



# Role of Bio-Based Synthesized Nanozinc Oxide in Ameliorating the Deleterious Effects Caused by Lead in *Vigna radiata* L

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## Abstract

The present work describes the bio-based green synthesis and characterization of zinc oxide nanoparticles (ZnO NPs) using leaf extract of *Tridax procumbens*; the synthesized nanoparticles were used to study their beneficial effect on the growth and metabolism of *Vigna radiata*. ZnO NPs were characterized using X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy, high-resolution transmission electron microscopy (HR-TEM), and ultraviolet–visible spectroscopy (UV–Vis spectra). Growth of *V. radiata* seedlings was measured in terms of shoot length and root length that were treated 20 and 40 mg/L concentrations of green synthesized ZnO NPs and constant concentration (50 mg/L) of PbCl<sub>2</sub>. These studies have shown the effect of ZnO NPs in the stimulation of growth as well as physiological and biochemical parameters. *Vigna* seedlings showed positive effects depending upon the increasing concentrations of ZnO NPs. This study suggests that ZnO NPs can be effectively used to ameliorate the toxicity of Pb in *Vigna* plants.

**Keywords** Zinc oxide nanoparticles · *Vigna radiata* · Green synthesis · Nanoparticles

## Introduction

Nanotechnology is the combination of technology and objects around nanoscale size. It is a budding area of science that involves the engineering of tiny particles of different materials. It provided the great benefits to the developing world, viz. agriculture, medicine, energy storage, and water treatment. Nanotechnology can be considered the major tool for the upcoming industrial revolution and regarded as the foundation for many biotechnological finding in this twenty-first century. The importance of nanoparticles (NPs) is due to their advanced physiochemical and biological properties against their bulk phase. The nanoscale

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material size range from 1 to 100 nm gives them broader ratio for surface to volume and thereby results in the high reactivity of surface. The higher reactivity and high surface to volume ratio properties of NPs allow their utility in different fields covering material science to biotechnology [1]. Due to the abovementioned uniqueness over bulk materials, NPs were consolidated for various innovative alternatives in numerous industries.

The metal and its oxide NPs are synthesized through various physical, chemical, biological, and eco-friendly approaches. Numerous nanostructures were formed from the oxides of zinc (ZnO) through the involvement of physical and chemical methodology incorporation with defined setup, non-ecological chemical formulations, significant expense, and specifically raised temperature and pressure. But involvement of toxic chemical release and high energy input makes them harmful in both environmental and health aspects. However, in recent years, various green approaches for NP synthesis have attracted the attention of scientists because of their eco-friendly properties. For example, the formation of NPs like ZnO can be done with other methods, namely, hydrothermal process, precipitation, and vapor transport.

Synthesis of NPs through bio-based has gained very much popularity in recent years due to their low energy inputs, non-toxic effects, and cost-efficiency [2]. The synthesis of NPs of ZnO with the usage of microorganisms or extract obtained from different plants is considered a new area of interest. The biological aspects for NP synthesis are more advantageous over the traditional physical and chemical methods [3]. Various studies have been conducted for the evaluation of ZnO NPs over several crop plants. The significant effect of ZnO NPs has been observed in the root cortex and epidermis of *Lolium perenne* in addition to NPs in vascular and endodermal tissues [4]. The pigment, protein, and sugar contents were enhanced with nanodimensional ZnO particles by increasing activity of antioxidant enzymes in various vegetable crops [5]. The contamination of soil and water with heavy metals (such as silver, cadmium, and lead) is increasing due to various human activities such as urbanization and industrialization.

Heavy metals such as lead (Pb), which is non-essential and does not have an essential role in the cell metabolism, easily accumulated as well as absorbed in various plant parts. Their uptake is regulated by particle size, pH, exudation from the root through different physical and chemical parameters, and cation exchange with soil. Pb shows numerous toxic symptoms in plants, viz. growth retardation, chlorosis resulting in photosynthesis retardation, root blackening, and various other symptoms [6]. The present study deals with mitigating the negative impacts of PbCl<sub>2</sub> on growth and metabolism of *Vigna radiata* via using ZnO NPs.

## Materials and Methods

### Synthesis of Green ZnO NPs with Leaf Extract of *Tridax procumbens*

The fresh and mature leaves of *Tridax procumbens* were collected from Ewing Christian College, University of Allahabad, Prayagraj, India. About 250 g of leaves was thoroughly washed several times with deionized water and further shade-dried at room temperature. The extract of *T. procumbens* leaves was obtained from 10 g of air-dried, finely chopped leaf powder dissolved under 100 mL of deionized water at 60 °C approximately for 30 min. The extract was cooled at ambient condition followed by its filtration concluding with transparent yellow color broth followed with 4 °C storage. Furthermore,

0.02 M zinc acetate dihydrate [ $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$ ] solution was homogenized with 100 mL DDW with continuous stirring. After 10 min of continuous stirring, extract of leaves (25 mL) was added in the prepared solution in a drop-by-drop manner followed by 2 M NaOH aqueous solution and the solution was maintained at pH 12. The prepared solution was stirred by magnetic stirring with a hot plate and the process was continued until the color of the milky white solution turned into pale yellow-colored paste. The resulting precipitate was separated and washed with DDW. Afterwards, impurities present in the final products were removed with ethanol and the remaining paste was collected in tubes followed with overnight heating in a hot air oven. The entire process was repeated in order to have sufficient nanomaterials (NMs). The powder of color lying between somewhere creamish to white was carefully collected and stored for characterization.

### Characterization of ZnO NPs and Biophysical Parameters

Formation of ZnO NPs was confirmed with the analysis done by a UV spectrophotometer (Shimadzu, UV-2450). The recorded spectra of absorbance using UV range from 200 to 800 nm. Additionally, the possible functional groups were identified using a FTIR spectrometer (PerkinElmer). Furthermore, X-ray diffraction was performed with an X-ray diffractometer (Rigaku Smart lab 50/60 Hz) in order to determine the zinc oxide nanoparticle crystal phase. The operational spectrum lies in the  $2\theta$  range of  $20\text{--}80^\circ$  with  $1.5406 \text{ \AA}$  by  $\text{CuK}\alpha$  at 100 mA and 3 kV. Selected area electron diffraction (SAED) and transmission electron microscopy (TEM) were used to measure the size and radius of NPs with Tecnai (G2 20 S-TWIN).

### Seed Germination with Petri Plate Bioassay

A preliminary screening experiment was done with seed germination with petri plate Bioassay. The suspension of ZnO NPs in DDW was followed by 30-min ultrasonic vibration (100 W, 40 kHz). The aggregation of particles was avoided with the usage of small magnetic bars. Furthermore, the concentrations, viz. 0, 20, and 40 mg/L of ZnO NPs, were used for the conduction of experiments. *V. radiata* seeds were procured from a certified seed agency (Prayagraj, India). The germination rate for the mentioned plant material lies around 70–75% in accordance with earlier studies. Thereafter, storage of seeds was done in a seed desiccator in order to conduct the planned experiments.

At the time of experiment, 10-min sterilization of seeds was done with 70% (v/v) ethanol and 1.5% (w/v) sodium hypochlorite solution followed by repeated DDW washing. Thereafter, 10 viable seeds in duplicate were soaked in 10-mL synthesized solution of ZnO NPs and Pb prepared with graded concentrations from stock solution. On the other hand, seeds soaked in DDW were taken as control.

Seeds of fixed counts, i.e., 10 from each treatment, were put at an equal distance in filter paper (Whatman No. 1)–lined petri plates with 29 cm and 15 cm. The moisturization of petri plate filter paper was done with the respective prepared solution as per treatment. Afterwards, the prepared petri plates were incubated for 8 days in a dark room followed by regular observation at an interval of 24 h. After 3 days of sowing, radicle length and plumule length were recorded at 24 h of regular interval up to 8 days.

## Seedling Vigor Index

The chelated ZnO NPs were considered as reference Zn source by farmers; this is because the hydrated form of Zn powder is insoluble in water and therefore was not absorbed by plants [7]. Furthermore, its synthesized form, i.e., ZnO NPs, was formed by direct suspension in DDW followed by 30-min ultrasonication. The two concentrations of ZnO NPs, viz. 20 mg/L and 40 mg/L, and Pb, viz. 50 mg/L, were used in single and in combination. The treatments were labeled as Pb, N1, N2, Pb + N1, and Pb + N2, respectively. Thereafter, 10 seeds in duplicate were soaked in solutions of Pb and ZnO NPs in single and in combination in 50 mL Pb for 5–6 h and immersed for 5–6 h in 20 mL of the abovementioned concentration.

The DDW-immersed seeds were regarded as control. The treated seeds of *Vigna* were transferred in filter paper (Whatman No. 1)-lined petri plates followed by addition of 2 mL of water in each plate. Thereafter, 8-day incubation of covered petri plates was done under dark condition. The result in the form of germination and seedling length was recorded with 1 day time interval. The formula used by Abdul Baki and Anderson (1973) [8] was used in order to calculate seedling vigor index (SVI).

$$\text{Seedling vigor index} = \text{Germination\%} \times (\text{radicle length} + \text{plumule length})$$

## Biochemical Parameters

### Photosynthetic Pigments (Total Chlorophyll and Carotenoids)

The quantification of photosynthetic pigments, i.e., total chlorophyll and carotenoids, was done following the protocol mentioned by Lichtenthaler (1987) involving some modifications [9]. For this, 10 mg of fully grown NP-treated leaves was homogenized with 80% acetone and kept in dark condition. Afterwards, the supernatant was observed under a UV spectrophotometer (PerkinElmer Lambda 35) at 663 nm, 645 nm, and 470 nm. The formulae used for estimation of photosynthetic pigments were as follows:

$$\text{Chlorophyll } a \text{ } (\mu\text{g/mL}) = 12.21 \times A_{663} - 2.81 \times A_{645}$$

$$\text{Chlorophyll } b \text{ } (\mu\text{g/mL}) = 20.13 \times A_{645} - 5.03 \times A_{663}$$

$$\text{Carotenoids } (\mu\text{g/mL}) = [1000 \times A_{470} - 3.27(\text{Chla}) - 104(\text{Chlb})]/198$$

where *A* is the observed OD.

## Protein

For the estimation of total protein content, 10 mg of fully grown leaves was homogenized for 5 min with 1 mL of 1 N NaOH at 100 °C. The obtained supernatant was mixed with an alkaline copper reagent and 0.5 mL of Folin-Ciocalteu reagent (FCR) was added after 10 min of incubation period. Thereafter, the estimation was done with absorbance recorded after 30 min of incubation period at 650 nm under a UV spectrophotometer as per standard curve obtained from the graded concentration of bovine serum albumin (BSA).

## Sugar Content

The estimation of total soluble sugar was done following Hedge and Hofreiter (1962) with some modifications. For this, 50 µg of fresh leaf was homogenized with 5 mL of 95% ethanol followed by centrifugation. The obtained 1 mL of supernatant was added to 4 mL of anthrone reagent followed by 10 min of water bath boiling. The estimation was done as per the standard curve obtained with D-glucose and the analysis was performed at 620 nm [10].

## Enzymatic Assays

### Superoxide Dismutase

The estimation of superoxide dismutase (SOD) (EC 1.15.1.1) activity was done by the nitroblue tetrazolium (NBT), photochemical assay following the method of Beyer and Fridovich (1987) [11]. The fresh plant leaves (500 mg) were homogenized using 0.1 M sodium phosphate buffer containing 1% polyvinyl pyrrolidone (pH 7.0). The extract was then centrifuged at 4 °C at 14,000 g for 30 min (Remi instruments C 24). Preparation of reaction mixture (4 mL) was done by 20 mM methionine, 0.15 mM ethylenediaminetetraacetic acid (EDTA), 0.12 mM NBT, and 0.5 mL supernatant. After that, test tubes were kept under fluorescent lamps for 30 min and identical un-illuminated assay mixture was added as blank. One unit of enzyme was calculated as the amount of enzyme which caused 50% inhibition of NBT reduction.

### Catalase Activity

For estimation of catalase (CAT; EC1.11.1.6) activity, the method of Cakmak and Marschner (1992) was adopted. H<sub>2</sub>O<sub>2</sub> dissociation rate was recorded for 1 min using extinction coefficient of 39.4 mM<sup>-1</sup> cm<sup>-1</sup> at 240 nm. One unit of enzyme activity is defined as 1 nmol H<sub>2</sub>O<sub>2</sub> dissociated-min<sup>-1</sup> [12].

### Nitrate Reductase Activity

The activity of nitrate reductase (EC 1.6.6.1) was determined using a modified Jawroski technique (1971). Fresh leaf samples (250 mg) from treated plants were incubated in 4.5 mL medium containing 100 mM phosphate buffer (pH 7.5), 20 mM KNO<sub>3</sub>, and 5% propanol for 3 h before being stored in the dark. Afterwards, 0.4 mL of aliquot was mixed with 1% sulphanilamide prepared in 3 N HCl and 0.02% N-1-naphthyl ethylene diamine dihydrochloride. The absorbance was recorded at 540 nm. NR activity was recorded with standard curve prepared from NaNO<sub>2</sub> and expressed as µ mol NO<sub>2</sub> g<sup>-1</sup> FW h<sup>-1</sup> [13].

### Electrolyte Leakage

Membrane integrity of the test seedlings was assessed in terms of electrolyte leakage (EC) as described by Lutts et al. (1996) [14]. Fresh leaf samples (100 mg) were cut and placed in test tubes containing 10 mL of DDW. The tubes were incubated at room temperature in dark for 24 h and the initial electrical conductivity of the medium (EC1) was measured

using a digital conductivity meter (type BCT-4308). The samples were then heated in water bath at 95 °C for 20 min to release all electrolytes, cooled down to 25 °C, and the final electrical conductivity (EC2) was measured. The EC was calculated according to the following formula:

$$EC = EC1/EC2 * 100$$

### Lipid Peroxidation

Oxidative damage of lipids was estimated by measuring the content of malondialdehyde (MDA) in leaf (200 mg) homogenates in 0.1% (w/v) trichloroacetic acid and centrifuged at 10,000×g for 10 min. Lipid peroxidation (LP) was measured as the amount of MDA determined by thiobarbituric acid (TBA) reactive substance as described by Heath and Packer (1968) on milliliter of supernatant was mixed with 4 mL of 0.5% TBA. The mixture was then heated at 95 °C for 30 min and again centrifuged after cooling. The absorbance of the supernatant was recorded at 532 nm and corrected by subtracting the non-specific absorbance at 600 nm. The MDA concentration was calculated using the extinction coefficient of 155 mM<sup>-1</sup> cm<sup>-1</sup> and expressed as n mol g<sup>-1</sup> FW [15].

### Free Proline

Extraction and determination of proline were done following Bates et al. (1973). Leaf samples were extracted with 3% sulphosalicylic acid. Aliquot was treated with acid ninhydrin and acetic acid, boiled for 1 h at 100 °C. The reaction mixture was extracted with 4 mL of toluene. Absorbance of chromophore containing toluene was determined at 520 nm. Proline content was expressed as mol g<sup>-1</sup> fresh weight [16].

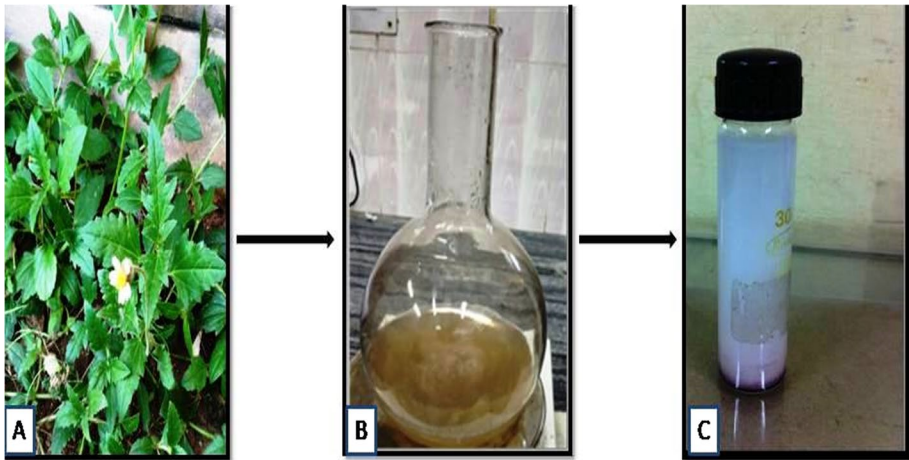
### Statistical Analysis

All data are presented as the mean value of triplicate randomized block design is used in field experiment. Data were statistically analyzed using analysis of variance (ANOVA) by using SPSS software (version 16, SPSS Inc., USA). Appropriate standard error of means ( $\pm$  SEM) was calculated for presentation of graphs. Since the results showed normal distribution, results were statistically analyzed by analysis of variance (one-way ANOVA) to test the significance level against the null hypothesis. The treatment means were then analyzed using Duncan's multiple range test (DMRT) at  $P < 0.05$ .

## Results and Discussion

### Green Synthesis of ZnO NPs

The photographs of the plant source, leaves in dried form, and the synthesized ZnO NPs are shown in Fig. 1. Leaf extract of *Tridax* has been used as reducing as well as surface stabilizing agents for the synthesis of ZnO NPs. The established and most widely used method for production of ZnO NPs is the wet chemical procedure. The method involves synthesis of ZnO NPs in a liquid medium containing different reactants, in particular reducing agents



**Fig. 1** Green synthesis of ZnO NPs using *Tridax procumbens* leaf extract. (A) Leaves of *T. procumbens*. (B) Color change observed by mixing of leaf extract of *T. procumbens*. (C) Color change during the synthesis of ZnO NPs

present in leaf extract. In this process, ZnO NPs are formed due to the reaction between  $Zn^{++}$  ions from zinc acetate and the reducing agent, i.e., leaf extract. The signature of biomolecules as evidenced by FTIR spectrum reveals that phytochemicals, viz. polyphenols, carboxylic acid, polysaccharides, amino acids, and proteins, are present on the surface of ZnO NPs.

The ZnO NPs have been synthesized using leaf extract of *Aloe barbadensis*, *Abutilon indicum*, *Melia azedarach*, *Indigofera tinctoria*, and bacterial and fungal culture [17–20]. Recently, zinc oxide nanoparticles have been green synthesized using leaf extract of *Psidium guajava* [21], extract of *Daucus carota* [22], plant extract of *Monsonia burkeana* [23], and leaf extract of *Mangifera indica* [24].

## Characterization of the Synthesized NPs

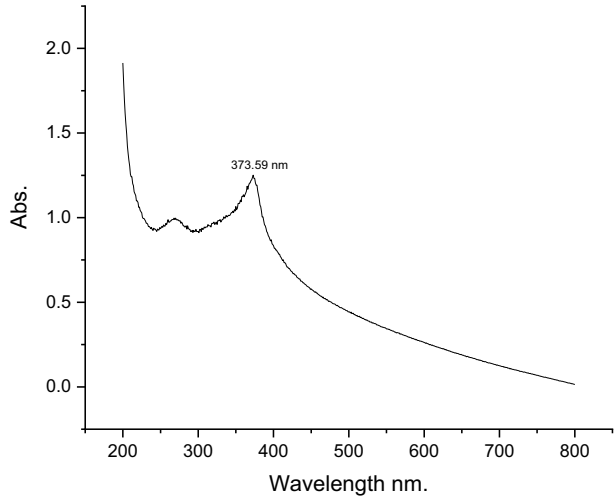
### UV–Vis Spectra Analysis

Preliminary characterization of ZnO NPs was carried out by the UV–Vis spectroscopy. The UV–Vis spectral study of ZnO NPs was monitored in the range of 200–800 nm (Fig. 1). Aqueous leaf extract of *T. procumbens* was added to the prepared zinc acetate solution. The apparent color change was observed from light yellow to creamish within 20 min. The samples showed a similar behavior as reported (ranging between 370 and 430 nm). The spectrum exhibits a characteristic absorption peak at 373.59 nm due to the electron transitions from the valence band to the conduction band (Fig. 2).

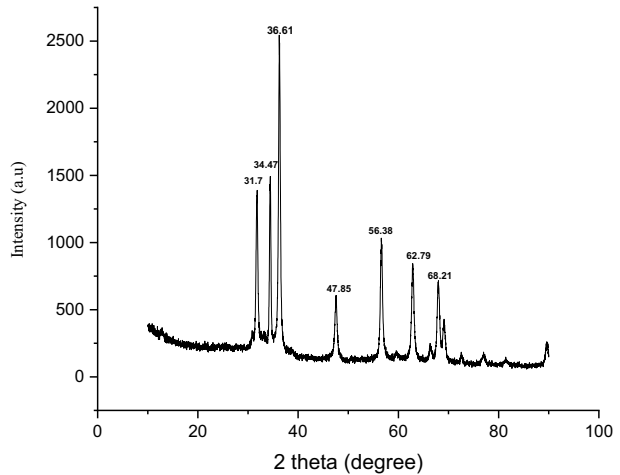
### Fourier Transform Infrared Spectroscopy

FTIR measurements were performed to identify the biomolecules responsible for capping, reducing, and stabilizing agents present in the leaf extract of *T. procumbens*. The FTIR spectra of the biological synthesized ZnO NPs are shown in Fig. 3 with spectral range

**Fig. 2** UV–Vis of synthesized ZnO NPs using *T. procumbens* leaf extract



**Fig. 3** X-ray diffraction patterns of green synthesized ZnO NPs using *T. procumbens* leaf extract



400–4000  $\text{cm}^{-1}$ . A typical FTIR spectrum of pure zinc oxide nanoparticles indicates absorption spectrum with possible assignments. The peak at 512  $\text{cm}^{-1}$  is the characteristic absorption peak of the Zn–O bond and the broad absorption peak at 3402  $\text{cm}^{-1}$  can be attributed to the characteristic absorption of the hydroxyl group. FTIR spectra of the bio-synthesized ZnO NPs showed a small shift with slight changes in some related peaks and in their intensities, suggesting that the major biomolecules from the extract were capped or bonded to the surface of ZnO NPs. The major absorption peaks were observed in the region of lower wave numbers.

### X-ray Diffraction

The X-ray diffraction (XRD) patterns were used to examine the size and degree of crystallinity of ZnO NPs. The XRD patterns of dried ZnO NPs synthesized using *T. procumbens*



leaf extract at room temperature are presented in Fig. 6. The X-ray diffraction pattern showed  $2\theta$  values at  $31.70^\circ$ ,  $34.47^\circ$ ,  $36.61^\circ$ ,  $47.85^\circ$ ,  $56.38^\circ$ ,  $62.79^\circ$ , and  $68.21^\circ$ . All evident peaks could be indexed as the zinc oxide wurtzite structure. Zinc oxide crystallizes in two main forms, hexagonal wurtzite and cubic zinc blend. The wurtzite structure is most stable at ambient conditions and thus most common. Hence, XRD results clearly indicated that ZnO NPs formed using *T. procumbens* leaves were essentially crystalline. The average nanocrystallite size has been calculated by using the Debye–Scherrer formula,  $D = K \lambda / \beta \cos$ , and it was found to be around 13.8 nm (Fig. 4).

## High-Resolution Transmission Electron Microscopy (HR-TEM)

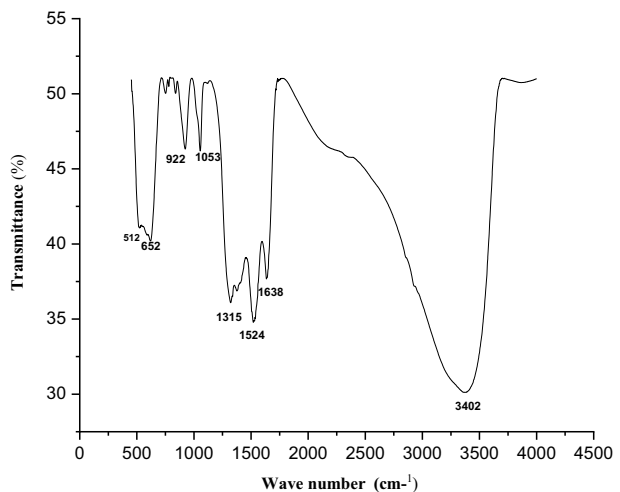
TEM analysis clearly confirmed that size of individual ZnO NP is in the range between 20 and 50 nm. The ZnO NPs were well dispersed and almost spherical in shape (Fig. 5). The results also describe that the average particle size of ZnO NPs is highly influenced by the concentration of leaf extract.

## Biophysical Parameters

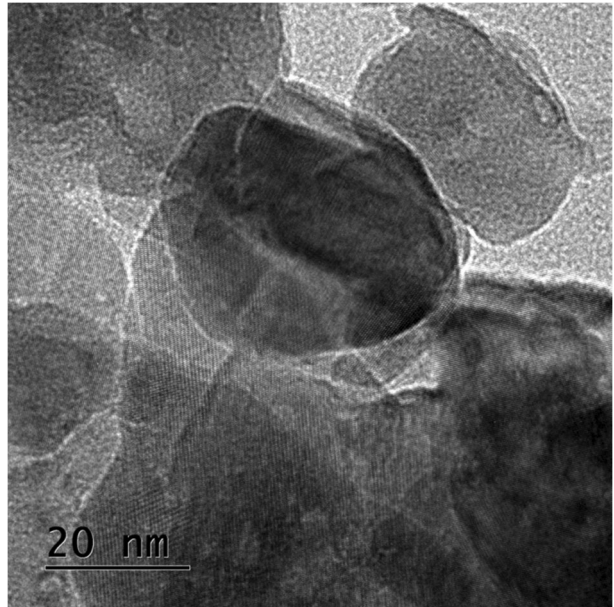
### Growth of Seedlings

Pb treatment adversely affected the seedling growth as compared with control. Root length (RL) and shoot length (RL) of seedlings significantly decreased under the influence of Pb. The treatment with ZnO NPs improved both root growth and shoot growth in comparison with Pb treatments. The fresh weight (FW) of seedlings decreased significantly caused ( $P < 0.05$ ) in bulk treatment as compared to control. In Pb treatment 34.5% and 36.4%, reduction in SL and RL was recorded respectively as compared to control. The ZnO NPs enhanced RL, SL, FW, and DW of the seedlings under the N1 and N2 treatment (Figs. 6 and 7). ZnO NPs seem to be transported to plant tissue when the roots absorb moisture from the sand treated with Hoagland's solution containing NPs. Raliya et al. [25] found that the presence of ZnO NPs increased shoot and root growth as well as biomass in *Solanum lycopersicum*. It has been demonstrated that the

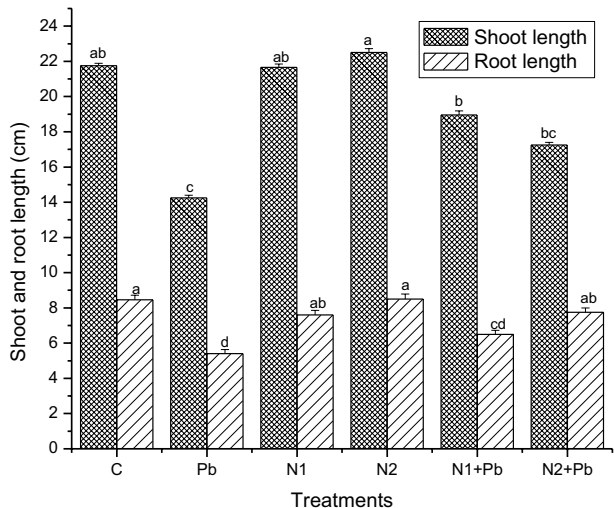
**Fig. 4** FTIR patterns of synthesized ZnO NPs using *T. procumbens* leaf extract



**Fig. 5** TEM images synthesized ZnO NPs using *T. procumbens* leaf extract

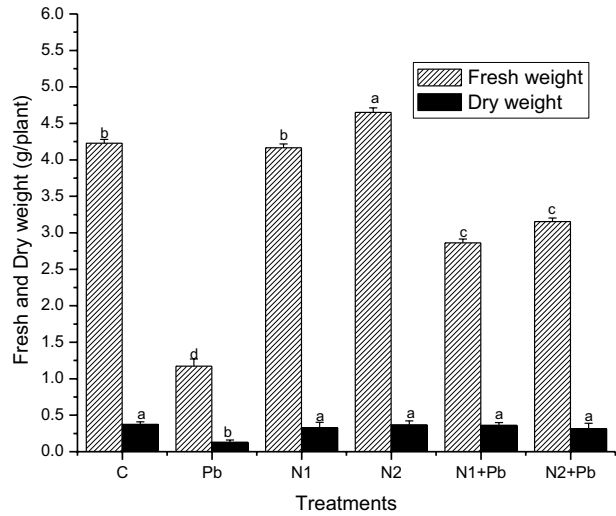


**Fig. 6** Effect of ZnO NPs and  $\text{PbCl}_2$  on shoot length, root length, and fresh and dry weight in *Vigna radiata*. Data are  $\pm$  standard error of three independent experiments with three replicates. Bars followed by different letters show significant difference at  $P < 0.05$  significant level between treatments according to the Duncan's multiple range test. Control: Pb (50 mg/L  $\text{PbCl}_2$ ); N1 (20 mg/L ZnO NPs); N2 (40 mg/L ZnO NPs); N1 + Pb (20 mg/L ZnO NPs + 50 mg/L  $\text{PbCl}_2$ ); N1 + Pb (40 mg/L ZnO NPs + 50 mg/L  $\text{PbCl}_2$ )



use of silicon nanoparticles reduced Pb-induced phytotoxicity and increased growth rate and biomass in rice seedlings [26]. The increased rate of seedling growth and biomass (fresh and dry weight) with the presence of ZnO NPs strongly suggests that phytomolecule-loaded ZnO NPs are playing an important role in enhancing seedling growth characteristics by regulating Cd and Pb metal tolerance potential through oxidative stress reduction [27].

**Fig. 7** Effect of ZnO NPs and PbCl<sub>2</sub> on fresh and dry weight in *Vigna radiata*. Data are ± standard error of three independent experiments with three replicates. Bars followed by different letters show significant difference at *P* < 0.05 significant level between treatments according to the Duncan’s multiple range test. Control: Pb (50 mg/L PbCl<sub>2</sub>); N1 (20 mg/L ZnO NPs); N2 (40 mg/L ZnO NPs); N1 + Pb (20 mg/L ZnO NPs + 50 mg/L PbCl<sub>2</sub>); N1 + Pb (40 mg/L ZnO NPs + 50 mg/L PbCl<sub>2</sub>)

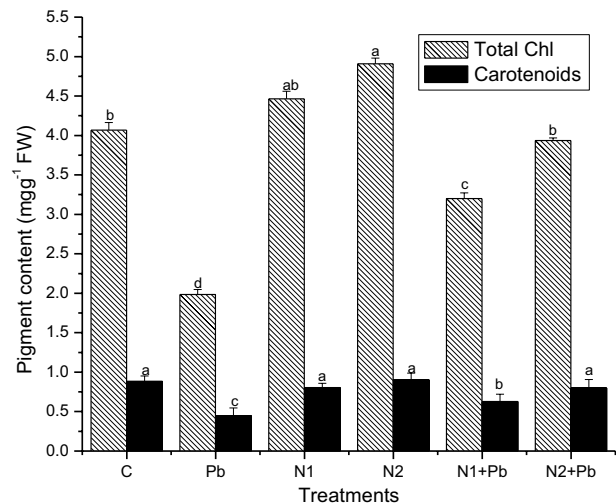


### Pigment Contents

The photosynthetic pigments declined under Pb treatments but ZnO NPs slightly increased in the pigment content in Pb+N1 treatment. The amount of total chlorophyll registered maximum value at N1 and N2 concentrations of ZnO NPs and found to be increased by 8.8% and 17%, respectively, as compared to control. Carotenoid content was found maximum 50.6% in N2 treatment of NPs as compared to Pb. The minimum inhibition of total chlorophyll in Pb and Pb+N1 treatments was recorded 51% and 21% respectively as compared to control (Fig. 8).

It has been suggested that heavy metal (Cd and Pb) exposure–associated reduction in seedling growth and biomass might be due to the alterations of various physiochemical mechanisms in plant cells including water deficit, photosynthetic machinery, and

**Fig. 8** Effect of ZnO NPs and PbCl<sub>2</sub> on pigments in *Vigna radiata*. Data are ± standard error of three independent experiments with three replicates. Bars followed by different letters show significant difference at *P* < 0.05 significant level between treatments according to the Duncan’s multiple range test. Control: Pb (50 mg/L PbCl<sub>2</sub>); N1 (20 mg/L ZnO NPs); N2 (40 mg/L ZnO NPs); N1 + Pb (20 mg/L ZnO NPs + 50 mg/L PbCl<sub>2</sub>); N1 + Pb (40 mg/L ZnO NPs + 50 mg/L PbCl<sub>2</sub>)



antioxidative defense system [28–30]. ZnO NPs can significantly promote the growth of plants by increasing chlorophyll content and total biomass.

### Sugar and Protein Content and Nitrate Reductase Activity

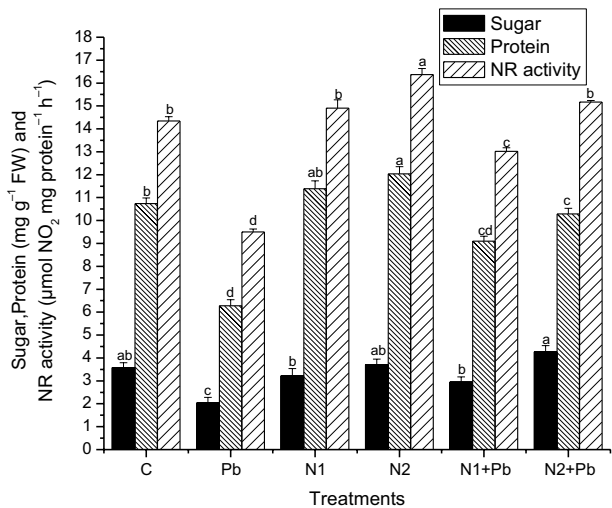
Sugar content inhibited by 43% in Pb treatment as compared to control. As per results, Pb treatment has more adverse effect on sugar content. Protein content decreased in the seedlings treated with Pb as compared to control. Plants treated with N2 concentration of synthesized NPs showed maximum increase in sugar, protein, and NR activity as compared to control while maximum inhibition was reported in the seedlings treated with the concentration of PbCl<sub>2</sub>, i.e., Pb 50 mg/L. The inhibition of protein content was concentration dependent. The inhibition of 41.5% in protein content was recorded in the seedlings treated with Pb as compared with control.

NR activity declined significantly ( $P < 0.05$ ) in the seedlings under the influence of 50 mg/L concentration of Pb. The reduction of 33.7% was observed in treatment with bulk Pb as compared to control. Nitrate is one of the major nitrogen sources for plants and promotes development and yield in plants. NR activity was slightly stimulated at 20 and 40 mg L<sup>-1</sup> of ZnO NPs in *Vigna* seedlings. NR is the only substrate-induced enzyme and decreased activity of NR may be corresponding to the low rate of absorption of NO<sub>3</sub><sup>-</sup> under the influence of ZnO NPs (Fig. 9).

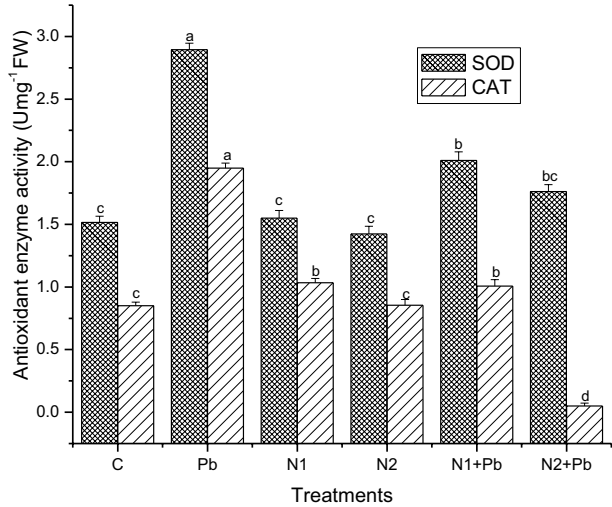
### Activities of Antioxidant Enzymes

The activity of antioxidant enzymes increased in a dose-dependent manner but the plant exposed to highest concentration of N2 and Pb + N2 decreased the activity of antioxidant enzymes. Maximum stimulation was recorded in Pb and N1 + Pb treatment in which the superoxide dismutase (SOD) and catalase (CAT) activity was found to be 47.6, 24.6%, 56%, and 15.7 respectively as compared to control. The application of excess amount of Pb might have enhanced reactive oxygen species (ROS) production which caused oxidative stress and induced antioxidant machinery in response to altered metabolic processes.

**Fig. 9** Effect of ZnO NPs and PbCl<sub>2</sub> on sugar content, protein content, and activity of nitrate reductase in *Vigna radiata*. Data are  $\pm$  standard error of three independent experiments with three replicates. Bars followed by different letters show significant difference at  $P < 0.05$  significant level between treatments according to the Duncan's multiple range test. Control: Pb (50 mg/L PbCl<sub>2</sub>); N1 (20 mg/L ZnO NPs); N2 (40 mg/L ZnO NPs); N1 + Pb (20 mg/L ZnO NPs + 50 mg/L PbCl<sub>2</sub>); N1 + Pb (40 mg/L ZnO NPs + 50 mg/L PbCl<sub>2</sub>)

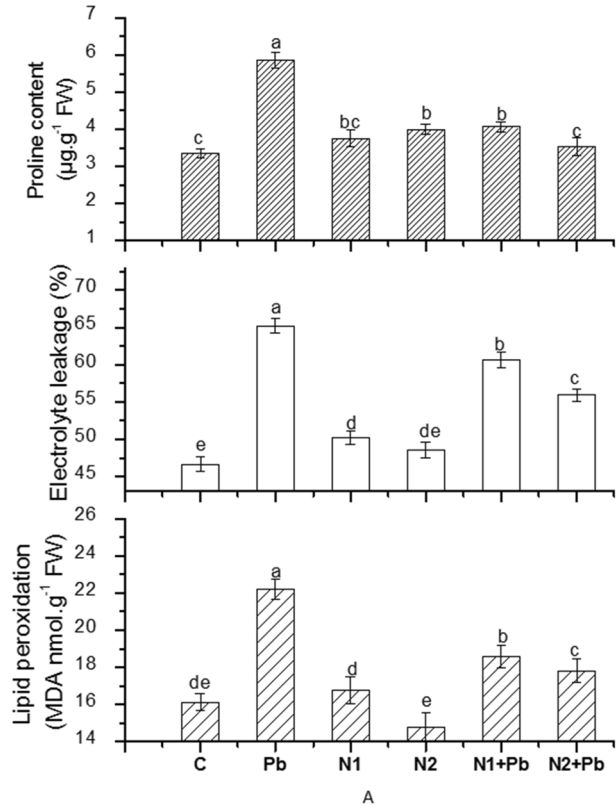


**Fig. 10** Effect of ZnO NPs and PbCl<sub>2</sub> on enzyme activities in *Vigna radiata*. Data are ± standard error of three independent experiments with three replicates. Bars followed by different letters show significant difference at *P* < 0.05 significant level between treatments according to the Duncan’s multiple range test. Control: Pb (50 mg/L PbCl<sub>2</sub>); N1 (20 mg/L ZnO NPs); N2 (40 mg/L ZnO NPs); N1 + Pb (20 mg/L ZnO NPs + 50 mg/L PbCl<sub>2</sub>); N1 + Pb (40 mg/L ZnO NPs + 50 mg/L PbCl<sub>2</sub>)



Minimum activity of SOD and CAT was recorded in Pb-exposed seedlings; however, application of nanoparticles ameliorated the effect of lead on the seedlings (Fig. 10).

**Fig. 11** Effect of ZnO NPs and PbCl<sub>2</sub> on lipid peroxidation, electrolyte leakage, and proline content in *Vigna radiata*. Data are ± standard error of three independent experiments with three replicates. Bars followed by different letters show significant difference at *P* < 0.05 significant level between treatments according to the Duncan’s multiple range test. Control: Pb (50 mg/L PbCl<sub>2</sub>); N1 (20 mg/L ZnO NPs); N2 (40 mg/L ZnO NPs); N1 + Pb (20 mg/L ZnO NPs + 50 mg/L PbCl<sub>2</sub>); N1 + Pb (40 mg/L ZnO NPs + 50 mg/L PbCl<sub>2</sub>)



## Electrolyte Leakage and Oxidative Stress Variables

Oxidative stress biomarkers, viz. EC, proline, and MDA content, were reported (Fig. 11). The integrity of membrane decreased in plants exposed to  $\text{PbCl}_2$  due to leakage of ions. Seedlings treated with Pb showed an accumulation of 42.8% proline in *Vigna* seedlings. The significant ( $P < 0.05$ ) increase in MDA content 27.3% over control was recorded in the *Vigna* seedlings under the influence of Pb treatment. However, the treatment with the high concentration of ZnO NPs, i.e., N2, decreased the accumulation of MDA content during the experiment. The exposure of plants to N2 treatment declined MDA content and inhibition was 33.3% respectively as compared with non-treated seedlings. Reduction in lipid peroxidation was 16 and 19% when plants were exposed to Pb + N1 and Pb + N2 treatments. Ion leakage enhanced up to 28% in Pb-treated seedlings. However, in N2-treated seedlings, stability of membrane increased significantly ( $P < 0.05$ ) when compared with the control and thus resulted in low ion leakage. In *Vigna*, content of proline was significantly elevated with application of  $\text{PbCl}_2$ .

## Conclusion

The current study investigated the effect of two different concentrations of ZnO NPs with constant concentrations of Pb on *Vigna*. For the sustainable production of commercial crop plants, various practices are in trend to neutralize the harmful impact of abiotic and biotic stresses. The application of NPs proved as an economical way to mitigate the detrimental effects of metal stress on crop plants. It is possible to deduce that higher dosages of ZnO NPs decrease Pb toxicity in *Vigna*. ZnO NPs altered the activity of antioxidant biomarkers and MDA levels in *Vigna*, indicating the induction of oxidative stress that alters plasma-membrane permeability. To assess the positive and negative impacts of nanotechnology in crop fields, further studies are required.

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**Data Availability** Not applicable.

## Declarations

**Ethical Approval** Not applicable.

**Consent to Participate** Not applicable.

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**Competing Interests** The authors declare no competing interests.

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