Online Journal of Biological Sciences

Original Research Paper

Morphological and Structural Reaction of *Avena sativa* Seedlings to the Effects of Nano and Microparticles of Zinc

[1]Arailym Serikbai, [1]Aidar Aitkulov, [2]Anna Stanco, [3]Margarita Ishmuratova, [4]Assylbek Zeinidenov and [5]Wojciech Pusz

[1]*Department of Physiology, Buketov Karaganda University, Karaganda, Kazakhstan*
[2]*Department of Materials Science, Tomsk Polytechnic University, Tomsk, Russia*
[3]*Department of Botany, Buketov Karaganda University, Karaganda, Kazakhstan*
[4]*Center of Nanotechnology and Functional Nanomaterials, Buketov Karaganda University, Karaganda, Kazakhstan*
[5]*Department of Plant Protection, Wroclaw University of Environmental and Life Sciences, Warsaw, Poland*

Article history
Received: 15-01-2024
Revised: 29-02-2024
Accepted: 18-03-2024

Corresponding Author:
Arailym Serikbai
Department of Physiology, Buketov Karaganda University, Karaganda, Kazakhstan
Email: arailym_serikbai@mail.ru

Abstract: Kazakhstan has a well-developed mining and metallurgical industry, which produces macroand nanoparticles of heavy metals, including zinc emitted to the environment. The nanoscale particles have a different biological activity, which requires some additional research. The effects of various concentrations of zinc macroparticles and nanoparticles on the anatomical structure of oat seedlings (*Avena sativa* L.) and their accumulation in vegetative organs have been comparatively studied. Oat seeds were treated with the suspensions of zinc macro and nanoparticles in concentrations from 50-2000 mg/L. The treated seeds were planted and grown in pots with a soil substrate with the suspensions of macroand nanoparticles added in concentrations from 1.25-50 mg/L. The anatomical features of the vegetative organs of plants and the accumulation of zinc in them were studied in 28 days. According to the results of the research, it was revealed that different concentrations of macroand nanoparticles of zinc cause multidirectional changes in the thickness of the epidermis of the stem and roots, the diameter of large and small conductive bundles of the stem, the diameter of the xylem vessels and the length of the trichomes in the root, as well as the thickness of the parenchyma and the diameter of the stem. Zinc added into the soil in the form of nanoparticles led to its accumulation in the roots at a concentration of zinc in the roots at 0.309±0.009 mcg/g compared with 0.067±0.002 mcg/g in the control (0.085±0.003 in the stem (0.021±0.003-in the control group) and only 0.022±0.005 mcg/g in the leaves (0.117±0.002 mcg/g-control group). The zinc content in the stems increases with an increase in the concentration of MPs from 1.25-2.5 mg/L, but at a concentration of MPs >2.5 mg/L, the zinc content does not depend on the concentration. The NP and MP zinc added into the substrate leads to its accumulation in vegetative organs and changes in their anatomy, but further study of the biological mechanisms of these phenomena and its effect on productivity and physiological parameters in the open ground is required for further deeper analysis.

Keywords: *Avena sativa*, Zinc Nanoparticles, Seedlings, Zinc Accumulation

Introduction

Zinc nanoparticles (hereinafter Zn NPs) are promising agents for improving the functional properties of resin coatings (Shi *et al*., 2009) soft tissue regeneration and wound healing (Paramita *et al*., 2021), development of drug carriers (Hameed *et al*., 2019), wastewater treatment (Mahmood, 2022) and agriculture (Ahmed *et al*., 2023). Due to their high demand, NP production is constantly increasing, which creates sources of NP release into the environment, including the lithosphere (Shrivastava *et al*., 2019). Therefore, studying the influence of NPs on soil and plant components is important (Remédios *et al*., 2012; Anjum *et al*., 2015). The high solubility of metal NPs

(Godymchuk *et al*., 2015), their high migration activity in the soil (Zhang *et al*., 2020), small size, and increased reactivity make NPs potentially hazardous to the plant world. On the other hand, they can act as suppliers of essential micronutrients to plants (Awan *et al*., 2021). It is known that Zn NPs can have both positive and negative effects on plant growth and development (Lin and Xing, 2007; Lee *et al*., 2008; Wang *et al*., 2012). NPs and their dissolution products have a high ability to penetrate plant roots (Rajput *et al*., 2021), accumulate in vegetative organs, and further participate in food chains (Rawat *et al*., 2018).

The Zn nanoparticles are currently used in agriculture both as the components of fertilizers and as an active agent to protect plants from diseases (Al Jabri *et al*., 2022). In recent studies, scientists have concluded that biosynthesized ZnO NPs with a size of 20 nm improved growth parameters, protein content, and leaf area of maize (Sabir *et al*., 2020). However, the protein content in the plant decreased in the following order of NP concentration: 8>16>4>2>0 mg/L.Under drought stress, the addition of 50 and 100 mg/L ZnO NPs (<200 nm) promotes the growth and fruit yield of eggplants by 12 and 23%, respectively, compared with fully irrigated plants and nonapplied ZnO NP (Semida *et al*., 2021). Foliar spraying with 100 mg/L ZnO NPs (<100 nm, among 75, 100, and125 mg/L) contributed to a 200% increase in tomato yield, with the MARDI tomato-3 variety showing better results compared to MARDI tomato-1 (Ahmed *et al*., 2023). Higher concentrations of ZnO NPs (1000 mg/L) increased also the number and average weight of pepper fruits whereas 2000 mg/Lsuppressed seedling development (García-López *et al*., 2019). It has been shown that with a decrease in size of ZnO NPs from 204-34 nm, the Zn content in the roots and shoots of maize significantly increases, with much higher accumulation observed under hydroponic conditions compared to soil cultivation (Ahmed *et al*., 2021). It has also been demonstrated that the toxicity of 50 nm ZnO NPs is influenced by plant species and soil pH: Cucumber and beetroot were the most resistant crops in an alkaline environment, while corn, wheat, and peas were more resistant in an acidic medium (García-Gómez *et al*., 2018). The combined effect of Zn NPs and plant growth regulators (IAA and GA3) on sunflowers under water stress conditions resulted in a significant increase in nutrient content in the plant leaves (Al-Dhalimi and Al-Ajeel, 2020). Sorahinobar *et al*. (2023) showed a positive influence of ZnO NPs in size 10-80 ppm on growth, nutrient uptake, and some physiological parameters of mung bean.

Also, the scientific literature describes that nanoparticles of metals and their oxides negatively affect the growth and physiology of important crops such as wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), beans (*Phaseolus radiatus*), peas (*Pisum sativum* L.), rice (*Oryza sativa* L.), etc., (Larue *et al*., 2012; Mukherjee *et al*., 2014; Farhat Yasmeen *et al*., 2015; Pena *et al*., 2022; Szablińska-Piernik *et al*., 2022; Stałanowska *et al*., 2023; Shaikhaldein *et al*., 2024).

The works of Munir *et al.* (2018) show that the addition of Zn and ZnO NPs can enhance their accumulation in vegetative organs of *Triticum aestivum* as an additional component of nutrition and improve photosynthesis of the plant. Vasilachi *et al.* (2023) showed the potential negative effect of heavy metal NPs including zinc, on some cereal crops (rice, maize, wheat, barley). Such effects as chlorosis and deformation of leaves, reduction of photosynthesis intensity, growth retardation, necrosis, disturbance of plant water metabolism, limitation of absorption of vital components from the soil, and delay of flowering and fruiting period, which leads to yield reduction, were noted.

In the last decade, both the positive and negative effects of NPs of metals and their oxides on the growth, yield, and physiology of the above crops have been widely studied (Adrees *et al*., 2015; Zhang *et al*., 2015; Rizwan *et al*., 2017; Du *et al*., 2017; Khan *et al*., 2019; Lahuta *et al*., 2022; Ghouri *et al*., 2024).

In this regard, it should be noted that the effects of NPs on agricultural plants vary greatly, depending on the type of plant, stages and conditions of growth, methods, dosage, duration of exposure to NPs and their oxides as well as some other factors.

Oats are an important agricultural crop used as a feed additive in animal husbandry, green manure in crop production, and raw materials for the food industry. Oat food products have a steady demand as the basis of a healthy diet (Al-Rawi and Abood, 2021). In this regard, oats were used as a model plant in this study.

In this regard, studying new methods to increase the productivity of oats remains a significant matter. One of the promising directions to increase agricultural yields is the use of metal nanoparticles and their oxides as stimulators of plant biological activity and/or protectors against diseases that increase plant productivity (Kaningini *et al*., 2022). Our increased interest in nanoobjects is caused by the fact that new-generation fertilizers as growth stimulators and plant protectors are being tested in our country.

The purpose of this study is to study the effect of nano- and microparticles of zinc on the anatomical structure and accumulation of Zn NPs in *Avena sativa* organs.

Materials and Methods

Nanoparticle Preparation and Characterization

Zn NPs were synthesized by grinding (Research Center for Ionoplasmic Technologies and Modern Instrumentation, Buketov Karaganda University, Kazakhstan) conducted using a laboratory full-directional planetary ball mill QXQM-1 (Tencan, China).

The microscale Zn powder (MPs, grade PCR-1, Interkhim LLC, Russia, Zn content-98.13 wt.%, bulk density-3.0 g/cm^3, size <40 μm) was ground for 5 h in the grinding shell made of WC using WC grinding balls (10 mm diameter, rotation speed of 500 rpm, ball mass exceeded the powder mass by 10 times). The MPs were also used in the experiment to assess the size effect. The particle morphology was determined using a Scanning Electron Microscope (SEM) MIRA 3 LMU (Tescan, Czech Republic) with a carbon substrate. Suspensions of particles with concentrations of 50, 100, 200, and 2000 mg/L were prepared using distilled water (pH = 6.7±0.6 with the conductivity of 0.2 μS/cm) through magnetic stirring (magnet size 8×39 mm, stirring speed-200 rpm). The particles were weighted by using an electronic balance As60/220.R2 (Radwag, Poland) with an accuracy of ±0.0001 g.

Experimental Setup, Seed Germination

The seeds of cultivated oats (*Avena sativa* L., Poaceae family, Mirny variety harvested in 2021, 1000 grain weight-41.5 g) were provided by the Akbulak farm (Osakarovskiy district, Karaganda Region, Kazakhstan). Oats are one of the leading food and feed grains with high nutritional properties, which are considered a source of potentially useful substances (Ahmad *et al*., 2023; Osbourn, 2003). Apart from being a leading food source, seeds and seedlings of oats as a monocotyledonous plant are a recommended test object in the study of phytotoxicity of chemical agents (US EPA, 1996). Ecological effects test guidelines. OCSPP 850.4100, seedling emergence and growth. Office of chemical safety and pollution prevention (7101) EPA 712-C-012.

Priming before sowing, 135 seeds were exposed to a 100 mL freshly prepared suspension on a multi-shaker PSU20 (BioSan, Latvia) for 240 min at the speed of 165 rpm. The distilled water-soaked seeds were used as control samples.The substrate was prepared by mixing soil (manufactured by a soil factory, in Russia) with coconut substrate (Listok, Russia) in a volume ratio of 1:1, resulting in a pH range of 5.5-6.5. The soil was a universal complex peat fertilizer with micronutrients, limestone materials, and loosening agents. Its composition included peat, limestone materials, loosening agents, and a complex fertilizer. The nutrient content per 100 g of dry soil was as follows: N-50-150 mg, P-100-250 mg, and K-150-300 mg; organic matter was≥70wt.%. The coconut substrate consisted of coconut peat (20 wt.%) and chips (80 wt.%).

In the experiment, a plastic pot (52.0×18.0×14.5 cm) was filled with 4,000 mL of soil. The seeds were planted with a planting depth of 0.5 cm and a spacing of 1 cm between them. Subsequently, the substrate was watered with 100 mL of freshly prepared suspension of Zn NPs. The final particle concentrations in the substrate were 1.25, 2.5, 5, and 50 mg/L. The seeds were germinated for 28 days with the photoperiod of "day: Night"- "6 h: 20 h"at room temperature under a phytolamp (LED T8 tube, 18 W, 120 cm in length). The soil was irrigated with 200 mL of water on days 7 and 14. After the experiment, the seedlings were rinsed with distilled water and dried at room temperature for 48 h. Some seedlings were subjected to zinc content analysis, while others were used for morphological studies.

Detection of Nano-and Microparticles of Zinc in Seedlings

The oat seedlings were divided into elements (roots, stems and leaves), dried at 105°C for 12 h, and used to prepare aqueous extracts. Approximately 0.05 g of the organ sample was added to a glass beaker along with 10 mL of 1 M HNO_3. The mixture was heated on a hot plate in the glass beaker for 3-4 h until complete sample decomposition. Then, the solutions were filtered and diluted with water to a volume of 100 mL. These solutions were used to determine Zn concentration related to the initial mass of the dry organ.

Zn concentration was determined using an atomic absorption spectrometer AA-140 Varian (Agilent Technologies Inc., USA) by burning the sample in the flame of an acetylene burner. The range of detectable concentrations was within 0.0001-4.0 mg/L.

Anatomical Structure of Seedlings Grown with Added Zn Nano-and Microparticles

To study the anatomical structure, the seedlings were fixed using the Strauss-flemming technique in a preparation composed of "92% ethanol: Glycerin: Distilled water" in a ratio of "1:1:1" (Henry *et al*., 2016). The cross-sectional cuts were then made using sterile blades of the aerial (leaves, stems) and underground (roots) parts of the plant. The fixed glycerin sections were placed on glass slides and covered with a cover slip.

Images were obtained using a digital optical microscope Bio 2,0 (Altami Russia) and microslide photographs were taken at the magnifications of ×20 and ×40. The photographs were processed and tissue structures were measured using the Altami Studio 3.3 software. The measured parameters included the thickness of the epidermis and parenchyma, diameter of large and small conducting bundles in the stem, thickness of the upper and lower epidermis, mesophyll, veins in the central part, and diameter of conducting bundles in the leaves, as well as the thickness of the epidermis, parenchyma and endodermis, diameter of the xylem vessel, conducting bundles and root hairs. Measurements were done on 10 microslides to obtain reproducible results. The values were expressed in μm.

Statistical Analysis

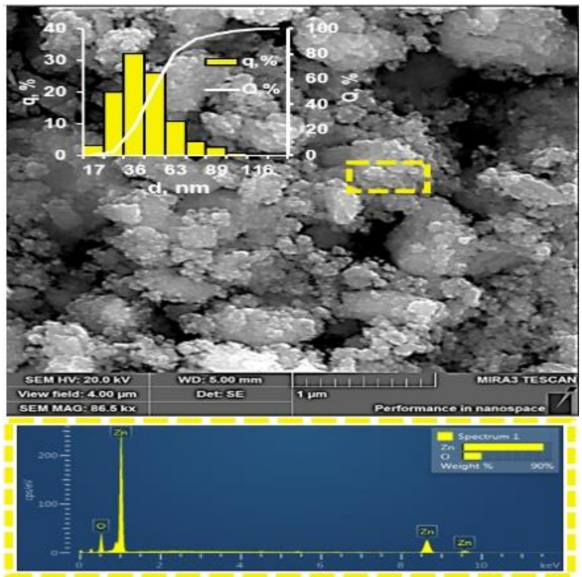
The statistical analysis of the experimental groups was conducted using a student's t-test at a significance level of 95% (r<0.05). All experiments with plant germination were carried out in 4-fold repetition and measurements of microscopic parameters were carried out in 10-fold repetition.

Results

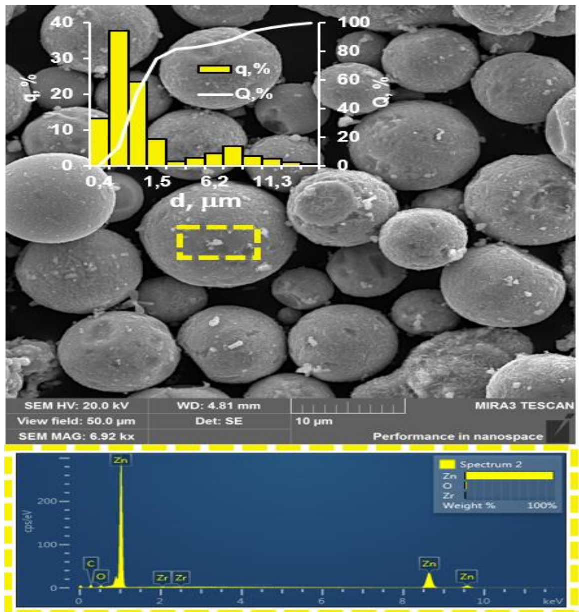
According to SEM data, the NPs consisted of particles with a roughly elliptical shape (Fig. 1a) that were aggregated into clusters ranging in size from 0.09-0.44 μm. The MPs were predominantly represented by dense, spherical granules (Fig. 1b).

The fractured granules indicated that all the granules were hollow. In addition, the fine particle fraction filled the damaged large granules. The analysis of the images allowed for the calculation of the average particle size, which was found to be 43.8 nm and 2.0 μm for NPs and MPs, respectively. The EDX spectra confirmed the presence of Zn at 1.0, 8.5, and 9.5 keV and an O-peak at 0.5 keV (with a C-peak from the carbon substrate). The quantitative analysis showed that Zn content was 90% and 100% for NPs and MPs, respectively. Therefore, the Zn content decreases as the particles are reduced in size due to the incorporation of oxygen into the Zn lattice and the formation of the ZnO phase.

After growing oats in soil containing NPs, Zn accumulated in the roots and stems, while Zn content in the leaves was not statistically significant. For the seedlings grown in soil with 2.5 mg/L of NPs, Zn concentration in the roots was 0.309±0.009 μg/g (compared to 0.067±0.002 μg/g in the control), in the stem-0.085±0.003 μg/g (0.021±0.003 μg/g in the control) and in the leaves-0.022±0.005 μg/g (0.117±0.002 μg/g in the control, Fig. 2a). With an increase in the NPs' concentration, a strong dose-dependent effect was marked in the roots: For a 2.5 mg/L suspension, the Zn content in the root exceeded the control by 3 times (~190%, Fig. 2b) and with an increase in the NPs' concentration in the soil from 2.5-50 mg/L, Zn content in the root increased by another 2.5 times. In the stems, Zn content increased with increasing NPs' concentration from 1.25-2.5 mg/L, but for >2.5 mg/L NPs, Zn content did not depend on the concentration. In an aqueous suspension in the presence of O_2, metallic Zn is capable of oxidizing to form the colloids of Zn $(OH)_2$ ($\Delta\varphi = 1.309$ V) (Godymchuk *et al*., 2015), whose size enables them to migrate into the root and subsequently move into the stem (Fig. 2a).

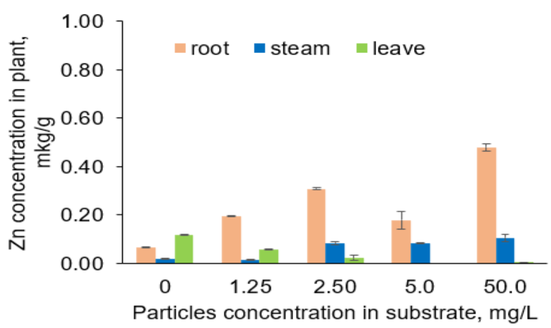


(a)

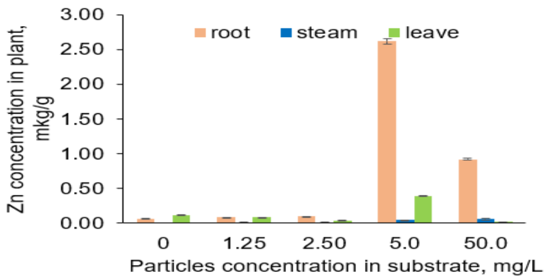


(b)

Fig. 1: SEM images, corresponding particle size distributions, and EDX spectra of Zn NPs; (a) and (b) MPs



(a)



(b)

Fig. 2: Effect of Zn NPs; (a) and MPs; (b) concentration on Zn concentration in roots, stems, and leaves of oats seedlings (root, stem, leaves)

Further, we found Zn uptake in MPs treated roots was shown when plants were exposed to doses of ≥5 mg/L (Fig. 2), while Zn content in the stems and leaves was statistically insignificant regardless of the MPs dose in the soil (Fig. 2b). For instance, in the seedlings treated with 5 mg/L MPs, Zn concentration in the roots was 2.613±0.036 µg/g compared to 0.067±0.002 µg/g in untreated roots, while Zn content in stems and leaves did not exceed the control group (Fig. 2b). With an increase in the MPs concentration from 2.5-5 mg/L, the Zn content increased by 11 times in the leaves and 29 times in the roots. For plants treated with 50 mg/L MPs Zn content in the roots decreased by 3 times, while there was no Zn detected in the leaves. Apparently, at high concentrations of MPs (50 mg/L), particle aggregation might occur, which could reduce the active surface area of MPs capable of oxidation, resulting in less Zn $(OH)_2$ reaching the roots. The results partially agree with the literature data, according to which Zn accumulation predominantly occurred in the root part of the plant. Using EDX spectrum analysis, the authors demonstrated Zn accumulation in the roots (4.9-7.6%), stems (0.36%), and leaves (0.21%) of corn seedlings grown in sandy clay loam soil after soil treatment with ZnO NPs suspensions (34 nm, 2000 mg/L) (Ahmed *et al*., 2021).

Observations of biological development showed that macro and nanoparticles of zinc had different effects on the seedlings of the cultures under the experiment. The macroparticles of zinc had a depressing effect on oat seedlings in all experimental groups, which led to a decrease in the root length compared to the control values (Fig. 3).

Measurement of the length of the aerial part of oat seedlings showed that treatment with zinc macroparticles in some concentrations has a depressing effect on their growth parameters and nanosized particles in all concentrations have a stimulating effect (Fig. 4). Compared with the control values, when treated with macroparticles at the concentrations of 5, 20 mg, the sizes of leaves and stems decreased by 57.6 and 13.2,%, respectively. At the same time, at the concentration of metal particulates of 10 mg, an increase in this indicator by 3.7% was noted. The treatment of oat seeds with nanoparticles at all concentrations led to an increase in the length of the aerial part compared to the control values.

The wet weight of the underground oats' organs treated with zinc nanoparticles had similar values for the experimental and control groups, while the weight of the roots of seedlings treated with suspensions of macroparticles of 5 and 10 mg was lower than the control values. A similar picture was observed by studying the parameters of the mass of the aerial part of seedlings.

The comparative study of the roots of seedlings grown with the addition of Zn NPs and Zn MPs showed that in the presence of NPs, the degree of zinc accumulation in the roots was higher at concentrations ≤5 mg/L and lower at concentrations >5 mg/L compared to the presence of MPs. Thus, at the particle concentrations in the soil ≤5 mg/L, the zinc content in the roots of seedlings grown with NPs exceeded that of MP-treated roots by 2.4 times, while at the particle concentrations >5 mg/L, it was lower by 2...14 times.

The outer ring of conducting bundles adjoined the ring of sclerenchyma, while the inner ones were located among parenchyma cells. The stem was hollow and there was no medullary parenchyma (Fig. 5a). The oat leaf, in the transverse section, was flat and belonged to the dorsiventral type. On the periphery, upper and lower epidermal cells were arranged in a single layer on both sides. Below the upper epidermis were the palisade mesophyll cells, while below the lower epidermis was the spongy mesophyll. The conducting bundles of rounded or oval shape, of a concentric type, were distributed throughout the leaf mesophyll (Fig. 5b). In the transverse section, the root wasround, and around the perimeter, there was a single-layered rhizodermis with root hairs reaching from half to the full diameter of the root. Below the rhizodermis, there was anextensive region of crustal parenchyma and the central partwas occupied by a radial conducting bundle (Fig. 5c).

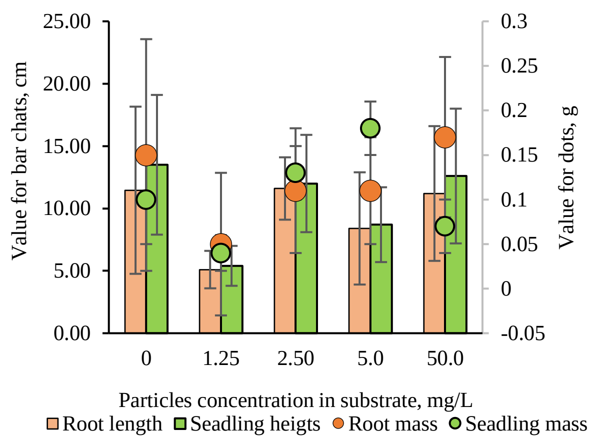


Fig. 3: Effect of Zn nanoparticles on *Avena sativa* morphological parameter and biomass

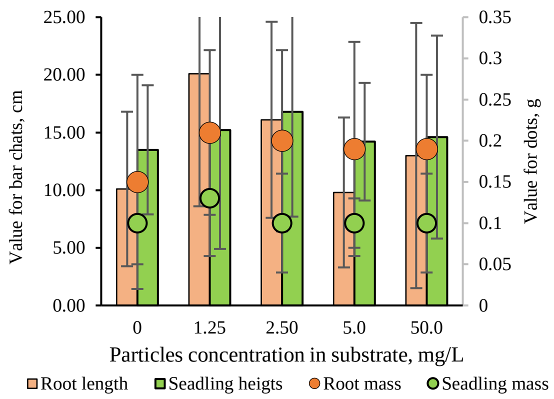


Fig. 4: Effect of Zn macroparticles in *Avena sativa* morphological parameter and biomass

The exposure of oat seeds to MPs and NPs caused specific changes in the anatomical structure of the stems, leaves, and root seedlings, for which the indicators are presented in the bar charts below (Figs. 6-8).

Regardless of the particle size, epidermis thickness and diameter of small conducting bundles in the stem decreased. In the stem of a plant treated with 1.25 mg/L NPs, the epidermis thickness and diameter of the small conducting bundles were 50 and 24% smaller, respectively, compared to the control (Fig. 6a).

The change in particle concentration did not have a statistically significant effect on these indicators. It was observed that the particle exposure increased parenchyma thickness and stem diameter. For instance, in a 1.25 mg/L of NPs suspension, the parenchyma thickness and stem diameter of the grown seedling were 280 and 186% larger, respectively, compared to the untreated stem. Increasingthe particle concentration in the substrate from 1.25-2.5 mg/L led to an increase in these indicators for both samples andat concentrations >2.5 mg/L, they decreased for NPs (Fig. 6a) but remained unchanged for MPs (Fig. 6b). In the concentration order of 1.25...2.5...50 mg/L, the stem diameter was 6.48...7.52...5.29 and 5.7...3.96...5.10 μm for NPs and MPs, respectively. Regarding the diameter of the large conducting bundles, the effect of NPs was statistically insignificant, while for MPs, it decreased by 20% regardless of the concentration after particle treatment (Fig. 6b).

Adding 1.25 mg/L particles to the substrate did not result in statistically significant changes in the morphology of the upper and lower epidermis, central vein, and mesophyll of the leaves (Fig. 7). Increasing the particle content in the substrate did not lead to statistically significant changes in the size of the upper and lower epidermis and central vein of the leaves for both particles. The thickness of the mesophyll did not change with increasing NPs content (Fig. 7a), while for MPs, it increased by 78% with an increase in concentration from 1.25-2.5 mg/L (Fig. 7b). At particle concentrations >2.5 mg/L, the thickness of mesophyll was independent of both the concentration and particle size.

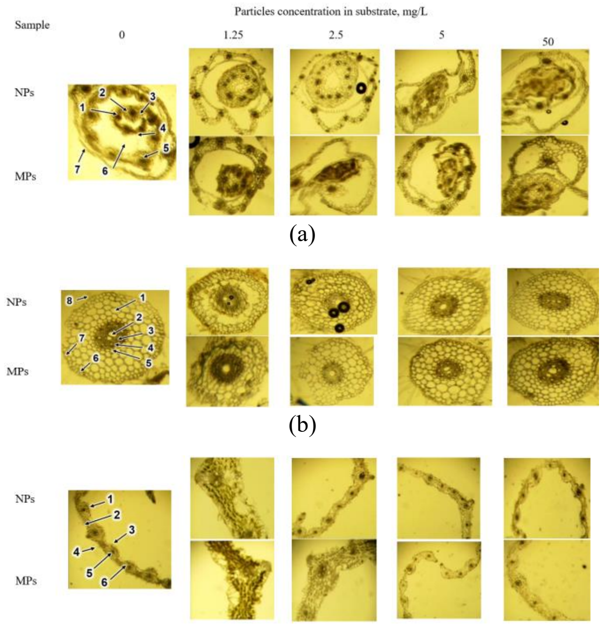


Fig. 5: Morphology of (a) stem (magnification ×40), (b) root (×20) and leave(×40) (c) of oats seedling; (a)1-xylem, 2-phloem, 3-medullary parenchyma, 4-crustal parenchyma, 5-chlorenchyma, 6-endoderm, 7-epidermis; (b) 1-medullary parenchyma, 2-xylem, 3-phloem, 4-sclerenchyma, 5-crustal parenchyma, 6-chlorenchyma, 7-epidermis, 8-simple multicellular trichomes; (c) 1-lower epidermis, 2-upper epidermis, 3-simple multi-cellular trichomes, 4-simple unicellular trichomes, 5- palisade mesophyll, 6-spongy mesophyll

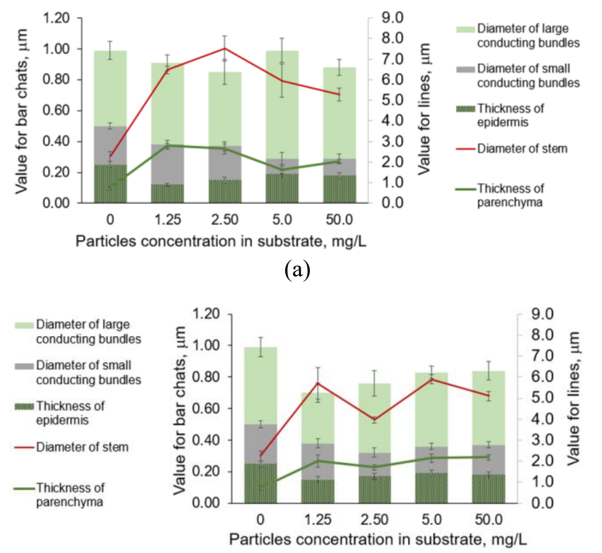
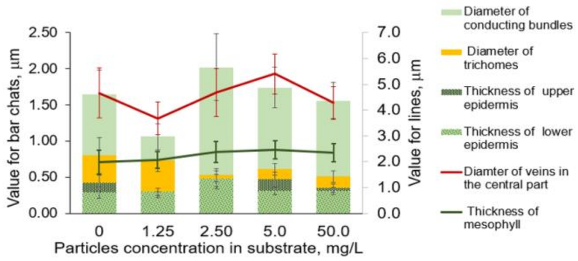
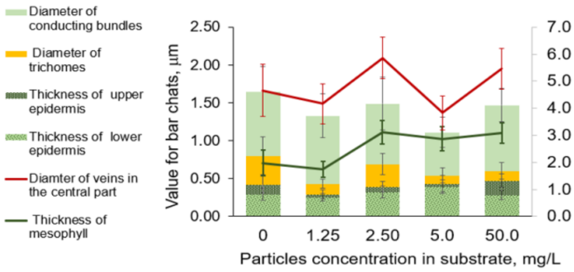


Fig. 6: Effect of particle concentration on the steam structures' size of seedlings exposed to NPs; (a) and MPs; (b) (top to bottom: Diameter of large conducting bundles, the diameter of small conducting bundles, the thickness of the epidermis, stem diameter, the thickness of parenchyma)

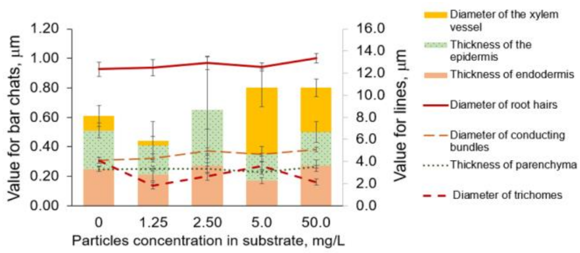


(a)

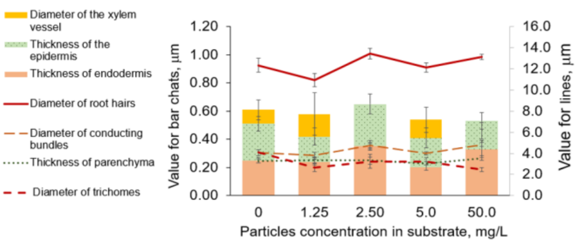


(b)

Fig. 7: Effect of particle concentration on the leaf structures' size of seedlings exposed to NPs; (a) and MPs; (b) (top to bottom: Diameter of conducting bundles, trichome, upper epidermis, lower epidermis, central vein, mesophyll thickness)



(a)



(b)

Fig. 8: Effect of particle concentration on the root structures' size of seedlings exposed to NPs; (a) and MPs; (b) (top to bottom: Xylem vessels, epidermis thickness, endoderm thickness, stem diameter, diameter of conducting bundles, thickness of parenchyma, trichome (root hairs)

It has been shown that the reduction in the diameter of leaf conducting bundles by 1.5 times compared to the control was statistically significant for 1.25 mg/L NPs (Fig. 7a) and 5 mg/L MPs (Fig. 7b). Under the influence of 1.25 mg/L of NPs, the trichome size remained unchanged, while for MPs, it decreased by 1.9 times compared to the untreated plant. At concentrations ≥2.5 mg/L, the trichome size did not change significantly in relation to the control for both particles.

In the roots of treated seedlings, regardless of the concentration and size of the particles, the morphological changes in the endodermis, parenchyma, conducting bundles, and the root itself were not statistically significant (Fig. 8). Additionally, for both particles, the root epidermis thickness decreased by 20-30% after 1.25-2.5 mg/L treatment compared to the control and did not differ from the control at higher concentrations.

It was evident that adding 1.25 mg/L of both particles led to a narrowing of xylem vessels and decreasing trichome length. In this regard, the NPs had a greater effect: The trichome size decreased by 55 and 39% for NPs and MPs, respectively. With an increase in particle concentration in the substrate, the morphological changes in trichomes were not statistically significant, while the vessel size increased under the influence of NPs (Fig. 8a), but remained statistically unchanged for MPs (Fig. 8b). When increasing the particle concentration from 1.25-5.0 mg/L, the vessel diameter increased by 1.8 and 0.8 times, respectively, for NPs and MPs.

Discussion

A comparative study of the seedlings' roots grown with added Zn NPs and MPs showed that in the presence of NPs, the degree of zinc accumulation in the roots at a particle concentration of ≤5 mg/L is greater and at>5 mg/L is less than in the presence of MPs. Thus, at a concentration of particles in the soil <5 mg/L, the zinc content in the seedlings' roots grown with added NPs exceeded 2.4 times. At a concentration of particles in the soil >5 mg/L-it was 2...14 times less than in the roots of seedlings grown in the presence of MPs.

When studying the effect of zinc particles on *Avena sativa*, it was found that high values of all morphological parameters of the areal and underground parts of seedlings were recorded when exposed to zinc nanoparticles at the concentration of 20 mg. The nanoparticles at the concentration of 5 mg caused a significant increase in the mass of the aboveground part of the plants and the length of the underground part, with a decrease in its mass, compared with the control values.

Thus, the treatment of *Avena sativa* seeds with aqueous suspensions of nano and micro-sized zinc particles with an average size of 43.8 nm and 2 microns, respectively, in various concentrations (1.25, 2.5, 5.0, and 50.0 mg/L of substrate) affects the anatomical parameters and distribution of zinc in the stems, leaves, and roots of 28-day-old *Avena sativa* oat seedlings grown in pots. The addition of <2.5 mg/L particles to the soil, regardless of their size and concentration, leads to a statistically significant

(<0.05) decrease in the thickness of the epidermis of the stem and roots, the diameter of large and small conductive bundles of the stem, the diameter of the xylem vessels and the length of the trichomes in the root, as well as an increase in the thickness of the parenchyma and the diameter of the stem. The effect of particles' concentration (an increase in concentration from 1.25-50.0 mg/L) on seedlings was pronounced only for the epidermis and small conductive bundles in the stem and the diameter of the xylem vessels in the root. At the same time, the effect of NPs was stronger than that of MPs, regardless of the accumulation of zinc in the seedlings. For example, with an increase in the particle concentration from 1.25-5.0 mg/L, the diameter of the vessel increased by 1.8 and 0.8 times, respectively, for both NPs and MPs.

These results are accompanied by data from other researchers, in which A.thaliana plant seeds treatment with ZnO NPs at a concentration of 300 mg/L reduced growth by 80% and chlorophyll content by 50% (Wang *et al*., 2016). A decrease in roots and shoot length in the radish and spinach treated with ZnO NPs was also noted (Singh and Kumar, 2016; 2018). The effect of lower concentrations of ZnO NPs on the growth and development of *A. thaliana* plants was revealed (Nair and Chung, 2017; Wan *et al*., 2019). In another study, both positive and negative effects on the plant of low-frequency ZnO were shown, depending on the concentrations used (Javed *et al*., 2017).

Atomic absorption spectroscopy of aqueous plant extracts revealed Zn uptake in the roots and stems of plants treated with NPs and only the root of the seedlings exposed to MPs. Furthermore, a dose-dependent effect was observed: At particle concentrations ≤5.0 mg/L in the soil, the Zn content in the roots was 2.8 times higher in the presence of NPs, whereas at concentrations >5.0 mg/L, it was 2...14 times lower compared to seedlings grown in soil containing MPs. Zinc can accumulate in plants and their tissues, in cellular and subcellular organelles such as chloroplasts, cell membranes, vacuoles, andnuclei (Bradfield *et al*., 2017). High accumulation of Zn in plant tissues in pots and hydroponic conditions has also been reported in the results of various studies performed using Zn NPs (Zhang *et al*., 2015; Peng *et al*., 2020; Zoufan *et al*., 2020).

Hernandez-Viezcas analyzed the effects of cerium and zinc oxide nanoparticles on some agricultural plants, in particular soybeans (Hernandez-Viezcas *et al*., 2013). Soybean seeds were planted in soil rich in cerium and zinc oxide NPs. Compared with control plants, soybean plants grown in soil containing a high concentration of zinc oxide NPs form fewer leaves. At the same time, cerium oxide NPS impairs plant growth at all tested concentrations. In this case, zinc oxide accumulates in the leaves of plants, and cerium oxide is retained at the level of nodules formed by the roots. At significant doses of cerium oxide, the nodules do not contain bacteria that bind nitrogen in the form of ammonium salts acting as fertilizers. Accumulation of zinc NPs and MPs in the root part of oats can affect changes in the rhizospheric microflora, this may be due to their bactericidal effect.

Conclusion

Thus, the experiment revealed a concentration effect in terms of Zn accumulation in the roots and anatomical features of oats, including an increase in stem diameter or xylem vessel diameter in the roots. Considering that anatomical changes in plants are the result of cellular and biochemical processes, further investigation is needed to understand the response of plant organisms to the effects of nano- and microparticles at the cellular and subcellular levels. The outcomes prove that the effect of zinc nanoparticles results in a change in the anatomy of the vegetative organs of oats, affecting their growth and development. This makes it possible to reasonably state the prospects for further research on the effects of zinc nanoparticles on oats and other agricultural crops.

Acknowledgment

Our acknowledgments go to Vitaliy Fomin and Daulietkhan Kaykenov (Laboratory of Physical and Chemical Research Methods and Engineering at Buketov Karaganda University, Kazakhstan) for conducting Atomic Absorption Spectroscopy (AAS).

Funding Information

We (all authors) confirmed that this study has no external/third-party sources of funding. All financial expenses are sourced from the author's private fund.

Author's Contributions

Arailym Serikbai: Investigate, the morphology of seedlings, visualized, originally drafted preparation.

Aidar Aitkulov: Conceptualized, methodology, supervised and proposed the topic.

Anna Stanco: Written-reviewed, edited, figures prepared, software, SEM data treatments, data curation.

Margarita Ishmuratova: Methodology, anatomy of seedlings, validated, discussion.

Assylbek Zeinidenov: Nanoparticles synthesis, SEM and BET analysis, project administration.

Wojciech Pusz: Provided expert and intellectual contribution.

Ethics

The authors declare no conflict of interest regarding the publication of this article. All authors have read and agreed to the published version of the manuscript.

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