THE COMBINED EFFECT OF ZnO NANO PARTICLES AND TOXICITY OF HEAVY METALS (ARSENIC AND CHROMIUM) ON THE MORPHOLOGICAL, BIOCHEMICAL ATTRIBUTES OF WHEAT (*TRITICUM AESTIVUM* L.) AS WELL AS SOIL AND WATER PROPERTIES

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Abstract. This research work was conducted in research area, Department of Botany, University of Gujrat, Pakistan during 2018-2019. The performance of research work was conducted to check the effect of heavy metal Chromium (Cr) and Arsenic (As) on two varieties (Faisalabad, Aas) of wheat and is the foliar application of Zinc oxide nanoparticles helpful to remove the stress effect of heavy metals. Heavy metal (Cr and Ar 10 ppm) were added to plants through the rooting medium. There were 10, 20, 30 levels of ZnO (Zinc oxide) nanoparticles for each heavy metal as applied foliarly after 14 days of germination. Experiments were set up in completely randomized design with four replicates. Heavy metal accumulation in soils is steadily growing, resulting in increased toxicity of this ingredient in a variety of crop plants. While Zn is an important nutrient for plants, some soils are deficient in Zn or have poor Zinc bioavailability. The viability of the project is the subject of this article. Soil amendments containing ZnO nanoparticles (NPs) to increase the amount of Zn in the plant. Results indicated that with the application of ZnO NPs, metal concentrations (As and Cr) declined in morphological, biochemical and gaseous attributes with soil and water properties. This reduction was more prominent in the Faisalabad 2008 variety as compared to Aas 2011. Based on the result we concluded that the foliar application of ZnO nano particles were suitable to boost up the biochemical and morphological characteristics, as well as gas exchange of the studied wheat varieties under heavy metal stress, including soil and water properties. As a result, our findings support the importance of ZnO–NPs in reducing heavy metals toxicity in wheat plants.
Keywords: *chlorophyll, foliar spray, zinc, stress, toxic*

Introduction

Historically, wheat has been the staple grain in western countries. In reality, it is now the world’s third-most-produced cereal (after rice and corn) and the second-most-consumed cereal (after rice) for human consumption (Caro et al., 2018). Wheat is the most commonly planted crop on the planet, with annual production in North America, Europe, Asia, Australia, and Africa totaling 600 million tonnes. Wheat supplies 20% of overall protein and calories in human nutrition for millions of people who depend on wheat-based diets, as well as 40% of dietary consumption of essential micronutrients including zinc, iron, manganese, magnesium (Sansaloni et al., 2020).

Soil is one of humanity’s most important and precious natural resources. Soil plays a huge role in societal civilization and agricultural sustainability (Mushtaq, 2021). Heavy metal pollution of soil, on the other hand, is a significant danger to humanity and a major global problem. Chromium is a toxic heavy metal which is used in the manufacture of electroplating, garment dyeing, stainless steel, as a radioactive coolant, and in the leather industry (Fu and Xi, 2020). Cr exposure has also been linked to

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metabolic changes in wheat, either through direct effects on enzymes or through its ability to produce reactive oxygen species (ROS), which may persuade oxidative stress and increased lipid peroxidation (Wakeel et al., 2020).

Arsenic is a hazardous metalloid and influential toxin that affects living things specially plants. Bio magnification of arsenic is becoming a growing concern around worldwide, especially in Southeast Asian countries. Human and anthropogenic sources also contribute to the metalloid's presence in the environment. Arsenic prevents root proliferation and expansion, while after translocation to the shoot, growth of plant is slowed and productivity as well as reproductive ability are reduced (Sil et al., 2019). Arsenic contamination in plants has been shown to inhibit growth, supplement of nutrients, protein content, photosynthetic pigments as well as biomass accumulation (Farouk and Al-Amri, 2019).

Nanotechnology has risen to prominence as a technological movement in recent years, with potential applications in a variety of fields. Nanoparticles have distinct physicochemical properties when compared to bulk particles and play an important role in transfer of chemical agents in plants to a molecules of targeted cellular organelle (Tripathi et al., 2017). ZnONPs are the most well-known of the many metal nanoparticles that are usually used for industrial purposes. As a result, widespread use of ZnONPs in a variety of materials increases the likelihood of their release into the atmosphere, which may have significant implications for plant productivity (Ifeanyichukwu, 2020).

Under stressful and non-stressful environments, nitric oxide which is a gaseous free radical, affects many physiological as well as biochemical responses in plants. It has been found to efficiently mitigate the toxic effects of various stresses on plants, including ultraviolet rays, salt, heavy metals, sun and light (Tripathi et al., 2017). ZnO NPs induced phytotoxicity in seedlings grown in acid soil, as shown by the inhibition of root elongation in wheat (Watson et al., 2015).

In recent years, the use of nanoparticles (NPs) containing micro- and macronutrients has been promoted as a promising approach for the crop growth and yield (Shang et al., 2017). This nanoparticle supplements may help to minimize nutrient loss and increase crop production in a long-term way. The use of ZnO NPs in agricultural sciences is gaining popularity among metal-based NPs. Despite this, only a few studies have looked into the effects of ZnO–NPs in the soil as well as plant environment. The availability of Zn in soils and the toxicity of ZnO–NPs in plants are influenced by the plant types, soil condition, and soil pH. (Rizwan et al., 2019). Surprisingly, the low abundance of Zn in soils can help foliar application of ZnO–NPs. Many studies have shown that zinc nanoparticle formulations and their use as a foliar spray are effective at reducing heavy metal accumulation in plants (Hussain et al., 2018; Rizwan et al., 2019; Wu et al., 2020).

In the light of above-mentioned literature the main objectives of this research work was to find out the effect of heavy metals and also determine the ZnO nano particles function under heavy metals stressed plant on wheat varieties.

Materials and methods

Pot experiments were carried out in the Botanical Garden, University of Gujrat, Gujrat Pakistan during 2018-2019. Two varieties of wheat were used in these experiments i.e. Aas (2011) and Faisalabad (2008). Heavy metals (Arsenic and

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Chromium) and Zinc Oxide Nanoparticles were used. Eleven level of treatment of Ar, Cr and ZnONPs that were:

T0 = Control 0 ppm (As, Cr, ZnO NPs)
T1 = 10 ppm As
T2 = 10 ppm Cr
T3 = 10 ppm ZnONPs
T4 = 20 ppm ZnONPs
T5 = 30 ppm ZnONPs
T6 = 10 ppm ZnONPs + 10 ppm As
T7 = 20 ppm ZnONPs + 10 ppm As
T8 = 30 ppm ZnONPs + 10 ppm As
T9 = 10 ppm ZnONPs + 10 ppm Cr
T10 = 20 ppm ZnONPs + 10 ppm Cr
T11 = 30 ppm ZnONPs + 10 ppm Cr

48 Pots was bought for each variety from market and filled with about 8 kg soil. Eight to ten healthy seeds of both varieties were sown in each pot. After 10^{th} day of germination thinning of the plants was made leaving only 5 plants per pot at equal distance and same height.

After two weeks Chromium in the form of chromium oxide and Arsenic in the form of arsenic oxide were added to plants. Levels of treatments, i.e., 0 (control), 10 ppm by plant rooting medium. ZnO nano particles was added by foliar after a few days of heavy metal stress. Water was supplied on daily basis to keep homeostasis. After 15 days of heavy metals stress ZnO nano particles was applied using a manual sprayer early in the morning. Experiments had four replicates and were set up in a completely randomized system (CRD). Data was collected at vegetative stage for the morphological, biochemical and gaseous exchange parameters such as number of leaves, leaf area, chlorophyll contents and gaseous exchange. Various soil and water analysis parameters were also examined. The number of leaves on each plant was counted first, and then the mean value was determined. To determine the leaf area, Carelton and Foote formula as described in Shahbaz and Ashraf (2008) was used. The formula was:

$$\text{Leaf Area (cm}^2\text{)} = \text{maximum leaf length} \times \text{maximum leaf width} \times 0.75$$
$$\text{(Correction factor = 0.75)}$$

Chlorophyll a, b and carotenoid contents were calculated by Arnon procedure as described by Nazeer et al. (2020). IRGA (LCA-4 ADC portable infrared gas analyzer (Analytical Production Firm, Hoddeson, England. Model C1-340) was used to estimate the gas exchange parameters such as photosynthetic and transpiration rates, stomatal conductance, and sub-stomatal CO_2 concentration. Data was collected between 10:00am and 02:00pm as mentioned by Khalid et al. (2017). The following adjustments of IRGA were made: leaf surface area (11.35 cm^2), leaf chamber temperature (Tch) which may varied from 29.2-37.50 °C, ambient CO_2 concentration (349.12 µmol mol^{-1}), ambient temperature 31-36 °C, gas flow rate of leaf chamber (397 ml min^{-1}), water vapor pressure in chamber 6-9.0 mbar, molar flow of air per unit leaf area (401.06 mol m^{-2} s^{-1}), ambient pressure (99.95) KPa, PAR at leaf surface was up to 1515 µ$molm^{-2}$.

Water use efficiency was estimated with formula described by Bacon (2009):

$$\text{WUE (g/l)} = \text{(dry wt. of final biomass – dry wt. of initial biomass) / Rainfall or water consumed (ml)}$$

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Sampling pattern and depth

250 g soil sample in each polyethylene bag were taken within the 8-10-feet radius randomly in order to avoid systematic patterns such as starter or preplant bands from experimental pots of university of Gujrat and analyzed in the Punjab Agriculture Soil Testing Labs Gujrat, Pakistan. Various soil characteristics were determined including soil texture, pH, Electric Conductivity, Organic Matter (%), saturation % and Nitrogen, Phosphorous and potassium in the soils. For the analysis water various water sample were collected from experimental pots of University of Gujrat and examined its physiochemical properties in Punjab Agriculture Water Testing Labs Gujrat, Pakistan. Various water characteristics such as Electric conductivity, Ca + Mg, sodium, HCO_3, Chloride, sodium adsorption ratio, and residual sodium carbonate were determined.

Green synthesis of ZnO nanoparticles

Fresh *Aloe vera* leaves were collected in the area of the University of Gujrat in Pakistan. Tiny fragments of the leaves were chopped. Electrical balancing was used to weigh 35 g of chopped parts. 100 ml purified water was used to boil these leaves. The extracted material was then purified and stored for future use.

To reduce Zinc nitrate into ZnO NPs, *Aloe vera* extract (30 ml) was used as a reducing agent. In *Aloe vera* extract, 3 g of zinc nitrate was added for this reason. After that, the sample was held at 60 °C with magnetic stirring until it dried. To extract moisture, the powder was dried at 400 degrees in a furnace (Singh et al., 2008).

The following methods were used to classify the structural and morphological properties of the sample.

X-ray diffraction analysis

This method was used to characterize nanoparticles and analyze its structure. Each crystalline solid has its own atom structure and powder pattern. Since X-rays interact with the atoms of the lattice planes, the interatomic distance between neighboring atoms is ‘d’. Constructive interference happens when the distance length of two rays approaches a whole multiple of the wavelength of radiation used. Bragg’s law explains the condition for constructive interference:

$$n\lambda = 2d\ Sin^{\theta}$$

where ‘n” is integral multiple, ‘d’ is inter atomic spacing, ‘λ’ the wavelength of X-ray used.

Scanning electron microscopy

A high-resolution image of zinc oxide nano particles with a size of less than 10 nm was obtained using a scanning electron microscope. It specifically tests very small features and artifacts, to put it another way. Nanoparticles range in size from 1 to 100 nm, with an interfacial coating made up mostly of organic and inorganic molecules. The agglomeration mass and irregular shape of nanoparticles is visible in SEM photographs.

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Fourier transform infrared spectroscopy

FTIR approach, or a basic failure analysis procedure, can detect molecular motions ranging from two atoms in a diatomic molecule moving in a simple coupled motion to each individual atom in a large poly functional molecule moving. The material knowledge concerning chemical bonding and materials composition can be derived from the research infrared spectra (Griffiths and Haseth, 2007).

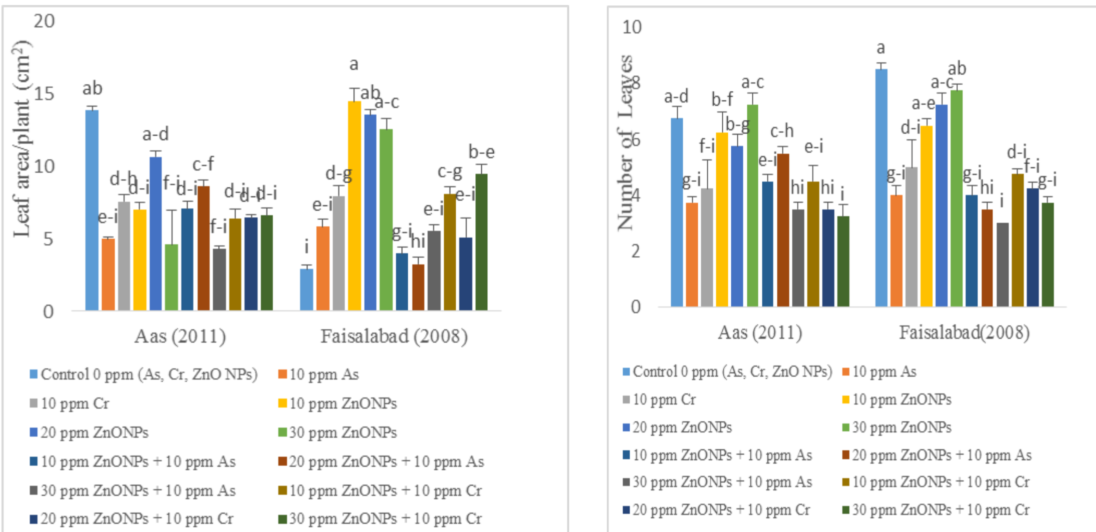
Statistical analysis

Data was subjected to two-way variance analysis (ANOVA) for different variables with two factors i.e. variety and treatments using Ministat-C software and means were compared using Tukey ‘s HSD test at $P \leq 0.05$, with a confidence interval of 95% (Silverman, 2018).

Results

Morphological attributes

Results of ANOVA for morphological parameters was recorded in *Table 1*. ANOVA indicated that the impacts of heavy metals and ZnO nano particles on leaf area and number of leaves of different varieties of wheat (*Triticum aestivum* L.) were highly significant. While effect of varieties showed non-significant results. However, the interaction between treatments and varieties were also highly significant as described in *Table 1*. Highest leaf area was noted at T3 (10 ppm ZnONPs) from Faisalabad variety. Result also narrated that leaf area of both varieties was improved with the foliar spray of 20 ppm ZnONPs. Low leaf area was recorded at 30 ppm ZnONPs + 10 ppm As from both varieties (*Fig. 1A*). Application of ZnONPs at concentration of 30 ppm gave the best result of number of leaves as compare to heavy metal stress at both varieties of wheat. However, control from Faisalabad variety also had high number of leaves as compare to other treatments (*Fig. 1B*).



(A) Leaf area (cm^2)   (B) Number of leaves

Figure 1. *Interactive role of zinc oxide nanoparticles and heavy metals (arsenic and chromium) toxicity on morphological parameters of wheat (Triticum aestivum L.)*

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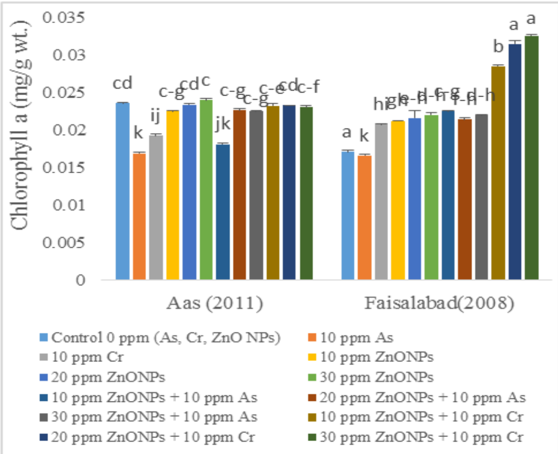
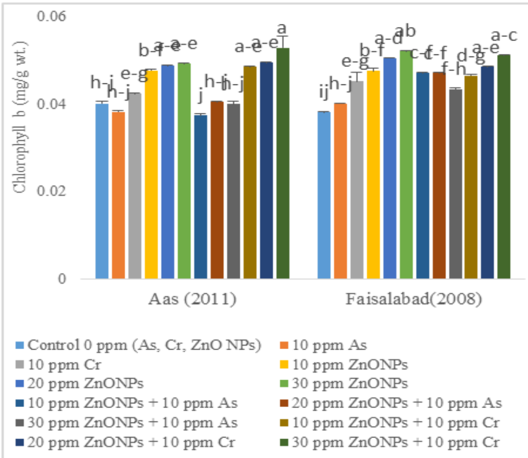
Biochemical attributes

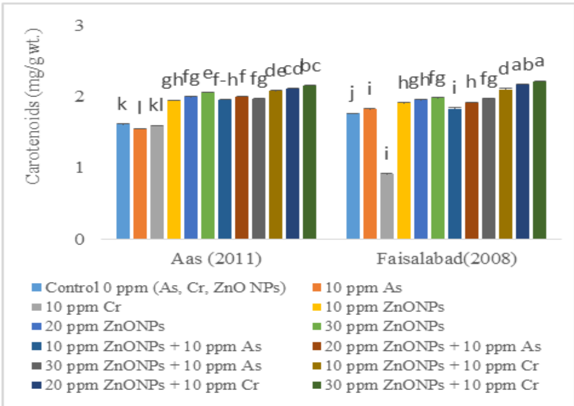
Analysis of variance ANOVA on biochemical parameters (chlorophyll a, b and carotenoids) was presented in *Table 1*. Results showed that effect of heavy metals and ZnO on chlorophyll and carotenoids content \were highly significant with highly significant interaction between treatments and varieties. Graphical representation showed that highest values of chlorophyll and carotenoids content were obtained at combination of 30 ppm ZnONPs + 10 ppm Cr in Faisalabad variety as compare to other treatments. On the other hand, heavy metals (10 ppm Cr and As) stress reduced chlorophyll contents in both varieties of wheat (*Fig. 2A, B, C*).



(A) Chlorophyll a (mg/g wt.)



(B) Chlorophyll b (mg/g wt.)



(C) Carotenoids (mg/g wt.)

Figure 2. *Interactive role of zinc oxide nanoparticles and heavy metals (arsenic and chromium) toxicity on biochemical parameters of wheat (Triticum aestivum L.)*

Table 1. *ANOVA (means squares) for morphological and biochemical parameters of wheat under interactive role of zinc oxide nanoparticles and heavy metals (arsenic and chromium) toxicity*

Source	df	Leaf area/plants (cm^2)	Number of leaves/plants	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Carotenoids content (mg/g)
Variety	1	3.523ns	2.041ns	0.00003***	0.00008***	0.0404***
Treatment	11	39.526***	19.053***	0.00008***	0.0001***	0.2265***
Variety × treatment	11	54.254***	1.928***	0.00004***	0.00002***	0.0350***
Error	72	2.804	0.652	0.0000	0.00000	0.0003
Total	95	1237.01	279.833	0.0014	0.0023	2.9398

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Gaseous exchange attributes

Analysis of variance ANOVA from *Table 2* showed interactive role of heavy metals and ZnO nano particle on gaseous exchange parameters of different varieties of wheat. Varietal effect of photosynthetic, transpiration and stomatal conductance rate were non-significant while, water use efficiency and CO_2 conc. showed highly significant results. However, effect of treatments on all gaseous exchange parameters were highly significant with its interactions except water use efficiency which showed non-significant results. From *Figure 3A* maximum photosynthetic rate was observed in Faisalabad variety at foliar spray of 10 ppm ZnO under As stress as compare to other variety and treatments. Treatment of Cr and As decreased transpiration rate in wheat plants. Application of ZnO concentration 20 ppm gave best results of transpiration rate to overcome heavy metals stress (*Fig. 3B*). It was also concluded from the results that heavy metal stress reduced CO_2 concentration and reduction was greater in Faislabad (2008) as compare to Aas (2011) (*Fig. 3C*). A significant reduction in the stomatal conductance was noticed when heavy metals (Cr and As) was applied on plants of both varieties (*Fig. 3D*). Graph also demonstrated that Faislabad (2008) showed less water use efficiency on all treatments it means that there was no effect of heavy metal and ZnO on plants of Aas (2011) (*Fig. 3E*). Foliar spray of ZnO under heavy metal (As) stress gave best results of relative water content at concentration of 10, 20, and 30 ppm in both varieties of wheat as compare to all other treatments (*Fig. 3F*).

Soil analysis attributes

ANOVA results of different soil analysis parameters of two varieties of wheat was recorded in *Table 3*. There was non-significant effect of heavy metals and ZnO on all soil analysis characteristics of wheat varieties. The interaction between variety and treatment also showed non-significant outcomes. Results from *Figure 4A* narrated that heavy metals did not affect soil pH of both varieties of wheat and plants grown under normal condition. Results showed that electric conductivity of soil also did not affect by heavy metals stress as ZnO nano particles enhanced the growth and productivity of plant in the soil. Electric conductivity reached up to normal levels after all treatments of ZnO under heavy metal stress (*Fig. 4B*). Organic matter in soil characteristics also not changed by heavy metals stress and increased growth of plant by ZnO Nano particles application on wheat varieties (*Fig. 4C*). The concentrations of phosphorous and potassium remain same at all treatments of both varieties of wheat as Cr and As not affected the ions concentration in soil of all plants (*Fig. 4D, E*). Saturation in soil also not changed by any kind of treatments (*Fig. 4F*).

Table 2. *ANOVA (means squares) for gaseous exchange parameters of wheat under interactive role of zinc oxide nanoparticles and heavy metals (arsenic and chromium) toxicity*

Source	df	Photosynthetic rate (µmol/m²/sec)	Transpiration rate (mmol/m²/sec)	Intercellular CO_2 concentration (µmol/m²/sec)	Stomatal conductance rate (mmol/m²/sec)	Water use efficiency (mmol/mol)	Relative water contents (%)
Variety	1	2.503ns	0.2430ns	25.318***	0.001ns	545930***	179.5ns
Treatment	11	23.854***	0.1529ns	2.958ns	0.002***	26267ns	1450.5***
Variety × treatment	11	32.942***	0.5060***	10.364***	0.0003ns	26158ns	1392.0***
Error	72	3.156	0.1667	2.335	0.0003	26223	234.0
Total	95	854.527	19.4940	339.95	0.0508	3010633	48293.7

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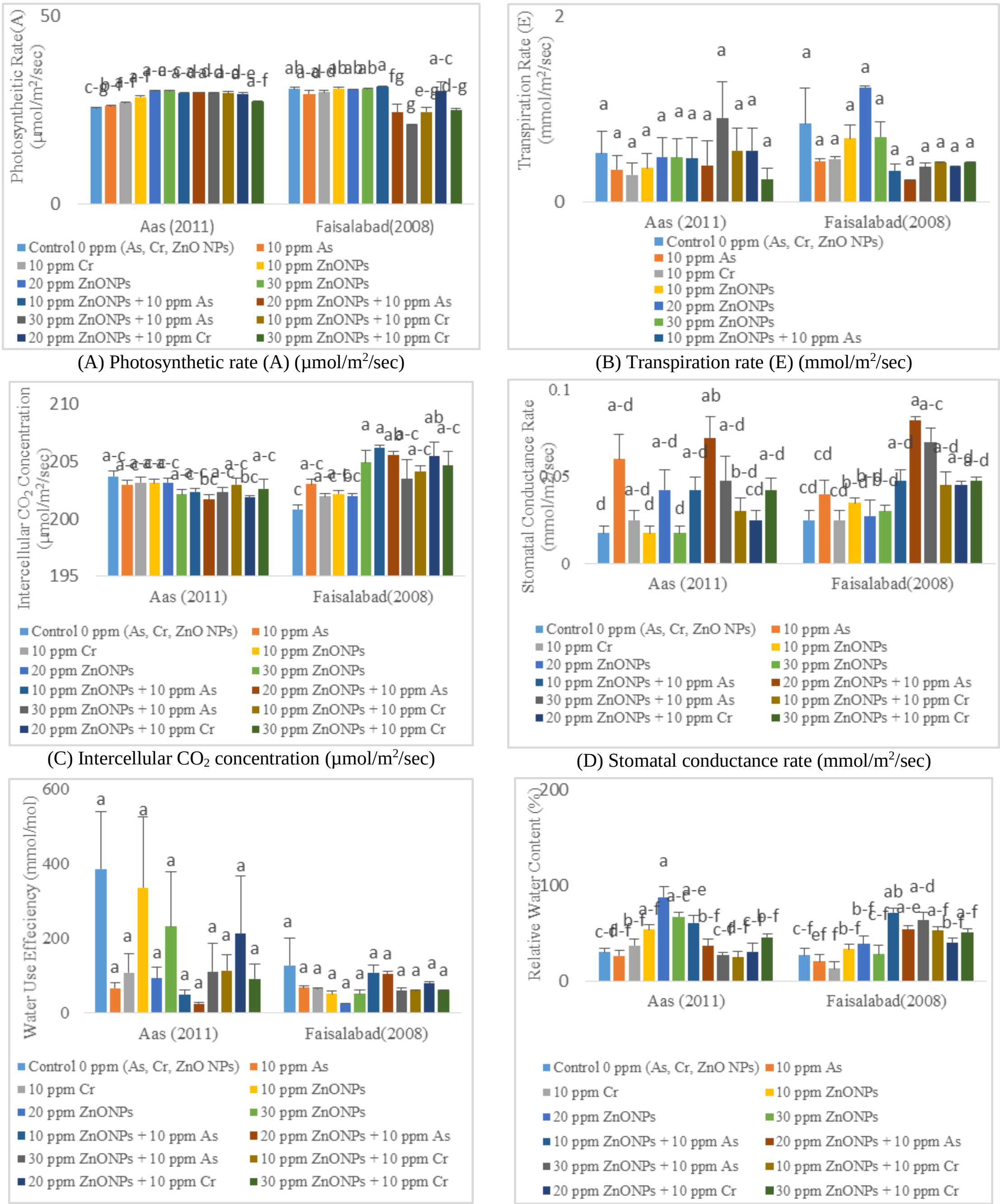

(A) Photosynthetic rate (A) (μmol/m²/sec) (B) Transpiration rate (E) (mmol/m²/sec)

(C) Intercellular CO_2 concentration (μmol/m²/sec) (D) Stomatal conductance rate (mmol/m²/sec)

(E) Water use efficiency (mmol/mol) (F) Relative water content (%)

***Figure 3.** Interactive role of zinc oxide nanoparticles and heavy metals (arsenic and chromium) toxicity on gaseous exchange parameters of wheat (Triticum aestivum L.)*

***Table 3.** ANOVA (means squares) for soil analysis parameters of wheat under interactive role of zinc oxide nanoparticles and heavy metals (arsenic and chromium) toxicity*

Source	df	Soil pH	Soil E.C	O.M %	Phosphorous (ppm)	Potassium (ppm)	Soil saturation %
Variety	1	0.0001ns	0.063ns	0.003ns	3.840ns	48490208ns	292494ns
Treatment	11	0.0112ns	0.058*	0.002ns	1.646ns	48172515ns	127244ns
Variety × treatment	11	0.0012ns	0.030ns	0.003ns	1.495ns	48214787ns	126842ns
Error	72	0.0101	0.031	0.002	1.629	4818662	140763
Total	95	0.8648	3.341	0.264	153.684	4578187366	13222393

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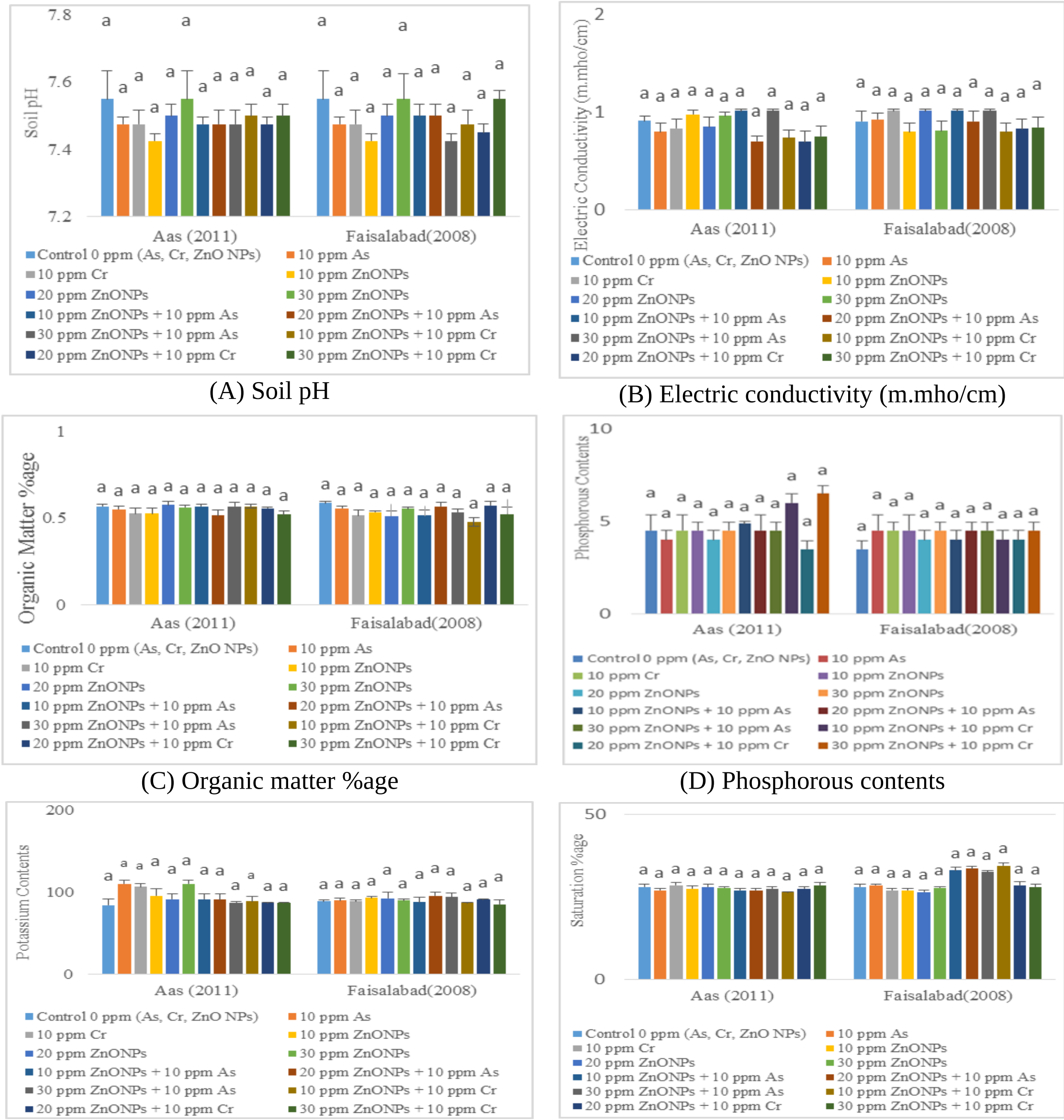


(A) Soil pH (B) Electric conductivity (m.mho/cm)

(C) Organic matter %age (D) Phosphorous contents

(E) Potassium contents (F) Saturation %age

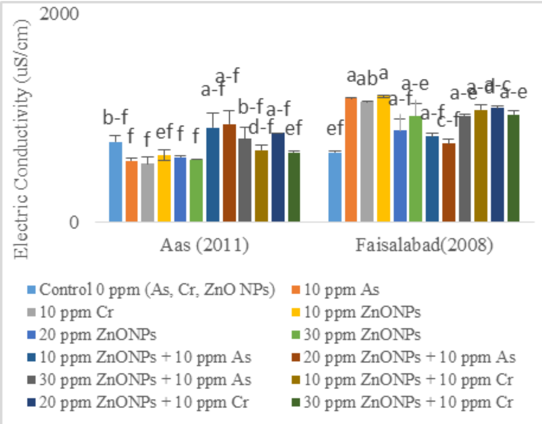
***Figure 4.** Interactive role of zinc oxide nanoparticles and heavy metals (arsenic and chromium) toxicity on soil analysis parameters of wheat (Triticum aestivum L.)*

Water analysis attributes

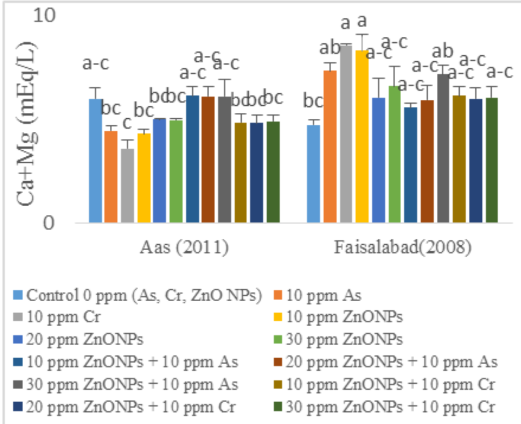
ANOVA results for water analysis parameters of wheat varieties was noted in *Table 4*. Results indicated that the effect of heavy metal and interactive role of ZnO nano particle was highly significant on water characteristics of both varieties of wheat. The interactions between treatments and varieties also showed highly significant results. Results also narrated that water analysis characteristics were highly observed in Faisalabad (2008) as compare to Aas (2011) at all treatments. In Faisalabad (2008), maximum values of water characteristics were detected in plants under heavy metal (Cr and As) stress as there was not effect of heavy metal on water characteristics. From the results of experimental data, it was concluded that heavy metal stress less reduced in water parameters. And this little reduction was more prominent in Faisalabad (2008) as compare to Aas (2011) (*Fig. 5A-G*).

Table 4. *ANOVA (means squares) for water analysis parameters of wheat under interactive role of zinc oxide nanoparticles and heavy metals (arsenic and chromium) toxicity*

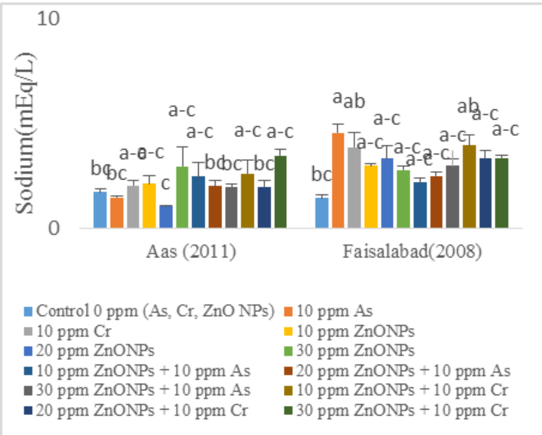
Source	df	EC (uS/cm)	Ca + Mg (mEq/L)	Na (mEq/L)	HCO_3 (mEq/L)	Cl (mEq/L)	Sodium adsorption ratio	Residual sodium carbonate
Variety	1	1769366***	49.163***	21.5651***	71.760***	2.070***	4.9641***	0.130*
Treatment	11	40289*	1.234 ns	2.0973**	1.049ns	0.041*	0.5374*	0.110***
Variety × treatment	11	147468***	6.372***	2.3631***	5.930***	0.179***	0.6468**	0.068**
Error	72	20987	1.369	0.883	1.439	0.023	0.281	0.034
Total	95	5345770	231.42	134.26	252.15	6.211	38.287	4.5459



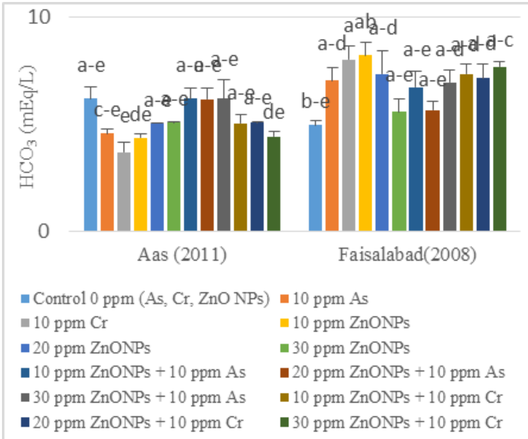
(A) Electric conductivity (uS/cm)



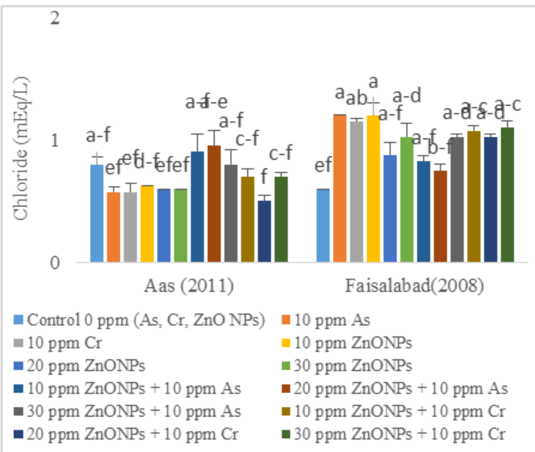
(B) Ca + Mg (mEq/L)



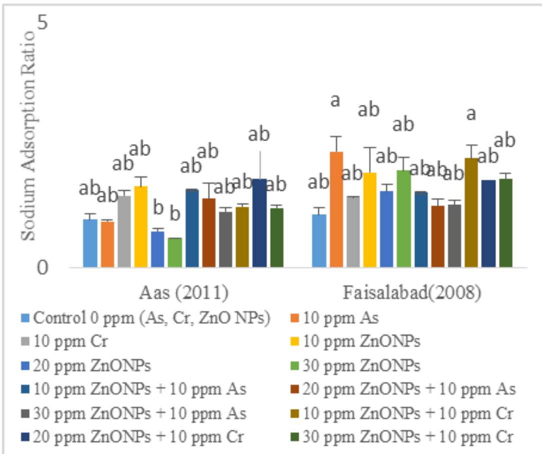
(C) Sodium (mEq/L)



(D) HCO_3 (mEq/L)



(E) Chloride (mEq/L)



(F) Sodium adsorption ratio

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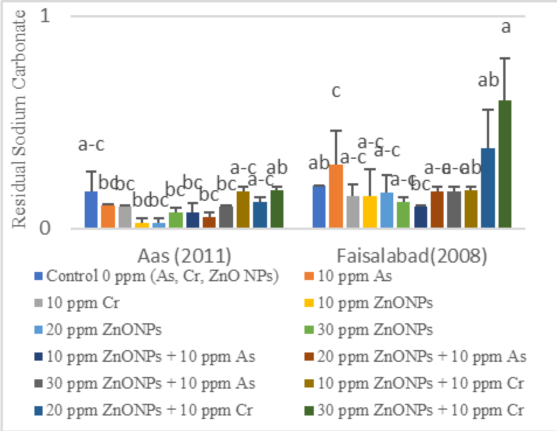

(G) Residual sodium carbonate

Figure 5. *Interactive role of zinc oxide nanoparticles and heavy metals (arsenic and chromium) toxicity on water analysis parameters of wheat (Triticum aestivum L.)*

Fourier transform infrared spectroscopy of ZnO nano particles

Figure 6 showed the analysis of ZnO nano particles under heavy metal stress in wheat plants. In order to analyze the structure of the bonds present in the ZnO prepared sample, the FTIR was used. The FTIR spectrum of ZnO nanoparticles was found to be in the 4000-500 cm^{-1} band. The extended modes of ZnO nano particles were defined as the peaks observed in the 500-1000 cm^{-1} region. The hydroxyl group of absorbed H_2O molecules caused the peak around 9705 cm^{-1}. C – O – C and O – H bonds caused the peaks around 14415 and 15523 cm^{-1}. Due to the bound H_2O molecules present on the surface of the ZnO nanoparticles, an extreme peak at 317877 cm^{-1} was assigned to O – H group stretching vibrations.

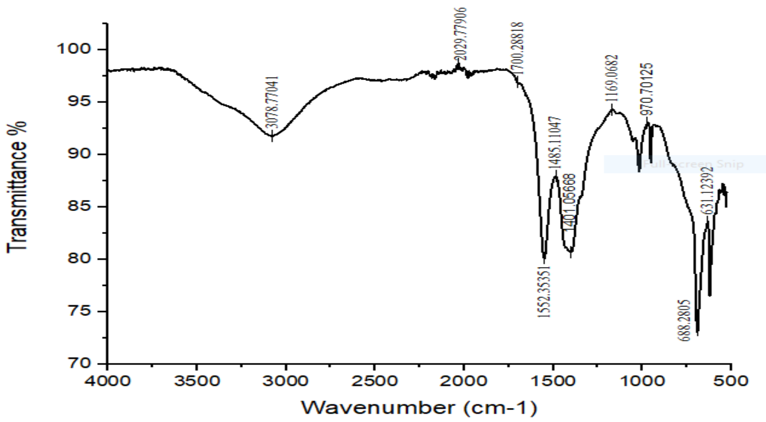

Figure 6. *Interactive role of zinc oxide nanoparticles and heavy metals (arsenic and chromium) toxicity on FTIR, of wheat (Triticum aestivum L.)*

Scanning electron microscopy of ZnO nano particles

Scanning electron microscopy was used to examine the surface morphology of a prepared sample of ZnO nano particles, as seen in *Figure 7*. The microscopy images clearly showed the homogenous and uniformly distributed ZnO nano particles.

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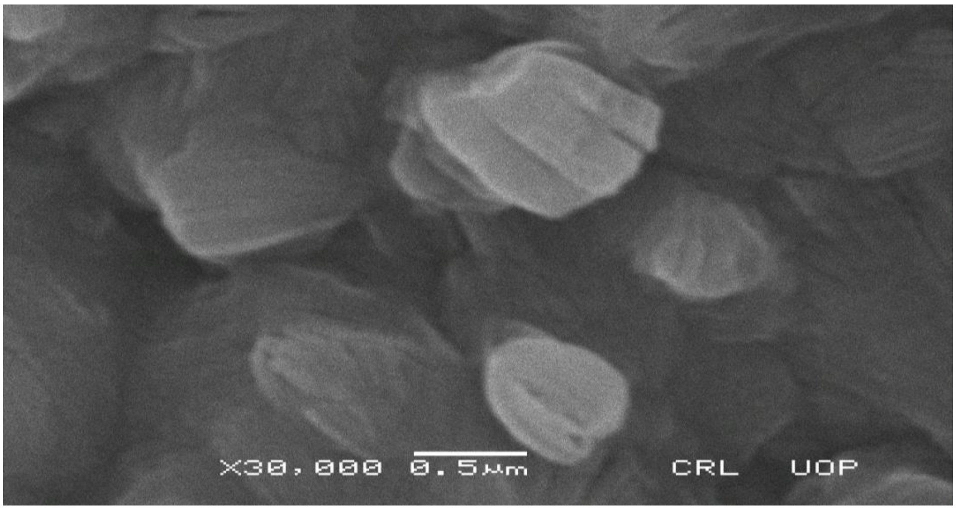

Figure 7. *Interactive role of zinc oxide nanoparticles and heavy metals (arsenic and chromium) toxicity on SEM of ZnO nanoparticles of wheat (Triticum aestivum L.)*

XRD analysis of ZnO nano particles

X-ray diffraction analysis confirmed the phase and crystal structure of ZnO nano particles made with *Aloe vera* extract, and the pattern was reported in the range of 200 to 600, as shown in *Figure 8*. In this pattern the peaks were detected at angles 20° of 25.3°, 36.1°, 37.5°, 41.5°, 42.3°, 45.2°, 46.1°, 47.2° corresponding to the lattice planes (100), (002), (101), (102), (110), (103), (200), (112), (201), (004) and (202) respectively.

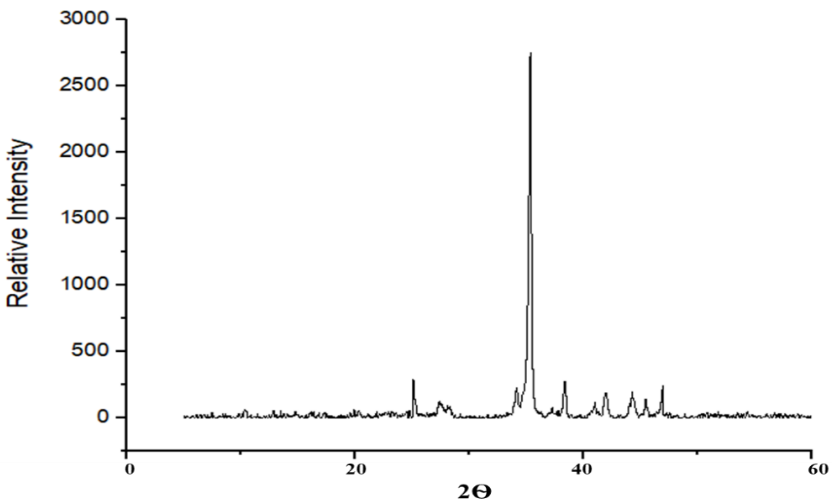

Figure 8. *Interactive role of zinc oxide nanoparticles and heavy metals (arsenic and chromium) toxicity on XRD pattern of zinc oxide nanoparticles of wheat (Triticum aestivum L.)*

Discussion

Arsenic induces oxidative stress in plants by starting a chain reaction that disrupts metabolic processes. Several toxic intermediates released during stress, including superoxide anion, hydroxyl radicals, hydrogen peroxide, and lipid peroxide, disrupt

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cellular processes and trigger growth arrest (Majumder et al., 2018). Since it is a key component for auxin synthesis and aggregation, indole-3-acetic acid, which is important for cell division and expansion, ZnO nanoparticles release Zn, which regulates development (Seleiman et al., 2021). Under stress conditions, Zn application preserves membrane integrity by binding to sulfhydryl groups as well as phospholipids, according to Soares et al. (2019). It was also found from the result that Zn is necessary for preserving the structural integrity of plants membranes under salinity stress. In reality, Zn supplementation improves the absorption of micro- and macronutrients that are depleted in stressful situations (Latef et al., 2017).

In our research, however, ZnO–NP supplementation greatly increased the biomass, growth as well as yield of arsenic stressed wheat plants. These findings are similar to those of Rizwan et al. (2017), who found that applying ZnO–NP to Cd-stressed maize plants significantly increased growth. In several other species, including *Leucaena leucocephala*, ZnO–NPs application has been shown to decreased oxidative stress caused by heavy metal (Venkatachalam et al., 2017).

Under As stress, photosynthetic attributes of the soybean plants were found to be reduced. Since arsenic interferes with nitrogen metabolism, it has a lower bioavailability (Rahman et al., 2007). The use of ZnO–nano particles significantly enhanced the amount of chlorophylls in leaves, as well as improving gas exchange attributes. These observations support those of Faizan et al. (2018), who found that applying ZnO–NP to rice plants increased photosynthetic rates. Results from our findings showed that leaf relative water content also reduced in As stressed wheat plants. On the other hand, there was a significant increase in water capacity and RWC of the leaves when arsenic-stressed wheat plants were supplemented with different concentrations of ZnO-NP. This may be attributed to improved water and mineral portion uptake in the vicinity of ZnO–NP (Vezza et al., 2018).

Damage to the photosynthetic apparatus caused by arsenate, as well as changes in pigment content and photosynthetic capacity, delayed growth. Increased ROS development under arsenate stress disrupted biochemical and metabolic processes in the test cultivar, resulting in a significant decrease in chlorophyll and carotenoid content and unfavorable associations with applied arsenate (Sil et al., 2019). In Gouan (*Aeluropus littoralis*), replacing Mg^{2+} with arsenic in the chlorophyll structure has been shown to reduce chlorophyll content, lowering photosynthetic rate (Rastgoo and Alemzadeh, 2011). Under arsenate stress, destruction of chloroplasts and photosynthetic apparatus decreased gaseous exchange characters in the cultivar. Heavy metal stress (Cr and As) has been shown to affect various gaseous attributes as well as chlorophyll content of various plants (Gill et al., 2016).

Chromium is more phytotoxic than other metals due to its higher electronegativity (Su et al., 2019). The presence of Cr6 + has an effect on plant growth because it prevents seed germination by interfering with amylase activity. Since chromate interfered with stomatal opening and closure, transpiration and stomatal process were also decreased. In the presence of chromium, photosynthesis, transpiration, and water usage efficiency both decreased by up to 31%, 30%, and 29% respectively (Zaheer et al., 2020).

To prevent Zn hidden hunger in humans, higher Zn concentrations in cereal grains are expected (Qaswar et al., 2017). This increased Zn content in cereal grains can be accomplished by a variety of methods, including the use of Zn fertilizers and the production of high Zn accumulating varieties. A level of > 40 mg Zn per kg of grains is

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required for Zn biofortification (Cakmak et al., 2017). Higher Zn concentrations can promote wheat growth, resulting in a reduction in heavy metal per unit biomass. Zn NPs and Zn salts were recently confirmed to enhance sorghum growth and nutritional stability with NPK inputs, as well as increase plant nitrogen contents (Dimkpa et al., 2017).

Conclusion

This research shows that ZnO–NPs are effective at reducing heavy metal (As and Cr) toxicity in wheat plants at various concentrations. ZnO–NPs could therefore prove to be an excellent foliar fertilizer in areas vulnerable to heavy metal toxicity if applied at specific doses. Therefore, it was concluded that the use of these nanoparticles will improve system of cropping performance by reducing As and Cr stress and increasing plant growth, yield and soil quality.

Nanotechnology is strengthening the performance and efficiency of common products, making our lives easier. It contributes to a healthy environment by delivering cleaner air and water, as well as clean renewable energy for a long-term future. Nanotechnology has gotten a lot of attention, and top institutions, industries, and organisations are investing more in research and development. Nanotechnology has established itself as a cutting-edge field of science in which extensive research is being conducted in order to put the technology into practice. This study further recommended that Nanotechnology has a bright future because of its efficiency and environmental benefits.

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