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# *In- vitro* Phytotoxic Effects of Cadmium on Morphological Parameters of *Allium cepa*

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**Abstract**

The aim of this study is to investigate the phytotoxic effects of Cadmium (Cd) on germination and the early seedling growth of *Allium cepa* in the Faridabad district, India. The cadmium poses a major threat to agriculture and human health. Seeds of three different onion cultivars were exposed to five different Cd concentrations (20; 40; 60; 80 and 100 ppm). The germination percentage, germination index and growth parameters such as plumule and radical growth were compared to the untreated seeds. The inhibitory effects on the germination of onion seedlings and the inhibition was observed at 80 ppm and 100 ppm. The increased concentration of cadmium is directly proportional to the inhibitory effects on the seedling germination, and the root and shoot length of *Allium.* Pusa Ridhi performed better under Cd stress, while Pusa Madhavi had poor performance. Later, there had been a maximum decrease in seedling length (3.97 %), germination percentage (53.33 %) and the germination index (1.353%) at 100 ppm. Tolerance index varied among the onion cultivars; maximum tolerance index was observed in Pusa Ridhi followed by Pusa Red and Pusa Madhavi. From these results, it is evident that Pusa Ridhi could germinate effectively in soils contaminated with Cd, however, further studies are needed to signify its effects on the growth and yield of onions.

**Keywords:** Onion, Agriculture, Germination index, Tolerance index, Germination percentage.

## 1. Introduction

Onions are of great importance among the staple food, and are consumed worldwide as part of daily diets. Common red onion (*Allium cepa L.*) is a vegetable crop of great economic importance cultivated globally (Mogren *et al.,* 2007). Heavy metals, such as cadmium, are the most significant threat to plant organisms. Cadmium is an unessential heavy metal, but it poses threats and can have a series of harmful effects when taken in excess by a plant (Guo *et al.,* 2016). Also, the quick absorption of cadmium via roots can lead to a high rate of accumulation of cadmium in the plant tissues (Shah *et al.,* 2017). Cadmium present in soil can inhibit plants metabolism, and cause significant retardation in their growth **(**Luo *et al.,* 1998). The earth's biosphere is distinctly affected by heavy metal contamination. Depending upon the origin of the soil, metal concentration in the soil can be less than 1mg/kg or as high as 100,000 mg/kg (Aydinalp, 2009). Rare or noticeable concentration of some heavy metals such as Cadmium (Cd), Chromium (Cr), Copper (Cu), Mercury (Hg), Nickel (Ni) and Zinc (Zn), in the soil has caused harms to the natural and terrestrial ecosystems to a disruptive level. The extent of metal toxicity and sensitivity depends on factors such as the amount or concentration of metal pollution and the period of exposure (Kabir *et al.,* 2008). Heavy metal can pose stress, which causes physiological and biochemical constraints thereby decreasing the plant vigor and inhibiting the plant growth (Subin *et al.,* 2013). As the metal ion accumulates in the roots before the shoots, the roots of a plant are likely to be affected more compared to the shoots of that plant. Thus, Cd toxicity inhibits the plant roots growth (Liu *et al.,* 2003) and affects the root morphology (Daud *et al.,* 2009). It has been reported that cadmium suppress the biosynthesis of chlorophyll, and also targets the function of the photochemical reaction centers (Chugh *et al.,* 1999). Hence, it markedly reduces the chlorophyll content and inhibits leaf photosynthesis. Cadmium can naturally enter air, water, soil and foodstuffs through the weathering of rocks, volcanic eruptions and forest fires. In water, Cd can be found in ionic form or in inorganic complexes. It is obvious that Cd concentrations will be higher in soils being directly treated with Cd -containing fertilizers, sewage sludge, and city waste (Williams *et al.,* 1973). Rapid industrialization has played a major role in exceeding the natural Cd limit to a toxic level. Cd is strongly absorbed by clay materials and organic substances. In this form it accumulates in the upper layers of the soil, while in acid soils this element is transported into the deeper layers due to their weaker absorption. The aim of this study is to determine the influence of Cd at different concentrations on seed germination, germination index and various other physical parameters including root, shoot and seedling length of *Allium cepa.*

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## 2. Materials and Methods

### *2.1. Plant Material*

Healthy seeds of three onion cultivars, namely Pusa Madahvi, Pusa Red and Pusa Ridhi were procured from the Indian Agriculture Research Institute, Pusa, New-Delhi.

### *2.2. Cadmium Treatment*

Cadmium nitrate [$Cd(NO_3)_2.H_2O$] (98 %) was procured from HiMedia Laborataties Chemicals, India and was used to prepare five cadmium concentrations viz. 20 , 40 , 60 , 80 and 100 ppm along with a control without Cadmium.

### *2.3. Seed Germination*

Seeds of all the three onion cultivars were surface sterilized with 0.1 N $HgCl_2$ solutions for two minutes to prevent fungal infections (Ramasubramanian *et al.,* 1993). The treated Seeds were washed under running water and then with double distilled water for ten minutes. Ten seeds of the selected onion cultivars were placed at an equal distance on a Whatman paper bed with an equal volume (30 mL) of the prepared concentrations of Cd solutions in sterilized petripates. All the petriplates were then covered with Whatman filter paper and to keep them wet with the Cd solution; they were sprinkled with respective Cd solutions. Control was prepared with distilled water sprinkled on Watman filter both at the base and at top of seeds. (Subin *et al.,* 2013).

The seeds were allowed to germinate under a photoperiod of twelve hours at 25 ºC / 18 ºC temperature in an incubator. The research experiments were arranged in CRD-factorial and replicated five times. The seedlings were harvested after two weeks and the germination percentage, germination index, radical length and plumule length were recorded.

### *2.4. Determination of Plant Parameters*

After a two-week exposure, the number of germinated seeds were counted and the seed germination was evaluated to estimate the viability of seeds. (Subin *et al.,* 2013; Stephen, 2009).

The germination percentage (GP) was determined by the method proposed by Stephen (2009) with the formula given below:

GP = Number of germinated seeds/Total number of seeds*100

The data in regard to root and shoot length was measured with the aid of meter rod (Khan *et al.,* 2006).

Seedling height = Mean of root length + Mean of shoot length

Germination index was determined according to the method described by Zhang (2012).

GI = Σ (Gt / Tt), where Gt is estimated as the no. of germinated seeds on Day (t) and Tt is the corresponding time to Gt in days.

Tolerance indices was estimated with the formula (Iqbal *et al.,* 1992):

$$\text{Tolerance indices (T.I)} = \frac{\text{Mean root length in metal solution}}{\text{Mean root length in control}} \times 100$$

### *2.5. Statistical Analysis*

The data were analyzed by one-way analysis of variance (ANOVA) using SPSS (SPSS Inc., USA, version 13.0) to determine the statistical significance of differences among means of treatments (Peralta *et al.,* 2001). Significant differences among the treatments were evaluated at $P < 0.05$.

## 3. Results

### *3.1. Effect of Cadmium on Germination of Seeds*

Cadmium has inhibitory effects among onion cultivars and drastic effects on the germination of seeds. The suppression of seed germination and germination index was observed above 40 ppm (Table 1). Among the three cultivars, the maximum reduction in the germination percentage and germination index was observed in Pusa Madhavi compared to Pusa Ridhi and Pusa Red. Inhibitory effect was maximum at the highest concentration (Cd-100 ppm). The germination index of Pusa Ridhi was remarkably affected by increasing the Cd concentration.

**Table 1**. Effect of different concentrations (20 ppm-100 ppm) of Cd on germination percentage (%) and germination index in three onion cultivarsGermination percent (%) Germination index.

| Treatment (ppm) | Pusa Madhavi | Pusa Ridhi | Pusa Red | Pusa Madhavi | Pusa Ridhi | Pusa Red |
|---|---|---|---|---|---|---|
| Control | 93.33±3.33 | 96.66±3.33 | 93.33±3.33 | 3.060±0.11 | 3.007±0.16 | 3.040±0.15 |
| Cd -20 | 90.00±5.77 | 90.00±5.77 | 93.33±3.33 | 2.877±0.11 | 2.800±0.15 | 2.887±0.14 |
| Cd -40 | 83.33±3.33 | 90.00±5.77 | 86.66±6.66 | 2.487±0.08 | 2.663±0.06 | 2.710±0.11 |
| Cd -60 | 80.00±5.77 | 86.66±3.33 | 76.66±3.33 | 1.677±0.27 | 2.563±0.06 | 2.497±0.1 |
| Cd -80 | 66.66±3.33 | 73.33±6.66 | 66.66±3.33 | 1.933±0.35 | 2.373±0.1 | 2.240±0.09 |
| Cd -100 | 53.33±3.33 | 60.00±0.00 | 56.66±3.33 | 1.353±0.13 | 1.953±0.12 | 1.867±0.03 |

Significant difference at p<0.05. Data are mean ± SD. Replicate (n) = 5

### *3.2. Effect of Cadmium on Root Length, Shoot Length and Seedling Size*

The root length, shoot length and seedling size revealed that the cultivars and the Cd concentration were statistically significant (0.05). The data presented indicates that the root length (RL), shoot length (SL) and seedling size (SS) are inversely proportional to the increase in the Cd concentrations.

#### *3.2.1. Root Length*

The data presented (Figure 1), indicates a decrease in root length with the increase in the cadmium concentration. The decline in root length ranged from 35 mm (with 20 ppm Cd) to 2.36 mm (with 100 ppm Cd). The root length of Pusa Madhavi was observed to be the least compared to the other onion cultivars. Pusa Ridhi and Pusa Red showed almost equal growth rates in the control, but at a higher concentration of cadmium, Pusa Red was more negatively affected and found to be as sensitive as Pusa Madhavi.

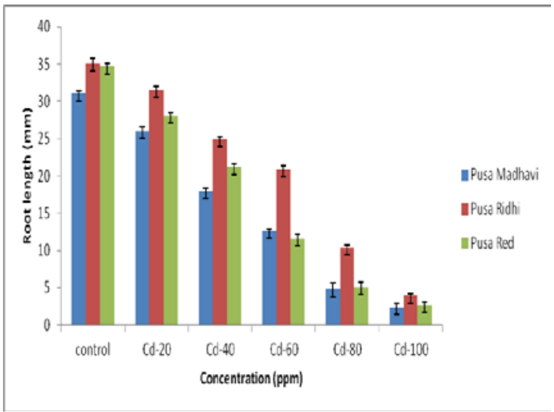


**Figure 1.** Effect of various Cd concentrations (ppm) on mean root length (mm) of three onion cultivars. Data are mean ± SD. Replicate (n) =5. Vertical bars show Standard error in root lengths.

*3.2.2. Shoot Length*

The data presented in (Figure 2) indicates the significant effect of cadmium toxicity to the shoot growth of onion seed cultivars. Similar to the case of root growth, Pusa Madhavi had the least growth rate among the three and is most affected by increasing the cadmium concentration. Significant decrease in the shoot growth was observed in all the three cultivars of onion. Shoot length of Pusa Ridhi was observed to be the highest at the concentration of 100 ppm when compared to the control.

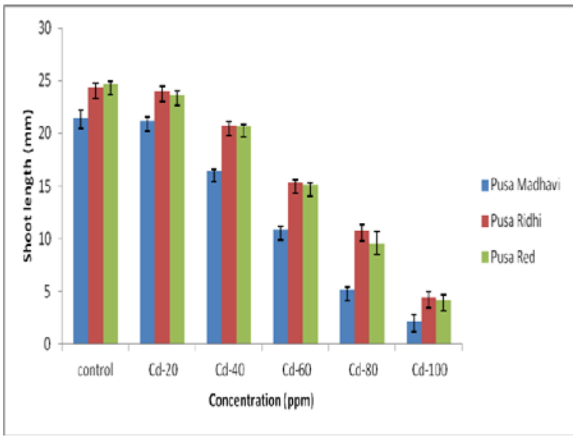


**Figure 2.** Effect of various Cd concentrations on mean shoot length (mm) of three different onion cultivars. Data are mean ± SD. Replicate (n) = 5. Bars show standard error in shoot length.

*3.2.3. Seedling Length*

There was an augmented effect of Cd stress on the seedling length as its inhibition was statistically significant compared to control. The seedling height of Pusa Ridhi was observed to be maximum in control and with a further addition of Cd from 20 to 100 ppm, but maximum inhibition occurred at 100 ppm (Figure 3). The seedling length for Pusa Madhavi and Pusa Red was almost the same at a higher concentration.

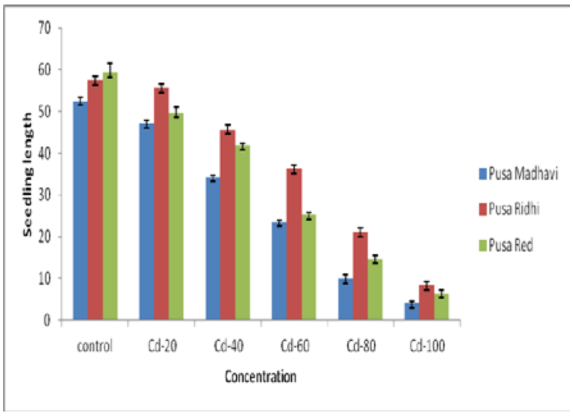


**Figure 3.** Effect of various Cd concentrations on seedling length (mm) of three different onion cultivars. Data are mean ± SD. Replicate (n) = 5. Bars show standard error in seedling length.

*3.2.4. Effect of Cadmium on Tolerance Indices*

All three cultivars showed different results in response to varied Cd concentration. Pusa Ridhi is found to be the most tolerant onion cultivar among the three tested. Tolerance of the three onion cultivars decreases in this order, Pusa Ridhi > Pusa Red > Pusa Madhavi (Figure 4).

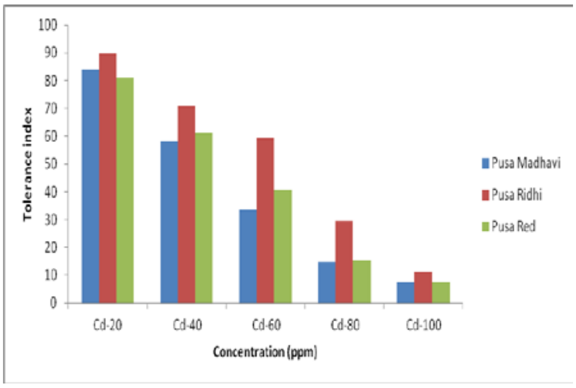


**Figure 4.** Tolerance indices of different onion cultivars to Cd stress

## 4. Discussion

The primary objective of this study is to assess the sensitivity of different cultivars of onion towards different cadmium concentrations. Cadmium along with lead and mercury are categorized as non-essential heavy metals as these are potentially toxic to humans. Also, the relative mobility of cadmium into the soil is high. The root system of onions is capable of absorbing cadmium easily from the soil or solution (Heidari *et al.*, 2011).

The growth performance of *Allium cepa* cultivars may have decreased as a result of meristematic cell reduction or hindrance to the functions of hydrolytic enzymes contained in the cotyledon and endosperms due to the toxicity of cadmium (Subin *et al.*, 2013). The mobilization of stored food materials to the plumule and radicle is a function of hydrolytic enzymes. The breakdown of stored food materials in seeds by the application of cadmium can also be one of the reasons for the retarded or inhibited growth (Katarzyna *et al.*, 2015).

Cadmium is detrimental to vegetables, cereals and fruit crops (Ahmad, 2012), and it induces innumerable health hazards in humans (Anon., 2004). Consequently there is an urgent need to determine the toxic level of Cadmium for onion growth. In the present investigation the increased Cadmium concentration resulted in highly significant inhibition of onion seed germination. Adverse effects of cadmium on seed germination determined its phytotoxicity to different rice cultivars due to the genetic diversity of cultivars (Zhang *et al.*, 2005).

Similar studies were earlier reported by Raziuddin *et al.,* 2011 on the seedlings of wheat. Cd showed a strong inhibitory effect on the root elongation and coleoptile growth of the evaluated onion cultivars, especially at high cadmium concentrations. The ranges of Cd toxicity to root and shoot length were as follows: 2.36 mm, 2.16 mm for Pusa Madhavi and 3.91 mm, 4.39 mm for Pusa Ridhi respectively, while for Pusa Red the ranges were 2.59 mm and 4.15 mm at 100 ppm. The results are in conformity with Ahmad *et al*., 2011 who stated that the seedling length, root and shoot are effected in *Brassica juncea* due to the presence of high Cd content. This study along with the research of Guo *et al.,* done on three different *Miscanthus* species, reflect the fact that cadmium toxicity varies among genotypes of the same species ( Guo *et al*., 2016).

Metal contamination disorganizes the metabolism of plants due to the enzyme interactions which leads to biochemical reactions which effects the seedling length (Ashraf *et al*., 2011). Several Researchers (Unyayar *et al*., 2006 and Shafi *et al*., 2010) reported the sensitivity of plant growth to Cd toxicity. Changes in the physiological mechanism as well the growth phases during germination might have contributed to the low tolerance indices of the different onion cultivars.

## 5. Conclusions

The current study concludes that root, shoot, seedling elongation and germination index of all the evaluated onion cultivars were observed to be positive indicators of Cd toxicity to onion. Different cultivars showed different adverse effect levels on onion growth indices at low concentrations of Cd, but the maximum inhibition occured at 100 ppm. Tolerance indices for Pusa Madhavi, Pusa Ridhi and Pusa Red were 7.47, 11.4 and 7.61 respectively. Pusa Madhavi is the most sensitive to Cd compared to other evaluated onion cultivars. In contrast, Pusa Ridhi could be well grown on cadmium-contaminated soils compared to others. The accumulation of heavy metals such as Cd in the soil and into the food chains is a worldwide concern. It can have damaging effects on human life and animal life as well.

## References


Ahmad I, Akhtar MJ, Zahir ZA and Jamil A. 2012. Effect of cadmium on seed germination of four wheat (*Triticum aestivum* L.) cultivars. *Pak J Bot.,* **44 (5):** 1569-1574.

Ahmad P, Nabi G and Ashraf M. 2011. Cadmium-induced oxidative damage in mustard [*Brassica juncea* (L.) Czern. & Coss.] plants can be alleviated by salicylic acid. *South African J Bot.*, **77(1):** 36-44.

Anon YJ. 2004. Soil ecotoxicity assessment using cadmium sensitive plants. *Environ Pollut*, **127:** 21-26.

Ashraf MY, Sadiq R, Hussain M, Ashraf M and Ahmad MSA. 2011. Toxic effect of nickel (Ni) on growth and metabolism in germinating seeds of sunflower (*Helianthus annuus L.*)- *Biol Trace Elem Res.*, **143**: 1695- 1703.

Aydinalp C. 2009. Concentration and speciation of Cu, Ni, Pb and Zn in cultivated and uncultivated soils. *Bulgarian J Agri Sci.*, **15 (2)** : 129-134.

Chugh LK and Sawhney SK. 1999. Photosynthetic activities of *Pisum sativum* seedlings grown in presence of cadmium. *Plant Physiol Biochem*, **37**: 297-303.

Daud MK, Sun YQ, Dawood M, Hayat Y, Variath MT, Wu YX, Raziuddin, Mishkat U, Salahuddin Najeeb U and Zhu SJ. 2009. Cadmium-induced functional and ultrastructural alterations in roots of two transgenic cotton cultivars. *J Hazard Mater,* **168**: 614-625.

Guo H, Hong C, Chen X, Xu Y, Liu Y, Dean J and Zheng B. 2016. Different growth and physiological responses to Cadmium of the three *Miscanthus* species, *PLoS ONE*. **11(4):** e0153475

Heidari M and Sarani S. 2011. Effects of lead and cadmium on seed germination, seedling growth and antioxidant enzymes activities of Mustard (*Sinapis arvensis L.*) ARPN. *J Agri Biol Sci.*, **6(1):** 44-47.

Iqbal MZ and Rahmati K. 1992. Tolerance of *Albizia lebbeck* to Cu and Fe application. *Ekologia (CSFR),* **11**: 427-430.

Kabir M, Iqbal MZ, Shafiq M and Farooqi ZR. 2008. Reduction in germination and seedling growth of *Thespesia populnea l.*, caused by lead and cadmium treatments. *Pak J Bot.*, **40**: 2419-2426.

Katarzyna S, Klaudia K, Jakub W and Urszula P. 2017.Evaluation of cadmium, lead, zinc and copper levels in selected ecological cereal food products and their non-ecological counterparts. *Current Issues in Pharm Medical Sci*., **30** (3); 147-150.

Khan NA, Ahmad I, Singh S and Nazar R. 2006. Variation in growth, photosynthesis and yield of five wheat cultivars exposed to cadmium stress. *World J Agri Sci.*, **2:** 223-226.

Liu J, Li K, Xu J, Liang J, Lu X, Yang J and Zhu Q. 2003. Interaction of Cd and five mineral nutrients for uptake and accumulation in different rice cultivars and genotype. *Field Crops Res*, **83**: 271-281.

Luo LX, Sun TH and Jin YH. 1998. Accumulation of superoxide radical in wheat leaves under cadmium stress. *Acta Sci Circum.*, **18**: 495–499.

Mogren LM, Olsson ME and Gertsson UE. 2007. Effects of cultivar, lifting time and nitrogen fertiliser level on quercetin content in onion (*Allium cepa L.*) at lifting. *J Sci Food Agri.,* **87**: 470-476.

Peralta JR, Torresdey JLG, Tiemann KJ, Gómez E, Arteaga S, Rascon E and Parsons JG. 2001. Study of the effects of heavy metals on seed germination and plant growth on Alfalfa plant (*Medicago sativa*) grown in solid media. *Bull Environ Contam Toxicol*, **66**:727.

Ramasubramanian V, Kannan N and Ravichandran V. 1993. Communications in soil science and plant analysis. *J Comm Soil Sci Plant Analysis,* **24**: 2241-2249.

Raziuddin, Farhatullah, Hassan G, Akmal M, Shah SS, Mohammad F, Shafi M, Bakht J and Zhou W. 2011. Effects of cadmium and salinity on growth and photosynthesis parameters of brassica species. *Pak J Bot*, **43(1):** 333-340.

Shafi M, Zhang GP, Bakht J, Khan MA, Islam E, Dawood MK and Raziuddin. 2010. Effect of cadmium and salinity stresses on root morphology of wheat. *Pak J Bot*., **42(4):** 2747-2754.

Shah K, Mankad AU and Reddy MN. 2017. Cadmium accumulation and its effects on growth and biochemical parameters in *Tagetes erecta L*, *J Pharmacog Phytochem*., **6(3):** 111-115

Stephen, G S. 2009 Primer on Seed Germination, https://employees.csbsju.edu/ssaupe/biol327/Lab/Seeds/ germination _percentage.htm, (01/07/2009).

Subin MP and Francis S. 2013. Phytotoxic effects of cadmium on seed germination, early seedling growth and antioxidant enzyme activities in *Cucurbita maxima* Duchesne. *Inter Res J Biol Sci.*, **2(9)**: 40-47.

Unyayar S, Celik A, Cekic FO and Gozel A. 2006. Cadmium-induced genotoxicity, cytotoxicity and lipid peroxidation in *Allium sativum* and *Vicia faba*. *Mutagenesis*, **21**: 77–81.

Williams CH, David DJ and Aust J. 1973. The effect of superphosphate on cadmium content of soils and plants. *Soil Res.*, **11**: 43-56.

Zhang H 2012. Seed germination and early seedling growth of *Cynanchum bungei* Decne (*Asclepiadaceae*) in response to photoperiod, temperature and seed size. *Hortsci.*, **47(9):** 1338–1341.

Zhang S, Hu J, Chen ZH, Chen JF, Zheng YY and Song WJ. 2005. Effects of Pb pollution on seed vigor of three rice cultivars. *Rice Sci*., **12**: 197-202.