OPEN ACCESS
Haya: The Saudi Journal of Life Sciences
Abbreviated Key Title: Haya Saudi J Life Sci
ISSN 2415-623X (Print) | ISSN 2415-6221 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article

Unraveling the Growth and Physiological Responses of Spinach to Cadmium Exposure

Muhammad Ahmad[1], Abdul Basit[2*], Muhammad Sheeraz Javed[3], Shazia Zulfiqar[4], Shafiq ur Rehman[4], Marva[4], Muhammad Sharjeel[5], Kainat[4]

[1]Department of Botany, University of Education Lahore (Vehari Campus), Pakistan
[2]Department of Botany, Abdul Wali Khan University Mardan, Pakistan
[3]School of Life Sciences, Hainan Normal University, China
[4]Department of Botany, Government College University Faisalabad, Pakistan
[5]Department of Zoology, Government College University Lahore, Pakistan

DOI: 10.36348/sjls.2024.v09i06.001 | **Received:** 29.04.2024 | **Accepted:** 01.06.2024 | **Published:** 04.06.2024

***Corresponding author:** Abdul Basit
Department of Botany, Abdul Wali Khan University Mardan, Pakistan

Abstract

Vegetables are a crucial part of the food chain. Vegetables are abundant sources of proteins, fats, minerals, carbohydrates, vitamins and antioxidants. The consumption of leafy vegetables is steadily rising in both urban and rural communities. The rising content of cadmium (Cd) in the food chain through the wastewater poses a significant threat to growth of plants and human health. Cadmium interferes with plant processes like transpiration, nitrogen assimilation, photosynthesis, and respiration. Main purpose of this experiment was to investigate the side effects of various exposure durations and various Cd concentrations on the physiology and growth of spinach. This research was conducted using a factorial design with five Cd doses and three exposure durations (25, 50, and 75 days), set up according to CRD and applying three replications. Results indicated that leaf area, root length and plant height were impacted by Cadmium concentrations up to 40 mg kg^{-1}. Additionally, biochemical, and photosynthetic parameters were also affected. Spinach showed tolerance to various levels of cadmium. The highest tolerance index for spinach was observed at 10 mg Cd kg−1 soil after 50 DoE, while the lowest tolerance index was recorded at 40 mg Cd kg−1 soil after 75 DoE. Cd accumulation in the vegetables, daily intake via consumption poses significant health risks. The findings suggest that both the duration of exposure and Cd dosage are critical in determining Cd toxicity, as evidenced by significant reductions in spinach growth and physiological parameters. Consequently, the consumption of such Cd-contaminated vegetables could pose serious health risks to humans.

Keywords: Heavy metals, Cadmium, Spinach, Photosynthetic pigments, Ecotoxicology.

1. INTRODUCTION

Around 27% of vegetables are irrigated with wastewater [1]. This wastewater is considered as an appealing irrigation source due to its content of important nutrients such as iron, zinc, nitrogen, phosphorus, copper and nickel. However, it also contains harmful heavy metals like cadmium, chromium, lead and arsenic, which can pose significant health risks if consumed beyond optimum levels [2,3]. Cadmium (Cd) is considered the seventh most toxic element. Soil toxicity with cadmium in Pakistan ranges from 3.47 to 6 mg/kg, depending on soil type and region [4].

In environment cadmium is released through various natural sources, such as weathering and volcanic eruptions as well as anthropogenic activities like the manufacturing of paint pigments and plastics, production of batteries containing cadmium, use of sewage sludge, mining and phosphate fertilizers [5]. Cadmium is readily absorbed by plants, and when these plants are eaten by humans, it can lead to various problems of health. For example, when rice toxicated with cadmium were utilized in Japan, it became cause of outbreak of itai-itai disease [6]. It is now recognized that cadmium can cause a range of diseases, including lung cancer, osteoporosis, anemia and hypertension [7].

The accumulation of cadmium in parts of vegetables depends on the concentration of cadmium present in the soil [8]. Cadmium interferes with plant processes like transpiration, nitrogen assimilation,

Citation: Muhammad Ahmad, Abdul Basit, Muhammad Sheeraz Javed, Shazia Zulfiqar, Shafiq ur Rehman, Marva, Muhammad Sharjeel, Kainat (2024). Unraveling the Growth and Physiological Responses of Spinach to Cadmium Exposure. *Haya Saudi J Life Sci, 9*(6): 207-213.

photosynthesis and respiration. Numerous studies have reported the harmful effects of cadmium on seed germination, plant growth [5], chlorophyll content and CO2 fixation [9]. Studies have also elaborated the toxicity of cadmium to plants, with its effects varying depending on factors such as soil type, plant species, metal concentration in the growth medium and exposure duration [10]. Effects of cadmium exposure on crops such as wheat are well understood. But it's effects on leafy vegetables are poorly explained.

Vegetables are abundant sources of proteins, fats, minerals, carbohydrates, vitamins and antioxidants. The consumption of leafy vegetables is steadily rising in both urban and rural communities [11]. Spinach (*Spinacia oleracea* L.) is an annual leafy vegetable known for its high nutritive value and short growth cycle. The cultivation and consumption of spinach are increasing annually in Pakistan, according to statistics of 2017-18 spinach is cultivated on 8820 hectares of land.

Wastewater is major source of irrigation for vegetables grown in peri urban areas. Furthermore, recent research has revealed elevated levels of cadmium concentration in both food crops and soil in Pakistan. Peoples who consume vegetables face an increased risk of developing cadmium-related illnesses [12]. Hence, it is crucial to evaluate the risks associated with cadmium accumulation in spinach. Thus, the specific aims of this study are to investigate (1) the dose- and time-dependent impact of cadmium on the growth and physiological processes of spinach and (2) the cadmium accumulation in parts of spinach.

2. MATERIALS AND METHODS

2.1. Experimental Details

The soil was processed and air dried to determine its basic properties. The experimental soil had a EC of 1.55 ± 0.47 dS m−1, pH of 7.77 ± 0.8, nitrogen content of 0.053 ± 0.0022% and phosphorus content of 9.39 ± 1.222 mg kg−1. The soil's textural class was sandy loam, consisting of 15% clay, 55% sand and 30%. 10 kg of sieved Cd-contaminated soil (with concentrations of 0, 10, 20, 30, and 40 mg kg−1) was placed in pots. 10 seeds od spinach were cultivated in each pot and irrigation was done with distilled water. At seedling stage, thinning of plants were done to uniform five plants per pot. Data on seedling growth attributes, as well as biochemical and physiological traits, were collected at three harvests: 25, 50, and 75 exposure duration.

2.2. Collection of Data

To record data three plants from each pot were tagged randomly. Growth parameters of these tagged such as height was measured with the help of measuring tape, portable area meter was used to measure leaf area. Then tagged plants were then harvested to measure root length. To measure physiological parameters like transpiration rate (E), stomatal conductance (Gs) and photosynthetic rate (A) an infrared gas analyzer was used. These measurements were taken during daytime. During data collection, the leaf chamber molar gas flow rate was set to 248 µmol s−1, with an ambient CO2 concentration of 352 µmol mol−1. The leaf chamber temperature ranged from 36.1 to 40.4 °C, ambient pressure was 98.01 kPa, and the molar flow of air per leaf area was 221.06 mol m−2 s−1. [13] protocols were used to measure chlorophyll content.

2.3. Cadmium analysis

After collection samples of each treatment were washed with water, dried with tissue paper and then dried in oven at 70°C. Then these samples were grounded with the help of mill to form a fine powder. Then according to [14] procedure a sample of grounded powder was wet digested in a diacid mixture of HNO_3 and $HClO_4$. Then with the help of spectrophotometer, cadmium concentration in the powder was measured.

2.4. Statistical analysis of data

his research was conducted using a factorial design with five Cd doses and three exposure durations (25, 50, and 75 days), set up according to CRD and applying three replications. To assess the effects of Cd levels and exposure times on physiology, growth and Cd accumulation, a two-way (ANOVA) was conducted using Statistics 8.01. The significance between treatment means was determined using the LSD test at a 5% probability level.

3. RESULTS

In this experiment five treatments of cadmium were used for three exposure durations. ANOVA showed significant differences for treatments of cadmium and treatments × exposure duration for all physiological and growth parameters. Mean squares of ANOVA for all traits are represented in tables.

3.1. Effect of Cd on growth

Spinach showed increased plant height, deeper roots, and larger leaf areas over time in normal soil conditions, with peak growth observed at 75 DoE (Fig-1,2,3). However, these growth parameters significantly declined when exposed to cadmium (Cd). Each increase in Cd concentration led to notable reductions in plant height, root length, and leaf area, regardless of the exposure duration (DoE). Particularly in the first 25 DoE, spinach showed high sensitivity to Cd toxicity. Nonetheless, as the exposure duration increased, spinach demonstrated improved adaptation to Cd stress (Fig-1,2,3). The data indicated that soil containing 40 mg Cd kg−1 led to a reduction in root length by up to 57% and leaf area by up to 60% [15, 16], highlighting these parameters as the most sensitive indicators of Cd toxicity beyond 25 DoE.

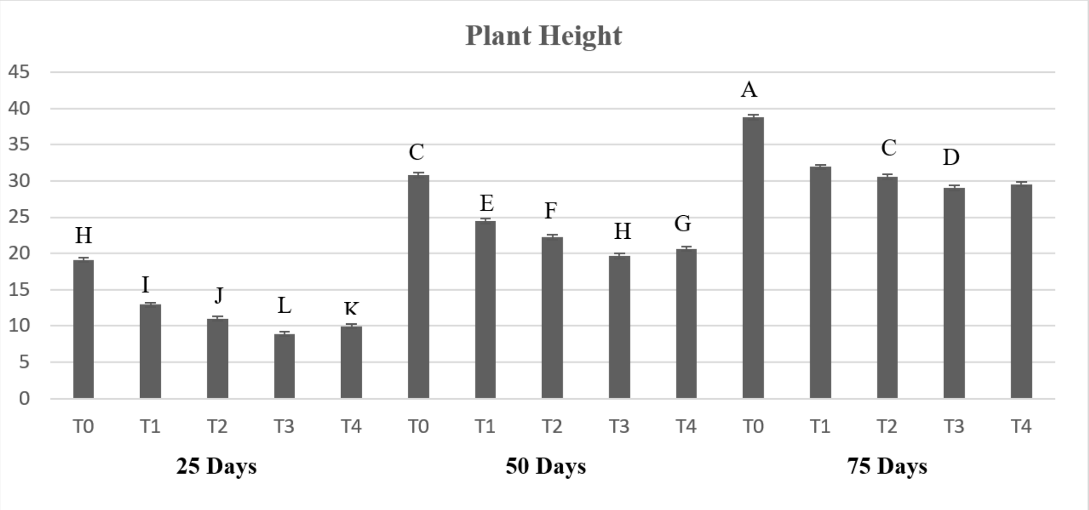

Fig-1: Graphical representation of LSD test for Plant Height

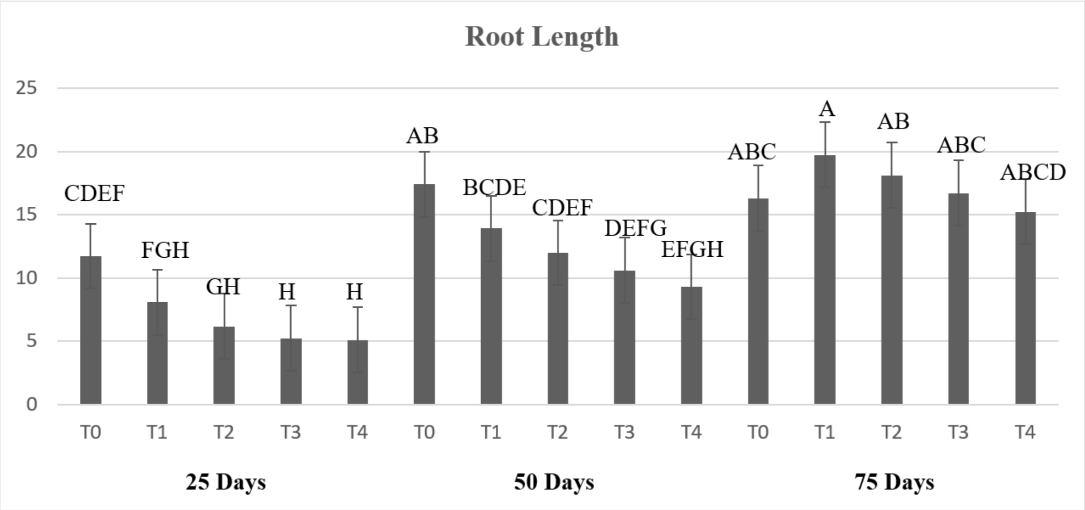

Fig-2: Graphical representation of LSD test for Root Length

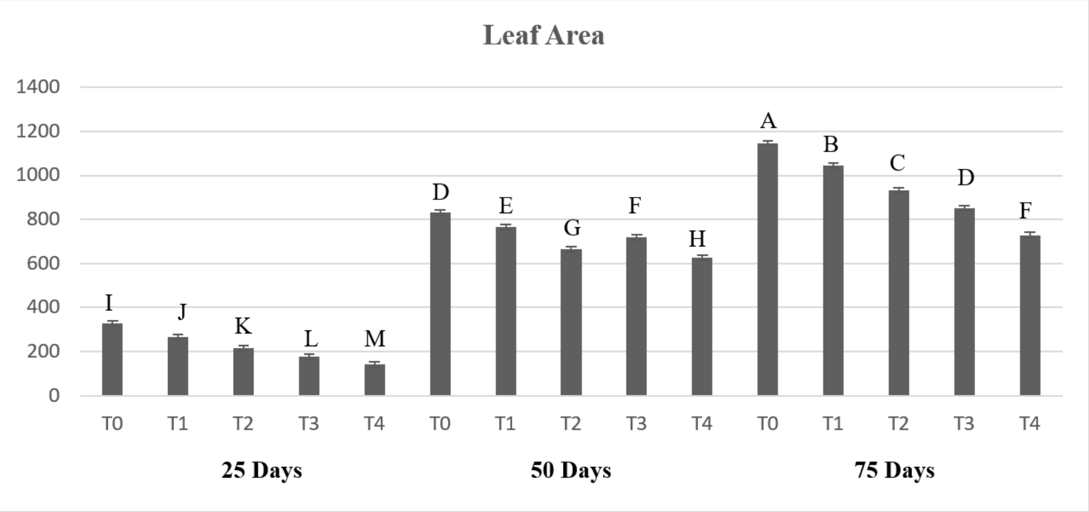

Fig-3: Graphical representation of LSD test for Leaf Area

3.2. Effect of Cd on Physiology

Spinach displayed varying responses in its physiological characteristics. Under normal soil conditions, spinach produced increasing amounts of Chl a and Chl b over time, with peak levels observed at 75 DoE. However, these chlorophyll indices significantly declined upon exposure to cadmium (Cd). As Cd concentrations increased, Chl a and Chl b levels significantly decreased at each exposure time compared to the controls (Fig-4,5). Chl a level gradually declined with increasing Cd concentrations, reaching their lowest at 40 mg Cd kg−1 soil, while Chl b exhibited different patterns, with the maximum reduction occurring at 20 mg Cd kg−1 soil [17]. Our findings suggest that Chl a and Chl b are highly sensitive indicators of Cd toxicity, irrespective of DoE.

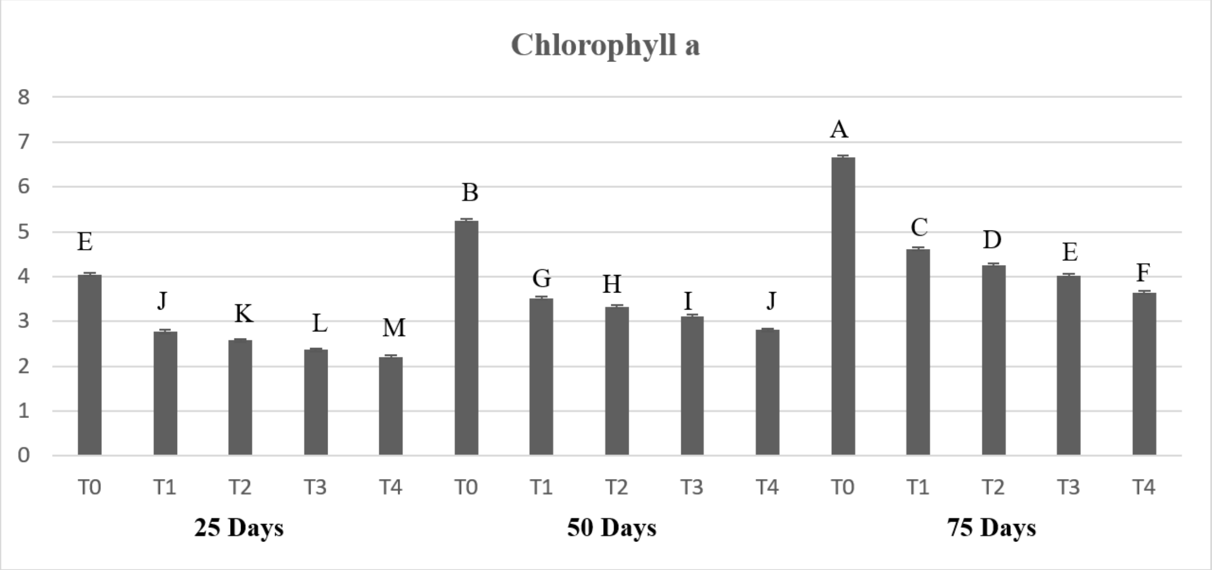

Fig-4: Graphical representation of LSD test for Chl a

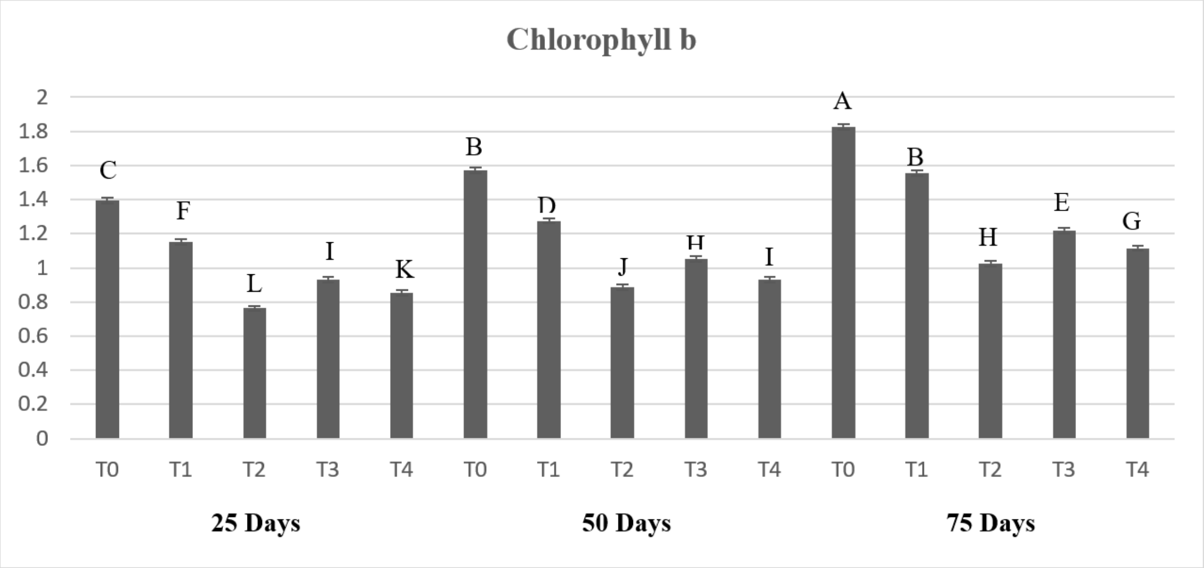

Fig-5: Graphical representation of LSD test for Chl b

Transpiration rate (E), stomatal conductance (Gs) and photosynthetic rate (A) showed a linear relationship with time irrespective of cadmium levels (Fig-6,7,8). The highest photosynthetic rate was recorded at 75 DoE, while the maximum rates for E and Gs were seen at 50 days of eposure in both normal (Cd0) and Cd-affected soils (Cd10). Notably, low Cd concentrations up to 10 mg kg−1 soil did not affect A and E; however, increasing Cd concentrations from 20 to 40 mg kg−1 soil significantly reduced A and E, regardless of the DoE. After 25 exposure duration, E were increased up to 1% at 10 mg cadmium level as compared to the control. At each exposure duration Gs showed significant decreased at 30 and 40 mg cadmium levels as compared to their controls.

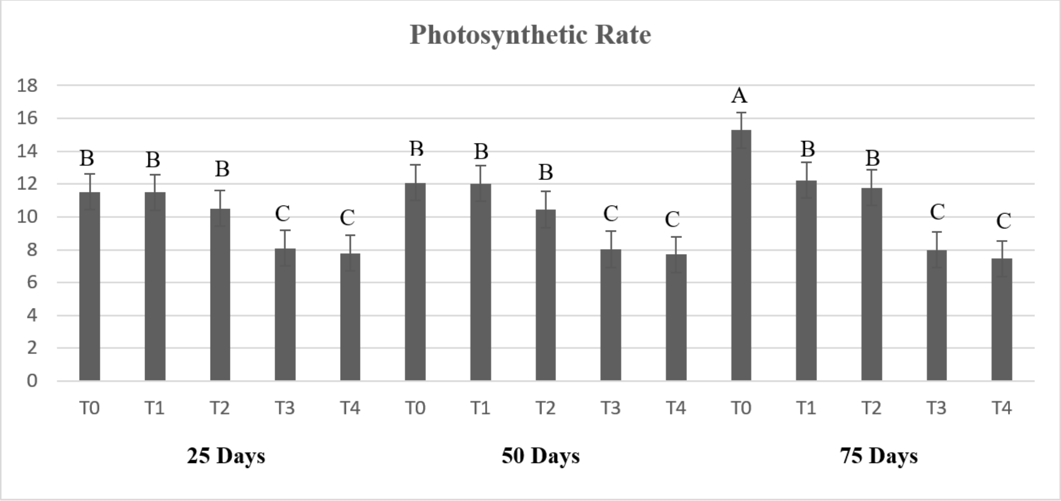

Fig-6: Graphical representation of LSD test for Photosynthetic Rate

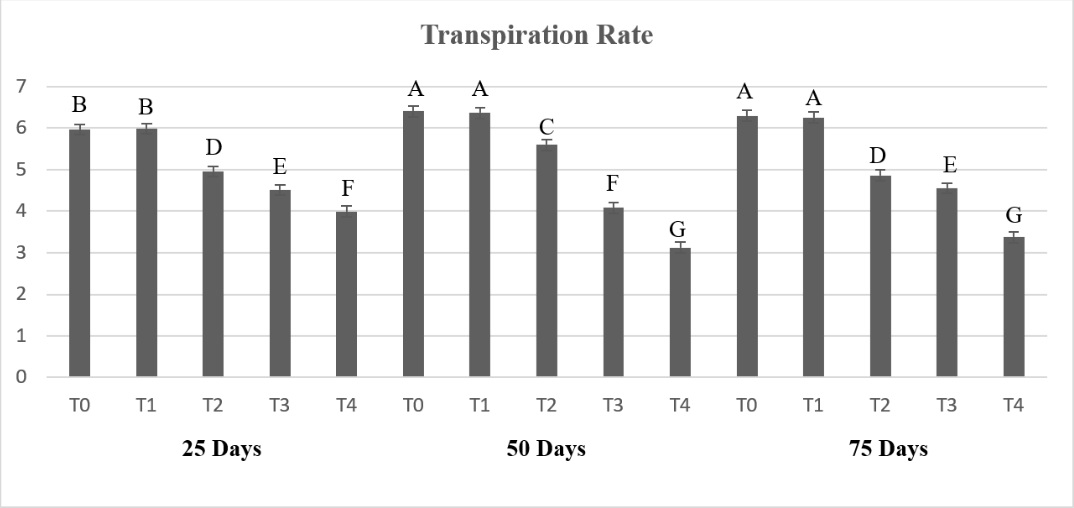

Fig-7: Graphical representation of LSD test for Transpiration Rate

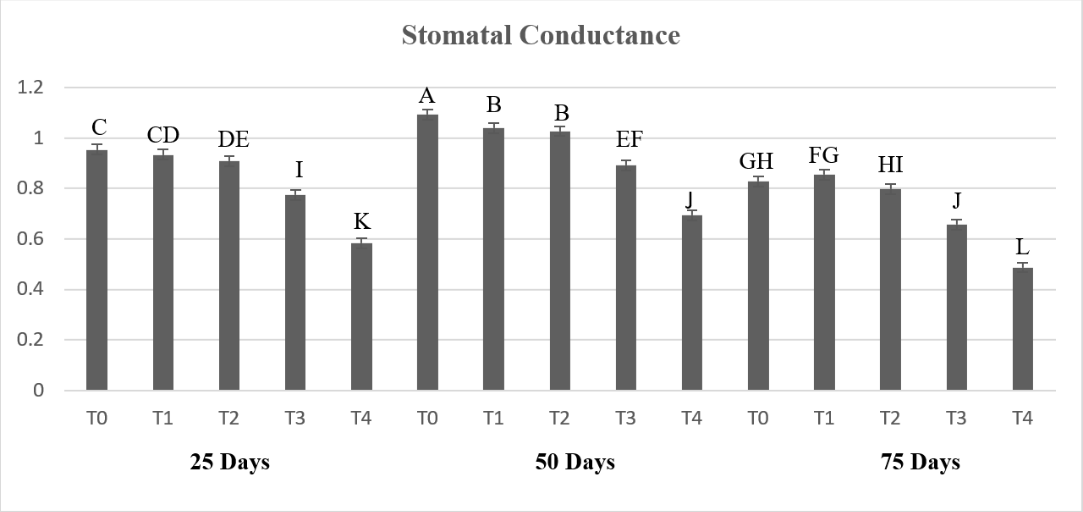

Fig-8: Graphical representation of LSD test for Stomatal Conductance

3.3. Effect of Cd on Shoot and Tolerance

Cadmium levels in shoot was dependent on both soil Cd availability and the duration of exposure (DoE) (Fig. 9). With increase of cadmium levels in soil, shoot cadmium levels were also increased, with the highest concentration observed at 40 mg Cd level [18]. Similarly, exposure duration also significantly affected on shoot cadmium levels showing maximum shoot cadmium level after 75 exposure duration.

Ratio of dry biomass in cadmium affected soil to normal soil is known as tolerance index, decreased with both increasing DoE and higher Cd doses (Fig. 10). The highest tolerance index for spinach (87.77%) was showed at 10 mg Cd level after 50 exposure duration, while the lowest tolerance index (22.87%) was recorded at 40 mg Cd level after 75 exposure duration.

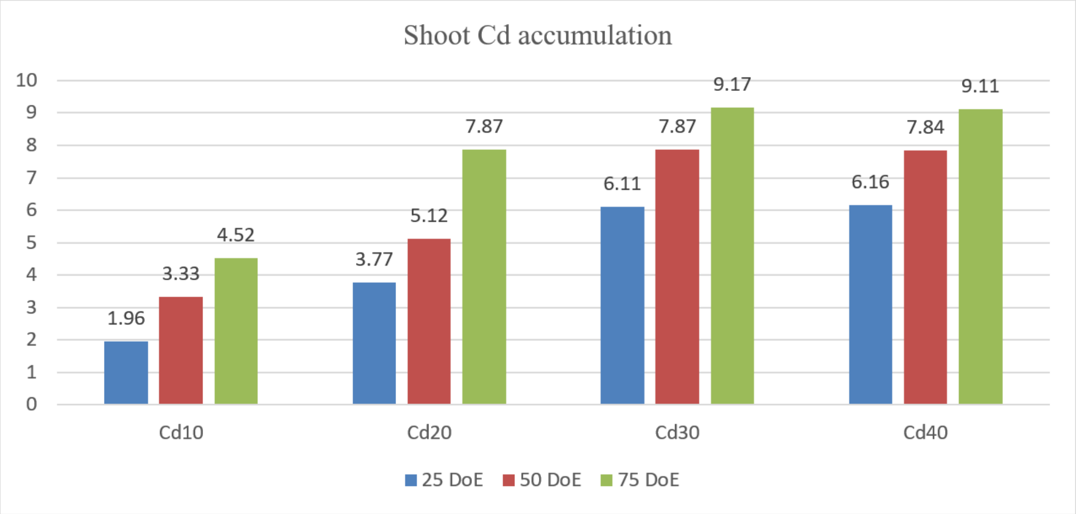

Fig-9: Graphical representation of Shoot Contamination of Spinach at various levels of Toxicity and DoE

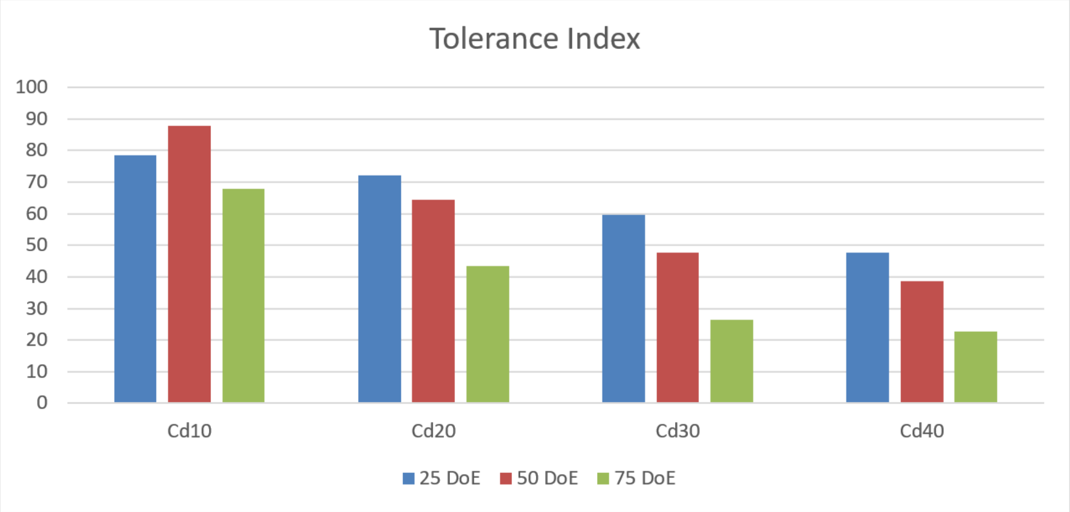

Fig-10: Graphical representation of Tolerance Indices of Spinach at various levels of Toxicity and DoE

CONCLUSIONS

This analysis concludes that the tested physiological parameters of spinach declined with an increase in Cd dose alone, rather than exposure duration. Additionally, growth attributes such as root, shoot, and leaf area of were influenced by both Cd dose and exposure duration. Similarly, these growth parameters were more sensitive to Cd, suggesting their potential as crucial markers for assessing Cd toxicity in vegetables. The highest tolerance index for spinach was observed at 10 mg Cd kg−1 soil after 50 DoE, while the lowest tolerance index was recorded at 40 mg Cd kg−1 soil after 75 DoE. Which showed that concentration of cadmium and DoE both adversely affects on spinach.

REFERENCES

1. Ensink, J. H., Mahmood, T., Van der Hoek, W., Raschid-Sally, L., & Amerasinghe, F. P. (2004). A nationwide assessment of wastewater use in Pakistan: An obscure activity or a vitally important one?. *Water policy*, 6(3), 197-206.
2. Hu, X., Jin, W., Lv, W., Cheng, S., & Jiang, Y. (2013). Investigation and evaluation on heavy metal copper and cadmium contaminations of vegetables grown in Huanggang city of China. *Advance Journal of Food Science and Technology*, *5*(2), 106-109.
3. Chen, Y., Wu, P., Shao, Y., & Ying, Y. (2014). Health risk assessment of heavy metals in vegetables grown around battery production area. *Scientia agricola*, *71*, 126-132.

4. Rehman, Z. U., Khan, S., Brusseau, M. L., & Shah, M. T. (2017). Lead and cadmium contamination and exposure risk assessment via consumption of vegetables grown in agricultural soils of five-selected regions of Pakistan. *Chemosphere*, *168*, 1589-1596.
5. Ahmad, I., Akhtar, M. J., Asghar, H. N., Ghafoor, U., & Shahid, M. (2016). Differential effects of plant growth-promoting rhizobacteria on maize growth and cadmium uptake. *Journal of plant growth regulation*, *35*, 303-315.
6. Nogawa, K., Sakurai, M., Ishizaki, M., Kido, T., Nakagawa, H., & Suwazono, Y. (2017). Threshold limit values of the cadmium concentration in rice in the development of itai-itai disease using benchmark dose analysis. *Journal of applied toxicology*, *37*(8), 962-966.
7. Zeitoun, M. M., & Mehana, E. E. (2014). Impact of water pollution with heavy metals on fish health: overview and updates. *Global veterinaria*, *12*(2), 219-231.
8. Anwar, S., Nawaz, M. F., Gul, S., Rizwan, M., Ali, S., & Kareem, A. (2016). Uptake and distribution of minerals and heavy metals in commonly grown leafy vegetable species irrigated with sewage water. *Environmental monitoring and assessment*, *188*, 1-9.
9. Ji, X., Liu, S., Juan, H., Bocharnikova, E. A., & Matichenkov, V. V. (2017). Effect of silicon fertilizers on cadmium in rice (Oryza sativa) tissue at tillering stage. *Environmental Science and Pollution Research*, *24*, 10740-10748.
10. Tran, T. A., & Popova, L. P. (2013). Functions and toxicity of cadmium in plants: recent advances and future prospects. *Turkish journal of Botany*, *37*(1), 1-13.
11. Sobukola, O. P., Adeniran, O. M., Odedairo, A. A., & Kajihausa, O. E. (2010). Heavy metal levels of some fruits and leafy vegetables from selected markets in Lagos, Nigeria. *African Journal of food science*, *4*(2), 389-393.
12. Yang, Y., Zhang, F. S., Li, H. F., & Jiang, R. F. (2009). Accumulation of cadmium in the edible parts of six vegetable species grown in Cd-contaminated soils. *Journal of environmental management*, *90*(2), 1117-1122.
13. Nagata, M., & Yamashita, I. (1992). Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit. *Nippon shokuhin kogyo gakkaishi*, *39*(10), 925-928.
14. Jones Jr, J. B., & Case, V. W. (1990). Sampling, handling, and analyzing plant tissue samples. *Soil testing and plant analysis*, *3*, 389-427.
15. Huang, Y., He, C., Shen, C., Guo, J., Mubeen, S., Yuan, J., & Yang, Z. (2017). Toxicity of cadmium and its health risks from leafy vegetable consumption. *Food & function*, *8*(4), 1373-1401.
16. Baruah, S., Bora, M. S., Sharma, P., Deb, P., & Sarma, K. P. (2017). Understanding of the distribution, translocation, bioaccumulation, and ultrastructural changes of Monochoria hastata plant exposed to cadmium. *Water, Air, & Soil Pollution*, *228*, 1-21.
17. Ci, D., Jiang, D., Wollenweber, B., Dai, T., Jing, Q., & Cao, W. (2010). Cadmium stress in wheat seedlings: growth, cadmium accumulation and photosynthesis. *Acta Physiologiae Plantarum*, *32*, 365-373.
18. Bauddh, K., & Singh, R. P. (2011). Differential toxicity of cadmium to mustard (Brassica juncia L.) genotypes under higher metal levels. *Journal of environmental biology*, *32*(3), 355.