Effect of nanoparticles of nickel on morphobiochemical parameters *Eisenia fetida*

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Abstract. We conducted comparative study of effects of NP Ni on vital characteristics, the microflora of the soil and intestine of *Eisenia fetida*, its antioxidant system and protein content. We formed 5 groups of worms ($n = 10$), respectively 50 were added to soil substrate; 100; 200 and 400 mg/kg of Ni nanoparticles. In our work, we showed that nanoparticles (NP) Ni had negative effect on growth of worms. We noted stimulation of growth at maximum concentration of Ni (500 mg/kg) on day 28. Disturbance of protein metabolism was manifested in decrease in level of protein in body of worm, maximum values were observed in Ni-containing substrate at doses of 250 mg/kg (53.3%) and 500 mg/kg (42.8%). The functioning of antioxidant protection in presence of NP Ni was manifested in increase in activity of SOD and CAT, depending on timing of exposure and dose of substance. Similar level was characterized by presence of MDA.

1. Introduction
In recent years, nanoparticles (NP) are of considerable interest both from scientific point of view and in connection with possible practical applications. Nanoparticles of nickel and its oxide also attract attention of researchers due to their unique biological properties [1], magnetic [2], catalytic properties [3]. Such widespread use of nickel nanoparticles also implies increase their production, as well as increased risk of potential toxicity to biological objects and systems [4], since it has been experimentally proven that nickel is toxic and has carcinogenic properties [5]. In general, experiments conducted with use of natural soils and soil inhabitants to study toxicity of nanoparticles, most realistically reproduce conditions of real environment, which allows to take into account complex of variable parameters and predict long-term effects of effects of certain substances [6]. Since invertebrates play important role in soil ecology, it is very important to pay special attention to risk assessment of NPs based on metals as source of dysfunction in this trophic group of soil organisms. That is why purpose of this study was to study effect of nickel nanoparticles and its oxide on state of morphometric, biochemical and antioxidant system of red Californian worm *Eisenia fetida*.

2. Materials and methods
Spherical nanoparticles (NP) of nickel (Ni) (94 nm) of Advanced Powder Technologies LLC (Tomsk, Russia) were used to conduct the research. *Eisenia fetida* (Oligochaeta: Lumbricidae) were cultivated in Federal Scientific Center for Biological Systems and Agrotechnologies (Orenburg, Russia), previously purchased at OOO BiOera-Penza (Penza, Russia). Worms were grown in horse manure at temperature of 22±2 °C. The soils were represented by texture-calcareous chernozem.
Test was conducted according to the guidelines for testing chemicals with OECD (2010) ground oligochaetes. We mixed soil with distilled water by means of electric stirrer until dense mass was formed without free water with moisture content of 40-45%. Substrates were left for 1 day, after which nanoparticles were added at concentrations of 50, 125, 250 and 500 mg/kg substrate, the latter, in turn, adjusted to moisture content of 65-70%. Preparation of NP was carried out in isotonic solution on ultrasonic disperser (f=35 kHz, N=300 W), by dispersing for 30 minutes.

The experiment was carried out for 28 and 56 days at air temperature of 22±2 °C and substrate temperature of 25±2 °C in five replicates. After cleansing digestive tract, worm tissues were homogenized on tissue homogenizer (QIAGEN, Germany). In homogenate, we determined content of malonic dialdehyde (MDA), catalase (CAT) and superoxide dismutase (SOD) on analyzer CS-T240 (Dirui Industrial Co., China) with biochemical sets of Randox (USA).

We performed the statistical analysis using standard ANOVA techniques followed by the Tukey criterion (SPSS ver. 17.0). Differences were considered statistically significant at P<0.05.

3. Results and discussion

3.1. The study of morphobiochemical parameters Eisenia fetida when NP Ni introducing into soil

At the 28th day of exposure, compared with initial concentration in control variant (without use of NP Ni), growth dynamics was positive. In variants with addition of NP Ni at concentrations of 50, 125 and 250 mg/kg, worm mass was below control value. In comparison with initial mass, we recorded decrease of 5.36% at a NP Ni concentration of 125 mg/kg (Figure 1). At concentration of 500 mg/kg, we established increase in mass of worm, which was 24.5% of background value.

![Figure 1. Change in weight of E. fetida with introduction of NP Ni in soil (% of control).](image)

Distinctive feature of protein metabolism is the absence in the body of this depot. The whole protein is included in the structure of cellular elements of tissues and liquids.

In control group on day 28 the protein level was 1.05 g/l, but its level with dose of NP Ni 50 mg/kg decreased by 31.4%, at 125 mg/kg - by 27.6 %; at 250 mg/kg - by 53.3%, at 500 mg/kg - by 42.8% compared to the control (Figure 2).

Catalase (CAT) and superoxide dismutase (SOD) act as a primary antioxidant defense system, catalyze the conversion of reactive oxygen species (ROS) to less active or inert compounds.
Analysis of activity of enzymatic antioxidants in soil environment showed, after exposure with NP Ni, slight decrease in SOD activity for 28 days compared to the control in all the experiment variants (Figure 3). The increase in the exposure time to 56 days was accompanied by significant increase in enzyme activity with use of NP Ni at concentrations of 250 and 500 mg/kg, while NP Ni at concentration of 125 mg/kg still provoked decrease in its activity (Figure 3).

Catalase activity, on the other hand, after 28 days of exposure in all the concentrations studied, increased significantly, with the trend remaining up to 56 days of exposure. The difference in CAT activity at concentrations of 250 and 500 mg/kg of NP Ni compared with the control reached 33.3% and 50%, respectively (Figure 4).
Product of lipid peroxidation (LPO) of membranes - malonic dialdehyde (MDA) is used as biological indicator of development of plant oxidative stress (Figure 5). ROS can initiate LPO, as result of which cell membranes become permeable to ions and organic acids.

According to modern concepts, NP of metals also contribute to mechanical destruction of membrane structures, which change probability of metals entering cells. We found that level of MDA did not have pronounced dynamics at the 28th day of exposure. Compared to the control, decrease in its amount was determined at dose of 50 and 250 mg/kg NP Ni, increase at dose of 125 mg/kg. However, on 56 days, stress effect of the metal was manifested in steady increase in amount of MDA when all concentrations of NP Ni were studied.

4. Conclusion
We established, as in the studies [7], violation in enzymatic and organ systems of worm regulation in response to stressful effects of metals, expressed by decrease in mass, protein metabolism. CAT and SOD act as primary system of antioxidant protection, catalyze active forms of oxygen and are important element of protecting cells from excess Ni˚. Heteropolar response of enzymatic system under conditions of prolonged exposure to metals was expressed in decrease in activity of SOD due to its increased expenditure on O₂ quenching, as well as destruction of enzyme by other ROS [8]. In turn, increase in activity of CAT indicates stimulation of oxidative processes and catalytic destruction of peroxides in cell, which in subsequent could be the cause of decline in SOD activity. Not excluded, and activation of latent forms of enzyme and synthesis of new molecules [9-11].

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