SOIL SCIENCE ANNUAL
2024, 75(3), 193449

https://doi.org/10.37501/soilsa/193449

Appropriate agro-environmental strategy for ZnO-nanoparticle foliar application on soybean

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Received: 2024-05-14
Accepted: 2024-09-17
Published online: 2024-09-17
Associated editor: Jacek Antonkiewicz

Keywords:

Sustainable agriculture
Soybean
ZnO nanoparticles
Fluvisols
Soil-metals phytoavailability
Soil microbial respiration
Pollen viability

Abstract

Although the nanoparticle (NP) utilization in agronomy is currently orientated to intensify crop yield, the potential negative effects on soil and plant reproductive organs, including effects on pollen are largely absent in the literature. For this reason, our study was set to evaluate the impact of ZnO nanoparticles (ZnO-NPs) on the selective properties of Fluvisol, on the direct microbial activity and zinc (Zn) phytoavailability, and crop yield after their foliar application on soybean [*Glycine max* (L.) Merril] under field conditions. Additionally, the potential hazardous impact to plant reproductive structures was evaluated, focusing on the agronomically and environmentally sensitive biomarker – pollen viability. The soil biological activity was evaluated through microbial respiration while Zn phytoavailability was determined using reaction agents with nutrients analysis conducted through flame atomic absorption spectroscopy (F-AAS). Pollen viability was evaluated using the iodine potassium iodide (IPI) test. The experiments were carried out at an experimental site of the Faculty of Agrobiology and Food Resources (FAFR) at the Slovak University of Agriculture (SUA) in Nitra, located in Central Europe, during the 2023 vegetation season. Depending on increasing concentrations of ZnO-NPs through order of 1.4, 14, and 140 $mg \cdot L^{-1}$, revealed no harmful effect on soil microbial activity or hazardous Zn accumulation in the context of its Fluvisol-phytoavailable distribution compared to NPs-free control. A positive impact on soybean pollen viability was observed at all applied ZnO-NP concentrations compared to the NP-free control. The highest pollen viability, reaching up to 97.04%, was achieved at a concentration of 1.4 $mg \cdot L^{-1}$, and, subsequently, it slightly decreased with increasing concentrations of ZnO-NPs. Moreover, the application of ZnO-NPs had a positive impact on soybean weight of thousand seeds and seed yield, where it's the highest concentration was the most effective. Thus, our results directly demonstrate the positive efficiency on selective properties of soil and reproductive structure – pollen, where ZnO-NP spray application acted positively and stimulatingly. Additionally, ZnO-NPs had positive impact on weight of thousand seeds (TSW) and seed yield. Therefore, the use of nanoparticles in foliar applications could be considered as kind of novelty in precision and sustainable agriculture.

1. Introduction

Progress in nanotechnology has been already integrated into various agricultural disciplines, particularly in the case of applying nano-fertilizers (NF) in the form of metal-based nanoparticles (NPs) containing essential micronutrients such as Zn (Liu and Lal, 2015). The application of NF with dimensions typically under 100 nm is currently significant due to their potential for good effect at low concentrations, gradual release, and more targeted effects compared to chemically analogues counterparts in the lager-sized forms or ionic solutions (Kolenčík et al., 2024). These size-diverse forms of NF have various advantageous physicochemical and biological properties (Mousavi Kouhi et al., 2014).

Zinc oxide nanoparticles (ZnO-NPs) are an ubiquitous material used in various industrial applications, including glass and ceramic production, food additives, and pigments (Kołodziejczak-Radzimska and Jesionowski, 2014). According to Gottschalk et al. (2009), its annual input into the environmental counterparts is estimated to exceed 500 tons. Due to zinc's essentiality, its NPs-application is becoming increasingly popular in agricultural usage, more than commonly applied ionically-soluble Zn fertilizers (Sturikova et al., 2018).

Contamination risk from metal-based NPs occurs when they exceed permissible limits in the soil, leading to adverse effects on soil biota and microorganisms (Ge et al., 2011), plant roots system (Yusefi-Tanha et al., 2022), plant development (Priester et al., 2017), and reproductive organs (Kandil et al., 2022).

The pollen is a key biomarker for environmental pollution assessment and biomonitoring and possesses high ability to absorb different kind of pollutants (Pfahler, 1992; Speranza et al., 2010). Despite its crucial role in fertilization, pollen's viability can be influenced by environmental factors, including fertilization methods (Pandey et al., 2006; 2009). However, there is limited research on the impact of different NPs concentrations even for self-pollinating crops, vital pollen is crucial for seed formation (Pandey et al., 2006; Yoshihara et al., 2021; Ali et al., 2020).

The several soil properties serve as markers reflecting both gradual and immediate changes, accumulating various toxins, including metal-based NPs (Rinklebe and Shaheen, 2017). Fluvisols, are particularly susceptible to heavy metal damage, have significant implications for water sources, soil biodiversity, ecosystems, yield potential, fertility, and food safety (Kočař et al., 2008; Kobierski, 2015).

NPs enter plants through foliar application on leaves or from the soil via the root system. Subsequently, NPs, such as ZnO-NPs, can be distributed, differentiate, metabolically transformed, and accumulated in various plant parts. The aim of their application is mostly to enhance crop yield (Kolenčík et al., 2019; 2020; 2022a).

In this context the soybean [*Glycine max* (L.) Merril] is a suitable model plant for studying metal-based nanoparticle interactions with crops (Koti et al., 2004; Yusefi-Tanha et al., 2022). The soybean belonging to the Fabaceae family, it's known for its protein-rich seeds, contributing significantly to global nutrition. Its versatile applications fall into food production, fodder or biofuel manufacturing (Chauhan and Joshi, 2005; Ali et al., 2020).

In academic research, the predominant focus is on laboratory investigations of NP toxicity (Ge et al., 2011) and greenhouse experiments (García-Gómez et al., 2018), rather than offering comprehensive insights under field agronomic conditions.

Therefore, our goal was to evaluate the effects of increasing concentrations of ZnO-NPs on environmentally important soil properties with soybean cultivation under field agronomic conditions, including evaluation of plant reproductive structures such as pollen viability and potential agronomical positive effect on seed yield.

2. Materials and methods

2.1. Origin and characterization of applied ZnO nanoparticles on soybean

A dispersion containing ZnO-NPs was obtained commercially from Sigma-Aldrich (Saint Louis, MO, USA). For the visualization of the size and morphology of ZnO-NPs, scanning electron microscopy (SEM) was utilized using a JEOL 7610 F+ instrument (JEOL, Akishima, Tokyo, Japan), and the chemical verification of zinc oxide was examined through energy-dispersive X-ray spectroscopy (EDS) using a Phillips XL30 device. Crystallinity and structure parameters of ZnO-NPs were confirmed in Kolenčík et al. (2019) via XRD, and the colloidal properties including zeta potential and hydrodynamic dimension were determined in (Kolenčík et al., 2022b).

2.2. Plant material

The experiment utilized the soybean [*Glycine max* (L.) Merril] variety Bettina, considered to be of medium-early maturity with initial rapid growth, high soil coverage, and the highest lodging resistance. It exhibits a medium height and high yield potential, particularly when cultivated in wider row spacings. The variety has a medium oil content, moderately high protein content (~38%), and relatively high heat stress tolerance. It is characterized by purple flower colour, large light-yellow seeds, and high resistance to shattering (Saatbau, 2024).

2.3. Monitoring of air temperature and precipitation at the experimental site during the vegetation season

Based on meteorological data from Meteoblue (Meteoblue, 2023), variability of precipitation in mm (Fig. 1a) and average daily temperature in °C (Fig. 1b) throughout the vegetation season in 2023 was recorded.

2.4. Description of natural settlement of locality, experimental design, and soybean yield

The experiment was conducted at the experimental site of the Slovak University of Agriculture in Nitra by the Institute of Agronomic Sciences, Faculty of Agrobiology and Food Resources (Fig. 2). The coordinates of the site are 48°1812"N, 18°5'42"E and at an altitude of 160 meters above sea level (Špánik et al., 2002).

The study was carried out on soils that correspond to Hortic Calcaric Fluvisols, as described by Polláková and Šimanský (2015). The groundwater level remains stable, fluctuating between 1.20 to 2.50 meters (Špánik et al., 2002). The climate in this area is warm and dry, with a long-term average temperature of 10.2°C and an annual precipitation of 539 mm. The selected soil characteristics for the experiment correspond to the data provided in Table 1.

The microplot single-factor field experiments were carried out according to Duflo and Banerjee (2017), with slight modification already described in Kolenčík et al. (2019), where four treatments were performed including control, each consisting of three replications (Fig. 2). The experimental field was deep ploughed in autumn. After spring pre-sowing tillage, NPK 15-15-

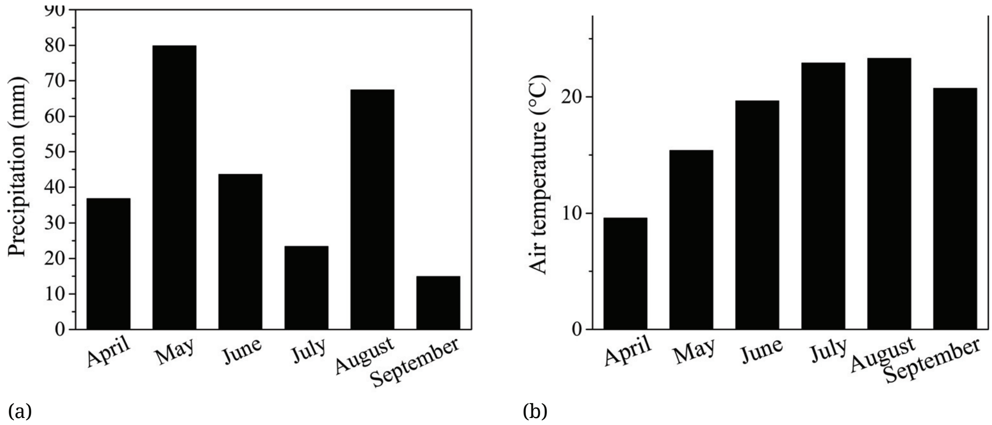

Fig. 1. Monthly variability from April to September in (a) air temperature and (b) precipitation during the vegetation season of 2023 at the experimental site in Nitra, Slovak Republic, Central Europe

Table 1
Initial soil parameter before sowing in the vegetative season of 2023 at the experimental locality, Slovak Republic, Central Europe

Soil parameters	
pH	7.2 ± 0.28
humus (%)	3.5 ± 0.2
N_{an} (mg·kg^{-1})	21.25 ± 8.4
P (mg·kg^{-1})	27.5 ± 7.07
K (mg·kg^{-1})	244 ± 43.8
Zn (mg·kg^{-1})	1.85 ± 0,35
Fe (mg·kg^{-1})	3 ± 0.84
Mn (mg·kg^{-1})	1.6 ± 0.56

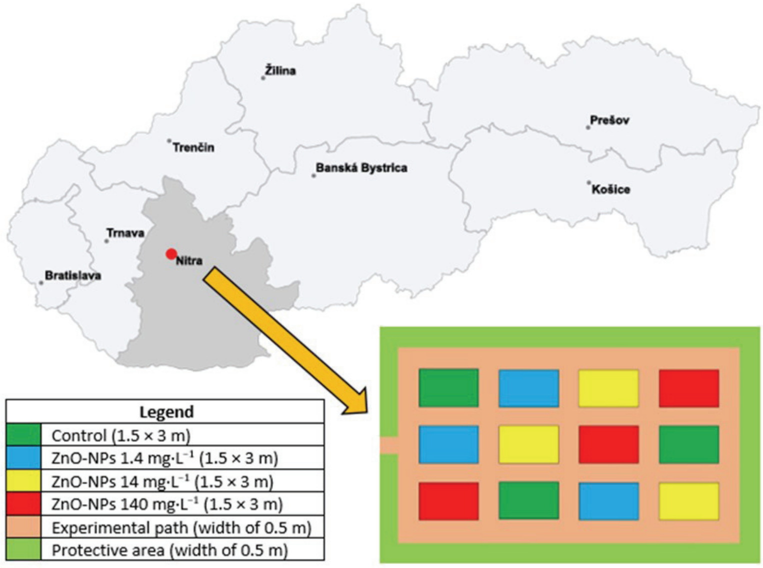

Fig. 2. Geographic location of the experimental locality within the Central European agronomic region of the Slovak Republic, along with a schematic representation of the arrangement of the different nanoparticles treatments

15 DUSLOFERT fertilizer (Duslo, a.s., Šaľa, Slovak Republic) was manually applied at a dose of 90 kg·ha^{-1}. Soybean sowing was carried out manually with the following parameters: plant spacing in the row 50 mm; row spacing 125 mm; sowing depth 40 mm. Foliar application of ZnO-NPs was applied only once during the vegetation season at the beginning of inflorescence emergence growth stage (Fig. 3). Foliar dispersions of NPs were applied using a handheld sprayer, Gamma 5 (Mythos Di Martino, Mussolente, Italy), at concentrations of 0 mg·L^{-1} for NPs-free control with water, followed by 1.4 mg·L^{-1}, 14 mg·L^{-1}, and 140 mg·L^{-1}.

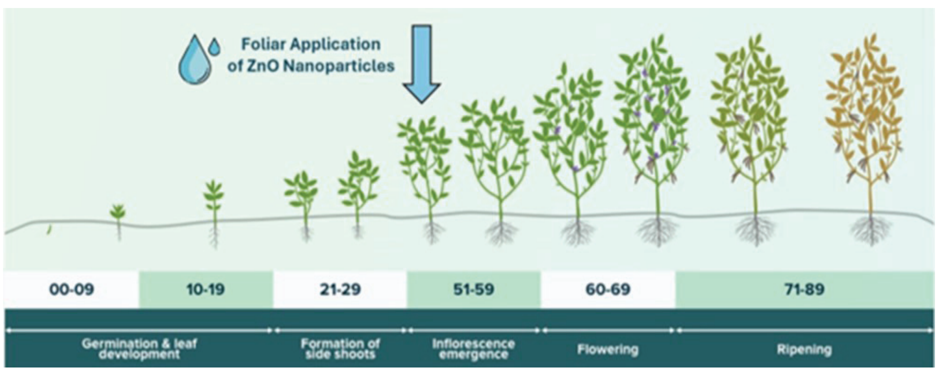

Fig. 3. Schematically illustrated foliar application of ZnO-NPs in growth phase BBCH 51 to soybean

At the end of the vegetative period of soybean, at the stage of full seed maturity, plant material was harvested, and the seeds were collected and evaluated for the seed yield ($t \cdot ha^{-1}$) (Kandel and Endres, 2019). Additionally, the weight of thousand seeds (g) was measured using the Kern PCB3500-2 lab scale (KERN & Sohn GmbH, Balingen, Germany) with the Numirex seed counter (MEZOS spol. s.r.o., Hradec Králové, Czech Republic).

2.5. Analysis of soil parameters of Fluvisols

2.5.1. Analysis of soil microbial activity based on released CO_2

Direct microbial activity as basal respiration in soil samples from each variant of ZnO-NPs application was measured in soil samples collected from the A-horizon immediately prior to harvest. Subsequently, it was determined as the amount of CO_2 released during the soil samples incubation in a semi-hermetically closed system. The basal respiration based on of CO_2 release was captured in a sodium hydroxide solution and subsequently titrated with HCl according to the methodology by Tobiašová et al. (2018).

2.5.2. Analysis of soil-mineral nutrients background and phytoavailability of selected nutrients including zinc content

The collected A-horizon soil samples were also evaluated for most typical soil minerals containing zinc. After removing larger organic matter and roots, the most typical soil minerals were identified. The morphology of the soil samples was investigated using the scanning transmission electron microscope (STEM) JEOL JSM-7610F Plus, Tokyo, Japan with a Schottky cathode at 30 keV in high vacuum chamber. Soil samples were prepared on copper-formvar grids for STEM observation or on Al-stubs and coated with gold. Elemental composition analysis was performed using the energy-dispersive X-ray spectroscope (EDS) AZtec Ultima Max 65 (Oxford Instruments, Abingdon, UK).

The determination of phytoavailable geochemical species of macro- and micronutrients from soils was achieved through an extraction process utilizing the chelating agent Melich III. The method involved the combination of 100 ml of the reagent with 10 g of soil within a 250 ml polyethylene vessel, followed by agitation for 10 minutes. The resulting solution was then filtered and subjected to colorimetric analysis for P, content, based on methodology by Cade-Menun et al. (2018). Additionally, soil extraction procedures utilizing different reagents and shaking conditions were also employed. DTPA was utilized for the extraction of the micronutrient Zn, Fe, and Mn that were subsequently analysed by atomic absorption spectrometry (AAS) (Haynes and Swift, 1983). The conductometer (DiST HANA HI 9831) was employed to analyze electrical conductivity, utilizing a soil to distilled water ratio of 1:2. Carbonate content was determined using a Jankov calcimeter through a volumetric method, measuring the CO_2 evolution upon reaction with HCl (diluted in a 1:3 ratio with water).

2.6. Analysis of pollen viability after foliar application of ZnO-NPs at different concentrations

Pollen grains from soybean flowers were collected at the full flowering stage, fixed in Carnoy's fixative solution (60% ethanol, 30% chloroform, and 10% glacial acetic acid) and released onto a microscope slide. Pollen viability was based on the presence of starch in the pollen grains which turns to brown after the reaction by adding iodine potassium iodide solution (IKI test). Calculation of pollen viability was conducted by using the formula: viable pollen (%) = number of viable pollen grains x 100 / total number of pollen grains in the sample (Chang et al., 2023).

2.7. Applied statistical methods

The observed values of phytoavailable distribution of macro- and micronutrients from soil samples, as well as soil microbial respiration, were statistically analysed using the Tukey's Honestly Significant Difference (HSD) test at a significance level of $\alpha = 0.01$. Statistical analysis for all other treatments was conducted using Statistica 10 (StatSoft, Inc., Tulsa, OK, USA) and Statistica 14 (TIBCO Statistica™ 14.0.0) software. A one-way analysis of variance (ANOVA) was performed on the experimental data, and Fisher's LSD test was employed for testing contrasts.

3. Results and discussion

3.1. Phytoavailability, mobility and destiny of ZnO-NPs in the soil matrix Fluvisol

The ZnO-NPs input to soil environment, their interactions, behaviour, and fate depend on the colloidal system properties of NPs and ambient soil specifics (Šebesta et al., 2017). In soil environment, ZnO-NPs may dissolve or interact with microbiota and affecting root system development (Yusefi-Tanha et al., 2022), thereby impacting plant vitality mainly due to the size, crystallinity, morphology and other physico-chemical nature of NPs (Priester et al., 2017; Reddy Pullagurala et al., 2018).

In our case, ZnO-NPs was deposited on plant leaves at different concentrations, under the hypothesis that foliar application will have higher impact on plant productivity with lower potential of negative impacts on selected environmental components involving soil. Based on SEM observations, the applied ZnO-NPs exhibited dominantly a spherical shape, rarely rod-like and cuboidal shape, with chemical verification of Zn in EDS spectra corresponding to the stoichiometry of ZnO nanoparticles (Fig. 4).

In the context of ZnO-NPs functionality in soil solutions, aggregation or agglomeration, sorption or precipitation with dominant ions, such as NO_3^-, SO_4^{-2}, PO_4^{-3}, may occur (Šebesta et al., 2017; Lv et al., 2019; Elhaj Baddar et al., 2019). Significant immobilization potential could also be associated with natural organic matter in the soil environment. From our results, it is evident that immobilization of Zn geochemical species could

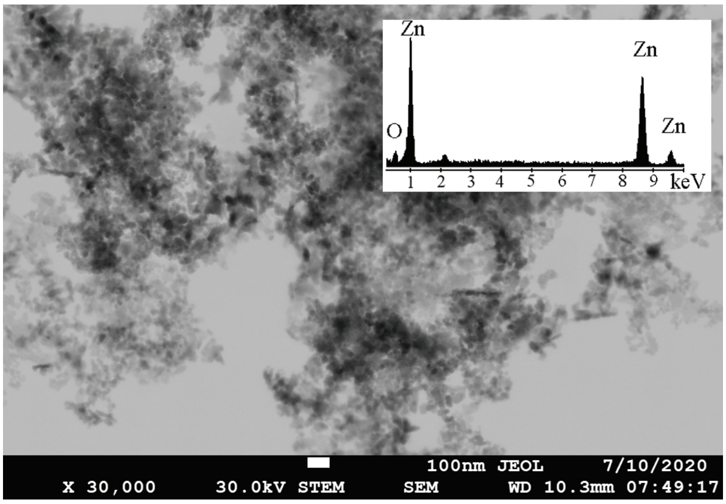

Fig. 4. Scanning electron micrograph (SEM) depicting the morphology and size distribution of ZnO-NPs, verified by chemical energy-dispersive spectra (EDS) of zinc and oxygen

have occurred with the slightly increasing organic content corresponding to the increase in ZnO application concentration, or potential inclusion into CO_3^{-2} or PO_4^{-3} forms, where both have shown a relative decrease in values (Table 2).

Also, this was confirmed by the reduction in electrical conductivity (Table 2), which the presence of all integrated ions of soil-solution, particularly at relatively higher alkaline the pH. The destiny of Zn at higher the pH values in soil environment, especially above pH > 7, was demonstrated primarily through sorption or precipitation either increasing carbonate content (Waalewijn-Kool et al., 2013) or aggregation in alkaline soil environments (Šebesta et al., 2020).

In the case of Zn, its phytoavailable forms or ZnO-NPs or their residues, higher concentrations were not detected with increasing application concentration at a statistically significant level (Table 2). This is a surprising fact because of the difference in our applied concentrations was up to 100-times than the control variant. This could be attributed to the soil buffering capacity or the presence of adequate number of functional groups able bind Zn into soil minerals, such as calcium carbonates (Fig. 5b), where Zn could substitute to Ca in structure via bacteria participation (Kim et al., 2021).

Potential Zn sites-specific absorption may be localized on the surfaces of Fe oxides and hydroxides (Fig. 5a) or within Mn oxide-hydroxides structures. However, the soil-phytoavailable Fe and Mn distribution did not statistically change in the observed variants (Table 2), where Zn could accumulate. The wide-range ability of the active surface of Fe minerals to immobilize metal contamination was reviewed by Prasad et al. (2016), a phenomenon commonly observed in soil settings such as Fluvisols (Rinklebe and Shaheen, 2017) or river sediments which originally Fluvisols come from (Kobierski, 2015).

In this context, the dissolved Zn^{2+} forms could also be sorbed to the aluminosilicate clay minerals (Fig. 5c), or the presence of organic matter (Fig. 5d). Here, in the case of humic acid, could potentially chelate, modify the crystallinity, surface morphology of ZnO-NPs, or even synergistically accommodate the adequate nutrition uptake and, thus, enhance plant growth (Rahale et al., 2021).

Table 2
Impact on selected soil parameters after ZnO-NPs foliar application on soybean at pre-harvest stage during the 2023 growing season

	Control	ZnO-NPs 1.4 $mg \cdot L^{-1}$	ZnO-NPs 14 $mg \cdot L^{-1}$	ZnO-NPs 140 $mg \cdot L^{-1}$
Content of C (%)	2.024 ± 0.035	2.052 ± 0.04 ^{ns}	2.066± 0.04 ^{ns}	2.152 ± 0.012 *
pH – H_2O	7.74 ± 0.011	7.643 ± 0.025 ^{ns}	7.557 ± 0.013 **	7.544 ± 0,02 **
pH – KCl	6.657 ± 0.045	6.555 ± 0.021 ^{ns}	6.484 ± 0.009 **	6.481 ± 0.012 **
CO_3^{2-}	0.41 ± 0.033	0.35 ± 0 ^{ns}	0.32 ± 0.017 ^{ns}	0.33 ± 0.017 *
Electrical conductivity (EC) (mS·m)	255.33 ± 9	222.67 ± 4.11 **	206 ± 1.633 **	200.67 ± 2.494 **
Content of P ($mg \cdot kg^{-1}$)	40 ± 2.5	36.30 ± 1.3 ^{ns}	37.5 ± 0 ^{ns}	31.30 ± 1.3 ^{ns}
Content of Zn ($mg \cdot kg^{-1}$)	2.26 ± 0.5	2.87 ± 0.16 ^{ns}	2.24 ± 0.37 ^{ns}	2.60 ± 0.11 ^{ns}
Content of Fe ($mg \cdot kg^{-1}$)	1.76 ± 0.025	1.68 ± 0.015 ^{ns}	1.67 ± 0.015 ^{ns}	1.61 ± 0.015 ^{ns}
Content of Mn ($mg \cdot kg^{-1}$)	5.70 ± 0.08	6.20 ± 0 ^{ns}	7.27 ± 0.02 ^{ns}	7.36 ± 0.41 ^{ns}

The significance: * P value < 0.01, ^{ns} non-significant

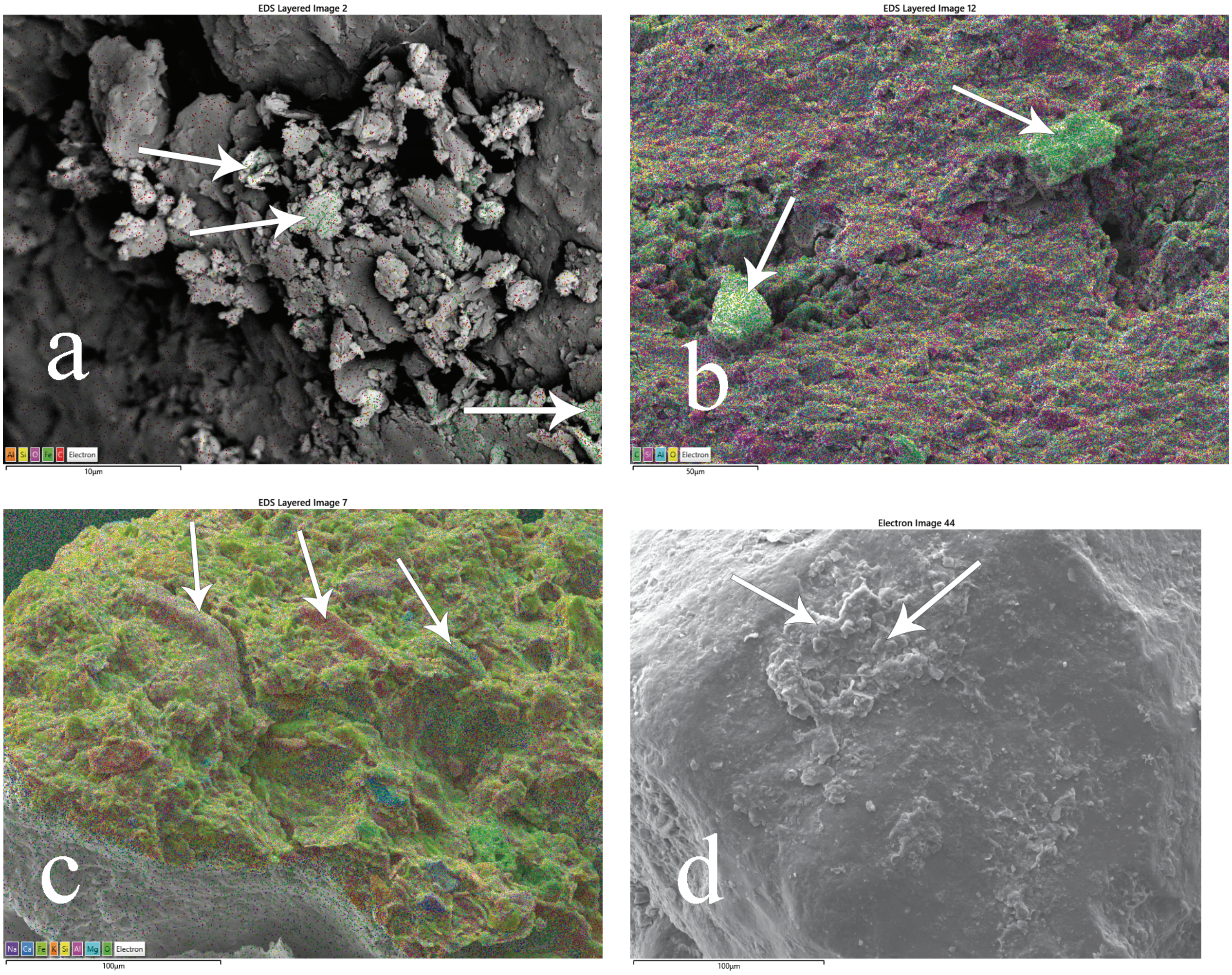

Fig. 5. Visualisation of soil minerals capable of integrating zinc to their structure a) iron oxides and hydroxides, b) secondary carbonates, c) alumosilicates corresponding to clay minerals, d) organic parts of soil

3.2. Impact of foliar application of ZnO-NPs on soil biological activity and seed yield of soybean

Soil biological activity reflects the current soil health status, its potential fertility, and the mineral nutrient cycles or its condition in the case of elimination of toxic heavy metals including Zn (Zamulina et al., 2021). Depending on the Zn distribution and its concentration, soil microbial activity could be affected positively, unaffected, or negatively influenced on the cytological level at relatively low Zn concentrations (Coman et al., 2019; Clemens, 2021). In our case, there was not evident the significant Zn releasing, distribution and exposure into soil environment (Table 2), with associated negative impact on microbial activity, based on basal respiration within pre-harvest analyzation (Table 3).

García-Gómez et al. (2018) carried out experiments applying wide-range of ZnO-NPs concentration (0, 0.1, 1, 10, 100, 1000 $mg \cdot kg^{-1}$) to soils in both acidic and alkaline environments, observing soil bacterial communities over a 180-day period. The results indicate that potential soil respiration was negatively affected only at the highest concentration 1000 $mg \cdot kg^{-1}$ partly confirming the beneficial stimulatory effect of the exposed Zn with relative stable microbial respiration observed in our study.

One of the appropriate soil change indicators is its microbial biomass. In the study conducted by Ge et al. (2011), alterations in microbial biomass composition and diversity were observed at relatively low concentrations of ZnO-NPs ranging from 0.05 to 0.5 $mg \cdot g^{-1}$ in slightly acidic soil (pH = 6.0) with a loam texture containing 2.2% C and 0.21% N over a 60-day period. Also, there was found that ZnO-NPs exhibited a higher negative response than non-essential TiO_2-NPs at the same concentration range. However, these findings do not correspond to our results, most likely due to the relatively lower concentrations of ZnO-NPs and different soil properties, such as pH, which in our case had a value of ~7.5 (Table 2) in comparison to the lower pH (~6) of the soil in the other study. In general, lower pH values are usually connected with more intense solubility, bioavailability, and reactivity of ZnO-NPs towards soil microbiota (Ge et al., 2011).

In the study by Chen et al. (2023), soybeans were cultivated under greenhouse conditions in farmland soil treated with three Zn species at concentrations of 0, 100, and 500 $mg \cdot kg^{-1}$ for the period of 70 days. All three Zn forms-$ZnSO_4$, ZnS-NPs, and ZnO-NPs-applied to the soil, reduced the soil diversity bacteria in both roots and nodules, likely due to Zn-induced oxidative stress. The higher concentrations had a more pronounced impact on bacterial beta diversity and metabolite production than lower doses for all applied Zn forms, directly affecting N and C metabolism. Conversely, our indicator of soil microbial activity-respiration was not statistically significant at all applied concentrations. However, soil C content gradually increased with increasing foliar application of ZnO-NPs concentration (Table 2). This suggests that their effect may have been slightly stimulatory for organic production and C content, thus the ZnO-NPs may have directly affected the carbon cycle in Fluvisols. If ZnO-NPs or their residues were more mobile in the soil environment during vegetation season, one hypothesis was their higher accumulation in roots, followed by their subsequent root-to-shoot distribution (Montanha et al., 2020).

Jiang et al. (2021) conducted research in farmland soil, cultivating wheat for 84 days, with high levels of ZnO-NPs corresponding to 500 mg·kg^{-1} and revealed a significant increase in Zn^{2+} soil uptake along with pH changes and inhibition of heterotrophic respiration and soil microbial diversity. Bacterial communities were more sensitive to these changes than fungal counterparts, with ascomycetes being the most dominant community at the end of the experiment.

From our results, it is evident that there was no significant disruption to biological activity, likely because the applied ZnO-NPs or their residues were directly metabolized by soybeans at all concentrations range, including the highest concentration. Here, the concept of effective Zn utilization is supported by the highest soybean production parameters such as weight of thousand seeds and seed yield per hectare observed at the concentration 140 mg·L^{-1} (Table 4), compared to the NPs-free control and other treatments with lower concentrations of ZnO-NPs.

These results are not surprising for our Central European agronomic region because similar yield and its forming components intensification trends were observed in different crops such as foxtail millet (Kolenčík et al., 2019), lentils (Kolenčík et al., 2022a) or sunflower (Kolenčík et al., 2020). Regarding the impact of ZnO-NPs on soil ecology, higher yields and soil buffering capacity will gradually change until the point of Zn exceeding chronic exposure levels (Jiang et al., 2021) or other negatively accelerated factors typical for field conditions including year-weathered condition (Liu et al., 2022).

3.3. Impact of ZnO-NPs on plant reproductive organs based on assessing pollen viability

Soybean flower anthers were collected at full bloom stage after the ZnO-NPs application at various concentrations to assess the impact on soybean reproductive organs. The indication of pollen grains changes serves as the analysis and monitoring of direct toxicological effects (Sharafi et al., 2017), as well as a marker for assessing environmental quality (Mičieta and Murín, 1996) or preferred agronomic practices, including affection of metal based-NP plant treatments (Ďurišová et al., 2023).

Soybean is widely cultivated, easily grown in diverse habitats, and genetically sensitive to toxicants involving metals (Koti et al., 2004). Although the soybean pollen analysis was largely absent in *"in vitro"* and field academic studies, it poses a kind of novelty compared to similarly cultivated species such as tobacco or apples, where pollen studies have been conducted for exposure to potential toxic elements such as Ni, Pb, Co, Cd, Hg, Al, Zn, Cu. Effects of reduced pollen germination and decreased fertilization ability due to insufficient pollen tube growth towards the eggs have been previously studied (Tuna et al., 2002, Munzurođlu and Gür, 2000).

In general, the metal-based NP application in agriculture has shown both positive and negative effects on plant growth and development. Zinc, as a micronutrient, plays a key role in positively influencing the development of plant reproductive

Table 3
Basal respiration analysis of Fluvisol using the Isermeyer method after foliar application of varying concentrations of ZnO-NPs on soybeans during the 2023 growing season, as part of pre-harvest assessment

	Control	ZnO-NPs 1.4 mg·L^{-1}	ZnO-NPs 14 mg·L^{-1}	ZnO-NPs 140 mg·L^{-1}
Employing of HCl (ml)	15 ± 0.40	14.9 ± 0.14 ^{ns}	14.98 ± 0.27 ^{ns}	14.92 ± 0.13 ^{ns}

The significance: ^{ns} non-significant

Table 4
Evaluation of yield and yield component of soybean after ZnO-NPs foliar application at various concentrations in the 2023 vegetation season

	Control	ZnO-NPs 1.4 mg·L^{-1}	ZnO-NPs 14 mg·L^{-1}	ZnO-NPs 140 mg·L^{-1}
Weight of thousand seeds (g)	28.46 ± 5.90	51.88 ± 17.26 *	70.89 ± 17.59 **	96.63 ± 16.33 **
Seed yield (t·ha^{-1})	0.63 ± 0.36	1.33 ± 0.17 ^{ns}	2.02 ± 0.54 ^{ns}	2.97 ± 1.65 *

The significance: ** P value < 0.01, * P value < 0.05, ^{ns} non-significant

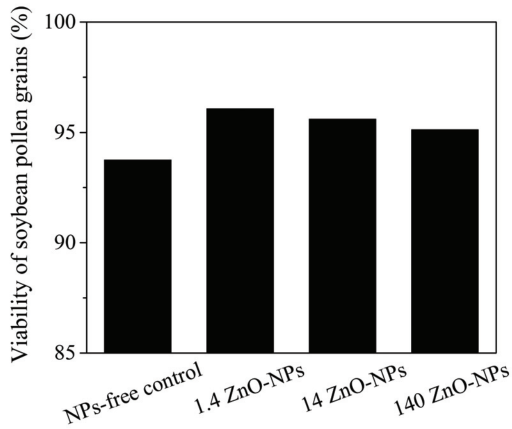

Fig. 6. Assessment of soybean pollen grains after ZnO-NPs foliar application at various concentrations range during the 2023 vegetation season in Nitra, Slovakia, Central Europe agronomic region

organs, pollen production and their quality, although at higher concentrations, it often exhibits toxic effects (Kandil et al., 2022; Pandey et al., 2006; 2009).

For beans, the threshold concentration that initiated disturbances during pollen development was observed was at 250 mg·L^{-1} of ZnO-NPs, with our slightly lower concentration still exhibiting beneficial, stimulatory effects (Fig. 6). Rapid pollen grain damage occurs with applications above 2000 mg·L^{-1} of Zn-NPs. Excessive concentrations of ZnO-NPs result in disturbances during developmental processes, leading to the production of deformed pollen grains with reduced viability (Salehi et al., 2021a; Salehi et al., 2021b). A similar scenario of ZnO-NP concentration with the same toxic effect was also observed in rice pollen (Kandil et al., 2022).

From our results, it is obvious that all applied ZnO-NP concentrations, namely 1.4, 14, and 140 mg·L^{-1}, had a positive – stimulatory effect (Fig. 6). The most effective ZnO-NP concentration for pollen viability was 1.4 mg·L^{-1}, while pollen viability gradually decreased with increasing ZnO-NP concentrations (Fig. 6).

Despite higher grain yields and improved grain quality, foliar application of ZnO-NPs at concentration exceeding 2000 mg·L^{-1} resulted in genotoxic effects, causing structural changes and degeneration of pollen grains (Kandil et al., 2022). However, our study revealed that our highest concentration corresponding to 140 mg·L^{-1} led to the highest seed yield of soybean without negatively affecting pollen viability.

In this context, sunflower plants that were treated with a concentration of 250 mg·L^{-1} of NPs had earlier flowering stage, exhibited higher starch content in pollen grains along with highest pollen viability at a concentration of 500 mg·L^{-1}. The inhibitory effect on germination was observed only at a concentration of 1000 mg·L^{-1} (Shukla et al., 2016). However, lower concentration of NPs also initiated either the pollen germination inhibition or mutual pollen tube-growth. It has been observed that the germination rate significantly decreased in lily pollen tube elongation exposed to low solubility ZnO-NPs at a concentration of 100 mg·L^{-1} (Yoshihara et al., 2021).

5. Conclusions

Our short-term results show no adverse effects on selected soil properties or its function during soybean cultivation in Fluvisol (A-horizon) in field conditions conducted in the Central European region in 2023. After foliar application, there was no indication of ZnO-NPs preconcentration or their residual phytoavailable distribution associated with the soil inorganic and organic parts along with no-harmful effects to soil microbial activity. Moreover, it can be deduced that with all applied concentrations of ZnO-NPs (1.4, 14, 140 mg·L^{-1}), there was a positive stimulation of pollen viability as selected biomarkers compared to NP-free control. Soybean pollen viability was beneficially stimulating by the all ZnO-NP concentrations. The highest stimulation occurred at 1.4 mg·L^{-1}, and slightly declined with application of higher concentrations of ZnO-NPs. The positive viability of soybean pollen was not the only positive effect. Seed yield also increased, where the highest concentration of ZnO-NP corresponded to the highest weight of thousand seeds and seed yield at most statistically significant levels. As a result, the application of ZnO-NPs has positioned them as a promising insight as new generation nanofertilizers in precision and sustainable agriculture while mitigating negative environmental impacts associated with traditional farming practices.

Acknowledgments

This research was funded by the Grant Agency of the Slovak Republic Ministry of Education and the Slovak Academy of Sciences under contract VEGA 1/0655/23, VEGA 1/0331/23, VEGA 1/0359/22 and also the project of Grant Agency of Faculty of Agrobiology and Food Resources, SUA in Nitra, GAFAPZ 8/2023 with title "*Evaluation of Field Crops Quality as Result of Application of Inorganic Nanoparticles as New Generation Foliar Nutrition*", and GAFAPZ 9/2023 with title "*Investigation of Effect of Foliar Application of Nanofertilizers on Selected Field Crop Production Indicators*".

Conflict of interest

The authors declare no conflict of interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. This research did not involve human or animal subjects.

Author Contributions

Conceptualization – DE, MK, MŠ, YQ; Data curation – DE, LT, GK, JG, NP, VS, MJ, LD; Funding acquisition – MK, DE, VŽČ; Investigation – MK, DE; Methodology – DE, LT, GK, JG, NP, VS, MJ, LD; Supervision – DE, MK; Validation – DE, MK; Visualization – IČ, VŽČ, IR; Writing – original draft – MK, DE; Writing – review & editing – MK, ĽĎ, YQ. All authors read and approved the final manuscript.

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