Effect of cadmium on oxidative enzymes activity in Persian clover (*Trifolium resupinatum* L.)

Homayun Ghasemi[1], Mojtaba Yousefirad*[1] and Mozhgan Farzami Sepehr[2]

1. Department of Agriculture, Saveh Branch, Islamic Azad University, Saveh, Iran
2. Department of Biology,Saveh Branch, Islamic Azad University, Saveh, Iran

---

Abstract

Heavy metals are among soil pollutant resources that in case of accumulation in the soil and absorption by the plant, enter into the food chain and poison the plants or the people who consume those plants. This research was performed in order to examine the role of cadmium as a heavy metal in the activity of catalase and peroxidase as well as protein concentration in *Trifolium resupinatum* L. based on a randomized block design with three repetitions. The used treatments included consumption of Cd $(NO_3)_2$at four levels, namely, 0, 100, 200, and 300 ppm. The plants under study were treated for 10 days. The results of the study showed that catalase activity decreased by the increase of cadmium. Moreover, peroxidase activity increased by an increase inthe consumption of cadmium. The analysis of protein level showed that plantlet protein decreased in high cadmium concentrations. The findings also demonstrated that cadmium concentration in roots was higher than in shoots.

Key words: Catalase, Heavy metal, Peroxidase, Protein,

Keywords:Persian clover; heavy metal; cadmium; catalase; peroxidase, protein;

Ghasemi, H., M. Yousefiradand **M. FarzamiSepehr.** 2014. 'Effect of cadmium on oxidative enzymes activity in Persian clover (*TrifoliumresupinatumL.*)'. *Iranian Journal of Plant Physiology*5 (1),1203- 1208.

Introduction

Persian clover is used as a forage crop and also for regeneration of pasture. Re-growth after grazing or cutting is excellent and it has a high nutritive value as pasture or hay (Lacy et al., 2003).

Nowadays there are around 400 plant species such as Violaceae, Poaceae, Caryophyllaceae, Brassicaceae, Astraceae, and Fabaceae that possess the ability to tolerate very high levels of heavy metals in the soil (Poschenrieder et al., 2006; Matthew and Leon, 2008). Phytoremediation is an alternative to traditional chemical methods of treating polluted soils (Mathur et al., 2007). Heavy metals contamination decreases crops production in agricultural lands (Smith, 2009). Soil pollution by metals differs from air or water pollution because heavy metals remain in soil much longer than in other compartments of the biosphere (Lasat, 2002).

At high concentrations, a number of heavy metals have been reported to inhibit the

*Corresponding author
E-mail address:m.yousefirad@iau-saveh.ac.ir
Received:April, 2014
Accepted: September, 2014

growth and decrease the productivity of garlic (Liu et al., 2003). Cadmium is well known as a highly toxic environmental element due to its great toxicity and high mobility from soil to plants and further down the food chain (Vig et al., 2003). High amounts of cadmium can be accumulated by all organisms and can break some of the physiological metabolisms in plants including transpiration, photosynthesis, evaporation, and stabilization of nitrogen (Zhou et al., 2006; Wang et al., 2008; Chugh and Sawhney, 1999). Increase in cadmium in the environment reducees chlorophyll and growth (Zhou and Qiu, 2005) and affects the performance of chloroplast or stabilization of CO_2 (Krupa and Baszynski, 1995; Seidlecka et al., 1997). Cadmium is the fourth element which has the highest level of toxicity for vascular plants (Qadir et al., 2004). One of the main effects of cadmium stress is the increased generation of Reactive Oxygen Species (ROS) that usually damage cellular membrane by reducing lipid peroxidation (Unyayar et al., 2006; Goncalves et al., 2007). Heavy metals such as cadmium, lead, copper, and zinc are easily absorbed by plants and cause toxicity and damage the plant by reducing enzymatic activity, protein level, and damaging nutrient materials (Arun et al., 2005). The plants grown in the soils with high cadmium concentration show symptoms of damage including chlorosis, impaired growth, brownness of root tips, and finally death (Wojcik and Tukiendorf, 2004; Mohanpuria et al., 2007). Therefore, in this research the effects of cadmium on enzymatic activity and protein level of Persian clover were examined.

Material and Methods

The research was conducted in The Research Farm Greenhouse of The Faculty of Agriculture, Islamic Azad University, Saveh Branch during the agricultural year 2011-12. The seeds were first disinfected for cultivation by NaClO5% for 10 minutes and then were washed for three times with distilled water. The seeds were placed on Whatman filter papers. They were then planted in vases with 12 cm in diameter and a height of 10 cm with cocopeat and peat moss substrates. Seeding depth was 2 cm. Cultivation was followed by irrigation (electrical conductivity of the used water was 0.5 ds/m²). On the 9th day after cultivation, Hoagland solution was added. From the 10th day until the 25th day of cultivation, 0.1, 0.2, and 0.3 g/l of Cd $(NO_3)_2.4H_2O$ were added to the Hoagland solution. Depending on the plant condition, a daily amount of 150 m/l of nutrient was used.

Level of catalase activity was measured by Pereira method (Pereira et al., 2002). Koroi method (Koroi, 1989) was used to determine peroxidase activity. Moreover, Bradford method (Bradford, 1976) was used to calculate protein level.

Transport factor (TF) shows the amount of transport of heavy metals from the root to aerial organ of the plant (Marchiol et al., 2004). TF amount higher than 1 indicates the transport of heavy metals from the root to the aerial organ (Jamil et al., 2009) while the TF lower than 1 indicates transport to the plant root. Transport factor is calculated by the following formula:

$$Translocation\ Factor\ (TF) = \frac{\text{Metal Concentration (stems + leaves)}}{\text{Metal Concentration (roots)}}$$

Statistical calculations and analysis of data variance were done by using SAS software. Comparison of means was conducted by using Duncan's Multiple Range test (P<0.05).

Results

Increase of cadmium in nutrient solution decreased catalase activity (Fig. I). Also, no significant difference was found between 100 and 200 ppm treatments. It was found in this research that cadmium in nutrient solution increased the level of peroxidase activity (Fig.II). However, no significant difference was found between the levels of consuming cadmium and only the difference with control was significant (P<0.05).

The results obtained from measuring protein level showed that the increase in consuming cadmium decreased the amount of protein (Fig. III). However, there was no significant difference between 200 ppm and 300 ppm treatments.

As is shown in Figs. (IV) and (V), cadmium concentration increased in root and shootsupon increase of cadmium in the nutrient solution.

The findings of this study indicated that cadmium concentration in roots was higher than in shoots and this suggests that cadmium transport were not conducted well in the plant. Transport factor decreased by the increase in cadmium concentration and on the whole, transport factor in the plants under studywas less than 1 (Table 1).Therefore, Persian clover lacks a suitable potential in cadmium transport from the root to aerial organs and the metal remains more in the plant root. Moreover, as cadmium increases in the nutrient solution, cadmium transport factor of Persian clover reduces and plant transport capability gets lower.

Discussion

Catalaseactivity was decreased as the cadmium increased in this study. The reduction in the catalase activity in the presence of cadmium can be attributed to plant's growth response to cadmium and this is consistent with the results obtained by Touiserkani and Haddad (2012).

Results of this study indicate that increasing consumed cadmium leads to an increase in peroxidase activity.This is consistent with the findingsof studies on beans (Shaw, 1995), barley (Erdie et al., 2002), tomato (Quiroga and Guerrero, 2000), peanuts (Li and Feng, 2001), radish (Chen and Chen, 2002), and sunflower (Laspina and Groppa, 2005). Peroxidase is one of the most significant enzymes engaged in the plant'sresponse to the stressful situations. It plays a role in destruction of H_2O_2 (Pandolfini et al., 1992). H_2O_2 is often consumed within membrane lipid peroxidation process as a cellular planned signal mediator (Cakmak and Horst, 1991). Moreover, H_2O_2 improves mechanical power and decreases plant cell wall potential stretch (Schopfer, 1996).

Thepresence of cadmium decreased protein content in the plants. A study on the effects of different concentrations of cadmium on *Brassicea jounica* was revealed that the levels of proline and proteins increased atlower

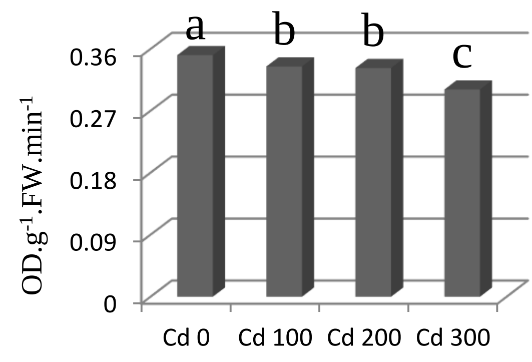

Fig. I. Effect of Cd on the catalase activity

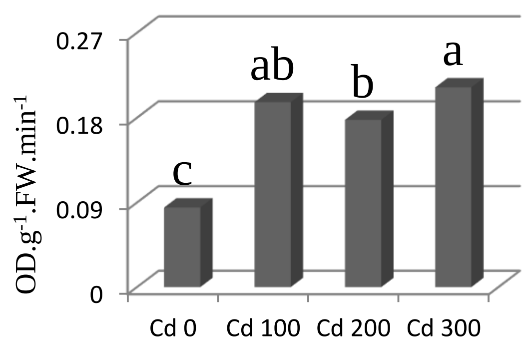

Fig. II. Effect of Cd on the peroxidase activity

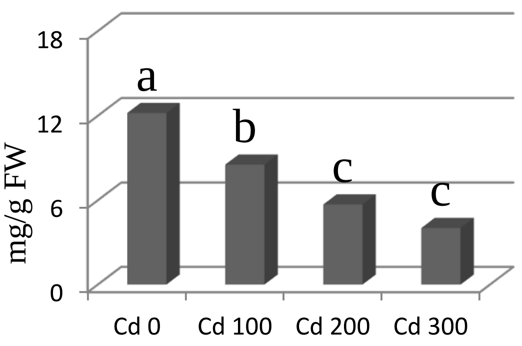

Fig. III. Effect of cadmium on protein

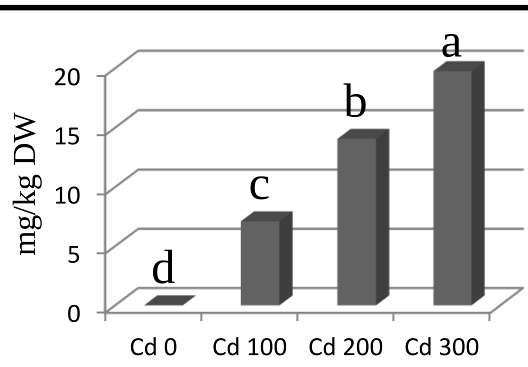

Fig. IV. Cd concentration in root

concentrations of heavy metals but at higher concentrations these parameters decreased (John et al., 2009).

Studies on the plants under the stress of heavy metals such as cadmium and nickel have shown decrease in absorption of water and nutrients, decrease in water transport, disruptionin water balance and enzyme activities, decreasein cellular metabolism, reduction in photosynthesis, respiration and transpiration, nitrogen and phosphor deficiency, controlled growth, accelerated senescence, and even death of the plant (Sanita and Gabbrielli, 1999).

Findings of the present study suggest that Iranian clove is not a right choice for phytoremediation as it could not tolerate long term cadmium concentration. Results showed that concentration of heavy metals in roots was higher than that of shoots. It has been reported that continuous absorption of metals by the plant depends on its ability for absorption, transfer, aggregation, and resistance to high levels of heavy metals within plant growth period (Abbott and Robson, 1991).

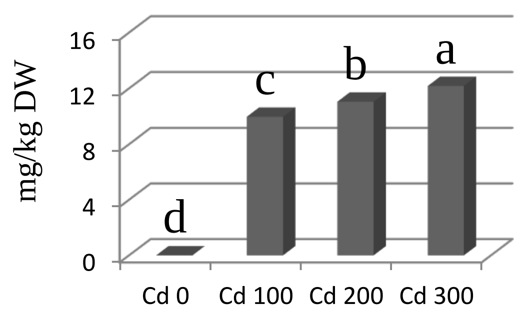

Fig. V. Cd concentration in aerial organ

Translocation factor (TF) of Cd in Persian clover

Treatment		Cd concentration of Root	Cd concentration of Shoot	TF
Cd	100 ppm	12.20	11.14	0.913
	200 ppm	12.82	12.95	0.726
	300 ppm	19.06	14.83	0.778

References

Abbott L. K. and **A. D. Robson**.1999. 'A quantitative study on the spores and anatomy of mycorrhizas formed by a species of Glom us, with special reference to its taxonomy'. *Australian Journal of Botany*, 27: 363-375.

Arun K. S., C. Cervantes, H. Loza-Tavera and **S. Avudainayagam**. 2005.' Chromium toxicity in plants'. *Environ Int.* 31: 739-753.

Bradford M. M. 1976. 'A rapid and sensitive method for the quantitation of microgram quantities ofprotein utilizing the principle of protein-dye binding'. *AnnalBioen*. 72: 225-260.

Cakmak I.and**W. Horst**. 1991.'Effect of aluminum on lipid peroxidation, superoxide dismutase, catalase and peroxidase activities in root tips of soybean (*Glycine max*)'. *Plant Physiol*. 83: 463-468.

Chen E. and **Y. Chen**. 2002. 'Effect of copper on peroxidase activity and lignin content in *Raphanussativus*'. *Plant physiol. Biochem*. 40: 439-444.

Chugh L. K and S. K. Sawhney. 1999. 'Photosynthetic activities of *Pisumsativum* seedlings grown in presence of cadmium'. *Plant Physiol. Biochem*. 37: 297-303.

Erdie S., A. Hegedus and **G. Hauptmann**. 2002. 'Heavy metal induced physiological changes in the antioxidative response system'. *Acta Biologica Szegediensis.* 46: 89-90.

Goncalves J. F., A. G. Becker, D.Cargnelutti, A. L. Tabaldi, L. B. Pereira, V. I.Battisti, R. M.Spanevello, V. M.Morsch, F. TNicoloso and **M. R. C. Achetinger**. 2007. 'Cadmium toxicity causes oxidative stress and induces response of the antioxidant system in cucumber seedlings'. *Braz J Plant Physiol.* 19(3): 24-26.

Jamil S.,P. C. Abhilash, N. Singh and P. N. Sharma. 2009. '*Jatrophacurcas*: A potential crop for phytoremediation of coal fly ash'. *J Hazard Mater.* 172: 269-275.

John R., P. Ahmad, K.Gadgil and **S. Sharma**. 2009. 'Heavy metal toxicity: Effect on plant growth, biochemical parameters and metal accumulation by *Brassica juncea* L. '*International Journal of Plant Prod*. 3 (3):1735-8043.

Krupa Z. and **T.Baszynski**. 1995. 'Some aspects of heavy metals toxicity towards photosynthetic apparatus-direct and indirect effects on light and dark reactions'. *Acta Physiol Plant*. 17: 177-190.

Koroi S. A. A. 1989. 'Gelelektropherstischeand spectral photometrischoe under uchungenzomeinfiuss der tempreture auf straktur and aktritat der amylase and peroxidase isoenzyme'. *Physiol.*20: 15-23.

Lacy J., B. Dear and **G. Sandral**.2003. 'Persian clover. Agfact, NSW Agriculture. http://www.agric.nsw.gov.au/reader/pastvarieties/p2522.htm'. 25 May 2004.

Lasat M. M. 2002.'Phytoextraction of toxic metals – A review of biological mechanisms'.*Journal of Environmental Quality,* 31: 109-120.

Laspina N. V and **M. D. Groppa**. 2005. 'Nitric oxide protects sunflower leaves against Cd-induced oxidative stress'. *Plant Sci*. 169: 323-330.

Li T. C and**T. Y. Feng**. 2001. 'The acute effect of copper on the levels of indole-acetic acid and lignin in peanut root'. *Aust J Plant Physiol.* 28: 1-6.

Liu D. H., W. S. Jiang and **X. Z. Gao**.2003.' Effects of cadmium on root growth, cell division and nucleoli in root tip cells of garlic'. *Biol Plant.* 47: 79-83.

Marchiol, L., S. Assolari, P. Sacco and G. **Zerbi,**2004.Agricultural research and education organization, Phytoextraction of heavy metals by canola andSoil and Water Research Institute, Technical Bulletin radish grown on multicontaminated soil. Environ.Pollut., 132: 21-27.

Mathur N., J. S. S.Bohra, A. Quaizi and **A. Vyas**. 2007. 'ArbuscularMycorrhizal Fungi: A Potential Tool for Phytoremediation'. *Journal of Plant Sciences.* 2: 127-140.

Matthew J. M and **V. K. Leon**. 2008. 'Investigating heavy metal hyperaccumulation using *Thlaspicaerulecens* as a model system'. *Ann Bot.* 102: 3-13.

Mohanpuria P.,N. K.Rana and**S. K. Yadav**. 2007. 'Cadmium induced oxidative stress influence on glutathione metabolic genes of *Camellia sinensis* (L.) O. Kuntze'. *Environmental Toxicology,*22: 368–374.

Pandolfini T., R. Gabbrielli and **C. Comparini**. 1992. 'Nickel toxicity and peroxidase activity in seedling of *Triticum aestivum* L. '*Plant Cell Environ*. 15: 719-725.

Pereira G. J. G., S. M. G.Molina, P. J. Lea and **R. A. Azevedo**. 2002. 'Activity of antioxidant enzymes in response to cadmium in *C.juncea'*. *Plant Soil,* 239: 123-132.

Poschenrieder C., R. Tolra and **J. Barcelo**. 2006. 'Can metals defend plants against biotic stress'? Review *article*. *Trends Plant Sci*. 11(6): 288-295.

Qadir S., M. I. Qureshi, S. Javed and **M. Z. Abdin** 2004. 'Genotypic variation in phytoremediation potential of *Brassica junceae* cultivars exposed to Cd Stress'. *Plant Sci.*167: 1171-1181.

Quiroga M. and **M. A. Guerrero**. 2000. 'A tomato peroxidase involved in the synthesis of lignin and suberin'. *Plant Physiol*. 122: 1119-1127.

Sanita di Toppi L. and **R. Gabbrielli**. 1999. 'Response to cadmium in higher plants. *Enviromental and Experimental Botany*, 4: 105-130.

Schopfer P. 1996.' Hydrogen peroxidase mediated cell wall stiffening in vitro in maize coleoptiles'. *Planta*, 199: 43-49.

Shaw B. P. 1995. 'Effect of mercury and cadmium on the activities of anioxidiative enzymes in the seedlings of *Phaseolus aureus*L.'.*Plant Biol.* 37: 587-596.

Seidlecka A., Z. Krupa, G. Samuelsson, G. Oquist and**P. Gardestorm**. 1997. 'Primary carbon metabolism in *Phaseolus vulgaris* plants under Cd/Fe interaction'. *Plant Physiol. Biochem*. 35: 951-957.

Smith S. R. 2009. 'A critical review of the bioavailability and impacts of heavy metals in municipal solid waste composts compared to sewage sludge: Review Article'.*Environ Int*.35: 142-156.

Touiserkani T. and**R. Haddad**. 2012. 'Cadmium-induced stress and antioxidative responses in different *Brassica napus* cultivars'. *J AgrSci Tech*. 14: 929-937.

Unyayar S., A. Celik,A. OzlemCekicand**A. Gozel**. 2006. 'Cadmium-induces genotoxicity, cytotoxicity and lipid peroxidation in *Allium sativum*and *Viciafaba'*. *Mutagenesis*, 21(1): 77-81.

Vitoria A. P., P.J. Lea and **R. A. Azvedo**. 2001. 'Antioxidant enzymes responses to cadmium in radish tissues'. *Photochemistry*, 57: 701-710.

Vig K.,M. Megharaj, N. Sethunathanand**R. Naidu.**2003.'Bioavailability and toxicity of cadmium to microorganisms and their activities in soil: a review'. *Adv. Environ. Res.* 8: 121-135.

Wang L., Q. X. Zhou, L. L. Dingand**Y. B. Sun**. 2008. 'Effect of cadmium toxicity on nitrogen metabolism in leaves of *Solanumnigrum* L. as a newly found cadmium hyperaccumulator'. *J Hazard Mater*, 154: 818-825.

Wojcik M. and **A. Tukiendorf**. 2004. 'Phytochelatin synthesis and cadmium localization in wild type of *Arabidopsis thaliana*'. *Plant Growth Regulation*, 44: 71-80.

Zhou W. B and **B. S. Qiu**. 2005. 'Effects of cadmium hyperaccumulation on physiological characteristics of *Sedum alfredii* Hance (Crassulaceae)'. *Plant Sci.* 169: 737-745.

Zhou W. B., J. Philippeand **B. S. Qiu**. 2006. 'Growth and photosynthetic responses of the bloom-forming cynabocterium *Microcystis aeruginosa* to elevated levels of cadmium'. *Chemoshphere*, 65: 1738-1746.