



Available Online at EScience Press

Plant Health

ISSN: 2305-6835

<https://esciencepress.net/journals/planthealth>

Enhancing Wheat (*Triticum aestivum* L.) Resilience to Hexavalent Chromium Stress through the Application of Farmyard Manure

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ARTICLE INFO

Article History

Received: January 03, 2024

Revised: March 21, 2024

Accepted: April 17, 2024

Keywords

Wheat

Hexavalent chromium

Phytotoxicity

Farmyard manure

Mitigation

ABSTRACT

Toxicity due to heavy metals is one of leading contributors of losses in crop fields in the whole world. In the current research work, the impact of organic soil amendment with farmyard manure on different growth and physiological parameters of wheat plants under the stress of Hexavalent Chromium (Cr-VI) was investigated. The experiment was conducted in plastic pots in the greenhouse. In the experiment, three different treatments concentrations of Cr(VI) (100, 200 and 300 mg kg⁻¹) were taken individually and in combination with 1% FYM. Wheat grown under the stresses of chromium exhibited the reduction in growth parameters like shoot length, root length, root biomass and shoot biomass. Whereas, the soil amendment with 1% farmyard manure mitigated the effect of both stresses and enhanced the growth of tested plant. Thus, soil amendment with farmyard manure reduced the chromium stress in plants. So far, further studies are required to investigate the efficacy of farmyard manure as a soil amendment in abiotic stresses in soil to enhance the plant growth in field trials.

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INTRODUCTION

Wheat (*Triticum aestivum* L.) is a fundamental staple crop contributing significantly to global food security. However, the sustainable production of wheat faces multiple challenges, with soil contamination being a critical concern. Among the various contaminants, hexavalent chromium (Cr(VI)) toxicity poses a substantial threat to wheat crops (Khan et al., 2016). According to the U.S. Environmental Protection Agency (EPA), the ecological soil screening level (Eco-SSL) for total chromium is set to protect plants and soil-dwelling organisms. The EPA has set the Eco-SSL for total chromium at 37.3 mg kg⁻¹ in soil (Sinduja et al., 2022). Chromium is a naturally occurring element that exists in multiple oxidation states, with hexavalent chromium

being particularly toxic to plants as well as other living organisms. This heavy metal contaminant enters the soil through anthropogenic activities such as industrial discharges, improper waste disposal, and the use of chromium containing fertilizers. Chromium contamination in soil is a significant environmental concern in Pakistan, particularly in industrial areas. The primary sources of chromium pollution are industrial activities such as leather tanning, electroplating, and the use of chromium-based compounds in various manufacturing processes. Once in the soil, hexavalent chromium can adversely affect the growth, development, and yield of wheat plants (Alloway, 2013). Chromium in polluted agricultural soils accumulates in the crop system; therefore, it carries human risks due to food

chain contamination (Ahemad, 2015).

Chromium toxicity in wheat manifests through several physiological and biochemical mechanisms. The metal interferes with essential plant processes, including nutrient uptake, photosynthesis, and enzymatic activities, leading to morphological abnormalities and reduced biomass. Additionally, the accumulation of hexavalent chromium in plant tissues may compromise the quality of wheat grains, impacting their nutritional value and safety for human consumption (Kabir and Rahman, 2019). The impact of chromium toxicity on wheat is not only an agronomic concern but also raises broader environmental and health issues. Contaminated wheat crops can enter the food chain, posing risks to both animal and human health. Addressing the challenges associated with chromium toxicity in wheat requires a multidisciplinary approach. Integrated knowledge from agronomy, soil science, and environmental science is required to develop sustainable and effective mitigation strategies (Tripathi *et al.*, 2017).

In addressing the challenges posed by hexavalent chromium stress, sustainable agricultural practices are essential. Farmyard manure (FYM), a rich source of organic matter derived from animal waste and other residues, has gained attention for its potential to mitigate the impact of heavy metal stress on crops. The incorporation of farmyard manure into the soil has been reported to enhance soil fertility, improve nutrient availability, and promote overall soil health (Meena *et al.*, 2014).

MATERIALS AND METHODS

Preparation of Cr(VI) solution

In a typical preparation of 1000 ppm Cr(VI) stock solution, 1000 mg Potassium dichromate ($K_2Cr_2O_7$) was dissolved in double distilled water while making 1 L of solution. Different concentrations containing 300 and 500 mg kg^{-1} Cr(VI) were prepared by the dilution's method using doubly distilled water.

Procurement of seeds

This study was executed on seeds of wheat plant (Ujala-2016) that were obtained from Ayub research institute, Faisalabad, Pakistan.

Soil preparation

The soil sterilization was performed by inserting formalin-soaked cotton plugs in soil at selected points. These selected points were protected by polythene for

7 days. Prior to sieving and metal spiking, soil was kept for another 7 days without plastic sheet and cotton plugs to come up with a sterilized and formalin free soil. Finally, the soil was sieved (mesh size: 2 mm) and utilized for experiment. The spiking was performed by spraying Cr (VI) solutions (100, 200, 300 mg kg^{-1} on sterilized substratum and sprayed soil, left for 2 days. Finally, the metal spiked soil was crushed and re-sieved. Furthermore, soil saturation (%) and water required for saturation were determined (Malik *et al.*, 1984).

Seed Sowing and treatment plan

About 350 g sterilized soil was filled in plastic pots. Furthermore, five surface sterilized healthy seeds were sown in each pot. The experiment was divided in three sets with seven different treatments. First set was named as negative control, second set was labeled as 100, 200 and 300 mg kg^{-1} of Cr(VI), third set of the experiment contained soil amendment with 1% FYM in each metal dose of contaminated soil.

Morphological assay

The morphological assay of wheat plant grown under Cr(VI) stress was evaluated after 14th day of seed germination while observing chlorosis, necrosis, reduction in root growth and decrease in shoot growth.

Growth assay

The sampling was done after 14th day of wheat seed germination while carefully uprooted the plants at harvesting. Prior to the separation of shoots and roots the uprooted plants were washed under running water and then dried with blotting paper. Moreover, the length (cm) and fresh weight (g) of each plant was recorded. In addition, roots and shoots were oven dried at 80 °C for 24 hours and re-weighed to determine the dry weight.

Statistical Analysis

The data obtained in this study was statistically analyzed by applying LSD Test using computer software Statistics 8.1. Moreover, the principal component analysis and heat map was analyzed by using Origin Pro.

RESULTS

In the present research work, the influence of farmyard manure on the growth of wheat plant grown under different levels of Cr(VI) stress was studied. The obtained results revealed remarkable increase in plant growth by soil amendment with 1% FYM in metal

contaminated soil. Moreover, the wheat plants that were growing in metal contaminated soil showed typical symptoms like stunted growth of root and shoot and yellowing of leaves were observed. However, soil amended with 1% FYM exhibited no symptoms in wheat plant.

Growth Parameters

Shoot length

In comparison to the shoot length of plant (5.6 cm) in control sample, the shoot length of wheat plant was significantly ($P \leq 0.05$) declined by 23 to 63% at 100 to

300 mg/kg of Cr(VI). However, a significant increase in shoot length (-81 to -190%) was noticed while applying 1% FYM than respective control as shown in Figure 1.

Shoot fresh weight

Results reveal that the shoot fresh weight of wheat plant was significantly ($P \leq 0.05$) declined by 48-78% at 100-300 mg/kg of Cr(VI) in comparison to control (5.6 cm). However, the use of 1% FYM significantly increased the said parameter of wheat plant by -75 to -84% as compared to respective control as provided in Figure 1.

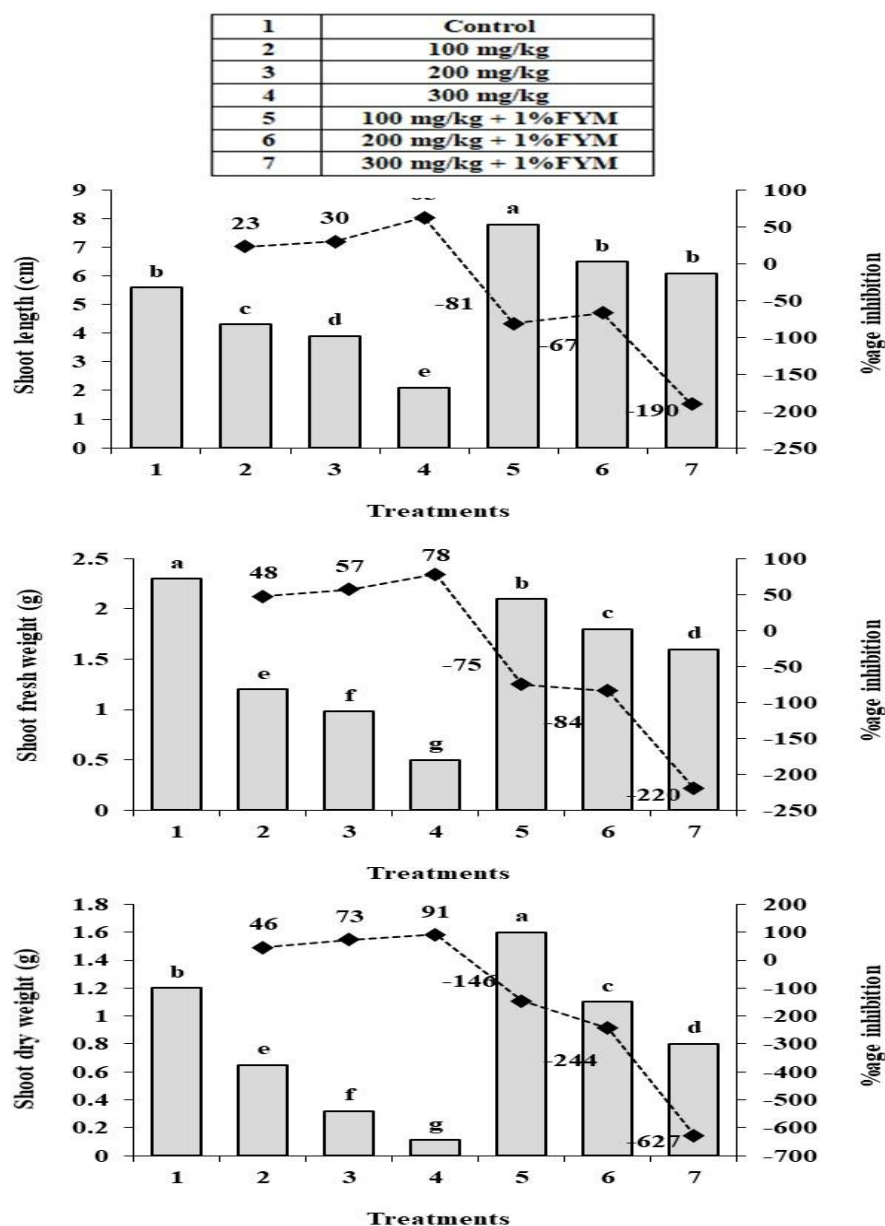


Figure 1. Influence of farmyard manure on the shoot parameters of wheat plant grown under Cr(VI) stress. Alphabets on bars: Least significant difference of three replicates.

Shoot dry weight

The shoot dry weight of wheat plant was also significantly ($P \leq 0.05$) reduced by 46-91% at 100-300 mg/kg of Cr(VI) as compared to control (5.6 cm). However, with the application of 1% FYM a significant increase in parameter of wheat plant by -146 to -630% as compared to respective control (Figure 1) was noticed.

Root length

In comparison to control (5.6 cm), the root length of wheat plant was significantly ($P \leq 0.05$) declined by 31-

77% at 100 to 300 mg/kg of Cr(VI). However, with the application of 1% FYM a significant (-16 to -112%) increase in the said parameter of wheat plant was observed as compared to respective control (Figure 2).

Root fresh weight

The root fresh weight of wheat plant was declined significantly ($P \leq 0.05$) 15 to 30% at 100 to 300 mg/kg of Cr(VI) in comparison to control (5.6 cm). However, a significant (-27 to -17%) increase in the said parameter of wheat plant while applying 1% FYM was noticed in comparison to respective control as provided in Figure 2.

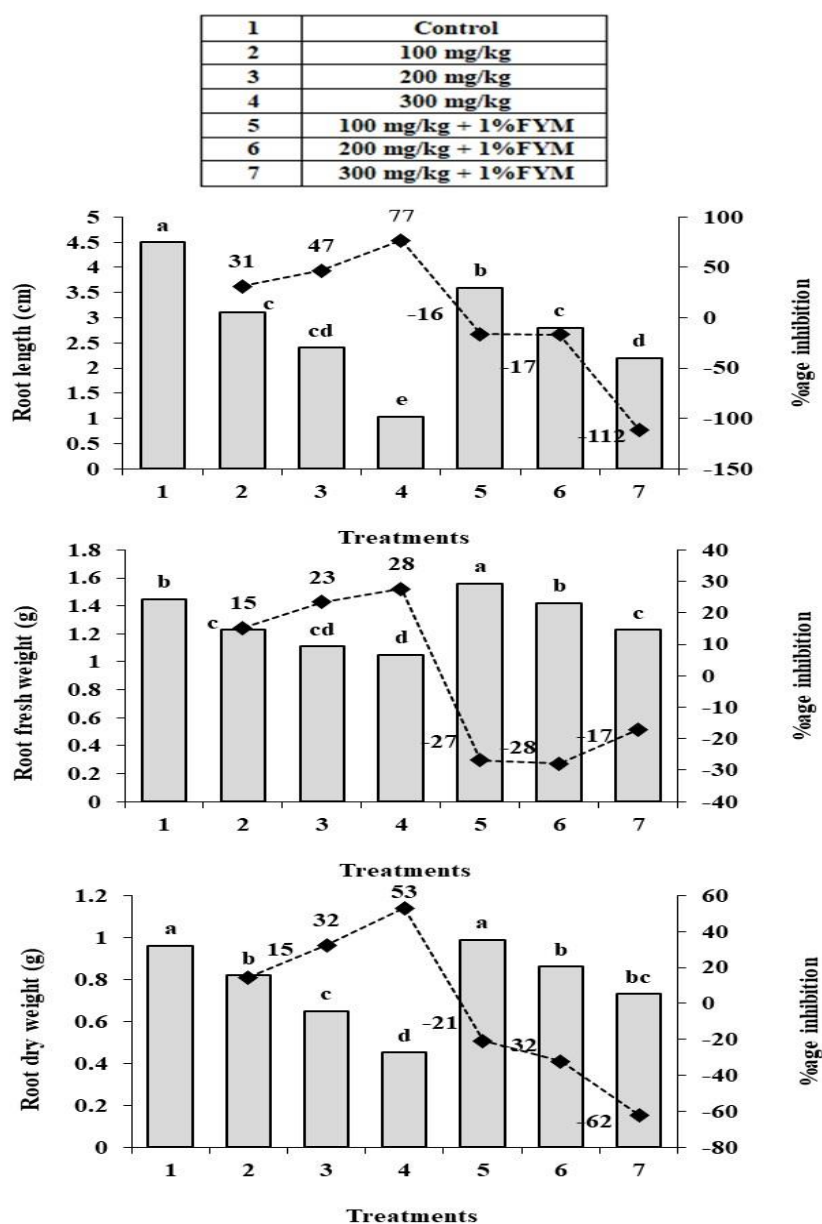


Figure 2. Influence of farmyard manure on the root parameters of wheat plant grown under Cr(VI) stress. Alphabets on bars: Least significant difference of three replicates.

Root dry weight

Results reveal that the root dry weight of wheat plant was significantly ($P \leq 0.05$) declined by 15 to 53% at 100 to 300 mg/kg of Cr (VI) as compared to control (5.6 cm). However, with the application of 1% FYM a significant increase in the said parameter of wheat plant by -21 to -62% was observed as compared to respective control as shown in Figure 2.

Principal component analysis and heat map

The principal component analysis (Figure 3 A) and heat map (Figure 3 B) showed that wheat plant that grown under the stress of metal alone exhibited negative association in plant growth while incorporation of farm yard manure revealed positive correlation in plant growth.

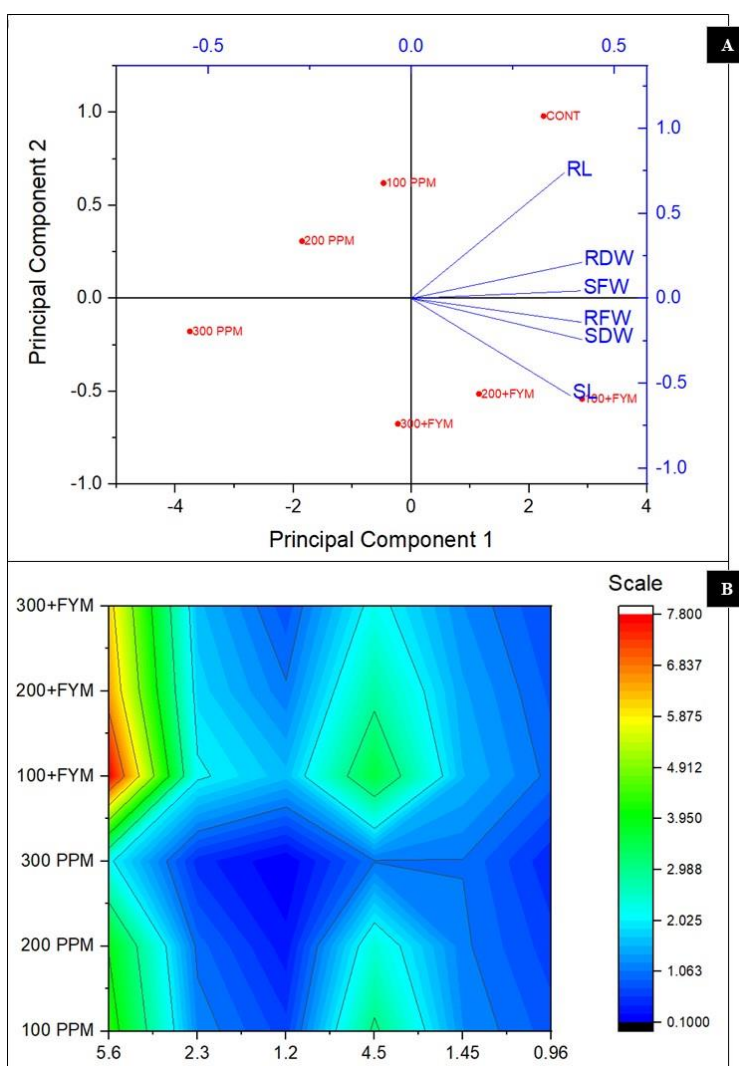


Figure 3 A and B. Principal component analysis and heat map among treatments and growth parameters of wheat plant.

CONCLUSION

The results revealed remarkable increase in plant growth by soil amendment with 1% FYM in metal contaminated soil was attributed to the presence of fertilizer (s) in farmyard manure whose continuous and

effective supply to the plants plays a vital role in increasing the growth of a plant (Akhtar *et al.*, 2023).

In the present study the toxicity posed by Cr(VI) in the soil was responsible for the decrease in shoot length (23 to 63%), shoot fresh weight (48 to 78%) shoot dry weight (46 to 91%), root length (31 to 77%), root fresh

weight (15 to 30%) and root dry weight (15 to 53%) of wheat plant in comparison of control. The negative impact of Cr(VI) contaminated soil on the shoots and roots of *Zea mays* was noticed and this soil was reclaimed with FYM by Kannan and his coresearchers (Kannan et al. 2014). In another study by Gurmeet and his coworkers, the toxic effect of Cr(VI) containing soil on the growth of spinach was studied by applying $K_2Cr_2O_7$ with different quantities of Cr(VI) (0, 1.25, 2.5, 5.0 and 10.0 mg) per kg of soil. Wherein, a significant decrease in dry matter yield of shoots was observed with increasing Cr(VI) content in the soil and they reported the positive role of FYM in decreasing the toxicity of Cr(VI) (Kavinder et al., 2019). In fact, the higher level of Cr(VI) retards the process of availability of nutrients to the plants by creating oxidative stress which ultimately leads to the reduction of these growth parameters (Khan et al., 2020).

The sufficient availability of FYM also increases the water and nutrient holding capacity of soil which is equally helpful in mitigating the toxic effects of heavy metals such as Cr(VI) in soil as well (Tadesse et al. 2013). In addition, FYM also improves the mineral supply of soil and soil fertility while enhancing its microbial activity (Kumar, et al., 2021). It is also reported that that FYM considerably improved growth of plant application of soil amendment with FYM also increased morphology and physiology of plant (Akhtar et al., 2023). Furthermore, according to they also said that the application of 1% FYM in metal contaminated soil possibly increased the resources for the self-protection of plants, may lead to restoration in chloroplast-to-nucleus communication, therefore accounting for improvement in crop yield. The application of FYM boosts microbial activity in the soil. These microorganisms can transform Cr(VI) into the less toxic Cr(III) form, thus reducing the toxicity and availability of chromium to plants. Therefore, the wheat plants when exposed to chromium stress in the presence of 1% FYM exhibited an increase in physiological and biochemical markers presenting metabolic cost for limiting the adverse effects of Cr(VI).

According to latest study, the toxicity of Cr(VI) contaminated soil was reduced with the use of 1% FYM which significantly increases the growth parameters of wheat plant by making the availability of nutrients and fertilizers to the plant in addition to modifying the quality of soil. Future research should focus on extensive

field trials to determine the optimal application rates and methods of FYM for different soil types and wheat varieties. This will help in developing practical guidelines for farmers.

CONCLUSION

Research has shown that FYM not only promotes overall plant growth and biomass but also reduces the uptake of Cr(VI) by plants, thereby decreasing its concentration in edible parts and minimizing potential health risks. Additionally, the enhancement of the plant's antioxidant defense system through FYM further mitigates oxidative stress induced by chromium, leading to healthier and more robust crops. Continued research and innovation in this area will help optimize these practices, ensuring their effectiveness across diverse agricultural landscapes.

CONFLICT OF INTEREST

The authors have not declared any conflict of interests.

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