



# Interaction of zinc oxide nanoparticles with soil: Insights into the chemical and biological properties

Yukti Verma · Satish Kumar Singh · Hanuman Singh Jatav  · Vishnu D. Rajput · Tatiana Minkina

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**Abstract** Widespread use of zinc oxide nanoparticles (ZnO-NPs) threatens soil, plants, terrestrial and aquatic animals. Thus, it is essential to explore the fate and behavior of NPs in soil and also its mechanism of interaction with soil microbial biodiversity to maintain soil health and quality to accomplish essential ecosystem services. With this background, the model experiment was conducted in the greenhouse to study the impact of ZnO-NPs on soil taking maize as a test crop. The X-ray diffraction, Fourier transform infrared spectroscopy, Scanning electron microscopy and Particles size analysis of engineered NPs confirmed that

the material was ZnO-NPs (particle size—65.82 nm). The application of ZnO-NPs resulted in a significant decrease in soil pH. Significantly high EC ( $0.13 \text{ dS m}^{-1}$ ) was recorded where ZnO-NPs were applied at the rate of  $2.5 \text{ mg Zn kg}^{-1}$  soil over control ( $0.12 \text{ dS m}^{-1}$ ). A significant increase in soil available phosphorus was observed on applying ZnO-NPs ( $15.29 \text{ mg kg}^{-1}$  of soil) as compared to control ( $11.84 \text{ mg kg}^{-1}$  of soil). Maximum soil available Zn ( $2.09 \text{ mg kg}^{-1}$ ) was recorded in ZnO-NPs-amended soil ( $T_{11}$ ) which was significantly higher than control ( $0.33 \text{ mg kg}^{-1}$ ) as well as treatments containing conventional zincatic fertilizers. The inhibition rates of dehydrogenase enzyme activity in the presence of 0.5 mg, 1.25 mg and 2.5 mg ZnO-NPs per kg soil were 31.3, 46.2 and 49.7%, respectively. Soil microbial biomass carbon was significantly reduced ( $103.33 \mu\text{g g}^{-1}$  soil) in soils treated with ZnO-NPs over control ( $111.33 \mu\text{g g}^{-1}$  soil). Soil bacterial count was also significantly lesser ( $12.33 \times 10^5$  CFU) in the case where 2.5 mg  $\text{kg}^{-1}$  ZnO-NPs were applied as compared to control ( $21.33 \times 10^5$  CFU). The corresponding decrease in fungal and actinomycetes colony count was 24.16, 37.35, 46.15% and 14.59, 17.97, 22.45% with the application of 0.5 mg, 1.25 mg and 2.5 mg ZnO-NPs per kg soil, respectively, as compared to control. Thus, the use of ZnO-NPs resulted in an increase in soil available Zn but inhibited soil microbial activity.

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Y. Verma · S. K. Singh · H. S. Jatav (✉)  
Department of Soil Science and Agricultural Chemistry,  
Institute of Agricultural Sciences, Banaras Hindu  
University, Varanasi, Uttar Pradesh 221005, India  
e-mail: hanumaniashbhu@gmail.com;  
hsjatav.soils@sknau.ac.in

*Present Address:*

Y. Verma  
ICAR-National Research Centre for Grapes, Pune,  
Maharashtra 412307, India

*Present Address:*

H. S. Jatav  
Department of Soil Science and Agricultural Chemistry,  
Sri Karan Narendra Agriculture University, Jobner,  
Jaipur 303329, India

V. D. Rajput · T. Minkina  
Southern Federal University, Rostov-on-Don, Russia  
344090

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## Introduction

In recent years, metal-based nanomaterials have received a great deal of attention due to their exceptional definite physicochemical properties and multiple potential applications in agriculture, medicine, engineering, food safety, environmental remediation and many others (Du et al., 2019; Malea et al., 2019). Among nanomaterials, zinc oxide nanoparticles (ZnO-NPs) are the third most commonly used metal-based nanoparticles (NPs) with an estimated worldwide annual production ranged between 550 and 33,400 tons (Bondarenko et al., 2013; Connolly et al., 2016; Peng et al., 2017; Rajput et al., 2019). Zinc oxide is considered as a biologically safe material having photocatalysis and photo-oxidizing effects on chemical and biological species (Vaseem et al., 2010; Sirelkhatim et al., 2015). The micronutrient zinc is one of the essential nutrients involved in the synthesis of phytohormone indole acetic acid (IAA) which controls plant growth. It has an indispensable role in many metabolic processes such as chlorophyll synthesis and carbohydrate formation, protein, lipid and nucleic acid synthesis (Vitosh et al., 1994). In addition, zinc (Zn) is the fundamental component of many enzymes and also the only metal to be present in all six enzyme classes, *i.e.*, transferases, lyases, hydrolases, isomerases, oxidoreductases and ligases (Auld, 2001). It facilitates the plants to tolerate lower air temperatures and aids in the biosynthesis of cytochrome pigment which is responsible for maintaining the integrity of the plasma membrane. Improving the Zn content of the plant enables it to deter the toxicity of heavy metals (HMs) like Cd.

Environmental levels of ZnO-NPs have been reported to be in the range of 3.1–31  $\mu\text{g kg}^{-1}$  in soil and 76–760  $\mu\text{g L}^{-1}$  in water (Ghosh et al., 2016; Rajput et al., 2019a). Properties of NPs which play an important role in understanding their behavior when they interact with the environment are size, shape, the surface-to-volume ratio, surface charge, crystalline behavior, porosity percentage, state of agglomeration, purity, redox potential and catalytic activity (Colvin, 2003; Hoet et al., 2004; Royal Society and The Royal

Academy of Engineering, 2004; Lead & Wilkinson, 2006; Powers et al., 2006; Handy & Shaw, 2007; Rajput et al., 2017a). Soil can be considered as an eco-friendly solid matrix containing an appreciable amount of natural colloidal material/NPs. It is also the major sink of environmentally released NPs in comparison with water and the atmosphere (Keller et al., 2013; Rajput et al., 2017a).

The interaction of metal-based NPs with different soil constituents is a complicated phenomenon comprising several physicochemical and biological processes that may affect the ecosystem. The different fate of NPs once released to the soil includes sorption onto soil constituents, runoff and leaching leading to transportation to groundwater and decomposition as well as degradation by numerous biotic and abiotic processes (Boxall et al., 2007). Nanoparticles due to their smaller size and high surface area are likely to be absorbed 15–20 times more as compared to their bulk units (Rajput et al., Rajput, Chaplygin, et al., 2020). The sorption of metals present in NPs onto different soil particles becomes stronger with increasing pH of soil. According to Tourinho et al. (2012), transport of NPs in soil occurs due to Brownian motion and gravitational force. Multifaceted aqueous mediums lead to the stabilization of NPs in high ionic strength, situations that augment aggregation and sedimentation of ZnO-NPs (Peng et al., 2017; Zhou et al., 2015). The ZnO-NPs exhibit low mobility at different ionic strengths (Zhao et al., 2012), and greater sorption compared to ionic  $\text{Zn}^{2+}$ . The properties of soil like pH, texture, organic matter content, soil structure, degree of compactness and soil microbial community play a key role in influencing the bioavailability of metal-based NPs (Fierer & Jackson, 2006).

Several research reports indicate that NPs have positive as well as a negative influence on soil properties such as nutrient content, porosity, organic carbon, changes in humic substances, hydraulic conductivity, enzyme activity and microbial diversity (Ben-Moshe et al., 2013; Rajput et al., 2017b). Therefore, assessing the impact of NPs on the soil is essential in order to preserve the soil and maintain its health and quality to fulfill vital ecosystem services. With this background, the present investigation was undertaken to understand the impact of ZnO-NPs as well as conventional zincatic fertilizers on the chemical and biological properties of soil taking maize as a test crop.

## Materials and method

### Dispersion of zinc oxide nanoparticles

Zinc oxide NPs were purchased from Sigma-Aldrich (mean particle size-65.82 nm) and were dispersed with the help of Ultrasonic Probe Sonicator: Model 200S (200 watts, 24 kHz) at the Department of Physics, Banaras Hindu University (BHU), Varanasi, India. The CAS number of procured ZnO-NPs was 1314-13-2. The device is suited for sample volumes ranging from 0.1 to 1000 mL. The 40 nm sonotrode transmits the ultrasound smoothly across a relatively large surface, and it is therefore suited to support wet sieving processes using very fine mesh sizes.

### X-Ray diffraction (XRD)

The exfoliation of NPs and the changes in the structure was analyzed using XRD analysis. X-ray diffraction was performed at Central Instrumentation Facility, Indian Institute of Technology (IIT), BHU, Varanasi, India. The XRD plots of ZnO-NPs were taken using a Rigaku Miniflex 600 X-ray diffractometer. The sample was scanned at room temperature at a voltage of 45 kV and a current of 15 mA with CuK $\alpha$  radiation ( $\lambda = 1.54 \text{ \AA}$ ). The diffraction angle ( $2\theta$ ) ranged from  $10^\circ$  to  $80^\circ$  with a scan step time of 22.4 s. Sample holder, made up of aluminum, was cleaned with acetone solution, and ZnO-NPs sample was filled in the space defined for specimen. The specimen holder was inserted in the X-ray diffractometer for the analysis of random oriented sample.

### Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared spectroscopy was attained using a Nicolet 5700 instrument, and ZnO-NPs sample was scanned in the region of  $4000\text{--}400 \text{ cm}^{-1}$  using KBr pellets. The measurements were completed in transmittance mode at room temperature having a resolution of  $4 \text{ cm}^{-1}$  in ATR mode. Approximately 100- $\mu\text{m}$ -thick samples were used.

### Scanning electron microscopy (SEM)

Surface morphology and size of ZnO-NPs particles were studied by SEM (Philips XL30 SEM, Netherlands) at 15 kV accelerating voltage and 1000X

magnification. The sample was placed on the sample holder and air-dried for coating. The sample was then vacuum sputtered with gold palladium mixture to improve its conductivity and viewed under SEM.

### Particle size analysis (PSA)

The particle size distribution of sample was measured using VASCO-FLEX nano-particle size analyzer (Cordouan Technologies, France). It works on the principle of dynamic light scattering (DLS) technique. When light is scattered from a solution or a suspension, it undergoes random changes in intensity. This phenomenon helps us to determine the size of the particles that scatter the light. The mean diameter of the particles was calculated from their Brownian motion via the Stokes–Einstein equation.

### Study area description

A greenhouse pot experiment was conducted with maize (variety: Malaviya Hybrid Makka-2) as a test crop. Seeds were procured from Institute Research Farm, Banaras Hindu University (BHU), Varanasi, India. The experiment was carried out in *Kharif* season, June–September 2017 in the Net house of the Department of Soil Science and Agricultural Chemistry of BHU, Varanasi, India, which is situated at an altitude of 80.71 m above mean sea level and located between  $25^\circ 18'$  north latitude and  $80^\circ 36'$  east longitudes. The climate of Varanasi is semiarid to subhumid type having a moisture deficit index in the range of 20–40. Ten kg of soil was filled in each pot and NPK were added to the soil through urea, diammonium phosphate (DAP) and muriate of potash (MOP) at the rate of 120:60:60 kg ha $^{-1}$ .

### Experimental soils and analyses

Typic Haplustept belonging to Inceptisol order (0–30 cm) was collected from the agricultural farm of a farmer named Bachhalal Pandey of village Karsara of Varanasi district, India ( $25^\circ 13' 74'' \text{ N}$   $82^\circ 54' 75'' \text{ E}$ ), for this experimental study. The initial physicochemical properties of experimental soils are given in Table 1.

The pH was determined in 1:2 (soil:water) suspension (Jackson, 1973). The electrical conductivity (EC) was determined in the supernatant liquid of the same

**Table 1** Methodology and some important physicochemical properties of the experimental soils

Parameter	Inceptisol (Varanasi, U.P.)	Reference
pH1:2.5	8.58	Jackson (1973)
EC 1:2.5 (dS m <sup>-1</sup> )	0.13	Jackson (1973)
Soil texture	Clay (%) 15.1 Silt (%) 23.9 Sand (%) 61.0	Bouyoucos (1962)
CaCO <sub>3</sub> %	1.25	Puri (1930)
Organic C (%)	0.59	Walkley and Black (1934)
Available N (kg ha <sup>-1</sup> )	93.29	Subbiah and Asija (1956)
Available P (kg ha <sup>-1</sup> )	12.73	Olsen et al. (1954)
Available K (kg ha <sup>-1</sup> )	99.37	Hanway and Heidel (1952)
Available Zn (mg kg <sup>-1</sup> )	0.39	Lindsay and Norvell (1978)
Available Cu (mg kg <sup>-1</sup> )	2.08	Lindsay and Norvell (1978)
Available Mn (mg kg <sup>-1</sup> )	3.44	Lindsay and Norvell (1978)
Available Fe (mg kg <sup>-1</sup> )	23.71	Lindsay and Norvell (1978)

extract. Bouyoucos hydrometer method was used to determine percent sand, silt and clay in test soil. Calcium carbonate was determined using Puri's method. Soil organic carbon was determined by the wet oxidation method (Walkley & Black, 1934). For determining mineral N (NH<sub>4</sub><sup>+</sup>-N + NO<sub>3</sub><sup>-</sup>-N), 2 M KCl solution was used as an extractant. The soil available P was extracted using Olsen's reagent. The intensity of blue color was measured using a spectrophotometer. Available K in soil was determined by extracting the soil sample with 1 N ammonium acetate and K content in the sample was measured using a flame photometer. The initial experimental soil was silty loam in texture. Micronutrient determination was done by the DTPA extraction method (Lindsay & Norvell, 1978).

#### Treatments details

Total eleven treatment combinations in Inceptisol were replicated thrice in a completely randomized design (CRD). Soil sampling was done at harvest stage of crop. Thus, a total of 33 pots were maintained for this experiment. The treatments were as follows: T<sub>1</sub>-RDF *i.e.*, N:P:K = 120:60:60 kg ha<sup>-1</sup>, T<sub>2</sub>-ZnSO<sub>4</sub>·7H<sub>2</sub>O (2.5 mg Zn kg<sup>-1</sup> soil + RDF), T<sub>3</sub>-ZnSO<sub>4</sub>·7H<sub>2</sub>O (0.5% foliar spray + RDF), T<sub>4</sub>-ZnO (2.5 mg Zn kg<sup>-1</sup> soil + RDF), T<sub>5</sub>-ZnO (0.13% foliar spray + RDF), T<sub>6</sub>-n-ZnO (0.03% foliar spray + RDF), T<sub>7</sub>-n-ZnO (0.08% foliar spray + RDF), T<sub>8</sub>-n-ZnO (0.13% foliar spray + RDF), T<sub>9</sub>-n-ZnO (0.5 mg

Zn kg<sup>-1</sup>soil + RDF), T<sub>10</sub>-n-ZnO (1.25 mg Zn kg<sup>-1</sup> soil + RDF), T<sub>11</sub>-n-ZnO (2.5 mg Zn kg<sup>-1</sup> soil + RDF). Three foliar sprays were applied at 20, 40 and 60 days after seedling emergence.

\*n - ZnO = Zinc oxide nanoparticles

\*RDF = Recommended dose of fertilizers

#### Dehydrogenate enzyme activity (DA)

One gram air-dried soil was taken in 15 ml of airtight screw capped test tube. 0.2 ml of 3% 2, 3, 5-triphenyltiazolium chloride (TTC) was added in each of the tubes to saturate the soil. Then 0.5 ml of 1% glucose solution was added to each tube. The test tubes were kept in an incubator at 28 °C for 24 h. After 24 h, 10 ml of methanol was added to each test tube and tubes were shaken vigorously. The soil in each tube was allowed to stand for 6 h. Clear pink colored supernatant was withdrawn and absorbance was observed at a wavelength of 485 nm in spectrophotometer (Klein et al., 1971). Dehydrogenase activity was expressed in mg triphenylformazan (TPF) produced g<sup>-1</sup> soil h<sup>-1</sup>.

#### Determination of microbial population

Bacterial, fungal and actinomycetes populations in soil were estimated by serial dilution and plate count techniques (Schmidt & Cadwell, 1967). Different

media were used to determine microbial population. Microbial population is presented in Table 2.

The 90 mL of sterilized water was added to a 250-mL Erlenmeyer flask and 10 g of fresh soil was suspended in it, and flasks were shaken on a horizontal shaker for 15 min. The suspension was further diluted to 10<sup>-5</sup> for bacteria and 10<sup>-4</sup> for actinomycetes and fungi. One mL aliquot was inoculated separately into five petri dishes (9 cm diameter), and 20 mL of their respective media was poured in each petri dish. The petri plates were incubated at 27 ± 2 °C in biological oxygen demand (BOD) incubator for 4 days, and developed microbial colonies were counted (Fig. 1) and multiplied with their dilution factor to obtain the microbial population in the soil.

#### Determination of soil microbial biomass carbon

Microbial biomass carbon (MBC) was determined by fumigation extraction method (Vance et al. 1987). Moist field soil sample (50 g) was weighed each in 3 sets, one for complete killing the microbes by fumigation and another for keeping in the refrigerator to predict the normal death rate, last one for the determination of soil moisture content gravimetrically. All the soil samples and 50 mL of purified chloroform were kept in vacuum desiccator. The pump

was switched on and it was kept on until boiling of chloroform, and then it was left for 5 min. After switching off the pump, it was kept in dark for 24 h. Back suction was performed five to six times to ensure the removal of any excess/adheres chloroform vapor. The fumigated and non-fumigated soil samples were taken in 500-mL conical flasks, and 100 mL of 0.5 M K<sub>2</sub>SO<sub>4</sub> (1:5:: soil:extractant) was added in each sample. It was shaken for half an hour, followed by filtration with Whatman No.1 filter paper, and 10 mL of the filtrate was transferred in 500-mL conical flask. Two mL K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> (0.2 N), 10 mL concentrated H<sub>2</sub>SO<sub>4</sub> and 5 mL of orthophosphoric acid were added to each flask. Two blanks were run with 10 mL distilled water each, along with the acids mentioned above. Funnels were kept in every flask (for refluxing), and all these were kept on a hot plate at 100 °C for ½ h. Flasks were taken out and about 250 mL of distilled water was added immediately. The contents were allowed to cool at room temperature. Two to three drops of ferroin indicator was added, and filtration was done against 0.05 N FAS to get a brick-red endpoint. The MBC was expressed in µg g<sup>-1</sup>soil.

$$MB - C(\mu\text{gg}^{-1}\text{soil}) = \frac{(\text{ECF} - \text{ECNF})}{\text{KEC}}$$

**Table 2** Culture media used for enumerating the microbial population

Asparagine Mannitol Agar <sup>a</sup> (for bacteria)		Rose-Bengal Agar <sup>b</sup> (for Fungi)		Kenknight and Munaier's (for actinomycetes)	
Component	Conc. (g L <sup>-1</sup> )	Component	Conc. (g L <sup>-1</sup> )	Component	Conc. (g L <sup>-1</sup> )
K <sub>2</sub> HPO <sub>4</sub>	1	Glucose	10	Dextrose	10
MgSO <sub>4</sub>	0.5	Peptone	5	KH <sub>2</sub> PO <sub>4</sub>	0.1
K <sub>2</sub> HPO <sub>4</sub>	0.2	KH <sub>2</sub> PO <sub>4</sub>	1	NaNO <sub>3</sub>	0.1
CaCl <sub>2</sub>	0.1	MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.05	KCl	0.1
FeCl <sub>3</sub>	0.1	Streptomycin	30	MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.1
KNO <sub>3</sub>	Trace	Agar	15	Agar	15
Asparagine	0.5	Rose-bengal	0.035	Dextrose	10
Mannitol	1	Glucose	10	KH <sub>2</sub> PO <sub>4</sub>	0.1
Agar-agar	15	Peptone	5	NaNO <sub>3</sub>	0.1
Distilled water	1000 mL	Distilled water	1000 mL	Distilled water	1000 mL

<sup>a</sup>(Thornton, 1922); <sup>b</sup>(Martin, 1950)



**Fig. 1** Colonies of bacteria, fungi and actinomycetes

where CF: carbon in fumigated soil, CUF: carbon in unfumigated soil, and KEC: efficiency of extraction (0.45).

#### Statistical analysis

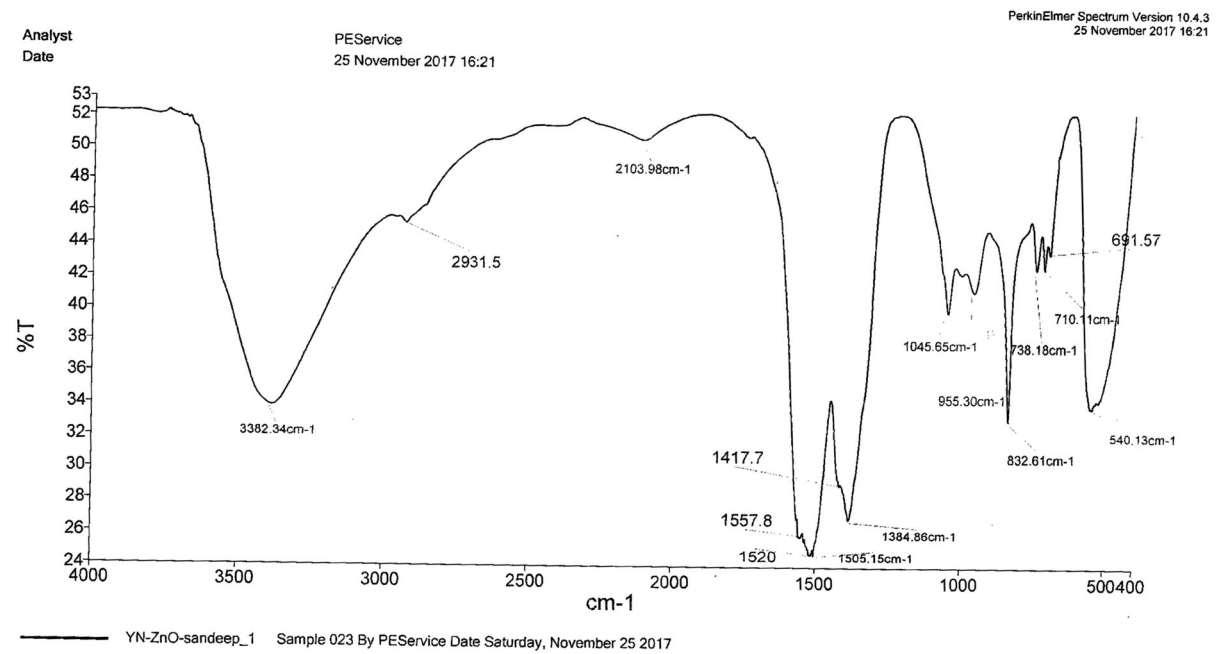
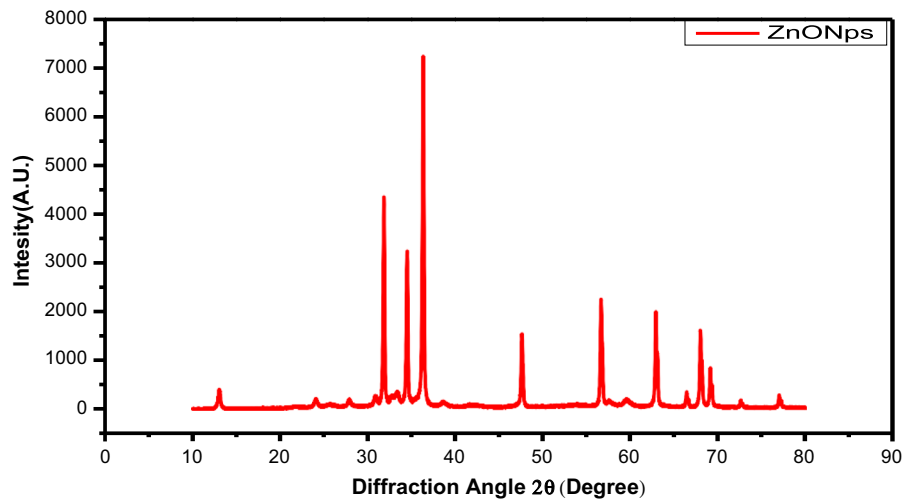
The raw data observed during the pot experiment were put for statistical analysis by following the CRD to draw the valid differences among the treatments using SPSS software. Significance of treatment on soil chemical and biological properties was tested by adopting the procedure for factorial completely randomized design (FCRD) for pot experiment as recommended by Federer (1967). Duncan's multiple range test (Duncan, 1955) was performed to test the significance of the difference between the treatments. All the data presented are an average with standard error (S.E.).

## Results and discussion

### Characterization of zinc oxide nanoparticles

Zinc oxide NPs were characterized by XRD patterns in which the intensity data were collected over a range of  $2\theta = 20\text{--}80^\circ$  using Cu-K $\alpha$  radiation and scan step time of 22.4 s (Fig. 2). The XRD patterns confirmed that the material was ZnO with wurtzite phase and no diffraction peaks were observed other than ZnO. The sharpness, intensity and narrow width of ZnO diffraction peaks in XRD patterns confirmed that the sample is well crystallized. The FTIR spectra of ZnO -NPs exhibited two strong vibrational bands at 540.13 and 832.61  $\text{cm}^{-1}$  assigned to stretching modes of Zn–O. A specific fingerprint was observed at 738.18 and 1045.65  $\text{cm}^{-1}$  (Fig. 3). A characteristic peak at 3382.34  $\text{cm}^{-1}$  was observed due to stretching vibrations of the hydroxyl group (Gavade et al., 2016; Meshram et al., 2017). Due

**Fig. 2** X-Ray diffraction pattern of zinc oxide nanoparticles

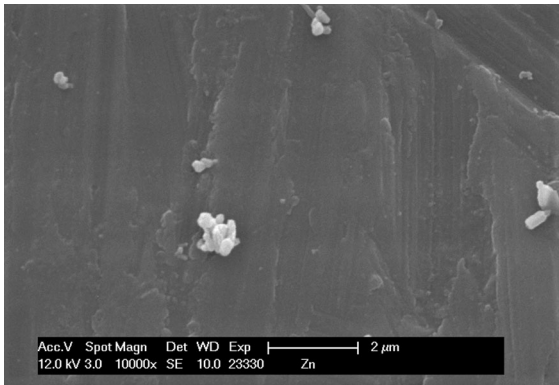


**Fig. 3** FTIR spectra of zinc oxide nanoparticles

to interatomic vibrations, metal oxides generally exhibit absorption bands in the fingerprint region below  $1000\text{ cm}^{-1}$  (Janaki et al., 2015).

Zinc oxide nanoparticles	(wave number, $\text{cm}^{-1}$ )
FTIR peaks	540.13, 691.57, 710.11, 738.18, 832.61, 955.30, 1045.65, 1384.88, 1505.15, 1417.7, 1520, 1557.8, 2931.5, 3382.34

Figure 4 shows SEM image of ZnO-NPs at higher magnification and it also gives clear idea about the particle separation, as it can be seen that the particles are separated smoothly and not highly affected by agglomeration. Figure 5 shows the particle size analysis of ZnO-NPs, and it is also visible that mean particle size is less than 100 nm, *i.e.*, 65.82 nm.



**Fig. 4** Scanning electron microscopy (SEM) micrographs of zinc oxide nanoparticles

### Distribution statistics

Dn 10%: 49.06 nm Dn 50%: 61.83 nm Dn 90%: 85.45 nm.

Mean Size (Number): 65.82 nm.

Solution index: 4

Peak: 1 Mode: 59.03 nm Mean: 65.87 nm Std Dev: 23.91% Intensity: 100%

Effect of zinc oxide nanoparticles on pH, EC, organic carbon and available phosphorus

The initial chemical properties of soil such as pH, EC, organic carbon, soil texture, available macro- and micro-nutrient contents were analyzed before starting the experiment (Table 3). The initial soil exhibited alkali pH (8.58), EC ( $0.13 \text{ dS m}^{-1}$ ), 0.59% OC and low Zn content ( $0.39 \text{ mg kg}^{-1}$ ). The soil as well as the foliar application of ZnO-NPs and concentration of ZnO-NPs, had a significant effect on soil pH, EC and available P (Table 4). A significant decrease in soil pH was observed in ZnO-NPs treatments. The maximum decrease in pH (8.22) was observed in treatment receiving soil application of ZnO-NPs at the rate of  $2.5 \text{ mg Zn kg}^{-1} \text{ soil} + \text{RDF}$  ( $T_{11}$ ) followed by soil application of ZnO-NPs at the rate of  $1.25 \text{ mg Zn kg}^{-1} \text{ soil} + \text{RDF}$  ( $T_{10}$ ) in comparison with control (8.59) at harvest of the crop (Table 3). The soil EC, which provides the measurement of total soluble salts, was significantly affected by ZnO-NPs applications.

Significantly high EC ( $0.13 \text{ dS m}^{-1}$ ) was recorded in  $T_{11}$  (ZnO-NPs at the rate of  $2.5 \text{ mg Zn kg}^{-1} \text{ soil} + \text{RDF}$ ) followed by  $T_{10}$  (ZnO-NPs at the rate of

$1.25 \text{ mg Zn kg}^{-1} \text{ soil} + \text{RDF}$ ) over control ( $0.12 \text{ dS m}^{-1}$ ). Soil organic carbon, an important indicator of soil fertility, was found to be the maximum (0.78%) in  $T_2$  ( $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  at the rate of  $2.5 \text{ mg Zn kg}^{-1} \text{ soil} + \text{RDF}$ ) and the minimum in  $T_{11}$  (ZnO-NPs at the rate of  $2.5 \text{ mg Zn kg}^{-1} \text{ soil} + \text{RDF}$ ), but no significant difference was observed among the treatments (Table 3). This could be attributed to very low microbial activity due to suppression of soil microorganisms in case of NPs application which is responsible for lesser decomposition of soil organic matter resulting in low organic carbon percentage.

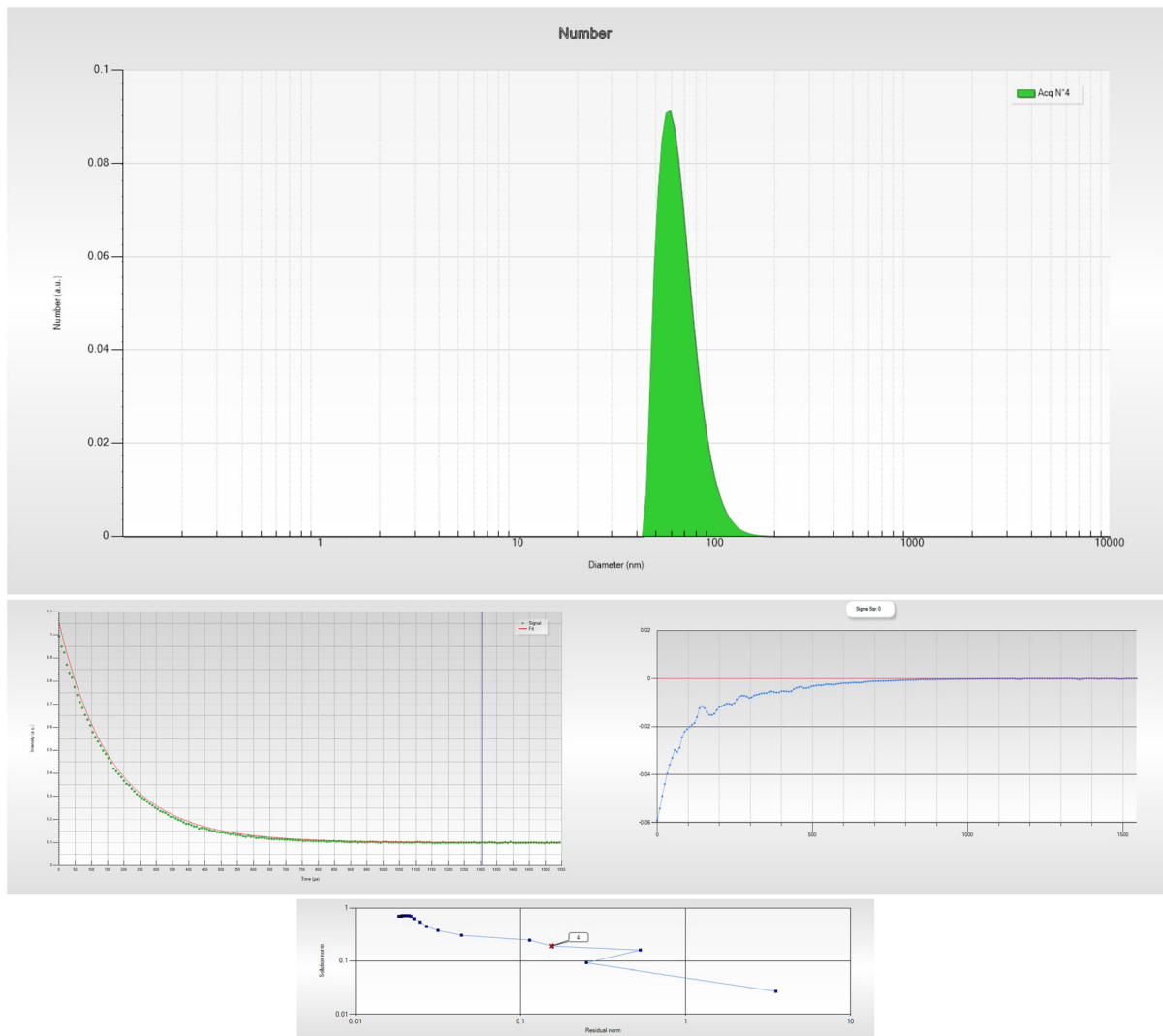
Similar results were reported by Garcia-Gomez et al. (2015) who found a significant decrease in the rate of carbon mineralization when ZnO-NPs were added to natural soils. The chemical properties of soil play a significant role in governing the adsorption-desorption phenomenon, thereby regulating the solubility of Zn in soil. Several published research reports indicate that the source of Zn-based fertilizers and their properties, time and method of application play an important role in affecting the bio-chemical properties of soil (Yuan et al., 2013).

Among the treatments, the maximum soil available phosphorus ( $16.83 \text{ mg kg}^{-1}$ ) was recorded in  $T_2$  ( $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  at the rate of  $2.5 \text{ mg Zn kg}^{-1} \text{ soil} + \text{RDF}$ ) followed by  $T_4$  ( $16.33 \text{ mg kg}^{-1}$ , ZnO at the rate of  $2.5 \text{ mg Zn kg}^{-1} \text{ soil} + \text{RDF}$ ) and  $T_9$  ( $14.18 \text{ mg kg}^{-1}$ , ZnO-NPs at the rate of  $2.5 \text{ mg Zn kg}^{-1} \text{ soil} + \text{RDF}$ ). Since Zn is one of the essential structural components of phytase and phosphatase enzymes which are involved in the native phosphorous mobilization, the application of Zn as  $\text{ZnSO}_4$  or ZnO resulted in more secretion of P-mobilizing enzymes, thus increasing soil available P (Tarafdar & Claassen, 2003). Phosphatases are involved in hydrolyzing esters and anhydrides of phosphoric acid and release  $\text{PO}_4^{3-}$  from immobile organic P. Research reports indicate higher phosphatase enzyme activities due to metals present in the soil that act as the cofactors of the respective enzyme (Rajput, Minkina, et al., 2020; Raliya & Tarafdar, 2013).

Effect of zinc oxide nanoparticles on soil available micronutrients

The method of application and concentration of ZnO-NPs had a significant effect on soil micronutrient contents. Among the different treatments, the highest





**Fig. 5** Particle size analysis of zinc oxide nanoparticles

**Table 3** Effect of zinc oxide nanoparticles on chemical properties of experimental soil

Treatments	pH	EC (Ds m <sup>-1</sup> )	OC (%)	Available P (kg ha <sup>-1</sup> )
T <sub>1</sub>	8.59 ± 0.023 <sup>a</sup>	0.12 ± 0.002 <sup>c</sup>	0.57 ± 0.165 <sup>a</sup>	11.84 ± 0.122 <sup>g</sup>
T <sub>2</sub>	8.33 ± 0.011 <sup>bc</sup>	0.13 ± 0.002 <sup>bc</sup>	0.78 ± 0.107 <sup>a</sup>	16.83 ± 0.20 <sup>a</sup>
T <sub>3</sub>	8.42 ± 0.088 <sup>b</sup>	0.12 ± 0.001 <sup>c</sup>	0.75 ± 0.061 <sup>a</sup>	13.69 ± 0.495 <sup>de</sup>
T <sub>4</sub>	8.30 ± 0.005 <sup>bc</sup>	0.13 ± 0.001 <sup>bc</sup>	0.78 ± 0.107 <sup>a</sup>	16.33 ± 0.282 <sup>a</sup>
T <sub>5</sub>	8.37 ± 0.011 <sup>b</sup>	0.12 ± 0.001 <sup>c</sup>	0.64 ± 0.076 <sup>a</sup>	13.07 ± 0.128 <sup>ef</sup>
T <sub>6</sub>	8.36 ± 0.035 <sup>bc</sup>	0.13 ± 0.001 <sup>bc</sup>	0.57 ± 0.165 <sup>a</sup>	12.96 ± 0.128 <sup>ef</sup>
T <sub>7</sub>	8.33 ± 0.017 <sup>bc</sup>	0.13 ± 0.001 <sup>bc</sup>	0.46 ± 0.203 <sup>a</sup>	12.89 ± 0.036 <sup>f</sup>
T <sub>8</sub>	8.34 ± 0.023 <sup>bc</sup>	0.13 ± 0.001 <sup>bc</sup>	0.44 ± 0.092 <sup>a</sup>	12.86 ± 0.094 <sup>f</sup>
T <sub>9</sub>	8.29 ± 0.012 <sup>bc</sup>	0.13 ± 0.002 <sup>ab</sup>	0.45 ± 0.203 <sup>a</sup>	14.18 ± 0.425 <sup>c</sup>
T10	8.28 ± 0.005 <sup>bc</sup>	0.13 ± 0.002 <sup>a</sup>	0.43 ± 0.218 <sup>a</sup>	14.51 ± 0.184 <sup>c</sup>
T11	8.22 ± 0.104 <sup>c</sup>	0.13 ± 0.001 <sup>a</sup>	0.42 ± 0.197 <sup>a</sup>	15.29 ± 0.234 <sup>b</sup>

Different letters for each parameter show significant difference at  $p < 0.05$  by Duncan's multiple range test

Zn content was observed in T<sub>11</sub> (2.09 mg kg<sup>-1</sup>) followed by T<sub>10</sub> (2.09 mg kg<sup>-1</sup>) which was significantly higher than control (0.33 mg kg<sup>-1</sup>) as well as treatments in which conventional Zn-based fertilizers were applied (Table 4). Similar results were reported by Bala et al. (2019). Similarly, ZnO-NPs had a significant effect on other micronutrients, *i.e.*, Mn, Cu, and Fe.

Among the treatments, the highest Mn and Cu were recorded in T<sub>2</sub> (ZnSO<sub>4</sub>·7H<sub>2</sub>O at the rate of 2.5 mg Zn kg<sup>-1</sup> soil + RDF), as compared to ZnO-NPs treatment. However, the Fe content was highest in ZnO-NPs treated soil (T<sub>11</sub>—22.65 mg kg<sup>-1</sup>) compared to control (T<sub>1</sub>—21.74 mg kg<sup>-1</sup>) as presented in Table 4. Bala et al. (2019) also reported a significant increase in soil Zn content on foliar application of ZnO-NPs. Different concentrations of ZnO-NPs and days after treatments significantly affected other micro-nutrients also, *i.e.*, Cu, Fe and Mn.

#### Effect of zinc oxide nanoparticles on soil microbial activity

Soil DA, MBC and soil microbial counts were significantly reduced in ZnO-NPs treatments indicating their negative effect on the test soil microbial activities (Table 5). The highest DA (3.88 µg TPF g<sup>-1</sup> h<sup>-1</sup>) and MBC (113.33) were recorded for T<sub>2</sub> (ZnSO<sub>4</sub>·7H<sub>2</sub>O at the rate of 2.5 mg Zn kg<sup>-1</sup> soil + RDF). Over the study period of four months, the DA in the soils treated with ZnO-NPs was significantly lower as compared to that in control. The inhibition rates in the presence of 0.5 mg, 1.25 mg and 2.5 mg ZnO-NPs per kg soil were 31.3%, 46.2% and 49.7%,

respectively. A higher application dose (2.5 mg kg<sup>-1</sup> ZnO-NPs) resulted in more drop-in enzymatic activity with respect to the lower application dose (0.5 mg kg<sup>-1</sup> ZnO-NPs), which may be ascribed to higher specific surface area resulting in higher adsorption. Dehydrogenase enzyme falls under oxido-reductase group and is mainly involved in the transfer of protons and electrons from substrates to acceptors. It reflects the oxidative capacities of all living microorganisms and is an essential part of the enzyme system.

Generally, Heavy metals present in metal oxide NPs can affect enzyme activities by interfering with the enzyme substrate complex or with the protein active groups through a variety of changes such as modifications in the proteins conformational configuration and displacements of metals at the active sites (Du et al., 2011; Ge et al., 2011; Hemida et al., 1997; Pan & Yu, 2011; Rahman et al., 2008; Simonin et al., 2015; Xu et al., 2015). Similarly, research reports indicated that metal oxide-based NPs significantly inhibited the catalase, peroxidase and protease enzyme activity in soil after 10 months of application (Reidy et al., 2013). Detrimental effects of metal oxide NPs on microbial activity, abundance and diversity have been proven, even at very low concentrations (< 1 mg kg<sup>-1</sup>). Lethal effects of ZnO-NPs have been witnessed in several *in-vitro* studies of ZnO-NPs having antimicrobial properties (Dinesh et al., 2012; Shen et al., 2015).

The results revealed that the soil MBC was significantly reduced in T<sub>11</sub> (103.33) and T<sub>10</sub> (106.33) as compared to that in the control (111.33). Soil bacterial count was also significantly lesser in

**Table 4** Effect of zinc oxide nanoparticles on DTPA extractable micro-nutrients of experimental soil

Different letters for each parameter show significant difference at  $p < 0.05$  by Duncan's multiple range test

Treatments	Zn (mg kg <sup>-1</sup> )	Cu (mg kg <sup>-1</sup> )	Fe (mg kg <sup>-1</sup> )	Mn (mg kg <sup>-1</sup> )
T <sub>1</sub>	0.33 ± 0.001 <sup>f</sup>	1.31 ± 0.025 <sup>c</sup>	21.74 ± 0.064 <sup>h</sup>	2.12 ± 0.022 <sup>c</sup>
T <sub>2</sub>	1.15 ± 0.023 <sup>d</sup>	1.39 ± 0.009 <sup>a</sup>	24.71 ± 0.029 <sup>e</sup>	2.42 ± 0.035 <sup>a</sup>
T <sub>3</sub>	0.34 ± 0.009 <sup>f</sup>	1.35 ± 0.005 <sup>abc</sup>	22.31 ± 0.158 <sup>g</sup>	2.27 ± 0.138 <sup>abc</sup>
T <sub>4</sub>	1.02 ± 0.009 <sup>e</sup>	1.38 ± 0.004 <sup>a</sup>	24.14 ± 0.025 <sup>d</sup>	2.30 ± 0.031 <sup>ab</sup>
T <sub>5</sub>	0.34 ± 0.013 <sup>f</sup>	1.35 ± 0.001 <sup>abc</sup>	22.54 ± 0.012 <sup>fg</sup>	2.27 ± 0.020 <sup>abc</sup>
T <sub>6</sub>	0.35 ± 0.014 <sup>f</sup>	1.34 ± 0.013 <sup>abc</sup>	22.56 ± 0.029 <sup>fg</sup>	2.24 ± 0.004 <sup>bc</sup>
T <sub>7</sub>	0.36 ± 0.005 <sup>f</sup>	1.34 ± 0.021 <sup>abc</sup>	22.65 ± 0.017 <sup>f</sup>	2.23 ± 0.055 <sup>bc</sup>
T <sub>8</sub>	0.37 ± 0.017 <sup>f</sup>	1.33 ± 0.012 <sup>bc</sup>	23.13 ± 0.042 <sup>e</sup>	2.18 ± 0.007 <sup>bc</sup>
T <sub>9</sub>	1.38 ± 0.012 <sup>c</sup>	1.37 ± 0.001 <sup>ab</sup>	25.07 ± 0.151 <sup>b</sup>	2.29 ± 0.012 <sup>ab</sup>
T <sub>10</sub>	1.73 ± 0.082 <sup>b</sup>	1.37 ± 0.020 <sup>ab</sup>	25.57 ± 0.238 <sup>a</sup>	2.27 ± 0.041 <sup>abc</sup>
T <sub>11</sub>	2.09 ± 0.018 <sup>a</sup>	1.37 ± 0.009 <sup>ab</sup>	25.73 ± 0.072 <sup>a</sup>	2.27 ± 0.021 <sup>abc</sup>

**Table 5** Effect of zinc oxide nanoparticles on soil microbial activity

Treatments	Dehydrogenase ( $\mu\text{g TPF g}^{-1} = \text{soil h}^{-1}$ )	Bacteria ( $\times 10^5$ CFU)	Fungi ( $\times 10^4$ CFU)	Actinomycetes ( $\times 10^4$ CFU)	MBC ( $\mu\text{g g}^{-1}$ soil)
T <sub>1</sub>	3.13 $\pm$ 0.029 <sup>c</sup>	21.33 $\pm$ 1.201 <sup>abc</sup>	30.33 $\pm$ 1.855 <sup>abc</sup>	29.66 $\pm$ 1.201 <sup>abc</sup>	111.33 $\pm$ 2.603 <sup>ab</sup>
T <sub>2</sub>	3.88 $\pm$ 0.038 <sup>a</sup>	23.66 $\pm$ 0.666 <sup>a</sup>	36.33 $\pm$ 1.201 <sup>a</sup>	32.66 $\pm$ 0.666 <sup>a</sup>	113.33 $\pm$ 0.333 <sup>a</sup>
T <sub>3</sub>	2.19 $\pm$ 0.029 <sup>d</sup>	22.33 $\pm$ 1.452 <sup>ab</sup>	30.00 $\pm$ 0.577 <sup>abc</sup>	29.33 $\pm$ 0.881 <sup>abc</sup>	110.66 $\pm$ 0.333 <sup>ab</sup>
T <sub>4</sub>	3.47 $\pm$ 0.064 <sup>b</sup>	23.00 $\pm$ 0.577 <sup>ab</sup>	31.33 $\pm$ 0.666 <sup>ab</sup>	30.33 $\pm$ 2.027 <sup>ab</sup>	113.00 $\pm$ 2.309 <sup>a</sup>
T <sub>5</sub>	2.19 $\pm$ 0.029 <sup>d</sup>	20.66 $\pm$ 0.333 <sup>abcd</sup>	29.00 $\pm$ 0.577 <sup>bcd</sup>	29.66 $\pm$ 1.855 <sup>abc</sup>	110.66 $\pm$ 1.452 <sup>ab</sup>
T <sub>6</sub>	2.20 $\pm$ 0.027 <sup>d</sup>	20.33 $\pm$ 0.882 <sup>abcd</sup>	26.33 $\pm$ 0.881 <sup>cde</sup>	29.00 $\pm$ 1.527 <sup>abc</sup>	110.66 $\pm$ 1.855 <sup>ab</sup>
T <sub>7</sub>	2.19 $\pm$ 0.029 <sup>d</sup>	19.66 $\pm$ 2.027 <sup>cd</sup>	26.66 $\pm$ 0.666 <sup>cde</sup>	27.33 $\pm$ 0.666 <sup>bcd</sup>	110.33 $\pm$ 2.603 <sup>ab</sup>
T <sub>8</sub>	2.20 $\pm$ 0.029 <sup>d</sup>	18.33 $\pm$ 1.452 <sup>cd</sup>	25.66 $\pm$ 0.88 <sup>de</sup>	26.66 $\pm$ 2.333 <sup>bcd</sup>	109.33 $\pm$ 1.452 <sup>ab</sup>
T <sub>9</sub>	2.15 $\pm$ 0.002 <sup>d</sup>	17.66 $\pm$ 1.201 <sup>de</sup>	23.00 $\pm$ 2.08 <sup>e</sup>	25.33 $\pm$ 1.20 <sup>cd</sup>	108.66 $\pm$ 0.666 <sup>ab</sup>
T10	2.14 $\pm$ 0.005 <sup>d</sup>	14.66 $\pm$ 0.666 <sup>ef</sup>	19.00 $\pm$ 1.527 <sup>f</sup>	24.33 $\pm$ 0.333 <sup>d</sup>	106.33 $\pm$ 1.452 <sup>c</sup>
T11	2.09 $\pm$ 0.013 <sup>d</sup>	12.33 $\pm$ 0.333 <sup>f</sup>	16.33 $\pm$ 1.452 <sup>f</sup>	23.00 $\pm$ 0.577 <sup>d</sup>	103.33 $\pm$ 0.333 <sup>c</sup>

Different letters for each parameter show significant difference at  $p < 0.05$  by Duncan’s multiple range test

case of ZnO-NPs treatment at the rate of 0.5 ( $17.66 \times 10^5$  CFU), 1.25 ( $14.66 \times 10^5$  CFU) and 2.5 mg kg<sup>-1</sup> soil ( $12.33 \times 10^5$  CFU) as compared to control ( $21.33 \times 10^5$  CFU) and other zincatic fertilizer treatments, *i.e.*, T<sub>2</sub> ( $23.66 \times 10^5$  CFU) and T<sub>4</sub> ( $23.04 \times 10^5$  CFU). The lesser colony count in NPs-amended soil would also be the reason for lower MBC in this treatment compared to control or conventional zincatic fertilizers. It might be attributed to the oxidative stress induced to the bacteria by ZnO-NPs and not Zn<sup>2+</sup> at the study concentration. In the experimental soil, the level of dissolved Zn<sup>2+</sup> was less than 10 mg kg<sup>-1</sup>, hence the toxicity might be due to ZnO-NPs themselves. According to Raliya and Tarafdar (2013), ZnO-NPs adhered to the bacterial membrane and some of them moved inside the bacterial bodies. More studies are required to elucidate the mechanism of ZnO-NPs interaction with soil microorganisms. Exposure to ZnO-NPs resulted in alterations in cellular morphology, cytoplasm leakage, shock and lysis of the microorganisms.

Zinc has also been validated to decrease the size of the MBC (Renella et al., 2002). The action of ZnO-NPs seems to be species dependent. The complex mechanism behind it is required to be investigated further. Similarly, ZnO-NPs treatment significantly reduced fungal and actinomycetes colony count as compared to control. The inhibition rates in the presence of 0.5 mg, 1.25 mg and 2.5 mg ZnO-NPs per kg soil were 24.16, 37.35 and 46.15% in case of fungi and 14.59, 17.97 and 22.45% in case of actinomycetes. Mandal et al. (2019) observed

significantly lower colony-forming units of heterotrophic bacteria and fungi in NPs-treated soil. A significant decrease in microbial diversity was recorded after 60 days of ZnO-NPs application to soil (Ge et al., 2011; Griffiths & Philippot, 2013). Chai et al. (2015) reported a reduction in number of colonies of Azotobacter, K-solubilizing and P-solubilizing bacteria as well as inhibition in enzymatic activities such as catalase, urease and fluorescein diacetate hydrolysis activity. Zinc oxide NPs were also found to be toxic to *Escherichia coli* (gram-negative) and gram-Staphylococcus aureus (gram-positive) bacteria (Reddy et al., 2007). Zinc oxide NPs are extensively used in environmental remediation, and its antimicrobial properties have been demonstrated on bacteria such as *B. subtilis*, *E. coli*, *P. fluorescens*, *S. aureus* and *S. typhimurium*, as well as on the fungi *A. flavus* and *A. fumigates* (Gajjar et al., 2009; Kairyte et al., 2013; Navale et al., 2015; Manzoor et al., 2016; Ahmed et al., 2017). Environmental parameters mostly affect the rate of chemical transformation of NPs, and depending on whether the toxicity caused by NPs to microbes is due to direct or indirect interaction with the cells, the fate governs the effect of NPs on soil microbial diversity (Reidy et al., 2013).

### Conclusion and future prospects

The results of our experiment indicated that exposure of soil to ZnO-NPs led to slight decrease in pH and organic carbon. However, EC and soil available P

increased in presence of ZnO-NPs as compared to control. The application of ZnO-NPs to soil increased the soil available Zn and Fe as compared to control and conventional zincatic fertilizers. A decrease in DA, MBC and microbial counts was recorded on treating the soil with NPs. Since the present examination was carried out in pot, the real scenario may be dissimilar under the field condition, where climatic as well as several other factors may vary and interact in a complex way. Moreover, it is essential to conduct additional research regarding long-term effects of ZnO-NPs on soil and primary producers before depicting a comprehensive conclusion about the impact of metal oxide-based NPs on soil chemical and biological properties.

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**Data availability** The data are from our conducted field experiment, and all the methods are used mentioned in the manuscript.

#### Declarations

**Conflict of interest** The authors declare no conflict of interest.

**Ethical approval** All the authors have been agreed to submit it.

**Consent to participate** Before the submission of paper, all the authors have given the consent to publish.

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