Study of the effect of nanoparticles Cu, Zn and Mo on vermiculture to create new technologies for bioremediation of contaminated soils

S V Lebedev and I A Vershinina

Laboratory of Biological Testing and Expertise, Federal State Budgetary Scientific Institution Federal Scientific Center for Biological Systems and Agricultural Technology, Orenburg, Russia

E-mail: lsv74@list.ru

Abstract. One of the manifestations of the toxic effect of nanoparticles on living organisms is the ability of nanoparticles to generate reactive oxygen species. At the same time, antioxidant protection enzymes should be considered as biomarkers of such an effect. In our study, we used nanoparticles (NPs) of Cu, Zn, Mo at concentrations of 50, 200, 500 and 1000 mg/kg of dry soil. Standardized soil of 70 % quartz sand, 20 % kaolin, and 10 % crushed peat was used as a substrate. The test objects were *E. fetida* worms. We obtained the following results: SOD activity predominantly decreased (to –69.5 % at 200 mg/kg nanoparticles Cu), the CAT activity increased (up to 110 % at a dose of 1000 mg/kg nanoparticles Mo). The MDA content was higher than the control at 500–1000 mg/kg nanoparticles Zn and Mo. In accordance with the revealed effects in the description of the comparative action of Cu, Zn, Mo nanoparticles on the antioxidant system, it was found that their toxicity to *E. fetida* progressively decreased in the order: Mo → Zn → Cu. Thus, we have shown the possibility of using vermiculture in the development of technologies and methods for the restoration of soils contaminated with metals, since worms have the ability to tolerate oxidative stress, activating defense mechanisms.

1 Introduction

As it is known, metal nanoparticles are increasingly used in various industries [1] and can get into the soil from sewage sludge, waterways, air, volcanic eruptions, mining and automobile exhaust [2].

At the same time, nanoparticles falling into living tissues can lead to oxidative stress in them. It is assumed that the main toxicity pathway for nanoparticles in soil organisms, including earthworms, is a violation of oxidative phosphorylation and the formation of reactive oxygen species (ROS) and lipid peroxidation (LPO) [3]. The activity of antioxidant enzymes plays a key role in the removal and deactivation of the excessive formation of toxic radicals formed in living organisms, and acts as the first line of defense against tissue damage mediated by reactive oxygen species. The balance between ROS production and the antioxidant system leads to regulated intracellular steady-state ROS levels in aerobic organisms [4].

As you know, earthworms make a significant contribution to the health of the ecosystem by changing the physical, chemical and biological properties of the soil, processing organic material, increasing the availability of nutrients, providing food for other soil organisms and improving the profile of the soil structure, and all this improves soil fertility. Therefore, the earthworm Eisenia fetida is the most common...
type of earthworm used to develop technologies for the restoration of contaminated land as a result of human activities [5].

The impact of nanoparticles on earthworms can lead to increased lipid peroxidation (LPO) and tension of antioxidant protective enzyme systems such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase (GST). Therefore, to assess the possibilities of using worms in bioremediation of lands contaminated with nanoparticles, it is necessary to assess the ability of worms to respond to oxidative stress, which is one of the negative aspects of the impact of nanoparticles [6].

The aim of this study was to study the activity of antioxidant enzymes and the content of lipid peroxidation products in the body of E. fetida when exposed to soil with different concentrations of nanoparticles Zn, Cu and Mo for the restoration of soils contaminated with metals, since worms have the ability to tolerate oxidative stress, activating defense mechanisms.

2 Materials and methods

2.1. Chemicals and substrates

The study used nanoparticles Zn (d = 70 ± 2 nm), Cu (d = 50 ± 0.5 nm) and Mo (d = 48 ± 1.3 nm), manufactured by Advanced Powder Technologies LLC (Russia).

Standardized soil (OECD, 1984) prepared by mixing 70 % quartz sand (dry weight) (Inesco LLC, Hydrotorf, Russia), 20 % kaolin (Novokaolinovsky GOK, Chelyabinsk, Russia) and 10 % of ground peat, (organic nitrogen – 5.8 %, Lama Torf LLC, Volokalamsk, Russia), the pH was adjusted to 6.0 ± 0.5 with powdered calcium carbonate (CaCO₃, Mineralprom LLC, Syzran, Russia).

2.2. Test organisms

The E.fetida worms used in the studies were 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), purchased at BiO Era-Penza LLC (www.bioeragrup.ru). E. fetida was cultivated in horse manure without any drugs at 22 ± 2 °C. In the experiment, we used worms weighing 350 to 450 mg.

2.3. Testing

The test was conducted according to guidelines for testing bioaccumulation of chemicals in OECD terrestrial oligochaetes (1984) in four concentrations. Doses of nanoparticles were selected taking into account the load: 50 mg/kg – background, 200–500 mg/kg – increased and 1000 mg/kg – threshold.

The starting lyosols of nanoparticles were prepared by adding a test metal (dry powder) at concentrations of 50; 200; 500 and 1000 mg / kg into deionized water (10 ml) followed by dispersion on an ultrasonic disperser (UZDN, f – 35 kHz, N –300 W, Russia) for 30 minutes. Next, the prepared Zn, Cu, and Mo lyosols for each repetition and concentration were mixed with moist artificial soil (humidity 45–50 %), then brought with distilled water to a moisture content of 75–80 % and mixed with a mixer. For each concentration, plastic containers of 5×4×4 cm filled with 500 g of dry artificial soil were used.

10 healthy worms were added to each container. All containers were covered with a perforated lid to prevent loss of moisture and kept in constant light. The test was carried out for 14 days, at t air 20 ± 2 °C, in five replicates.

2.4. Determination of the activity of antioxidant enzymes in E.fetida

After cleansing the digestive tract, the tissues of the worms were homogenized on a tissue homogenizer TissueLyser LT, QIAGEN (QIAGEN, Germany). The resulting homogenate was centrifuged for 10 minutes at 15,000 rpm. The resulting supernatant was diluted with a buffer mixture to 10 % homogenate (Li et al, 2011).

The content of lipid peroxidation products – malondialdehyde (MDA), as well as the activity of the key units of the antioxidant defense system – catalase (CAT) and superoxide dismutase (SOD) on a CS-T240 automatic biochemical analyzer (Dirui Industrial Co., Ltd, China) was determined in the
homogenate of the worm tissues using commercial Randox biochemical kits (USA). For this, hoods were prepared by homogenization in a buffer medium (Tris 50 mmol/L, DTT 1.0 mmol/L, EDTA 1.0 mmol/L, sucrose 250 mmol/L, pH 7.5), which was added in a ratio of 1: 9.

2.5. Statistical analysis
Statistical analysis was performed using standard ANOVA techniques, followed by the Tukey test (SPSS ver. 17.0). The differences were considered statistically significant at \( P<0.05 \).

3 The discussion of the results
The increase in the level of active forms of oxidizing agents under the influence of a toxic agent promotes the activation of protective mechanisms, which is an important component of the body's response to the presence of toxic dosages of a substance in the environment.

In our experiment, the adaptive reactions of the organism E. fetida to the introduction of Cu, Zn, or Mo nanoparticles in the medium was manifested in a change of antioxidant enzymes activity and the content of malondialdehyde with the existing dependence on the applied dosage (Figure 1–3).

We showed that when Cu nanoparticles were added, the SOD activity decreased below the control values (by 54.1–69.5 \%), with the exception of the concentration of nanoparticles 20 mg/kg of dry soil, where the enzyme activity did not differ from the control (Figure 1).

In turn, the activity of catalase against the background of the introduction of nanoparticles Cu increased by 91–99 \% at a dose of 500–1000 mg/kg nanoparticles, compared with the control values. Also, at 50 mg/kg nanoparticles, CAT activity was 86.7 \% higher than control. When 100 mg/kg nanoparticles were added, the CAT activity did not show a significant increase in its activity.

The content of MDA in the body of E. fetida decreased in all experimental groups with the addition of Cu nanoparticles by 60.7–73.8 \%.

Figure 1. Level of MDA, CT and SOD in the homogenate of E. fetida tissues on day 14 of incubation with Cu nanoparticles

The activity of superoxide dismutase (SOD) against the background of the presence of Zn nanoparticles in the substrate tended to decrease (Figure 2). Only when making nanoparticles concentrations of 500 mg/kg of dry soil did the enzyme activity increase by 22.5 \%, compared with control values.

CAT activity upon introduction of Zn nanoparticles in all experimental groups was higher than the control by 22.1–25.2 \%, with the exception of the concentration of 500 mg/kg, where we noticed an increase of 98.1 \%.
The lipid peroxidation product – MDA content was lower than the control values in the concentration range of 50–100 mg/kg nanoparticles (by 35.7–78.5 %), while an increase in the applied concentration of nanoparticles to 500 mg/kg of dry soil led to increasing the content of this substance in the body of the worm to 35.7 %.

Peak SOD values were observed for Mo nanoparticles at a dose of 200 mg/kg (Figure 3). A further increase in dosage contributed to a decrease in the value of SOD activity (by 10.7–40.4 %).

The activity of catalase when applying Mo nanoparticles in all experimental groups was higher than the control values by 86.5–110 %. At the minimum dosage of nanoparticles (50 mg/kg), the MDA content decreased relative to the control values, while with an increase in concentration it increased by 13.3–50.3 %.

When assessing the antioxidant status and determining the content of the secondary molecular product of lipid peroxidation, malondialdehyde, the activity of antioxidant enzymes, superoxide dismutase, and catalase, it was established that, under the action of nanoparticles, the catalase activity increases compared to the control. This may happen due to an increase in the level of work of this antioxidant link protection and the availability of adaptation mechanisms.
In addition, comparable dosages of various metal nanoparticles with respect to E. fetida have a multidirectional effect. So, Mo nanoparticles had the highest toxicity compared to Zn and Cu nanoparticles.

However, even at high concentrations, the body of the worm is able to level the effect of the metal by activating the body’s antioxidant systems.

4 Conclusion
Soil restoration technologies imply the use of organisms that are resistant to the effects of certain pollutants. In our experiment, we used widely used nanoparticles of copper, zinc and molybdenum, which, being in different environments, are capable of provoking the onset of oxidative stress [7].

The increasing release of metallic nanoparticles (NPs) or their sulfidized forms into soils have raised concerns about their potential risks to soil ecosystems. Hence, there is a need for novel strategies to remediate metallic NPs pollution in soils. In this study, to explore the feasibility of using earthworm *Eisenia fetida* to manage soils contaminated with metallic NPs, we simultaneously investigated the ability of worms to adapt under oxidative stress to the effects of reactive oxygen species generated by nanoparticles.

Metals of variable valency, including their nanoforms are one of the reasons for the development of oxidative stress in the body. An increased level of malondialdehyde, as a marker of lipid peroxidation, can be a confirmation of the development of these processes under the influence of nanoparticles [8]. However, an assessment of its level against the background of the introduction of various doses of Cu and Zn nanoparticles into the medium showed a decrease in values compared to the control (Figure 1, 2). At the same time, the addition of nanoparticles Mo at high concentrations (200 mg/kg, 500 mg/kg and 1000 mg/kg) leads to an increase in MDA, compared to the control. In this regard, nanoparticles Mo can be considered as inducers of prooxidant effects, nanoparticles Zn in second place, and Cu nanoparticles in third place.

A similar dependence of the biological activity of nanoparticles was described [9], which claimed that SOD activity increases with moderate environmental stress and decreases with strong environmental stress. In addition, another enzyme, catalase, is also an important enzyme in the antioxidant defense system of organisms, which protects cells from damage. In *Eisenia fetida*, CAT activity increased when the concentration of nanoparticles in Zn artificial soil reached 500 mg/kg and decreased at a dose of 1000 mg/kg. A similar effect of Zn NPs has been reported [10]. This is possible due to the high activity of catalase in the soil substrate and the high fluidity of the metal between the substrate and the body of the worm [11].

Along with MDA, markers of the development of oxidative stress (OS), diene conjugates and other TBA-active products can act. Possibly, the accumulation of precisely these products was the reason for the activation of the antioxidant system against the background of the introduction of nanoparticles and metals of variable valence [12].

In accordance with the revealed effects, when describing the comparative action of Cu, Zn, Mo nanoparticles on the antioxidant system, it was found that their toxicity towards E. fetida progressively decreased in the order: Mo → Zn → Cu. Thus, when developing technologies for the restoration of contaminated soils, including nanoparticles, it is worth considering the data on the tolerance of worms to oxidative stress.

Acknowledgments
The studies were carried out in accordance with the research plan for 2019-2020 of the Federal Research Centre for Biological Systems and Agrotechnology’s of the Russian Academy of Sciences (№ 0761-2019-0003).

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