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Inhibitory Effect of Cadmium on the Growth of Mung Bean (*Vigna radiata* (L.) R. Wilczek) and the Removal by Chelating Agent

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Abstract

This research studied the effect of cadmium (Cd) on the growth of mung bean (*Vigna radiata* (L.) R. Wilczek). The mung bean was cultured in a Hoagland solution containing different concentrations of Cd (0, 0.1, 0.3 and 0.5 mg/L) for 5 days. The result showed a significant decrease in the lengths of the roots and shoots of mung bean that was grown in cadmium solution. This effect was proportional to the concentrations of Cd. To assess cell death in the root of mung bean, Evan's blue staining technique was used in this study. The results showed that the concentrations of Evan's blue dye taken up by Cd-exposed mung beans at 0.1, 0.3, and 0.5 mg/L were 1.5612 ± 0.5417, 6.8641 ± 1.7447, and 8.0850 ± 2.6336 mg/L, respectively. A concentration-dependent increase of dead cells was found in the Cd-treated group, mostly at the root cap zone. With respect to this result, the level of dead cells that was stained with Evan's blue dye could be used as a biomarker to indicate cadmium contamination in water. Furthermore, the effects of chelating agents (EDTA) on cadmium removal were also studied. The results showed the possibility of using EDTA as a cadmium treatment agent and promoted plant growth in cadmium contamination areas.

Keywords: Cadmium toxicity, Mung bean, Evan's blue, Chelating agent

1. Introduction

Various technologies, particularly in the industrial and agricultural sectors, have been developed recently for convenience working. However, these developments, including mining, plating and agricultural industries, have had an impact on the environment. These industries have polluted the environment with toxic metals, which have been found in the soil, water, and air (Rafiq et al., 2014; Zhang & Reynolds, 2019). Some of these metals, like cadmium (Cd), are the hazardous substances in ecosystems that are most abundantly and omnipresently concerned with human health (Zhang & Reynolds, 2019). Due to their industrial applications, such as Ni-Cd batteries, color pigments, plastic stabilizers, smelters, and alloys, the amount of Cd output in the environment is dramatically increasing (Kubier, Wilkin, & Pichler, 2019). There have been reports of Cd contamination in soil and water in countries such as France, Belgium, China, South Korea, and Japan (Rizwan, Ali, Rehman, & Maqbool, 2019). The concentration of contamination varies by location, for example France has the highest Cd concentration in soil (16.7 mg/kg), followed by Belgium (7.61 mg/kg) and China (7.43 mg/kg). Cd concentrations in Taiwan were

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found to be 0.4 mg/kg however, after the establishment of the electroplating industry, it increased to 30 mg/kg (Rafiq et al., 2014). Pluemphuak, Mala, and Kumlung (2014) conducted a study on the contents of Cd that contaminated rice fields in Tak Province, Thailand, and found that Cd concentrations were greater than the standard limit (11.45-46.87 mg/kg).

Cd is absorbed and accumulated by plants, which can cause damage to the cell. This can be a physical and genetic influence on the plant (He, Yang, He, & Baligar, 2017; Parmar, Kumari, & Sharma, 2013). A number of studies have been conducted to investigate the effects of Cd on plants (Rizwan et al., 2019). Riceberry rice (*Oryza sativa*), for example, exhibited lower growth rate and pigmentation, as well as increased root cell toxicity, when exposed to high levels of Cd (Thaenghin, Pewnim, & Nakphayphan, 2017). A study of pea roots in Cd-contaminated wastewater treatment revealed that at Cd concentrations more than 30 mM, root growth changed significantly (Lima, Pereira, Figueira, Caldeira, & de Matos Caldeira, 2006). Furthermore, the influence of Cd on photosynthesis, nutrition, and growth of mung bean was studied, and the results showed that mung bean was resistant to low concentrations of Cd, and it was accumulated in roots, leaves, and stems (Wahid, Ghani, & Javed, 2008). Several studies have shown that Cd is hazardous to plants and other organisms, hence limiting consumption and minimizing Cd are crucial. Furthermore, Cd contamination should be detected early before it has a deleterious impact on ecosystems and public health.

Although measuring Cd concentration in water is an important method for determining the safety of water sources, it does not indicate the safe level of Cd concentration in aquatic organisms. A sensible approach for measuring the biological effect and assessing environmental quality is recently presented using cell or molecular biomarkers. Plants are sessile organisms susceptible to a range of stress factors such as Cd (Jaskulak & Grobelak, 2019). The ability of the cell to maintain changing external conditions is determined by the plant membrane, which can be used as a biomarker in the evaluation of cell damage or stress-induced death (Rizwan et al., 2019). Evan's blue staining technique has been established for the simultaneous monitoring of stress by cell death and membrane damage (Oprisko, Green, Beard, & Gates, 1990; Smith, Reider, & Fletcher, 1982; Vemanna et al., 2017). Evan's blue is a non-permitting, acidic exclusion dye that stains dead or damaged cells. The dye does not enter living cells with strong membranes. These stained cells can be characterized qualitatively by a light microscope. The blue dye can also be extracted from stained cells and quantified using a spectrophotometer. The amount of cells stained with blue dye under various conditions can be used as a measure of cellular stress by the correlation of dye in positively stained cells with the level of cell membrane damage (Vijayaraghavareddy, Adhinarayanreddy,Vemanna, Sreeman, & Makarla, 2017).

Mung bean (*Vigna radiata* L.) is a common legume crop throughout Asia and Thailand, and it is an important component of many cropping systems (Asim, Aslam, Hashmi, & Kisana, 2006). It is a food source and is cultivated for consumption and export. Thailand's exports to the Philippines, India and Singapore are approximately 1.26 million USD. According to the Organization for Economic Cooperation and Development (OECD) and the American Society for Testing Materials (ASTM), mung bean is an appropriate plant for toxicity experiments because it is sensitive to metal contamination and is also easy to grow and cultivate in a short period of time (Lee, Kwak, & An, 2012). As a reason, mung bean was chosen as the experiment plant for Cd toxicity in water in this study.

A chelating agent plays a key role in environmental adsorption of heavy metals. Ethylenediaminetetraacetic acid (EDTA) is a common chelating agent that is widely used in industry to sequester metal ions in aqueous solutions (Peraferrer, Martínez, Poch, & Villaescusa, 2012). EDTA is a hexadentate ligand that forms highly stable complexes with most transition metals, including Cd. It bonds these metals by forming a stable coordination complex with the donor atoms, two N atoms, and four O atoms of the unidentate carboxylate groups (Zheng, 2001). Because of its ability to bind metal ions, EDTA is frequently used

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for a range of applications, including reducing metal concentrations in contaminated environments. Studies on the adsorption of Cd and nickel metals in sunflowers by EDTA and HEDTA have been published. The study demonstrated the high efficacy of EDTA and HEDTA, although the plant species had limits (Chen et al., 2001). In addition, EDTA and citric acid were utilized for Cd water treatment with water hyacinth (*Eichornia speciosa*) (Kongmuang & Sampanpanish, 2010). Thus, the proposed study's aim was to investigate the effects of different Cd concentrations on mung bean growth and Cd toxicity using Evan's blue technique. This study also focused on the role of chelating agents (EDTA) in reducing the Cd effect.

2. Materials and Methods

2.1 Mung bean cultivation and treatment

Mung bean (*Vigna radiata* (L.) R. Wilczek) seeds were purchased from a supermarket in Chombueng, Ratchaburi, Thailand. Mung bean seeds germinated on wet tissue paper at room temperature and in the dark. After 24 hours, seedlings with root lengths of 1-2 mm were chosen and cultivated on diluted liquid Hoagland solution (Vijayaraghavareddy et al., 2017), containing $CdCl_2$ (Sigma-Aldrich Co., USA) concentrations of 0, 0.1, 0.3, and 0.5 mg/L. Cultivating the seedlings was accomplished by passing the roots through a plastic mesh disc (approximately 10 seedlings per 5 cm diameter disc). The discs were floating in 250 mL of solution that was kept under a light and dark cycle of 12:12 hours at room temperature for 5 days. The length of the root and shoot was measured every day of the experiment.

2.2 Quantification of membrane damage/ cell death using Evan's blue staining technique

According to Lehotai et al. (2011) Evan's blue staining was used to determine the toxicity of Cd on mung bean root. Evan's blue (Sigma-Aldrich Co., USA) solution at 0.1% (w/v) was being prepared. The mung bean root was cut into 1 cm lengths on ice and incubated in Evan's Blue solution for 15 min. As a positive control, the root was incubated in 1% Triton-X solution for 15 min. They were then rinsed thrice in distilled water or until unbound dye washes out from the root surface. Each mung bean root was photographed at 100x magnification using a light microscope (Olympus BX50). To quantify Evan's blue stain taken up due to membrane instability, the dye must be extracted from the roots using a mortar and pestle and homogenized in a destaining solution containing 1% Sodium Dodecyl Sulfate (SDS). Centrifuge the extract at 3,000 *x g* for 5 min at room temperature to elute the dye into the solution and to remove the debris. The absorbance of a blue supernatant was measured at 600 nm with a spectrophotometer using the destaining reagent as a blank. Concentration of Evan's blue can be estimated by referring to a standard curve. The amount of dye accumulated in positively stained cells correlates with the extent of cell membrane damage, hence the number of cells stained with Evan's blue dye can be used as a cellular death indicator. Samples from three independent experiments were measured with a pool of 10 plants.

2.3 Efficiency of Cd detoxification by chelating agents

In this study, EDTA was used as a chelator to evaluate the effects of chelating agents in reducing the Cd effect on Mung bean seedlings. There were five groups in the experiments. As a control group, the germinated mung bean was placed in a Hoagland solution. For the first experiment group, the germinated mung beans were placed in a Hoagland solution containing 0.5 mg/L EDTA. Secondly, the germinated mung beans were placed in a Hoagland solution containing 0.5 mg/L Cd. Thirdly, the germinated mung beans were placed in a Hoagland solution containing 0.5 mg/L EDTA and 0.5 mg/L Cd. Finally, the germinated mung beans were placed in a Hoagland solution containing 1 mg/L EDTA and 0.5 mg/L Cd. Root and shoot lengths were measured and recorded for 3 days in each experiment.

2.4 Statistical analysis

All the experiments were carried out in three replicates. The results are shown as mean ± SD.

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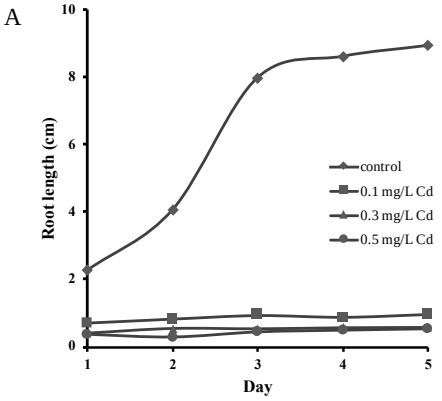
3. Results and Discussion

3.1 The effects of Cd on mung bean growth

According to Figure 1, the results showed that the growth of mung bean in the control and Cd treatment groups was clearly different, and the Cd concentration was inversely related to the growth. When compared with the control group, the length of the roots and shoots was inhibited in seedlings exposed to lower Cd concentrations and decreased significantly in seedlings exposed to higher Cd concentrations. This suggested that Cd had an effect on the root and shoot when it germinated, resulting in a decrease on mung bean growth. According to Figure 1A and 1B, Cd inhibited root growth since day one and caused brown stains on the roots. The shortening and browning of the roots exhibited in plants may suggest root cell damage caused by Cd. This can be explained by the fact that the root entered into direct contact and absorbed Cd before transferring it to the shoot. When Cd enters plant cells, Cd could inhibit protein synthesis, such as phosphoenol-pyruvate carboxylase (Shanmugaraj, Malla, & Ramalingam, 2019; Thaenghin et al., 2017), as well as bind to biological molecules, causing conformational and functional changes (Holubek et al., 2020). Cd, which is present in roots, shoots, and leaves, can accumulate in plant tissue and harm the plant throughout time (Wahid et al., 2008). According to this study, Cd may accumulate in a variety of mung bean parts, particularly the root. In this regard, the mung bean, like other leguminous plants, was sensitive to Cd (Aqeel et al., 2021; Geuns et al., 1997) and can be used to monitor the level of Cd in contaminated soil and water.



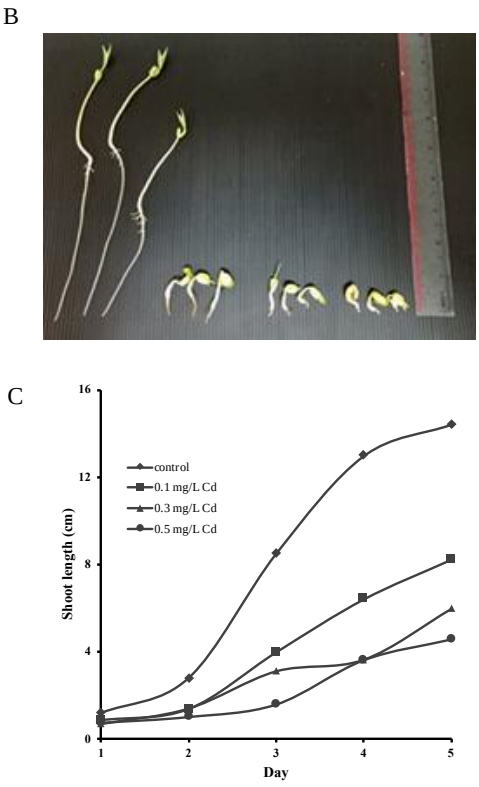


Figure 1. The growth of mung bean under various $CdCl_2$ concentrations for 5 days. A) Mung bean root growth decrease; B) Root and shoot morphology at day 5 ($CdCl_2$ concentrations of 0, 0.1, 0.3, and 0.5 mg/L from left to right); C) Mung bean shoot growth decrease.

3.2 Cell death and membrane integrity

Evan's blue staining technique was used to evaluate root cell damage caused by Cd exposure. The result showed that the roots of Cd-exposed mung bean seedlings uptake more blue dye than the control, suggesting that Cd caused membrane damage, permitting the dye to permeate through the cell. The higher concentration of Cd also resulted in a statistically significant increase in blue dye uptake, indicating an increase in the number of membrane damaged or dead cells. The Cd-exposed mung bean exhibited a concentration-dependent increase in dead cells, most of which were found in the root cap zone (Figure 2A). Plant cell membranes are semipermeable membranes that allow only certain substances to pass through cells. As a result, Evan's blue dye cannot pass through a living cell, but it can pass through death cells (Holubek et al., 2020). Furthermore, the concentration of Evan's blue dye in the root could well be conducted to assess Cd-induced root cell death (Thaenghin et al., 2017). The

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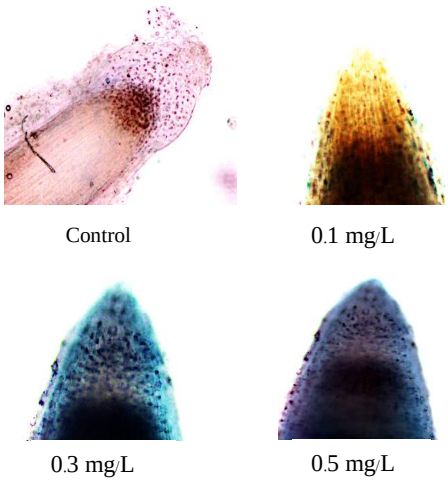
spectrophotometric method was used to assess root cell death by measuring the quantity of Evans' blue dye in the root. The results showed that the concentrations of Evan's blue dye taken up by Cd-exposed mung beans at 0.1, 0.3, and 0.5 mg/L were 1.5612 ± 0.5417, 6.8641 ± 1.7447, and 8.0850 ± 2.6336 mg/L, respectively (Figure 2B). According to the results of this study, the Cd-treated group absorbed more dyes than the control group. It also showed an increase in dye uptake that was relative to the higher concentration of the Cd-treated group, indicating a large increase in dead cells. This suggests that Cd causes cell death.

3.3 Efficiency of Cd detoxification by chelating agent

As a chelating agent for heavy metal adsorption, EDTA was employed in this study. According to Figure 3, the results showed that when chelators EDTA were mixed with Cd in a 1 to 1 ratio (0.5 mg/L Cd and 0.5 mg/L EDTA), there was a slight increase in growth of root and shoot of mung bean compared to the Cd treatment group on day 1 and a considerable increase on day 2 and day 3. When the ratio of EDTA and Cd was increased to 1 to 2 (0.5 mg/ L Cd and 1. 0 mg/ L EDTA) , there was a significant increase in root and shoot growth since day 1. This demonstrated that EDTA can minimize Cd toxicity and allow mung beans to grow normally in Cd- contaminated solution. According to the findings of several studies, EDTA could bind and remove heavy metals from Cd- contaminated conditions, which cause plant growth. Similarly, Bacaha et al. (2015) investigated the effect of Cd and EDTA treatment on plant growth (*Sorghum bicolor*).

A



Control 0.1 mg/L

0.3 mg/L 0.5 mg/L

B

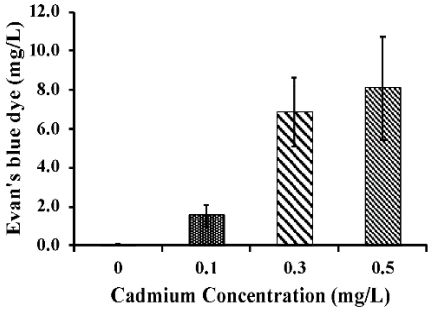


Figure 2. A) Microscopic detection of Evan's blue dye stain on mung bean root tips and B) the level of Evan's blue dye uptake in relation to the control by spectrophotometer measurements.

The results revealed that application of Cd and EDTA adversely affected shoot length, fresh weight and dry weight of *S. bicolor*. This implies that the hexadentate chelating agent EDTA could form a complex with Cd by bonding to it at six different sites. EDTA functions as a strong chelator to bind Cd, forming a Cd-EDTA complex which can reduce Cd accumulation and toxicity in plants. Additionally, Kongmuang and Sampanpanish (2010) investigated whether EDTA and citric acid may improve the performance of water hyacinth as a Cd adsorption agent in synthetic wastewater. The results showed that EDTA treatment could remove Cd more effectively than citric acid treatment.

The result of this study also found that EDTA alone had no effect on mung bean growth. Despite the fact that EDTA is a useful agent for Cd detoxification, it has a low harmful impact on both plants and the environment. Since EDTA degrades slowly and is a persistent organic pollutant, it can cause significant environmental problems. There have been some reports of concerns if we use it in high concentrations (Gonsior, Sorci, Zoellner, & Landenberger, 1997; Xie, 2009).

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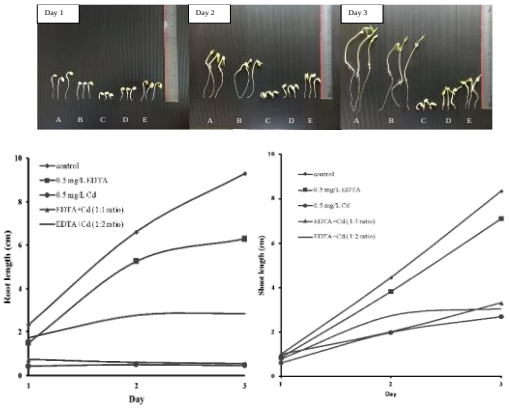

Figure 3. Morphology, root and shoot length of mung bean under EDTA and Cd treatments for 3 days, when mung bean germinated in A) distilled water, B) 0.5 mg/L EDTA, C) 0.5 mg/L Cd, D) 0.5 mg/L Cd and 0.5 mg/L EDTA (1:1 ratio), E) 0.5 mg/L Cd and 1.0 mg/L EDTA (1:2 ratio).

4. Conclusions

The results demonstrate clearly that Cd has an effect on mung bean root and shoot growth and is even harmful to plants in small concentrations. Following Cd exposure, the number of cell death stained with Evan's blue dye was found to be high in the mung bean root cap zone, indicating that Cd caused cell death. In a chelating agent application study, EDTA effectively detoxified Cd while also promoting root and shoot growth in mung beans. This is confirmed using chelators such as EDTA in the detoxification of heavy metals from the environment. According to Oviedo and Rodríguez (2003), EDTA has poor biodegradability, hence the study should use another chelator with greater biodegradability than EDTA. Mung bean plants grow well and much faster than other plants, and also show a reduction in root growth and cell death after Cd exposure. Instead of just employing costly and sophisticated methods to determine Cd, mung bean has the potential to be a sensitive tool or as a bioindicator for detecting Cd and monitoring Cd contamination in the environment. The most sensitive parameters to measure when using mung bean as a bioindicator for Cd exposure are root and shoot growth reduction, change in root tip brown color, and, most importantly, the quantity of root cell damage. However, additional study is needed to consider the possibility of Cd stress having an effect on pigment reduction, Cd accumulation, and other stress-induced changes in mung bean.

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Conflict of Interest

No conflict of interest.

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References

Aqeel, M., Khalid, N., Tufail, A., Ahmad, R. Z., Akhter, M. S., Luqman, M., ... Noman, A. (2021). Elucidating the distinct interactive impact of cadmium and nickel on growth, photosynthesis, metal-homeostasis, and yield responses of mung bean (*Vigna radiata* L.) varieties. *Environmental Science and Pollution Research*, *28*(21), 27376-27390. doi:0.1007/s11356-021-12579-5

Asim, M., Aslam, M., Hashmi, N. I., & Kisana, N. S. (2006). Mungbean (*Vigna radiata*) in wheat based cropping system: An option for resource conservation under rainfed ecosystem. *Pakistan Journal of Botany*, *37*(4), 1197-1204.

Bacaha, N., Shamas, R., Bakht, J., Rafi, A., Farhatullah, & Gillani, A. (2015). Effect of heavy metal and EDTA application on plant growth and phyto-extraction potential of Sorghum (Sorghum bicolor). *Pakistan Journal of Botany*, *47*(5), 1679-1684.

Chen, H., & Cutright, T. (2001). EDTA and HEDTA effects on Cd, Cr, and Ni uptake by *Helianthus annuus*. *Chemosphere*, *45*(1), 21-28. doi:10.1016/S0045-6535(01)00031-5

Geuns J. M. C., Cuypers, A. J. F., Michiels, T., Colpaert J. V., Laere, A., Van Den Broeck, K. A. O., & Vandecasteele, C. H. A. (1997). Mung bean seedlings as bio-indicators for soil and

water contamination by cadmium. *Science of The Total Environment*, *203*(3), 183-197. doi:10.1016/S0048-9697(97)00146-0

Gonsior, S. J., Sorci, J. J., Zoellner, M. J., & Landenberger, B. D. (1997). The effects of EDTA on metal solubilization in river sediment/water systems. *Journal of Environmental Quality*, *26*(4), 957-966. doi:10.2134/jeq1997.00472425002600040005x

He, S., Yang, X., He, Z., & Baligar, V. C. (2017). Morphological and physiological responses of plants to cadmium toxicity: A review. *Pedosphere*, *27*(3), 421-438. doi:10.1016/S1002-0160(17)60339-4

Holubek, R., Deckert, J., Zinicovscaia, I., Yushin, N., Vergel, K., Frontasyeva, M., ... Chmielowska-Bąk, J. (2020). The recovery of soybean plants after short-term cadmium stress. *Plants*, *9*(6), 782. doi:10.3390/plants9060782

Jaskulak, M., & Grobelak, A. (2019). Cadmium phytotoxicity– Biomarkers. In M. Hasanuzzaman, M. N. V. Prasad, & K. Nahar (Eds.), *Cadmium tolerance in plants* (pp. 177-191). Academic Press.

Kongmuang, K., & Sampanpanish, P. (2010). Effect of EDTA and citric acid on cadmium uptake by water hyacinth. *Proceedings of the Maefahluang Symposium*. Chiangrai province, Thailand.

Kubier, A., Wilkin, R. T., & Pichler, T. (2019). Cadmium in soils and groundwater: A review. *Applied Geochemistry*, *108*, 104388. doi:10.1016/j.apgeochem.2019.104388

Lee, W. M., Kwak, J. I., & An, Y. J. (2012). Effect of silver nanoparticles in crop plants *Phaseolus radiatus* and *Sorghum bicolor*: Media effect on phytotoxicity. *Chemosphere*, *86*(5), 491-499. doi:10.1016/j.chemosphere.2011.10.013

Lehotai, N., Pető, A., Bajkán, S., Erdei, L., Tari, I., & Kolbert, Z. (2011). In vivo and in situ visualization of early physiological events induced by heavy metals in pea root meristem. *Acta Physiologiae Plantarum*, *33*(6), 2199-2207. doi:10.1007/s11738-011-0759-z

Lima, A. I. G., Pereira, S. I. A., Figueira, E. M. A. P., Caldeira, G. C. N., & de Matos Caldeira, H. D. Q. (2006). Cadmium detoxification in roots of *Pisum sativum* seedlings: Relationship between toxicity levels, thiol pool alterations and growth. *Environmental and Experimental Botany*, *55*(1-2), 149-162. doi:10.1016/j.envexpbot.2004.10.008

Oprisko, M. J., Green, R. L., Beard, J. B., & Gates, C. E. (1990). Vital staining of root hairs in 12 warm□season perennial grasses. *Crop Science*, *30*(4), 947-950. doi: 10. 2135/ cropsci1990. 0011183X003000040039x

Oviedo, C., & Rodríguez, J. (2003). EDTA: The chelating agent under environmental scrutiny. *Quimica Nova*, *26*, 901-905. doi:10.1590/S0100-40422003000600020

Parmar, P., Kumari, N., & Sharma, V. (2013). Structural and functional alterations in photosynthetic apparatus of plants under cadmium stress. *Botanical Studies*, *54*(1), 1-6. doi:10.1186/1999-3110-54-45.

Peraferrer, C., Martínez, M., Poch, J., & Villaescusa, I. (2012). Toxicity of metal–Ethylenediaminetetraacetic acid solution as a function of chemical speciation: An approach for toxicity assessment. *Archives of Environmental Contamination and Toxicology*, *63*(4), 484-494. doi:10.1007/s00244-012-9788-x

Pluemphuak, T., Mala, T., & Kumlung, A. (2014). Cadmium contents in rice grown in Cd contaminated paddy fields in Mae Tao floodplains Tak Province Thailand. *Journal of Science and Technology*, *3*(2), 26-38.

Rafiq, M. T., Aziz, R., Yang, X., Xiao, W., Rafiq, M. K., Ali, B., & Li, T. (2014). Cadmium phytoavailability to rice (*Oryza sativa* L.) grown in representative Chinese soils. A model to improve soil environmental quality guidelines for food safety. *Ecotoxicology and Environmental Safety*, *103*, 101-107.

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doi:10.1016/j.ecoenv.2013.10.016

Rizwan, M., Ali, S., Rehman, M. Z., & Maqbool, A. (2019). A critical review on the effects of zinc at toxic levels of cadmium in plants. *Environmental Science and Pollution Research*, *26*(7), 6279-6289. doi:10.1007/s11356-019-04174-6

Shanmugaraj, B. M., Malla, A., & Ramalingam, S. (2019). Cadmium stress and toxicity in plants: An overview. In M. Hasanuzzaman, M. N. V. Prasad, & M. Fujita (Eds.), *Cadmium toxicity and tolerance in plants* (pp. 1-17). doi:10.1016/B978-0-12-814864-8.00001-2

Smith, B. A., Reider, M. L., & Fletcher, J. S. (1982). Relationship between vital staining and subculture growth during the senescence of plant tissue cultures. *Plant Physiology*, *70*(4), 1228-1230. doi:10.1104/pp.70.4.1228

Thaenghin, P., Pewnim, T., & Nakphayphan, A. (2017). Effect of cadmium to growth rates, cytotoxicity and pigment contents in Riceberry (*Oryza sativa* L.). *Veridian E-Journal Science and Technology Silpakorn University*, *4*(3), 10-20.

Vemanna, R. S., Babitha, K. C., Solanki, J. K., Reddy, V. A., Sarangi, S. K., & Udayakumar, M. (2017). Aldo-keto reductase-1 (AKR1) protect cellular enzymes from salt stress by detoxifying reactive cytotoxic compounds. *Plant Physiology and Biochemistry*, *113*, 177-186. doi:10.1016/j.plaphy.2017.02.012

Vijayaraghavareddy, P., Adhinarayanreddy, V., Vemanna, R. S., Sreeman, S., & Makarla, U. (2017). Quantification of membrane damage/cell death using Evan's blue staining technique. *Bio-protocol*, *7*(16). doi:10.21769/BioProtoc.2519

Wahid, A., Ghani, A., & Javed, F. (2008). Effect of cadmium on photosynthesis, nutrition and growth of mungbean. *Agronomy for Sustainable Development*, *28*(2), 273-280. doi:10.1051/agro:2008010

Xie, C. Z. (2009). *Environmental impacts of effluent containing EDTA from dairy processing plants* (Doctoral dissertation). The University of Waikato, New Zealand.

Zhang, H., & Reynolds, M. (2019). Cadmium exposure in living organisms: A short review. *Science of The Total Environment*, *678*, 761-767. doi:10.1016/j.scitotenv.2019.04.395

Zheng, Z. (2001). Ligand-controlled self-assembly of polynuclear lanthanide-oxo/hydroxo complexes: From synthetic serendipity to rational supramolecular design. *Chemical Communications*, *24*, 2521-2529. doi:10.1039/b107971a