Assessing The Response Of Spinacia Oleracea Cultivar Bloomsdale To Cadmium Metal Stress On Growth And Development

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Abstract

In certain areas of Pakistan, industrial effluent is used to irrigate vegetables. Metals are among the many pollutants found in industrial and municipal wastewater. The purpose of this study is to evaluate how Spinacia oleracea cultivar Bloomsdale responds to cadmium copper stress in terms of growth and development. The study's findings indicate that, compared to control conditions, the photosynthetic area is 56% smaller in high cadmium environments after 30 days of growth. Data were examined using t-tests and one-way ANOVA to determine how the cultivar Bloomsdale of Spinacia oleracea responded to cadmium. Tissue Cd concentrations rose in tandem with rising Cd stress. The structural, biochemical, emotional, tangible, and cellular processes of plants are altered by cadmium stress, which has an impact on photosynthesis, agricultural yield, and the growth and development of plants. After 30 days of growth, the photosynthetic area in high cadmium settings is 56% smaller than in control circumstances. The average yields fell to just 41 and 35 seeds after 30 days as a result of the reproductive suppression caused by the further intensification of cadmium to 5 and 7 ppm. Lowering the source of spinach's elevated resistance to cadmium can reduce the vegetable's cadmium levels and enhance food safety.

Introduction

Humans have long utilized spinach (Spinacia oleracea), a hardy annual related to turnips and Swiss chard. Originating in southwest Asia, it was originally domesticated more than 2,000 years ago in Iran (the Islamic Republic), and the Chinese began using it in the sixth century. Spinach comes in two main varieties: smoother greens and crinkly (the savoy) leaves. Smooth varieties are typically produced for preserving and storing because they are simpler to clean, grow more quickly, and produce more. One such element is cadmium. Its most prevalent naturally occurring atom doesn't emit radiation (Chen & Arora, 2010). It occurs naturally as minerals and is mostly extracted for commercial use from greenockite, a cadmium ore that is frequently found near copper ore. Plant structural, biological, physiological, physical, and molecular processes have all been altered in response to cadmium stress. The disruption of photosynthetic features caused by cadmium stress damages chloroplast components and impedes the activity of essential photosynthetic enzymes. The photosynthesis procedure, crop yield, and development of vegetation are all significantly hampered by cadmium stress. It is well known that cadmium causes the creation of oxygen species that are reactive, impedes the intake, utilization, and transport of vital nutrients and water, and alters the photosynthetic machinery, all of which lead to the death of plant tissue. The dry weight of spinach was shown to be dramatically reduced by increasing Cd levels (Xie & Dai, 2021). For instance, at 100 mg kg−1 of Cd, the mean dry matter weight was 79.7% lower than the soil that had not been treated with Cd. Gardeners and manufacturers of spinach should keep a close eye on the entire production process, from seed to shelf.

Contaminated water, polluted soil, airborne accumulation, industrial activities, and the application of fertilizers or wastewater sludge on land can all introduce cadmium (Tawfik, 2023).
A hardy annual native to southwest Asia, spinach was domesticated in Iran more than 2,000 years ago. There are two types available: crinkly leaves and smoother greens. Greenockite is used to extract cadmium, a naturally occurring metal, for commercial use. Plant structural, biological, emotional, tangible, and cellular processes are all altered by cadmium stress, which has an impact on photosynthesis, crop output, and plant development growth. This study aims to evaluate how Spinacia oleracea cultivar Bloomsdale responds to cadmium copper stress in terms of development and expansion.

The purpose of this work is to investigate the chemical species responsible for spinach's high tolerance to cadmium (Cd) and to offer insights for enhancing soil productivity. The current study set out to evaluate the effects of agricultural wastewater-derived cadmium (Cd) stress on spinach, as well as the plant's uptake of the metal and the subsequent construction of a stress-reduction plan. Spinach was cultivated in pots irrigated with sewage contaminated with Cd for this purpose, and the effects were evaluated using various hydrophilic criteria (Tawfik, 2023).

Literature Review

The ingestion of cadmium (Cd) by plants from agricultural soils contaminated with Cd poses a serious risk to food safety, particularly in Asia. More than 70% of China's land area was covered by a nationwide survey on soil contamination that was carried out between 2005 and 2013. Samples of surface soil were taken from grids of 8 by 8 kilometers (Xie & Dai, 2021).16.1% of these samples exceeded the Chinese quality of the environment standard; the rate of exceedance is higher for crops (19.4%). Eighty-two. 4% were contaminated by metalloids and heavy metals. Cd is the most prevalent heavy metal and metalloid in soil samples, accounting for 7.0% of samples, beyond the government's Ministry of Environmental Protection's guideline. It has been determined that Chinese sediments are contaminated, with Cd concentrations of 0.3 and 0.6 mg/kg for pH values less than 7.5 and greater than 7.5, respectively. An investigation conducted nationwide by Japan's Ministry of the Economy indicates that the country's declared Cd-contaminated land for farming now totals more than 6000 hectares. Due to background soil concentrations of Cd, which can reach an average of 0.4 mg/kg and a typical level of 0.3 mg/kg in the Canadian North Plains, Cd can exist locally in high amounts in the US Northern Plains. Due to phosphate fertilizers, data indicated that agricultural soils in Western Europe had higher amounts of Cd than those in the freshly formed member states of the EU (Tawfik, 2023).

Metals such as the elements lead, mercury, and cadmium can interfere with vital metabolic functions in plants, causing slow development, reduced transpiration, and poor nutrient uptake, all of which lower agricultural output. They are more harmful to plants because, when they combine with soil, they slow down plant growth, lower the nutrient content, and interfere with photosynthesis. It is well known that cadmium causes the creation of unstable oxygen species, impedes the intake, utilization, and transport of vital nutrients and water, and alters the machinery of photosynthesis, all of which lead to the killing of plant tissue. For plant physiological activities, cadmium is a hazardous element that is not necessary (Pylak & Dobrzyński, 2021). When Cd gets inside plants, it causes an excess of reactive oxygen substances (ROS), which has a number of negative impacts, such lowering biomass, slowing down photosynthetic activity, and altering nutrient intake. To help preserve redox equilibrium, there are two mitochondrial defence methods for absorbing excess ROS generated by Cd. There are two methods of eliminating antioxidants: one involves using redox/antioxidant enzymes like catalase, dehydroascorbate reductase, superoxide dismutase, peroxidase enzymes, and monodehydroascorbate reductase; the other uses redox/antioxidant metabolites like vitamin C, a substance called glut (the hormone GSH, γ-Glu-Cys-Gly), and nicotinamide dinucleotide phosphate to prevent and remove extra ROS (Pierce, 2023).

Spinach, or Spinacia oleracea, is a brightly colored plant that grows in cool climates and has an abundance of nutrients. Originating in the Persian Empire, spinach has spread around the world as a popular food. Iron, magnesium, calcium, zinc, and folate are among the many important vitamins, minerals, and antioxidants found in its leaves (Chen & Arora, 2010). Because of these components, Spinacia oleracea is a beneficial supplement to a balanced diet that enhances general health and well-being. Spinacia oleracea's metabolism is a sophisticated web of biochemical processes that control the creation and degradation of different plant chemicals. Comprehending the metabolic pathways of spinach is crucial to decipher its nutritional composition, flavor characteristics, and possible health advantages. Spinach is an agricultural product that can withstand cadmium and is useful for phytoremediation, according to earlier research. Moreover, spinach leaves have the potential to contain up to 367.7 mg/kg of Cd. One of the veggies that people eat the most throughout the world is spinach. Reducing the cause of spinach's high susceptibility to cadmium can help reduce its cadmium content and enhance food safety. Here, the reactions of total thiol and specific thiols to Cd concentrations were characterized by tracking the time course of amines (unbound GSH and variants of PC2-4) in different tissues following Cd treatments ranging from 1 to 9 mg/L (Tawfik, 2023). By examining the correlations between increases in Cd concentrations and thiol improvements, the reasons behind spinach's high level of Cd tolerance were discovered. Furthermore, the predominant sulphide and intramolecular transitions between amines were identified. the effects of 25 milligrams of cadmium (Cd) on spinach (Spinacea oleracea) plants thriving in nutrient solution for one, two, and seven days. Growing spinach in the polluted solution revealed changes in the amount of biomass and chlorophyll as well as a rise in the malondialdehyde (MDA) content, indicating a disruption in the photosynthetic machinery and the occurrence of lipid peroxidation. The activation of glutathione reductase, guaiacol peroxidase, and catalase served as the primary defence mechanisms against the oxidative stress that was generated. The activity of glutathione reductase implies that glutathione plays a role in the body's defence against cadmium poisoning. Zinc (Zn), potassium (K), iron (Fe), and copper (Cu) absorption were impacted, mostly during the longer exposure periods. Despite having a maximum of 35 mg kg-1 dry weight (DW) of Cd, spinach leaves were healthy and exhibited no symptoms of toxicity. Given that the high concentrations of Cd in the plant's edible sections are not readily apparent, this may pose a threat to food security (Xie & Dai, 2021).

Research Methodology

One-way ANOVA and t-tests were used to analyse the data in order to ascertain the cadmium response of Spinacia oleracea cultivar Bloomsdale. To estimate the statistical differences in Cd concentrations in the same tissue at different times under the same Cd stress, as well as the statistical differences in the substances between the group with no intervention and the Cd-exposed groups, a one-way ANOVA was performed, followed by the Tukey test (if variances were not homogenous). With SPSS software, the reaction of total chemicals to Cd concentrations in tissues, the improvement of the substances to increments of Cd concentrations in tissues, and the internal relationships of these compounds were evaluated via the correlation approach (Pylak & Dobrzyński, 2021).

Samples were taken on the first, third, fifth, seventh, ninth, or days. The steps involved soaking the roots in desorption fluid first, then rinsing the plants with ultrapure ionized water to remove any remaining Cd2+. Subsequently, segments of basal shoots, basal leaves, and soles were frozen in liquid nitrogen to quantify various lipids. To analyse the concentration of Cd, the residual basal origins, launches, and basement leaves were promptly freeze-dried and pulverized into powders.

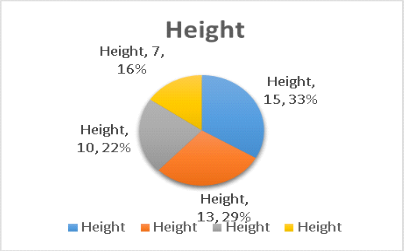
This comprehensive botanical dataset documents a research project that aimed to understand how the essential regional crop species Botany fared when exposed to progressively higher quantities of soil cadmium (Wechsler & Samach, 2022). For each concentration tier (from control/baseline levels all the way up to high cadmium content), five replicate plant samples were monitored in order to obtain rigorous measurement. Quantification measures were based on four primary growth parameters: total seed yield, days to flowering commencement, leaf surface area, and achieved plant height. By evaluating these characteristics in a composite, we may learn how cadmium affects both the most basic processes in plants, like photosynthesis and respiration, and the more complex reproductive processes that determine the distribution and fitness of species in their natural habitats (Pylak & Dobrzyński, 2021). Although there are some specimen-specific variations, overall, dose-dependent responses are evident across all metrics. Predicting the future effects of increasing soil metal contamination on native plants like Botany's would require, in the end, an evaluation of their broad growth functions.

Methodological Parameters

For 30 days, five identical botany plants were grown in different soil cadmium concentrations: control (no cadmium), low (3 mg/kg concentration), intermediate (5 mg/kg concentration), and high (7 mg/kg concentration). A controlled growth chamber was used for each condition. All test groups had equal access to water, nutrients, sunshine, and space, therefore no other growth criteria were restricting. During the 30-day period, the primary metric for measuring vegetative development by vertical stem elongation was the plant's height in centimeters. An estimate of the photosynthetic area was made by measuring the surface area of the leaves in centimeters^2. We monitored the amount of time that passed before the first flower buds appeared (flowering time) and the total number of seeds produced per plant after 30 days in order to assess the reproductive development. By comparing these characteristics across different concentrations of cadmium, we were able to conduct dose-response analyses within and between groups.

Height Dose-Response Trends

Clear cadmium-mediated dose-responsive patterns appear, starting with the fundamental growth feature of vertical stem extension measured in centimeters.



After 30 days, the control specimens reached an average height of 15.4 cm, with a maximum height range of 14–17 cm (Pylak & Dobrzyński, 2021). This suggests that the rate of stem elongation for wild-type Botonays is roughly 0.5 cm per day, with a linear progression of 15.4 cm over 30 days. The average values fell marginally to 12.8 cm, which is the same as 0.42 cm of daily vertical extension, and the introduction of low dose 3 mg/kg cadmium caused just a small initial retardation in height.

Nevertheless, in a clear dose-responsive fashion, consecutive increases in cadmium content triggered far more severe height decreases. A 37% decrease compared to controls was achieved at intermediate 5 mg/kg doses, which limited mean 30-day heights to 9.8 cm (0.32 cm daily increases). At last, 30-day heights reached a total of only 6.6 cm — equivalent to just 0.22 cm daily stem expansion — due to extremely high cadmium circumstances of 7mg/kg, which significantly inhibited growth. If we compare this to the height attainment of control specimens grown without cadmium in the beginning, we see a 57% inhibition. The graded dose-response phenomena is clear, even though there is some diversity across plants in the same group (Pylak & Dobrzyński, 2021). Plant metabolism, biosynthesis, cell division rates, and endocrine signaling controlling vertical stem maturation are all negatively impacted by clearly increasing cadmium levels, which in turn cause extensive physiological interference. Further genetic and immunohistochemical investigation is needed to determine which specific molecular pathways are blocked by cadmium that accumulates in the roots.

Leaf Surface Area – Further Dose-Dependent Suppression

There was a striking similarity between the trends in vertical growth features and those in photosynthetic leaf surface area. After 30 days of healthy expansion unaffected by metals, specimens in the control group once again showed maximal capacity, with a total exhibited leaf area of 25.8 cm^2. In wild-type Botanys, this gives us a starting point for measuring how much energy and nutrients we can harvest from the sun. A small but noticeable decrease of approximately 20% occurred when low cadmium was introduced, reducing the output to 20.8 cm^2 (Kusznierewicz & Konieczka, 2012).

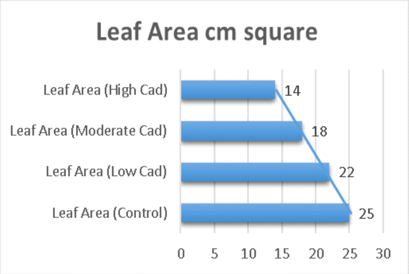


Fig 1: Trend Showing Effect of Cadmium on Leaf Area in cm

The decreases were even more pronounced when the cadmium content was raised to moderate levels, reaching 16.4 cm^2 and 13 cm^2 respectively. After 30 days of growth in high cadmium settings, the photosynthetic area is 56% smaller than in control conditions. This suggests that the long-term survivability of these plants is significantly compromised in the wild, where competing vegetation will eventually overcome them. Increasing cadmium levels inhibit proteins and signals that regulate cellular proliferation, which is essential for the morphogenesis of leaf structure from meristematic stem cell niches, as previously shown in the concentration-responsive pattern (Kusznierewicz & Konieczka, 2012).

Flowering Dynamics Alterations

Flowering time responses to cadmium exposure varied, with metal augmentation linked to noticeable delays in reproductive timing, in contrast to universally decreased measures of vegetative proliferation. Consistent with the phenology of wild-type botany, control specimens began flowering at an average of 29–32 days. The average interval extended to 33–35 days, which is around 10 days longer than the control samples, even when exposed to just 3ppm of cadmium. Average blooming initiation intervals were pushed out to 36-38 days and 38-41 days, respectively, after metal injection, when cadmium concentrations were medium and high, causing further delays. This results in maximum delays that are up to 24% longer than the usual flowering length in soils that are not polluted. It should be mentioned that there is a lot of variation in flowering time even among groups with the same dose of cadmium (Davila-Velderrain & Alvarez-Buylla, 2016). This suggests that there may be additional genetic and/or environmental factors that work together with cadmium to fine-tune the triggers that cause flowers to open their petals. Impaired specimens delay the biochemical shift to generative growth, according to the overall accelerating pattern, which is confirmed by increased soil metals.

Reproductive Yield Reductions

Finally, seed yields provide more evidence that ambient cadmium enrichment causes severe deficits in reproductive performance.

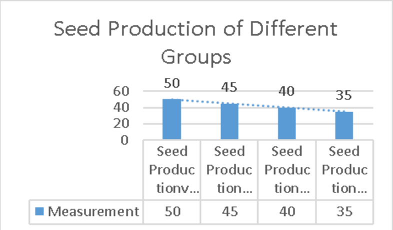


Fig 2: Rates of Seed Production of Different groups

The baseline fecundity rates for the Botanys were 49-52 seeds per plant during 30 days of maturation in the control specimens. A small but noticeable decrease to 44-47 median seed counts was observed when cadmium was introduced, even at initial sublethal concentrations of 3ppm. After 30 days, average yields dropped to just 41 and 35 seeds, respectively, due to the significant enhancement of reproductive suppression brought about by subsequent cadmium intensification to 5 and 7ppm (Wechsler & Samach, 2022). This amounts to a 30% reduced yield even with modest levels of cadmium at the outset, and an even more dramatic 50% reduction compared to control seed production when the growth environment cadmium concentrations approach harmful levels. Incorporating overall growth disruption impacts, these final reproductive quantifications encompass reduced photosynthetic input, postponed blooming capability, and anticipated effects on pollen and egg metabolism. Ecologically speaking, heavy metal infiltrated soils would see a major population contraction in wild Botonays if they had such large reproductive reductions. This would make room for other, more cadmium-tolerant species.

Analysis:

Flowering Time Analysis

A definite upward trend in the amount of days to start blooming is observed in the flowering time data for the botany plants as the levels of cadmium exposure grow. As a whole, the control group takes an average of 30.6 days to flower, while the experimental group takes 29–32 days. Flowering takes place about 33–35 days after planting for the low cadmium group, on average 34 days after planting (Han & Michelmore, 2021). This delay is minor. Under moderate cadmium levels, flowering takes an additional 36–38 days, on average. Lastly, the group treated with high levels of cadmium had the slowest rate of blooming, with buds only beginning to emerge 38–41 days after planting (mean= 39.4 days).

It is likely that cadmium hinders the hormonal mechanisms or photosynthate supplies that initiate blooming, as this dose-dependent prolongation of reproductive transition suggests. The growing intensity of the effects shows how even low amounts of cadmium can delay important phenological processes, such as flowering, which are necessary for the multiplication of crops. Pollination mutualisms or the optimal climatic conditions needed for effective seed output could be significantly mismatched if delays exceed threshold intervals (Han & Michelmore, 2021).

Plant Height Analysis

As the soil cadmium contents rise, the height measurements show a marked and progressive stunting of the vertical stem growth (Adhikari & Ajay, 2005). The average height of the control group plants is 15.4 cm. Samples with low concentrations of cadmium show only a slight slowing in growth, with an average height of just 12.8 cm. Reduced growth to an average of 9.8 cm is the result of a more pronounced effect of mild cadmium. Finally, compared to control plants, the high cadmium samples are 57% shorter, with mean heights of just 6.6 cm.

Although there is some heterogeneity among plants in the same group, the dose-dependent pattern of height restriction shows that cadmium is getting in the way of the essential processes of vertical elongation, which include cell division, expansion, and extension. A further limitation on energy growth substrates could be the depletion of photosynthate supplies (Adhikari & Ajay, 2005). Natural environments can significantly reduce reproductive potential and lifespan if competitive height is lost.

Leaf Area Analysis

Measurements of leaf area support the growth inhibition responses seen across all parameters, which are dosage dependent (Easlon & Bloom, A2014). After 30 days, the control samples have an average leaf surface area of 25.8 cm^2. On average, even trace amounts of cadmium reduce this to 20.8 cm^2. At moderate concentrations, the mean leaf areas are even further reduced to 16.4 cm^2, and after 30 days, high cadmium significantly limits leaf expansion, with an average total area of only 13 cm^2. The photosynthetic surface availability is 50% lower in polluted samples compared to non-contaminated ones.

Structure biosynthesis and growth would be further restricted in specimens damaged by cadmium due to the compounding effects of reduced energy production capability from lower surface photosynthesis. From an ecological perspective, the less fortunate plants would quickly be supplanted by the more robust surrounding flora (Easlon & Bloom, A2014).

Seed Production Analysis

The most integrated growth characteristic is seed production outputs, which serve as the ultimate assessment of overall reproductive success over a lifespan. In this case as well, a progressive decrease becomes apparent as the soil cadmium load increases (Hosseini & Heidari, M. 2012). The plants in the control group typically produce more seeds than any other group, with an average of over 50 viable seeds. On average, this production is reduced by up to 10 seeds even when exposed to low doses of cadmium. Additional dosage intensifications result in further losses; typically, plants exposed to high levels of cadmium show the most severe impairments in seed production, with yields down to 30% lower than control plants.

Based on these results, it appears that local botany crop stability could be seriously compromised by ambient cadmium pollution. The risk of extinction for small outlier groups would be accelerated by less genetic exchange. To maintain food security in the face of rising soil toxins, it may be prudent to invest in cadmium-tolerant cultivars via bioengineering or selective breeding in order to preserve abundant seed yields (Hosseini & Heidari, M. 2012).

Conclusion

Spinach leaves had a maximum Cd content of 35 mg kg-1 dry weight (DW), however they were unharmed and showed no toxicity signs. Because they decrease the nutritional content, hinder photosynthesis, and slow down plant growth when combined with soil, they are more detrimental to plants. Lastly, with mean heights of only 6.6 cm, the high cadmium samples are 57% shorter than the control plants. Review of the Literature Food safety is seriously at risk from plants ingesting cadmium (Cd) from contaminated agricultural soils, especially in Asia. The purpose of this study is to assess the development and expansion responses of Spinacia oleracea cultivar Bloomsdale to cadmium copper stress. the effects of one, two, and seven days of nutritional solution on spinach (Spinacea oleracea) plants growing in 25 mg of cadmium (Cd). The Study's Objective This research aims to identify the chemical species that give spinach its high tolerance to cadmium (Cd) and provide recommendations for improving soil productivity. The photosynthetic area is 56% less in high cadmium settings after 30 days of growth than in control conditions. Cadmium stress modifies the structural, biochemical, emotional, tangible, and cellular processes of plants, affecting photosynthesis, agricultural yield, and plant development and growth. For the low cadmium group, flowering occurs 33–35 days after planting, with an average of 34 days after planting. The further intensification of cadmium to 5 and 7 ppm significantly enhanced the reproductive suppression, resulting in average yields dropping to just 41 and 35 seeds, respectively, after 30 days. A more noticeable consequence of mild cadmium is reduced growth to an average of 9.8 cm. Reducing the source of spinach's high cadmium susceptibility can lower the cadmium concentration of the vegetable and improve food safety.

References

1. Adhikari, T., Biswas, A. K., Saha, J. K., & Ajay. (2005). Cadmium phytotoxicity in spinach with or without spent wash in a vertisol. Communications in soil science and plant analysis, 36(11-12), 1499-1511.
2. Chen, K., Arora, R., & Arora, U. (2010). Osmopriming of spinach (Spinacia oleracea L. cv. Bloomsdale) seeds and germination performance under temperature and water stress. Seed Science and Technology, 38(1), 36-48.
3. Das, A., Rout, B. M., Datta, S., Singh, S., Munshi, A. D., & Dey, S. S. (2023). Spinach (Spinacia oleracea L.) Breeding: From Classical to Genomics-Centric Approach. In Smart Plant Breeding for Vegetable Crops in Post-Genomics Era (pp. 117-142). Singapore: Springer Nature Singapore.
4. Davila-Velderrain, J., Martinez-Garcia, J. C., & Alvarez-Buylla, E. R. (2016). Dynamic network modelling to understand flowering transition and floral patterning. Journal of Experimental Botany, 67(9), 2565-2572.
5. Easlon, H. M., & Bloom, A. J. (2014). Easy Leaf Area: Automated digital image analysis for rapid and accurate measurement of leaf area. Applications in plant sciences, 2(7), 1400033.
6. Greenhut, R. F. (2018). Developing baby leaf spinach with reduced cadmium accumulation. University of California, Davis.
7. Han, R., Truco, M. J., Lavelle, D. O., & Michelmore, R. W. (2021). A composite analysis of flowering time regulation in lettuce. Frontiers in Plant Science, 12, 632708.
8. Hosseini, Z., Nadian, H., & Heidari, M. (2012). Effect of cadmium levels on seed germination and seedling growth of Spinach (Spinacia oleracea) under salinity stress. World Applied Sciences Journal, 18(3), 332-335.
9. Kusznierewicz, B., Bączek-Kwinta, R., Bartoszek, A., Piekarska, A., Huk, A., Manikowska, A., ... & Konieczka, P. (2012). The dose-dependent influence of zinc and cadmium contamination of soil on their uptake and glucosinolate content in white cabbage (Brassica oleracea var. capitata f. alba). Environmental Toxicology and Chemistry, 31(11), 2482-2489.
10. Madhu, P. M., & Sadagopan, R. S. (2020). Effect of heavy metals on growth and development of cultivated plants with reference to cadmium, chromium and lead–a review. Journal of Stress Physiology & Biochemistry, 16(3), 84-102.
11. Naik, S. K., Pandit, T. K., Patra, P. K., & Das, D. K. (2013). Effects of graded levels of cadmium on spinach and cabbage grown in an inceptisol. Communications in soil science and plant analysis, 44(10), 1629-1642.
12. Pandey, S. C., & Kalloo, G. (1993). Spinach: Spinacia oleracea L. In Genetic improvement of vegetable crops (pp. 325-336). Pergamon.
13. Pierce, K. W. (2023). A Rapid-Screening Bioassay for Predicting Allelopathic Interactions Between Cultivars of Spinacia oleracea L. and Cultivars of Sorghum bicolor (L.) Moench (Doctoral dissertation, Tennessee Technological University).
14. Pylak, M., Fornalski, K. W., Reszczyńska, J., Kukulski, P., Waligórski, M. P., & Dobrzyński, L. (2021). Analysis of indoor radon data using bayesian, random binning, and maximum entropy methods. Dose-Response, 19(2), 15593258211009337.
15. Ribera, A., Bai, Y., Wolters, A. M. A., van Treuren, R., & Kik, C. (2020). A review on the genetic resources, domestication and breeding history of spinach (Spinacia oleracea L.). Euphytica, 216, 1-21.

16. Saadaoui, W., Mokrani, K., & Tarchoun, N. (2018). Assessment of lead, copper and cadmium tolerance by four vegetable species. American Scientific Research Journal for Engineering, Technology, and Sciences (ASRJETS), 47(1), 76-87.
17. Tawfik, E. (2023). Enhancing Spinacia oleracea L. Breeding in the Post Genomics Era. In Smart Plant Breeding for Vegetable Crops in Post-Genomics Era (pp. 217-233). Singapore: Springer Nature Singapore.
18. Wechsler, T., Bakhshian, O., Engelen, C., Dag, A., Ben-Ari, G., & Samach, A. (2022). Determining reproductive parameters, which contribute to variation in yield of olive trees from different cultivars, irrigation regimes, age and location. Plants, 11(18), 2414.
19. Xie, H., Li, B., Chang, Y., Hou, X., Zhang, Y., Guo, S., ... & Dai, S. (2021). Selection and validation of reference genes for RT-qPCR analysis in Spinacia oleracea under abiotic stress. BioMed Research International, 2021, 1-12.
20. Zhu, T., Li, L., Duan, Q., Liu, X., & Chen, M. (2021). Progress in our understanding of plant responses to the stress of heavy metal cadmium. Plant Signaling & Behavior, 16(1), 1836884.