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Original Article

Detrimental Effects of Metal Nanoparticles on Microorganisms and Biochemical Activities through Soil Quality Index Analysis

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ABSTRACT

The expanding use of nanoparticles broachs their environmental impacts, but their effects on ecosystem functions and the organisms remain largely unknown. Few studies have targeted soil biochemical activities which are regarded as sensitive soil health indicators. Thus, present study designed to estimate the ecotoxicological effects of Al_2O_3 and ZnO NPs at various concentrations (30, 60, 90, 120, 150 and 180 mg/l) in a 60-day microcosm incubation experiment under 25±3°C. Microbial count, enzyme activity, basal respiration rate and microbial biomass were estimated, besides the inhibition rate, toxicity index, potential soil fertility index and soil quality index (SQI). Significant differences in pH, EC, TDS and SOM were observed after application of NPs. Microbial activity was generally increased in response to both NPs, whereas *Azotobacter* was not affected by Al_2O_3 NPs. Various results were observed in biochemical activities. Dehydrogenase, catalase, nitrate reductase and biomass C were generally decreased, while urease and respiration rate were increased in comparing with control. The greatest inhibition rates shown by both Al_2O_3 and ZnO NPs were in dehydrogenase activity by 159.5% and in free-living nitrogen-fixing bacteria by 213.7% respectively. Referring to toxicity index, both NPs showed toxicity on free-living nitrogen-fixing bacteria, dehydrogenase, catalase and nitrate reductase. The highest SQI recognized as 0.792 in 90 mg/l treated soils with Al_2O_3 NPs, while the highest SQI recognized as 0.978 in 150 mg/l treated soils with ZnO NPs. Consequently, NPs may measurably impact soil microbiological and biochemical properties and all the used bioindicators be advantageous in monitoring soil contamination by NPs through analysis of soil quality index.

Keywords: Enzymes, Nanoparticles, Pollution, Soil Quality Index, Toxicity Index.

1 Introduction

Extensively, pesticides are used for plant protection against pests. Uncontrolled application may contaminate soil and kill non-target organisms through damaging soil biomass and microorganism [1]. The majority of food safety researches focus on climate change, resistant pathogens and need for sustainable agriculture while addressing human health and environmental concerns [2]. Changing the pesticide policy programs aim to reduce pesticide risks on human health and the environment [3] through their replacement with alternative plant protection techniques [4]. The market of green chemicals worldwide have been placed [5] and the interest in nano-biopesticides have increased due to their efficiency in small quantities [6], reduce pesticide application and increase their degradation, detect and remediate heavy metals in the soil and increase crop yields [7,8]. The release of NPs into the ecosystem, particularly the soil, exhibit both positive and negative effects [9,10]. NPs can be prepared by several techniques [11], among them biological synthesis from plants which can be helpful in the large-scale production of metal oxide NPs without using of any toxic chemical [12,13]. Plant components like seed, stem, root, and leaf are commonly used in the production of metal-based NPs. Plant extract including phenolic acids, terpenoids, proteins, sugars, bioactive polyphenols and alkaloids are significantly contributing to the reduction of metallic ions and their stabilization [14]. The two most commonly utilized NPs are zinc oxide (ZnO NPs) and aluminum oxide nanoparticles (Al_2O_3 NPs) which have wide ranges of biological and agricultural applications [15], including wastewater treatment, biofiltration, drug delivery, the production of therapeutic cancer vaccines [16], increasing drought tolerance in plants via increasing leaf water contents and above-ground biomass [17]. Most studies demonstrated that ZnO can have different toxicological effects on some insect species which could serve in agriculture and pest control feild [18]. The largest reservoir of microorganism on the earth is referring to the soil microbial communities. They serve crucial functions to sustain soil health such as the biogeochemical cycling and decomposition

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of organic matter in the ecosystems. The disturbance of these processes can lead to compromised ecosystem functioning [19]. Soil population densities depends on pH changes, temperature, moisture content and physico-chemical characteristics of plant exudation bedside the agricultural management practices [20]. However, soil enzymes are participating in food chain cycle, organic matter decomposition, and contaminants degeneration. Therefore, it is very important to protect activity and stability of soil enzymes [21]. The capability of soil functioning within the ecosystems is known as soil quality which responsible for water and air quality maintenance, sustainability of plant and animal productivity, and supporting human well-being and heritage [22]. The approach of soil quality index (SQI) has been used as a quantitative tool for determination of the association between soil health and management goals [23]. The most frequent used biological indicators of soil quality are soil enzymes, microbial activity and biomass, soil respiration, nitrogen mineralization rates and ratios of bacteria to fungi [24]. Biological indicators considered as the main factor that govern soil quality because they are directly influence soil ecosystem processes [25] and their protection is a major challenge for greater nutrient turnover and soil disease quelling [26]. However, the sick soils are those with low carbon reservoir, nutrient and greater levels of xenobiotic chemicals like NPs [27]. In the environments, the concentration of most NPs still remains unknown and according to exposure modeling soil could become the vital sink of released NPs into the environment [28-30]. The toxicity of NPs to microorganisms is doubtless that raises serious concerns to microorganisms promoting plant growth and benefit nutrient cycling in soils such as nitrifying and denitrifying bacteria [31]. Whereas, previous studies demonstrated that the exposure to ZnO NPs has insignificant effects on soil physico-chemical features, including pH, electrical conductivity (EC) and organic matter content [32]. Presently the metal oxide NPs and their derivatives pose potential risks to human activity and to the soil ecosystems, but their effects on soil and microbial communities in the field condition-studies stayed unknown. Accordingly, ZnO and Al_2O_3 NPs were synthesized to confirm their possible safe use in modern agriculture alternative to chemical pesticides in a detailed study through their impacts on soil microbial communities, enzyme activities, biomass and respiration rate. The paper also uncovers more details on the effect of NPs on inhibition rate, toxicity index and soil quality index which are considered as the originality of the work.

2 Methods and Materials

2. 1 Soil sampling and processing

The used soil for the present study was taken from the upper layer at three points in an agricultural land with no history of pesticide use at Aski-Kalak area, Erbil, Iraq (36˚15' 22.9" N, 43˚38' 20.6" E) using GPS Accuracy ± 4m. This region is well-known by a semi-arid continental climate as very hot and dry in summer, and cold and wet in winter, with median yearly temperature 27.83°C and median precipitation 564mm. The soil type was classed as calcareous. The topsoil may contain 1-2% organic matter [33,34]. Samples were taken randomly at the depth (5-30 cm depth). The soil was characterized by pH (7.79); organic matter (12.5 g/kg); electrical conductivity (695 µS/cm); TDS 992.86 mg/l; particle size distribution: clay (52.1g/kg), sand (251.1g/kg) and sand (696.8g/kg) [35]. Eventually, rocks, plant matter, and wood chips were manually removed from the soil and they were composite into a single sample. The soil was sieved using 2mm mesh, and immediately preserved at 4°C until use.

2. 2 Experimental design and microcosm setup

Forty-two microcosms of 500 g dry soils were incubated in glass jars for 60 days, after adding of 0, 30, 60, 90, 120, 150 and 180 mg/l of biogenically produced Al_2O_3 NPs and ZnO NPs in two set of completely randomized design experiment. Each microcosm type was placed in triplicates and incubated at room temperature 25°C, during October 2022. The soil moisture was preserved at 60% field capacity using distilled-deionized water.

2. 3 Nanoparticle preparation and characterization

2. 3. 1 Aluminum oxide NPs (Al2O3 NPs)

The prepared Al_2O_3 powder by the method of [36] from the calcination of Iraqi kaolin via evaporation process was used during this study. X% of Al_2O_3 was prepared (X = 17.85, 28.05, 87.98 and 95.63). The annealed powder temperature-dependence of various Al_2O_3 dielectric constant was examined at the frequency-range of 1KHz – 1MHz which varied between 9.21 – 12.29 for 87.98% and 6.99 – 12.063 for 95.63% Al_2O_3 and changed between 22.195 – 151 for 17.85% and 13.015 – 21.09 for 28.05% Al_2O_3 powder-content samples. For the present study, different concentrations of Al_2O_3 NPs were prepared (30, 60, 90, 120, 150 and 180 mg/l).

2. 3. 2 Zinc oxide NPs (ZnO NPs)

The used method of ZnO NPs biosynthesis from *Musa paradisiaca* L. leaf extract was followed according to (Karim et al. 2025) [37]. The leaves were rinsed then dried, chopped, pasted, heated and inspired, filtered and air-dried (Figure 1). After that, zinc acetate di-hydrate [$Zn(CH_3CO_2)_2{\cdot}2H_2O$] was boiled with the plant extract, heated and stirred, pH calibrated to 8 by NaOH, past produced, nanoparticles assessed by calcination at 400°C, and then stored until use. Different concentrations were prepared as the same as mentioned above for Al_2O_3 NPs. The NPs then identified by UV-Visible spectroscopy, X-ray diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR), and Transmission electron microscopy (TEM) techniques having particle size of ~ 47 nm.

2. 4 Laboratory analysis

2. 4. 1 Physico-chemical properties

The soil samples were assessed for chemical properties in the laboratory at the Departments of Biology and Environmental Science and Health/ Salahaddin University. Soil pH, EC and TDS were determined according to (Ryan et al., 2001) [38] in 1:1 soil solution using electrochemical methods by pH, EC, TDS-meter

(JENWAY-Model). Walkley-Black method was applied for SOM as described by (Ryan et al., 2001) [38].

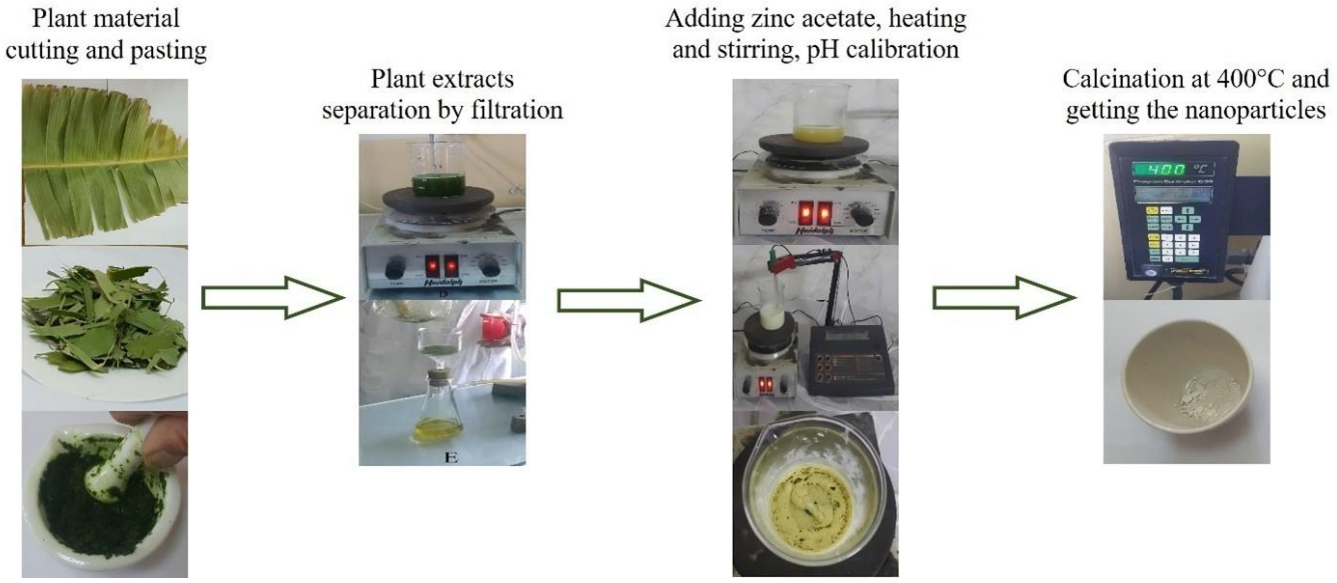

Figure 1: Preparation. of ZnO NPs from M. paradisiaca L. leaf extract.

2. 4 Laboratory analysis

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2. 4. 2 Microbiological analysis

Serial dilution from 10^{-1}-10^{-7} was performed for each treated soil in aseptic condition as given in (Bieganowski et al., 2015) [39]. Standard plate count was used for enumerating total bacteria, fungi and actinomycetes on nutrient agar, Sabouraud dextrose agar incorporated 0.3mg penicillin and water agar media respectively [40]. Total bacterial count explicated as bacterial number $\times 10^6$ cfu/g dry soil, while fungi and actinomycetes as total fungi and actinomycetes $\times 10^5$ cfu/g dry soil. However, free-living nitrogen-fixing bacteria and total *Azotobacter* sp. were counted using most probable number (MPN) technique [41] using nitrogen-free Jensen's broth and Ashbey's Mannitol broth respectively as described by (Khudhur and Sarmamy, 2016) [42]. The incubation period for total bacteria, fungi and actinomycetes, nitrogen-fixing bacteria and *Azotobacter* sp. were 24 h, 5 days and 2 weeks at 28±0.5°C respectively.

2. 4. 3 Estimation of biochemical parameters

2. 4. 3. 1 Dehydrogenase

Dehydrogenase activity was determined according to the method of Casida *et. al.*, 1964 given by (Małachowska-Jutsz and and Matyja, 2019) [43]. In test tubes, 10 g soil was mixed with 2.5 ml of phosphate buffer, 0.2 g $CaCO_3$, and 1 ml substrate (3% v/w TTC triphenyl tetrazolium chloride). Similarly, a blank sample was prepared with differencing in 1 ml of a 3% TTC solution phosphate buffer being instituted. The samples were then centrifuged at 3000 rpm for 10 min after incubation at 25°C for 24h. The liquid supernatant was discarded and the formed TPF was extracted with 5 ml of methanol for a few minutes. The liquid supernatant absorbance was measured for λ = 485 nm. A calibration curve designed in accordance with the standard method was used for estimation of TPF concentration and the results demonstrated as µg TPF/g dry soil/24h.

2. 4. 3. 2 Urease

The activity of urease was found out by Hoffmann and Teicher's modified method given by (Khudhur, 2018) [44]. Inside test tubes, 0.25 ml toluene, 0.75 ml citrate buffer (pH 6.7) and 1 ml of 10% urea substrate solution were added to a gram of soil, and incubated for 3h at 37°C. The formed ammonium solution was determined at λ = 636 nm [45]. Final results demonstrated as µg N-NH_4^+/g dry soil/3h.

2. 4. 3. 3 Nitrate reductase

The method of (Nath and Samanta, 2012) [46] was applied for nitrate reductase determination. 5 g of soil samples were inoculated with 50 ml of peptone water medium amended with 1% KNO_3 into 150 ml conical flasks, and then the flasks were incubated at 30°C for 3h. After centrifugation of each soil suspension at 5000 rpm for 10 min, the supernatants (1 ml) were

treated with 1 ml of sulphanilamide and left for 20 min. Then, 1 ml of N (naphthyl) Ethelene Diamine Dihydrochloride (NEDD) was poured to each sample and left to produce pink colour which then measured at λ = 540 nm. Media without inoculation by sulphanilamide and NEDD were used as blank. Results expressed in µg N-NO_2/g dry soil/3h.

2. 4. 3. 4 Catalase

Hydrogen peroxide decomposition method was utilized for catalase activity determination using $KMnO_4$ titration [47]. 40 ml of distilled water and 2g of oven-dried soil were mixed and put on rotary shaker followed by the addition of 5 ml of 0.3% H_2O_2 and shaking the slurry for 20 min at 150 rpm. To stabilize the remaining peroxide, 5 ml 3N H_2SO_4 was added and then 25 ml of filtered aliquots were titrated with 0.1N $KMnO_4$. The expressed results were as ml of 0.1N $KMnO_4$/g dry soil/20 min, equivalent to the decomposed peroxide per a gram of oven-dry soil.

2. 4. 3. 5 Basal soil respiration and biomass C

Soil respiration was estimated according to (Khopkar, 1998) [48]. The released CO_2 was absorbed into NaOH. Into glass jars, 100g of soil was placed and small flasks containing 0.05M NaOH were placed inside the soil and then the jars were sealed for 3 days. To the flasks, 2 ml of $BaCl_2$ and 1-2 drops of phenolphthalein indicator were added and then titrated with 0.1M HCl until the color changed from pink to colorless. The calculation of soil respiration was done according to this equation:

$$RCO_2 = \frac{2.2\,(Vb - Vp)}{Ms\,Wsd} \quad (1)$$

where:
RCO_2 is the CO_2 evolution rate as mg CO_2/g dry soil.
V*b* is the consumed HCl volume in the control as (ml).
V*p* is the consumed HCl volume in the test sample as (ml).
M*s* is the soil mass as (g).
2.2 is the factor in which 1 ml of 0.1M HCl corresponding to 2.2 mg of CO_2 as (mg/ml).
W*sd* is the dry mass fraction of the soil.
Final basal soil respiration (BAS) expressed as µgCO_2-C/g/h.
Biomass (*B*) µgC/g was determined in accordance with [49] by applying the below equation:

$$B\ (\mu g\ C/g\ soil) = 433 \times log_{10} BAS\ (CO_2/g\ soil/h) + 59.2 \quad r^2 = 71\ \%\ \text{at } 25\ ^\circ C. \quad (2)$$

where:
B is the biomass
BAS is the basal soil respiration

2. 5 Mathematical calculations

2. 5. 1 Calculation of inhibition rate %

The inhibition rate (*I*) was calculated according to [50] using the below equation:

$$I = \frac{Co - Ct}{Ct} \times 100 \quad (3)$$

where:
I is the inhibition rate, *Co* is the values of parameters in the control, and *Ct* is values of parameters in the NPs treatment.

2. 5. 2 Calculation of toxicity index (TI)

The toxicity index (TI) of the nanoparticles was calculated according to the below equation given by [51]:

$$TI = \sum \frac{Ci}{EC50i} \quad (4)$$

where:
C*i* is the concentration of NPs (mg/l), and EC50*i* is the NPs producing 50% reduction in *i* th parameter (inhibition data taken from Table 2 and 3).

2. 5. 3 Calculation of biochemical potential soil fertility coefficient (Mw)

Based on soil enzyme activity and *C* content, the biochemical potential soil fertility coefficient was calculated as given by [52] using the below formula:

$$Mw = \left(\frac{Urease}{10} + Dehydrogenase + Nitrate\ reductase + Catalse\right) \times C\% \quad (5)$$

2. 5. 4 Calculation of soil quality index (SQI)

Soil Quality Index (SQI) was calculated based on the normalization summation, bringing average of the studied soil quality indicator characteristics into a single uniter [53] using the below equation:

$$SQI = \sum (X\,Xmax^{-1})\, n^{-1} \quad (6)$$

where:
X is the value of any individual soil characteristic, *X* max is greatest value of that individual soil characteristic, and *n* is the total number of soil characteristics applied in the calculation (13 included in this study). The ranges of SQ index is from > 0 to <1.

2. 5. 5 Statistical analysis

Statistical expressions were done using Microsoft Office Excel 2019 and the data represented as mean ± standard error. For comparison, control was used for every experimental value. Results were resolved by one-way ANOVA accompanied by Duncan test at significant level 0.05 [54] applied on Statistical Package for Social Sciences (SPSS) Version 26.

3 Results and discussion

3. 1 Physico-chemical parameters

Physico-chemical parameters are presented in Table 1. Significant differences in pH values were observed in different treatments following application of Al_2O_3 NPs and ZnO NPs. The highest pH values were 8.23 in both 150 and 180 mg/l amended soils with Al_2O_3 NPs and amended soils with 180 mg/l ZnO NPs. However, the lowest values were 8.19 in control and 30 mg/l amended soils with ZnO NPs. As nanoparticle concentrations increased, the soil pH was also increased and this refer to the dissolution of the ions of Zn^{2+} and Al^{+3} from nanoparticles that declined protons and increased soil pH. Similar pH increment after ZnO Nps addition were described by (Waalewijn-Kool et al., 2013) [55] and (Tourinho et al., 2013) [56]. The relation between the nanoparticle concentrations with soil pH may refer to the sorption of Zn and Al to the soil and their solubility beside to the content of clay in the calcareous soil which raised the conservation of Zn and Al in the soil [57].

The total soluble salts provide by EC and TDS were significantly affected by NPs application. Greater EC and TDS values were observed in 180 mg/l amended soils with both Al_2O_3 NPs and ZnO NPs (Table 1). EC values were 180 and 193.9µS/cm 25 °C and TDS values were 277 and 257.2 mg/l for both Al_2O_3 and ZnO NPs treatments respectively. Similarly, (Verma et al., 2022) [58] recorded significantly high EC upon ZnO NPs application at the rate of 2.5 mg/l. The main experimental results showed EC go up with increasing NP concentration. Basically, the mechanisms expressed as electric double layer effect and nanoparticles' conductance increase. Moreover, the rise in the electrical conductivity may also refer to the ionic concentration dependence and other physico-chemical properties, the conduction improvement mechanisms inside the dissolution, the elevated electrophoretic potency of the NPs caused by the organized particles' nano-dimensions and the elevation in the concentration of NP direct a raised conducting pathways in the nanofluid [59].

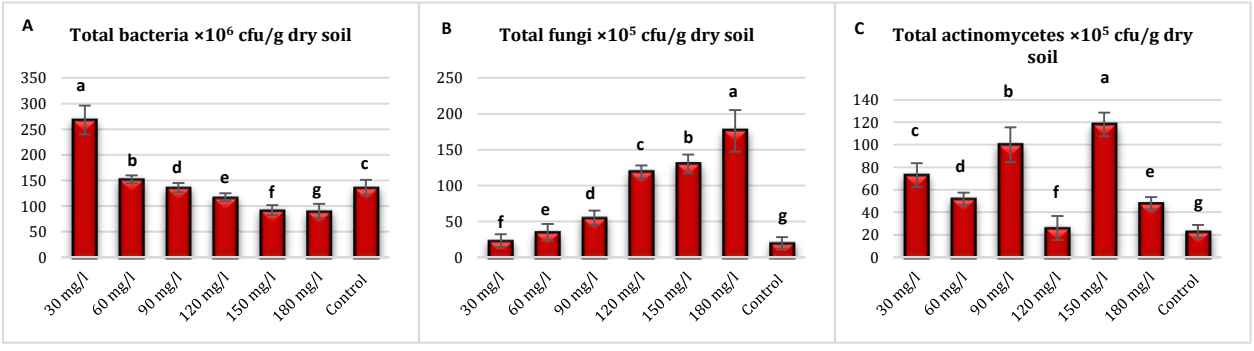
Soil organic matter (SOM) may influence nanoparticle fate and effects in soil. Highest soil organic matter was 7.8 gm/kg amended soils with 180 mg/l of Al_2O_3 NPs and 8.7 gm/kg amended soils with 180 mg/l of ZnO NPs (Table 1). The researchers (Ben-Moshe et al., 2013) [60] stated that the effects of nanoparticles on the properties of soil organic matter are rare. However, the relation of SOM with nanoparticles may refer to their sorption to soil particles strongly [31]. As a consequence of their high surface-area and smaller size they are properly be more absorbable about 15–20 times greater in comparing to their ion's share units (Rajput et al., 2020) [61].

Table 1: Soil physico-chemical parameters under the effect of different Al2O3 and ZnO NPs concentrations.

Type of NPs		Al_2O_3 NPs							ZnO NPs						
Concentrations		30 mg/l	60 mg/l	90 mg/l	120 mg/l	150 mg/l	180 mg/l	Control	30 mg/l	60 mg/l	90 mg/l	120 mg/l	150 mg/l	180 mg/l	Control
Parameter	pH	8.20^{cd}	8.21^{bc}	8.21^{bc}	8.22^{ab}	8.23^{a}	8.23^{a}	8.19^{d}	8.19^{e}	8.2^{cd}	8.2^{cd}	8.21^{bc}	8.22^{ab}	8.23^{a}	8.19^{de}
	EC µS/cm 25 °C	147.3^{g}	151.4^{e}	155.5^{d}	166.3^{c}	175.9^{b}	180.0^{a}	171.0^{f}	167.7^{g}	174.6^{e}	188.0^{d}	188.4^{c}	189.5^{b}	193.9^{a}	171.0^{f}
	TDS mg/l	239.5^{g}	249.4^{e}	268.6^{d}	269.1^{c}	270.7^{b}	277^{a}	244.3^{f}	210.4^{g}	216.3^{e}	222.1^{d}	237.5^{c}	251.3^{b}	257.2^{a}	244.3^{f}
	SOM gm/kg	5.4^{e}	4.9^{f}	7.3^{d}	7.4^{c}	6.4^{b}	7.8^{a}	4.7^{g}	4.4^{f}	6.2^{d}	6.2^{d}	7.4^{c}	8.4^{b}	8.7^{a}	4.7^{e}

3.2 Microbiological parameters

Amended soils with 30 and 60 mg/l of Al_2O_3 NPs showed significant increases in total bacterial counts by 268 and 152×10^6 cfu/g dry soil as compared with control (Figure 2-A). All concentrations of Al_2O_3 NPs showed significant fungal count increase in comparing with control (Figure 2-B) in which 180 mg/l of Al_2O_3 NPs showed the highest total fungal count by 176×10^5 cfu/g dry soil. While actinomycetes count showed different results, highest count was in 150 mg/l amended soils with count of 118×10^5 cfu/g dry soil (Figure 2-C).



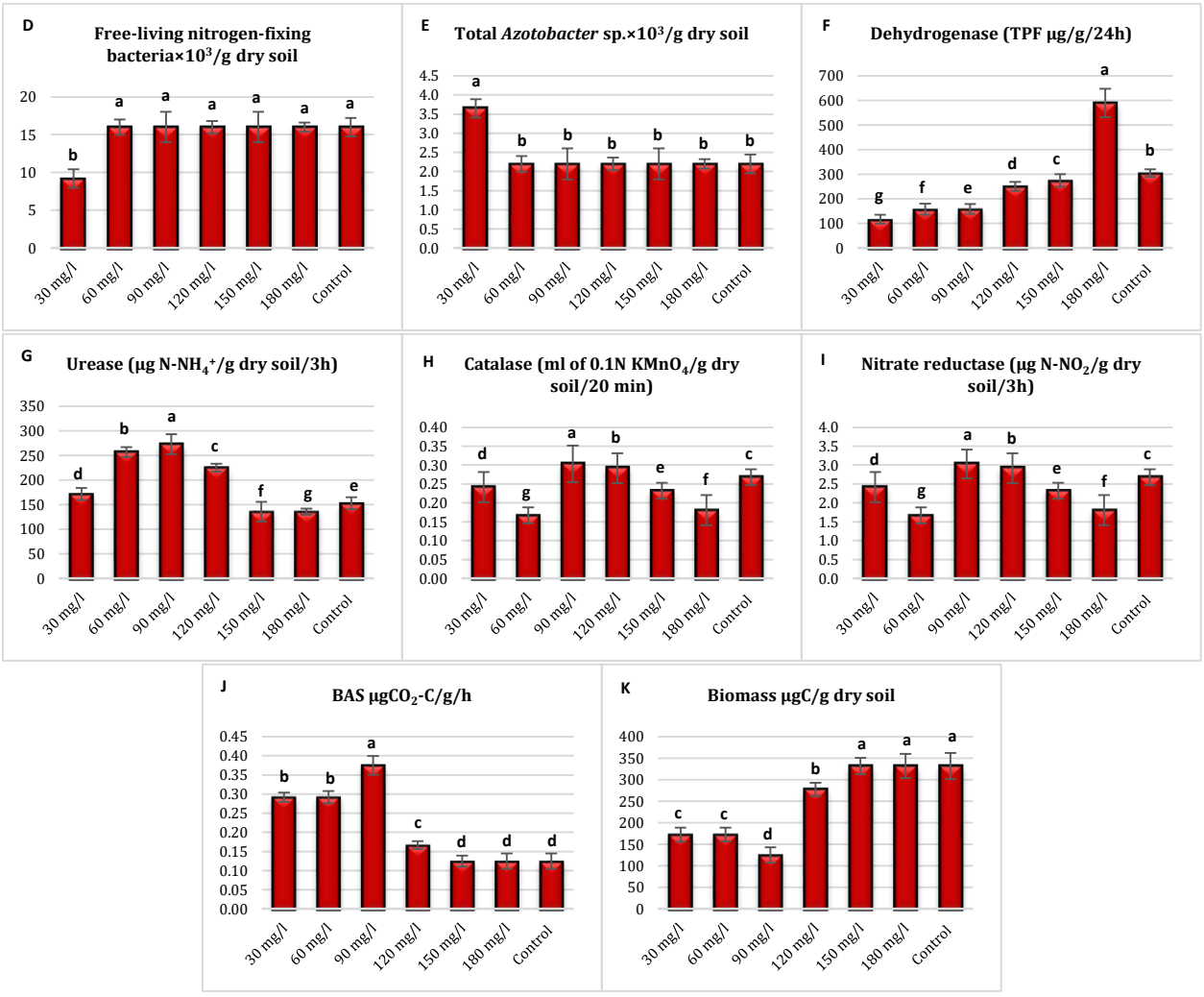
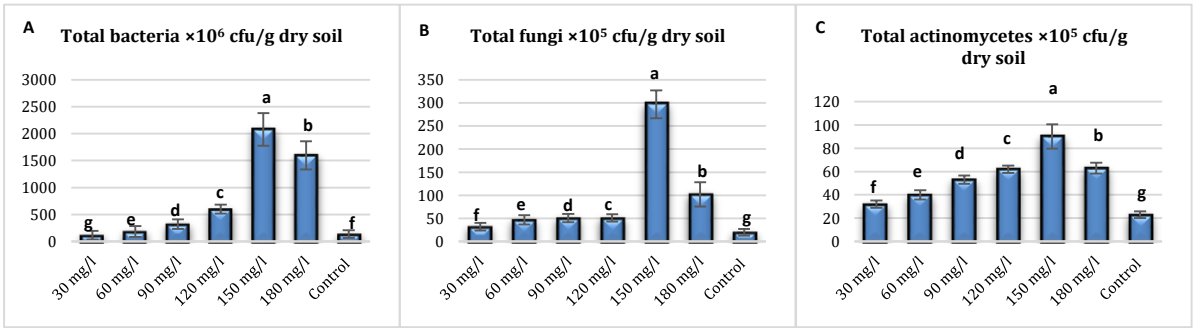


Figure 2 (A-K): Different concentrations' effect of Al2O3 NPs on soil biological and biochemical properties.

However, all the used concentrations of ZnO NPs have significantly increased total bacterial counts except for 30 mg/l. The highest count was observed in the amended soils with 150 mg/l of ZnO NPs by a count of 20.8×10^8 cfu/g dry soil as compared with control (Figure 3-A). All the amended soils with ZnO NPs concentrations showed significant fungal and actinomycetes count increase in comparing with control (Figure 3-B and C). Whereas, 150 mg/l treated soils showed the highest counts of both fungi and actinomycetes were 297 and 90×10^5 cfu/g dry soil respectively.



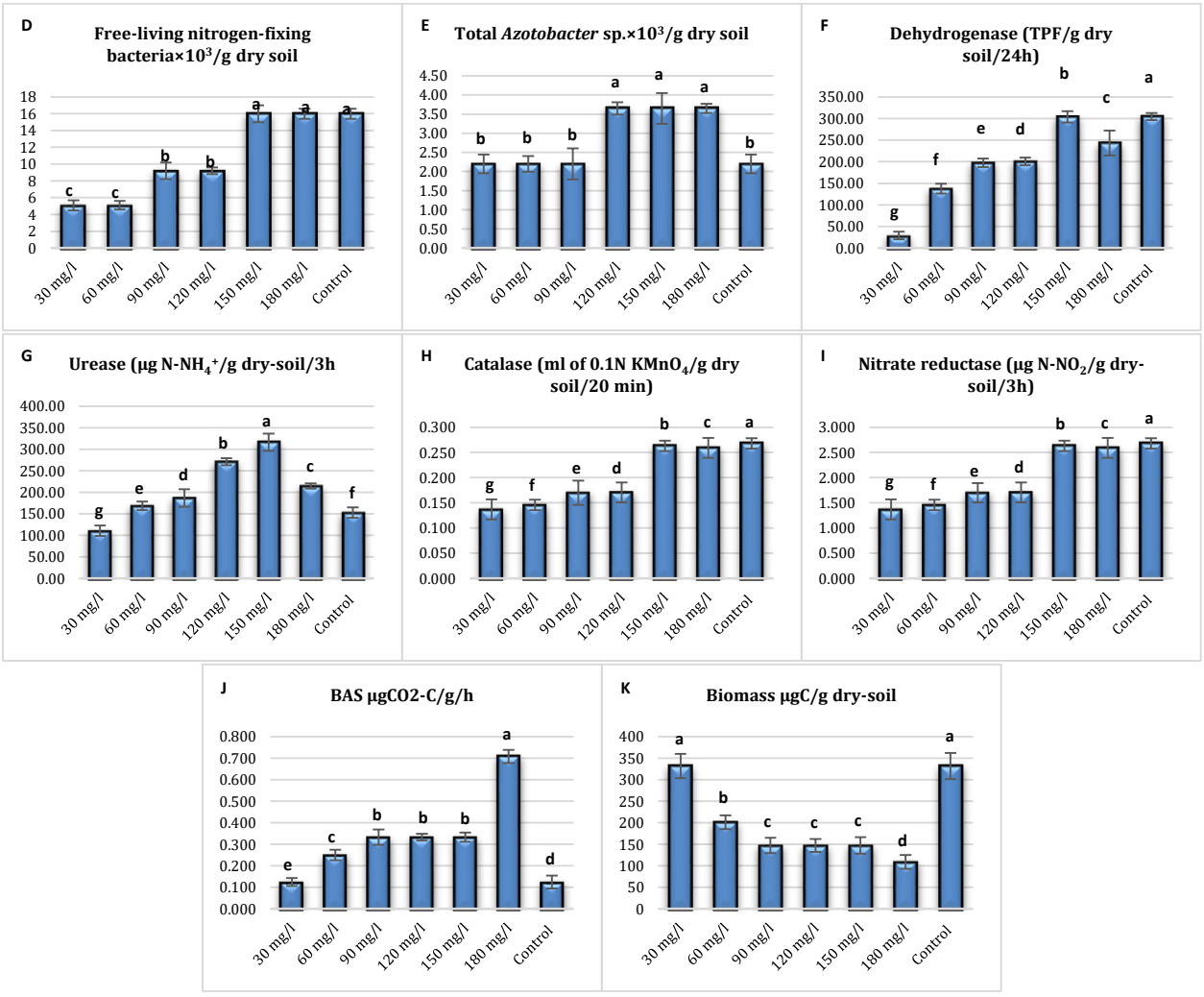


Figure 3 (A-K): Different concentrations' effect of ZnO NPs on soil biological and biochemical properties.

Generally, there is no sufficient literature on the effect of Al_2O_3 NPs on soil microbial activities, whereas most of the studied showed the unfavorable effects of Al_2O_3 NPs on the microorganisms of soil [62]. We can say that the present observations are in line with those of (Li et al., 2021) [63] who observed that 50 mg/l Al_2O_3 NPs has reduced microbial population and enzymatic activity. While (Fajardo et al., 2014) [64] observed no significant toxicity at any concentration of Al_2O_3 NPs or time assayed on soil microorganisms in an in vitro study. In contrast, (Verma et al., 2022) [58] observed significantly lesser soil bacterial count (12.33×10^5 cfu) in case of applying 2.5 mg/l of ZnO NPs when compared with control (21.33×10^5 cfu). Moreover, the consonant reduction in the colonies of fungi and actinomycetes was 24.16, 37.35, 46.15% and 14.59, 17.97, 22.45% with the application of 0.5, 1.25 and 2.5 mg/l, respectively, as compared to control. This antimicrobial activity may refers to the ability of ZnO NPs to argue the production of reactive oxygen species (ROS) and can bring on the cell death in case of exceeding the antioxidative capacity of the cell [65]. Whereas, the statement of [57] may confirm our findings who stated that microbial communities were self-adapted over time. They found that 1000 mg/l of ZnO NPs improved bacterial growth unaccompanied by obvious alterations in their metabolic profiles in time with an adequate correlation between respiration rates and metabolic activities of microorganisms. The view of this research suggests further studies to find out the effects of ZnO NPs in natural soil microorganism populations for prolonged periods.

The soils treated with 30 mg/l of Al_2O_3 NPs revealed the lowest free-living nitrogen fixing bacterial count of 9.2×10^3 bacteria/g dry soil in comparing with the other treatments (Figure 2-D). While in case of ZnO NPs, the free-living nitrogen fixing bacterial count were reduced following the treatment of the soils with 30-120 mg/l of ZnO NPs. However, 150 and 180 mg/l showed no differences from control (Figure 3-D). The toxicity of both Al_2O_3 and ZnO NPs on soil free-living nitrogen fixing bacteria remain unexplained and need further investigation.

The concentration 30 mg/l of Al_2O_3 NPs revealed the highest significant increase in total *Azotobacter* sp. count which was 3.65×10^3 bacteria/g dry soil in comparing with the other treatments (Figure 2-E). Whereas, the concentrations 120-180 mg/l of ZnO NPs significantly increased total *Azotobacter* sp.

count with values of 3.65×10^3 bacteria/g dry soil when compared with control (Figure 3-E). In contrast [66] showed that ZnO NPs was diminished the numbers of soil *Azotobacter*, K-solubilizing, and P-solubilizing bacteria. However, [67] observed that prevalent soil nitrogen-fixers like *Azotobacter*,*Rhizobium*, *Beijerinckia*, and *Bradyrhizobium* were not affected by the existence of Ag and ZnO NPs. Whereas, (Chavan and Nadanathangam, 2019) [66] observed that ZnO NPs to impede thermogenic metabolism, decline *Azotobacter*, K-solubilizing, and P-solubilizing population and hinder the activity of enzymes.

3.3 Biochemical parameters

Various results were shown in soil enzymatic activities in response to Al_2O_3 NPs concentrations. The concentration 180 mg/l showed the greatest significant increase in dehydrogenase activity with value of 589.2 µg TPF/g dry soil/24h (Figure 2-F), while the other concentrations reduced dehydrogenase activity when compared with control. However, all the concentrations of ZnO NPs revealed significant decrease in dehydrogenase activities when compared with control (Figure 3-F). The concentration 30 mg/l showed the greatest reduction in dehydrogenase activity to 28.96 µg TPF/g dry soil/24h. Previous studies showed that metal NPs could immediately penetrate the cells and greatly destroy the DNA and the synthesis of protein, as well as damage redox reactions and organelle functions. These mechanisms are likely lead to decrease dehydrogenases activity in the soil which depend on NP size and the class of the enzyme [68]. By the present study, it was observed that the activity of dehydrogenases was more susceptible to ZnO than Al_2O_3 NPs pollution. The higher sensitivity of dehydrogenases activity to ZnO NPs among the other types of NPs was also confirmed by (Lin et al., 2022) [69] and (Ben-Moshe et al., 2013) [58].

The concentrations 30-120 mg/l of Al_2O_3 NPs showed significant increases in urease activities when compared with control (Figure 2-G). The highest urease value was 272.88 µg N-NH_4^+/g dry soil/3h in 90 mg/l amended soils. Whereas 150 and 180 mg/l of Al_2O_3 NPs reduced urease activities to 135.9 µg N-NH_4^+/g dry soil/3h. All the concentrations of ZnO NPs revealed significant increase in urease activities except for 30 mg/l which reduced urease activity to 111.3 µg N-NH_4^+/g dry soil/3h in comparing with control (Figure 3-G). The highest urease value was 316.44 µg N-NH_4^+/g dry soil/3h in 150 mg/l amended soils. (Qian et al., 2016) [70] observed that of 1-500 mg/l Al_2O_3 NPs have no significant detrimental impact on soil microbial community and the activity of urease. Moreover, studies of (Du et al., 2011) [71], (Qian et al., 2016) [70], (Asadishad et al., 2018) [72] and (Lin et al., 2022) [69] showed no effect of ZnO NPs on urease activity. While, [73] showed that concentrations of NPs upon or equal to 100 mg/l inhibited β-glucosidase and urease activities. A consent order of potential toxicity of NPs to soil enzyme have infered due to the complexity of soil environments and wide variety of NPs. There is no agreement on whether NPs have negative or positive effects on soil enzymes. NP effects on soil enzymes rely on their type, shape, size, concentration, and stability [69]. Other types of NPs like Ag NPs showed to decrease urease activity in soil as in the studies of (Shin et al., 2012) [74], (Huang et al., 2018) [75] and (Xiaohong et al., 2021) [76].

Different responses of both catalase and nitrate reductase activities were observed toward Al_2O_3 NPs concentrations (Figure 2-H and I); so, the concentrations 90 and 120 mg/l increased both catalase to 0.303 and 0.292 ml of 0.1N $KMnO_4$/g dry soil/20 min and nitrate reductase to 3.030 and 2.920 µg N-NO_2/g dry soil/3h respectively, while the remaining concentrations reduced both catalase and nitrate reductase activities in comparing with control and the state of nitrate reductase inhibition come in line with [77]. While, all the concentrations of ZnO NPs have reduced both catalase and nitrate reductase activities when comparing with control (Figure 3-H and I). Similar observations given by (Du et al., 2011) [71] who observed that ZnO NPs was repressed the activities of soil catalase, protease, and peroxidase. Whereas, no literatures were found on ZnO NPs effects on nitrate reductase activity. While studies on the reducing effects of CuO and Ag NPs on nitrate reductase concluded by (Zhao et al., 2020) [78] and (Xiaohong et al., 2021) [76] respectively. The mechanism whereby NPs inhibit soil enzymatic activities may directly refer to their interaction with soil extracellular enzymes without disrupting the microbes. Whereas, the intracellular enzyme activity is complex and repeatedly associated with the viable microbes, tiny NPs can penetrate cell-membrane and associate with intracellular enzymes promptly. While, indirectly NPs may interrupt intracellular enzymes through impairing microbial cell and terminating enzyme synthesis by via apoptosis to microbial cell [69].

Basal soil respiration (BAS) showed various responses to Al_2O_3 NPs. The concentrations 30-120 mg/l of significantly increased BAS when compared with control, and 90 mg/l showed the greatest BAS increase (0.375 µgCO_2-C.g^{-1}.h^{-1}) in comparing with control (Figure 2-J). However, all the concentrations of ZnO NPs have increased basal soil respiration (BAS) except for 30 mg/l. The increase was shown by 180 mg/l which was 0.708 µgCO_2-C.g^{-1}.h^{-1} (Figure 3-J). In contrary, studies of (Rashid et al., 2017 and García-Gómez et al., 2018) [79], [57] showed inhibition of soil respiration during NPs application. Different responses to NPs may refer to different types of NPs and their size, exposure concentration, duration of exposure to NPs and soil attributes such as SOM, pH and texture particularly clay content. The toxic NPs can decline the associated soil enzymes to element cycling and biochemical reactions, and provoke soil respiration and microbial biomass [69]. Accordingly, in the study of (Hänsch and Emmerling, 2010) [80] basal soil respiration was grow up with growing up the application-rate of Ag NP.

Microbial biomass is a delicate constituent of SOM and play crucial role in the cycling of nutrients and elements in the soil [1,81]. During the present study, the biomass C was reduced by the concentrations 30-120 mg/l of Al_2O_3 NPs in comparing with control (Figure 2-K). While the concentrations 150 and 180 mg/l showed no significant difference from the control. However, biomass C was reduced by all the concentrations of ZnO NPs except 30 mg/l which showed no significant differences from control and the value was 332 µgC/g (Figure 3-K) as the same results of [80] who showed no treatment impacts for microbial

biomass and soil organic C. Furthermore, results of [50], [82], [83], [69] and [58] also come in agreement with the present observations in regard to the biomass reduction by ZnO NPs. This could refer to inadequate microbial activity during NP application due to inhibition of soil microorganisms which cause slighter decomposition of SOM and resulting in low percentage of organic carbon. In this regard, (Lin et al., 2022) [69] performed a global meta-analysis on the base of 73 publications and 1820 matched experimental detections on alterations in soil enzyme, microbial biomass carbon (MBC) and respiration in response to various type, concentration and duration of exposure to NPs as well as and soil characteristics. They supposed that toxic NPs can reduce soil enzyme activities due to biochemical reactions and elemental cycles, thus reducing MBC and soil respiration.

3.4 Indices results

The inhibition rate of the studied properties following soil treatment with Al_2O_3 and ZnO NPs were determined and presented in Tables 2 and 3. About Al_2O_3 NPs, the concentration 30 mg/l of reduced free-living nitrogen-fixing bacteria by 73.91%, dehydrogenase by 159.5%, catalase 10.7%, nitrate reductase 9.6% and biomass C by 92.4% (Tables 2). The concentration 60 mg/l reduced dehydrogenase by 92.4%, catalase 60.5%, nitrate reductase 61.6% and biomass C by 92.4%. However, 90 mg/l reduced dehydrogenase and biomass C by 91.4. While, 120 mg/l reduced total bacteria by 16.2%, dehydrogenase by 21.1% and biomass C by 19.5%. On the other hand, 150 mg/l reduced all of total bacteria and the enzymatic activities of dehydrogenase, urease, catalase and nitrate reductase by 47.8, 10.8, 12.5, 15.6 and 15.5% respectively. While the concentration 180 mg/l reduced total bacteria by 51.1% and urease, catalase and nitrate reductase by 12.5, 48.1 and 49.1% respectively.

Table 2: Inhibition rate % in soil biological and biochemical properties under the effect of different concentrations of Al2O3 NPs.

	Concentrations	30 mg/l	60 mg/l	90 mg/l	120 mg/l	150 mg/l	180 mg/l
Parameter	Total bacteria $\times10^6$ cfu/g dry-soil	-	-	-	16.2	47.8	51.1
	Total fungi $\times10^5$ cfu/g dry-soil	-	-	-	-	-	-
	Total actinomycetes $\times10^5$ cfu/g dry-soil	-	-	-	-	-	-
	Free-living nitrogen-fixing bacteria$\times10^3$/g dry-soil	73.9	-	-	-	-	-
	Total *Azotobacter* sp.$\times10^3$/g dry-soil	-	-	-	-	-	-
	Dehydrogenase (TPF/g dry-soil/24h)	159.5	92.4	91.4	21.1	10.8	-
	Urease (µg N-NH_4^+/g dry-soil/3h)	-	-	-	-	12.5	12.5
	Catalase (ml of 0.1N $KMnO_4$/g dry-soil/20 min)	10.7	60.5	-	-	15.6	48.1
	Nitrate reductase (µg N-NO_2/g dry-soil/3h)	9.6	61.6	-	-	15.5	49.1
	BAS µgCO_2-C/g/h	-	-	-	-	-	-
	Biomass µgC/g dry-soil	92.4	92.4	165	19.5	-	-

Moreover, ZnO NPs concentrations showed different inhibition rate percentages (Table 3). The concentration 30 mg/l reduced all of total bacteria, free-living nitrogen-fixing bacteria, dehydrogenase, urease, catalase and nitrate reductase by 18.3, 213.7, 136.1, 37.3, 95.6 and 94.5% respectively. Regarding to this, (Jiang et al., 2021) [32] showed that soil bacterial count are more sensitive to ZnO NPs than fungal population may confirm the finding of total bacterial count reduction. The concentration 60 mg/l reduced free-living nitrogen-fixing bacteria, dehydrogenase, catalase, nitrate reductase and biomass C by 213.7, 120.9, 82.6, 83.5, 64.7% respectively. In this regard, (Wyszkowska, 2002) [50] showed different inhibition pattern. They observed inhibition in dehydrogenase activity in various ZnO NPs treatments including 10, 50, and 100 mg/l of ZnO NPs. The concentration 90 mg/l reduced free-living nitrogen-fixing bacteria, dehydrogenase, catalase, nitrate reductase and biomass C by 73.9, 54.4, 57.7, 56.6, 125.1% respectively. The observations of (Khudhur and Sarmamy, 2019) [82] seem to confirm the finding of inhibition of microbial biomass C by 27.0 to 33.5% in 100 mg/l ZnO NPs treated soil. This is also endorsed by the fact that the ZnO NPs induced higher soil respiration rate under the effect of ZnO NPs treatments. The concentration 120 mg/l reduced free-living nitrogen-fixing bacteria, dehydrogenase, catalase, nitrate reductase and biomass C by 73.9, 51.9, 55.7, 56.7, 125.1% respectively. The concentration 150 mg/l reduced dehydrogenase, catalase, nitrate reductase and biomass C by 0.3, 1.9, 1.9, 125.1% respectively. However, the concentration 180 mg/l reduced dehydrogenase, catalase, nitrate reductase and biomass C by 25.1, 3.5, 3.5, 203.8% respectively. The study of (Kooch and Sohrabzadeh, 2024) [84] revealed that the presence of ZnO NPs in the soil produced a remarkable decline of 4.44%to54.5% in microbial biomass C when compared to the fresh soil. These observations greatly recommend that soil microbial communities were threatened by raised detrimental effect caused by ZnO NPs.

Results of toxicity index (TI) of Al_2O_3 NPs are presented in (Table 4). 30 mg/l showed toxicity on free-living nitrogen-fixing bacteria and dehydrogenase activity by values 0.41 and 0.19 respectively. However, 60 mg/l showed toxicity on dehydrogenase, catalase and nitrate reductase and 90 mg/l showed toxicity index value of 0.33 on dehydrogenase enzyme. Whereas, the toxicity index of 180 mg/l was 3.52 on total bacteria.

Table 3: : Inhibition rate % in soil biological and biochemical properties under the impact of different concentrations of ZnO NPs.

	Concentrations	30 mg/l	60 mg/l	90 mg/l	120 mg/l	150 mg/l	180 mg/l
Parameter	Total bacteria $\times 10^6$ cfu/g dry-soil	18.3	-	-	-	-	-
	Total fungi $\times 10^5$ cfu/g dry-soil	-	-	-	-	-	-
	Total actinomycetes $\times 10^5$ cfu/g dry-soil	-	-	-	-	-	-
	Free-living nitrogen-fixing bacteria$\times 10^3$/g dry soil	213.7	213.7	73.9	73.9	-	-
	Total *Azotobacter* sp.$\times 10^3$/g dry soil	-	-	-	-	-	-
	Dehydrogenase (TPF/g dry soil/24h)	136.1	120.9	54.4	51.9	0.3	25.1
	Urease (µg N-NH_4^+/g dry soil/3h)	37.3	-	-	-	-	-
	Catalase (ml of 0.1N $KMnO_4$/g dry soil/20 min)	95.6	82.6	57.7	55.7	1.9	3.5
	Nitrate reductase (µg N-NO_2/g dry soil/3h)	94.5	83.5	56.6	56.7	1.9	3.5
	BAS µgCO_2-C/g/h	-	-	-	-	-	-
	Biomass µgC/g dry soil	-	64.7	125.1	125.1	125.1	203.8

Table 4: Toxicity index (TI) of Al2O3 NPs on soil microbial and biochemical parameters.

	Concentrations	30 mg/l	60 mg/l	90 mg/l	120 mg/l	150 mg/l	180 mg/l
Parameter	Total bacteria	-	-	-	-	-	3.52
	Free-living nitrogen-fixing bacteria	0.41	-	-	-	-	-
	Dehydrogenase	0.19	0.32	0.33	-	-	-
	Catalase	-	0.50	-	-	-	-
	Nitrate reductase	-	0.50	-	-	-	-

ZnO NPs showed toxicity on dehydrogenase, catalase, nitrate reductase and free-living nitrogen-fixing bacteria at concentrations 30-120 mg/l (Figure 4). The highest TI was observed in 120 mg/l on free-living nitrogen-fixing bacteria by a value of 1.62. The toxicity of NPs on the microorganisms and enzymatic activity refer to several mechanisms including the nano-size of NPs that lead to membrane dis-organization, creation of reactive oxygen species (ROS), and oxidative DNA damage and liberate of the toxic-ions [65].

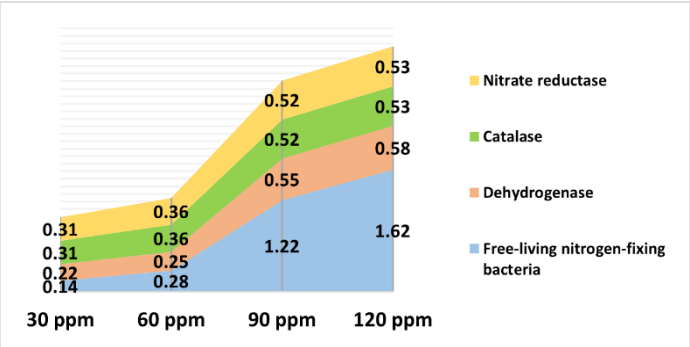


Figure 4: Toxicity index (TI) of ZnO NPs on soil microbial and biochemical parameters.

Biochemical Potential Soil Fertility Coefficient (Mw) was calculated from the enzymatic activities and organic carbon contents of the treated soils with different concentrations of both Al_2O_3 and ZnO NPs. The highest Mw was 274 observed in the amended soils with 180 mg/l of Al_2O_3 NPs followed by 150 mg/l which showed Mw of 209 (Figure 5). The concentrations 30 and 60 mg/l showed significantly lower Mw values when compared with control.

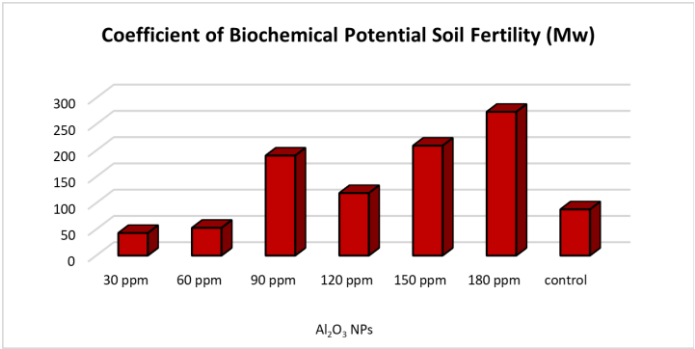


Figure 5: Coefficient of biochemical potential soil fertility under the effect of different concentrations of Al2O3 NPs.

Whereas, Mw showed different results in response to ZnO NPs concentrations. The highest Mw was 331 observed in the amended soils with 90 mg/l (Figure 6). The concentrations 30, 60 and 120 mg/l showed significantly lower Mw values when compared with control. Present results reported that the studied NPs produced biochemical changes in the soil on the base of more

precise index known as potential biochemical soil fertility index (Mw) comprising the activities of dehydrogenase, catalase, urease, nitrate reductase and organic carbon content in the soil.

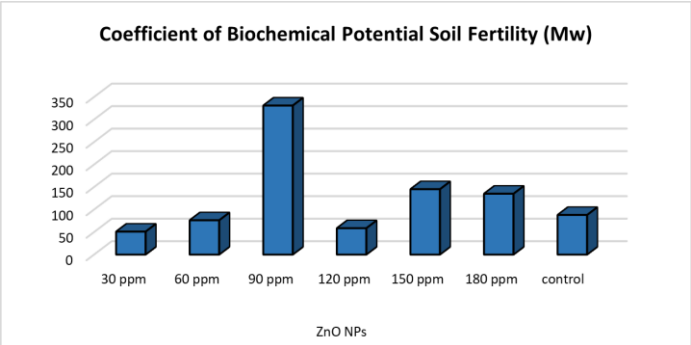


Figure 6: Coefficient of biochemical potential soil fertility under the effect of different concentrations of ZnO NPs.

Soil quality index (SQI) values showed various results in response to both Al_2O_3 and ZnO NPs concentrations (Figure 7). Highest SQI of 0.792 was observed in the amended soils with 90 mg/l of Al_2O_3 NPs, while the highest SQI in response to ZnO NPs was 0.978 observed in the amended soils with 150 mg/l. Biological indicators are considered as the factors affecting soil quality, they are markedly important because soil organisms instantaneously affect the operations of soil ecosystem, particularly SOM decomposition and nutrient cycling. Thus, any factor that affects soil microbial biomass, microbial action and population would surely affect soil quality and sustainability [85].

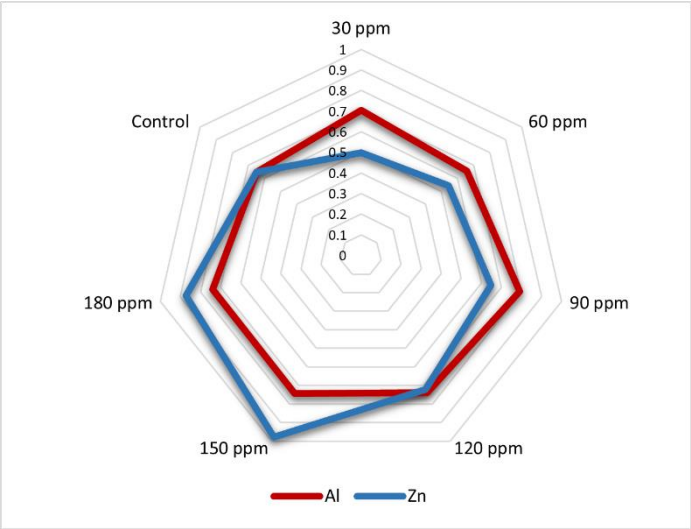


Figure 7: Soil quality index (SQI) under the effect of different concentrations of Al2O3 and ZnO NPs.

4 Conclusion

Expanded application of NPs may threaten beneficial microorganisms like free-living nitrogen-fixing bacteria. The examining of microbial activity and biomass can throw light on the deleterious effects of NPs on soil enzyme activity. Microbial biomass and soil respiration are well-known sensitive indicators of soil microbial response toward environmental disorders on soil quality and functioning. Up to now a consensus has not been attained on soil enzyme activity feedbacks toward NPs exposure. Therefore, the effects of exposure to NPs on the correlations of soil enzymes with microbial activity and biomass should be further synthetically checked out, alarmingly the use of NPs could have deleterious impacts on biodegradative operations of microorganisms in a scene of soil remediation. There is a need for more investigation by using more environmentally sensitive concentrations of NPs and more realistic exposure conditions. Furthermore, the discovery of soil parameters controlling the bioavailability of NPs is primitive for a better environmental risk assessment. NPs may affect the soil properties (SOM, pH, and clay content) which intern may affect the behavior of NPs in the soil. Nevertheless, there is a progressing debate about the influences of NPs on soil enzymes, and there is no NPs-specific universal meta-analysis yet. Present discoveries are of grateful help towards building a comprehension about the inherent environmental risks of metal oxide NPs with referring to soil quality index and coefficient of biochemical potential soil fertility results.

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Authors contribution

The author developed and wrote the original paper text, achieved the data analysis, appraised the contents of the manuscript and offered perceptive editing recommendations.

Conflict of interests

The authors declare no conflicts of interest.

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