e-ISSN No.: 2582-4228

Journal of Indian Association for Environmental Management

Journal homepage: www.http://op.niscair.res.in/index/php/JIAEM/index

Studies on the Effect of Different Concentrations of Cr Stress on Seed Germination and Seedling Growth of *Triticum aestivum* L. and *Vigna radiata* (L.) Wilczek

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Submitted:27 August 2022 Revised:13October 2022 Accepted: 22October 2022

Abstract:**Heavy metals possess many distressing effects on living organisms, either directly or indirectly. One of the indirect effects is the change in plant nutritional values. Plants, in their life cycle, are usually exposed to different types of heavy metals, which readily find their way inside the cells from contaminated soils. Chromium (Cr) is one such metal which affects many physiological processes of plants and contributes to severe toxicity. But there are plant species which have remarkable metabolic activities, absorption capabilities, and transport systems that facilitate the uptake of contaminants selectively from the growth matrix (soil or water). The present study was aimed at understanding the effect of Cr on the growth of Triticum aestivum L. and Vigna radiata L. Wilczek and to investigate their bioaccumulation efficiency. The seeds were sown in soil supplemented with different concentrations of Cr (10-100 ppm), and the effect of metal on the growth parameters was examined. Results revealed that the increasing concentrations of Cr impaired the germination and growth of the seedlings, which was marked by a decrease in root and shoot length, biomass, and chlorophyll content. Significant accumulation was also observed in the cells, as proved by the anatomical and colorimetric studies. The future lies in exploring metabolically active and genetically modified plants as an effective approach towards the remediation of heavy metal contaminated ecosystems and in establishing vegetation in metal stressed soil.**

Keywords**:Chromium; phytotoxicity; bioaccumulation; phytoremediation; *Triticum aestivum;* Vigna radiata**

I. INTRODUCTION

Metals are opaque, lustrous, malleable, and ductile elements that are natural constituents of the ecosystem. They are substances which are good conductors of heat and electricity. They are present in the atmosphere, water, earth crust and can accumulate in biological organisms such as plants and animals. The metals which have a specific density above 5 g/cm^3 with an atomic weight higher than 40 are termed as Heavy metals (Li et al., 2017). Some of the examples are antimony, tellurium, bismuth, tin, thallium, gold, arsenic, cerium, gallium, cadmium, chromium, cobalt, copper, iron, lead, mercury, manganese, nickel, platinum, silver, uranium, vanadium, and zinc (Duffus, 2002). Among them, some of the heavy metals are essential, and some of them are toxic to the human body. Heavy metals namely, cobalt, copper, manganese, selenium, magnesium, iron, molybdenum, nickel, chromium, and zinc are necessary for the physiological and biochemical functioning of the body. The insufficient quantities of these essential metals causes deficiency diseases, however their excess may cause acute or chronic toxicities.

The elements are released into the environment naturally through erosion, volcanic eruptions, spring waters, bacterial activity, and human activities such as fossil fuel combustion, industrial processes, and agriculture (Florea et al., 2004). When these heavy metals are utilized in industries, they can be released in the air, water or soil. Commercial products like paints, cosmetics, pesticides, and herbicides can also be a source of heavy metals (Engwa et al., 2019).

The heavy metals released into the environment are taken up by the plants; through which they enter into humans and other animals. Prolonged consumption of such affected plants or animals leads to the accumulation of metals in different cells and tissues, causing adverse effects (Yu et al., 2011).

When these heavy metals enter the human body, they are compartmentalized into the cells where they bind to proteins and nucleic acids, causing their destruction and, in turn disrupting the cellular functions. Continuous accumulation causes damage to DNA, mutation and mimicking of hormones, leading to destruction of the endocrine and reproductive system, causing cancer (Florea and Büsselberg, 2006). This affects the Central Nervous System (CNS), leading to mental disabilities, causes damage to the lungs, blood components, kidney, liver and other organs, causing diseased conditions. Long-term accumulation of these elements can cause Parkinson's disease and Alzheimer's disease (Jaishankar et al., 2014).

Chromium (Cr) is a heavy metal most commonly found in coal and petroleum, steel, catalysts, fertilizers, pigment oxidants, and metal plating tanneries. Industrially, chromium is used in wood preservation, chemical production, tanning, electroplating, metallurgy, production of paints and pigments, and pulp and paper production. These industries cause chromium pollution with a negative effect on biological and ecological species. Human activities such as disposal of sewage and use of fertilizers may lead to chromium pollution of the environment (Ghani and Ghani, 2011). Most frequently, the environment is polluted with hexavalent chromium, which is the more toxic species of chromium when compared to Chromium (III), which is less toxic and causes little or no health problems (Shahid et al., 2017). Chromium (VI) is corrosive and causes allergic reactions in the body. Inhaling high levels of chromium (VI) can lead to irritation in the lining of the nose and also to nose ulcers. Continuous exposure can also cause anaemia, irritations and ulcers in the small intestine and stomach, and damage sperm. The allergic reactions caused by chromium show severe redness and swelling of the skin. High exposure to chromium (VI) compounds can result in severe cardiovascular, respiratory, haematological, gastrointestinal, renal, hepatic, and neurological effects and possibly death. It also causes ulcers and inhibition of erythrocyte glutathione reductase, lowering the reduction of methaemoglobin to haemoglobin. Chromate components have the ability to induce DNA damage, causing chromosomal aberrations (Matsumoto et al., 2006).

Till now, there has been no evidence of Cr having any role in plant physiology. Excess chromium levels in plant tissues have provoked many morphological, physiological and biochemical processes in plants. Chromium toxicity has been attributed to a complex series of metal interactions with genetic processes, cellular pathways, and signal transduction (Shahid et al., 2017).Therefore, chromium toxicity affects plant growth and the metabolic pathways of plants (Moral et al.,1995). It decreases plant growth by inducing ultrastructural modifications of the cell membrane, chloroplast and chlorosis in leaves, damaging root cells, reducing pigment content, transpiration, nitrogen assimilation and mineral nutrition by altering different enzymatic activities (Shanker et al., 2005; Sundaramoorthy et al., 2010). The toxic effects of Cr are due to the over production of reactive oxygen species (ROS), which alter the redox balance in plants (Sharma et al., 2020).

Phytoremediation is a fast-growing, low-cost and eco-friendly alternative for the removal of metal contaminants from the environment (Singh and Sinha, 2011). This technique engages plants to absorb, accumulate and detoxify contaminants in the air, soil and water through physical, chemical or biological processes (Zhou et al., 2013; Saha et al., 2017). Factors such as properties of soil, plant and microbial exudates, bioavailability of metals and the ability of plants to uptake, accumulate and detoxify metals account for phytoremediation efficiency (Dheri et al., 2007; Hooda, 2007).

In the present study, the phytotoxic effects of chromium on seed germination and seedling growth of *Triticum aestivum* L. and *Vigna radiata* L. Wilczek were studied. Further, the bioaccumulation efficiency and the anatomical changes in seedlings after chromium exposure were investigated.

II. MATERIALS AND METHODS

Collection of soil samples

The soil samples were collected from the green house of Kristu Jayanti College, Bangalore, India. The collected samples were dried and sieved using a mesh to remove the undesired particles and stored in an airtight container. The soil was then subjected to pH testing and estimation of chromium.

Selection of plant material

The seeds of *Triticum aestivum* L. (Wheat) and *Vigna radiata* L. Wilczek (Mung bean) were selected to study the effects of chromium on germination and seedling growth. Wheat is a cereal and is consumed as food worldwide. Wheat is the second major crop produced and its world trade is the largest. It has acquired global demand due to its viscoelastic and adhesive nature of gluten protein, which facilitates in the production of packed foods. Mung bean is a legume which is cultivated for its seeds and sprouts in Asia. This is an annual plant, which is erect and has a height of 0.15-1.25 m. The leaves are alternate, trifoliolate, with elliptical to ovate leaflets. The flowers are pale yellow or greenish. The pods of this plant are long, cylindrical and hairy, consisting of 7-20 small cube-shaped seeds. The seeds are green or yellow and consist of high protein content in nature.*Triticum aestivum* L. is the staple food which is a major source of carbohydrates in India and *Vigna radiata* L. Wilczek is a legume with edible seeds which are sources of protein. Both these plants have rapid growth, and hence they were used as the model plants.

Preparation of Cr stock solution

Potassium dichromate ($K_2Cr_2O_7$) salt was used to prepare the stock solution of 100 ppm (w/v) concentration in distilled water, and it was stored in an airtight reagent bottle in the refrigerator. Working solutions of desired concentrations were prepared by diluting the stock solution.

Experimental design

About 10g of soil was sieved and transferred into a clean test tube and sterilized by autoclaving. The selected seeds

were washed properly with distilled water and were surface sterilized with 0.1% mercuric chloride in laminar air flow. Ten seeds each of wheat and Mung bean were inoculated in the test tubes supplemented with different concentrations of chromium (0, 10, 50, 100 ppm) (Table 1) in triplicates and were incubated for 10 days under 16/8 photoperiod.

TABLE 1
The Treatment Protocol used for Wheat and Mung Bean

Treatments	Chromium concentration (ppm)	Wheat	Mung bean
Control	0 ppm	10 seeds	10 seeds
Treatment 1	10 ppm	10 seeds	10 seeds
Treatment 2	50 ppm	10 seeds	10 seeds
Treatment 3	100 ppm	10 seeds	10 seeds

Growth parameters

Estimation of length and biomass

After 10 days of incubation, the plant samples were harvested and washed thoroughly with distilled water. Using a scale, the length of the shoot and root was measured and recorded. Furthermore, the roots and shoots were separated and the fresh biomass was measured for each seedling using an electronic weighing balance.

Chlorophyll estimation

The chlorophyll content of the seedlings was estimated using the acetone method (Arnon, 1949). Shoot samples were weighed, cut into small pieces and homogenized using extraction buffer [0.1N (w/v) NH_4OH solution with reagent grade acetone in the ratio of 1:9]. Then 5 ml of 80% (v/v) aqueous acetone was added and transferred into a centrifuge tube. The tubes were centrifuged at high speed for 20 minutes, and the supernatant was collected. To the supernatant, 10 ml of 80% (v/v) acetone was added and the absorbance was measured at 645 nm and 663 nm calorimetrically (Sudhakar et al., 2016). Chlorophyll was estimated by using the following standard calculation:

Chlorophyll a (mg/ml) = $12.7\ A_{663} - 2.69\ A_{645}$

Chlorophyll b (mg/mL) = $22.9\ A_{645} - 4.68\ A_{663}$

Total Chlorophyll = Chlorophyll a + Chlorophyll b

Measurement of survival index

The Survival index (SI) for each plant was calculated by determining the number of plants survived in each treatment divided by the sample size. It could be represented by the following equation:

$$Survival\ Index\ (SI) = \frac{\text{Number of plants survived}}{\text{Sample size}} \times 100$$

Anatomical studies

Thin cross-sections of the root and shoot were obtained using a sharp razor blade and washed with distilled water. The sections were stained with 0.05% safranin, followed by a thorough washing with distilled water. The sections were covered with a cover slip and observed under a light microscope for anatomical changes.

Estimation of Cr in plant tissues

The standard graph of Cr was prepared using potassium dichromate and sulphuric acid by the method described by Lace et al. (2019). Chromium in the root and shoot of different seedlings was determined by taking 1 g samples, which were grinded using distilled water. Aliquots of 1 ml from each sample were taken in test tubes, while the blank consisted of 1 ml of distilled water. Then, 5 ml of 0.01M H_2SO_4 was added to all the tubes and incubated at room temperature for 15 minutes. The optical density was measured at 540 nm and the concentration of Cr was determined by putting the values in standard graph.

III. Results and Discussions

Determination of germination rate

From the first day of incubation, the rate of germination was recorded on a daily basis. For both the seedlings (wheat and mung bean), maximum germination was observed in the control while the least was observed in the 100 ppm Cr concentration (Fig. 1). In *T. aestivum*, germination was recorded to be 93% in control, 80% in 10 ppm Cr, 73% in 50 ppm Cr and 13% in 100 pm Cr concentration. In *V. radiata*, germination was found to be 95% in control, 71% in 10 ppm, 57% in 50 ppm and 23% in 100 ppm Cr concentration (Fig. 2).

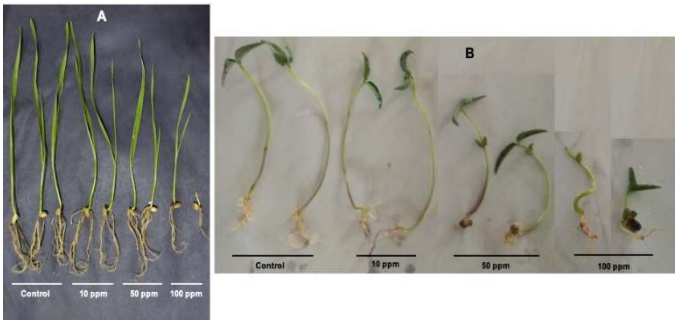

Fig.1. Effect of Cr on the growth of (A) *Triticum aestivum* and (B) *Vigna radiata* seeds.

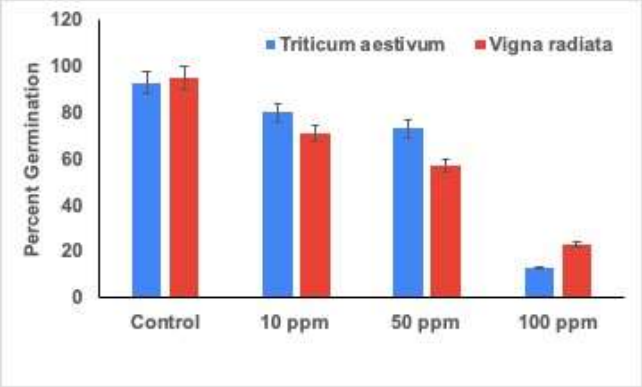

Fig.2. Effect of Cr on germination rate of *Triticum aestivum* and *Vigna radiata* seeds.

Determination of Shoot length

The mean shoot length of *T. aestivum* was found to be 17.54 cm in control, 14.25 cm in 10 ppm, 10.6 cm in 50 ppm and 3.15 cm in 100 ppm Cr concentrations, respectively. The average shoot length of *V. radiata* was found to be 10.57 cm in control, 7.55 cm in 10 ppm, 4.9 cm in 50 ppm and 3.45 cm in 100 ppm Cr concentrations, respectively (Fig. 3).

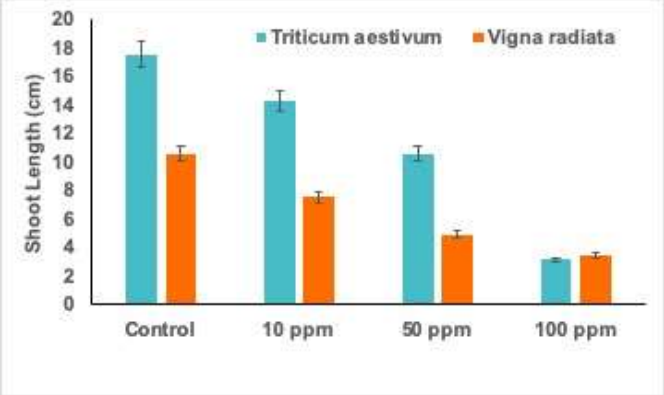

Fig.3. Effect of different concentrations of Cr on shoot length of *Triticum aestivum* and *Vigna radiata*seedlings.

Determination of Root Length

The root length of *T. aestivum* was estimated to be 8.09 cm in control, 6.9 cm in 10 ppm, 5.3 cm in 50 ppm and 1.0 cm in 100 ppm Cr concentrations, respectively. The mean root length of *V. radiata* was found to be 4.26 cm in control, 3.74 cm in 10 ppm, 2.45 cm in 50 ppm and 2.03 cm in 100 ppm Cr concentrations, respectively (Fig. 4).

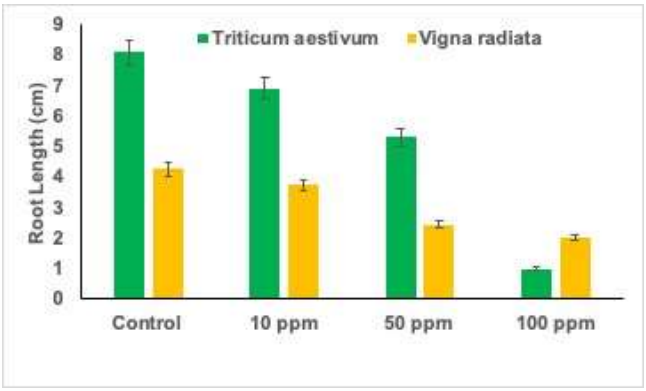

Fig.4. Root length of *Triticum aestivum* and *Vigna radiata* under different Cr concentrations.

Determination of Shoot Biomass

The mean shoot biomass of *T. aestivum* was found to be 0.11g in control, 0.08 g in 10 ppm, 0.07 g in 50 ppm and 0.02 g in 100 ppm Cr concentrations, respectively. The average shoot biomass of *V. radiata* was estimated to be 0.47 g in control, 0.3 g in 10 ppm, 0.19 g in 50 ppm and 0.12 g in 100 ppm Cr concentrations, respectively (Fig. 5).

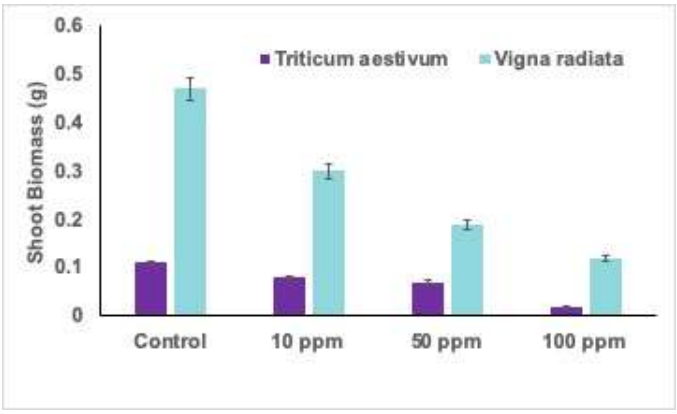

Fig.5. Effect of Cr on shoot biomass of *Triticum aestivum*, and *Vigna radiata* seedlings.

Determination of Root Biomass

The mean shoot biomass of *T. aestivum* was found to be 0.106 g in control, 0.08 g in 10 ppm, 0.05 g in 50 ppm and 0.01 g in 100 ppm Cr concentrations, respectively.The average shoot biomass of *V. radiata* was estimated to be 0.38 g in control, 0.15 g in 10 ppm, 0.14 g in 50 ppm and 0.03 g in 100 ppm Cr concentrations, respectively (Fig. 6).

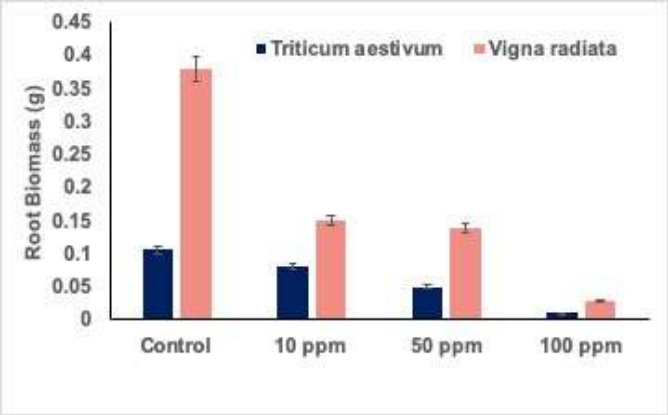

Fig.6. Effect of Cr on root biomass of *Triticum aestivum* and *Vigna radiata*seedlings.

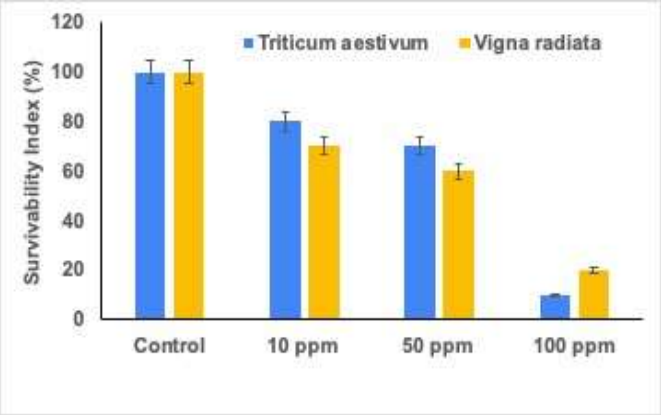

Fig.8. Survival Index of (a) *Triticum aestivum*, and (b) *Vigna radiata* in the presence of different Cr concentrations.

Chlorophyll estimation

The chlorophyll content (a and b) of the shoots of different treatments was analysed calorimetrically. As the concentration of chromium increased, the chlorophyll content was found to decrease gradually.

The chlorophyll a in *T. aestivum* was found to be 1.49 in control, 1.06 in 10 ppm, 0.86 in 50 ppm, and 0.56 in 100 ppm Cr concentrations, respectively; while in *V. radiata* it was found to be 2.17 in control, 1.47 in 10 ppm, 0.87 in 50 ppm, and 0.57 in 100 ppm Cr concentrations, respectively (Fig. 7a).

T. aestivum had a chlorophyll b content of 7.9 in control, 7.49 in 10 ppm, 7.13 in 50 ppm and 6.58 in 100 ppm Cr concentrations, whereas *V. radiata* had a chlorophyll b content of 8.4 in control, 7.12 in 10 ppm, 6.03 in 50 ppm and 5.486 in 100 ppm Cr concentrations (Fig. 7b).

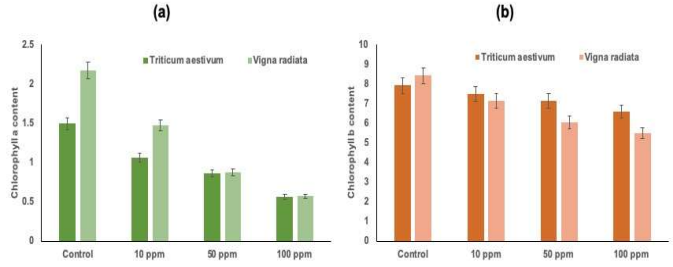

Fig.7. Chlorophyll a content in (a) *Triticum aestivum* and *Vigna radiata*seedlings; chlorophyll b content in (b) *Triticum aestivum* and *Vigna radiata* seedlings.

Survivability Index

The survivability index of *T. aestivum* was estimated to be 93% in control, 80% in 10 ppm, 73% in 50 ppm and 13% in 100 ppm Cr concentrations, respectively, while that of *V. radiata* was found to be 95%, 71%, 57% and 23% respectively, in control, 10 ppm, 50 ppm and 100 ppm Cr concentrations (Fig. 8).

Anatomical Study

Anatomical structural changes in the roots and shoots of *T. aestivum and V. radiata* plants were observed under chromium stress conditions. The cross-section of the shoots of the chromium-treated plants was marked by the blackening of the cells all along the walls of the cortex, indicating severe chromium toxicity. There were no black spots observed in the control. However, as the chromium concentration increased, the intensity of the spots also increased in both *T. aestivum* and *V. radiata*. The shoot micrographs also revealed distorted shapes due to the breakdown of the epithelial and cortex cells. Significant changes in the shape and size of the cells were observed in Cr-treated seedlings when compared to control (Fig. 9).

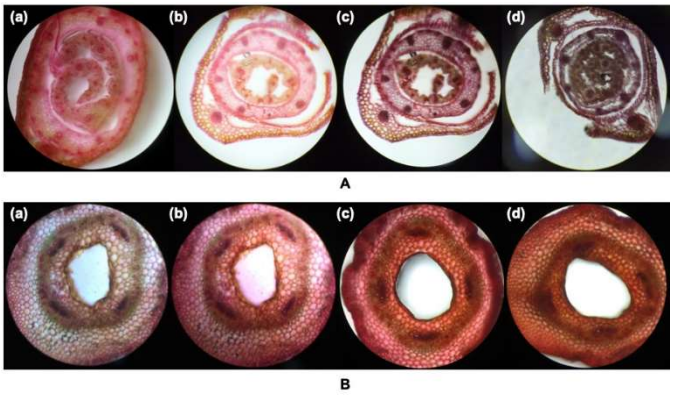

Fig.9. A. Anatomical changes in the shoot structure of *Triticum aestivum* L. (a) Control, (b) 10 ppm, (c) 50 ppm, and (d) 100 ppm Cr concentrations. **B.** Anatomical changes in the shoot structure of *Vigna radiata* (a) Control, (b) 10 ppm, (c) 50 ppm, and (d) 100 ppm Cr concentrations.

Chromium content in plant tissues

The seedlings exposed to Cr concentrations showed significant levels of accumulation in the tissues. As the concentration of Cr increased, the accumulation also increased. The maximum accumulation was seen in roots when compared to shoots. There was no Cr content in the control shoots and roots, while maximum accumulation was

recorded in the seedlings exposed to 100 ppm Cr concentration. The Cr content in the shoots of *T. aestivum* was found to be 25, 85 and 220 µg/ml respectively, when subjected to 10, 50 and 100 ppm Cr concentrations. Further, in the roots, the accumulation was reported to be 53, 190 and 460 µg/ml respectively, in the presence of 10, 50 and 100 ppm Cr concentrations (Fig. 10a).

The concentration of Cr in the shoots of *V. radiata* was estimated to be 12, 70 and 210 µg/ml, while in roots it was calculated to be 53, 180 and 300 µg/ml respectively, when exposed to 10, 50 and 100 ppm Cr concentrations (Fig. 10b).

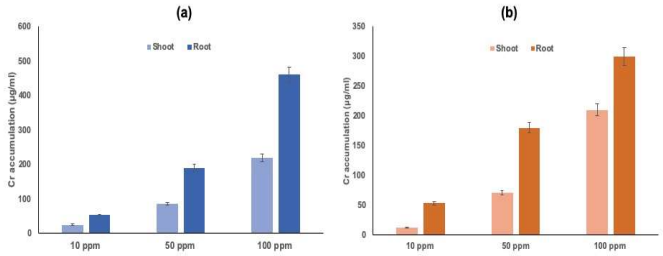

Fig.10. Accumulation of Chromium in (a) *Triticum aestivum*L., and (b) *Vigna radiata* seedlings.

Discussion

Chromium is a toxic element which causes adverse effects in humans, animals and plants. The accumulation of Cr in living organisms is a global threat and needs immediate attention. Phytoremediation is a promising, eco-friendly, cost-efficient and appealing method to prevent heavy metal accumulation (Revathi et al., 2011; Saha et al., 2017). The present is an attempt to understand the correlation between the concentration of Cr and the plant's growth. The data suggests that there is a negative correlation; as the concentration of Cr increased, the growth of *Triticum aestivum* L. and *Vigna radiata* seedlings decreased. In their studies, similar trends were observed by Bishnoi et al. (1993), Moral et al. (1995) and Dotaniya et al. (2014).

The treatment of seedlings with different concentrations of Cr clearly indicated that the increased concentration had a more detrimental effect on the plant growth. The close observation of the seedlings for various growth parameters showed that the maximum growth occurred in the control while minimum growth was achieved in the presence of 100 ppm Cr concentration. The trend was similar in all the growth parameters such as germination rate, shoot length, root length, shoot biomass, root biomass, survivability index, and chlorophyll content (Figs. 1-8). Effect of Cr toxicity on the morpho-physiological characteristics, growth and yield of *Cicer arietinum* L. has been reported by Singh et al. (2021). The study showed that increasing concentrations of Cr is correlated with the increased oxidative damage in seedlings. The toxic effects of Cr on seed germination and seedling growth have also been reported in other plant species like *Brassica oleracea botrytis* L. (Ahmad et al., 2020) and *Brassica napus* L. (Zaheer et al., 2020). The toxicity of Cr to plants depends on its valence state: Cr(VI) is highly toxic and mobile whereas Cr(III) is less toxic. Chromium negatively affects crop growth, total yield and grain quality. Exposure of chromium even at low concentration enhances its accretion in cells (Kapoor et al., 2022).

Chromium, when present in a higher concentration, also causes deleterious effects on plant physiological and metabolic processes. The alterations caused by Cr exposure in plants are described by its ability to generate reactive oxygen species which may cause oxidative stress (Shanker et al., 2005). Investigation of the anatomical behaviour of the seedlings exposed to Cr clearly indicated severe Cr toxicity, which was marked by the excessive generation of black spots in different regions of the cells, and it gradually increased with increasing chromium concentration (Fig. 9). Furthermore, there were regions marked with cell damage to a great extent. Chromium was capable of reaching to the innermost region of the tissues and causing cell distortion. As evident from the light micrographs, there were noticeable changes in the structures of the seedlings exposed to Cr when compared to the control ones. Many previous findings suggest that heavy metals are responsible for much of the tissue damage in a variety of plant species to a great extent (Pandey and Bhatt, 2016; Soumya et al., 2022).

Plants have an exceptional ability to tolerate heavy metals by transporting them through their cells. This leads to the accumulation of metals in the plant tissues. Because plants lack a specific transport system for Cr, it is primarily transported to plant parts via specific and non-specific carriers of essential ions such as sulphate or iron (Shahid et al., 2017). The present study gives insight into the capability of *Triticum aestivum* L. and *Vigna radiata* seedlings to accumulate Cr. Investigation of the Cr concentration in the tissues of these plants showed that both the varieties accumulated Cr both in their roots and shoots (Fig. 10). However, the concentration of Cr was found to be higher in the roots when compared to the shoots, which is likely because the roots are in direct contact with the soil. This could also be due to the less translocation of Cr towards the shoot tissues, which could be considered as one of the reasons that the seedlings withstand high Cr pressure (100 ppm), as most of the Cr is restricted to the roots. This is one of the defensive mechanisms of the plants to survive high metal concentrations (Pandey and Bhatt, 2016; Sinha et al., 2018).

It is evident from the present study that the increase in chromium concentration is detrimental to the plants. Some of the previous studies also suggest the same correlation with different plant species (Corradi et al., 1993; Sundaramoorthy et al., 2010). In the future, the plants can be grown to the flowering and fruiting stage. The chromium accumulation in the flowers and seeds can be studied. It is a challenging project because both the chosen plants are edible and the study needs to be conducted in an ethical manner. The study helped to figure out how much of the chromium can be taken up by different parts of the plant. Extending the study with different edible plants with the least effect on the seeds can be used to remediate the polluted soils.

IV. Conclusion

Industrial waste is one of the main sources of toxic heavy metals released into the environment, causing serious environmental hazards. The present study gives a clear indication that the heavy metal Cr is harmful to all life forms, though, it can be remediated naturally using plants. The results indicated that *Triticum aestivum* L. and *Vigna radiata* could tolerate lower concentrations of Cr, but with the increase in concentration, physiological and anatomical changes were distinctly observed. Phytoremediation is an eco-friendly and cost-effective approach for the successful mitigation of chemical pollutants in a beneficial way. More research in this field is needed, however, to fully understand the mechanisms involved in metal tolerance in plants and to find an appropriate way to eliminate the toxic heavy metal from the environment. Further investigations are required for the proper implementation of sustainable remediation strategies with their real-time applicability on contaminated sites.

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