Physiological and Growth Responses of Castor (Ricinus Communis L) Under Cadmium Stressed Environment

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Materials and Methods

Collection of soil samples and experiment design:
Surface soil samples (0-20 cm) were collected from an arable field of the Huazhong Agricultural University (HZAU) Wuhan, P.R. China. Five sub-samples were collected randomly and composited into one sample. The Cd salt, Cd (NO$_3$)$_2$, 4H$_2$O was used as the pollutant source and it was added to the soil at different
concentrations of Cd salt i.e. 0, 10, 25 and 50 mg kg\(^{-1}\) soil.

**Conducting pot experiment:** This pot experiment was conducted in a greenhouse condition having 25°C temperature and 65% average relative humidity. Seeds of castor plant were obtained from the TONGLYSHEAN mine, Daye city, Hubei Province, China. Castor plant grown in the treated soil for 30 days in various levels of 0, 10, 25 and 50 mg kg\(^{-1}\) soil treatments.

**Determination of soil and plant samples:** The soil samples were consisted of five sub-samples at randomly. The plants related materials present in soil were removed. Soil was air dried, ground into fine powder to pass through a 0.15 mm nylon mesh were measured physico-chemical properties of soil and the plant samples (leaf, shoot and roots) were washed thoroughly to remove soil and dust particles, oven dried at 75°C for overnight and ground into fine powder for processes to determine the Cd concentrations using an atomic absorption spectrophotometer (Spectr-AA 220FS, Varian, USA).

**The determination of enzymatic activities of castor plant:** To examine the enzymes superoxide dismutase (EC 1.15.1.1), SOD, Enzyme classification number (ECN) peroxidase (EC 1.11.1.11), POD and malondialdehyde MAD (CH\(_2\)(CHO)\(_2\)) content was determined by measuring the inhibition of the reduction of nitro-bluetetrazolium (NBT) described by (Lacan and Baccou, 1998). The plant leaf samples (0.5 g) were homogenized in 5 ml of 0.2 mol L\(^{-1}\) of sodium phosphate buffer (pH 7.8). The homogenized mixture was centrifuged at 12,000 rpm for 15 minutes at 4°C and aliquot was collected in falcon tubes so as to proceed with the enzyme determination absorbance tests was measured at 470 nm as described by (Wu et al., 2015). SOD in the plant samples was determined by analyzing the ability to reduce the photochemical activity of nitro blue tetrazolium as described by (Wu et al., 2015). The MDA content was determined as described by (Heath andacker, 1968).

By using following formula:

\[
\text{MDA (Emol/l)} = 6.45 \times (\text{OD}_{340} - \text{OD}_{500}) - 0.56 \text{OD}_{450}.
\]

**Chlorophyll and Proline contents:** Chlorophyll was extracted in 85% (v/v) aqueous acetone and absorption measured in an atomic absorption spectrophotometer model (Spectr-AA 220FS, Varian, USA) at 664 nm. Shimadzu UV-1201 model spectrophotometer at 465 and 663 nm.

**Proline Determination**

Proline determination was extracted from a sample of 0.5 g fresh shoot material samples in 3% (w/v) aqueous sulphosalycylic acid and estimated using the ninhydrin reagent according to the method of (Turkan and Bor, M and Ozdemir, 2003: Bates et al. 1973). The absorbance of fraction with toluene aspirated from liquid phase was read at a wave length of 520 nm. Proline concentration was determined using a calibration curve and expressed as \(\mu\)mol proline g\(^{-1}\) FW.

**Statistical analysis:** All data were analyzed with (SPSS IBM Statistics version 21) and Microsoft Office Excel 2013, as the mean value with the standard deviation was found significant using analysis of variance (ANOVA) \(p < 0.05\) value was considered as a significant difference test.

**Results**

Increased industrialization and urbanization have decidedly contributed to soil contamination by Cd which impairs plant growth development and antioxidant enzymes in castor under stress condition. Castor is a suitable plant used in contaminated soils for remediation and tolerate metal toxicity environments.

**The development of growth parameters & accumulation of cadmium:** Plant height and biomass of the castor plant was significantly reduced under Cd stress environment as compared to control. Although all the Cd toxicity stresses exposed that castor was extremely affected by the plant growth and biomass under 25-50 Cd mg kg\(^{-1}\), which caused 71% - 116%, 166% - >200% and 48% - 84% reduction in stem, root and plant height respectively, as against to control (Table 1).

| Cd Stress (mg kg\(^{-1}\)) | Stem weight (g pot\(^{-1}\)) | Root weight (g pot\(^{-1}\)) | Plant height (cm) |
|-----------------------------|-------------------------------|-------------------------------|-------------------|
| 0 | 15.77 ± 0.42 | 10.77 ± 0.40 | 56.10 ± 0.06 |
| 10 | 11.60 ± 0.31 | 8.71 ± 0.35 | 42.12 ± 0.58 |
| 25 | 9.22 ± 0.38 | 6.23 ± 0.35 | 38.40 ± 0.10 |
| 50 | 7.30 ± 0.25 | 4.30 ± 0.35 | 30.50 ± 0.21 |

Data are presented as the mean ± SD (n = 3). Significant differences from the control are indicated as \(p < 0.05\).

![Table 1. To evaluate the effect of Cd stress on plant height and the biomass of castor plant under contaminated soil](https://www.joarps.org/data/Table1.png)

Cd uptake in the castor plant such as (root, stem and leaves) was examined to assess the ability of castor to remove Cd, when exposed different levels of Cd in the soil at 25 and 50 mg kg\(^{-1}\) Cd stress treatments as against control as shown in (Table 2). The increased level of Cd toxicity can significantly increase its concentration in all parts of the plant. However, the increment was more marked, when Cd was applied at 25 and 50 mg kg\(^{-1}\). The increment of castor plant tissue in the order of roots <stem < leaves was significantly increased Cd concentration with increasing Cd concentrations in the soil at 25 and 50 mg kg\(^{-1}\) Cd stress as against control. The Cd stressed castor plants treated with 50 mg kg\(^{-1}\)Cd were exhibited 2 fold, 1.8 fold and 1.5 fold in leaves,
stem and roots respectively as compared to control treatments (Table 2)

Table 2. The distribution of castor plant tissue grown under Cd stress condition in Cd contaminated soil

| Cd Stress (mg kg⁻¹) | Leaves (µg pot⁻¹) | Stem(µg pot⁻¹) | Root (µg pot⁻¹) |
|---------------------|------------------|---------------|-----------------|
| 0                   | 0.04±0.001       | 0.05±0.01     | 0.14±0.01       |
| 10                  | 99.03 ±0.01     | 124.82±0.01   | 130.45±0.14     |
| 25                  | 145.05±0.05     | 167.03±0.08   | 170.54±0.28     |
| 50                  | 160.09±0.06     | 188.04±0.10   | 200.34±0.56     |

Data are presented as the mean ± SD (n = 3). Significant differences from the control are indicated as p<0.05.

Table 3. The response of chlorophyll and carotenoid content and proline content of castor plant grown under contaminated soil.

| Cd Stress (mg kg⁻¹) | Chlorophyll (µg-1) | Carotenoid (µg-1) | Proline (µg-1) |
|---------------------|-------------------|-------------------|---------------|
| 0                   | 2.23 ± 0.001a     | 0.15 ± 0.002c    | 64 ± 0.001a    |
| 10                  | 2.01 ± 0.002b    | 0.19 ± 0.002b    | 59 ± 0.002b    |
| 25                  | 1.82 ± 0.003c    | 0.23 ± 0.002ab   | 57 ± 0.002bc   |
| 50                  | 1.75 ± 0.002d    | 0.27 ± 0.002a    | 49 ± 0.002c    |

Data was analyzed by one way analysis of variance Duncan multiple range at p < 0.05. Different values are means ± SD of thrice replicates. Indicate significant difference between the treatments.

Table 4. Relationship of plant height with physiological parameters

| PH | RT | ST | Clf | Crt | Pr | MAD | POD | Sod  |
|----|----|----|-----|-----|----|-----|-----|------|
| RT | 0.91** |    |     |     |    |     |     |      |
| ST | 0.92** | 0.99** |     |     |    |     |     |      |
| Clf| 0.26ns | 0.56ns  | 0.57ns |     |    |     |     |      |
| Crt| 0.14ns | 0.45ns  | 0.45ns | 0.99** |    |     |     |      |
| Pr | 0.99** | 0.89** | 0.89** | 0.26ns | 0.15ns |     |     |      |
| MAD| 0.78*  | 0.92** | 0.93** | 0.80** | 0.72*  | 0.78* |      |      |
| POD| -0.92** | 0.85**  | -0.84** | -0.04ns | 0.08ns | -0.88ns | -0.60* |      |
| Sod| -0.97** | -0.81*** | 0.02ns | 0.15ns | -0.80** | -0.89** | -0.89** | 0.55ns |
| Rpt| 0.08ns | 0.24ns  | 0.24ns | 0.49ns | 0.49ns | 0.99ns | 0.37ns | 0.02ns |

Chlorophyll content of castor plant expressed as chlorophyll a, b, proline and carotenoid contents value was shown in Table 3. The results indicate the chlorophyll and proline content of castor plants were dramatically decreased under Cd stress. The castor plant experienced significant reduction exposed in chlorophyll content under 50 mg kg⁻¹, treatments as compared to control (Table 3). Chlorophyll contents were

Relationship of plant height with physiological parameters

According to Pearson’s correlation, the strongly positive antioxidant enzymes inhibition in Cd contaminated soil: Antioxidant enzymes play a key role in the management of oxidative stress. Accordingly, the present parameters were evaluated to the effect of Cd levels on the antioxidant enzyme activities under Cd-stressed. Cd stress lead to a significant variation into the antioxidant defense in the castor plants. The antioxidant enzymes activity in the castor plant was significantly increased under Cd stress as compared to control (Figure 1). The maximum increase occurred at 50 mg Cd kg⁻¹ soil treatment as compared to the control. In this study, the significantly decreased under 25- 50 mg kg⁻¹ Cd stress were noted values 2.23 to 1.75 µg⁻¹ as compared to control. Another hand, proline contents of castor plants were also decreased under Cd stress was noted having values 64 to 49 µg⁻¹ in 0 and 50 mg kg⁻¹ treatments, respectively. In Table 3, while, it can be seen that the carotenoid significantly increases under 50 mg kg⁻¹ treatments as compared to control relationship exposed between root, shoot, proline, malondialdehyde (Table 1). Although a destructive relationship was demonstrated by PoD and SOD. results related to two key antioxidant enzymes activities such as peroxidase (POD) super oxide dismutase (SOD) was significantly greater (215.30 U g⁻¹ and 53.20 U g⁻¹) under Cd in 50 mg kg⁻¹ treatment as compared to control. Contrary malondialdehyde (MAD) activity in the castor plant was significantly decreased under 50 mg kg⁻¹ Cd stress as against control (Figure 2). While, the investigation of alteration results indicated that the MAD was significantly decreased 3.11% under Cd stress as against to control (p < 0.05)
Figure 1 a&b: Peroxide (POD) and superdismute (SOD) antioxidant enzymes content under Cd stress. Data was analyzed by one way analysis of variance Duncan multiple range at p < 0.05. Different letters indicate the significant difference between the treatments.

Figure 2. The malonaldehyde (MDA) antioxidant enzymes of castor plants grown under Cd stress. Data was analyzed by one way analysis of variance Duncan multiple range at p < 0.05. Different Values are means ± SD of thrice replicate. Indicate significant difference between the treatments.

Discussion
The effects of Cd on biomass, protein, antioxidative enzymes and proline were examined. High Cd level in soil cause significant reduction in malondialdehyde (MAD), Chlorophyll and proline content. Metal toxicity by addition of Cd has been widely found in different plant species and it has shown that Cd can cause a selection of phototoxic symptoms (Farooq et al., 2013). Recently (Arshad et al., 2016) found that the Cd concentrations in shoot and root of wheat were observed greater under 100 μM Cd treatment than in the 0 μM Cd treatment. (Shi and Cai, 2009), also reported that the Cd inhibited the
The present study indicated that castor (Ricinus communis L.) plant might be a vigorous potential to build up and tolerate the Cd stress in polluted soil and Cd has negative effects on the morphological and physiological changes in R. communis. Cd induced the oxidative stress via increasing antioxidative enzymes activities such as (SOD and POD), which build up the higher Cd under 50 mg kg⁻¹ Cd stresses. The plant development, MDA enzyme activity, chlorophyll content and proline content of castor plants were significantly reduced under 50 mg kg⁻¹ Cd stress. Thus, this study showed that 50 mg kg⁻¹ Cd could be a safer option for increasing the Cd uptake by Ricinus Communis L. and minimizing the Cd toxicity under polluted soil. Our results highlighted that the castor is a good choice to remediate and tolerate the Cd stress environment.

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References

Adriano, D. C. (2001). Trace elements in terrestrial environments: biogeochemistry, bioavailability, and risks of metals (Vol. 860). New York: Springer. https://doi.org/10.1007/978-0-387-21510-5

Arshad, M., Ali, S., Noman, A., Ali, Q., Rizwan, M., Farid, M., & Irshad, M. K. (2016). Phosphorus amendment decreased cadmium (Cd) uptake and ameliorates chlorophyll contents, gas exchange attributes, antioxidants, and mineral nutrients in wheat (Triticum aestivum L.) under Cd stress. Archives of Agronomy and Soil Science, 62(4), 533-546.

Baudddh, K., & Singh, R. P. (2012). Growth, tolerance efficiency and phytoremediation potential of Ricinus communis (L.) and Brassica juncea (L.) in salinity and drought affected cadmium contaminated soil. Ecotoxicology and Environmental safety, 85, 13-22.

Chaoui, A., Mazhoudi, S., Ghorbal, M. H., & El Ferjani, E. (1997). Cadmium and zinc induction of lipid peroxidation and effects on antioxidant enzyme activities in bean (Phaseolus vulgaris L.). Plant Science, 127(2), 139-147.

Chhajro, M. A., Rizwan, M. S., Guoyong, H., Jun, Z., Kubar, K. A., & Hongging, H. (2016). Enhanced accumulation of Cd in castor (Ricinus communis L.) by soil-applied chelators. International journal of phytoremediation, 18(7), 664-670.

Chhajro, M. A., Fu, Q., Shaaban, M., Rizwan, M. S., Jun, Z., Salam, A., & Jamro, G. M. (2018). Identifying the functional groups and the influence of synthetic chelators on Cd availability and microbial biomass carbon in Cd-contaminated soil. International journal of phytoremediation,
Effects of cadmium on plant growth and physiological traits in contrast wheat recombinant inbred lines differing in cadmium tolerance. *Chemosphere*, **77**(11), 1620-1625.

Farooq, M. A., Ali, S., Hameed, A., Ishaque, W., Mahmood, K., & Iqbal, Z. (2013). Alleviation of cadmium toxicity by silicon is related to elevated photosynthesis, antioxidant enzymes; suppressed cadmium uptake and oxidative stress in cotton. *Ecotoxicology and environmental safety*, **96**, 242-249.

Gallego, S. M., Benavides, M. P., & Tomaro, M. L. (1996). Effect of heavy metal ion excess on sunflower leaves: evidence for involvement of oxidative stress. *Plant Science*, **121**(2), 151-159.

Guo, H., Hong, C., Chen, X., Xu, Y., Liu, Y., Jiang, D., & Zheng, B. (2016). Different growth and physiological responses to cadmium of the three *Miscanthus* canes. *PloS one*, **11**(4), e0153475.

Heath, R. L., & Packer, L. (1968). Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of biochemistry and biophysics*, **125**(1), 189-198.

Huang, H., Yu, N., Wang, L., Gupta, D. K., He, Z., Wang, K., & Yang, X. E. (2011). The phytoextraction potential of bioenergy crop *Ricinus communis* for DDTs and cadmium co-contaminated soil. *Bioresource Technology*, **102**(23), 11034-11038.

Khan, N. A., Samiullah, Singh, S., & Nazar, R. (2007). Activities of antioxidant enzymes, sulphur assimilation, photosynthetic activity and growth of *Triticum aestivum* cultivars differing in yield potential under cadmium stress. *Journal of Agronomy and Crop Science*, **193**(6), 435-444.

Lacan, D., & Baccou, J. C. (1998). High levels of antioxidant enzymes correlate with delayed senescence in nonnetted muskmelon fruits. *Planta*, **204**(3), 377-382.

Liu, S. L., Yang, R. J., Ma, M. D., Dan, F., Zhao, Y., Jiang, P., & Wang, M. H. (2015). Effects of exogenous NO on the growth, mineral nutrient content, antioxidant system, and ATPase activities of *Trifolium repens* L. plants under cadmium stress. *Acta Physiologiae Plantarum*, **37**(1), 1-16.

Roychoudhury, A., Basu, S., & Sengupta, D. N. (2012). Antioxidants and stress-related metabolites in the seedlings of two indica rice varieties exposed to cadmium chloride toxicity. *Acta Physiologiae Plantarum*, **34**(3), 835-847.

Shi, G., & Cai, Q. (2009). Cadmium tolerance and accumulation in eight potential energy crops. *Biotechnology Advances*, **27**(5), 555-561.

Shi, G., Xia, S., Ye, J., Huang, Y., Liu, C., & Zhang, Z. (2015). PEG-simulated drought stress decreases cadmium accumulation in castor bean by altering root morphology. *Environmental and Experimental Botany*, **111**, 127-134.

Szőlősi, R., Varga, I. S., Erdei, L., & Mihalik, E. (2009). Cadmium-induced oxidative stress and antioxidant mechanisms in germinating Indian mustard (*Brassica juncea* L.) seeds. *Ecotoxicology and environmental safety*, **72**(5), 1337-1342.

Bor, M., Özdemir, F., & Türkan, I. (2003). The effect of salt stress on lipid peroxidation and antioxidants in leaves of sugar beet *Beta vulgaris* L. and wild beet *Beta maritima* L. *Plant science*, **164**(1), 77-84.

Vaculik, M., Lux, A., Luxová, M., Tanimoto, E., & Lichtschaidl, I. (2009). Silicon mitigates cadmium inhibitory effects in young maize plants. *Environmental and Experimental Botany*, **67**(1), 52-58.

Wu, Z., Zhao, X., Sun, X., Tan, Q., Tang, Y., Nie, Z., ... & Hu, C. (2015). Antioxidant enzyme systems and the ascorbate–glutathione cycle as contributing factors to cadmium accumulation and tolerance in two oilseed rape cultivars (*Brassica napus* L.) under moderate cadmium stress. *Chemosphere*, **138**, 526-536.

Xi, T., Xing, H., Shi, W., Wu, Y., & Zhou, P. (2012). Preparation and characterization of artificial antigens for cadmium and lead. *Biological trace element research*, **150**(1), 411-417.

Xu, X., Liu, C., Zhao, X., Li, R., & Deng, W. (2014). Involvement of an antioxidant defense system in the adaptive response to cadmium in maize seedlings (*Zea mays* L.). *Bulletin of environmental contamination and toxicology*, **93**(5), 618-624.

Zhang, H., Guo, Q., Yang, J., Shen, J., Chen, T., Zhu, G., & Shao, C. (2015). Subcellular cadmium distribution and antioxidant enzymatic activities in the leaves of two castor (*Ricinus communis* L.) cultivars exhibit differences in Cd accumulation. *Ecotoxicology and environmental safety*, **120**, 184-192.

Zhang, X., Chen, D., Zhong, T., Zhang, X., Cheng, M., & Li, X. (2015). Assessment of cadmium (Cd) concentration in arable soil in China. *Environmental Science and Pollution Research*, **22**(7), 4932-4941.

Zhou, W., & Qiu, B. (2005). Effects of cadmium hyperaccumulation on physiological characteristics of *Sedum alfredii* Hance (Crassulaceae). *Plant Science*, **169**(4), 737-745.