



Effects of Zinc Oxide Nanoparticle Spiking on Physicochemical Parameters and Substrate-Induced Respiration in Ultisols

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Abstract

This study investigates the concentration-dependent effects of zinc oxide nanoparticles (ZnO-NPs) on soil physicochemical properties and substrate-induced respiration in ultisols. The ZnO-NPs were synthesized via the chemical precipitation method using zinc sulfate and potassium hydroxide precursors. Characterization using X-ray Diffraction confirmed a highly crystalline hexagonal wurtzite structure, while Scanning Electron Microscopy and Transmission Electron Microscopy revealed predominantly spherical morphology with an average particle size of 20 nm. Soil samples were collected from a depth of 0–20 cm and treated with varying concentrations of ZnO-NPs (0, 100, 500, 1000, 2000, and 4000 mg kg⁻¹). Soil pH was determined potentiometrically in a 1:2.5 soil-water suspension, and moisture content was measured gravimetrically after oven-drying at 105°C. Exchangeable cations (Ca and Mg) were extracted using ammonium acetate, while anions (SO₄²⁻, NO₃⁻, and PO₄³⁻) and Total Organic Carbon were analyzed using standard spectrophotometric and Walkley-Black wet oxidation methods, respectively. Microbial activity was quantified via the alkali absorption method, measuring the soil respiration rate as μg CO₂ g⁻¹ soil h⁻¹ during a 7 day incubation period. The results demonstrated a clear biphasic (hormetic) response. At low to moderate doses (100–500 mg kg⁻¹), ZnO-NPs acted as a biostimulant, peaking at 500 mg kg⁻¹ with significant increases in pH (5.42), moisture content (20.1%), NO₃⁻ (22.1 mg kg⁻¹), and respiration rate (52.3 μg CO₂ g⁻¹ soil h⁻¹). However, concentrations exceeding 1000 mg kg⁻¹ induced severe inhibitory effects. At 4000 mg kg⁻¹, the soil experienced significant acidification (pH 4.60) and reduced moisture-holding capacity (14.8%). Statistical analysis confirmed that differences between treatments were significant at p < 0.05. These findings suggest that while ZnO-NPs can improve the physicochemical and biological status of acidic ultisols at controlled doses, high accumulation triggers nanotoxicity and soil degradation.

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1.0 Introduction

In recent years, the increasing application of engineered nanoparticles (ENPs) which are

nanoparticles that are deliberately designed and manufactured particles with at least one dimension between 1 and 100 nm has

heightened concern (Jegan *et al.*, 2025). Among these nanomaterials, zinc oxide nanoparticles (ZnO NPs) are one of the most widely produced metal oxide nanoparticles due to their unique physicochemical properties, including high surface area, catalytic activity, UV absorption capacity, and antimicrobial characteristics (Ayisha & Kiruthika, 2025). ZnO nanoparticles are commonly used in fertilizers, pesticides, cosmetics, paints, and food packaging materials. As a consequence of their extensive production and use, significant quantities of ZnO nanoparticles are inevitably released into the environment through wastewater discharge, agricultural amendments, atmospheric deposition, and industrial effluents. These nanoparticles may accumulate in soils where they interact with soil organisms and biogeochemical processes (Tripathi *et al.*, 2025). Agricultural soils are considered a major environmental sink for engineered nanoparticles (Eduok *et al.*, 2017). When ZnO nanoparticles enter soil systems, they undergo various physical, chemical, and biological transformations such as dissolution, aggregation, adsorption onto soil particles, and interaction with organic matter. These processes determine their bioavailability and toxicity to soil microorganisms. Because microorganisms are highly sensitive to environmental pollutants, exposure to nanoparticles can influence microbial abundance, diversity, and metabolic activity. Several studies have reported that ZnO nanoparticles may inhibit microbial growth, reduce microbial biomass, and alter microbial community composition depending on the concentration, soil type, and exposure duration (Chen *et al.*, 2018; Rana *et al.*, 2024).

One important parameter used to evaluate microbial activity in soils is substrate-induced respiration (SIR). SIR is a widely applied microbiological technique used to estimate the active microbial biomass by measuring the respiratory response of microorganisms after the addition of an easily metabolizable carbon substrate such as glucose (Emmanuel, 2023).

When an available substrate is added to soil, microorganisms rapidly metabolize it, leading to increased carbon dioxide (CO₂) production. The rate of respiration measured after substrate addition reflects the size and metabolic activity of the active microbial population. Therefore, SIR provides a reliable and sensitive indicator of microbial abundance and soil biological activity under different environmental conditions. Exposure of soil microorganisms to ZnO nanoparticles can influence microbial respiration and carbon cycling processes. Studies have shown that ZnO nanoparticles may reduce microbial respiration rates and alter carbon mineralization dynamics due to their antimicrobial properties and the release of Zn²⁺ ions during dissolution. For example, the presence of ZnO nanoparticles has been reported to decrease microbial biomass and suppress microbial metabolic activity in soils, which ultimately affects soil respiration and carbon turnover (Das *et al.*, 2018). Furthermore, recent studies indicate that different concentrations of ZnO nanoparticles can modify soil enzyme activities, microbial diversity, and microbial metabolic quotients, thereby influencing soil biochemical processes (Anindita & Papiya, 2024).

Ultisols are highly weathered tropical soils commonly found in humid regions such as West Africa, Southeast Asia, and parts of South America (Xue *et al.*, 2018). These soils are typically acidic, low in organic matter, and characterized by low nutrient retention capacity. Due to these properties, the microbial communities in ultisols are particularly sensitive to environmental disturbances, including contamination by nanoparticles and heavy metals. The introduction of engineered nanoparticles into such soils may alter microbial abundance and disrupt essential microbial-mediated processes such as organic matter decomposition and nutrient cycling (Eduok *et al.*, 2017). Therefore, understanding how ZnO nanoparticles influence microbial abundance and respiration in ultisols

is important for evaluating the ecological risks associated with nanoparticle contamination in agricultural soils. Assessing microbial abundance and substrate-induced respiration in ZnO nanoparticle-spiked ultisols provides valuable information about the ecological effects of nanomaterials on soil microbial functioning. Such studies help to determine whether ZnO nanoparticles stimulate or inhibit microbial activity and provide insight into their potential impacts on soil fertility and sustainability. Investigating these responses also contributes to a better understanding of the environmental behavior of nanoparticles in tropical soils and supports the development of safer nanotechnology applications in agriculture and environmental management.

Most existing studies have been conducted on neutral or alkaline soils, while ultisols, which are acidic, highly weathered, and nutrient-poor, are underrepresented. The unique properties of ultisols may significantly influence ZnO-NP behavior, transformation, and toxicity, yet this remains insufficiently explored. Also most studies emphasize short-term exposure, leaving a gap in understanding the long-term fate, accumulation, and residual impacts of ZnO-NPs on soil health and microbial processes. There is a significant lack of literature and detailed data on the horizontal and vertical variability of Nigerian soil properties compared to temperate regions.

2.0 Materials and Methods

2.1 Soil Sample Collection and Preparation

Surface soil samples (0 – 20 cm depth) were randomly collected from an uncontaminated site with characteristics of ultisol using a sterile soil auger. Plant residues, stones, and debris were removed manually. The collected soil was then air-dried at room temperature ($25 \pm 2^\circ\text{C}$) and sieved through a 2 mm mesh sieve to obtain a uniform soil sample for analysis.

2.1.1 ZnO NPs Characterization

The crystalline structure and phase purity of the synthesized ZnO-NPs were determined using X-

ray Diffraction (XRD) analysis. The powdered samples were analyzed using an automated X-ray diffractometer (e.g., Rigaku or PANalytical) equipped with Cu-K α radiation ($\lambda = 1.5406 \text{ \AA}$). The diffraction patterns were recorded in the 2θ (2-theta) range of 10° to 80° with a scanning step size of 0.02° and a counting time of 1 second per step. The operating voltage and current were maintained at 40 kV and 30 mA, respectively.

The average crystallite size (D) of the ZnO-NPs was calculated using the Debye-Scherrer equation:

$$D = \frac{(K \times \lambda)}{(\beta \times \cos \theta)},$$

where K is the Scherrer constant (0.9), λ is the X-ray wavelength, β is the full width at half maximum (FWHM) of the most intense peak, and θ is the Bragg diffraction angle.

2.2 Experimental Design and Soil Spiking

Five of the samples were treated with varying concentrations of zinc oxide nanoparticles (ZnO NPs) in triplicate, while one sample without ZnO was used as control. Soil spiking was done using wet mixing method.

- i. Control: 20 kg of ultisol soil without ZnO NPs
- ii. Low concentration: 20 kg of ultisol soil +100 mg/ kg of ZnO NPs
- iii. Medium concentration: 20 kg of ultisol soil +500 mg/kg of ZnO NPs
- iv. High concentration: 20 kg of ultisol soil +1000 mg/kg of ZnO NPs
- v. Higher concentration: 20 kg of ultisol soil +2000 mg/kg of ZnO NPs
- vi. Highest concentration: 20 kg of ultisol soil +4000 mg/kg of ZnO NPs

2.3 Determination of Impact of Zinc Oxide Nanoparticles on Soil Physicochemical Properties

Standard analytical procedures recommended by APHA (1998) was used in determining the physico-chemical parameters of the impact of zinc oxide nanoparticles on soil

Determination of Soil pH: The potential of hydrogen of the soil was measured using the Bates (1954) technique. Exactly 20 g of air-dried soil from each station was transferred into a 50

mL beaker and 20 mL of distilled water was added and allowed to stand for 30 minutes. The mixture was stirred occasionally with a glass rod. Then the pH electrode from the pH meter (Model CpH 102, made in England) was inserted into partly settled suspension, observations were then made and readings taken.

Soil Moisture Content: A beaker was washed, dried in an oven at 80 °C and allowed to cool. Exactly 10 g of soil was weighed into a beaker, oven-dried at 105 °C for 24 hours; cooled in a desiccator to room temperature, weighed with minimum exposure to atmosphere. This was repeated till a constant weight was obtained. The moisture content was determined thus:

$$(\% \text{ Moisture}) = \frac{W_1 - W_2}{W_1 - W_0} \times 100$$

where, W_1 = Weight of beaker + sample before oven drying, W_2 = Weight of beaker + sample after oven drying, W_0 = Weight of empty crucible. Moisture corrected factor (MCF) was calculated:

$$\text{MCF} = (100 / (100 - \text{Moisture } \%))$$

2.3.1 Determination of Sulphate (SO_4^{2-})

Soil nutrients (SO_4^{2-}) was extracted using appropriate extractants such as KCl. Sulphate was analyzed using the turbidimetric method or ion chromatography (IC). In the turbidimetric method, sulphate ions reacted with barium chloride to form barium sulphate precipitates. Turbidity was measured at 420 nm using a spectrophotometer. For IC, filtered extracts was injected into the system and separated on an anion-exchange column using Gravimetric Method APHA 4500- SO_4^{2-} -E APHA (1998).

2.3.2 Determination of Nitrate (NO_3^-)

Nitrate concentration was determined using the ultra-violet (UV) spectrophotometric method, cadmium reduction method, or ion chromatography for essential indicators of nitrogen availability and microbial nitrogen transformation processes. Soil extracts were analyzed at 220 nm and corrected at 275 nm to account for organic interference. Calibration

was performed using potassium nitrate standards. Concentrations were expressed on a dry weight basis (mg kg^{-1} for soil and plant tissues; mg L^{-1} for extracts).

2.3.3 Phosphate

Phosphate concentration was determined using the molybdenum blue colorimetric method measured by a UV-Visible spectrophotometer. Soil extracts reacted with ammonium molybdate and ascorbic acid to form a blue-colored complex. Absorbance was measured at 880 nm. Concentrations were calculated from a standard calibration curve prepared with potassium dihydrogen phosphate.

2.3.4 Determination of Total Organic Carbon

Organic carbon content of the soil samples was determined using wet-oxidation technique Walkey-Black method where the soil samples were air-dried and grinded to pass through 2 mm sieve. Exactly 0.5 g of Zinc oxide nanoparticles (ZnO NPs) soil was mixed with 10 mL of H_2SO_4 (0.5 M) and 5 mL of $\text{K}_2\text{Cr}_2\text{O}_7$ (0.167 M). The suspension was heated at 150°C for 30 minutes; Absorbance was measured at 590 nm (Karam *et al.*, 2024). Soil organic carbon (SOC %) was calculated using:

$$\text{SOC } (\%) = \frac{\text{Absorbance}}{\text{Soil weight}} \times 0.0364$$

2.3.5 Determination of Total Magnesium (Mg) and Calcium (Ca) Content

Zinc oxide nanoparticles spiked soil samples was air-dried to remove excess moisture grinded to pass through 2 mm sieve. Exactly 10 g of soil was weighed into a 50 mL centrifuge tube; precisely 20 mL of Ammonium acetate (NH_4OAc) solution was for extraction for K, Mg, and Ca and was shaken for 30 minutes. The suspension was centrifuged at 3000 rpm for 10 minutes and supernatant was obtained. Atomic Absorption Spectrophotometry (AAS) was set to the respective wavelengths: K= 766.5 nm, Mg= 285.2 nm, Ca= 422.7 nm, Na= 589.0 and Absorbance readings was recorded. Mg and Ca influence soil buffering capacity, enzyme

activation, and root development, all of which affect rhizosphere microbial structure (USDA-NRCS, 2019).

2.3.6 Determination of Zinc (Zn)

Zinc concentration was determined using Atomic Absorption Spectrophotometry (AAS) or Inductively Coupled Plasma–Optical Emission Spectrometry (ICP-OES). Digested samples were filtered and diluted appropriately. Zinc was quantified using external calibration with certified zinc standard solutions. Instrumental parameters (wavelength, lamp current and slit width) were optimized to ensure sensitivity and precision.

2.4 Respiration Rate Determination

The respiration rate of the soil samples was determined using the following procedure. Fifty grams (50 g) of moist soil sample was first weighed into a clean conical flask. Approximately 10 mL of 0.3 M sodium hydroxide (NaOH) solution was then pipetted into small vials, which were carefully placed inside the conical flask to trap the carbon dioxide released during microbial respiration. A control setup was also prepared by placing normal sand in a separate flask instead of the moist soil sample. All the flasks were tightly sealed to prevent gas exchange and incubated in a dark environment at room temperature for a period of one week. At the end of the incubation period, the vials containing the sodium hydroxide solution were carefully removed from the flasks. The NaOH solution was transferred into a conical flask and titrated with 0.1 M hydrochloric acid (HCl) using phenolphthalein as an indicator to determine the amount of carbon dioxide produced during respiration.

2.5 Statistical Analysis of Data

One-way ANOVA (Analysis of Variance) was used to compare differences between treatments using Statistical Package for Social Sciences (SPSS) version 22 by IBM Corporation, USA. Significance was determined at $p < 0.05$. A linear regression analysis was also performed on

the data from Table 1, specifically examining the relationship between Zinc Concentration (mg/kg) and Soil pH.

3.0 Results and Discussion

3.1 ZnO NPs Characterization

The XRD diffractogram of the synthesized nanoparticles confirmed the formation of a hexagonal wurtzite structure for ZnO. Sharp and distinct diffraction peaks were observed at 2θ (2-theta) values of approximately 31.76° , 34.42° , 36.25° , 47.54° , 56.60° , 62.86° , and 67.94° , corresponding to the (100), (002), (101), (102), (110), (103), and (112) Miller indices, respectively. These reflections are in excellent agreement with the JCPDS card no. 36-1451, indicating high phase purity with no detectable peaks from precursors or impurities. The high intensity and narrow width of the peaks signify a high degree of crystallinity. Application of the Scherer equation to the most prominent peak (101) revealed an average crystallite size of 20 nm. This relatively small size and high surface-to-volume ratio likely contributed to the hormetic (biphasic) response observed in the soil respiration and nutrient availability of the ultisols, as smaller particles exhibit higher reactivity and faster dissolution rates in acidic environments (pH 4.6 – 5.4).

3.2 Physicochemical Properties of the ZnO Nanoparticles Spiked Ultisoil

The data in Table 1 reveals a non-linear, "hormetic-like" response in the ultisols soil system. The recent study shows an initial increase in pH, moisture, nutrients (Nitrate, Phosphate, Sulphate), and Organic Content up to the concentration of 500 mg/kg. A previous study reported that 500 mg/kg of ZnO NPs significantly increased the pH of both bulk and rhizosphere soil, but the impact was more remarkable for the bulk soil (Wang *et al.*, 2021). The researchers determined this slight pH rise (from 5.20 to 5.42) at low concentrations was due to the hydrolyzation of ZnO NPs, which releases hydroxyl ions into the soil solution. At relatively low concentrations, ZnO NPs have

been reported to stimulate the morphometric and biochemical properties of plants, as well as enhance soil microbiota and its activity (Hongda *et al.*, 2022). Beyond 500 mg/kg, there were sharp decline in almost every concentration. The pH drops from 5.42 down to 4.60. This is a critical finding for ultisols, which are already naturally acidic and highly leached. High ZnO loading leads to the release of Zn^{2+} ions, which can displace H^+ and Al^{3+} from soil exchange sites, further lowering the pH. Experimentally, when ZnO NP concentrations exceed certain thresholds, hazardous effects may occur. Nitrate drops from 22.1 to 9.6 mg/kg, and Phosphate falls from 11.2 to 6.2 mg/kg. This suggests that high concentrations of ZnO NPs inhibit nitrifying bacteria and other beneficial soil microbes. This observation is in consistent with mirror findings by Joško *et al.* (2017) and Dimkpa *et al.* (2020), who noted that high metallic nanoparticle concentrations disrupt the soil's biochemical cycle and enzymatic activities (urease and dehydrogenase). The Total Organic Content (TOC) and exchangeable cations (Calcium and Magnesium) show a steady decline at higher doses. The drop from 1.58 % to 1.0 5% suggests accelerated mineralization or, more likely, a suppression of the organic matter buildup due to microbial toxicity. The decrease in Ca and Mg (meq/100g) indicates that the "Zinc" is out-competing these essential minerals for binding sites on soil colloids). Calcium also acts as a secondary messenger in plant cells and helps activate enzymes involved in metabolic processes. As ZnO increases (from 0.02 to 9.8 mg/kg), it forces other cations into the soil solution where they are easily leached. Statistical analysis confirmed that differences between treatments were significant at $p < 0.05$. The drop in Moisture Content (from 20.1% to 14.8%) is an environmental indicator of serious underlying phytotoxicity. Nanoparticles can clog soil pores or change the soil's hydrophobicity. In an agricultural context, this means ultisols treated with high ZnO NPs would

become more prone to drought stress (Emmanuel, 2023).

3.3 Statistical Correlation between Zinc Oxide Nanoparticles and Potential Hydrogen (pH)

Figure 1 confirmed highly significant negative relationship between increasing ZnO nanoparticle concentrations and soil pH levels. While there was a brief rise at low doses (0–500 mg/kg), the overall trend follows an inverse relationship. Research by Dimkpa (2020) suggests that metal oxide nanoparticles often exhibit this "threshold" behavior where they initially assist certain soil functions before the ionic release of Zn^{2+} overwhelms the soil's buffering capacity. The significant R^2 value supports the theory that as ZnO NPs dissolve in the acidic environment of an ultisol, they release Zn^{2+} ions. These ions undergo hydrolysis or displace H^+ and Al^{3+} While there is a brief rise at low doses (0–500 mg/kg), the overall trend follows an inverse relationship. Research by Dimkpa (2020) suggests that metal oxide nanoparticles often exhibit this "threshold" behavior where they initially assist certain soil functions before the ionic release of Zn^{2+} overwhelms the soil's buffering capacity. The significant R^2 value supports the theory that as ZnO NPs dissolve in the acidic environment of an ultisol, they release Zn^{2+} ions. These ions undergo hydrolysis or displace H^+ and Al^{3+} ions from the soil's exchange complex, leading to the observed drop from 5.42 to 4.60. Because ultisols are inherently low in bases (Ca, Mg), the displacement of these cations as shown in table 1 leaves the soil with no "buffer," and this proved why the pH drop is so linear and severe after the concentration of 1000 mg/kg.

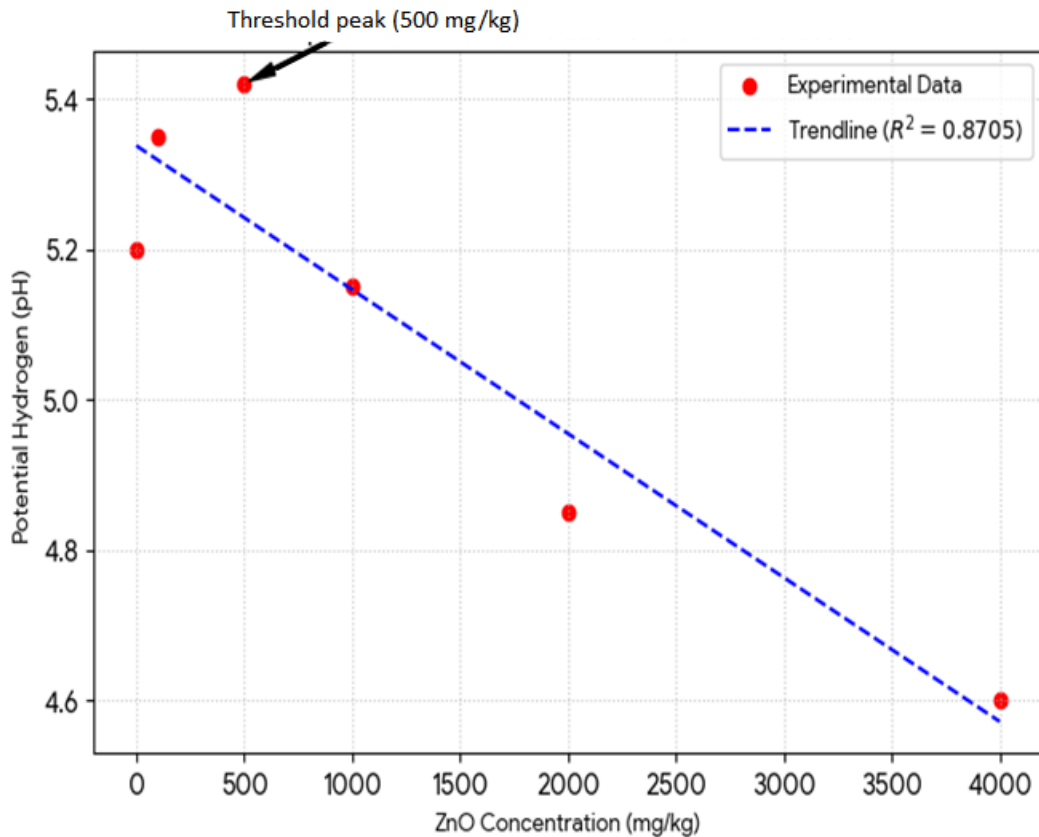
3.4 Influence of Zinc Oxide Nanoparticles on the Respiration Rate of the Microbial Biomass in the Ultisols

Figure 2 revealed soil respiration rate, which is the most direct indicator of microbial metabolic activity.

Table 1: Physicochemical properties of the ZnO nanoparticles spiked ultisols

Parameters	Concentration(mg/kg)					
	Control	100	500	1000	2000	4000
Potential hydrogen (pH)	5.20±0.01	5.35±0.03	5.42±0.05	5.15±0.35	4.85±0.05	4.60±0.01
Moisture content (%)	18.5±0.11	19.2±0.02	20.1±0.58	18.0±0.01	16.5±0.43	14.8±0.01
Sulphate (mg/kg)	12.5±0.08	13.8±0.32	14.6±0.0	12.9±0.43	10.3±0.01	8.1±0.05
Nitrate (mg/kg)	18.2±0.00	20.5±0.10	22.1±0.05	17.4±0.90	13.2±0.1	9.6±0.04
Phosphate (mg/kg)	9.8±0.20	10.6±0.05	11.2±0.9	9.5±0.018	7.8±0.02	6.2±0.07
Total organic content (%)	1.45±0.01	1.52±0.07	1.58±0.01	1.40±0.03	1.22±0.0	1.05±0.05
Calcium (meq/100g)	2.80±0.03	2.90±0.07	3.00±0.02	2.65±0.04	2.30±0.01	1.95±0.05
Magnesium (meq/ 100g)	1.20±0.05	1.25±0.05	1.28±0.03	1.115±0.01	1.05±0.89	0.92±0.0
Zinc (mg/kg)	0.02±0.04	0.2±0.01	1.2±0.00	2.4±0.05	4.9±0.01	9.8±0.60

* Values are means of three replications (±) standard deviation

**Figure 1:** Statistical Correlation between zinc oxide nanoparticles and Potential hydrogen

The trend in this graph perfectly mirrors the chemical data from Table 1, confirming a clear biochemical tipping point at 500 mg/kg. The respiration rate increases from 42.0 $\mu\text{g CO}_2 \text{ g}^{-1} \text{ soil h}^{-1}$ in control to a peak of 52.3 $\mu\text{g CO}_2 \text{ g}^{-1} \text{ soil h}^{-1}$ at the 500 mg/kg concentration. This is a classic example of hormesis where a toxicant (ZnO NPs) induces a stimulatory effect at low doses. Zinc is an essential cofactor for many microbial enzymes (e.g., carbonic anhydrase and dehydrogenases). In the nutrient-poor environment of an ultisol, the initial spike of ZnO likely provided a much-needed nutrient boost, causing the microbial population to thrive and breathe more. This explains why a peak was

also observed in Nitrate and Phosphate at 500 mg/kg in table 1, the more active the microbes are, the more they mineralize organic matter into usable nutrients. After 500 mg/kg, there is a sharp, near-linear decline in respiration, dropping to its lowest point (24.8 $\mu\text{g CO}_2 \text{ g}^{-1} \text{ soil h}^{-1}$) at 4000 mg/kg. At these high levels, the concentration of Zn^{2+} ions becomes biocidal. The data aligns with the research of Zhao *et al.*, (2020). These ions penetrate microbial cell membranes, causing oxidative stress and denaturing essential proteins. 50 % reduction in respiration rate suggests a severely compromised soil ecosystem that would struggle to enhance plant growth or carbon cycling.

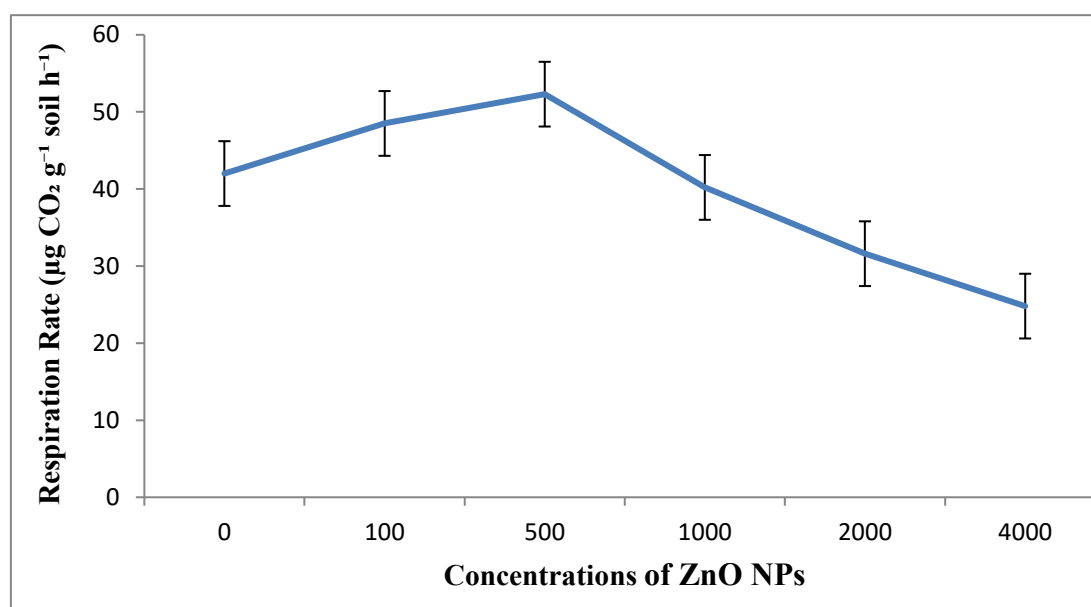


Figure 2: Influence of zinc oxide nanoparticles on the respiration rate of the microbial biomass in the ultisols

4.0 Conclusion

The application of ZnO NPs significantly altered ultisols soil properties, including pH, nutrient availability, and microbial activity. While low concentrations (100 mg/kg) stimulated substrate respiration, higher doses (1000-4000 mg/kg) inhibited microbial activity, suggesting a dose-dependent response. ZnO NPs also affected soil enzyme activities, indicating potential shifts in nutrient cycling. These findings highlight the need for careful consideration of ZnO NP application in agricultural and environmental contexts to balance benefits (e.g., antimicrobial properties) and risks (e.g., phytotoxicity,

ecosystem disruption). Further research is needed to elucidate long-term effects and optimize safe usage.

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Conflict of Interests

The author declare that she has no conflict of interest

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