



Impact of Zinc Oxide Nanoparticles Amended Organic Manure on *Arachis hypogaea* Growth Response and Rhizosphere Bacterial Community

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Authors' contributions

This work was carried out in collaboration among all authors. Authors PO and SE designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors BI and OJ managed the analyses of the study. Author IB managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The effect of zinc oxide nanoparticle-organic manure amended ultisol and loam soils on plant growth response and rhizosphere bacterial community of peanut (*Arachis hypogaea*) was evaluated using standard methods under greenhouse conditions. Results indicate germination rates ranged between 30 and 100% in the amended soils compared to 50 and 70% in the controls. ZnO nanoparticles exerted concentration-dependent and varying effects on the plant root and shoot lengths, weights, nodules and pod formation in the two soil types. Heterotrophic bacterial counts ranged from 7.21 ± 0.51 to 7.38 ± 0.5 $\text{Log}_{10}\text{CFUg}^{-1}$ in the amended ultisol and 6.99 ± 0.55 $\text{Log}_{10}\text{CFUg}^{-1}$ in the control with a log reduction to 6.70 ± 0.39 $\text{Log}_{10}\text{CFUg}^{-1}$ in 500 mgkg^{-1} ZnO spiked soil. Counts in the amended loam soil ranged between 6.59 ± 0.48 and 7.22 ± 0.41 $\text{Log}_{10}\text{CFUg}^{-1}$ relative to 6.80 ± 0.58 $\text{Log}_{10}\text{CFUg}^{-1}$ in the control. ZnO induced concentration-dependent effect on oxygen uptake rate relative to the controls. The organisms were members of

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the genera *Lactobacillus*, *Pseudomonas*, *Bacillus*, *Rhizobium*, *Xanthobacter*, *Enterobacter*, *Citrobacter*, *Nitrosomonas* and *Agromyces*. ZnO nanoparticle exerted concentration-dependent stimulatory and inhibitory effects on the plant growth response, oxygen uptake rate and induced temporal shifts in soil microbial abundance. It is challenging to generalize a consistent response of the plant or microorganisms because ZnO nanoparticles interacted with *A. hypogaea* and soil bacterial community in ways that differ in the ultisol and loam soil.

Keywords: ZnO nanoparticles; ultisol; loam soil; *Arachis hypogaea*.

1. INTRODUCTION

The development of nanotechnology and interest to incorporate engineered nanoparticles (ENPs) into consumer products over the last decade is associated with concerns for human and environmental health. The increased concern arises from potential toxic implications of ENPs released into the environment with varying effects on microbial-dependent processes [1,2] ENPs are designed and synthesized with specific properties to impart special functionalities on consumer products and include enhanced thermal and electrical conductivity, high sorption capacity and photocatalytic reactions [3].

Environmental input of ENPs includes the use of nano-enabled fertilizers, plant protection products applied directly to the land, disposal of nano waste and excretion of nanomedicines in veterinary products, wastewater effluents used for irrigation [4], synthesis and improper disposal of nano waste, sorption to biosolids and transport into wastewater treatment plants [5]. In the soil, ENPs aggregate with the naturally occurring nanoparticles, undergo redox reactions, dissolution, exchange of surface moieties, and reactions with biomacromolecules [6]. ENPs are transported to different parts of plants and exert a toxic effect by altering morpho-anatomical, physiological, biochemical and genetic constitutions which affects their productivity [7]. However, ENPs are also associated with positive effects on plants, for example increased germination and enhanced growth rate [8].

ENPs such as zinc oxide (ZnO) nanoparticles are implicated in reduced microbial biomass, altered soil bacterial community diversity and composition especially at higher concentrations [9,10]. It is common knowledge that the legumes harbour nitrogen-fixers such as *Rhizobium* in their nodules, therefore, it is important to know the effect of ENPs on the plant nodulation and the associated symbiotic bacteria. ENPs can accumulate in roots and leaves of plants [11], which are typical parts of the plant in association

with the symbiotic bacteria. We evaluated the influence of ZnO nanoparticles on peanut (*Arachis hypogaea*) growth response using indicators such as germination rate, shoot and root length, nodulation and pod formation, microbial abundance and respiration rate. Here, we provide comparative insights into the area of limited empirical data using the peanut and the associated rhizosphere bacterial community as a model in tropical ultisol and loam soils.

2. MATERIALS AND METHODS

2.1 Soil Sample Collection and Preparation

The ultisol and loam soil were excavated from the University of Uyo Farm, Nwaniba Road, Uyo and transported to the greenhouse. The soils were mixed with organic manure 14 kg of soil and 6 kg of organic manure (poultry dropping) placed into seven (7) wooden troughs (30 cm x 30 cm) lined with polyethene in duplicate. The soil samples and organic manure were homogenized and moistened by adding 300 mL. The wetting process was repeated once a week for three weeks. Planting was done in 28 troughs containing ultisol and loam soil under greenhouse conditions. Twenty-one days after soil preparation, fresh peanut (*Arachis hypogaea*) was seeded into each of the troughs at 3 cm depth with 8 cm distance from each seed.

The zinc oxide (ZnO, 99%, 30 nm) nanoparticles were purchased from Nanostructured & Amorphous Materials Inc. (Texas, USA), the properties were provided by the manufacturer and used without further characterization. Choice of ZnO was based on the wide application in a variety of consumer products. Before planting, the soil was spiked with ZnO nanoparticles and repeated 30 days after germination of the groundnut seeds to achieve chronic dose application with a total of 100, 400, 1000, 2000, 4000 mg kg⁻¹ of ZnO nanoparticles for each treatment.

2.2 Measurement of Plant Growth Response

The number of sprouted seeds on Day 8 was recorded and the germination rate determined using the formula: No of germinating seed/total seeds planted x 100. Shoot length was measured weekly from ground node to tip of the shoot using a meter rule and root length measured after harvesting. At ten (10) weeks, the plants were harvested by uprooting and the number of pods and nodules produced by the plants in each of the treatments were counted using a hand lens. The fresh weight and dry weight of the roots were measured using the gravimetric method.

2.3 Measurement of Oxygen Uptake Rate (OUR)

The OUR was measured by acid titration technique [12]. Briefly, fifty grams of moist ultisol and loam soil from each treatment in duplicates were placed in respiration flasks and 10 mL of approximately 0.3M sodium hydroxide (NaOH) solution in vials were suspended inside the flasks and sealed with a bung. The set-up was placed in the dark for 5 days at room temperature. Thereafter, the NaOH solution in each of the respiration flasks was transferred to 250 mL conical flasks and the vial was rinsed with distilled water. 10 mL of barium chloride (BaCl) and six drops of phenolphthalein was added to the conical flasks. This was titrated with 0.1M hydrochloric (HCl) acid until the colour changed from red to colourless. The titration value was used to calculate the OUR by the formula: Respiration rate = Mass of respiring soil x titration value x time (seconds).

2.4 Characterization and Identification of Bacteria

At maturity, the plants were uprooted and rhizosphere soil was obtained by carefully shaking the root to release the soil particles into a clean sample container. Bulk soil samples were collected by the use of hand trowel. One gram each of the rhizosphere and bulk soil samples was suspended in 9 mL of sterile water and vigorously shaken and ten-fold serial dilutions carried out. Precisely 1.0 mL aliquot from dilutions 10^{-5} and 10^{-6} were used for total heterotrophic bacterial counts. The dilutions were inoculated in duplicates using the pour-plate technique on Nutrient Agar (Oxoid, UK),

incubated at $28 \pm 2^\circ\text{C}$ for 24 hours and discrete colonies enumerated.

The bacterial isolates were characterized based on their cultural and morphological attributes and response to standard biochemical tests as described by [13]. Twenty-four hours old bacterial cultures were subjected to Gram's staining and biochemical tests such as catalase, citrate utilization, motility, spore stain, indole, urease, methyl red, Vogues Proskauer and sugar fermentation. The characteristics of the isolates were compared with those of known taxa using Bergey's Manual of Determinative Bacteriology [14].

2.5 Statistical Analysis

The data were subjected to analysis of variance (ANOVA) and Kruskal Wallis test on log-transformed data using Statistical Package for the Social Science (SPSS version 20.0, IBM Corp, USA). Results are presented as mean \pm standard deviation with levels of significance maintained at 95% for each test.

3. RESULTS AND DISCUSSION

3.1 Effect on Plant Growth Response

The effect of ZnO nanoparticles on germination rates differed across two soil types and concentrations. The germination rate of *A. hypogea* in the control ultisol and 100 mg kg^{-1} amendment was 60% compared to 30% in the 400, 500 and 1000 mg kg^{-1} , 80% in the 2000 mg kg^{-1} and 40% in the 4000 mg kg^{-1} (Fig. 1). The germination rate in the control soil was higher than the 400, 500, 1000 and 4000 mg kg^{-1} amended ultisol with a difference of 1.6 to 2.5, whereas the 2000 mg kg^{-1} amendment was however 1.2 times higher than that of the control. The germination rate of plants in control loam soil was 50% compared to 40% in 1000, 2000 and 4000 mg kg^{-1} amendments (Fig. 1). In the 100, 400, and 500 mg kg^{-1} concentrations, germination rate ranged from 60 to 100% and indicate 1.2 to 2 times higher outcome than the control. The differences in the germination rate of the control plants and the treatments were significant at $p = .05$. ZnO stimulated the germination rates at low concentrations with the reverse outcome at higher concentrations compared to the control. Similarly reduced germination rates on the exposure of *Cier arietinum* L. to 100 and 1000 ppm ZnO

nanoparticles have been reported [15]. The ZnO nanoparticles probably generated reactive anions which influenced oxygen and water uptake required for germination [16].

3.2 Effect on the Shoot and Root Length of *A. hypogaea*

The shoot length in 400 mg kg⁻¹ treatment was 43.17 ± 3.88 cm compared to 42.75 ± 4.02 cm in the control ultisol (Fig. 2a). In the 100, 500 to 4000 mg kg⁻¹ concentrations, shoot length ranged from 36 ± 3.1 cm to 41.8 ± 6.23 cm and denotes 1.02 to 1.19 times longer shoot in the control than the treatments. The root length of the plant in 4000 mg kg⁻¹ treatment was 18 ± 5.9 cm compared to 13.25 ± 1.91 cm in the control ultisol (Fig. 2a) and indicates 1.36 times longer root length than the control. However, the 100 to 2000 mg kg⁻¹ amendments ranged from 7.6 ± 1.71 to 11.5 ± 3.81 cm and suggests a difference of 1.2 to 1.74 times higher root lengths in the control. The differences in the root and shoot length of the plants in control ultisol and the treatments were significant at p = .05.

The mean shoot length of plants in the control loam soil was 33.83 ± 2.21 cm compared to 38 ± 0.0 and 35.57 ± 4.86 cm in the 100 and 400 mg kg⁻¹ ZnO amendments respectively (Fig. 2b) indicating 1.12 and 1.05 higher plant shoot length than in the control. In the 500 to 4000 mg kg⁻¹ ZnO amendments, shoot length ranged from 28 ± 8.19 to 32.33 ± 2.21 cm and represents 1.05 to 1.31 times longer plant shoot length in the control. In 500 mg kg⁻¹ ZnO amendments, root length of *A. hypogaea* was 12.67 ± 2.73 cm and 12.33 ± 3.55 cm in the control (Fig. 2b). The results indicate that the root length for the 500 mg kg⁻¹ treatment was 1.03 times longer than the control. With a mean root length that ranged from 9.67 ± 4.13 cm to 11.67 ± 4.24 cm in the 100 to 4000 mg kg⁻¹ ZnO nanoparticles amendments, the plant in the control soil was 1.06 to 1.28 times longer in length. The differences in the shoot and root length relative to the control were significant at p = .05. ZnO nanoparticles exerted a concentration-dependent stimulatory and inhibitory effect on the shoot and root length of *A. hypogaea* grown in the ultisol and loam soils. In a related study, ZnO nanoparticles induced elongation of the root length and shoot length of peanut at 400 to 2000 ppm in red sandy loam soil [17]. The results suggest that the concentration, soil properties and type of plant are factors that influence the outcomes of nanoparticle contact with plants.

3.3 Effect on Weights of *A. hypogaea* Roots

The mean wet weight of the plant roots in the control ultisol was 2.67 ± 0.65 g relative to 1.04 ± 1.83 g and 2.54 ± 0.75 g in the 100 and 500 mg kg⁻¹ ZnO amendments (Fig. 3a) indicating 2.57 and 1.05 times higher wet weight in the control than the respective amendments. The wet weight of the plant in 400, 1000, 2000 and 4000 mg kg⁻¹ amendments were 4.80 ± 3.24 g, 5.18 ± 3.20 g, 3.36 ± 2.71 g and 9.39 ± 3.68 g respectively with a difference that ranged between 2.44 to 9.02 compared to the control. The mean dry weight of the plant root from the control ultisol was 0.87 ± 0.57 g (Fig. 3a) and 0.42 ± 0.21 g in the 100 mg kg⁻¹ ZnO amendment indicates the control weighed 2.07 times higher than the plants in 100 mg kg⁻¹ amendment. The dry weight of the plant roots from the 400, 500, 1000, 2000 and 4000 mg kg⁻¹ amendments were 4.80 ± 1.38 g, 2.54 ± 0.27 g, 5.18 ± 1.17 g, 3.36 ± 1.14 g and 9.39 ± 1.42 g. These weights were higher than the control with a difference that ranged between 1.21 to 4.54. The differences in the wet and dry weights of the plant root about the control were significant at p = .05.

The mean wet weight of the plant root in the control loam soil was 2.91 ± 4.38 g and 1.21 ± 0.5 g, 1.13 ± 0.5 g, 1.10 ± 0.5 g, 1.55 ± 0.5 g, 2.12 ± 1.85 g and 1.71 ± 0.5 g in the 100, 400, 500, 1000, 2000 and 4000 mg kg⁻¹ ZnO amendments respectively (Fig. 3b). The wet weight of the plant root in control loam soil was higher than that of plants exposed to ZnO with a difference that ranged from 1.70 to 2.65. The mean dry weight of the of the plant root in the control loam soil was 1.18 ± 1.72 g in relation to 0.41 ± 0.09 g, 0.45 ± 0.09 g, 0.36 ± 0.19 g, 0.61 ± 0.52 g, 0.67 ± 0.12 g and 0.64 ± 1.72 g in the 100 to 4000 mg kg⁻¹ ZnO amendments respectively (Fig. 3b). The results indicate that the dry weight of the plant root in the control loam soil was 1.76 to 3.28 times higher than those exposed to ZnO nanoparticles. The differences between the mean wet and dry weights of plant root in the control compared to the ZnO amendments were significant at p = .05.

About the plant biomass, the results suggest that 100 and 500 mg kg⁻¹ ZnO caused a decrease in the wet and dry weight of the root in the amended ultisol compared to the control. However, higher concentrations of 1000 to 4000 mg kg⁻¹ exerted a positive effect on the wet and

dry weights compared to the control (Fig. 3a). In contrast, there was reduced wet and dry weights of plants in the ZnO nanoparticle amended loam soil (Fig. 3b). The results indicate that lower concentration of ZnO nanoparticles reduced plant biomass in the ultisol whereas higher concentrations increased the plant biomass. ZnO nanoparticles negatively impacted plant growth leading to low plant biomass in the loam soil. A reduction in wheat biomass exposed to 5 and 10 g kg⁻¹ ZnO and TiO₂ nanoparticles respectively [18] and increase in plant biomass of cluster bean on exposure to 10 mg L⁻¹ of ZnO nanoparticles [19] corroborate the findings in this study.

3.4 Influence on the Formation of Pods and Nodules

The mean number of pods produced by *A. hypogaea* in the control ultisol was 1.5 ± 1.07 compared to 1.0 ± 0.42 in 100 mg kg⁻¹ ZnO amendments (Fig. 4a). However, the number of pods from the plants in 400, 500, 1000, 2000 and 4000 mg kg⁻¹ ZnO amendments were 1.67 ± 1.4, 1.67 ± 0.5, 3.86 ± 1.6, 2.8 ± 2.5, and 7 ± 3.29 respectively. These values were higher than the plants in the control with a difference that ranged from 1.67 to 3.86. ZnO exerted a stimulatory effect on the number of pods produced by the plants and the difference was significant at p = .05. Nodules produced by *A. hypogaea* in the control ultisol was 1.0 ± 0.46 and none in the 100 mg kg⁻¹ and 2000 mg kg⁻¹ ZnO amendments (Fig. 4a). The number of nodules produced by the plants in the ultisol

amended with 400, 500, 1000 and 4000 mg kg⁻¹ were 2 ± 2.26, 30.33 ± 12.08, 2.57 ± 2.26 and 21.25 ± 9.69 respectively and were higher than the control ultisol with a difference that ranged from 2 to 30.33 which was significant at p = .05.

The mean number of pods produced by *A. hypogaea* in the control loam soil was 1.67 ± 2.34 and 1 ± 0.51 to 1.33 ± 0.55 in the ZnO amended soils (Fig. 4b). The number of pods produced by the plant in the control loam soil was 1 to 1.21 times higher than those in the treatments and significant at p = .05. Absence of nodules was observed in all the plants exposed to ZnO and the control loam soil. The results were consistent with the study by [17], in which higher concentrations of 1000 and 2000 ppm ZnO nanoparticles increased number of pods produced by peanuts and was inhibited at 400 ppm. Further to this, the result suggests that the loam soil was not deficient in nitrogenous substances for plant uptake. Plant growth response indicates that the ZnO nanoparticles exerted pronounced concentration and soil type-dependent effects on *A. hypogaea* probably through uptake and accumulation. Uptake and accumulation of ZnO nanoparticles by plants have been reported elsewhere to exert toxic effects [20]. The absence of nodulation in the loam soil is attributed to *A. hypogaea* negatively regulating nodule formation due to availability of 0.075% nitrate content compared to 0.05% in ultisol. Legumes regulate their nodule number, size and nitrogen fixation activity in environments with adequate nitrogen [21].

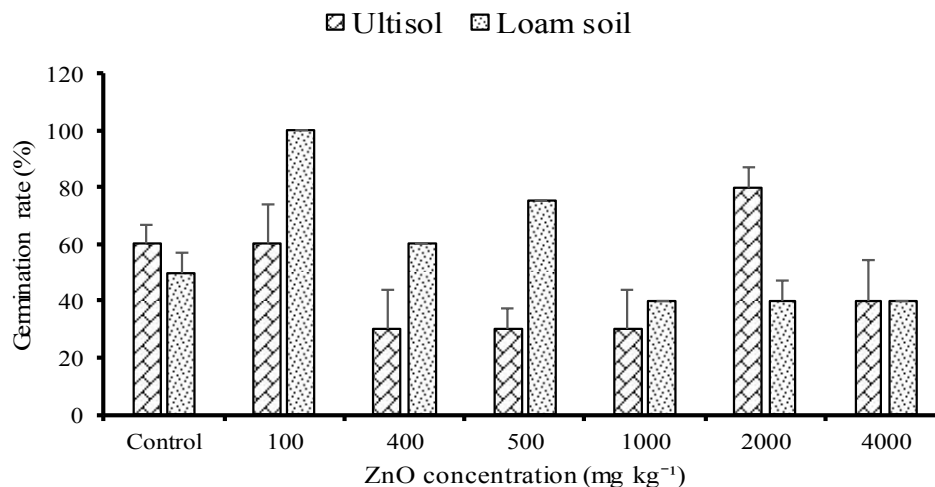


Fig. 1. Effect of ZnO nanoparticles on germination rates of *A. hypogaea*

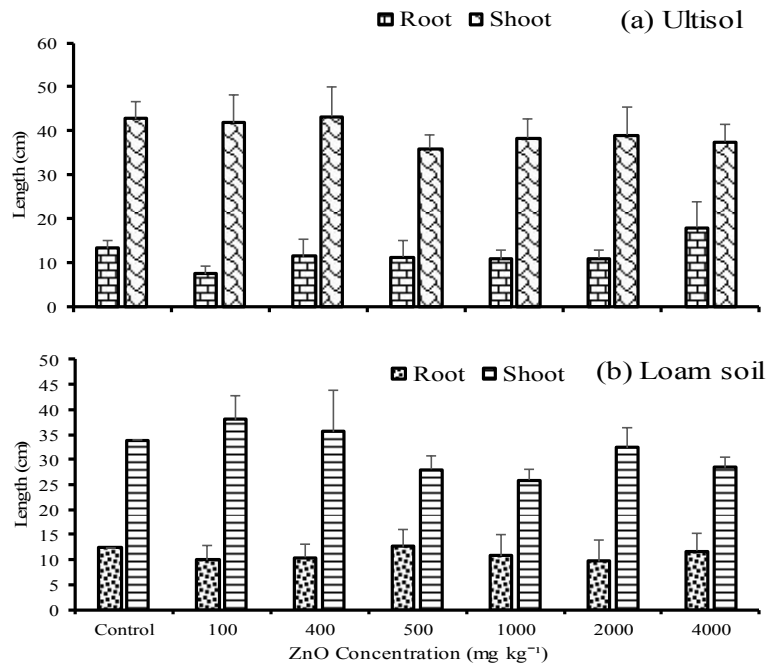


Fig. 2. Effect of ZnO nanoparticles on root and shoot lengths of *A. hypogaea*

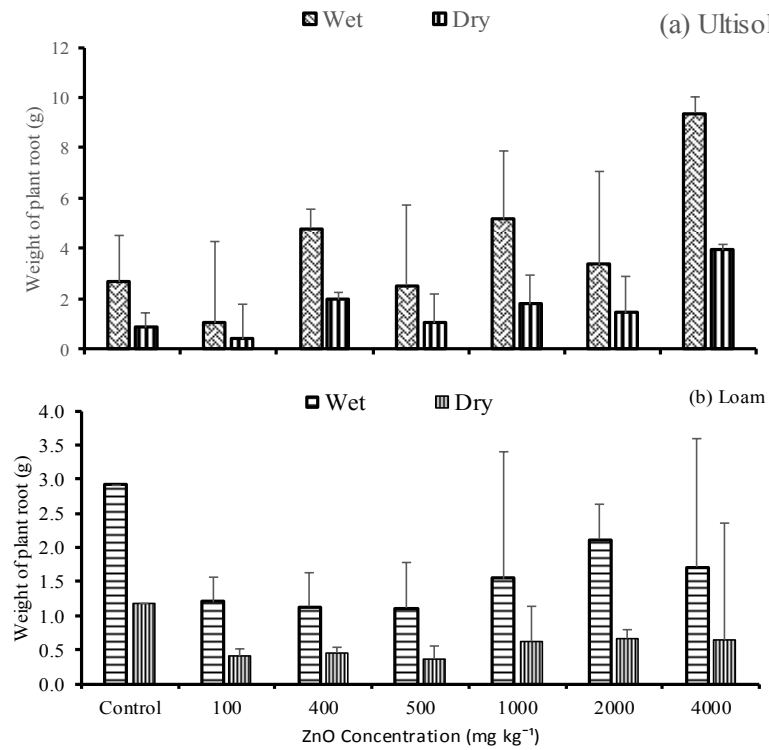


Fig. 3. Effect of ZnO nanoparticle on wet and dry weights of *A. hypogaea* roots

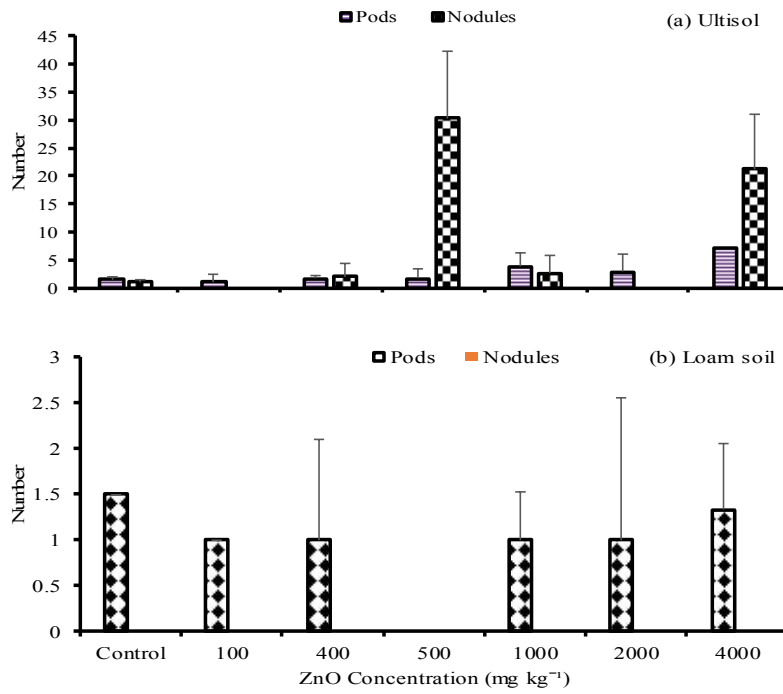


Fig. 4. Influence of zinc oxide nanoparticles on pod formation and nodulation by *A. hypogaea*

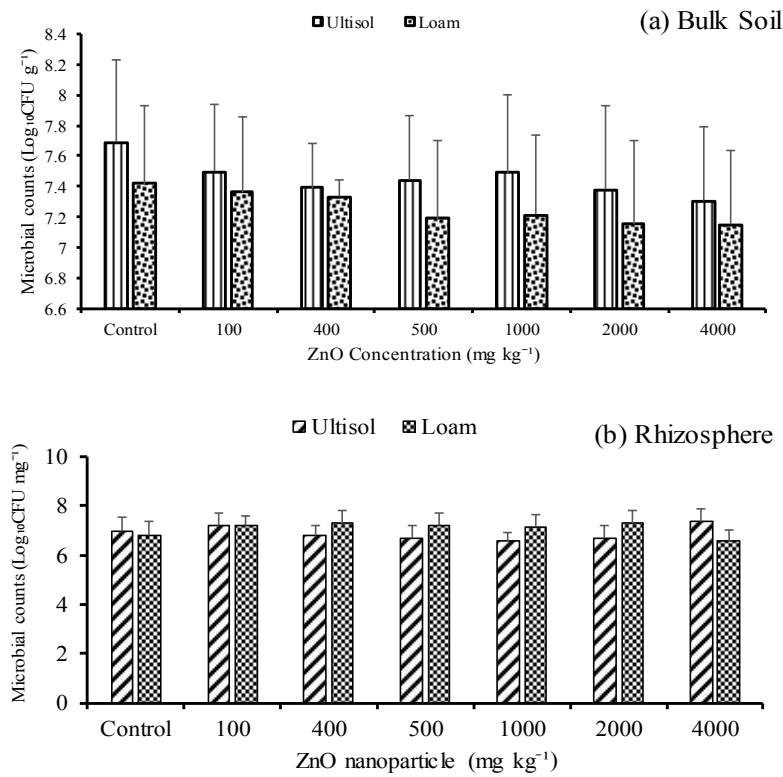


Fig. 5. Effects of ZnO nanoparticles on the heterotrophic bacterial abundance in the bulk and *A. hypogaea* rhizosphere

3.5 Effects on Heterotrophic Bacterial Abundance

Bacterial abundance varied across the soil type influenced by the different concentrations of ZnO nanoparticles. The counts of heterotrophic bacteria in the bulk ultisol (control) was $7.69 \pm 0.54 \text{ Log}_{10}\text{CFU g}^{-1}$ compared to 7.49 ± 0.44 , 7.39 ± 0.29 , 7.44 ± 0.43 , 7.49 ± 0.42 , 7.37 ± 0.51 and $7.30 \pm 0.55 \text{ Log}_{10}\text{CFU g}^{-1}$ in the 100, 400, 500, 1000, 2000 and 4000 mg kg^{-1} zinc oxide nanoparticles amended ultisols respectively (Fig. 5a). The results indicate 1.03 to 1.05 higher abundance in the control than the amended soils and the difference was however significant at $p = 0.05$. The mean counts in the control loam soil was $7.41 \pm 0.51 \text{ Log}_{10}\text{CFU g}^{-1}$ relative to 7.36 ± 0.49 , 7.33 ± 0.41 , 7.19 ± 0.51 , 7.21 ± 0.52 , 7.15 ± 0.54 and $7.15 \pm 0.48 \text{ Log}_{10}\text{CFU g}^{-1}$ in the 100 to 4000 mg kg^{-1} ZnO amended soil respectively (Fig. 5). The difference between the control and amended soil was low and ranged from 1.01 to 1.04 but significant at $p = 0.05$.

The counts in the bulk soil indicate that ZnO nanoparticles reduced the bacterial community abundance in the amended ultisol and loam soil (Fig. 5a). The result is consistent with other studies in which ZnO nanoparticles caused in a decrease in abundance of soil bacterial community [22], and bacterial load in plate counts of soil samples [23]. However, the effect of ZnO nanoparticles on rhizosphere bacterial abundance was concentration and soil type dependent. For instance, in the ultisol, ZnO nanoparticles stimulated growth and increased bacterial abundance at 100, 400, 2000 and 4000 mg kg^{-1} , whereas bacterial population was reduced at 500 and 1000 mg kg^{-1} . In the loam soil, 100 to 1000 mg kg^{-1} ZnO increased bacterial abundance, whereas 2000 and 4000 mg kg^{-1} ZnO nanoparticles inhibited the population density. ENPs such as ZnO and silver oxide are known to exert concentration-dependent inhibitory effects on soil bacterial, fungal and nitrifying communities by the release of zinc ions (Zn^{2+}) to induce oxidative stress, cell membrane damage and cytoplasmic leakage [24]. The effect was pronounced in the rhizosphere than the bulk soil and the reasons are unclear at the moment, but suspected to be caused by adsorption and accumulation of ZnO nanoparticles by root exudates.

The THB count in the *A. hypogaea* rhizosphere of the control ultisol was $7.00 \pm 0.55 \text{ Log}_{10}\text{CFU g}^{-1}$ whereas 6.83 ± 0.29 , 6.70 ± 0.37 , 6.55 ± 0.59

and $6.72 \pm 0.41 \text{ Log}_{10}\text{CFU g}^{-1}$ in the 400, 500, 1000 and 2000 mg kg^{-1} were recorded in ZnO amended ultisol respectively. The results indicate 1.02 to 1.07 higher counts in the control than the 400 to 2000 mg kg^{-1} amended ultisol (Fig. 5b). However, the 100 and 4000 mg kg^{-1} ZnO amended soils had counts of 7.21 ± 0.51 and $7.28 \pm 0.30 \text{ Log}_{10}\text{CFU g}^{-1}$ which were higher than the control with a difference of 1.03 and 1.05 respectively. The THB abundance in the *A. hypogaea* rhizosphere of control loam soil was $6.80 \pm 0.58 \text{ Log}_{10}\text{CFU g}^{-1}$ and $6.59 \pm 0.48 \text{ Log}_{10}\text{CFU g}^{-1}$ in 4000 mg kg^{-1} ZnO amended soil (Fig. 5b) and indicates 1.03 times higher than the 4000 mg kg^{-1} ZnO amendment. Counts of 7.21 ± 0.51 , 7.33 ± 0.11 , 7.22 ± 0.41 , 7.12 ± 0.43 and $7.34 \pm 0.28 \text{ Log}_{10}\text{CFU g}^{-1}$ were recorded for the 100, 400, 500, 1000, 2000 mg kg^{-1} ZnO amendments respectively with 1.06 to 1.09 times higher than the control. The differences in the control and amended soils were significant at $p = 0.05$

The rhizosphere is a dynamic habitat with different interactions with microorganisms associated with the release of plant exudates and growth-stimulating substances by microorganisms. However, altered environmental condition of the rhizosphere enhances or reduce the release of plant roots exudates with a corresponding stimulatory or inhibitory effect on rhizosphere microbial population [25]. About the control ultisol, the bacterial abundance in the rhizosphere increased at 100, 400, 2000 and 4000 mg kg^{-1} , but reduced at 500 and 1000 mg kg^{-1} ZnO amendments (Fig. 5b). In the loam soil, 100 to 1000 mg kg^{-1} amendments increased bacterial abundance, whereas 2000 and 4000 mg kg^{-1} ZnO nanoparticles reduced the population. The concentration-dependent effect on the bacterial abundance indicates an interference of ZnO nanoparticles with the production of root exudates by *A. hypogaea*. Also, hormesis, the biphasic growth response indicated by low dose stimulation and high dose inhibition [26] is the probable mechanism for the stimulatory effect on the bacterial abundance at low ZnO concentrations. It is possible that the plant root, clay fraction and organic matter in the two soil types and associated physicochemical properties interacted with the ZnO nanoparticles to either mitigate or enhance the effect on soil rhizosphere bacterial community. The influence of organic matter and clay content in soils is implicated in reduced ZnO nanoparticles toxic effects on soil microbial community [27]. However, in most of the *A. hypogaea* rhizosphere, a low relative

abundance of bacteria was observed compared to the bulk soil and indicates the possible interaction and influence of the plant exudate on ZnO nanoparticles. Indeed, the finding is in agreement with related studies in which ZnO [28] and copper oxide nanoparticles [29] exerted a concentration-dependent phytotoxic effect by interfering with the production of root exudates.

3.6 Effect on Bacterial Biomass Composition and Distribution

We evaluated the soil heterotrophic bacterial composition by culture-dependent methods and the microbial biomass recovered were *Pseudomonas aeruginosa*, *P. alcaligenes*, *Bacillus subtilis*, *Rhizobium leguminosarum*, *Xanthobacter autotrophicus*, *Enterobacter aerogenes*, *Lactobacillus*, *Citrobacter*, *Nitrosomonas* and *Agromyces species* (Table 1). In both control ultisol and loam soils, all the organisms were recovered, however, there were differences in the ZnO nanoparticles amended soils. *Lactobacillus* sp. was recovered in all soils

including the treatment and control. The result agrees with previous studies in which *B. subtilis* survived long term exposure to ZnO nanoparticles, although competence of *B. subtilis* was modified by the nanoparticles [30,31]. These organisms are usually present in the soil and implicated in increased crop yield, plant protection, bioremediation and contribute to plant diseases [32-35]. In the amended loam soils, *Rhizobium*, *Xanthobacter*, *Enterobacter*, *Citrobacter*, *Nitrosomonas* were inhibited by the ZnO nanoparticles whereas *Enterobacter* and *Citrobacter* were inhibited in the treated ultisols. The sustained abundance of *Agromyces* sp. in the ultisol and loam soil is attributed to their high metabolic rate and physiology adapted to tolerate stress [36].

3.7 Effects on Soil Microbial Oxygen Uptake Rate (OUR)

We assessed the impact of ZnO nanoparticles on soil microbial respiration by measuring the oxygen uptake rate (OUR) and the results varied

Table 1. Distribution of bacterial biomass in rhizosphere and bulk soil

Probable organism	Control ultisol and loam soil	ZnO-amended ultisol	ZnO-amended loam soil
<i>Pseudomonas aeruginosa</i>	+	+	+
<i>Pseudomonas alcaligenes</i>	+	+	+
<i>Bacillus subtilis</i>	+	+	+
<i>Rizobium leguminosarum</i>	+	+	-
<i>Xanthobacter autotrophicus</i>	+	+	-
<i>Entrobacter aerogenes</i>	+	-	-
<i>Lactobacillus</i> sp	+	+	+
<i>Citrobacter</i> sp.	+	-	-
<i>Nitrosomonas</i> sp	+	+	-
<i>Agromyces</i> sp.	+	+	+

+ = present, - = not recovered

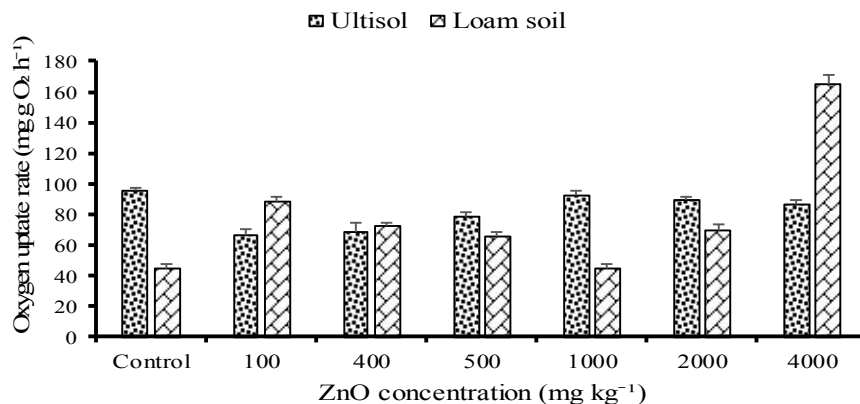


Fig. 6. Effect of ZnO nanoparticles on oxygen uptake rate of microbial biomass in the ultisol and loam soil

based on the ZnO nanoparticles concentration. In the control ultisol, the OUR was 95.5 ± 0.57 mg g O₂ h⁻¹ compared to the amended ultisols which ranged from 66.5 ± 0.57 to 92.5 ± 0.57 mg g O₂ h⁻¹ (Fig. 6). The result indicates that OUR in the control was 1.03 to 1.44 times higher than in the ZnO nanoparticles amended ultisol and the difference was significant at $p = .05$.

The OUR in the control and 1000 mg kg⁻¹ ZnO amended loam soils was 44.5 ± 0.57 mg g O₂ h⁻¹ and ranged from 65.8 ± 0.57 to 165.5 ± 0.57 mg g O₂ h⁻¹ in the amended loam soils (Fig. 6). The OUR in the amended loam soil, except the 1000 mg kg⁻¹ was 1.48 to 3.72 higher than the control loam soil and the difference was significant at $p = .05$. The mean OUR in the control ultisol was 95.5 ± 0.57 mg g O₂ h⁻¹ compared to 44.5 ± 0.57 mg g O₂ h⁻¹ in the loam soil and indicates 2.15 times higher uptake by microbial biomass in the control ultisol than loam soil and this difference was significant $p = .05$.

Aerobic organisms require oxygen as a terminal electron acceptor and the rate of oxygen consumption serves as an indicator of microbial activity in a growth medium and it is measured as the oxygen uptake rate [37]. Usually, a high respiration rate signifies increased microbial activity whereas a low respiration rate indicates reduced microbial activity about the control. The OUR of microbial biomass in the ultisol reduced in all the soil containing different ZnO concentrations (Fig. 6) which suggests that the ZnO nanoparticles inhibited microbial activity. The result corroborates with other findings in which ENPs such as silver oxide [38] and titanium oxide [39] reduced the respiration rates of microorganisms in wastewater treatment. OUR increased in the ZnO amended loam soil compared to the control except for 1000 mg kg⁻¹ which was similar to the control (Fig. 6). The results suggest that, while microbial activity was reduced in ultisol, ZnO induced an increase in the microbial activity in the loam soil. The composition of complex matrices such as the soil and wastewater either enhance or attenuate the inhibitory/toxic effect of ENPs. The increase in OUR induced by the ZnO nanoparticles is consistent with prior studies in which a combination of zinc, titanium and silver nanoparticles enhanced the oxygen uptake rate of microorganisms in wastewater treatment plant [40]. The increase in OUR was probably a response by the organisms to physiological stress.

4. CONCLUSION

The ZnO concentration- and soil type-dependent effect on microorganisms in a complex matrix such as the ultisol and loam soil provided a relevant environmental condition for microbial interaction with the nanoparticle and plant in real-time. The results provide insights on the effect of ZnO nanoparticles on the growth response of *A. hypogaea* and associated rhizosphere bacteria. ZnO nanoparticles exerted concentration-dependent and varying stimulatory and inhibitory effects on the germination, growth response and nodulation of *A. hypogaea*, induced a shift in the soil bacterial community abundance and stimulated the OUR of soil microbial biomass. Overall, the results infer that ZnO nanoparticles modified soil health and function and the effect on *A. hypogaea* and associated microbial community abundance was concentration-dependent and varied according to the type of soil.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by the personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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