



Physiological Effects of Chromium (Cr) on *Spinacia oleracea* L. under Controlled Conditions

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Abstract

Heavy metal contamination in agricultural soils is a significant environmental concern that jeopardises crop yield and food safety. This study examined the phytotoxic effects of hexavalent chromium (Cr^{6+}), provided as potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$), on many physiological parameters of *Spinacia oleracea* L. (spinach). Plants were subjected to several doses of Cr (0, 50, 100, and 150 mg/L) in a controlled environment. The findings indicated a concentration-dependent reduction in germination %, root and shoot lengths, leaf area, biomass (both fresh and dry weight), and chlorophyll content. In contrast, proline accumulation rose considerably under Cr stress, demonstrating an active physiological response to metal-induced oxidative stress. The highest level of chromium (150 mg/L) caused a 30.76% drop in germination, a 41.5% drop in total chlorophyll, and a 71.2% rise in proline content compared to the control. These results indicate that Cr stress adversely affects spinach growth and metabolism, but proline accumulation may function as a protective adaptation strategy. The study underscores the susceptibility of spinach to chromium toxicity and advocates for its possible application as a bioindicator for environmental monitoring in damaged agroecosystems.

Keywords: *Spinacia oleracea*, chromium stress, germination, biomass, chlorophyll, proline, physiological response, bioindicator.

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Introduction

Spinacia oleracea L. (spinach), belonging to the Amaranthaceae family, is a widely cultivated leafy green vegetable esteemed for its substantial nutritional value and agricultural significance. Spinach is abundant in vitamins A, C, E, and K, as well as iron, calcium, magnesium, and antioxidants including flavonoids and carotenoids, hence facilitating essential physiological activities such as immune defence, bone health, and the mitigation of oxidative stress (Bergquist *et al.*, 2006; Adress *et al.*, 2021). Heavy metals, both natural and anthropogenic, can significantly impact sustainable agricultural productivity due to their non-biodegradability (Shabaan *et al.*, 2021; Mustafa *et al.*, 2023). They persist in soils, inhibiting plant growth, chlorosis, and root browning, and even causing plant death. Chromium, a hazardous metal, also negatively impacts plants, humans, and soil, including its microbial fauna. Therefore, it is crucial to address these issues to ensure sustainable agricultural practices (Amber Abid, 2023; Zulfiqar *et al.*, 2023b). Due to its rapidly growth and development, substantial water absorption, and sensitivity to soil conditions, spinach serves as an exemplary model organism for evaluating environmental stress, particularly heavy metal contamination (Hirayama & Shinozaki, 2010). Heavy metals such as chromium (Cr), cadmium (Cd), lead (Pb), and mercury (Hg) significantly jeopardise plant growth and human health by interfering with photosynthesis, food uptake, and cellular metabolism (Shanker *et al.*, 2005; Zhu, 2016).

Chromium (Cr), especially in its hexavalent state (Cr^{6+}), is acknowledged as a significant environmental contaminant owing to its elevated mobility, solubility, and bioavailability in terrestrial and aquatic ecosystems. It is predominantly released into the environment via human activities, including leather tanning, electroplating, dye production, and industrial discharges. Potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) is a frequently found poisonous chromium compound that easily infiltrates plant tissues. Upon absorption, Cr^{6+} precipitates significant physiological and biochemical disruptions, encompassing the production of reactive oxygen species (ROS), lipid peroxidation, DNA damage, enzyme inhibition, and dysfunction of photosynthetic apparatus. Cr exposure significantly reduces chlorophyll content and photosynthetic efficiency, consequently impeding plant development and productivity (Shanker *et al.*, 2019; Zayed *et al.*, 2020; Alam *et al.*, 2021).

Spinach's capacity to absorb and respond to Cr^{6+} renders it a significant bioindicator in environmental monitoring and phytoremediation studies. Investigating spinach under potassium dichromate stress enhances comprehension of its antioxidant defence mechanisms, stress physiology, and viability for safe farming in contaminated soils (Zayed & Terry, 2003; Alharby *et al.*, 2016). This research is essential for addressing food safety issues and developing sustainable farming techniques in contaminated environments (Vajpayee *et al.*, 2000). Exposure to chromium (Cr) markedly interferes with numerous physiological and biochemical processes in plants, especially in *Spinacia oleracea* L. (spinach), a species notably susceptible to heavy metal stress owing to its delicate leaf and elevated metabolic activity.

The initial consequences noted include diminished germination and seedling vigour, chiefly attributable to oxidative damage to embryonic tissues and compromised water absorption (Zayed & Terry, 2003; Panda & Choudhury, 2005). Root and shoot development are negatively impacted since chromium disrupts cell division, nutrient absorption, and hormonal equilibrium, with roots exhibiting greater susceptibility (Shanker *et al.*, 2005; Peralta-Videa *et al.*, 2009). As a result, Cr buildup results in decreased biomass, indicating reduced photosynthetic activity and increased oxidative stress levels (Kumar *et al.*, 2019; Rai *et al.*, 2004). A significant reduction in chlorophyll a and b levels has been noted in spinach subjected to Cr treatment, signifying a direct effect on the plant's photosynthetic apparatus (Sharma *et al.*, 2021; Singh *et al.*, 2013). In reaction to chromium toxicity, spinach increases proline levels, serving as an osmoprotectant and antioxidant (Kumar *et al.*, 2019). Moreover, Cr compromises membrane integrity and water relations, resulting in electrolyte leakage and diminished relative water content, which finally induces cellular dehydration (Peralta-Videa *et al.*, 2009). The detrimental effects of chromium on spinach are linked to the inverse relationship between chromium accumulation and both dry biomass and physiological parameters, as demonstrated in the current study. The simultaneous application of compost and rhizobacteria enhanced spinach biomass under chromium stress, as previously reported in past research (Gibilisco *et al.*, 2022; Sarathchandra *et al.*, 2022; Sun *et al.*, 2023).

Materials and Methods

Experiments were conducted from December 2024 to January 2025 at Botanical Garden, Narain College, Shikohabad under ambient conditions (25–30 °C max, 5–15 °C min, 52–68% RH). Analytical-grade potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) was used as a source of hexavalent chromium (Cr^{6+}) at concentrations of 0, 50, 100, and 150 mg L⁻¹.

The initial method entailed positioning surface-sterilized seeds on filter paper saturated with Cr^{6+} solutions, whereas the subsequent method comprised sowing seeds in acid-washed soil enriched with nutrient solution and administering Cr^{6+} biweekly.

Morphological parameters were assessed to evaluate the early physiological response of *Spinacia oleracea* under chromium (Cr) stress. Germination percentage was recorded daily up to 12 days; seeds with radicle length ≥ 1 mm were considered germinated (Kabir *et al.*, 2008). Germination percentage was calculated using:

$$\text{Germination percentage (\%)} = \frac{\text{Total No. of germinated seeds}}{\text{Total No. of germinated and non-germinated seeds}} \times 100$$

Root and shoot lengths were quantified with a ruler in both treated and control seedlings. Leaf area was quantified utilising the graph paper approach by tracing leaves and calculating the average area of five leaves per treatment (Taghipour & Saheli, 2008). For biomass analysis, root and shoot dry weights were measured following oven-drying at 60 °C for 48 hours until a consistent weight was attained.

Biochemical parameters were assessed to determine Cr-induced physiological alterations. Chlorophyll content was estimated using Arnon's method (1949) by extracting 1 g of fresh leaf in 80% acetone. After 4–5 days in darkness, absorbance was measured at 663 and 645 nm. Chlorophyll concentrations were calculated using:

$$\text{Chl 'a'} = \frac{(9.78 \times \text{OD } 663) - (0.99 \times \text{OD } 645)}{1000 \times \text{Fresh Weight}} \times V$$

$$\text{Chl 'b'} = \frac{(21.4 \times \text{OD } 645) - (4.65 \times \text{OD } 663)}{1000 \times \text{Fresh Weight}} \times V$$

$$\text{Total Chl} = \text{Chl 'a'} + \text{Chl 'b'}$$

The proline content was quantified according to the methodology established by Bates *et al.* (1973). Five hundred milligrams of fresh leaf tissue were homogenised in 3% sulfosalicylic acid, followed by reaction with ninhydrin and glacial acetic acid, then boiled and cooled. The chromophore was extracted using toluene, and absorbance was measured at 520 nm. Proline was measured utilising:

$$\text{Proline} = x \times 0.346 \mu\text{g g}^{-1} \text{FW}$$

where x is the reading derived from the standard curve. Data were statistically analyzed using mean and standard error (Steel & Torrie, 1984; Duncan, 1995) to determine treatment effects.

Results and Discussion

This study shows that high concentrations of chromium (Cr), especially as potassium dichromate, severely disrupt the physiological and biochemical characteristics of *Spinacia oleracea*. The suppressive effects on seed germination, root and shoot development, biomass accumulation, pigment concentration, and increased proline accumulation highlight the phytotoxic characteristics of hexavalent chromium [Cr (VI)].

Impact of Chromium on Germination Rate- Exposure to elevated amounts of chromium (Cr) markedly impeded seed germination in *Spinacia oleracea* L. (Table 3.1). The germination percentage declined systematically from 86.67% in the control to 83.33%, 73.33%, and 60.00% at 50, 100, and 150 mg/L Cr, respectively. This pattern indicates a dose-dependent phytotoxic effect of chromium on seed germination capacity. The decline in germination may be ascribed to changes in membrane permeability, suppression of enzyme activities crucial for metabolic reactivation, and oxidative injury induced by reactive oxygen species (ROS), corroborating earlier research (Shanker *et al.*, 2005; Panda and Choudhury, 2005).

Impact on Root and Shoot Growth- A notable decrease in both root and shoot lengths was found with elevated chromium contents (Table 3.1). Root length diminished from 8.72 ± 0.62 cm in the control group to 5.10 ± 0.79 cm

at 150 mg/L Cr, whereas shoot length reduced from 14.53 ± 2.11 cm to 9.83 ± 1.02 cm. The inhibitory impact of chromium on root extension may stem from altered cell division and elongation, whereas diminished shoot growth likely indicates compromised photosynthetic efficiency and nutrient transfer (Gill and Tuteja, 2011).

Effect on Leaf Area- Leaf area shown a significant reduction under chromium stress, decreasing from 15.56 ± 1.22 cm² in the control group to 8.97 ± 0.98 cm² at the maximum chromium concentration (Table 3.1). Chromium exposure may disrupt cellular proliferation, chlorophyll synthesis, and stomatal activity, ultimately resulting in diminished leaf development (Alharby *et al.*, 2016).

Biomass Accumulation- Chromium substantially influenced plant biomass. Shoot fresh weight diminished from 15.80 ± 2.85 g in the control group to 11.22 ± 2.64 g at 150 mg/L Cr, whereas root fresh weight reduced from 10.92 ± 1.62 g to 7.66 ± 1.66 g (Table 3.1). A comparable trend was noted in dry biomass (Table 3.1), with shoot dry weight decreasing from 3.54 ± 0.92 g to 1.16 ± 1.06 g, and root dry weight diminishing from 1.75 ± 0.76 g to 0.88 ± 0.94 g. The noted decrease in biomass is ascribed to chromium-induced disturbances in cellular metabolism, photosynthesis, and water relations (Zayed and Terry, 2003; Costa and Klein, 2006).

Alterations in Chlorophyll Content- Chromium stress resulted in a significant reduction in chlorophyll a, chlorophyll b, and overall chlorophyll concentration (Table 3.1). The total chlorophyll content decreased from 2.10 mg g⁻¹ FW in the control group to 1.43 mg g⁻¹ FW at a concentration of 150 mg/L Cr. Chlorophyll a and b exhibited analogous patterns of diminution. This reduction may stem from suppressed chlorophyll biosynthesis, enhanced breakdown, or chloroplast damage induced by oxidative stress (Shanker *et al.*, 2005).

Proline Accumulation as a Stress Marker- Proline content exhibited a concentration-dependent rise under chromium stress (Table 3.1).

It increased from 0.32 mg g⁻¹ FW in the control to 0.55 mg g⁻¹ FW at 150 mg/L Cr. Proline functions as an Osmo protectant and reactive oxygen species (ROS) scavenger, frequently being increased in reaction to abiotic stress. The buildup indicates the activation of stress response pathways and potential tolerance mechanisms in spinach (Bates *et al.*, 1973; Ali *et al.*, 2013). The aggregated physiological reactions of *S. oleracea* to chromium stress demonstrate that potassium dichromate (K₂Cr₂O₇), a source of Cr (VI), has a pronounced deleterious impact even at moderate concentrations. Decreases in germination, growth, biomass, and pigment concentrations indicate the metal's disruption of essential metabolic processes. In contrast, increased proline buildup signifies a defensive adaptation to alleviate stress. These findings validate the effectiveness of spinach as a bioindicator of soil contamination and underscore the necessity for monitoring heavy metal contaminants in agricultural ecosystems.

Physiological Effects of Chromium on *Spinacia oleracea*

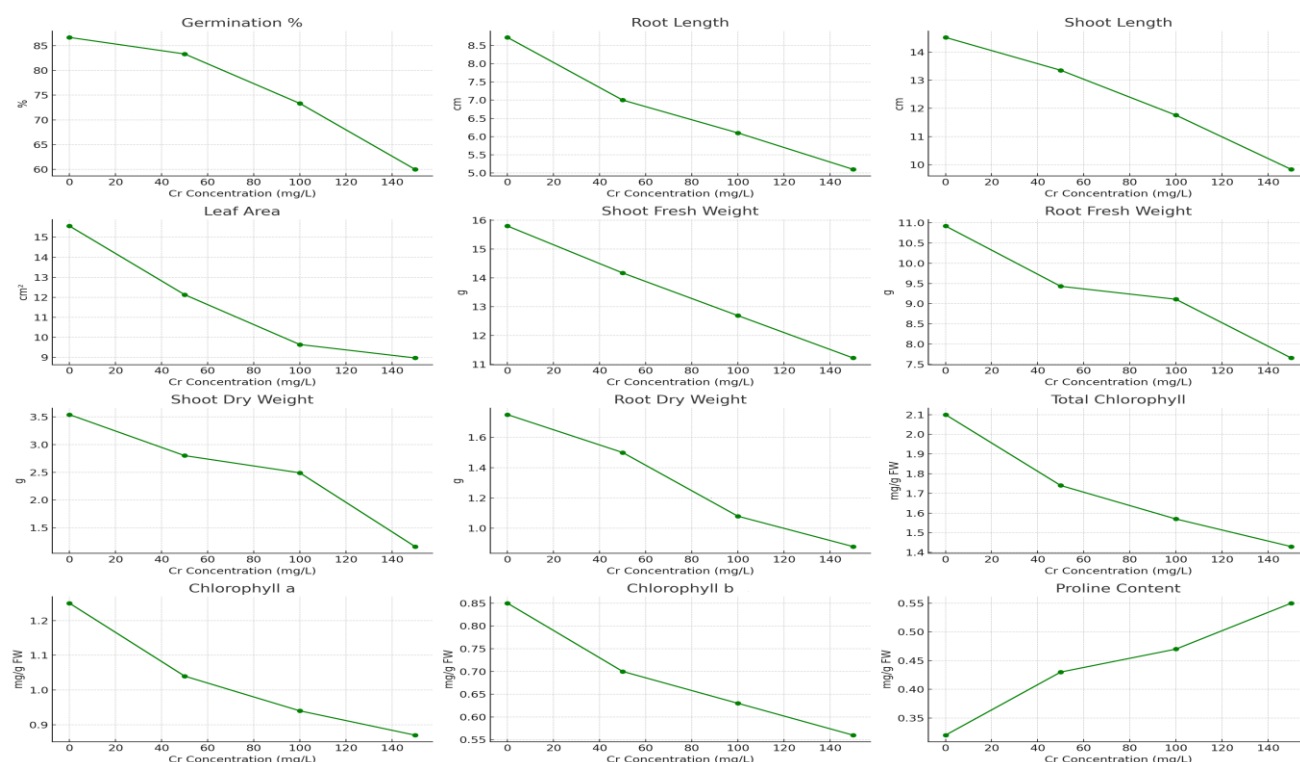


Fig 3.1- Physiological effects of Cr on *Spinacia oleracea*.

Table 3.1 Combined Effect of Chromium (Cr) on Morphological, Biomass, Pigment, and Proline Content of Spinach (*Spinacia oleracea*)

Metal	Conc. (mg/L)	Germination (%)	Root Length (cm)	Shoot Length (cm)	Leaf Area (cm ²)	Shoot Fresh Wt (g)	Root Fresh Wt (g)	Shoot Dry Wt (g)	Root Dry Wt (g)	Chl a (mg/g FW)	Chl b (mg/g FW)	Total Chl (mg/g FW)	Proline (mg/g FW)
Control	0	86.67	08.72±0.62	14.53±2.11	15.56±1.22	15.80±2.85	10.92±1.62	3.54±0.92	1.75±0.76	1.25	0.85	2.10	0.32
Cr	50	83.33	07.00±0.95	13.36±1.67	12.13±1.46	14.17±2.12	09.43±1.97	2.80±0.72	1.50±0.85	1.04	0.70	1.74	0.43
Cr	100	73.33	06.10±0.75	11.76±0.75	09.64±1.04	12.69±3.20	09.11±1.49	2.49±0.95	1.08±0.51	0.94	0.63	1.57	0.47
Cr	150	60.00	05.10±0.79	09.83±1.02	08.97±0.98	11.22±2.64	07.66±1.66	1.16±1.06	0.88±0.94	0.87	0.56	1.43	0.55

Conclusion

This study demonstrates that elevated levels of chromium, especially potassium dichromate, markedly impair the physiological and biochemical properties of *Spinacia oleracea*. The phytotoxic properties of hexavalent chromium are emphasised, exhibiting inhibitory effects on seed germination, root and shoot growth, biomass accumulation, pigment concentration, and elevated proline levels.

The study revealed that exposure to high levels of chromium significantly hindered seed germination, leading to a reduction in germination %. The reduction in germination may result from alterations in membrane permeability, inhibition of essential enzyme activities for metabolic reactivation, and oxidative damage caused by reactive oxygen species

(ROS). Chromium adversely impacted root and shoot development, resulting in a significant reduction in both root and shoot length. Chromium-induced disruptions in cellular metabolism, photosynthesis, and water interactions resulted in a substantial decrease in leaf area. Chromium stress led to a marked decrease in chlorophyll content, with total chlorophyll diminishing from 2.10 mg g⁻¹ FW in the control group to 1.43 mg g⁻¹ FW at a concentration of 150 mg/L Cr. The accumulation of proline as a stress marker was observed to rise during chromium stress, suggesting the activation of stress response pathways and possible tolerance mechanisms in spinach. These findings confirm the efficacy of spinach as a bioindicator of soil pollution and highlight the imperative for monitoring heavy metal pollutants in agricultural ecosystems.

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