



# Influence of Zinc Oxide Nanoparticles on Soil Physicochemical Properties and *Arachis hypogaea* Rhizosphere Microbial 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, OJ and IB 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

We evaluated the effect of 4000 mg zinc oxide (ZnO, 99%, 30 nm) nanoparticle on the physicochemical and microbiological properties of organic manure amended ultisol and loam soil cultivated with *Arachis hypogaea* using standard methods. The results indicate varying effects on the physicochemical properties in relation to the soil type. The pH of the control ultisol at  $7.85 \pm 0.17$  and  $8.3 \pm 0.12$  in the amended ultisol whereas, the control loam was  $7.15 \pm 0.17$  and  $7.41 \pm 0.11$  in the amended soil indicating 1.06- and 1.04-times higher difference than the controls respectively. Phosphorus concentration at  $57.82 \pm 0.54\%$ ,  $50.81 \pm 0.22\%$  and  $55.97 \pm 0.04\%$ ,  $59.97 \pm 0.02\%$  was 1.14 times lower in the ZnO amended ultisol and 1.07 times higher in amended loam soil compared to the respective controls. The organic matter content in the control and amended ultisol was  $2.28 \pm 0.32\%$  and  $0.91 \pm 0.02\%$ ,  $3.68 \pm 0.36\%$  and  $0.36 \pm 0.02\%$  in the control and amended loam soil. The concentration of nitrate in the control ultisol was  $0.05 \pm 0.01\%$  and  $0.03 \pm 0.01\%$  in the amended soil. The nitrate in the control loam soil was  $0.08 \pm 0.01\%$  relative to

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0.02 ± 0.01% in the treated soil and these differences were significant at  $p = 0.05$ . The concentration of nutritive salts was reduced and in contrast iron, copper, exchangeable acids, exchange capacity, clay and silt increased in the amended soils. Further to this, heterotrophic ammonia and nitrate-oxidizing bacterial population were inhibited in the amended soils and denitrifying organisms were stimulated. The organisms were members of the genera *Pseudomonas*, *Xanthobacter*, *Enterobacter*, *Bacillus*, *Lactobacillus*, *Citrobacter*, *Nitrosomonas*, *Agromyces* and *Rhizobium*. ZnO nanoparticles altered the soil physicochemical properties which exacerbated the negative effect on microbial abundance and varied with the soil type.

**Keywords:** Ultisol; loam soil; ZnO nanoparticle; bacterial abundance; soil properties.

## 1. INTRODUCTION

The ecological role of the soil ranges from providing habitat for plants and animals, to site for biogeochemical processes mediated by microorganisms and influenced by changes in the soil physicochemical properties. These changes include alteration in the presence and composition of organic matter, environmental conditions and soil organisms [1] and contamination from anthropogenic and emerging xenobiotic substances such as engineered nanoparticles (ENPs). ENPs have a range of sizes less than 100 nm with novel physical, thermal, optical and biological capabilities and are incorporated into a number of consumer products. Most of these products including personal care products, release ENPs into the soil at the end of their life cycle and disposal [2]. Thus, the soil becomes the largest receptor and sink for aged nanoparticles.

ENPs undergo different forms of interactions with soil properties and organisms which in turn alter the ecological function of the soil [3]. The soil properties that makeup up quality of the soil include: organic matter, pH, nitrogen content, cations, cation exchange capacity and minerals among others. Soil-ENPs interactions include aggregates formed with colloids, absorption, change in oxidation state, precipitation or formation of complexes with ligands [4,5]. The effect on soil properties can vary, for example, the soil pH is influenced by the accumulation of different types of nanoparticles [6]. Several studies [7,8,9,10] have reported increased pH of soils on exposure to different nanoparticles. Soil pH determines the function of a soil and is closely related to presence of, and absorption of nutrients, microbial activity and plant growth [8]. The ability of the soil to maintain the presence of positively charged ions (cation exchange capacity) is an important soil property that reflects on the structure, nutrient utilization and pH of soil [11].

ENPs can also indirectly affect soil properties through the effect on soil microbial activities. For instance, copper oxide nanoparticles increased iron (II) oxide ( $Fe^{2+}$ ) in paddy soils exposed to CuO nanoparticles [8]. Increased  $Fe^{2+}$  was attributed to the nanoparticles exerting a stimulatory effect on iron reducing bacterial community in the soil. In addition, heavy metals in the soil contribute to plant growth although high concentrations exert harmful effects [12]. Iron in soils is reduced by microbial respiration and involves electron transfer from organic matter to  $Fe^{3+}$  which serves as the electron acceptor. Reduction of iron in soils affects the carbon, nitrogen and sulphur cycles [13].

However, several studies indicate the presence of ENPs in the soil environment may exert no negative effect on soil properties. For instance, the porosity of soil was not affected by bimetallic (Fe/Pd) nanoparticles [14], copper oxide and magnetite ( $Fe_3O_4$ ) effect on clogging was minimal [6]. Also, copper oxide and magnetite nanoparticles exerted no effect on sorption capacity and organic matter although there was a change in dissolved organic matter attributed to short exposure time [15]. Here, we evaluated the effect of zinc oxide nanoparticles on the physicochemical properties of tropical ultisol and loam soils and bacterial abundance in groundnut (*Arachis hypogaea*) rhizosphere under greenhouse conditions.

## 2. MATERIALS AND METHODS

### 2.1 Soil Sample Collection and Preparation

The ultisol and loam soils were excavated from the University of Uyo Farm, Nwaniba Road, Uyo and transported to the greenhouse in the Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria. The soils were amended with organic manure and spiked with

zinc oxide nanoparticles as previously reported [16]. Briefly, 14 kg of soil and 6 kg of organic manure (poultry dropping) were placed into seven wooden troughs (30 cm x 30 cm) lined with polyethene in duplicate. The organic manure amended soils were allowed to stand for 21 days and seeded with *Arachis hypogea* under greenhouse conditions. The plants were harvested after 10 weeks of growth and the soil sampled for analysis. 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.

### 2.1.1 The greenhouse

The greenhouse was constructed over a land space of 3.6 x 4m and 2.7 m in height to accommodate the troughs with adequate space for walkway. Transparent roofing sheets resting on galvanized pipes as support were used and the perimeter covered with wire net to prevent rodents and trespassers. The whole structure was reinforced with transparent polyethene for enhanced illumination during the day and maintain optimum temperature during the rainy and cold conditions. The ambient temperature of the soil and greenhouse was monitored throughout the period of planting and growth of the plant using a mercury thermometer.

### 2.2 Soil Physicochemical Analysis

The control ultisol and loam soil and the 4000 mg kg<sup>-1</sup> ZnO nanoparticles amended soils were selected to represent a pristine soil condition and a worst-case scenario of ZnO nanoparticles input into the garden soil ecosystem through fertilizer application. The bulk ultisol and loam soils were collected from the control and 4000 mg ZnO nanoparticles amendments and analyzed for the following physical and chemical properties. Physicochemical analysis of the soil was carried out according to standard procedures [17,18]. The samples were digested and analysed for hydrogen ion, exchangeable cation and basic salt, potassium, sodium, magnesium, calcium, iron, copper, exchangeable acids, cation exchange capacity, organic matter, available nitrate and phosphate. The Bouyoucos (Hydrometer) method was employed for particle size distribution analysis. The conductivity meter was used to measure the electrical conductivity

and the pH was determined by the electrometric method in 1:1 soil/water ratio.

### 2.3 Enumeration, Characterization and Identification of Bacteria

The enumeration, characterization and identification of heterotrophic bacterial abundance were carried out as previously reported [16]. Briefly, matured plants were uprooted to obtain the rhizosphere soil and bulk samples collected, serially diluted, inoculated by pour-plate onto Nutrient Agar (Oxoid) and incubated at 28 ± 2°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 [19] and compared with those of known taxa using the Bergey's Manual of Determinative Bacteriology [20].

### 2.4 Statistical Analysis

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

## 3. RESULTS AND DISCUSSION

### 3.1 Greenhouse Condition

Temperature is an important environmental requirement for enzyme activity and microbial growth and different soil microorganisms and plants have varying temperature regime for optimum performance. The average temperature of the greenhouse throughout the period of plant growth was 26.18 ± 0.85°C, 30.36 ± 2.36°C and 28.09 ± 1.66°C in the morning, afternoon and evening respectively. In the ultisol, the average temperature was 26.73 ± 0.63°C, 30.82 ± 1.89°C and 29.09 ± 1.11°C, whereas in the loam soil, 26.73 ± 0.63°C, 31 ± 1.9°C and 29.09 ± 1.11°C were recorded in the morning, afternoon and evening respectively.

### 3.2 Effect of ZnO Nanoparticles on Soil pH, Hydrogen Ion, Exchangeable Cation and Basic Salt

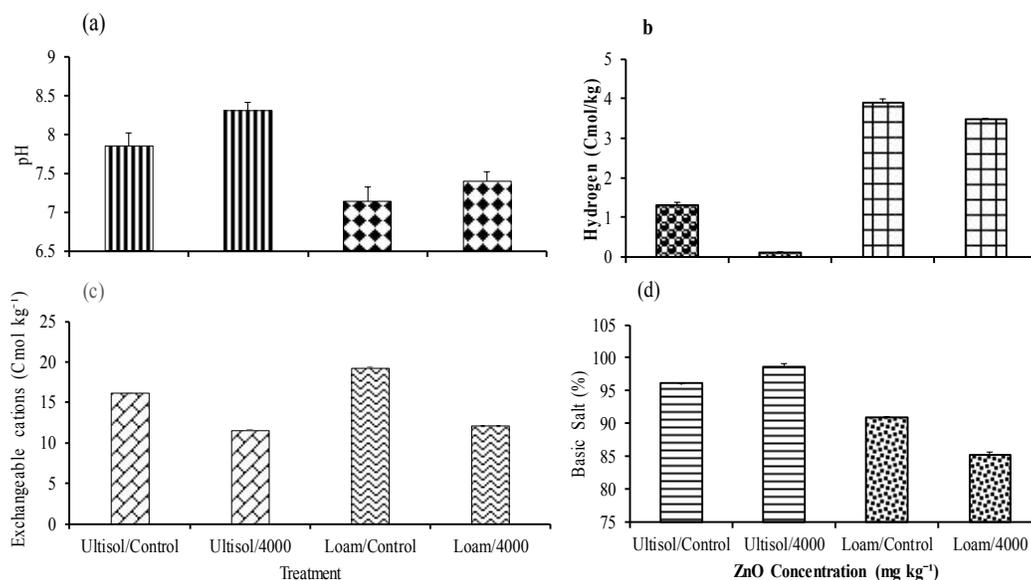
The soil pH determines the function of a soil and relates to presence of and absorption of

nutrients, microbial activity and plant growth [8]. ZnO nanoparticles induced a higher pH in the treated ultisol and loam soils in contrast to the control. The pH of the control ultisol at  $7.85 \pm 0.17$  was lower than  $8.3 \pm 0.12$  of the amended ultisol indicating 1.06 times higher value than the control. The pH of the control loam was  $7.15 \pm 0.17$  and  $7.41 \pm 0.11$  in the ZnO treated soil and suggests 1.04 times higher difference than that of the control. The differences between the pH of the treated soils and the controls were significant at  $p = 0.05$ . The results indicate that the ZnO nanoparticles induced changes that increased the pH of the ultisol and loam soils. Several studies indicate that ENPs such as copper oxide nanoparticles [8], zero-valent iron nanoparticles [10] and ZnO nanoparticles [7,9] induced changes in soil pH during acute and chronic exposures. These results are consistent with the present study in which ZnO nanoparticles raised the pH of the ultisol and loam soils.

The mean hydrogen ions in the control ultisol was  $1.3 \pm 0.09$  compared to  $3.89 \pm 0.12$  Cmol  $\text{kg}^{-1}$  in the control loam soil indicating 2.99 times higher content than the ultisol (Fig. 1b). The hydrogen ions in control ultisol was  $1.3 \pm 0.09$  relative to  $0.12 \pm 0.01$  Cmol  $\text{kg}^{-1}$  in the ultisol amended with ZnO nanoparticles suggests 10.83 times higher concentration in the control ultisol

than the ZnO amended ultisol. The control loam soil with  $3.89 \pm 0.12$  and  $3.49 \pm 0.01$  Cmol  $\text{kg}^{-1}$  in the ZnO amended loam soil indicates 1.11 times higher concentration than the treated loam soil. The difference in the control and amended ultisol and loam soils were significant at  $p = 0.05$ . The empirical evidence suggests reduced hydrogen ions in the treated ultisol and loam soils, and subsequent increase in pH compared to the control soils. Thus, ZnO induced a higher pH in the amended soils. The probable reason is that the ZnO nanoparticles consumed  $\text{H}^+$  in the soil solution with the production of  $\text{Zn}^{2+}$  [21].

The mean exchangeable cations content of the control ultisol was  $16.09 \pm 0.12$  relative to  $11.52 \pm 0.12$  Cmol  $\text{kg}^{-1}$  in the amended soil (Fig. 1c) indicating 1.4 times higher content than the treated soil. In the control loam soil, the mean exchangeable cations content was  $19.18 \pm 0.19$  Cmol  $\text{kg}^{-1}$  in relation to  $12.08 \pm 0.03$  Cmol  $\text{kg}^{-1}$  in amended soil. The exchangeable cation content in the control soil was 1.59 times higher than the treated soil. These differences were significant at  $p = 0.05$ . In the ultisol and loam soils exposed to ZnO nanoparticles, exchangeable cations content was reduced compared to the controls which caused an increase in the potassium concentration.



**Fig. 1. Influence of zinc oxide nanoparticles on the (a) pH (b) hydrogen ion (c) exchangeable cation and (d) basic salt concentration in ultisol and loam soils**

The mean basic salt content of the control ultisol was  $96.08 \pm 0.03$  in relation to  $98.74 \pm 0.30$   $\text{Cmol kg}^{-1}$  in the treated soil (Fig. 1d) which was 1.03 times higher than the control. In loam soil, the mean basic salt content of the control was  $90.92 \pm 0.09$  compared to  $85.29 \pm 0.34$   $\text{Cmol kg}^{-1}$  in the treated soil and indicates 1.07 times higher than the treated soil. In the control soils, the mean basic salt content of the ultisol was 1.13 times higher than the loam soil and these differences were significant at  $p = 0.05$ . The salinity of the control ultisol was higher than the control loam soil, however, salinity increased on amendment with ZnO nanoparticles indicating that the plants and microorganisms in the ultisol were exposed to higher salt stress caused by the ZnO nanoparticles. In the loam soil, the salinity was however reduced on amendment with ZnO nanoparticles (Fig. 1d). Salinity stress usually leads to reduced soil osmotic potential, nutritional imbalance and negative impact on biochemical and physiological processes in the soil [22]. Nevertheless, nanoparticles have been associated with increased plant growth despite salinity stress [23].

### 3.3 Effect of Zinc Oxide Nanoparticles on Soil Potassium, Sodium, Magnesium and Calcium Content

The mean potassium content of the control and ZnO amended ultisol was  $0.09 \pm 0.01$   $\text{Cmol kg}^{-1}$  and  $0.05 \pm 0.01$   $\text{Cmol kg}^{-1}$  respectively (Fig. 2a). The result indicates that the potassium content in

the control soil was 1.8 times higher than the treated soil and the difference was significant at  $p = 0.05$ . In loam soil, the mean potassium content of the control was  $0.08 \pm 0.01$   $\text{Cmol kg}^{-1}$  compared to  $0.08 \pm 0.01$   $\text{Cmol kg}^{-1}$  in the treated soil. There was no difference between the potassium content in the treated soil and the control.

The mean sodium content of the control ultisol at  $0.18 \pm 0.01$   $\text{Cmol kg}^{-1}$  was higher than  $0.12 \pm 0.01$   $\text{Cmol kg}^{-1}$  in the ZnO amended soil (Fig. 2b). The result shows that the sodium content in the control soil was 1.5 times higher than the ZnO amended soil. The difference was low but significant at  $p = 0.05$ . In loam soil, the mean sodium content of the control was  $0.21 \pm 0.01$   $\text{Cmol kg}^{-1}$  in relation to  $0.22 \pm 0.01$   $\text{Cmol kg}^{-1}$  in the ZnO nanoparticle treated soil indicating that the sodium content in the treated soil was 1.05 times higher than the control. The difference between the sodium content in control and the amended soil was however not significant ( $p = 0.05$ ). In the present study, calcium, potassium and sodium ions concentration in the ZnO amended ultisol were low relative to the control ultisol. The calcium ions were lower in the amended loam soils compared to the control, whereas sodium and potassium ions were stable despite the amendment with ZnO nanoparticles. The reduced cations content indicates that ZnO nanoparticles induced a rapid uptake by soil biota as a response to oxidative stress [24].

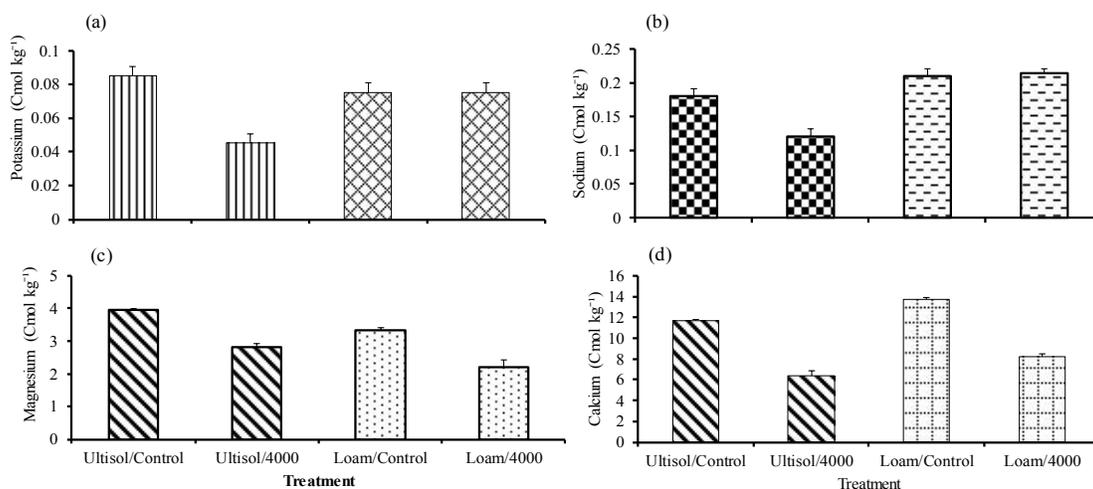


Fig. 2. Effect of zinc oxide nanoparticles on the concentrations of (a) potassium, (b) sodium, (c) magnesium and (d) calcium in the ultisol and loam soils

The mean magnesium content of the control ultisol was  $3.95 \pm 0.06$  Cmol  $\text{kg}^{-1}$  and  $2.80 \pm 0.12$  Cmol  $\text{kg}^{-1}$  in the treated soil (Fig. 2c) which was 1.41 times higher than ZnO nanoparticles amended soil. The content in the control loam soil was  $3.35 \pm 0.01$  Cmol  $\text{kg}^{-1}$  relative to  $2.20 \pm 0.23$  Cmol  $\text{kg}^{-1}$  in the ZnO nanoparticles amended soil suggesting 1.52 times higher value than the amended soil. The differences between the magnesium content in the control and amended soils were significant at  $p = .05$ . Magnesium serves as a cofactor for most enzymes, maintain membranes and nucleic acid formation, stabilize ribosome, cell growth and division [25]. Transport of magnesium ion ( $\text{Mg}^{2+}$ ) into cells is usually based on requirements and regulated in response to changing environmental conditions. The concentration of  $\text{Mg}^{2+}$  in the control ultisol and loam soil was higher than the treated soils (Fig. 2c) and indicates that ZnO nanoparticles induced the uptake of  $\text{Mg}^{2+}$  by both microorganisms and *Arachis hypogae*. ENPs such as  $\text{TiO}_2$  nanoparticles induced the activities of  $\text{Mg}^{2+}$ -ATPase of spinach chloroplast and in turn, lead to increased uptake  $\text{Mg}^{2+}$  [26]. An increase in  $\text{Mg}^{2+}$  intracellularly is associated with susceptibility of plants to pathogens, inhibition of cell division and ribosomal activities [27], whereas influx of  $\text{Mg}^{2+}$  is a coping mechanism of microorganisms to stress [28]. Thus, the results suggest that the ZnO nanoparticles was a stressor for the plant and soil microorganisms and led to increased uptake of  $\text{Mg}^{2+}$ .

The mean calcium content of the control ultisol was  $11.7 \pm 0.12$  Cmol  $\text{kg}^{-1}$  and  $6.4 \pm 0.16$  Cmol  $\text{kg}^{-1}$  in the treated soil (Fig. 2d) which was 1.83 times higher than zinc oxide nanoparticles amended soil. In the loam soil, the mean calcium content of the control was  $13.75 \pm 0.17$  Cmol  $\text{kg}^{-1}$  compared to  $8.25 \pm 0.29$  Cmol  $\text{kg}^{-1}$  in the ZnO nanoparticles amended soil and indicates 1.67 times higher value than the amended soil. The differences in the calcium content in both soils were significant at  $p = 0.05$ . Calcium is an important element in soil which helps in plant growth. Magnesium and calcium ions interact competitively in cells in relation to enzyme activity [25]. Calcium uptake and translocation by plants are influenced by presence or absence of cations, nitrates, exchange capacity, root temperature and metabolic inhibitors in the soil [29]. Here, the control ultisol and loam soils recorded higher calcium ions compared to the soils amended with ZnO nanoparticles (Fig. 2d) and suggest induced use of calcium by the soil biota.

### 3.4 Effect of ZnO Nanoparticles on Iron, Copper, Exchangeable Acids and Exchange Capacity

The iron content of the amended ultisol soil at  $93.21 \pm 0.23$  mg  $\text{kg}^{-1}$  was 8.63 times higher than the control with  $10.80 \pm 0.24$  mg  $\text{kg}^{-1}$  (Fig. 3a). In loam soil, the mean iron content of the amended soil was  $90.9 \pm 0.16$  mg  $\text{kg}^{-1}$  compared to the control at  $9.79 \pm 0.5$  mg  $\text{kg}^{-1}$  and indicated 9.29 times higher value than the control. In the control ultisol, the mean copper content was  $0.032 \pm 0.001$  and  $0.064 \pm 0.002$  mg  $\text{kg}^{-1}$  in the ZnO nanoparticles treated soil (Fig. 3b) indicating that copper content in the treated soil was 2 times higher than the control. In contrast, the control loam soil was  $0.042 \pm 0.001$  mg  $\text{kg}^{-1}$  and  $0.071 \pm 0.001$  mg  $\text{kg}^{-1}$  in the treated soil (Figure) which was 1.69 times higher than the control. The differences between the iron and copper contents in the control and treated soils were significant at  $p = 0.05$ .

Iron and copper are both important elements in soils and contribute to plant growth although high concentrations exert harmful effects [12]. Iron in soils is reduced by microbial respiration and involves electron transfer from organic matter to  $\text{Fe}^{3+}$  which serves as the electron acceptor. Reduction of iron in soils affects the carbon, nitrogen and sulphur cycles [13]. The results show an increase in iron ( $\text{Fe}^{2+}$ ) content in the ultisol and loam soil amended with ZnO nanoparticles (Fig. 3a) and indicate negative effect of ZnO nanoparticles on the activity of iron reducing bacteria. Similar findings which  $\text{Fe}^{2+}$  rapidly increased in paddy soils exposed to copper oxide nanoparticles have been reported [8]. The increase in  $\text{Fe}^{2+}$  content is attributed to the nanoparticles exerting a stimulatory effect on dissimilatory iron reduction in soil.

Copper in soils assist the enzymes responsible for photosynthesis, respiration and formation of lignin and deficiencies inhibit nodulation and nitrogen-fixation, delay flowering and plant yield [30]. Copper content in the ZnO nanoparticle amended ultisol and loam soils were higher than the control soils (Fig. 3b) and signify induced accumulation of copper ions. High copper content in soils exacerbates the toxic effect of xenobiotics evidenced in delayed germination and reduced shoot vigor [30]. The germination and growth response of *A. hypogaea* in the ultisol and loam soils were negatively impacted [16] and accumulation of copper ions, in part,

probably contributed to the poor plant performance.

The mean exchangeable acid content of the control ultisol was  $0.62 \pm 0.03 \text{ Cmol kg}^{-1}$  and  $0.6 \pm 0.07 \text{ Cmol kg}^{-1}$  in the amended soil (Fig. 3c) and indicates 1.03 times higher value than the treated soil. In loam soil,  $1.77 \pm 0.02 \text{ Cmol kg}^{-1}$  mean exchangeable acid content of the control was similar to  $1.77 \pm 0.05 \text{ Cmol kg}^{-1}$  of the amended soil. The differences between the exchangeable acid content were not significant at  $p = 0.05$ . The result indicates that the ZnO nanoparticles did not alter the exchangeable acids present in both soils and the reasons are unclear at the moment.

The mean exchange capacity for the control ultisol at  $0.15 \pm 0.06 \text{ d s}^{-1}$  and  $0.23 \pm 0.01 \text{ d s}^{-1}$  in the soil amended with zinc oxide nanoparticle (Fig. 3d) indicates 1.53 times higher value in the amended soil than the control. The mean exchange capacity for the control loam soil at  $0.35 \pm 0.03 \text{ d s}^{-1}$  was lower than  $0.53 \pm 0.03 \text{ d s}^{-1}$  in the ZnO nanoparticles amended soil with 1.51 times higher value than the control. The differences between the exchange capacity of the ZnO nanoparticles amended and the control soils were significant at  $p = 0.05$ . The presence and maintenance of positively charged ions is an essential property with influence on the structure, nutrient utilization and pH of soil [11]. The cation exchange capacity of the ultisol and loam soil exposed to ZnO nanoparticles was higher than the control soils and in agreement with related studies. For instance, volcanic ash nanoparticles increased the cation exchange capacity of Andisol soil which increased at high decomposition rate [31]. Here, there was increased decomposition rates indicated by the reduced organic matter content, in addition, to the enhanced exchange capacity in ultisol and loam soils amended with ZnO nanoparticles.

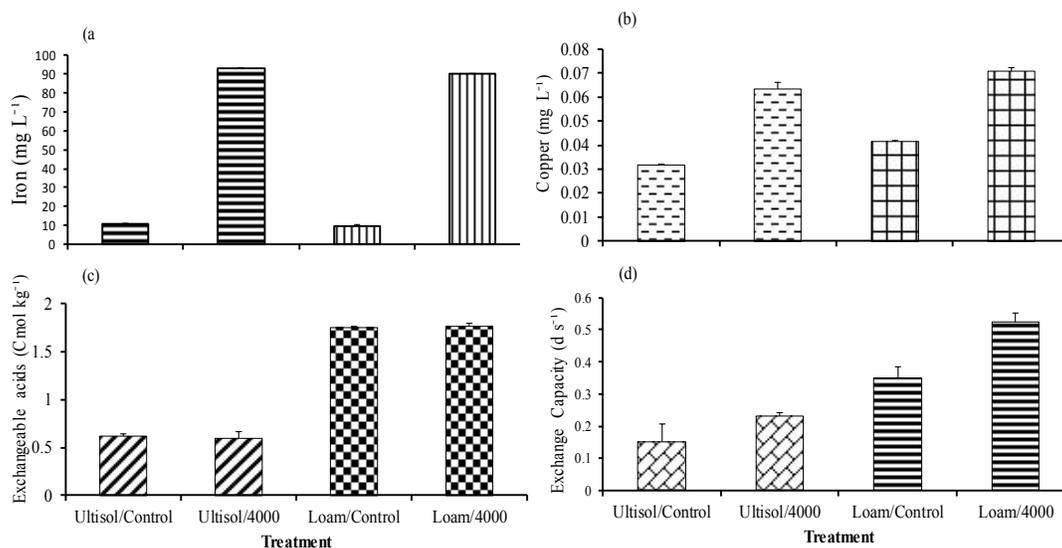
### 3.5 Effect of ZnO Nanoparticles on Particle Fraction, Organic Matter and Available Nitrate

The particle size of the control and ZnO amended ultisol was  $91.91 \pm 1.06 \%$ ,  $2.98 \pm 0.27\%$ ,  $4.47 \pm 0.26\%$  and  $83.04 \pm 0.32$ ,  $8.08 \pm 0.91\%$ ,  $8.58 \pm 0.23\%$  for sand, silt and clay fractions respectively (Fig. 4a). The sand content in the control ultisol was 1.11 times higher than the treatment, whereas the silt and clay content

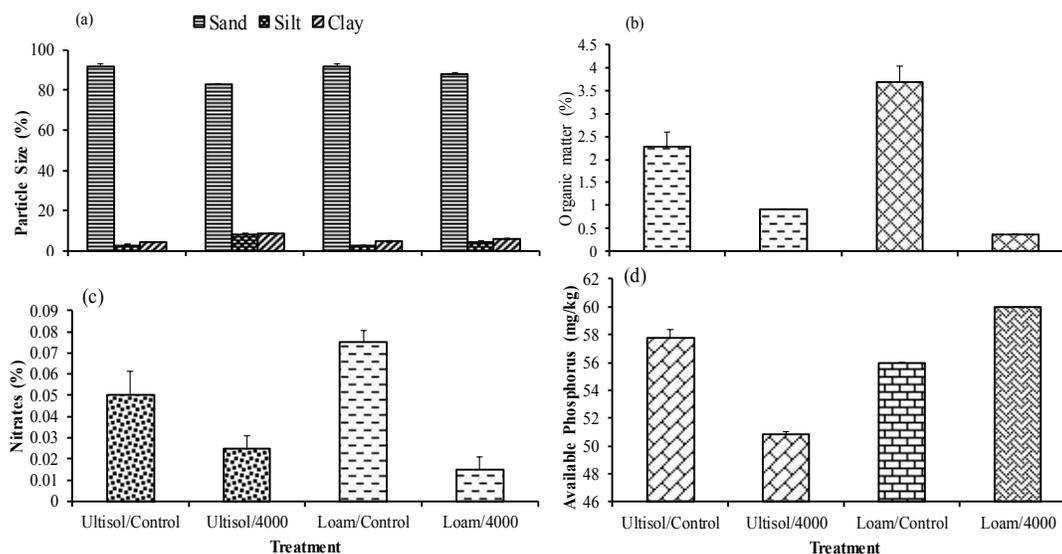
in the treated soil was 2.71 and 1.91 times higher than the control respectively. The particle size of the control and ZnO amended loam soil was  $90.83 \pm 0.94\%$ ,  $2.99 \pm 0.01\%$ ,  $4.45 \pm 0.41\%$ , and  $87.86 \pm 0.76$ ,  $4.65 \pm 0.52\%$  and  $6.19 \pm 0.22\%$  for sand, silt and clay fractions respectively (Fig. 4a). The sand content in the control was 1.03 times higher than the treated soil, whereas the silt and clay content in the ZnO amended soil was 1.56 and 1.39 times higher than the control. The results indicate changes in the particle fractions of the two soil types because of ZnO nanoparticles interaction, and the differences were significant at  $p = 0.05$ . Soil particles confer a structure that cause variations of oxygen availability over a small distance, leading to a presence of both aerobic and anaerobic conditions [32] and ZnO nanoparticle interaction with the soil particles increased this effect.

The organic matter content of the control ultisol was  $2.28 \pm 0.32\%$  and  $0.91 \pm 0.02\%$  in the treated soil (Fig. 4b) indicating 2.51 times higher value than the amended soil. The organic matter content of the control loam soil was  $3.68 \pm 0.36\%$  and  $0.36 \pm 0.02\%$  in the amended loam soil which was 10.22 times higher than the soil exposed to ZnO nanoparticles. These differences were significant at  $p = 0.05$ .

Soil organic matter synthesized primarily from plant remains degraded by microorganisms plays important roles in how the soil is formed, aggregates stabilized and soil fertility maintained [33,34]. The organic matter content in the control ultisol and loam soils were relatively higher than the soils amended with ZnO nanoparticles (Fig. 4b). The ZnO nanoparticles induced an increase in decomposition of organic matter in both the ultisol and loam soils probably because of the catalytic properties of the nanoparticles. This is consistent with other studies in which ENPs are reported to be highly specific and reactive because of their nanoscale size and catalytic properties [35]. Here, the ZnO nanoparticles exerted catalytic effect in the ultisol and loam soils and increased decomposition of the organic matter. Other ENPs such as iron oxide nanoparticles have been associated with increased decomposition of organic matter and soil enzyme activities [36]. The results suggest that the ZnO nanoparticles, in part, stimulated microbial activities that resulted in breakdown and use of the organic matter and, in part, induced catalytic degradation in the soil.



**Fig. 3. Influence of zinc oxide nanoparticles on the concentrations of (a) iron, (b) copper, (c) exchangeable acid and (d) exchange capacity in the ultisol and loam soils**



**Fig. 4. Effect of ZnO nanoparticles on soil (a) particle fraction, (b) organic matter (c) available nitrate and (d) phosphorus in the ultisol and loam soil**

The concentration of nitrate in the control ultisol was  $0.05 \pm 0.01\%$  compared to  $0.03 \pm 0.01\%$  in the treated soil (Fig. 4c) and indicates 1.7 times higher than the soil exposed to zinc oxide nanoparticles. However, the differences between the nitrate content in both soils was not significant at  $p = 0.05$ . Nitrates in the control loam soil were  $0.08 \pm 0.01\%$  relative to  $0.02 \pm 0.01\%$  in the treated soil. The concentration of

nitrate in the control loam soil was 4 times higher than the ZnO nanoparticles amended soil and this difference was significant at  $p = 0.05$ . Soil microorganisms immobilize and convert nitrate ions to organic forms used by plants for growth and generally contributes to soil fertility.

The nitrate concentration in the control ultisol and loam soils were higher than the ZnO amended

soils (Fig. 4c). The result suggests in part, that the ammonia- and nitrate-oxidizing organisms were inhibited in the amended soil, and in part, that ZnO nanoparticles induced nitrate reduction by stimulating the growth of denitrifying organisms in both soils. The reduced nitrate content in both soils treated with ZnO nanoparticles is consistent with other studies, for instance, there was a decrease in denitrifying enzyme activity on exposure of five different soils to copper oxide nanoparticles [37]. Furthermore, nanoparticles act as catalyst for nitrate reducing bacteria such as gamma-*Proteobacteria* or *Acidobacteria*, [38] to stimulate growth evidenced in the increased use of nitrate through denitrification and/or dissimilatory nitrate reduction.

### 3.6 Influence on Available Phosphate and Phosphate-solubilizing Bacterial Community

The mean available phosphorus of the control ultisol was  $57.82 \pm 0.54\%$  in relation to  $50.81 \pm 0.22\%$  of the treated soil (Fig. 4d) which was 1.14 times higher the zinc oxide nanoparticles amended soil. In loam soil, the mean available phosphorus of the control was  $55.97 \pm 0.04\%$  in contrast to  $59.97 \pm 0.02\%$  in the treated soil. The available phosphorus content in the ZnO nanoparticle treated soil was 1.07 times higher than the control and this difference was significant at  $p = 0.05$ . Phosphorus plays different roles in photosynthesis, energy transfer and metabolism of carbohydrates, either solubilized or mineralized from insoluble soil phosphate by microorganisms and/or addition of phosphate fertilizers. Examples of some phosphate-solubilizing microorganisms include members of the genera *Pseudomonas*, *Agrobacterium*, *Aspergillus*, *Pythium*, *Azotobacter* and *Bacillus* [39]. Depletion of phosphorus in agricultural soil causes deficiencies in plant growth. Here, the available phosphorus content in the control ultisol was higher than the treated soil indicating inhibition of phosphate-solubilizing bacteria by the ZnO nanoparticles. In contrast, phosphate-solubilizing bacterial activity was stimulated in the loam soil by the ZnO nanoparticles leading to increase in available phosphorus content compared to the control loam soil (Fig. 4d).

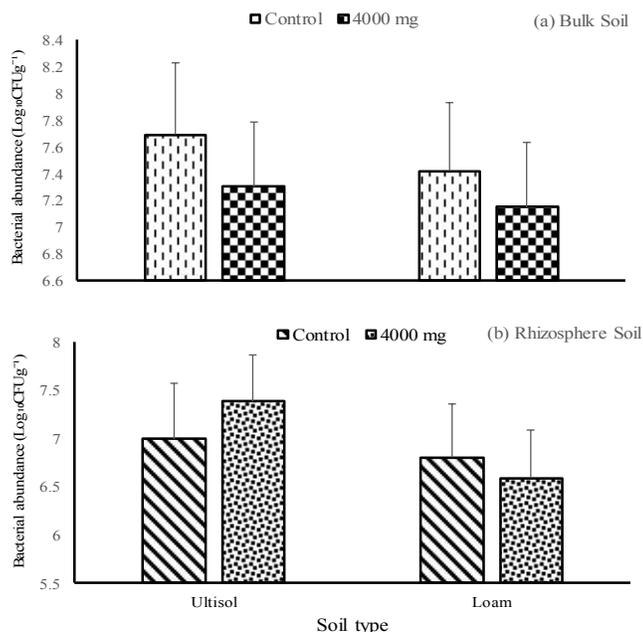
Different soil stressors such as increase in sodium chloride and pH influences phosphorus solubilization [40]. Despite the negative effect of ZnO nanoparticles on phosphate-solubilizing

bacteria which directly correlated with reduced phosphate content in the amended ultisol, *Pseudomonas aeruginosa* and *P. alcaligenes* were isolated from the ultisol and loam soils. ENPs such as ZnO are known to exert selective and varying inhibitory effects on microbial activity. For example, ZnO nanoparticles inhibited biofilm formation, production of pyocyanin, *Pseudomonas* quinolone potential and hemolytic activity of *Pseudomonas aeruginosa* with no effect on the cell growth [41]. Toxicity of nanoparticles was likely influenced by soil properties [42,43] which is consistent with the different effect exerted by the ZnO nanoparticles in the ultisol and loam soils. It is safe to assume that the soil component interacted with the nanoparticles for an additive, synergistic or potentiative effect on the microbes in the ultisol, whereas the effect was mitigated in the loam soil.

### 3.7 Influence of ZnO Nanoparticles on Total Heterotrophic Bacterial Abundance in *A. hypogaea* rhizosphere and Bulk Soils

The counts of total heterotrophic bacteria (THB) in the bulk ultisol (control) was  $7.69 \pm 0.54$  relative to  $7.30 \pm 0.49 \text{ Log}_{10}\text{CFU g}^{-1}$  in the  $4000 \text{ mg kg}^{-1}$  ZnO nanoparticles amended ultisol (Fig. 5a). The results indicate that the THB counts in the control was 1.05 times higher than the ultisol amended with  $4000 \text{ mg kg}^{-1}$  ZnO nanoparticles. The mean THB counts in the control loam soil at  $7.41 \pm 0.51$  compared to  $7.15 \pm 0.48 \text{ Log}_{10}\text{CFU g}^{-1}$  in the  $4000 \text{ mg kg}^{-1}$  zinc oxide nanoparticles amended loam soil (Fig. 5a) and was 1.04 times higher than the amended soil. The differences between the THB counts for in the control and ZnO nanoparticle amended soils were significant at  $p = 0.05$ .

The ecological role of microorganisms in promoting plant growth and nutrient cycling are essential and influenced by microbial abundance, diversity, intrinsic and extrinsic factors in the soil [44]. Here, the impact of ZnO nanoparticles on the bulk soil indicates that ZnO nanoparticles reduced the bacterial community abundance in both the ultisol and loam soil at  $4000 \text{ mg kg}^{-1}$  (Fig. 5a). The result is consistent with other studies where ZnO nanoparticles caused in a decrease in abundance of soil bacterial community [45]. ZnO nanoparticles exerted inhibitory effect on soil bacterial population by the release of zinc ions ( $\text{Zn}^{2+}$ ) to induce oxidative stress, cell membrane damage and cytoplasmic leakage [46].



**Fig. 5. Effects of ZnO nanoparticles on microbial abundance in the bulk ultisol and loam soil and *A. hypogaea* rhizosphere**

The THB counts in the *A. hypogaea* rhizosphere from control ultisol was  $7.00 \pm 0.55$  and  $7.28 \pm 0.30$   $\text{Log}_{10}\text{CFU g}^{-1}$  in the 4000  $\text{mg kg}^{-1}$  ZnO amended ultisol. The results indicate that the counts in the *A. hypogaea* rhizosphere from ZnO amended ultisol was higher than the control with a difference of 1.04. The THB abundance in the *A. hypogaea* rhizosphere for control loam soil was  $6.80 \pm 0.58$  and  $6.59 \pm 0.48$   $\text{Log}_{10}\text{CFU g}^{-1}$  in the 4000  $\text{mg kg}^{-1}$  ZnO amended loam soil. (Fig. 5b) and indicates a 1.03 times higher counts in the control than the 4000  $\text{mg kg}^{-1}$  amended soil. The differences between the THB counts in the amended and control soils was significant at  $p = 0.05$ . Indeed, the decomposition of organic matter had effect on bacterial composition [34] and correlated with the differences in the composition of *A. hypogaea* rhizosphere bacterial biomass and abundance in the amended and the controls ultisol and loam soil [16]. Further to this, nanoparticles are implicated in enhanced plant growth in environments with high salt concentration [23] which plausibly accounted for some of the positive impacts on the bacterial and *A. hypogaea* growth response [16] in the ultisol and loam soil despite the salt stress.

The effect of ZnO nanoparticles on rhizosphere microbial abundance was however soil type

dependent. For instance, in the ultisol, ZnO nanoparticles stimulated growth and increased bacterial abundance whereas in the loam soil, the ZnO nanoparticles reduced the abundance (Fig. 5b). Soil properties such as pH, texture, particle size distribution, structure, and organic matter content play a key role in the effect of nanoparticles on microorganisms [47]. The coating of soil organic matter on the surface nanoparticles reduced the direct contact between the microbial cells and nanoparticles [8] which probably accounted for the different effect of the nanoparticles on microorganisms in both soils.

#### 4. CONCLUSION

The outcome of the interaction between the soil physicochemical properties, biomass and ZnO nanoparticles was inconsistent and varied according to soil type. ZnO nanoparticles exerted differential reduction and oxidation effects to alter the soil physicochemical properties. In addition, microbial mediated processes were either stimulated or inhibited based on these alterations. The reduced nitrate concentration in both soils and phosphorus in ultisol reflects the inhibitory effects on phosphate-solubilizing and nitrate-fixing bacteria. ZnO nanoparticles induced an increased pH, exchangeable acids and exchange capacity, iron and copper, whereas the

concentration of nutritive salts was reduced and thus, modified the soil health and ecosystem function.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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