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Changes in microflora of soil and intestines of Eisenia fetida upon exposure to nickel nanoparticles

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Abstract. To date, nanoparticles (NP) are quite promising in science and technology. However, the widespread practical use of nanoparticles requires the assessment of their effects on living organisms, including soil organisms. This study analyzes the impacts of NiO nanoparticles (50, 100, 200 and 400 mg/kg dry soil) on morpho-biochemical parameters of Eisenia fetida. In the experiment, we determined the content of lipid peroxidation products - malondialdehyde (MDA), as well as the activity of catalase (CAT) and superoxide dismutase (SOD). The catalase activity was expressed within bipolar activity, which can be attributed to the non-specific response of living organisms and metal activity in a substrate. The MDA content was increased at a dose of 250 mg and 125 mg/kg of NiO NP on the 28th and 56th day, respectively. Thus, the biochemical parameters varied depending on the dose of NiO NP. One mechanism of NiO NP toxicity in animals is the oxidative stress.

1. Introduction

Today, nanoparticles (NP) present a considerable interest both from a scientific point of view and due to their wide practical application [1]. Nickel-containing dispersions are not an exception due to their unique biological properties [2].

The increase of production and use of nickel nanoparticles inevitably increases the risk of potential toxicity to different living organisms, where soils may become the main pollution target [3]. Earlier it was found that in the concentration of 0.1 M, Ni NP lead to oxidative stress and random degradation of DNA in the Triticum vulgare roots [4].

From these perspectives, there is a need to indicate the impact of nickel oxide nanoparticles on the physiological state of Eisenia foetida earthworms. This species is recognized as a suitable biological model for ecotoxicological studies [5]. For example, earthworms were shown to be the primary link of organic material decomposition, as well as the model organisms for toxicity studies of various substances. Besides, earthworms may directly contact with NP in the soil environment [6].

Thus, the purpose was to study the impact of nickel oxide nanoparticles on the morpho-biochemical indicators of Eisenia fetida worms.

2. Materials and methods

2.1. Chemicals and substrates

Commercially available spherical nickel oxide (NiO) NP (d=85±15 nm) manufactured by Advanced Power Technologies LLC (Tomsk, Russia) and Plasmotherm LLC (Moscow, Russia) were used for the study.



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The test metal represented by dry powder was added to deionized water (10 ml) followed by dispersion on UZDN-2T sonicator (Russia); f-35 kHz, N-300 W, A-10 µA) for 30 minutes.

The NP agent was prepared using the above sonicator (f-35 kHz, N-300 W) by dispersing for 30 minutes.

The soil collected from the upper humus horizon (0 to 30 cm) 40 km from Orenburg (51°28'55.3"N 55°03'49.9"E) representing textural carbonate chernozem, pH 7.7, organic C 3.9 % and N 0.24 % was used as the substrate. The soil material was then dried, inclusions were taken out, mixed, thoroughly dround in a porcelain mortar and sieved through a 2 mm mesh sieve.

2.2. Test object

Eisenia foetida (Oligochaeta: Lumbricidae) worms purchased at BiOEra-Penza LLC (Penza, Russia) were used as test organisms. The worms were grown in horse manure without any medicinal agents at $22\pm 2^{\circ}$ C in the Laboratory of Agroecology of Technogenic Nanomaterials of the Federal Scientific Center for Biological Systems and Agricultural Technologies of the Russian Academy of Sciences (Orenburg, Russia). Then mature worms weighing 300-400 mg were selected, which were pre-acclimatized for 7 days in pure soil.

2.3. Scheme of the experiment

The experiment was conducted according to the guidelines for chemical bioaccumulation testing by ground oligochaetes OECD (2010). Before the experiment, the worms were washed with distilled water and kept for 24 hours on a wet filter paper in petri dishes to evacuate the gut contents. After acclimatization, 3 individuals purified on a wet filter paper were selected, weighed and the NP content, which was considered the initial concentration of metal in the worm body, was analyzed.

To prepare the substrate, the soil was mixed with distilled water using an electric stirrer to form a thick mass without free water with the humidity of 40-45 %. The substrates were left for 1 day, after which NPs at the concentrations of 50, 100, 200 and 400 mg/kg of the substrate were added, and the humidity was adjusted to 65–70 %. The group without NP (0 mg/kg) was taken as the control.

The worms were kept in plastic containers 5 cm long, 4 cm wide and 4 cm high filled with 500 g of soil. Ten healthy worms were placed in each container. All containers were covered with a perforated lid to prevent moisture loss and kept in a dark place. The experiment was conducted for 56 days at an air temperature of 22 ± 2 °C and a substrate temperature of 25 ± 2 °C, in 5 replications. The number of dead individuals was monitored daily.

2.4. Biochemical study

The tissues of worms were ground on a TissueLyser LT tissue grinder (QIAGEN, Germany), centrifuged over 10 minutes at 15000 rpm. The content of products of lipid peroxidation products – malondialdehyde (MDA), as well as the activity of catalase (CAT) and superoxide dismutase (SOD) were defined in the received homogenate via CS-T240 automatic biochemical analyzer (Dirui Industrial Co., Ltd, China) using Randox commercial biochemical kits (USA).

2.5. Statistical analysis

The statistical analysis was carried out using standard ANOVA procedures followed by the Tukey test (SPSS ver. 17,0). The differences were considered statistically reliable at P<0.05.

3. Results and discussion

We found that *E. foetida* masses were changed when NiO NP were added to the soil (Figure 1). In particular, on the 28th day of exposure, the weight reduction was established at a dose of NiO NP amounting to 125 mg/kg dry soil. The extension of the cultivation period to 56 days was accompanied by the reduction in weight from 17 to 33.2 %, with the maximum value at a dose of 50 mg/kg (Figure 1). In medium and high concentrations, the weight indices had a smaller range of body weight loss.

Thus, the increase in exposure time with nickel-based nanomaterials to 56 days contributed to the total reduction of *E. foetida* weight as a whole. A similar pattern is shown in other metal nanoparticles (Heckmann et al., 2011).



Figure 1. Change of *E. foetida* weight at different NiO NP concentrations in soil substrate (% of control (0 mg/kg), cultivation time – 28 and 56 days)

The ambiguous impact of NiO NP was expressed on the 28^{th} day when the level of protein in *E. foetid*a was decreased reaching the maximum at the NP dose of 500 mg/kg (Figure 4). On the contrary, on the 56th day with increased load from 125 mg/kg and higher, the protein level exceeded the control value from 22.2 % to 68.2 %, and only a dose of 50 mg/kg of NiO NP negatively affected the protein synthesis (Figure 2).



Figure 2. Difference in protein content in *E. foetida* at different concentrations of NiO NP in soil (% of control (0 mg/kg), cultivation time – 28 and 56 days)

The introduction of NiO NP into soil was accompanied by the reduction of SOD activity on day 28 at all exposure periods against the background of weak enzyme activation at a dose of 250 mg/kg on day 56. The increase of CAT level on day 28 at low and high concentrations was accompanied by the decrease to the level of control values. If the incubation period was increased to 56 days, no significant impact was established (Figure 3).



Figure 3. Difference in the activity of antioxidant enzymes and the MDA level in *E. foetida* when different concentrations of NiO NP (% of control (0 mg/kg) were added to soil, cultivation time -28 (a) and 56 (b) days)

On day 28, MDA as a marker of fatty peroxidation and oxidative stress showed a marked decrease to 50 % at the maximum concentration (500 mg/kg). The increase of exposure period to 56 days was accompanied by the decrease of MDA content to control values, while maintaining high values at a dose of 125 mg/kg NiO NP.

Thus, our study showed that SOD and CAT as an important element of cell protection under exposure conditions and NiO was expressed in the bipolar activity of enzymes, which can be attributed to the non-specific response of living organisms and metal activity in the substrate.

4. Conclusion

The study showed that the biochemical indicators varied depending on the dose of NiO NP. According to researchers, one of the mechanisms of NiO NP toxicity for animals is the oxidative stress. At the same time, cellular antioxidant protective systems are unable to reduce the excess production of active oxygen forms and oxidative stress in earthworms. In his study Gomes et al. [7] showed that relatively low SOD activity in earthworm tissues was observed at 500 mg/kg NiO NP, which is consistent with the results of our study. Similar results were obtained on *E. fetida* when silver nanoparticles were exposed to CAT activity for 28 days [7]. Besides, Mwaanga et al. [8] determined that the impacts of CuO- and ZnO-NP on the worm organism had significant changes (increase/decrease) in the activity of SOD in urban soils. The studies show that various environmental pollutants, including NP metals, cause MDA increase leading to progressive oxidative damage and eventually cell death [9].

Thus, the results of our study introduce new ideas about the impact of heavy metal NP on the biological functions of vermiculture using the example of nickel. The work described the relationship between the level of NiO NP and the development of *E. foetida* responses, which allowed estimating the dynamics of direct biological impacts of excessive concentrations of these metals on the cells of soil organisms. Similar to the studies (Heckmann et al. [10–14]), a disorder in the enzymatic and organ systems of worm regulation in response to metal stress resulting from a decrease in weight and the level of protein exchange was established. The reduction of protein in soil organisms may be related to the interaction of Ni²⁺ with -SH-groups of proteins [15].

The applied significance of the study includes the development of biotechnologies for the effective management of organic wastes, including agricultural wastes, as well as the possibility to neutralize toxic substances in soil, including metal nanoparticles.

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