdSO_4 treatments

(Fig. 3). Except for 200 μM of treatment, all concentrations of Cd showed a decline of root fresh weight compared to control group though these declines were not statistically significant. However, the highest decrease in root fresh weight was observed in 90 μM of cadmium treatment (Fig. 4).

Results obtained from analysis of variance revealed that treatment with different concentrations of cadmium solution show a significant effect on shoot dry weight of *Brassica napus* L., whereas it does not significantly affect its root dry weight. Shoot dry weight experienced a decrease in all Cd treatments compared to control group so that the highest decrease was at 10 μM of CdSO_4 (Fig. 5). Although it was not significant, the highest decrease in root dry weight was observed in treatment with 90 μM of Cd (Fig. 6).

Shoot and root showed height decrease in all treatments compared to control group. Minooi *et al.* (2008) concluded that up to 50 mg/l of Cd does not put any trace on wheat leaves while roots get browner. These phenomena act as the beginning threshold of macroscopic changes in this concentration. Moreover, similar macroscopic changes happened simultaneously in roots and leaves for concentration more than 100 mg/l such that root length begins to decrease. A study on saffron also revealed that Cd for 5 mg/l causes a decline in root growth (Ostad Sharif *et al.*, 2001).

Forced disturbances in photosynthetic processes, cellular inspiration, and nitrogen metabolism, cadmium causes a substantial decrease in plant growth (Gouia, 2001). There are some reports about the negative effect of Cd on water absorption results in decrease in turgor pressure. This and reduction in cell wall flexibility leads to a decrease in cellular volume and intercellular space (Vassilev *et al.*, 1997). Indeed cadmium inhibits cell division in meristem (Fusconi, 2007). Our study proved that Cd has a significant effect on rapeseed shoot dry weight while it did not put any influence on root dry weight. Cd treatment led to decrease in root and shoot dry weights compared to control group. However, Cd did not show any significant variation in either shoot or root fresh weights. In general, any decreases in plant organs result from disruptions in cellular metabolism disruptions (Jeliazkova, 2003). Furthermore, Shariat *et al.* (2010) showed that in *Eucalyptus*, plant weight

loss appears in reduction in leaves and stems. Soltani *et al.* (2010) also showed that Cd causes decreases in leaves and roots dry weights. It has been proved that any decrease in bean upper-ground organs under Cd stress results from disturbances in underground water and nutrition absorption (Gouia, 2001). Some researchers proved that lipid peroxidation in plants treated with cadmium result from increases in hydrogen peroxidase content in cells and resulting in misbalanced water and nutritional status in plant tissue which is regarded as one of the most important causes of plants weight loss (Sanita di Toppi, 1999; Vassilev and Yordanov, 1997). As it is completely clear in this study, these disturbances are more sensibly observed in leaves and shoots of *Brassica napus* L.

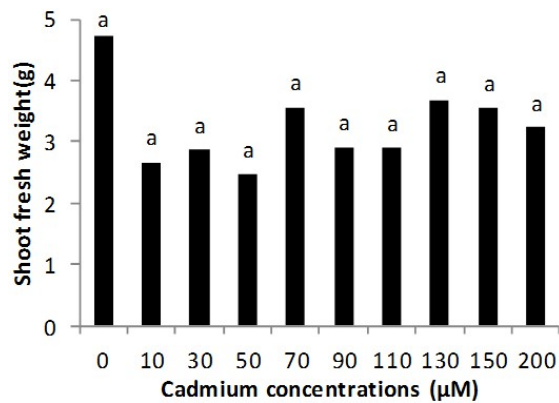


Fig. 3. Effects of cadmium on shoot fresh weight of *Brassica napus* L.

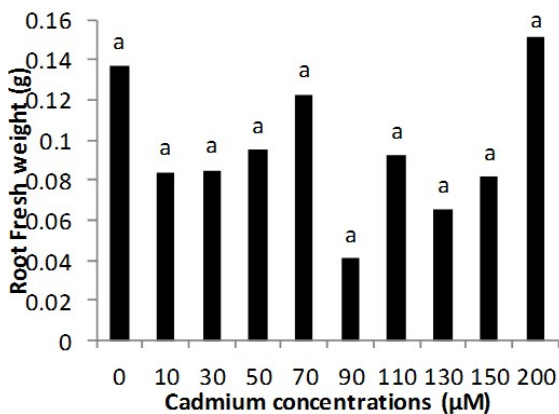


Fig. 4. Effects of cadmium on root fresh weight of *Brassica napus* L.

After four weeks of the beginning of the experiment, all the SPAD numbers decreased in amount so that the highest significant decrease observed in plant treated with 200 and 90 µM of Cd, respectively compared to control group though these treatments did not

revealed a significant difference with other treatments of CdSO₄ (Fig. 7).

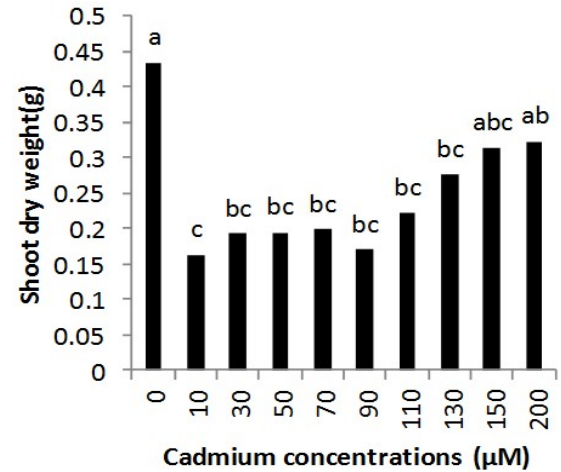


Fig. 5. Effects of cadmium on shoot dry weight of *Brassica napus* L.

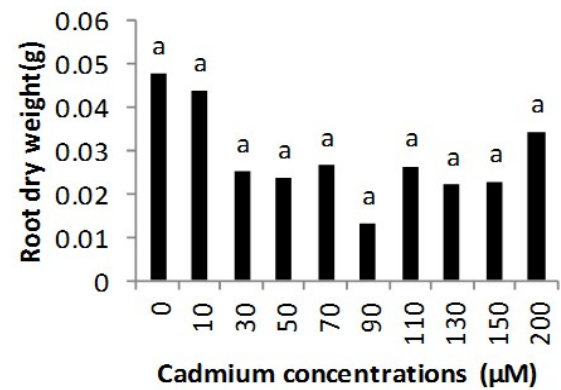


Fig. 6. Effects of cadmium on root dry weight of *Brassica napus* L.

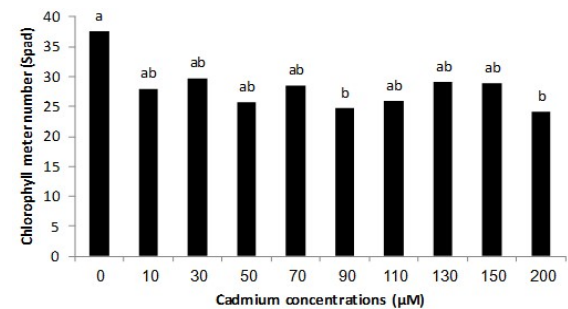


Fig. 7. Effects of cadmium on SPAD of *Brassica napus* L.

Comparison of chlorophyll *a* and *b* in plants treated with different concentrations of cadmium showed that Cd has no significant effect on these photosynthetic pigments. However, though it was non-significant, 50, 90 and 200 µM of CdSO₄ showed a slight

decrease compared to control group. Results also showed that Chlorophyll b may more affected by Cd than chlorophyll a (Fig. 8). In plants treated with cadmium, we did not observed any significant variation in total chlorophyll compared to control group though a non-significant decrease seen in plants treated with 50, 90 and 200 μM of CdSO_4 (Fig. 9).

Mean difference analysis showed that Cd had no significant effect on carotenoid concentrations in *Brassica napus* L. However, the highest concentration of the pigment was observed in 30 μM of CdSO_4 treatment and the least content was seen in plants treated with 50 and 90 μM of the cadmium. Though it was not significant, carotenoid content decrease with Cd concentration (Fig. 10).

This results in concordance with other researchers in which showed the same results in higher plants (e.g. Sanita di Toppi, 1999). Cd treatment resulted in significant decrease in these pigments in *Eucalyptus* (Shariat, 2010). In another study conducted on *Cyperus* and *Digitaria* (two weeds), cadmium caused a decrease in total chlorophyll for 20 mg/kg of concentration (Ewaise, 1997). In addition, Greger (1991) observed a decrease in chlorophyll and photosynthetic activities in sugar beets treated with cadmium. Soltani *et al.* (2006) showed that Cd in concentrations less than 200 μM does not significantly affect the content of chlorophyll a and b as well as of carotenoid content but it substantially changes their contents on concentrations more than 400 μM (400, 600 and 800 μM) compared to control group. Photosynthesis is sensitive to Cd because this element aims directly chlorophylls as well as the enzymes regulating CO_2 fixation (Stoyanova and Tchakalova, 2001). The chlorophyll and carotenoid contents of treated leaves show significant decrease that are concordance. These are consistent with our results. It has been proposed that the interaction between Mg^{2+} and Cd^{2+} ions in cells are likely the main cause that lead to prevention of electron transport in water breaking up complexes and eventually leads to a decrease in photosynthetic pigments contents in leaves (Jason, 2003). Additionally, any disruption in chlorophyll biosynthetic pathways because of haltering δ - amino levulinic acid and protochlorophyllide reductase biosynthesis lead to haltering chlorophyll biosynthesis in leaves (Vassilev and Yordanov, 1997). Furthermore, in

cadmium-treated leaves, production of LHCII is teched that finally lead to photooxidation of newly produced chlorophylls (Hegedus *et al.*, 2001). Non-photochemical suppression of excited chlorophylls also results in reduction of carotenoid content (Sanita di Toppi, 1999). Cadmium treatment also cause a reduction of Mg^{2+} and Fe^{2+} ions in sugar beet resulting in prevention of chlorophyll synthesis (Lee *et al.*, 2005).

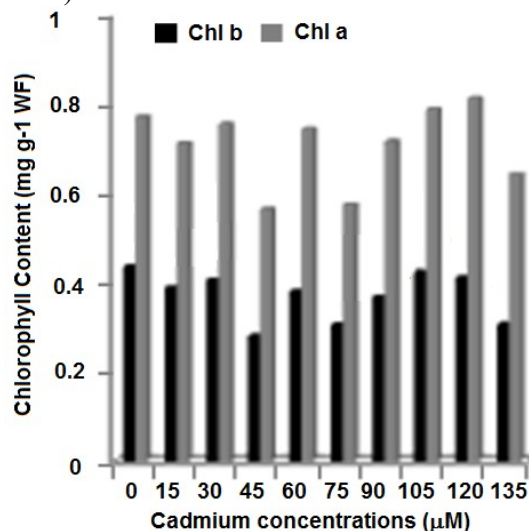


Fig. 8. Effects of cadmium on chlorophyll a and b content of *Brassica napus* L.

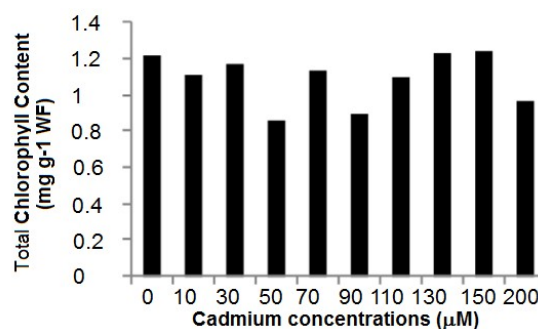


Fig. 9. Effects of cadmium on total chlorophyll content of *Brassica napus* L.

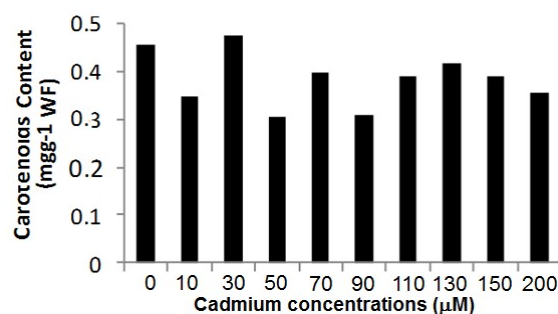


Fig. 10. Effects of cadmium on carotenoid content of *Brassica napus* L.

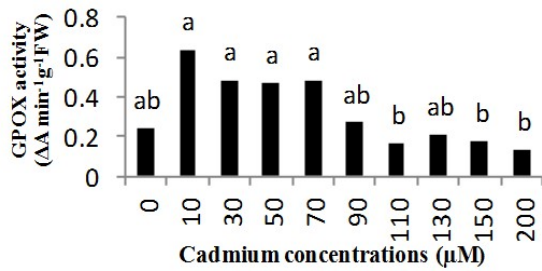


Fig. 11. Effects of cadmium on guaiacol peroxidase activity of *Brassica napus* L.

Results showed that Guaiacol peroxidase activity in *Brassica napus* L. leaves is significantly affected by various concentrations of CdSO₄. Cd treatment caused the non-significant increase in guaiacol peroxidase activity in plants treated with 10, 30, 50 and 70 μM of the material compared to control group. The element also caused a non-significant increase in enzyme activity in plants treated with 110, 150 and 200 μM of CdSO₄ compared to control group. The lowest activity was observed in plants treated with 200 μM of cadmium in which showed a significant difference with 10, 30, 50 and 70 μM of CdSO₄ (Fig. 11).

Results obtained from our study clarified that Cd treatment in different concentrations result in a decrease in chlorophyll a and b, total chlorophyll and carotenoid contents compared to control group though these variations did not showed to be significant.

Guaiacol peroxidase activity:

Our results showed that cadmium significantly affect Guaiacol peroxidase activity in leaves. We observed that the enzyme activity increased in plants treated with concentrations less than 90 μM of cadmium and decreased in concentrations more than 110 μM of cadmium. An important consequence of exposure to heavy metals is the production of reactive oxygen species that results in tissue damages (Cho and Park, 2000). This leads to an increase in lipid peroxidation and cell membrane damages (Zhang *et al.*, 2003). The plant responses to cadmium exposure depends to cadmium concentration, and the amount of thiol groups in plant that possess strong antioxidant characteristics (Sanita di Toppi, 1999). Cd cause an increase in dark inspiration and activity of isocitrate dehydrogenase, Glutamate dehydrogenase and malate dehydrogenase (Lee *et al.*, 2005). Van Assch and Clijsters (1990) observed an increase in activities of malic enzymes as well as other key enzymes including pentose phosphate

oxidative –glucose 6 phosphate dehydrogenase in beans treated with cadmium solutions. Dark inspiration is regarded as an accelerating tool for a mechanism that compensates ATP pools through oxidative phosphorylation (Ernst, 2002). Under Cd stress, an imbalance between production and excretion of different types of active oxygen species including superoxide radical, free oxygen and hydrogen peroxide observed (Okuda *et al.*, 2004). In addition, OH free radicals cause damages to cell membranes under Cd treatment. In this state, a significant increase in activities of antioxidative peroxidase, peroxidase and super oxide dismutase as end productions of dark inspiration processes happened (Lee *et al.*, 2005). There are some reports about the increased activity of antioxidant enzymes in plants like ocimum, cauliflower and paddy (Sing Anil, 2006). Higher concentrations of cadmium result in less enzyme activities through direct deterring influences of Cd ions on enzymes systems (Shanker *et al.*, 2005). While plants are exposed to toxic levels of heavy metals, they are faced with irreversible changes on cellular and physiological conditions. Therefore, Cd is capable to inhibit enzyme activities either directly or indirectly through reactions with –SH groups or disrupting the intracellular cationic balances, respectively. In addition, there are some reports that show the inhibitory effect of Cd on the activity of ATPase and α and β hydrolytic amylase enzymes (Chugh and Sowhney, 1996).

Acknowledgements

This work was supported by Mashhad Branch, Islamic Azad University, Mashhad, Iran.

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