Studying the vital signs of *Eisenia Fetida* after introducing copper-containing nanoparticles into the culture medium

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Abstract. Today, the attention of many researchers is turned to nanoparticles (NPs) and their use in practice. At the same time, nanoforms can exhibit toxic effects in relation to living organisms. One of the promising models for assessing the effect of nanoparticles is the *E. fetida* worm. In this regard, the aim of our study was to study the responses of the *E. fetida* worm in artificial soil with the introduction of NPs of Cu and CuO. In our work, we evaluated mortality, the mass of worms, the activity of antioxidant enzymes, and the content of malondialdehyde in the worm when introducing NPs of Cu and CuO at a concentration of 0; 50; 100 and 500 mg/kg of dry soil. In the course of our study, we found the stimulating effect of the minimum of the studied dosages of CuO NPs (50 mg / kg), which in the future may be of an applied nature. It has been established that Cu NPs have the highest toxicity, causing high mortality rates (80 %), a 50 % weight reduction (p ≤ 0.05) at a dosage of 500 mg/kg against the background of increased catalase activity and malondialdehyde content with a decrease in superoxide dismutase activity.

1. Introduction

To date, nanoparticles (NPs) are subjects of active study by scientists of various fields: medicine, technology, agriculture, etc. [1]. At the same time, there is a need to study the effects that occur when nanoparticles can enter the soil. Currently, there are a number of studies showing that metal NPs adversely affect soil communities [2]. At the same time, there are reports in the literature that low concentrations of NPs can have a stimulating effect on soil organisms. Nevertheless, NPs are potentially toxic (dangerous) for soil and soil organisms, since they can accumulate in the soil and interact with various substances in it for a long time [3].

Among a number of metal nanoparticles copper and copper-containing nanoparticles, are worth highlighting. Scientists' interest in copper is due to the fact that copper is a necessary element for the life of animals and plants. Interestingly, copper as an element is considered one of the most important elements that pollute the environment [4]. To study the effect of nanoparticles on soil objects, an important role is played by the choice of an object for conducting experiments. So, earthworms are high-quality bioindicators of soil pollution [5].

They are capable of accumulating various chemical elements, which can lead to a direct disruption of biological processes in the soil and local biodiversity, and indirectly through the transfer of metals by earthworms to organisms inhabiting different trophic levels [6].

Thus, the aim of our study was to study the responses of the *E. fetida* worm in artificial soil with the addition of Cu and CuO nanoparticles.
2. Materials and Methods
The study used nanoparticles (NPs) of Cu (80 ± 9 nm) and CuO (70 ± 5 nm), manufactured by Advanced Powder Technologies LLC (Russia).

For research, a standardized artificial soil was prepared (OECD, 1984) by mixing 70 % of quartz sand (dry weight), 20 % of kaolin and 10 % of ground peat (organic nitrogen amounted to 5.8 %, pH was adjusted to 6.0 ± 0.2 by powdered calcium carbonate (CaCO$_3$)).

Initial solutions of NPs (2 ml) were prepared by adding a dry sample to distilled water, after which suspensions of NPs were placed in an ultrasonic bath for 30 minutes. The solutions were stored without access to light.

_Eisenia fetida_ worms used in the studies were purchased at BioEra-Penza LLC (bioeragrup.ru) and grown in the nursery of the Laboratory of Agroecology of Technogenic Nanomaterials of the All-Russian Research Institute of Beef Cattle Breeding of the Russian Agricultural Academy (Orenburg, Russia). The worms were in horse manure without the use of any chemicals or drugs. Before the experiment, the worms were kept on moistened filter paper in a Petri dish.

For each repetition, mature worms weighing from 400 to 450 mg were selected, of which 4 groups were formed (n = 10): group I was cultivated with the addition of NPs at a concentration of 50 mg/kg of soil; soil for II group contained 100 mg/kg of soil; soil of the III group, 500 mg/kg of soil; IV (control) was cultivated without NPs in the substrate. The worms were placed in plastic containers (0.4 × 0.15 × 0.02 m) with 10 prepared substrates (Taylor et al., 2004).

The test was carried out for 14 days at an air temperature of 22±2 °C and a substrate temperature of 25±2 °C in 5 replicates. At the end of the experiment, the worms were selected, washed with distilled water, and then weighed. The contents of the digestive system were removed by dissection of the digestive tract.

Determination of antioxidant activity of enzymes (catalase (CAT), superoxide dismutase (SOD), content of malondialdehyde (MDA) in worms was carried out on an automatic biochemical analyzer CS-T240 (Dirui Industrial Co., Ltd, China) using commercial Randox biochemical kits (USA).

For this, extracts from worms were prepared by homogenizing them in a buffer mixture (Tris 50 mmol/L, DTT 1.0 mmol/L, EDTA 1.0 mmol/L, sucrose 250 mmol/L, pH 7.5).

All experiments were processed by methods of variation statistics using the Statistika V8 software package (StatSoft Inc., USA).

3. Results and Discussion
In our study, the introduction of Cu NPs had a strong effect on _E. fetida_, which was shown at a high mortality rate (70 %) at a dose of Cu NPs of 500 mg/kg. At the same time, the introduction of CuO NPs even at a concentration of 500 mg/kg was not higher than the test validity limit (20 %) (Fig. 1).

The mass of earthworms was evaluated on the 7th and 14th day of our experiment. Weight loss was detected in all variants of the experiment. The reason for weight loss was, firstly, the lack of organic matter for worms (10 % of sphagnum peat is contained in the substrate) and, secondly, in a decrease in locomotorm activity at high concentrations of Cu NPs. So, at the maximum Cu concentration, the decrease in the mass of the worm was 45 % (p ≤ 0.05), and at a similar concentration of CuO NPs it was only 28 %. Note that at a dose of 50 mg/kg CuO NPs, a stimulating effect was established, which was expressed in a significant superiority over the control by 21.3 %.

The activity of antioxidant enzymes, depending on the concentration of Cu nanoparticles and the exposure time, was changing in our experiment (Figure 2.3).

When introducing Cu NPs into the soil, it was shown that the MDA content (Figure 2) was higher than the control values (by 33.3 and 67 %, compared with the control, for concentrations of 100 and 500 mg/kg, respectively), except for the concentration of 50 mg/kg, where the content of MDA was reduced by 12.3 %, compared with the control group.

In the presence of Cu NPs, the enzymatic activity of CAT in the _E. fetida_ worm was higher than the control values. This phenomenon was most pronounced at a concentration of 50 and 500 mg/kg of dry soil (83 and 69.9 %, respectively, compared with the control).
Figure 1. Mortality of E. fetida upon application of NPs of Cu and CuO on days 7 and 14

Figure 2. Antioxidant enzymatic activity in E. fetida and MDA content after a 14-day exposure in artificial soil with different concentrations of Cu NPs

In our study, the activity of SOD in the worm decreased at concentrations of Cu NPs of 100 and 500 mg/kg of dry soil, while with 50 mg/kg of Cu NPs, the SOD activity was at the level of control values.

In the presence of CuO NPs in the soil, SOD activity (Figure 3) was higher than the control in all studied concentrations. The highest value (98.1 %, compared with the control) was obtained with the addition of 500 mg/kg of dry soil of CuO NPs.

CAT activity upon introduction of CuO NPs decreased relative to the control at dosages of 50 and 100 mg/kg of dry soil and increased at 500 mg/kg (by 18.7 % compared to the control).

The content of MDA upon application of CuO NPs to the soil was lower than the control values in all studied concentrations.

Thus, the trophometabolic potential of Eisenia fetida, due to the presence of copper and its oxide nanoparticles in the soil, was studied. It has been established that in relation to the studied object, copper NPs have the highest toxicity, causing high mortality rates (80 %), weight reduction by 50 % (P ≤ 0.05) at a dosage of Cu NP of 500 mg/kg against the background of increased catalase activity and malondialdehyde content with a decrease in the activity of superoxide dismutase.
Figure 3. Antioxidant enzymatic activity in E. fetida and MDA content after 14-day exposure in artificial soil with different concentrations of CuO NPs

In the course of our study, we found the stimulating effect of the minimum of the studied dosages of CuO NPs (50 mg/kg), which in the future may be of an applied nature. The experiments performed indicate that the high sensitivity of the organisms presented allows them to be used as biological tests (bioindicators) in order to assess the state of the pedosphere.

4. Conclusion
In our study, we showed that Eisenia fetida worms are a good model for monitoring the toxicity of ecological xenobiotics, in particular, nanoparticles and for assessing chemical toxicity in soil [7]. A large volume of soil passes through their digestive tract, which is why they are most susceptible to the toxic effects of environmental pollutants than any other terrestrial invertebrate. In addition, worms serve as an important component of the food chain and diet for many organisms. Due to the edaphic type of habitat, they are in close contact with the water and solid phases of the soil and can accumulate pollutants in the environment in their bodies [8].

Researchers believe that the generation of superoxide anion and nitric oxide are established cytotoxic reactions in annelides. Environmental-mediated modulation of these agents has been reported to affect many cell functions, including apoptosis, damage to nucleic acids, and lipid peroxidation [9].

Thus, it was shown that inhibition of superoxide dismutase activity was recorded in the earthworm, E. fetida, after prolonged exposure to the herbicide fomesafen [10]. We found similar effects in our study. According to Zhang et al. [10], the excessive formation of reactive oxygen species under experimental exposure to fomesafen led to the inhibition of superoxide dismutase activity and, thus, inactivated the mechanism of antioxidant protection of the earthworm. When studying the immunotoxicological effect of imidacloprid on the antioxidant buffering capacity of E. fetida, Zhang et al. [10] reported the induction of catalase activity.

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