



# EVALUATION OF THE EFFECTS OF ZINC APPLICATION METHODS ON TRITICUM AESTIVUM L. YIELD AND GRAIN QUALITY UNDER CHROMIUM **STRESS**

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# Abstract

Zinc (Zn) malnutrition is widespread in developing countries where cereal grains are used as staple food. Zn efficient wheat genotypes have been developed to minimize Zn malnutrition; however, there is another threat of accumulation of heavy metals in grains from heavy metal contaminated soils which would contaminate the food chain. Present study was conducted to check the impact of different Zn application methods on growth, yield and accumulation of Zn and chromium (Cr) in grains of a Zn efficient wheat cultivar grown under Cr contamination. The experiment was comprised of two factors including three contamination levels of Cr (0 ppm, 5 ppm, 15 ppm) and three Zn application methods (seed coating, soil application and foliar spray). Akbar-19 was used in this experiment which is a recent Zn efficient wheat cultivar developed for improving Zn concentration in grains of wheat. Observations including plant height, shoot length, shoot fresh weight and dry weight, spike length, 100 grains weight, photosynthetic efficiency, relative water content were recorded. Results showed that due to Cr stress, growth parameters, biological yield, photosynthetic yield and yield of wheat cultivar reduced at higher Cr level, i.e, 15 ppm. It was observed that application of Zn through seed coating, soil application and foliar spray significantly ameliorates the toxicity of Cr. Overall seed coating, foliar spray and soil application of Zn



improved all parameters but grain Zn content was significantly increased through foliar spray. Soil application was also effective and play mitigative role in the presence of Cr toxicity.

#### Keywords

Malnutrition, Heavy Metals, Contamination and Mitigation, Biofortification, Cereal crops, Cr toxicity, Zn fortification

### INTRODUCTION

Wheat *(Triticum aestivum L.)* being a major cereal crop around the world and in developing countries is the basic source of protein, calories and micronutrients. In Pakistan, wheat accounts for over 40% of the country's cultivated land. In human, Zn is an important micronutrient which is required for maintaining normal health. It is found in over 300 enzymes and is involved in a variety of metabolic activities. In humans, Zn deficiency is a major issue, with 17% of the world's population being Zn deficient, including 30% of the population in South Asia [1,2]. Zn deficiency causes anorexia, cognitive dysfunction, childhood mortality, hypogonadism, impaired immune function, diarrhea, pneumonia, stunted growth, and skin disorders [3]. About 35% of the world's population eats bread wheat as their primary source of nutrition and wheat is a poor supplier of Zn due to low Zn concentration and low Zn bioavailability which is further hindered by high phytic acid and fiber contents in grains. Zn concentrations in wheat grains ranges from 20 to 35 mg/kg while required Zn content is 45mg/kg to meet the daily Zn requirement of human body [4]. Zinc deficiency in agricultural soils is a worldwide issue that has been identified several countries. In Pakistan, several studies support the subject of rising zinc deficiency (ZnD) levels; for example, 22.1 and 18.6 percent of women and children, respectively, are Zn deficient. In terms of ZnD, Punjab province has the greatest share (24.1%) among them (Ministry of National Health Services Nutrition Wing, 2018). Biofortification of wheat with Zn has become an essential way to overcome Zn deficiency in humans. Biofortification is a process that uses genomic, biotechnology, and breeding approaches to improve essential vitamins and minerals in crops. Genetic and agronomic biofortification are the two main methods of biofortification however, agronomic biofortification is cost-effective [2] and an instant way to boost micronutrient content in edible part of food crops compared to genetic biofortification [5]. Five important staple food crops rice, wheat, maize, potato and cassava in Africa, Asia and Latin America have been Biofortified [6]. Wheat *(Triticum aestivum L)* is an excellent Zn Biofortification crop. Wheat, like most plants, is at the top of the food chain and absorbs both essential and non-essential nutrients from soil. Heavy metal contamination in soil is a worldwide issue. During past years, quantification of total content of heavy metals and their toxicity threats in the soil has been a subject of concern among environmental experts [7]. Because of increase in industries the concentration of heavy metals in the soil increases which in turn contaminates the soil. Cr is a silvery white transition metal and environmental pollutant which is categorized as  $7<sup>th</sup>$  most abundant heavy metal in earth's crust. Unlike other heavy metals such as Cd and Cu, Cr has only 2 valence states: Cr (VI) and Cr (III).Cr (VI) is more stable, mobile, and poisonous than Cr (III) [8]. Because Cr is highly soluble it readily dissolves in groundwater through land surface and enters into plants causing toxicity through its carcinogenic and mutagenic properties. Cr is easily transported and accumulated in various plant parts which are ingested by humans and animals. Cr contamination has a significant impact on wheat growth, development, and yield. Wheat is susceptible to Cr stress like other crops [9] Cr stress causes considerable impairment in mineral uptake, water absorption, seed germination and growth of plants because many metabolic processes are disrupted by the oxidative damage to mitochondrial and photosynthetic apparatus [10].

Interactions may occur when two or more heavy metals are introduced to the environment at the same time. By interacting with heavy metals in various ways, Zn can help to detoxify heavy metals stress and competes with heavy metals for plant uptake. Zn also helps in the improvement of a plant's Zn status by enhancing Zn translocation in edible parts of the crop. Consumption of Zn biofortified wheat cultivars support in the reduction of malnutrition and is critical for food security; however, possibility of higher accumulation of toxic metals by biofortified cultivars is a drawback of genetic Biofortification and in addition, impact of different Zn application methods on Cr uptake is missing. Therefore, a study was conducted to check the impact of different Zn application methods on growth, yield and accumulation of Zn and Cr in grains of a Zn efficient wheat cultivar grown under Cr contamination.

### MATERIALS AND METHODS

The pot experiment was conducted at wirehouse of field Allelopathy lab, Agronomic Research Area, University of Agriculture Faisalabad in Pakistan to check out the behavior of a biofortified wheat cultivar under Cr contamination when Zn is applied through different methods. Wheat genotype Akbar-19 was used to check Cr toxicity at 0 ppm, 5 ppm and 15 ppm levels. Biofortification of Zn was done by soil application, seed coating and foliar spray. Potassium dichromate  $(K_2Cr_2O_7)$  and Zn sulphate (ZnSO<sub>4</sub>) were the source of Cr and Zn respectively. Each pot was filled with 6kg Cr contaminated soil and labeled according to treatments. The treatments were as  $T_0$  (control without Zn application),  $T_1$  (soil application of ZnSO<sub>4</sub> at the rate of 8kg Zn ha<sup>-</sup> <sup>1</sup>),  $T_2$  (seed coating with ZnSO<sub>4</sub> at the rate of 2.25g Zn per kg seeds),  $T_3$  (foliar spray of 1% and 0.5% of Zn in solution form). Ten seeds were sown per pot and thinned to 3 plants per pot. The pots were randomized weekly and watered daily to maintain soil at field capacity by tap water. Physiological, biochemical, growth and yield related attributes was recorded during and after the experiment.

#### Data collection

#### Plant growth parameters

Plants were harvested and shoot length of the plants was measured with the help of measuring scale after 45 days of germination. Fresh weight of shoots was recorded in grams using electrical weight balance after harvesting them. The small pieces of the shoots sample were packed into labeled brown paper bags and sun dried. After the sun dry process sample bags were placed into the oven at  $65^{\circ}$ C for further drying. After the complete drying of the shoots their weight were observed with the usage of an electric balance. Final plant height was measured of standing plants before harvesting with the help of measuring scale. Spike length of 3 spikes was measured with the help of measuring scale after harvesting of crop.

#### Post-harvest parameters

 For 100 grain weight (g), hundred grains were counted from each replication and weight of the grains was taken using electrical balance. After harvesting, plants were threshed with hand and grain yield was measured using electrical weight balance. For the determination of total biomass after the harvesting, the weight of the wheat plant was weighed. Harvest index of the wheat was calculated as below formula.

Harvest index = (Grain yield/Biological yield)  $\times$  100

### Physiological parameters

SPAD value was recorded by the use of SPAD-meter after 45 days of germination.

Relative water content of leaf was determined by the [11] method. Fresh flag leaf samples were taken from plant and its fresh weight (FW) was measured and then this sample was dipped into distal water for 24 hours. The sample from water was taken out and excess water was removed with the help of tissue paper and turgid weight of sample was calculated immediately. Dry weight was taken after oven drying the sample at  $65\text{-}70\text{°C}$  for 5-6 hours. RWC was calculated by using following formula:

$$
RWC (%) = [(FW-DW)/(TW-DW)]x 100
$$

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## Biochemical parameters

Photosynthetic yield of PII (m molm<sup>-2</sup>s<sup>-1</sup>), ETR (m molm<sup>-2</sup>s<sup>-1</sup>) (electron transfer rate), and Ft (m molm<sup>-2</sup>s<sup>-1</sup>) (momentary fluorescence) of leaf were recorded after 65 days by using MINI-PAM.

#### Nutrients (Zn,Cr) content

For the determination of Zn and Cr content in grains, the grains were grinded first. After grinding, 0.1 g sample from each replication was weighted with the help of the electrical balance.  $5mL$  of Concentrated HNO<sub>3</sub> was poured into the samples and placed them over night. Next day after pre-digestion, 5mL of di-acid HNO3-HCLO4 was added in each sample. The samples were digested on hot plate at 150°C at start and then the temperature was increased to 240°C till a colorless solution appears. After that the samples were cooled, solution was filtered using whatsman filter paper and maintained the solution up to 50 mL with the help of distilled water. The samples were transferred into labeled bottles and stored. Zn and Cr content in samples were determined with the help of an atomic absorption spectrophotometer.

#### Statistical analysis

The data was analyzed by using Fisher's analysis of variance. Means of treatment were compared at a probability level of 5% by using the least significant difference (LSD).

## RESULTS

#### Growth parameters

Overall results showed (Table 1) that contamination of chromium negatively impacted the plant shoot fresh and dry weight, shoot length, and spike length. In addition, application of Zn had ameliorative impact. Results show that highest value (67.50cm) of plant shoot was recorded when treated with Zn (soil application) at 0 ppm level Cr. However, lowest value (61.08 cm) was observed in plants when treated with no Zn (control) and Cr at concentrations of 15 ppm. Lowest value (1.68 g) of shoot fresh weight was observed in plants when grown in controlled condition without application of Zn under Cr contamination at the level of 15 ppm. Highest value (0.49g) of shoot dry weight was observed in plants when treated with Zn (soil application) and Cr at the level of 0 ppm. Lowest value (0.22g) was observed in plants when treated with no Zn (control) and Cr at concentrations of 15 ppm. Highest value of spike

length was observed in plants when treated with Zn (seed coating) and Cr at the level of 0 ppm. Lowest value (9.23) of spike length was observed in plants when Zn was not applied (control) and Cr at concentrations of 15 ppm (Table 1).

Table 1: Effects of Zinc (Zn) treatments and Chromium (Cr) levels (0, 5 and 15 ppm) on wheat cultivar growth (Shoot Length, Spike Length, Shoot Fresh Weight, Shoot Dry Weight). Data represent mean ±SD, followed by different letters indicate significant differences as per Tukey's LSD test (P≤0.05).



## Post-harvest parameters

The findings indicate that Zn had ameliorative impact and reduces Cr toxicity while Cr stress had significant impact on grain weight (Table 2). Highest value (4.77g) of 100 grains weight was observed in plants when treated with Zn (soil application) and Cr at the level of 0 ppm. Lowest value (3.47g) was observed in plants when treated with no Zn (control) and Cr at concentrations of 15 ppm. Highest value (6.68 g) of grain yield was observed in plants when treated with  $Zn$  (soil application) and  $Cr$  at the level of 0 ppm. Lowest value  $(3.56 \text{ g})$  was observed in plants when treated with no Zn (control) and Cr at concentrations of 15 ppm. Highest value (35.60) of harvest index was observed in plants when treated with Zn (foliar spray) and Cr at the level of 0 ppm. Lowest value (21.27) was observed in plants when treated with no Zn (control) and Cr at concentrations of 15 ppm (Table 2).

Table 2: Effects of Zinc (Zn) treatments and Chromium (Cr) levels (0, 5 and 15 ppm) on wheat cultivar Post-harvest parameters (100 grain weight (g) and Grain yield (g)). Data represent mean ±SD, followed by different letters indicate significant differences as per Tukey's LSD test  $(P \leq 0.05)$ .



#### Physiological parameters

## SPAD-value

Highest value (57.23) of total SPAD-value was recorded in plants at 0ppm Cr level when Zn was applied in the form of foliar spray while the lowest value (34.57 and 35.33) of SPADvalue was recorded at 15ppm Cr level when Zn was applied in the form of foliar spray and without Zn application (Table 3). These values were recorded before the foliar application of Zn. So from the results it is clear that Zn application in foliar form had significant impact on SPADvalue and mitigates the negative impact of chromium.

Table 3: Effects of Zinc (Zn) treatments and Chromium (Cr) levels (0, 5 and 15 ppm) on wheat cultivar Physiological parameter (SPAD-value and Relative water content). Data represent mean ±SD, followed by different letters indicate significant differences as per Tukey's LSD test  $(P \le 0.05)$ .



## Relative water content

 Results indicate that chromium had highly negative impact on relative water content of plant. Moreover, Zn works as alleviator of chromium toxicity (Table 3). Highest value (92.93 and 92.21) of Relative water content was observed in plants when treated with Zn through soil application and seed coating at 0 ppm Cr level, respectively. Lowest value (72.78) was observed in plants when Zn was not applied (control) and Cr at concentrations of 15 ppm.

## Biochemical parameters

## Electron transfer rate (m mol  $m<sup>-2</sup>s<sup>-1</sup>$ )

Highest value (190.18) of electron transfer rate was observed in plants when treated with Zn (soil application) and Cr at the level of 0 ppm. Lowest value (107.00) was observed in plants when treated with no Zn (control) and Cr at concentrations of 15 ppm (Table 4).

Table 4: Effects of Zinc (Zn) treatments and Chromium (Cr) levels (0, 5 and 15 ppm) on wheat cultivar Biochemical parameter (Electron transfer rate and Momentary fluorescence rate). Data represent mean ±SD, followed by different letters indicate significant differences as per Tukey's LSD test  $(P \le 0.05)$ .



## Momentary fluorescence rate (m mol  $m^{-2}s^{-1}$ )

Overall, results indicate that chromium had negative impact on momentary fluorescence rate while foliar treatment of Zn is significant in ameliorating it instead of other treatments at higher levels of Cr, i.e, 15 ppm. Highest value (958.17) of momentary fluorescence rate was observed in plants when treated with Zn (foliar spray) and Cr at the level of 0 ppm. Lowest value (704.83) was observed in plants when treated with Zn (soil application) and Cr at concentrations of 15 ppm (Table 4).

# Photosystem II Yield  $(Y(II))$  (m mol m<sup>-2</sup>s<sup>-1</sup>)

Y(II) is effective photochemical yield of photosystem II. Highest value (0.6783) of photosynthetic yield was observed in plants when treated with Zn (foliar spray) and Cr at the level of 0 ppm. Lowest value (0.1882) was observed in plants when treated with Zn (seed coating) and Cr at concentrations of 15 ppm (Figure 1).



Figure 1: Effects of Zinc (Zn) treatments and Chromium (Cr) levels (0, 5 and 15 ppm) on wheat cultivar Biochemical parameter Photosystem II Yield (Y(II)). Data represent mean  $\pm SD$ , followed by different letters on top of error bars indicate significant differences as per Tukey's LSD test  $(P \le 0.05)$ .

## Zn content in grains

The results show that foliar spray of Zn is effective in increasing grain Zn content during heavy metal toxicity. Highest value (82.68) of Zn content was observed in plants when treated with Zn through foliar spray at 0 ppm Cr level. Lowest value (43.43) was observed in plants when Zn was not applied (control) and Cr at concentrations of 15 ppm (Figure 2).



Figure 2: Effects of Zinc (Zn) treatments and Chromium (Cr) levels (0, 5 and 15 ppm) on Zn content in wheat cultivar Grains. Data represent mean ±SD, followed by different letters on top of error bars indicate significant differences as per Tukey's LSD test (P≤0.05).

## Cr content in grains

The findings indicate that Cr accumulates more in grains when it is present at higher concentration in soil. It causes toxicity and reduced the plant yield. Highest value (0.45) of Cr content was observed in plants when no Zn was applied at 15 ppm Cr level. Lowest value (0.05) was observed in plants when Zn was applied through soil application and Cr at concentrations of 0 ppm (Table 5).

Table 5: Effects of Zinc (Zn) treatments and Chromium (Cr) levels (0, 5 and 15 ppm) on Cr content in wheat cultivar Grains. Data represent mean ±SD, followed by different letters on top indicate significant differences as per Tukey's LSD test (P≤0.05).

<b>Zn Treatments</b>	<b>Cr</b> levels		
	0ppm	5ppm	15 <sub>ppm</sub>
Control	$0.05 \pm 0.01$ <sup>g</sup>	$0.25 \pm 0.01$ <sup>d</sup>	$0.45 \pm 0.01$ <sup>a</sup>
Soil application	$0.05 \pm 0.01$ <sup>g</sup>	$0.20 \pm 0.01$ <sup>e</sup>	$0.40 \pm 0.1^b$
<b>Seed coating</b>	$0.10 \pm 0.01$ <sup>f</sup>	$0.23 \pm 0.03$ <sup>de</sup>	$0.38 \pm 0.03$ <sup>c</sup>
<b>Foliar spray</b>	$0.10 \pm 0.01$ <sup>f</sup>	$0.23 \pm 0.01$ <sup>de</sup>	$0.38 \pm 0.03$ <sup>c</sup>

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## **Discussion**

Plants water uptake and water ratio are modified by the existence of metals and metalloids, which limits plant growth [12,13]. Cr is a hazardous metal for plants and has no any important role in the growth and metabolism of plants [14]. Cr (VI) is easily available to plants by being more soluble and toxic in nature [14,15] Stress of heavy metals severely affected the morphological features of wheat, including the fresh and dry weight of the plant. Overall, the physical, biological, and morphological functions of many plants were affected by heavy metals [16]. According to Genter, (1996), interactions between heavy metals may have an impact on how they are absorbed by and distributed throughout plants. Increases in productive tillers, 100 grain weight, grains per spike, grain yield, and harvest index under Cr contamination shows that Zn treatment improved wheat yield and yield components. In late growth phases, such as booting and 7 days after flower initiation [18], heading and milk stages [19], as well as during the start of stem elongation and flowering, similar outcomes were reported for two foliar Zn applications [20]. The concentration of chlorophyll has a strong correlation with the photosynthetic activity, which is significant for crop productivity, particularly in times of stress [21]. As a result of metal contamination the amount of chlorophyll and the photosynthetic area decreases, resulting in early senescence. The kind of contaminating metal, the species of plant, the amount of metal, and the time of the stress are all associated to changes in metabolism of plant and photosynthetic characteristics [22,23]. Metal phytotoxicity can cause numerous changes in plant metabolism at the cellular and molecular level by interfering with metabolic processes. Increased formation of reactive oxygen species (ROS) is one effect of metal toxicity because metals prevent electron transport, particularly in chloroplast membranes. Increases in reactive oxygen species impose oxidative stress to plant cells and may cause DNA strand breaks, lipid peroxidation, and changes to various components and membranes. In response, plants use a variety of defence mechanisms to limit the entry, accumulation, and translocation of toxic metals as well as for detoxification [24]. For example, plants may reject free ionic forms of metals from the cytoplasm. Consequently, metal toxicity blocks PSII and decrease the efficiency of photosynthetic processes.

Chelonian Conservation and Biology https://www.acgpublishing.com/ Y(II) is effective photochemical yield of photosystem II. The Y(II) value estimates the photochemical use of excitation energy in the light. It is lowered by partial closure of PS II centres and various types of non-photochemical energy losses induced by illumination. Other researchers investigating the effects of Cr(VI) on PSII in Chlorella pyrenoidosa came to similar conclusions; they hypothesized that Cr may damage cells' reaction centres or interfere with the photosynthetic apparatus, reducing PSII activity [25]. Under stress condition photosynthetic efficiency is greatly affected because of reduction in chlorophyll content. Cr mainly accumulates in the roots, but it also hinders the development of all plant organs, as well as the yield and production of biomass [13,26]. Leaf relative water content is an important indicator of water status in plants; it reflects the balance between water supply to the leaf tissue and transpiration rate. Plants water uptake and water ratio are modified by the existence of metals and metalloids, which limits plant growth [12,13]. In studies carried out in a controlled environment, Vernay et al. (2007) noted that Cr reduced the amount of water that Lolium perenne plants could access. Modifications in the water intake and reduced water distribution to the aerial regions of the plants were caused by decreased relative water content of the leaves and restricted root and shoot growth. As seen in various plants treated with Cr, metal-induced damage to the plasmatic membrane and cellular ultrastructure of plants may also disrupt the water status of plants and cause progressive wilting [27].

Biofortification of wheat with Zn has become an important tool to combat Zn deficiency in humans. Over the past two decades, foliar Zn application has been recognized to be effective for biofortification of wheat grain with Zn. Zn accumulation in wheat grains is highest during the early stage of grain development [28] and foliar application of Zn at late growth stages increases wheat grain Zn, especially in the endosperm part, and thus in flour [18,29].

Zn supplied to wheat plants by foliar treatment is easily translocated via the phloem [30], and according to [31], Zn reserves in vegetative parts are quickly depleted during the development of grain, indicating that this is associated with Zn translocation to the grain. Therefore, foliar Zn application in the late growth stages can provide important reserves for Zn accumulation in grain. Zn foliar application has been shown to be more efficient than soil application for biofortification of wheat grain with Zn [20,32,33].

Chelonian Conservation and Biology https://www.acgpublishing.com/ Chromium joins soil through natural paths and disturbs normal functioning of soil and plant development [34]. It reduces root and shoot length, decrease rate of transpiration and photosynthesis, decrease plant biomass, lowers stomatal conductance, drops plant height etc. The reduction in chromium uptake by plant can occur by reactions with other ions, metallic, mineral surfaces and organic molecules [35]. Cr toxicity-imposed competition of food with other nutrient cations due to which plumule and radicle length reduces, zinc sulphate decreased this competition by resulting improvement in germination. The alleviators addition would enhance plant tolerance to toxic metals through reducing the uptake and translocation of heavy metals like chromium. A petri plate experiment was conducted in which different toxicity alleviators were used to check that which alleviator is more effective in reducing Cr toxicity. Findings showed that ZnSO4 was most effective of all in minimizing Cr toxicity as Zn hinders the uptake of Cr and works antagonistically [36].

## Conclusion

Cr stress causes impairment in mineral uptake, water absorption, seed germination and growth of plants because many metabolic processes are disrupted by the oxidative damage to mitochondrial and photosynthetic apparatus. Cr contamination significantly reduced the wheat growth, development, and yield. Overall results showed that Cr had significant impact on plant growth, metabolism and lowered the yield; however, Zn treatments especially soil application mitigated the toxic effects of Cr.

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