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Original Article

Studying the Impact of Chromium Contamination on Seed Germination, Phytotoxicity and Growth Dynamics in *Vigna Radiata Seeds*

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Abstract *- Soils contaminated with heavy metals pose a substantial threat to sustainable agriculture. Among these toxic elements released in the environment, chromium (Cr) is particularly hazardous, severely inhibiting plant growth. The current investigation examines the impact of varying chromium concentrations on the germination of seeds and the growth performance of seedlings in Vigna radiata. Exposure to higher chromium concentrations significantly affected the rate of germination, as the seeds exposed to 5 % concentration showed a 40% rate of germination. The root and shoot length decreased significantly with the increase in the concentration of Cr. The average shoot length of the seed exposed to 5% Cr concentration was 2.53 cm, while the average shoot length for the control group was 12.69 cm. Similar results were evident in the root length as the least development was visible in the 5% concentration, which only grew 2.82cm, while the longest root length was recorded in the control, i.e. 8.28 cm. These results prove that heavy metal contamination negatively affects plant growth. This could be due to the oxidative stress that plants face upon exposure to heavy metals, which leads to cellular damage.*

Keywords *- Vigna Radiata, Phytotoxicity, Contamination, Concentration, Germination.*

1. Introduction

In sustainable agriculture, heavy metal contamination has become a major problem that affects crop development, food safety, marketability, and soil health. Heavy metal bioaccumulation and biomagnification have expanded dramatically as a result of rapid anthropogenic activities such as mining, industrialization, and agricultural chemical usage, all of which have a deleterious influence on the ecosystem and food chain [1]. Heavy metals get absorbed by the plants through the roots and binding to interfere with vital plant components causing retardation in plant growth, which can also affect photosynthesis. Heavy metals and other air contaminants that are disposed of on the soil have an immediate impact on root function. They reduce the amount of nutrients that the plant can take up from the soil. With lesser resource acquisition, plant development and growth also reduce. Some plant varieties have demonstrated the ability to tolerate relatively high amounts of pollutants without toxic effects [2]. In low dosages, a few heavy metals are essential micronutrients for plants; nevertheless, at elevated doses, they are known to cause metabolic issues and growth suppression in the majority of plant species [3]. For example, chromium's concentration is fatal at 0.5 mg L^{-1} to 5.0 mg L^{-1} in nutrient solution and 5 mg g^{-1} to 100 mg g^{-1} in soil metals. Despite the fact that heavy metals occur naturally in all regions of the Earth's crust, human activities such as industrial production and the use of metals and metal-concentrated compounds in agricultural conditions are primarily responsible for an increase in heavy metal contamination and human exposure [4].

Naturally occurring chromium is ubiquitous in soil and vegetation, although its concentration is very low. Chromium is found chiefly in chromic-iron ore. Major industries in which chromium is used are alloys, electroplating steelworks, organic chemicals, leather tanning, textile mills, paper and pulp industries, etc. Chromium ions are widely employed in industrial operations and could enter water supplies through waste removal or discharge. Chrome compounds are frequently introduced into cooling water systems to control corrosion. Chromates act as effective corrosion inhibitors by forming a protective oxide layer on metal surfaces, which reduces the electrochemical reactions that lead to corrosion[5]. Both the hexavalent and trivalent forms of chromium can be found in water supplies, while drinking water seldom contains the trivalent form.

According to recent studies, chromium induces significant modifications in the ultrastructure of chloroplasts and cell membranes, leading to impaired photosynthetic function and resultant chlorosis, followed by the yellowing of leaves due to disrupted chlorophyll synthesis. Moreover, chromium exposure causes damage to root cells, compromising their integrity and functionality, which impedes water and nutrient uptake. This damage manifests as reduced concentrations of key pigments, such as chlorophylls and carotenoids, thereby reducing photosynthetic efficiency significantly. Furthermore, chromium disrupts water relations by altering cell turgor and stomatal function, leading to impaired transpiration and water balance. [6]. Furthermore, increased Cr accumulation in plants has a major impact on seed germination. It affects the total biomass and yield by slowing down the growth rates of the roots and shoots [6].

Vigna radiata, popularly referred to as Chickasaw, moong, green gram, and golden gram, is a major short-duration pulse crop. Mungbean grain has significant quantities of crucial elements such as protein (up to 31%), iron (about 8.7 mg/100 g), and zinc (around 6.2 mg/100 g) [7]. Mung beans are a popular edible legume seed in Asia (particularly India, Southeast Asia, and East Asia), Southern Europe, and the southern United States.

While much research has been conducted on the effects of heavy metals such as chromium on different plant species, much is still unknown about the precise physiological, biochemical, and molecular mechanisms by which leguminous plants, especially Vigna radiata (mung bean), are impacted by chromium toxicity. The majority of previous studies focus on non-leguminous plants or analyze chromium's impacts at the surface rather than delving deeply into how this metal disturbs processes such as photosynthesis, nitrogen uptake, and stress responses in legumes. Additionally, there is limited information on the differential responses of mung bean cultivars to varying chromium concentrations, which could shed light on potential tolerance and toxicity mechanisms. Using *Vigna radiata* (mung bean) as a model, this work attempts to fill the current knowledge gap by methodically investigating the effects of chromium on plant growth.

2. Materials & Methods

2.1. Aim of the Research

The objective of this research is to investigate the influence of heavy metals on the germination of seeds and the growth of seedlings by measuring phytotoxicity in the roots and shoots at various concentrations.

2.2. Objectives

- Use a range of chromium concentrations to see if it affects seedling growth and germination.
- Use multiple *Vigna radiata* seeds for higher accuracies.
- Control as many external variables that could affect the results, such as volume of water, exposure to sunlight, etc.
- Design an experiment in a suitable way to assess the impact of chromium.
- Calculate the phytotoxicity of shoot and shoot-for seedlings over some time by using different concentrations.
- Observe the development of *Vigna radiata* seeds.

Certified *Vigna radiata* seeds and chromium were obtained from a local market in Jakarta, Indonesia. A modified version from [8] was adopted for the study. A 10% chromium stock solution was produced by dissolving potassium dichromate in distilled water. From this stock solution, serial dilutions were conducted to obtain working solutions with chromium concentrations ranging from 1% to 5%[8]. This solution was used to investigate the impact of chromium on seedling growth. Seeds were exposed to the chromium solutions and allowed to germinate and grow for a duration of 12 days. During this period, seed germination and subsequent growth parameters were systematically recorded at 24-hour intervals to monitor and assess the effects of chromium exposure on seedling development throughout [8]. The experiment was carried out in three separate batches. The observed and recorded data were seed germination %, root length, and shoot length [8]. The following formulas were used to calculate the percent phytotoxicity of shoots and roots:

2.3. Formula & Calculation

According to Chou and Lin's (1976) formula, the percentage of phytotoxicity for the shoot and root of Vigna Radiata was evaluated.

$$\% \text{ Phytotoxicity of Shoot} = \frac{(\text{Shoot length of control} - \text{Shoot length of treatment}) \times 100}{\text{Shoot length of control}}$$

$$\% \text{ Phytotoxicity of Root} = \frac{(\text{Root length of control} - \text{Root length of treatment}) \times 100}{\text{Root length of control}}$$

2.4. Method

2.4.1. Chromium Main Stock Preparation (10%)

Take 20 grams of Potassium dichromate in a 250 ml flask and add 200 ml of distilled water. Mix well.

2.4.2. Working Chromium Stock Preparation

Prepare a range of 1-5% Chromium solution by pipetting an appropriate amount of working chromium solution (10%). Make up the volume to 100 ml with distilled water.

Formula: V=RT/G
Where:

V= Volume of stock
R= Required concentration
T= Total Volume
G= Given main stock solution

Calculation

To prepare a 1% Chromium working solution:
V= 1*100/10= 10 ml stock +90 ml of d/w= 100ml of 1% solution

Table 1. Dilution table for preparation of chromium working stock solution (%)

Conc of cr stock solution (%)	Vol of Cr stock solution (ml)	Vol of Distilled water (ml)	Total Vol (ml)
1%	10	90	100
2%	20	80	100
3%	30	70	100
4%	40	60	100
5%	50	50	100

2.4.3. Procedure for Experimental Setup

- Take the 6 containers and bore small holes at the bottom of the container
- Fill half the containers with garden soil.
- Label each container as 1%, 2%, 3%, 4%, 5%, and control respectively.
- Add 10 pre-soaked *Vigna radiata* seeds to each container.
- Add a thin layer of soil covering the *Vigna radiata* seeds. Leave some headspace for watering.
- Leave the container with the seeds in the garden where sufficient sunlight and ventilation are available.
- Water each container with respective Chromium solutions daily.
- Monitor the shoot growth for each set for 12 days.
- Record the data in an Excel sheet.

2.5. Safety Considerations

Safe storage of heavy metal solutions is crucial. These stock solutions were stored away from incompatible materials (such as strong acids or bases) to prevent unwanted reactions. Containers were handled with care to prevent breakage or leakage. This was done using secondary containment, such as trays, to mitigate spills. The container labels were conspicuous for safety and proper identification. These labels included information such as solution name and chromium concentration.

The chromium containers were smartly coated with aluminum, as sunlight exposure can lead to photochemical reactions, affecting the chromium concentrations. Aluminium shields the solutions from harmful UV radiation. Chromium stock is susceptible to photodecomposition. In this process, molecules break down in the presence of photons. Potassium dichromate is a chromium hexavalent compound. The photodecomposition of potassium dichromate involves the production of highly reactive radicals.

- Hydroxyl radicals (•OH)
- Iodate radicals (IO_3•)
- Periodate radicals (IO_4•)

Shielding these chromium stock solutions from light helps maintain stability and accuracy.

2.6. Ethical Considerations

To safeguard the vitality of the seeds, a deliberate choice was made to use lower chromium concentrations that do not exceed more than 5% of the stock solution. This prudent approach was followed to reduce any chance of contamination to the surroundings while ensuring the continued growth and viability of the *Vigna radiata* seeds under study.

Long-term sustainability was kept in mind while setting up this experiment. Maximum efforts were made to reduce the use of resources and waste disposal as much as possible. Safely disposing of the stock solution was important due to the possible harm these solutions pose to the environment and human health, as chromium contamination can cause severe negative effects. This is because heavy metals have the potential to persist in the environment for long periods, which leads them to accumulate in the soil, water bodies, and potentially ecosystems. These heavy metals can bioaccumulate in the food chain, leading to contamination, which can also pose a threat to wildlife.

3. Results and Discussion

Table 2. % shoot germination in *Vigna radiata* exposed to different concentrations of chromium

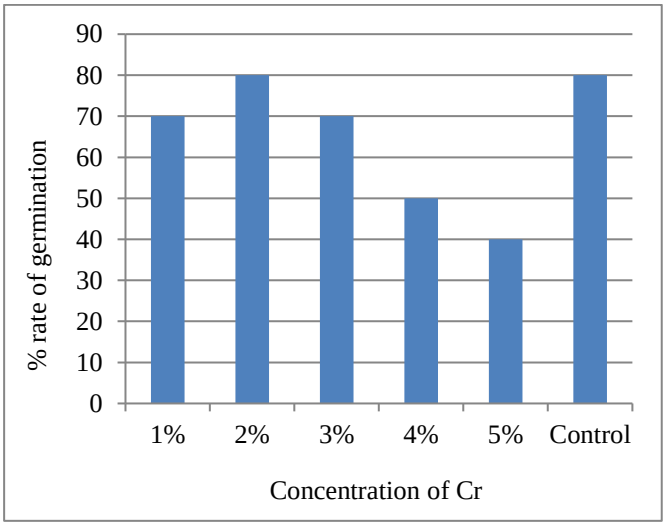
Concentration of Cr	% rate of germination
1 %	70
2 %	80
3 %	70
4 %	50
5 %	40
Control	80

From Table 2, it can be seen that the rate of germination was the maximum in the control group (80%). High germination percentages were shown by the 2% concentration (80%), while also, the 1 and 3% chromium concentrations had 70% successful germination.

Moreover, evident differences were shown from the 4% concentration that had a germination rate of 50%. The rate of germination was seen least in Mung seeds irrigated with 5% Cr stock solution (40%). This could be because Cr is a heavy

metal, and an increase in its concentration has detrimental effects on seed germination. High metal concentrations in soil can be hazardous to plants. Chromium's toxic effects on plant growth and morphogenesis include germination delays and changes, as well as lower root, stem, and leaf growth, which may affect the overall dry matter and crop yield [9]. Chromium is known to interfere with physiological and biochemical mechanisms in seeds.

In higher concentrations, chromium also inhibits root elongation and disrupts the structure of the roots, which may prevent root hair formation in the seeds. Thus, the seeds are unable to anchor themselves to the soil, preventing them from gaining access to vital mineral ions required for growth. At higher concentrations (4-5%), chromium is shown to exhibit genotoxicity, causing DNA damage and chromosomal aberrations in seeds. These chromium ions directly interact with the DNA, leading to DNA strand breaks and cross-links. This causes the germination percentage in seeds to decrease at higher concentrations.

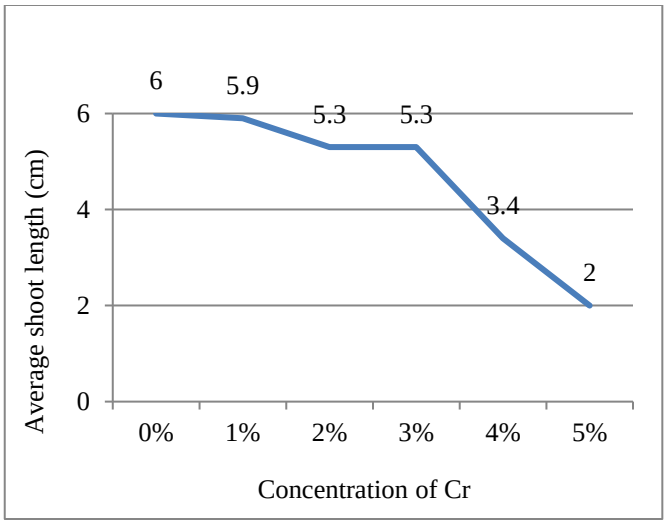


Graph 1. % seed germination in Vigna Radiata seeds exposed to Cr

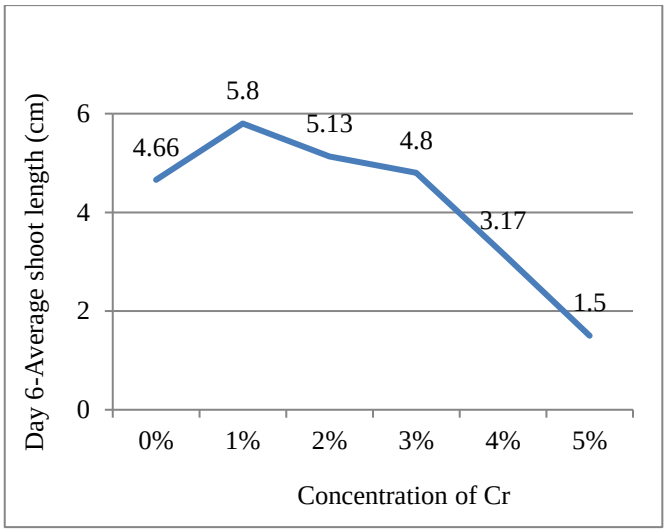
Delays in seed germination were observed in different sample sets, and hence, the shoot length was determined after the 6th day of the experiment's start date. *Vigna Radiata* seeds usually take 4-5 days to start germinating while having warm temperatures and moisture through this time frame.

More specifically, the seeds irrigated with 4% & 5% concentrations took longer to germinate than compared to the other Cr concentrations, as noticed, and there is a general trend in slow germination rates with higher levels of salinity conditions.

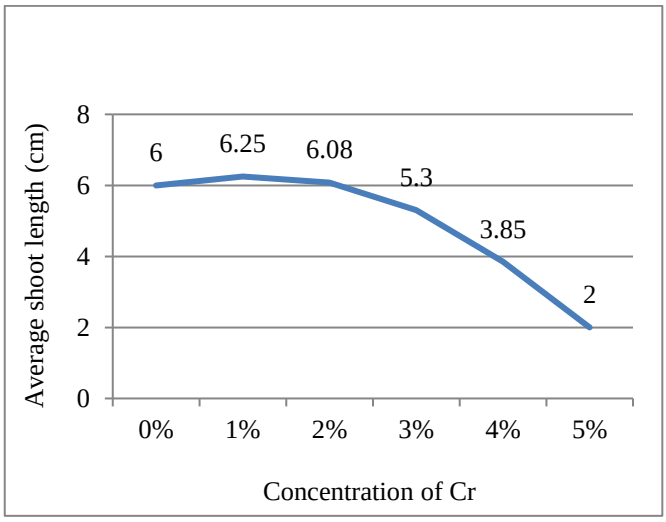
Graphs 3- 9 represent the growth of the shoot length of Mung plants from day 6 to day 12 were exposed to varying concentrations of Cr.



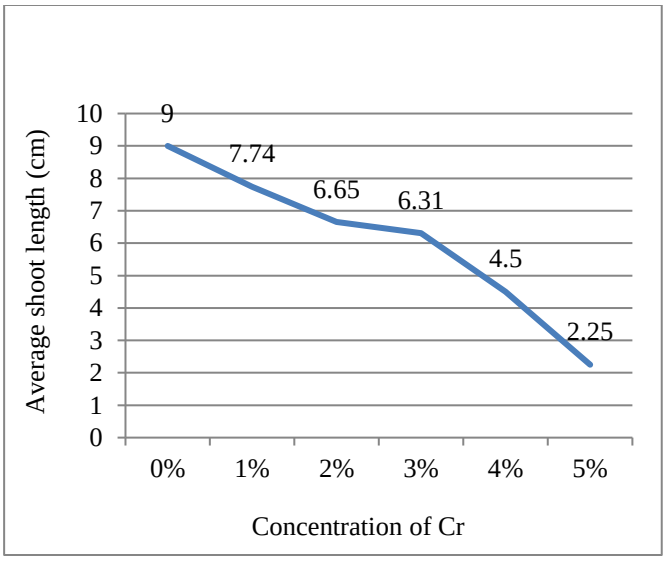
Graph 2. Mean shoot length of *Vigna radiata* on day 6.



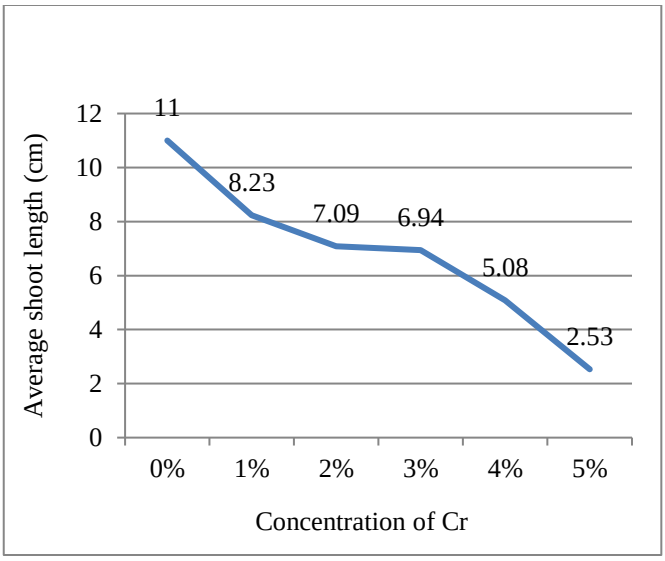
Graph 3. Mean shoot length of *Vigna radiata* on day 7



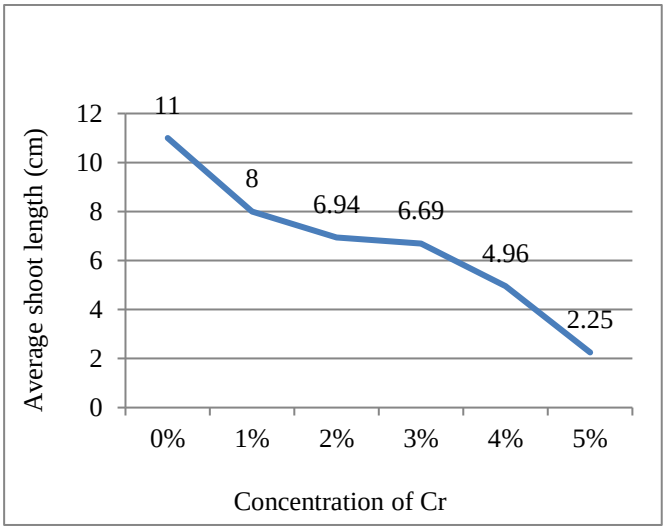
Graph 4. Mean shoot length of *Vigna radiata* on day 8



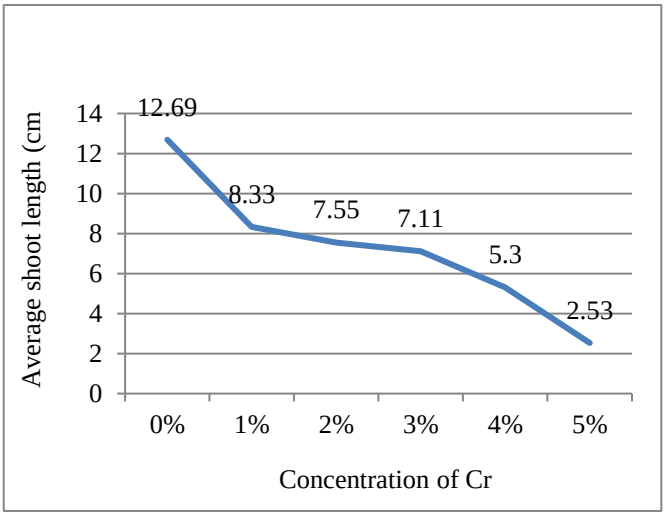
Graph 5. Mean shoot length of *Vigna radiata* on day 9



Graph 7. Mean shoot length of *Vigna radiata* on day 11



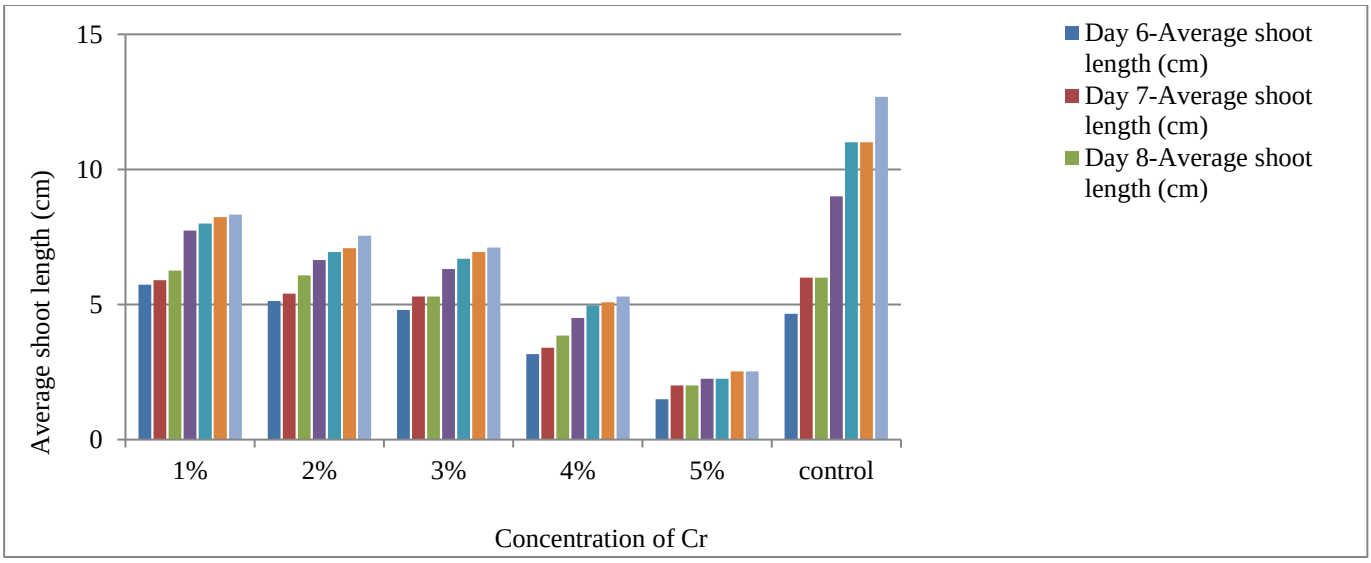
Graph 6. Mean shoot length of *Vigna radiata* on day 10



Graph 8. Mean shoot length of *Vigna radiata* on day 12 exposed to different concentrations of Cr

Table 3. Mean shoot length from days 6-12

	Average Shoot Length (cm)						
Conc.of Cr	**Day 6**	**Day 7**	**Day 8**	**Day 9**	**Day 10**	**Day 11**	**Day 12**
1 %	5.74	5.9	6.25	7.74	8	8.23	8.33
2 %	5.13	5.4	6.08	6.65	6.94	7.09	7.55
3 %	4.8	5.3	5.3	6.31	6.69	6.94	7.11
4 %	3.17	3.4	3.85	4.5	4.96	5.08	5.3
5 %	1.5	2	2	2.25	2.25	2.53	2.53
control	4.66	6	6	9	11	11	12.69



Graph 9. Mean shoot length from days 6-12

The impact of varying chromium concentrations on the height of Mung plants was monitored over 6-12 days post-sowing. In this pot culture experiment, the control group exhibited the highest biomass accumulation, whereas the group exposed to 5% chromium concentrations showed the least growth. An increase in chromium concentrations corresponded with a reduction in morphological development (shoot length) and root length growth at all the sampling days. From graphs 3-8, it can be observed that the shoot length has increased from 4.66 cm to 12.69cm. Similarly, for 1% Cr, the shoot length has increased from 5.71 cm to 8.29cm; for 2% Cr, the shoot length has increased from 5.12 cm to 7.625cm; for 3% Cr, the shoot length has increased from 4.80 cm to 7.20cm, 4% Cr the shoot length has increased from 3.14 cm to 5.0cm, 5% Cr the shoot length has increased from 1.50 cm to 2.50cm, respectively.

Currently, there is no recognized biological role for Cr in plant physiology. Plant growth may be impeded by Cr toxicity through the formation of a complex between the metal and protein, which inhibits protein synthesis and ultimately restricts plant growth [10]. Chromium, particularly in its hexavalent form (Cr(VI)), can generate reactive oxygen species within plant cells. These reactive oxygen species, such as superoxide radicals and hydrogen peroxide, cause oxidative stress, which causes damage to the cellular structures like membranes, proteins, and DNA in the *Vigna Radiata* seeds. This oxidative damage impedes normal cell functioning and leads to impaired growth and development. Chromium can also interfere with the activity of various enzymes involved in essential metabolic and fundamental processes, such as photosynthesis, respiration, and nutrient uptake. By inhibiting enzyme functionalities within the seeds, chromium disrupts key cellular processes, compromising the plant's capacity to generate energy and manufacture essential chemicals required for growth, such as nitrates, resulting in lesser amino acid development in the seed. Chromium also disrupts the uptake of other essential macro and micronutrients in the seeds. This is because chromium competes with other nutrients for uptake sites on root surfaces, leading to nutrient imbalances and deficiencies. As mentioned above this chromium entering the plant has no biological role in the plant. Lastly, chromium is known to induce changes and alter the expression of genes involved in detoxification mechanisms and growth regulations. These alterations in the expression of genes represent the plant's attempt to cope with the higher chromium concentration [11].

3.1. Phytotoxicity

Table 4. Effect of Chromium on the *Vigna Radiata* root and shoot length (% phytotoxicity)

Sample	Mean root length on Day 12 (cm)	% Phytotoxicity of root	Mean Shoot length on Day 12 (cm)	% Phytotoxicity of Shoot
Control	8.28	–	12.69	–
1%	6.69	19.20289855	8.33	34.35776202
2%	6.13	25.96618357	7.55	40.50433412
3%	5.87	29.10628019	7.11	43.97163121
4%	3.91	52.77777778	5.3	58.23483058
5%	2.82	65.94202899	2.53	80.06304177

The term "percent phytotoxicity" (%) refers to the way in which the presence of specific chemical substances inhibits the growth and development of plants [12]. Table 9 represents the % root and shoot Phytotoxicity in *vigna radiata* seeds exposed to different concentrations of chromium stock solutions ranging from 0% to 5%. The effect of chromium contamination is observed and visible in the above tables and graph as the % root Phytotoxicity ranged from 0-65.9% while the shoot phytotoxicity ranged from 0-80%. There were significant changes even with the 1% chromium concentration on the phytotoxicity of the root being 19.2% and on the shoot being 34.35%. This trend was maintained with the 2% chromium concentrations having the percentage root phytotoxicity at 25.97% and shoot phytotoxicity at 40.5%. The higher concentrations of 3% chromium had root phytotoxicity percent at 29.1% and shoot phytotoxicity percent at 43.97%.

Moreover, from this point, the chromium contamination increased significantly, which could cause serious damage to the plant's shoot and root system. The 4% concentration had the root phytotoxicity at 52.78% and shoot phytotoxicity at 58.23%. Lastly, the 5% concentration had the root phytotoxicity at 65.9% and shoot phytotoxicity at 80.1%.

4. Conclusion

The pathway of Cr uptake in plants still needs to be elucidated. However, since Cr is a non-essential element, it depends on Cr speciation and has no particular mechanism for absorption. Plants do not need to use any energy during the passive process of taking up Cr(III). Since Hexavalent Chromium is more soluble than Trivalent Chromium, which tends to form stable complexes in the soil, it is more hazardous at lower doses. According to the results, the seed germination of the *Vigna Radiata* plant is drastically affected as the chromium concentration increases. The study can be conducted under controlled conditions, such as controlling amounts of sunlight, humidity, nutrient concentration, etc, which will provide more insight into factors that contribute to heavy metal toxicity. Sunlight was majorly controlled as there were similar conditions in which the seeds were kept.

However, there could be slight differences as sometimes trees may be blocking the light from reaching the seedlings, which could lead to varied amounts of sunlight for different seedlings which may lead to varied growth outcomes. Although a sprayer was used to control the volume of chromium stock solution poured into the sample, sometimes a higher volume of stock solution may have been taken in for each spray, causing a higher volume of stock solution to be poured in. Natural garden soil was used for the experiment, and this soil already contains some nutrients in different concentrations, such as nitrogen, magnesium, etc. These nutrient concentrations in each sample could have been different, affecting the outcome of the study. Although the readings were accurate, there was still room for improvement throughout the investigation. As the experiment was carried out at home, there could be a difference in sunlight at one point over another, so there could have been varied and altered growth for the seeds. Secondly, as garden soil was used, there might have been additional nutrients within the solid, such as nitrogen and magnesium, which could have been in varied concentrations between the different samples. This may have ended up causing one sample to grow more than another sample. Furthermore, in the future, measures could be followed to improve the experiment, such as carrying out the investigation in a laboratory with controlled amounts of sunlight, humidity, nutrient concentration, etc. As a result, the growth of the seeds would be controlled, and any chance of error or growth alteration would be removed.

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