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INTERACTION EFFECT OF TITANIUM DIOXIDE (TiO_2) AND SILICA DIOXIDE (SiO_2) NANOPARTICLES ON BEAN (*Phaseolus vulgaris* L.) IN THE PRESENCE OF EARTHWORM (*Aporrectodea calignosa)*

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ABSTRACT

The manufactured nanoparticles used in the treatment of the agricultural soils, present an impact on the human health since their residues were found to be transferred into the food chain via plants. The present work was carried out in order to evaluate the effect of some nanoparticles on a plant bean, *Phaseolus vulgaris,* widely consumed by humans via a chronic treatment with titanium dioxide (TiO_2) (40 mg / l) and silica dioxide (SiO_2) (30 mg/l) separately and in mixture with or without earthworms. At first we made a half of the seeds of bean seeds "*Phaseolus vulgaris* L." variety MGT NELSON under optimal conditions of humidity and temperature (25-26° C), with a watering every other day using distilled water for a period of 25 days, beyond is the treatment for a period of 20 days every other day alternating with distilled water. Tests and measurements were carried out on the soil, the plant and the earthworm: An analysis of some physico-chemical determinants of soil: pH, electrical conductivity, and cation content. Several analyzes and assays carried out on the roots and leaves of plants treated or not namely, a total protein assay, soluble sugar levels, quantification of polyphenols, catalase activity and chlorophyll content associated with this a statistical study and a histological study. A final part concerns some analyzes on this earthworm, the protein content, lipid and carbohydrate levels, CAT activity, as well as a statistical study and a histopathological study. The obtained results show a significant disturbance at the level of treated plants lightened by the presence of earthworms, since the histological study confirms it by the reduction of its sieve-vascular system. While for earthworms the presence of oxidative stress which results in the damage observed in the epidermis and the intestinal tissue studied.

Key words: NPs, *Phaseolus vulgaris* L., soil, *Aporrectodea calignosa*, interaction, stress.

INTRODUCTION

The nanotechnology consists of developing particles; such as, nanoparticles (NPs), nanomaterials or nanotubes, with dimensions varying between 1 and 100 nm (ISO/TR 18401: 2017), used in many industrial fields and agriculture. Because of their small size, these particles have very specific physico-chemical properties, like resistance, chemical reactivity, electrical conduction, magnetism and optical effects, which also are giving an increase to new industrial electronic, textile and pharmaceutical, cosmetic and even food transformations. These nanoparticles which are currently available and marketed on a wide scale; present a toxicological effects that are still poorly understood (Vukosav et al. 2023). The size of nanoparticles is not only the factor that plays a role in the cellular responses and toxicity, bur other factors, including surface area, purity, crystallinity, surface reactivity, adsorbed groups, coatings, solubility and shape (Van 2008; Narayan 2018). However, in reality their toxicity depends on a number of other factors like the interaction between the living organism, the biotope and these xenobiotics.

In the agricultural field, nanoparticles are usually incorporated into pesticides, fertilizers and nano-formulated biosensors for crop protection and frequently are used for plant growth (Gokila et al. 2014). Although nanoparticles occur naturally in the environment and have been used intentionally for centuries, the manufactured artificially ones present an impact on human and environmental health (Wang et al. 2022), since the main part (63%) of their product ended in the agriculture land (Keller and *al*., 2013) and the remaining quantities are being released into the different biotopes; soil, water and atmosphere from the anthropogenic activities (Telo da Gama, 2023). A number of toxicological studies have clearly demonstrated that the very small size of nanoparticles is a key factor in their penetration of living organisms, and their ability to release some ions, such as free radicals that can also have a substantial influence on their toxicity (Zhao et al. 2019). Several authors have mentioned that the nanoparticles application in agriculture showed an increase crops production such as maize, cucumber and beans (Yuvakkumar et al. 2011; Servin and al. 2015; Jeelani et al. 2017; Wang et al. 2022). At present, various silica-based fertilizers are commonly used to promote crop growth; where many studies have shown that the use of nano-silica can improve the physical and chemical properties of the soil and consequently stimulate plant growth, and resistance to various biotic and abiotic stresses. They enhance nutrient absorption, photosynthesis and the activation of defense mechanisms in plants (Alsaeedi et al. 2019; Wang et al. 2022). For these reasons have encouraged the industrials to produce the titanium dioxide nanoparticles (TiO_2) which will be incorporated into various plant protection products and fertilizers. Studies indicate that these nanoparticles can enhance root and shoot length, as well as overall biomass, by improving physiological parameters such as chlorophyll content and antioxidant enzyme activities. This enhancement is linked to increased resilience against environmental stresses, including salinity and drought conditions (Ebrahimi et al. 2016). The nanoparticles are still xenobiotic and their mode of action within living organisms remain complex (Casals 2017) and the response suggests a careful management of these products, in order of minimizing potential phytotoxic effects (Ebrahimi et al. 2016a; Nguyen and Falagan-Lotsch 2023). However, the cumulative effect in organism or in biotopes, mainly soils, tends to result in imminent toxicity (Khan et al. 2023). Soils are complex and play a central role in the balance of ecosystems (Tran et al. 2024). The anthropogenic activities, particularly the agriculture, are using quantities of nanoparticles and consequently contaminate the soil, with subsequent damage to the non-target living organisms, including agricultural products, which constitute a gateway via the food chain, mainly the bean "*Phaseolus vulgaris* L." that represents the third most important legume crop in the world (Aydin et al. 1997) because of its resistance to various types of stress due to its high antioxidant content, more specifically polyphenols (Wetwitayaklung et al. 2006).

Nanoparticles can enter food webs, through their transfer to plants and terrestrial organisms and consequently reach toxicity thresholds at the end of the trophic chain. For this reason, it is important to determine the fraction that circulates in the biological compartment and also to choose a good representative of this compartment at soil level. This is the case with earthworms, which play a very important role in the soil. They have the ability to modify their environment and even create new habitats through various actions (Edwards and Bohlen 1996; Römbke et al. 2005; Jones et al. 1994). Monitoring their populations can be used to characterize the state of the soil ecosystem and highlight the impact of soil and climatic conditions and/or human activities on soil life (Goven et al. 2005).

The present work was focused on the use of two nanoparticles, titanium dioxide TiO_2) and silicon dioxide (SiO_2), which are widely used in industry, biotechnology and agriculture (Paulkumar et al. 2011; Ali et al. 2024). Hence, the main objective is to examine the impact of plant/worm interaction in the presence of these xenobiotic (NPs) that is abundant in the environment and to evaluate their impact on these two living organisms. Also to bio-remediate this presence of nanoparticles, by using an ancestral, economical and nature-friendly method of bringing earthworms into crop fields to minimize the transfer of these NPs to the plant consequently towards the food chain.

MATERIALS AND METHODS

Chemical material: The used nanoparticles is presented by the titanium dioxide (TiO_2) which is a superfine white powder, it has a 25nm in size, trace metals basis and was supplied from the physics laboratory Badji Mokhtar Annaba, while the fumed silica nanoparticles (SiO_2-NPs) also it is a white superfine powder acquired from Sigma-Aldrich. The particle size is 14 nm with a surface area of $200 \pm 25\ m^2/g$ and a purity level greater than 98% (RT).

Soil sampling: The soil used in this study comes from an area, Seraidi department, on the slopes of the Edough massif located in the Northern-East of Algeria. This region is considered to be an unpolluted area. The samples were taken at random from 10 to 20 cm from the surface of the soil and brought directly to the laboratory, and prepared according to the ISO 11464: 2006 (E) standard.

Biological materials: The plant model used in this trial is the green bean "*Phaseolus vulgaris*. L", variety "HARICOT MGT NELSON", their seeds come from the trade as seed intended for sowing in the regions of Annaba, -El Tarf, and Guelma. The seeds chosen were in good condition, without breakage or contamination and of the same size. Before use, the seeds were rinsed in briefly immersed in a 2% hypochlorite solution (for disinfection purposes) and rinsed three times with deionized water. The earthworms used in this trial come from small-scale rearing on a farm in the El-Tarf region. Their rearing medium is moistened peat and dung (cattle waste) (50/50). The rearing is therefore made up of batches of organisms of the same age group. The earthworms were depurated for 48 hours before treatment began. Depuration involves emptying the worms' digestive tract by placing them in glass jars containing moist filter paper. The filter paper was rinsed 3 times a day to avoid ingesting turricules. The worms were then weighed and selected for a weight of between 500 and 1000 mg.

Experimental design and Greenhouse Experiment: The experiments are realized in the laboratory Firstly, the seeds were sown in pots (12.5 cm diameter, 14 cm height) and filled with a mixture (50% compost and 50% soil), at a rate of 6 seeds per pot. For this trial, two batches were used (batch no. 1, batch no. 2), each containing 12 pots (Figure 1). The seeds germinated in a mini greenhouse at a temperature of 25-26°C, a 14/10 h photoperiod, with optimum humidity. Each pot was watered with 100 mL of distilled water every other day. The crop lasted twenty days (third leaf stage).

From day 21, 6 earthworms per pot were added to batch n° 2 and the two batches (batch n° 1, batch n° 2) were treated with the two types of Nanoparticles separately (Table 1) and in a mixture (10ml) every other day, alternating with distilled water during (three repetitions for each treatment) at the concentrations mentioned in the table 1and the duration of The treatment is twenty-five days.

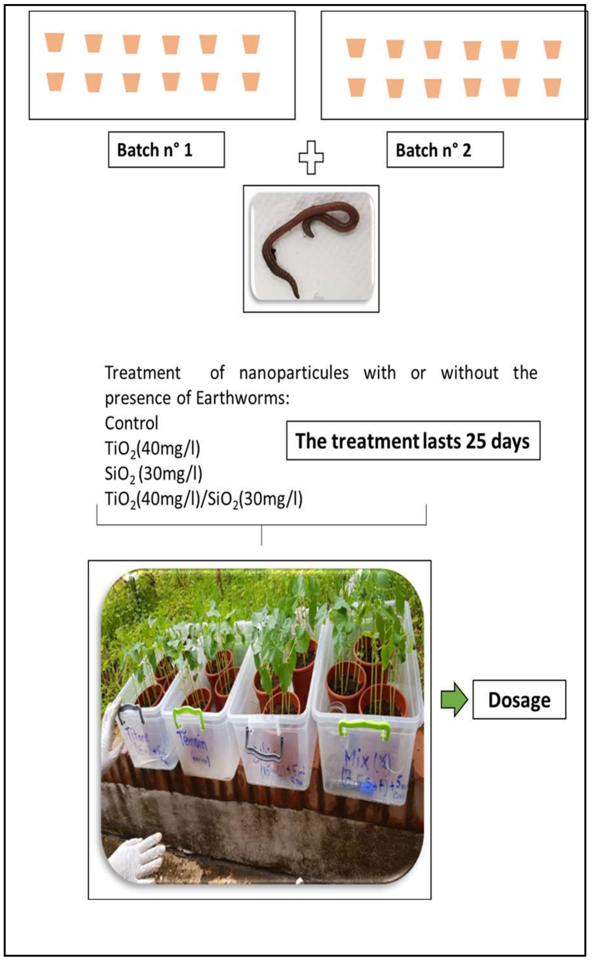


Figure 1. Experimental design of the essay.

*Table 1. **The protocol Nanoparticle essay used in the experiments.***

Treatment	Control	TiO_2	SiO_2	Mixture
Concentration (mg/l)	**0**	**40**	**30**	**40(TiO_2)/30(SiO_2)**

Soil parameters : The pH measurement gives an indication of the acidity or basicity of the soil. pH is measured directly, using a pH meter, in accordance with standard NF ISO 10390:2005(F). The electrical conductivity (EC) measurements allow to evalute the degree of the salinity of the soil. Electrical conductivity is determined in following the standard NF ISO 11265: 1994(F), using a conductivity meter on a soil extract. The results obtained are compared with a salinity scale. Colorimetric determination of exchangeable cautions Mg^+, Ca^+, K^+, Na^+ (of calcium: ammonium acetate extract is mixed with 8-hydroxyquinoline), which complexes with magnesium and then will be dialyzed and finally the calcium was complexed with cresol phthalic complex in an alkaline medium. The complex is measured at 580nm. Colorimetric determination of magnesium: after dialysis, the extract is mixed with a buffer solution and a coloring reagent, the intensity of which is measured at 505nm. Determination of calcium and magnesium by flame photometry (NF X 31-108).

Biochemical plant parameters : During the study different parameters of the used plant were considered. Soluble sugars are determined using the method of Shield's and Burnett (1960). Absorbance is measured with a spectrophotometer at a wavelength of 585 nm.

The total protein is determined by colorimetry using the method of Bradford (1976). The change in color is measured at a wavelength of 595nm using a spectrophotometer (JENWAY 3600) and bovine serum albumin (BSA) as the standard.

The Total polyphenols were determined according to the Singleton et *al*., 1999 method using the Folin-Cicalteu reagent; maximum absorption is between 725 and 750 nm and is proportional to the quantity of polyphenols present in the plant extracts.

Contents of the Chlorophylls a, b and a+b are estimated by the extraction from the plant according to the Holden (1975) method; where the values were read at two wavelengths 645 nm and 663 nm, after the calibration of the apparatus with the 80% acetone control solution. The used equation to calculate the chlorophyll values a, b and a+b (t) was according to Arnon (1949) as follows :

$$\left.\begin{array}{l}\textbf{Chl.a} = 12.70\ DO\ (663) - 2.69\ DO\ (645) \\ \textbf{Chl.b} = 22.90\ DO\ (645) - 4.68\ DO\ (663)\end{array}\right\}\ \mu g/g\ FM$$

Catalase activity (CAT) was determined using the method of Cakmak and Horst (1991). The reaction is triggered by the addition of hydrogen peroxide. The assay is carried out by following the absorbance kinetics at 240 nm for 1 minute. Catalase activity is expressed in nmol/min/mg protein (ε = 39400 M-1 cm-1).

Earthworm metabolite contents: The total, of the carbohydrates in the earthworm body, was determined using the method of Duchateau & Florkin (1959) and the absorbance reading was taken at a wavelength of 620 nm. The total of the proteins was determined using the Bradford method (1976) and the absorbance was read on a spectrophotometer at a wavelength of 595 nm. Also, the total, of the lipids was determined using the method of Goldsworthy and *al.* (1972) and the optical density was read in a spectrophotometer at a wavelength of 530 nm.

The catalase activity (CAT)was measured by following the kinetic reactions for 1 min at 240 nm in quartz UV cells, using a spectrophotometer (Aebi 1984).

Statistical analysis: Data were calculated as Mean ± SD and analyzed using Student's test followed by the analysis of variance (ANOVA) in one way of classification. The probability of 0.05 or less was considered significant. All statistical analysis was done using the software MINITAB of analysis and data processing version 17 Ink (Dagnelie 1998).

RESULTS AND DISCUSSION

Effect of NPs on the physical and chemical characteristics of the soil: Soils are both complex and extremely diverse living environments; they are home to a quarter of all identified terrestrial life. However, little research has been carried out into the interactions present in this ecosystem, despite their importance in environmental diagnostics, and in monitoring the fate of pollutants (Yotova 2018). The results obtained in this trial show that are presented in the table (2) and according to the soil pH Soltner's scale (1981), the addition of nanoparticles does not bring about any visible change in the absence of earthworms, a slightly acidic pH with values between 6.5 and 6.57, whereas in the presence of earthworms the pH obtained is between 6.34 and 6.46; This slight drop can be explained by the dejection of Earthworms into the soil, which is very rich in organic matter, at the same time enriching the soil with organic matter and, therefore more acidity (Kamboj 2021; Sunkara 2023). Soil pH is a major control parameter that has a direct influence on the distribution mechanisms of elements in the soil (McLaughlin et *al*., 2000), and therefore plays a key role in the mobility of NPs, particularly in their dissolution. The decrease in pH of the soil solution is accompanied by an increase in the number of protons in the solution, which enter into competition with the adsorbed metal cations and ultimately cause their release into the aqueous phase.

Table 2. Effect of nanoparticles (TiO_2 and SiO_2) on soil pH in the presence or absence of earthworms (control: 0 mg/l, TiO_2: 40mg/l, SiO_2: 30mg/l, mixture: TiO_2: 40mg/l and SiO_2: 30mg/l).

	In the absence of earthworms				**In the presence of earthworms**			
Sample	Control	TiO_2	SiO_2	Mixture	Control	TiO_2	SiO_2	Mixture
pH	6.50	6.50	6.56	6.57	6.46	6.44	6.34	6.46

Electrical conductivity gives a general idea of the concentration of electrolytes in the soil solution and its degree of salinization; the results obtained for electrical conductivity (table 3) show an unsalted soil for all the batches according to the Electrical Conductivity Scale (USSL, 1954). An increase is observed for the E.C. of the batches treated with TiO_2 NPs with the value of 43 compared with the control and the other treatments in the absence of Earthworms; whereas with the presence of Earthworms, these values increases for all the batches treated or not, with a maximum for the batches treated with silica (66.2).

The figure (2a&b), shows an increase in Calcium Ca^+ levels was recorded for batches treated with TiO_2 NPs in the absence (a) and presence (b) of Earthworms compared with the other treatments. In Earthworms, calcium requirements differ from one species to another, and it is a key element in the proper functioning of the Morren's glands, which secrete calcium carbonate in the form of small calcite concretions expelled into the digestive tract (Bachelier, 1978); once ejected into the environment, this enriches the soil with a form of calcium that can be assimilated by other organisms, such as plants.

Table 3. ***Effect of nanoparticles (TiO_2 and SiO_2) in the presence or absence of Earthworms on electrical conductivity (control: 0 mg/l, TiO_2: 40mg/l, SiO_2: 30mg/l, mixture: TiO_2: 40mg/l and SiO_2: 30mg/l).***

	In the absence of Earthworms				**In the presence of Earthworms**			
Sample	Control	TiO_2	SiO_2	Mixture	Control	TiO_2	SiO_2	Mixture
Electrical conductivity	36.6	**43**	36.4	37.4	55.6	56.5	**66.2**	**46 .7**

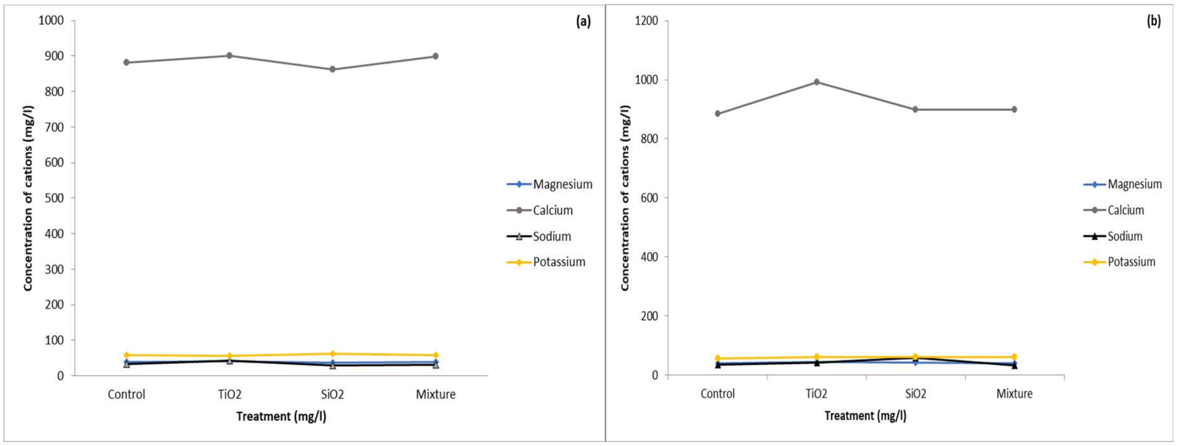


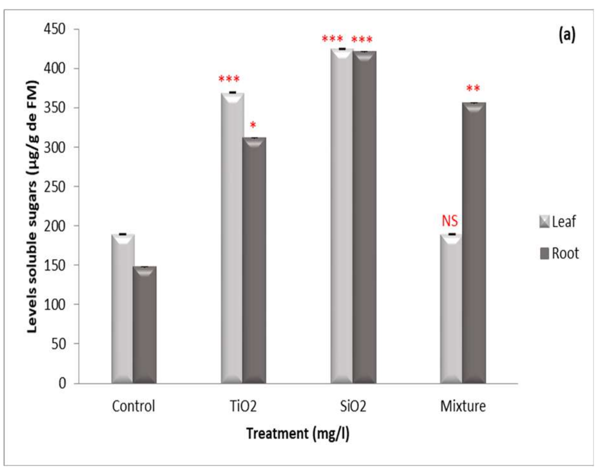
Figure 2. Effect of TiO_2 and SiO_2 nanoparticles on cation levels (Mg^+, Na^+, Ca^+, K^+) in treated and untreated soil in the absence (a) or presence (b) of Earthworms (control: 0 mg/l, TiO_2: 40mg/l, SiO_2: 30mg/l, mixture: TiO_2: 40mg/l and SiO_2: 30mg/l).

Today's agriculture contributes by adding huge quantities of these nanomaterials as fertilizers, plant protection products or as soil decontaminants, without worrying about their introduction into our food through their transfer via plants intended for human consumption (Babu et al. 2022). The integration of nanomaterials into soils presents both opportunities for improving agricultural production and challenges relating to environmental safety (Tran and *al*., 2024). While nanomaterials can improve nutrient availability and pest control, their long-term effects on soil health and the communities that support it need to be carefully considered, requiring ongoing monitoring to ensure sustainable agricultural practices with the greatest possible benefits and the least possible risks (Sharma et al. 2022; Sharma et al. 2015).

Several studies have shown that the biological models used in this trial, the bean "*Phaseolus vulgaris* L." and the Earthworm "*Aporrectodea calignosa*", are of great interest and can be used for bio-indication and/or bio-assessment of the quality of the ecosystem in which they live (Zerari et al. 2016; Akhila 2022; Sunkara 2023). One of the characteristics of soil is its ability to retain substances of various kinds. Cations and anions can be retained by the soil's absorbent complex, i.e. all the colloids (humic substances, clay, etc.) with exchangeable negative and/or positive charges. This confirms the results obtained for the batches treated in the absence of Earthworms, where there was a slight disturbance compared with the control batch.
The action of Earthworms on soil chemistry, by bringing to the surface some of the leached elements, particularly calcium, tends to limit soil decalcification, but even more important seems to be their role as an intermediary between the soil and the plant. Their droppings, known as 'castings', are considerably richer in assimilable potassium, phosphorus and magnesium. These castings have a high nutrient content in NH_4^+, NO^{3-}, Mg^{2+}, K^+ and HPO_4^{2-} compared with non-ingested soil (Syers et al. 1979; Mackay et al. 1983; Tiwari et al. 1989; James 1991); This enrichment results from the attack of minerals by digestive enzymes and the decomposition of incorporated organic matter. This data confirms the increase in the concentration of exchangeable calcium cations in all batches in the presence of Earthworms.

Effect of NPs on metabolites of the Bean "*Phaseolus vulgaris* L: The response to toxicity in living organisms via the presence of xenobiotic depends on several external and internal factors (Gil and Pla 2001), but in the case of nanoparticles the concentration, size, nature and shape of the particles are a very important factor in this reaction (Khan et al. 2019). In a large number of studies (Rameshaiah et al. 2015; Fraceto et al. 2016; Abd El Moneim et al. 2021) on the subject, it has been shown that exposure to nanoparticles improves free radical scavenging potential and antioxidant enzymatic activities, and modifies the expression of micro RNAs that regulate various morphological, physiological and metabolic processes in plants; or conversely, this exposure can cause enormous damage even if these nano-molecules are not toxic (Frazier et al. 2014; Abd El Moneim et al. 2021). At the same time, plant metabolism produces two types of metabolites: primary metabolites, whose main role is to ensure vital physiological activities (growth, development, respiration, photosynthesis and reproduction), as well as secondary metabolites, which play an indirect role in adaptation and tolerance between plants and their environment; however, in stressful situations, these two types of molecule play a complementary role (Wu and *al*., 2023).

In the first trial in absence of earthworms (figure 3 a), with treatment of 40 mg/l of TiO_2 NPs and 30 mg/l of SiO_2, the evolution of the soluble sugar content in the batches of bean seedlings treated at leaf and root level showed a notable increase between 310 to 423 µg/g FM compared with 147 to 188 µg/g FM in the batches of control seedlings: Note that this increase is more pronounced in batches treated by silica, with a non-significant difference recorded in the leaves with the mixed treatment. This result concord those obtained by Salama (2012) where 60 mg/l of Ag NPs increased carbohydrate content in *P. vulgaris* (57 % more than the control); this also corresponds to the work of Moshirian Farahi and *al*. (2023) where he obtained a significant increase in soluble sugar content (0.408 mg/g FM) in the leaves, with a concentration of 200 ppm in the treatment of *Vitex agnus* by TiO_2 NPs. At the same time, in the batches treated in the presence of earthworms (figure 3 b), the evolution of soluble sugar content followed the same trajectory, but with values clearly lower than those noted for the first treatment.



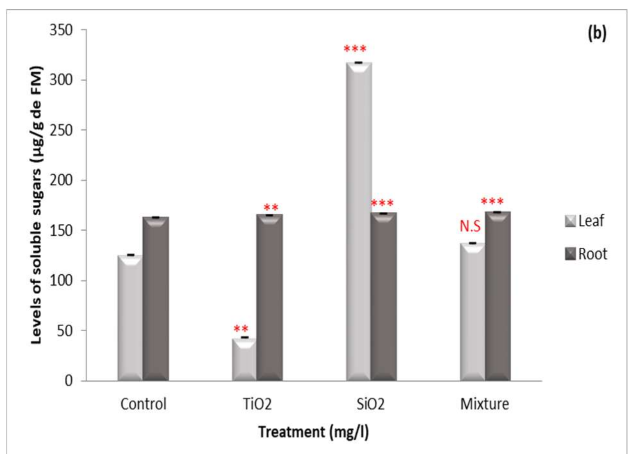


Figure 3a&b: ***Effect of TiO_2 and SiO_2 nanoparticles on the level of soluble sugars in the leaves and roots of* Phaseolus vulgaris *L., variety MGT NELSON in the absence (a) or presence (b) of Earthworms (control: 0 mg/l, TiO_2: 40mg/l, SiO_2: 30mg/l, mixture: TiO_2: 40mg/l and SiO_2: 30mg/l). FM: fresh matter.***

Proteins play a crucial role in the physiological processes and defense mechanisms of plants. They are integral to various cellular functions, signaling pathways, and responses to biotic and abiotic stresses. Under stress conditions, plants can exhibit either an increase or decrease in the total soluble protein content. This dynamic response is vital as proteins are involved in various functions, including metabolic processes and protective mechanisms against stress. However, the interaction of nanoparticles with biological molecules, particularly proteins, has raised concerns regarding their safety and toxicity.

In this essay(Figure 4a), no significant difference was recorded for batches treated with SiO_2 NPs in the absence of earthworms, these results correspond to the results of previous work (Moshirian Farahi et al. 2023), where indicated that the effects of different concentrations of TiO_2 NPs used on the amount of soluble proteins leaf of Vitex plant was not significant. Concurrently, the presence of earthworms (figure 4b) resulted in a markedly elevated total protein content, with a maximum of 79.42 µg/g FM observed in the treated leaf samples. This outcome aligns with the findings of prior studies indicating that earthworm activity can lead to substantial enhancements in soil nitrogen levels, which are crucial for protein synthesis in plants (Lemtiri et al. 2014).

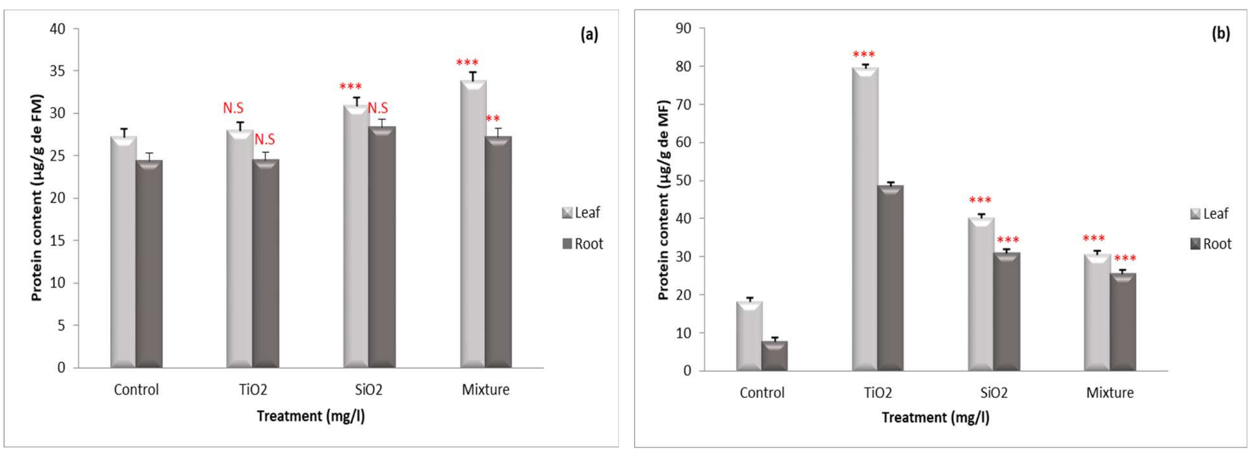


Figure 4a&b: Effect of TiO_2 and SiO_2 nanoparticles on total protein content in leaves and roots of "*Phaseolus vulgaris* L." variety MGT NELSON in the absence (a) or presence (b) of Earthworms (control: 0 mg/l, TiO_2: 40mg/l, SiO_2: 30mg/l, mixture: TiO_2: 40mg/l and SiO_2: 30mg/l). FM: fresh matter.

The results of this study (Figure 5a&b) demonstrate in the absence of Earthworms, a very highly significant increase in polyphenol levels in the leaves compared with a significant to highly significant increase in the roots of batches treated with SiO_2 and TiO_2 nanoparticles compared with the control, while batches treated with the mixture showed no significant difference; but in the presence of earthworms (figure 5b) a clear improvement in the polyphenol content of the roots of the two batches treated with TiO2 and SiO2 NPS compared with the control batches, with a non-significant response for the batches treated with the mixture. These findings are in accordance with the research conducted by numerous other scholars in the field (Khattak et al. 2021; Šebesta et al. 2021).

It should be noted that biochemical parameters in living organisms exposed to toxic substances are good diagnostic tools and can be considered as early biomarkers of exposure to chemical contaminants, stimulating the synthesis of enzymes in the detoxification system. High levels of soluble sugars in stressful situations enable the plant to regulate osmotically (osmoregulation); this action is considered a tolerance mechanism by a large number of authors (Garcia 1997; Gangola et al. 2018); this increase in soluble sugar levels maintains cell turgidity, which is the basis for the preservation of several physiological functions through the accumulation of solutes; it also protects membranes and enzyme systems, particularly in young organs (Jeandet et al. 2022). It must be remembered that sugars and amino acids are the major components of this adjustment in many plant species (Al-Tamimi et al. 2016; Singh et al. 2022).

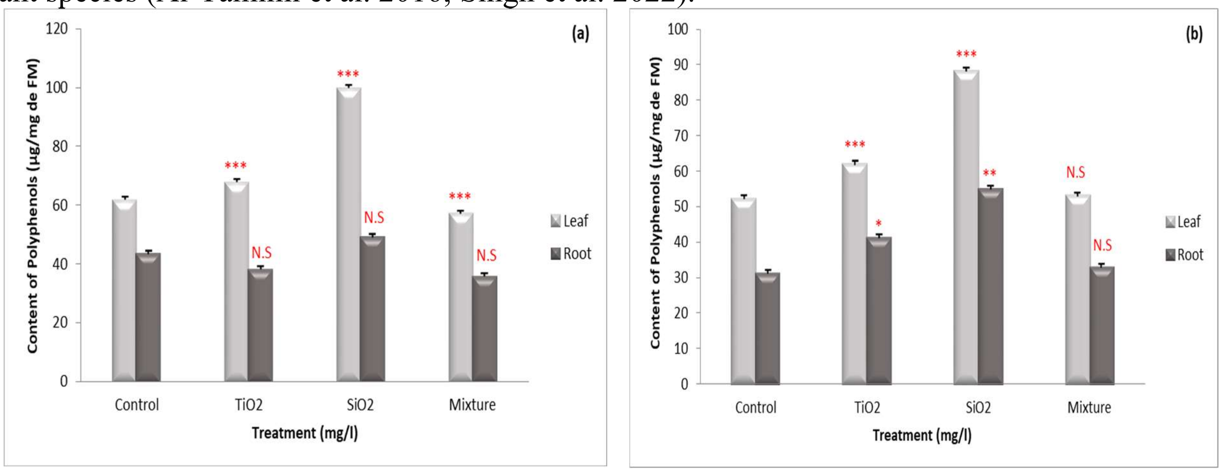


Figure 5a&b: ***Effect of TiO_2 and SiO_2 nanoparticles on polyphenol levels in leaves and roots of*** **Phaseolus vulgaris** ***L., MGT NELSON bean in the absence (a) or presence (b) of Earthworms (control: 0 mg/l, TiO_2: 40mg/l, SiO_2: 30mg/l, mixture: TiO_2: 40mg/l and SiO_2: 30mg/l). FM: fresh matter. Several studies have demonstrated that SiO_2 and TiO_2 NPs can exert a significant influence on secondary metabolite production, including polyphenols (Khater et al. 2015; Šebesta et al., 2021); which are crucial for plant defense mechanisms and possess antioxidant properties. (Uddin et al. 2023).***

The application of TiO_2 and SiO_2 NPs, either individually or in a mixture, has been demonstrated to enhance chlorophyll content in a range of plant species, including beans(Figure 6a&b). This enhancement is attributed to improved nitrogen absorption and the stimulation of chlorophyll biosynthesis pathways, which are critical for photosynthesis. Conversely, an excessive application of this nanoparticles can induce oxidative stress in plants, potentially leading to a decrease in chloroplast density and impairing the chlorophyll biosynthesis pathway (Raliya and *al*., 2015; Ebrahimi and *al*., 2016b).

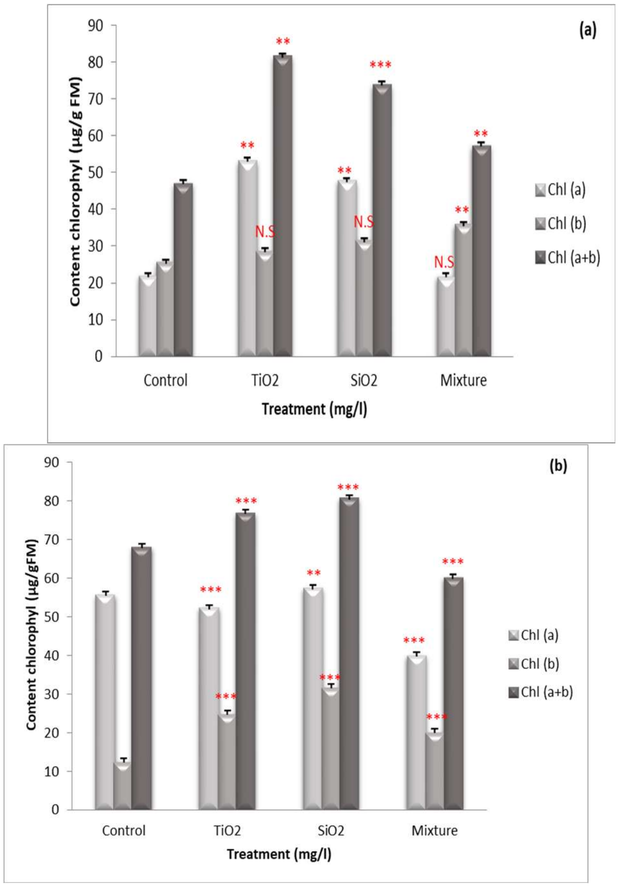


Figure 6a&b: ***Effect of TiO_2 and SiO_2 nanoparticles on chlorophyll levels in "Phaseolus vulgaris L." bean leaves, MGT NELSON variety in the absence (a) or in presence (b) of Earthworms (control: 0 mg/l, TiO_2: 40mg/l, SiO_2: 30mg/l, mixture: TiO_2: 40mg/l and SiO_2: 30mg/l). FM: fresh matter.***

In the absence of earthworms, a highly significant increase in chlorophyll content a in the leaves of plants treated with TiO2 and SiO2 NPs separately was observed compared with the control, However for chlorophyll content (b) no significant change was noted for all the batches treated; Conversely, the treatment with SiO2 NPs resulted in a highly significant increase in chlorophyll a+b. A highly significant increase (P=0.000) in chlorophyll a, b and a+b content was observed in the leaves of treated plants in comparison with the control, with the greatest response occurring in the TiO2 NPs treatment. Whereas, the presence of earthworms was observed to stimulate chlorophyll biosynthesis in all the treatments, with a highly significant increase compared with the control batches. The maximum recorded content of chlorophyll a+b was observed for the treatment with SiO_2 NPs. This result is in perfect accordance with the works of Ebrahimi and *al*. (2016), where the highest amount of chlorophyll a in *Phaseolus vulgaris* L. was obtained by following the application of nano titanium dioxide at the concentration of 0.02% during the flowering stage.

The Catalase is an essential enzyme in plants, particularly under conditions of abiotic stress, as it plays a critical role in the detoxification of hydrogen peroxide (H2O2), a reactive oxygen species (ROS) that can accumulate during environmental stressors such as drought, salinity, and pollution. The interest in catalase arises from its capacity to mitigate oxidative stress, thereby enhancing plant resilience and growth. At the same time, the plant has an integrated defense system against oxidative stress, or in instances of moderate stress, it is capable of functioning without catalase. For example, Plant phenolic compounds are powerful antioxidants that can mediate scavenging of harmful reactive oxygen species (ROS) in plants under different abiotic stressors (Šamec et al. 2021).

The results of the catalyse enzyme analysis are presented in the figure 7a, where no significant differences were observed in the roots of the various treated batches in comparison to the control series. Conversely, a significant to high significant increase was noted in the leaves with the absence of earthworms (Figure 7a). One potential interpretation of these results is that the nanoscale dimensions of these nanomolecules (Sharma et al. 2024) have facilitated their direct migration to the leaves, thereby triggering the accumulation of reactive oxygen species and increased production of stress enzymes such as catalase. Similarly results of previous works have demonstrated the induction of oxidative stress in Pinto bean (*Phaseolus vulgaris* L.), lentils (*Lens culinaris* M.) and pea (*Pisum sativum*), following treatment with TiO2 NPs, which confirm the found observations in these experiments. Upon conducting all treatments in the presence of earthworms, no significant difference observed with control series (Figure 7b). In the presence of earthworms, no significant difference in catalase activity measured between treated and control batches (Figure 7b)was recorded. This results can be explained by the fact that these small nematodes ingest soil (Lemtiri 2014), consequently nanomolecules, leading to a significant reduction in their concentration in the soil solution and less availability for our treated plants.

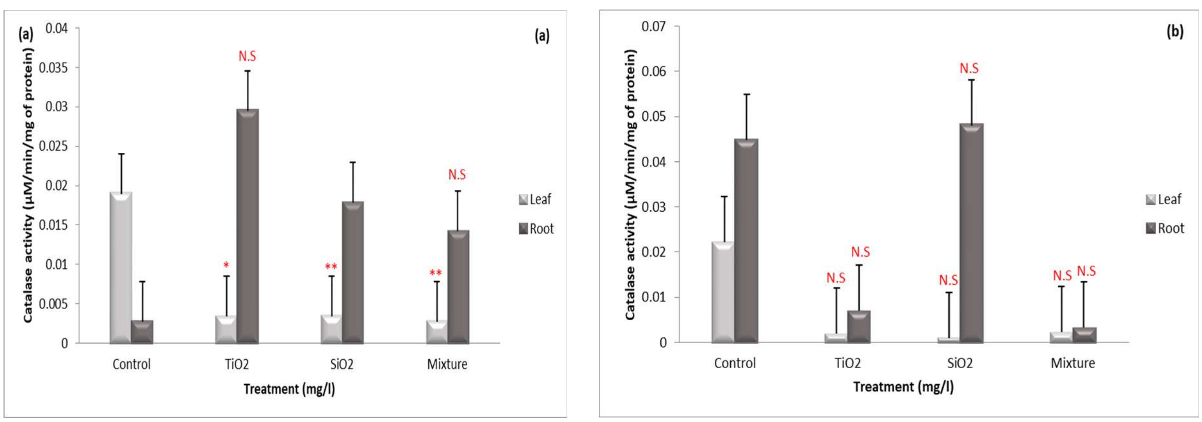


Figure 7a&b: Effect of TiO_2 and SiO_2 nanoparticles on CAT activity in the leaves and roots of *Phaseolus vulgaris* L. beans, MGT NELSON variety, in the absence (a) or presence (b) of Earthworms (control: 0 mg/l, TiO_2: 40mg/l, SiO_2: 30mg/l, mixture: TiO_2: 40mg/l and SiO_2: 30mg/l).

Effects of NPs on metabolite contents of the earthworm:

Metabolites have a fundamental role in the physiology and ecological function of earthworms, particularly in their response to environmental stressors and their interactions with soil ecosystems. Recent research has highlighted various aspects of earthworm metabolomics, emphasizing how these small molecules can serve as indicators of health, stress responses and ecological interactions.

Carbohydrates content of the earthworm under the presence of NPs are presented in figure 8. The result illustrates a highly significant increase in carbohydrate content in earthworms from all series treated with TiO_2 (4.20µg/mg of tissue) and SiO_2 NPs (5.76 µg/mg of tissue) in comparison to the control ones (1.94 µg/mg of tissue), with a maximum recorded value, which is observed for the combined treatment (8.17 µg/mg of tissue). While, direct studies, on the impact of TiO_2 and SiO_2 nanoparticles specifically on carbohydrate levels in earthworms are limited. therefore, it is suggested that exposure to these nanoparticles may disrupt normal biochemical processes. This disruption could manifest as changes in carbohydrate content, due to alterations in metabolic pathways or stress responses induced by nanoparticle exposure. The same for the series, of seeds treated in the presence of Earthworms in the second trial, a high significant increase was noticed, in comparison to the control series. This is probably due to the ingestion of these nanomolecules by the earthworms and, consequently, a reduction in their toxicity.

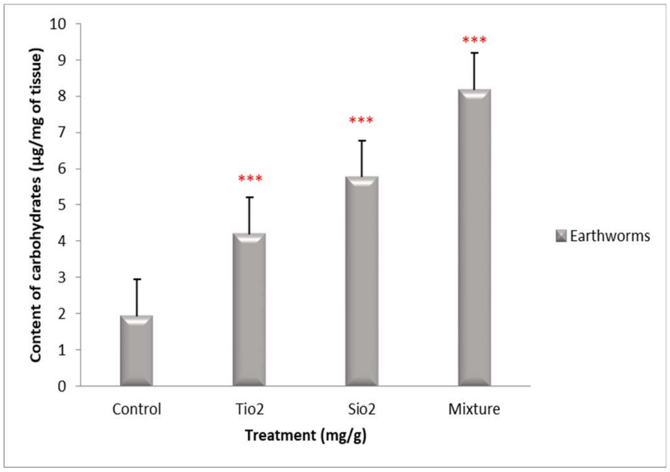


Figure 8. Effect of TiO_2 and SiO_2 nanoparticles on carbohydrates content in Earthworms (Aporrectodea calignosa » (control: 0 mg/l, TiO_2: 40mg/l, SiO_2: 30mg/l, mixture: TiO_2: 40mg/l and SiO_2: 30mg/l).

Protein content estimated from the earthworm, exposed to NPs are illustrated in figure 9. According to statistic analysi, the protein content (Figure 9) of the treatedseries with 40mg/l of TiO_2 NPs presented a high significant increase (4.86 µg/mg of tissue), while the ones treated with 30 mg/l of SiO_2 NPs showed a high significant decrease (1.71µg/g MF). The mixture treatment did not show a significant difference (2.53µg/g MF) compared to the control batches (2.83%g/g MF).

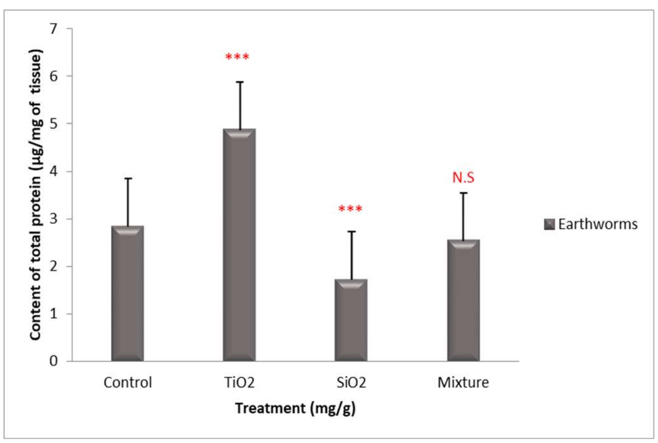


Figure 9. Effect of TiO_2 and SiO_2 nanoparticles on protein levels in Earthworms « *Aporrectodea calignosa »* (control: 0 mg/l, TiO_2: 40mg/l, SiO_2: 30mg/l, mixture: TiO_2: 40mg/l and SiO_2: 30mg/l).

Lipid content evaluated from the earthworm under effect of NPs are presented in figure 10. The interaction between earthworms and beans has resulted in a significant increase (P=0.000) in the lipid levels of earthworm in the treated batches, when compared with the control ones.

Maximum levels were observed in the batches treated with 30 mg/l of SiO_2 NPs. However, the results, of previous study (Halaimia et al. 2021) demonstrate that the application of conventional fertilizers such as NPK, causes a notable decline, in the concentration of metabolites in *Aporrectodea calignosa*.

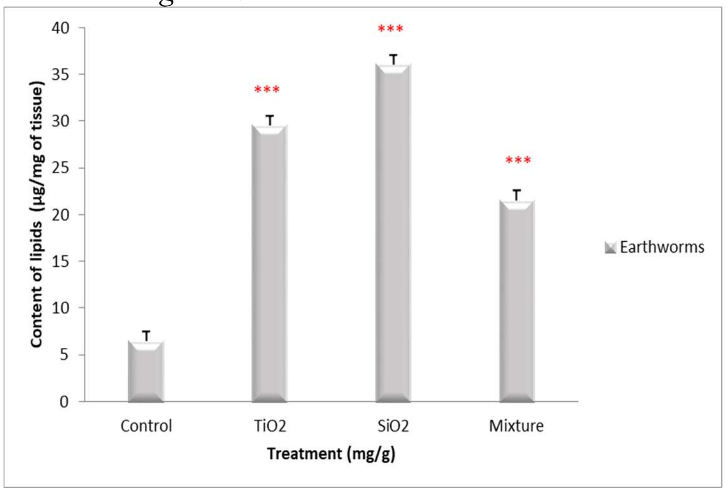


Figure 10. Effect of TiO_2 and SiO_2 nanoparticles on total lipid levels in Earthworms "*Aporrectodea calignosa*" (control: 0 mg/l, TiO_2: 40mg/l, SiO_2: 30mg/l, mixture: TiO_2: 40mg/l and SiO_2: 30mg/l).

The results effect of NPs on the enzymatic activity (CAT) on earthworm are shown in figure 11. It was noticed a high significant (P=0.000) reduction in Catalase activity in the treated earthworms by SiO_2 NPs (17349.20µmol/min/mg of tissue) and mixture (18594.03µmol/min/mg of tissue), compared to the control series (355336.4720µmol/min/mg of tissue). However, no significant difference in catalase activity observed in the series, treated with TiO_2 NPs. TiO_2 nanoparticle exhibits an effect on the structure and the activity of catalase in earthworms. The results of previous studies (Du et al. 2011; Zhang et al. 2014) indicate that, the earthworm exposure to TiO_2 can lead to alterations in catalase activity, which is in perfect agreement with the results obtained in the present work. However, the specific effects are contingent upon the concentration and duration of exposure, with higher concentrations typically resulting in more pronounced impacts on enzymatic activity.

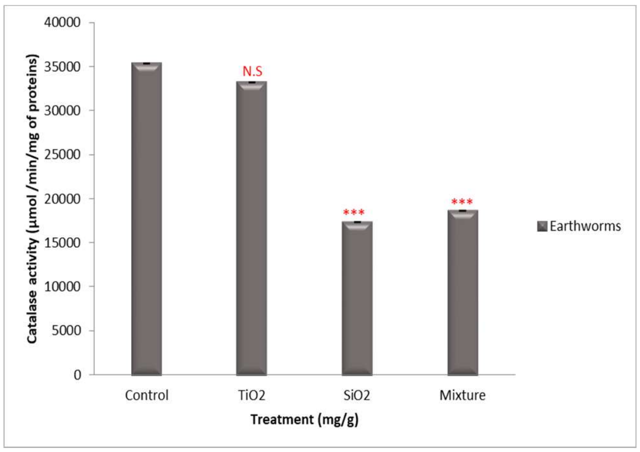


Figure 11. Effect of TiO_2 and SiO_2 nanoparticles on Catalase enzymatic activity in the Earthworm "*Aporrectodea calignosa*" (control: 0 mg/l, TiO_2: 40mg/l, SiO_2: 30mg/l, mixture: TiO_2: 40mg/l and SiO_2: 30mg/l).

CONCLUSION

The interaction of nanoparticles with plants in crop fields is a dynamic process, involving a multitude of complex uptake mechanisms and transport pathways. Although they offer the potential for benefits in terms of improving plant growth and resistance, their effects on biochemical and physiological processes, necessitate careful consideration of their use in agricultural practices; otherwise the application of these nanoparticles presents a challenge related to toxicity and bioaccumulation. The obtained results of the assays, carried out on *Phaseolus vulgaris*, revealed that the application of TiO_2 and SiO_2 NPs increase the soluble sugar levels, total protein content, total polyphenol levels and chlorophyll a content, and moderate catalase activity in absence the of earthworms. Consequently the use of Nps confirm the presence of oxidative stress, which is alleviated for the series where the earthworms were added.

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