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Toxicity Assessment of Molybdenum Nanooxide in Relation to Various Components of the Agroecosystem in a Model Experiment

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Abstract: (1) Background: The rapid growth in the number of nanoparticles today raises questions about studying their impact on the environment, including the soil, as the main absorber of nanoparticles. The purpose of our research was to study the effect of MoO_3 nanoparticles (NPs; 50, 100, 250, 500, and 1000 mg/kg of soil) on the physiological and biochemical parameters of *Eisenia fetida*, the number of certain ecologo-trophic groups of soil microorganisms, and enzymatic soil activity. (2) Methods: We used 92 ± 0.3 nm nanoparticles of MoO_3 at concentrations of 50, 100, 250, 500, and 1000 mg/kg dry soil. Texture-carbonate chernozem was used in the study. *Eisenia fetida* worms were used as test objects. (3) Results: The introduction of MoO_3 nanoparticles showed a weak toxic effect towards the animal and microbiological components of the soil at a concentration of 50–250 mg/kg, a medium toxic effect at 500 mg/kg, and a strong or unacceptable toxic effect at 1000 mg/kg. The oxidative stress response of *E. fetida* depended on the concentration of the NPs. MoO_3 NPs at a concentration of up to 100 mg/kg reduced the number of amylolytic bacteria, oligotrophs, and *Azotobacter*. In soil, urease and catalase showed mild activity, whereas the activity of invertase decreased by 34%. (4) Conclusions: The entry into the environment and the further deposition of nanoparticles of Mo and its oxides in the soil will lead to the suppression of the vital activity of beneficiary soil animals and the activity of soil enzymes. This phenomenon presents special kinds of ecological risks for the ecosystem.

Keywords: nanoparticles; molybdenum oxide; soil; pollution; *Eisenia fetida*; microorganisms; soil exoenzymes

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1. Introduction

The rapid development of nanotechnology and the production of new nanomaterials currently gives researchers the impetus to study their properties, and most importantly, the toxicity of these new materials. Among these new materials used in science and technology are molybdenum-containing nanoparticles (NPs). Molybdenum is found in natural soil at various concentrations from 0.2 to 100 mg/kg, and its background concentration is from 0.2 to 6 mg/kg in igneous and sedimentary rocks [1]. EC50 values for the reproduction toxicity of Mo were 129–2378 mg/kg for earthworms, 72–>3396 mg/kg for Collembola, and 301–>2820 mg/kg for enchytraeids [2]. Accordingly, environmentally relevant concentration could be comparable with toxic concentrations.

NPs of Mo and their oxides are used for the mediator-free electrochemical detection of dopamine [3], in the chemical [4] and electronic industry [5], and in other industries.

The study of the toxicity of nanoparticles, including molybdenum, on living organisms is the main aspect in the study of the effect of nanoparticles on living objects. It is known that molybdenum in soluble form is considered a substance with low toxicity; it does not significantly affect the metabolic activity or integrity of the cell membrane, at least

at a concentration of 40 μg/mL (EC50 = 90 μg/mL). At the same time, toxicity increases with an increase in concentration of over 50 μg/mL. Braydich-Stolle et al. suggested that molybdenum NPs contribute to the disruption of the plasma membrane and the release of the cell cytoplasm at concentrations of 5 μg/mL and 10 μg/mL (Braydich-Stolle et al., 2005) [6]. Also, they showed that molybdenum NPs had the lowest cytotoxicity compared to other metals.

However, we note that a significant number of studies on the toxicology of NPs are aimed at the health and safety of animals and/or humans. At the same time, less attention has been paid to the assessment of nanometal toxicity on the remaining links of the ecological chain [7,8].

On the other hand, different organisms in the ecological hierarchy may react differently to the introduction of nanoparticles into their habitat. Plants, vermiculture, and microorganisms are essential environmental components in terrestrial and aquatic ecosystems and serve as important living indicators for studying the general metabolic effects and toxicity of nanoparticles. NanoMoO_3 has been shown to inhibit seed germination and the growth of plants [9] and destroy bacterial cell walls [10]. Also, the antifungal activity of bulk and NP samples was recorded against Aspergillus sp. and Trichoderma sp. in 50 μL concentrations [10]. At the same time, most of the research is focused on studying the effect of molybdenum oxide NPs in vitro [11–13], and also affects only one object of research, which makes it relevant to carry out complex studies on this question.

To establish the toxic effects of metals, they must be biologically available. The bioavailability of metals in soil includes at least three dynamic processes, namely, the desorption of soil, absorption into living organisms, and the toxicodynamics of redistribution inside the body [14]. All three processes have their own kinetics and, therefore, toxicity cannot be determined without considering exposure time.

Researchers consider that the assessment of the degree of impact of nanoparticles on the soil microbial community is of scientific and practical interest. Researchers suggested that the *E. fetida* earthworm is a promising model to study the introduction of nanomaterials into soil, and therefore, the ecotoxicological assessment of newly created substances and preparations [15]. This is also facilitated by the fact that *E. fetida* has a high permeability of the body surface to pollutants [16].

Taken together, a comprehensive study of nano-sized particles of molybdenum oxide in a wide range of concentrations from the standpoint of assessing their toxicity on a number of living organisms is especially important.

Thus, at the moment, researchers are expressing concern about the expected ecological consequences of the impact of nanoparticles on the environment and impacts on species, populations, communities, etc. In this case, scientists are focusing their efforts on solving problems such as the characterization of nanoparticles in the environment, as well as elucidation of the biological mechanism associated with their toxicity and their effect on the adaptation of living objects, etc. The aim of this work is to assess the effect of MoO_3 NPs on *E. fetida* and the activity of soil exoenzymes under the conditions of a model experiment.

2. Materials and Methods

2.1. Chemicals and Substrates

MoO_3 nanoparticles (NPs) (92 ± 0.3 nm, Z-potential 42 ± 0.52 mV, Specific = 12 m^2/g) were obtained from Plazmotherm (Russia) (Figure 1). The study used MoO_3 NPs at concentrations of 50, 100, 250, 500, and 1000 mg/kg. The concentrations of nanoparticles used in the work in the range of 50–100 mg/kg of soil reflected the level of molybdenum inherent in ordinary soil, and increased doses of nanoparticles up to 1000 mg/kg of soil were used to assess the possible toxic effect of the applied concentrations. At the same time, it was impractical to use doses above 1000 mg/kg of soil due to the large agglomeration of nanoparticles in the solution.

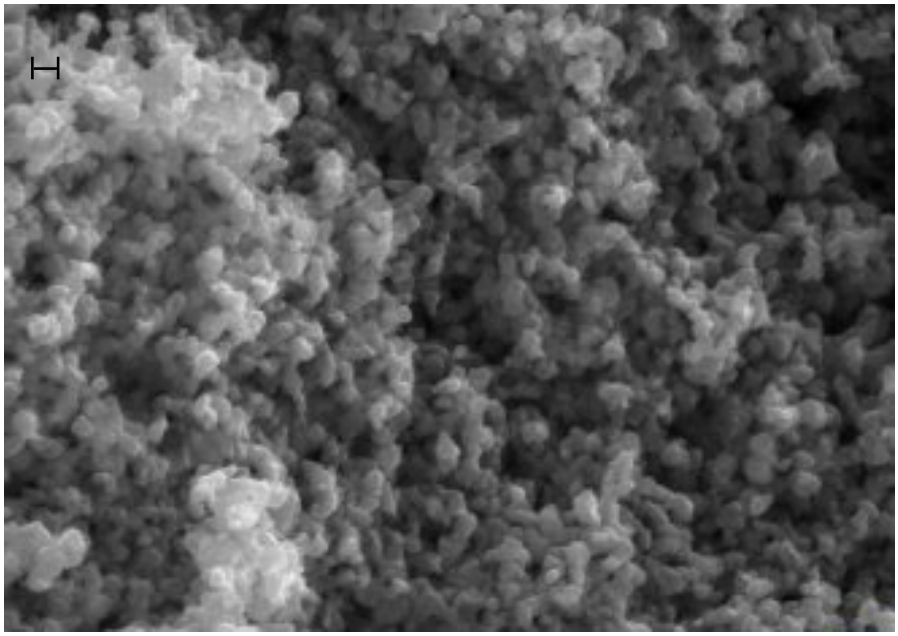

Figure 1. MoO_3 nanoparticles (scale corresponds to 100 nm).

Texture-carbonate chernozem was used in the study. Soil was sampled in the south of the Orenburg region (Russia, Volga Federal District). Soil acidity was 7.7. The organic matter in the soil was 3.89%, and nitrogen was 0.24%. The soil was prepared for use in the experiment based on the method described earlier [17]. The soil characteristics are presented in Table 1.

Table 1. Characteristics of the soil.

Index	Value
pH	7.72 ± 0.03
Clay content, %	30.60 ± 0.2
SOM, %	3.89 ± 0.5
CEC, mmol/100 g soil	42.21 ± 0.3
N_{total}, %	0.24 ± 0.07
P_2O_5, mobile, mg/kg soil	21.20 ± 0.1
K_2O, exchangeable, mg/kg soil	315.54 ± 0.02

2.2. Objects of Study

E. fetida worms (obtained from LLC BioEra-Penza, Penza, Russia) were used as test objects. In the preparatory period, the worms were kept in horse manure at a temperature of 22 ± 2 °C without the introduction of chemicals. The worms weighed 300–400 mg.

2.3. Experimental Design

Distilled water was added to the soil, and the soil was mixed with a mixer to a moisture content of 40–45%. This soil moisture was chosen for a number of reasons: the recommendations of the company that provided the worms, as well as our own observations of the behavior of the worms. This moisture was optimal for the worms, in which they did not crawl to the surface, but were in the soil. The soil was left in containers for one day.

NP solutions were prepared as described by Scott-Fordsmand et al. (2008) [18]. MoO_3 NP powder was added to distilled water. The resulting NP solutions were placed in an ultrasonic bath (LLC Sapphire, Moscow, Russia) for 30 min immediately before being introduced into the soil.

The following concentrations of MoO_3 NPs were used: 0 (control group); 50; 100; 250, 500, and 1000 mg/kg of dry soil. Then, the soil with the nanoparticles was mixed with a mixer.

The worms were placed in plastic containers (15 × 10 × 12 cm) with 250 g of soil after mixing the soil with a mixer.

The experiment was carried out according to the OECD methodology (2010, N° 317). We based our work on the principles proposed in the guide. At the same time, based on the available practical experience in studying the effect of nanoparticles on worms, we made our own adjustments. In particular, the duration of our experiment was 28 days. Feeding of the worms was not carried out, due to the possible undesirable interaction of the introduced feed and nanoparticles, which by their nature differ from ordinary bulk chemical substances. In addition, our experiment included microbiological studies. Therefore, food for the worms was not introduced, since atypical microflora could have been introduced.

In preparation, the worms were washed with distilled water and placed on moistened filter paper in Petri dishes to cleanse the intestines of the worms within 3 days. Then, the worms were weighed and divided into groups with the same weight—310 ± 10 g.

Ten worms were placed into each soil-containing vessel. The vessels with the soil and worms were covered with a lid with holes to avoid moisture loss from the soil. The experiment was conducted over 28 days at 22 ± 2 °C in the dark. The study was conducted with a 5-fold repetition.

The mass of the worms was measured on days 0, 14, and 28 of the experiment. After 14 and 28 days, we sampled the soil and worms for microbiological and biochemical analyses [17].

2.4. The Study of the Content of Mo in the Soil and E. fetida

The content of Mo in the *E. fetida* and in the soil was studied with atomic emission and mass spectroscopy at the experimental laboratory of the Center for Biotoc Medicine, Moscow, Russia (Registration Certificate of ISO 9001: 2000, Number 4017–5.04.06). Soil sample and *E. fetida* body ashing was performed with the microwave decomposition system MD-2000 (Thief River Falls, MN, USA). The element content was determined with the mass-spectrometer Elan 9000 and the atomic emission spectrometer Optima 2000 V (PerkinElmer, Shelton, CT, USA).

2.5. Study of of the Activity of Antioxidant Enzymes, Protein

We studied the activity of catalase (CAT), superoxide dismutase (SOD) activity, and the content of lipid peroxidation products—namely, malondialdehyde (MDA)—in the body of a worm. The determination of superoxide dismutase activity (SOD) levels was carried out using a commercially available kit (Ransod; Randox Laboratories Ltd., Crumlin, County Antrim, Northern Ireland, UK) using a CS-T240 automatic biochemical analyzer (Dirui Industrial Co., Ltd., Changchun, China). The determination of malondialdehyde (MDA) levels was estimated using the ELISA Kit for Malondialdehyde (MDA) (Cloud-Clone Corp., Houston, Texas, USA). Catalase activities were assayed using the ELISA Kit for Catalase (Cloud-Clone Corp., Houston, TX, USA). Protein content was determined by the automatic biochemical analyzer CS-T240 (Dirui Industrial Co., Ltd., Changchun, China) using commercial biochemical kits of Randox (Randox Laboratories Ltd., Crumlin, County Antrim, Northern Ireland, UK).

For this, we homogenized the worms using TissueLyser LT dispersant (QIAGEN, Hilden, Germany) [19]. The worm tissues were homogenized on a TissueLyser LT tissue homogenizer, QIAGEN (QIAGEN, Germany). For this, the extracts were prepared by homogenization 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) in a 1:9 ratio. The resulting homogenate was centrifuged for 10 min at 15,000 rpm. The resulting supernatant was diluted with a buffer mixture of up to 10% homogenate.

2.6. Studying Microbiological Community of Soil

The microbiological activity of the soil was determined by studying the ratio of the quantity of different ecological and physiological groups.

Our study assumed that each physiological group determines the intensity of certain physiological and biochemical processes carried out by taxonomically different microorganisms [20].

During the experiments, we investigated the number of colony-forming units (CFUs) when plating on solid nutrient media. The following culture media were used: MPA (meat-peptone agar) for determining the total number of microorganisms, nutrient media CAA (starch-ammonia agar) for amylolytic microorganisms, Czapek medium for the isolation of microscopic fungi, and Hutchinson's medium for cellulolytic microorganisms on Hutchinson's medium. Azotobacter bacteria were isolated on Ashby medium.

For sowing, soil samples were taken and dilutions were made to 10^{-4} (for microscopic fungi) and 10^{-6} (for bacteria). Next, 100 µL of the dilution was added to a solid nutrient medium.

Nystatin was added to all the media except Czapek to prevent growth of fungi, and penicillin was added to Chapek's medium to prevent bacterial growth.

The number of microorganisms was expressed as colony-forming units (CFUs) in grams of dry soil. The experiment was carried out with 3 replicates.

2.7. Study of the Number of Microorganisms of Various Physiological Groups in Intestine of E. fetida

The number of intestinal microflora of the worms and the microbial number of the soil were studied at day 28 after the start of the experiment. Intestines were selected from worms using a sterile scalpel, after which the intestines were transferred to a sterile tube. Soil samples were taken in sterile tubes. Microbiological studies of the intestines of the worms and soil were carried out on the first day after sampling using solid nutrient media (see above) [17].

2.8. Study of Soil Enzyme Activity

Catalase activity (CAT) in the soil was studied on day 28 of the experiment, in line with Rani et al. [21]. CAT activity was reported in mL of O_2 released in 1 min from 1 g of soil.

The activity of peroxidase (PO) and polyphenol oxidase (PPO) was determined according to the [22].

Soil urease (U) activity was determined spectrophotometrically by determination of the concentration of ammonia by using a modified Berthelot's method [23]. The activity of soil invertase (I) was measured by the method described by Balasubramanian et al. [24].

2.9. Statistical Analysis

The results were processed using the computer software Statistica for Windows 10.0 and Microsoft Office Excel 2010. Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. The Effect of MoO_3 Nanoparticles on the Morphological and Biochemical Parameters of E. fetida

In the first 14 days, the introduction of 50–250 mg/kg MoO_3 led to a higher body mass of *E. fetida*, while 500 and 1000 mg/kg led to a smaller increase in body mass compared to the controls (Figure 2). Further exposure demonstrated a weight decrease over the next 14–28 days. Worms in all the concentration groups lost body mass, including the controls. The control group also lost weight (−6, 3%), while the other groups lost (significantly) more mass. The loss in mass was dependent on the concentration, reaching maximum values of 30.8% and 34.2% after the treatment with 1000 mg/kg MoO_3 NPs.

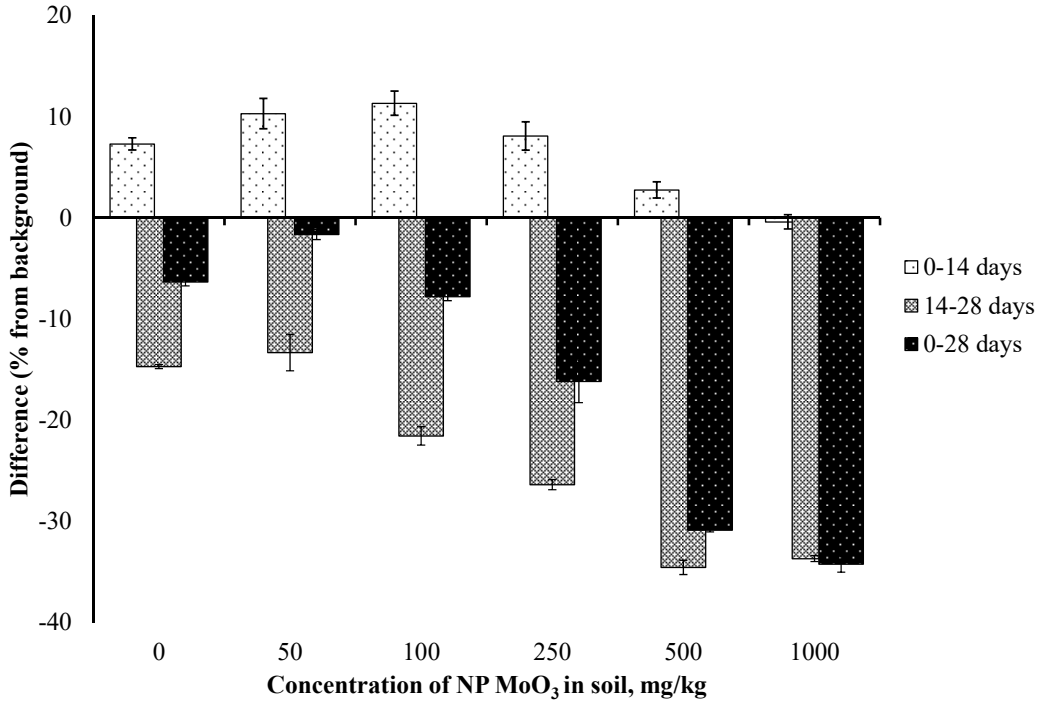

Figure 2. Mass change in *E. fetida* at different concentrations of MoO_3 NPs in soil substrate (% of initial mass).

A decrease in the protein content in the body was observed both on the 14th and on the 28th day, while the greatest decreases in the protein content of 91.3, 91.3, and 96.8% were observed in concentrations of 100, 250, and 500 mg/kg NPs compared with the control group, respectively (Figure 3).

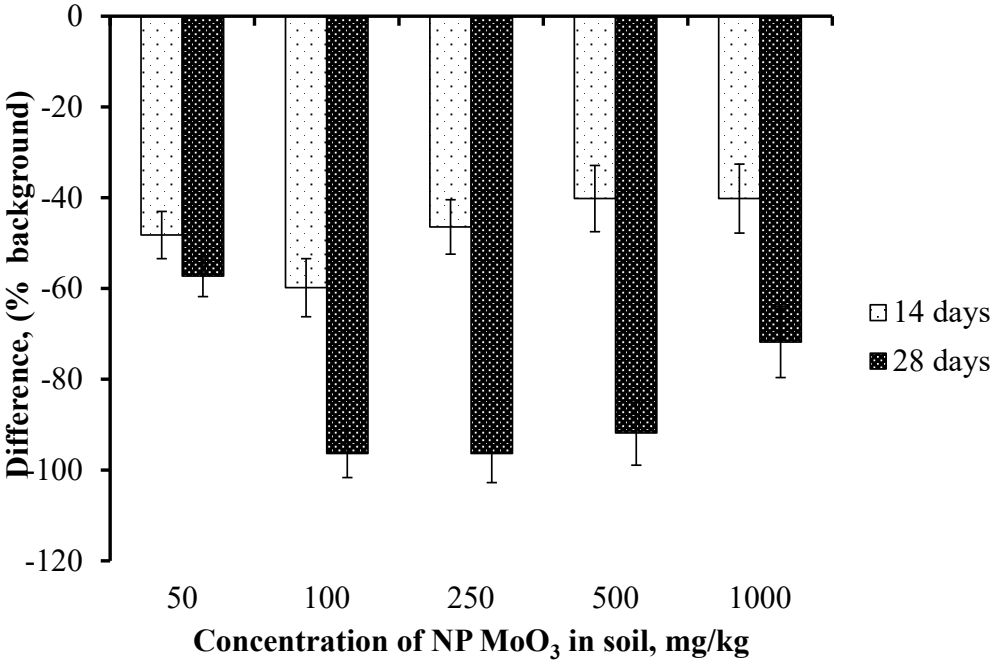

Figure 3. The difference in the protein content in the body of *E. fetida* with different concentrations of MoO_3 NPs (% of control, 0 mg/kg). Bars reflect standard deviations of the mean from three replications.

3.2. Enzyme Activity in *E. fetida* Exposed to MoO_3 NPs

The activity of the enzyme system of the worm on day 14 was expressed in an increase in SOD activity depending on the concentration of NPs from 8 to 25.8% (Figure 4).

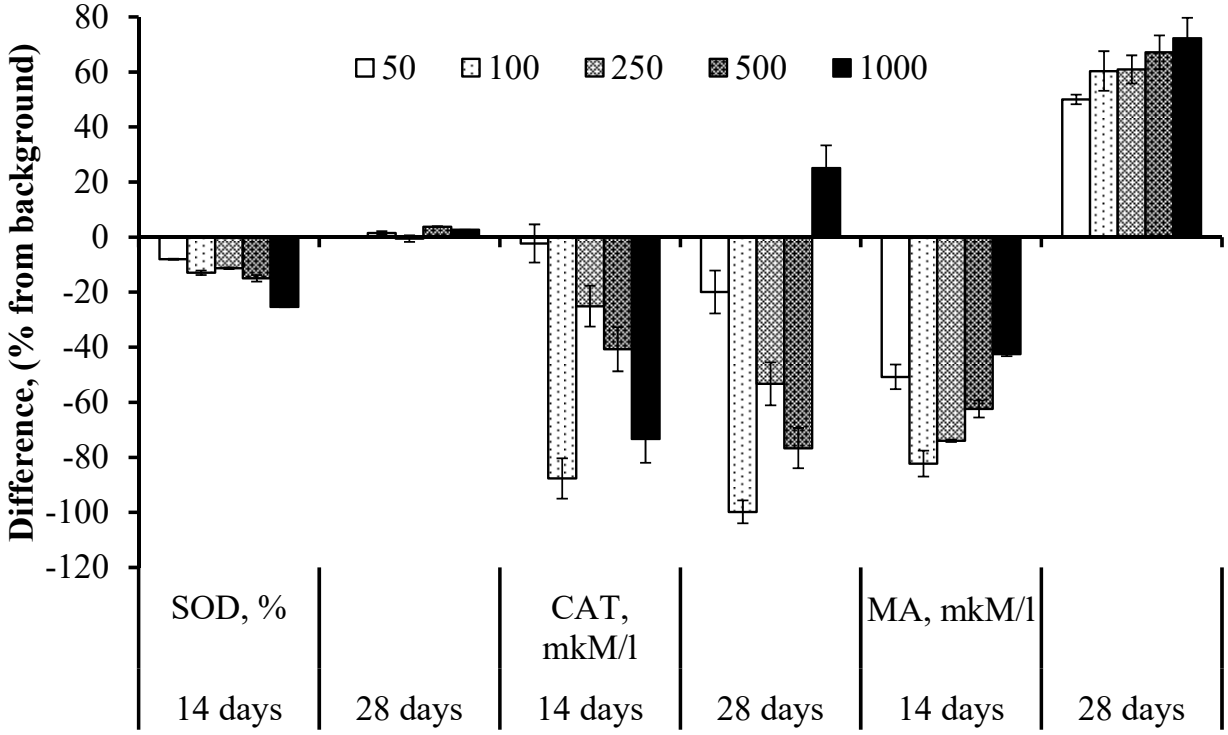

Figure 4. The difference in the activity of antioxidant enzymes in *E. fetida*, with different concentrations of MoO_3 NPs (50; 100; 250; 500; 1000 mg/kg) in the soil (% of control).

The level of CAT activity during the entire incubation period showed low activity (up to 91% lower than the controls at a concentration of 100 mg/kg NPs on day 28), except for a concentration of 1000 mg/kg on day 28, where the level of CAT exceeded the controls by 20.9%. The level of malondialdehyde on the 14th day in all variants of the experiment was lower than the controls. An increase in the exposure time to 28 days led to an increase in the content of malondialdehyde in the experimental groups. The difference with the controls ranged from 50 to 72%.

3.3. The Effect of MoO_3 Nanoparticles on the Intestinal Microbiocenosis of *E. fetida*

The experiment showed that MoO_3 NPs at a concentration of 50 mg/kg did not affect the total number of microorganisms in the worm's intestine. At the same time, the number of bacteria using mineral forms of nitrogen, which are potentially important for agriculture, increased 1.52 times; the number of bacteria of the genus *Azotobacter* increased 1.48 times compared to the controls (Table 2).

Table 2. Dynamics of the number of physiological groups of intestinal microbes *E. fetida* when cultivated with MoO_3 NPs on day 28, million CFU/g of dry soil (M ± SD, n = 10).

Concentration of Nanoparticles	The Number of Microorganisms per MPA	The Number of Bacteria in CAA	The Number of Fungi in the Chapek Medium	The Number of Microorganisms in the Getchinson Medium	The Number of Microorganisms in the Ashby Medium
0 mg/kg	26.02 ± 4.02	17.12 ± 2.2	0.0012 ± 0.0003	0.72 ± 0.22	6.10 ± 0.62
50 mg/kg	21.12 ± 1.60	26.07 ± 2.1 *	0.0007 ± 0.0002	0.49 ± 0.09	8.92 ± 0.57 *
100 mg/kg	15.79 ± 1.42 *	17.0 ± 1.8	0.0006 ± 0.0001	0.32 ± 0.12	2.75 ± 0.42 *
250 mg/kg	10.44 ± 1.05 *	13.21 ± 1.4 *	0.0004 ± 0.0001 *	0.31 ± 0.17	2.50 ± 0.53 *
500 mg/kg	5.58 ± 0.64 *	8.11 ± 1.2 *	0.0002 ± 0.00001 *	0.24 ± 0.14 *	2.17 ± 0.82 *
1000 mg/kg	4.00 ± 0.57 *	4.09 ± 0.6 *	0.0003 ± 0.0001 *	0.20 ± 0.11 *	1.42 ± 0.20 *

* The difference is significant compared with the value of the control variant with $p \leq 0.05$.

A further increase in the concentration of NPs to 1000 mg/kg was accompanied by a decrease in the groups of microorganisms mentioned above. When compared to the controls, the total number of microorganisms decreased 1.64–6.5-fold, the number of bacteria in the CAA increased 1.3–4.25 times, microscopic fungi decreased 3–4 times, cellulose-decomposing microbes decreased 3.5 times, and nitrogen-fixing microorganisms decreased 2.4–4.28 times.

3.4. Accumulation of Molybdenum in the Body of E. fetida

The introduction of MoO_3 NPs into the soil was accompanied by the active accumulation of molybdenum (Figure 5) in the worm (dry weight). On the 14th day of observation, the concentration of molybdenum per dry body weight of worms in the control group was 6.0 ± 0.3 mg/kg, and on the 28th day, it was 10.1 ± 0.7 mg/kg. An increase in the concentration of MoO_3 NPs in the range from 50 to 250 mg/kg soil promoted an increase in the bioavailability of molybdenum. In this case, at a concentration of 250 mg/kg of MoO_3 NPs, the concentration of molybdenum was the maximum value (17.1 ± 1.3 mg/kg). With the addition of 500 mg/kg and 1000 mg/kg NPs, the concentration of molybdenum in the worm was 15.4 ± 1.1 mg/kg and 14.6 ± 1.2 mg/kg, respectively.

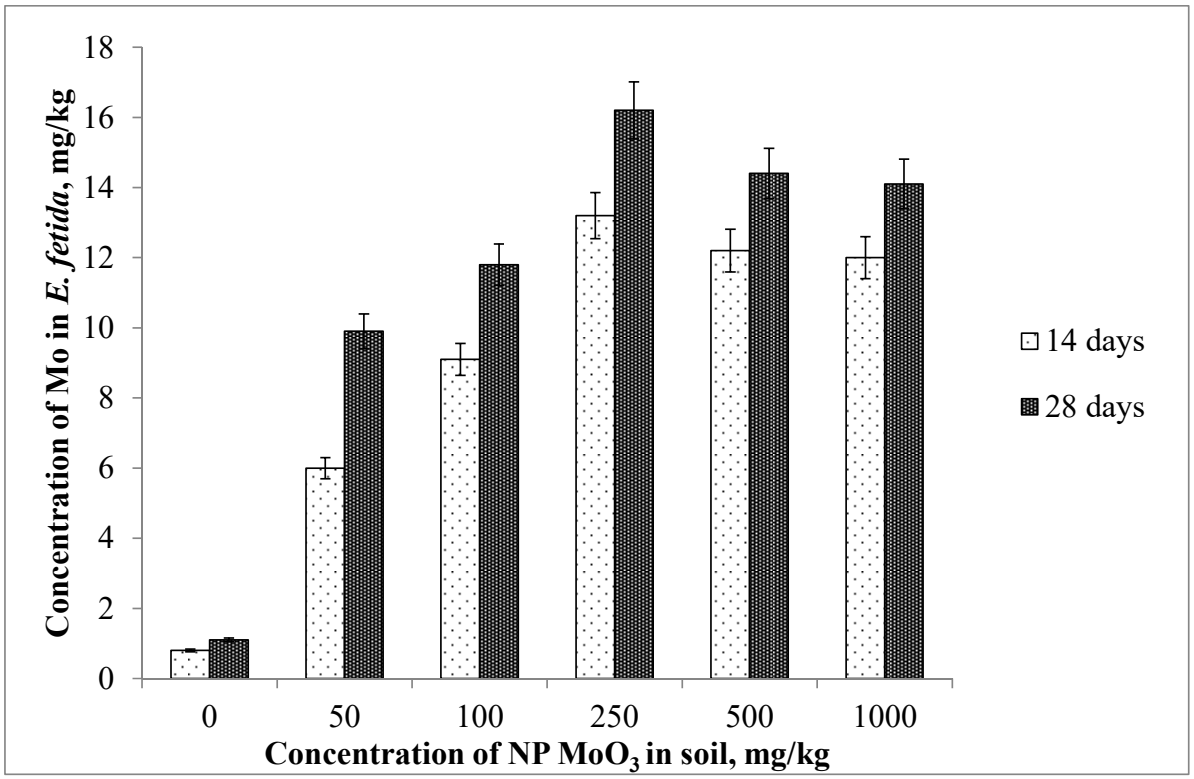

Figure 5. Concentration MoO_3 in soil, mg/kg.

3.5. The Influence of MoO_3 Nanoparticles on the Qualitative and Quantitative Composition of the Soil Microbial Community

All variants of the model experiment are characterized as very rich in microorganisms (in terms of the number of amylolytic and total number of microorganisms in the soil) (Table 3).

Table 3. The number of microorganisms of physiological groups of microorganisms in soil, million CFU/g of dry soil (M ± SD).

Concentration of Nanoparticles	The Number of Microorganisms per MPA	The Number of Bacteria in CAA	The Number of Fungi in the Chapek Medium	The Number of Microorganisms in the Getchinson Medium	The Number of Microorganisms in the Ashby Medium
0 mg/kg	28.47 ± 0.30	42.58 ± 0.85	0.016 ± 0.023	1.56 ± 0.32	82.84 ± 0.33
50 mg/kg	63.60 ± 0.21	21.67 ± 2.87	0.0061 ± 0.0003 *	1.03 ± 0.34	-
100 mg/kg	72.33 ± 0.62	135.67 ± 3.61 *	0.0067 ± 0.0004 *	1.05 ± 0.59	13.3 ± 1.30 *
250 mg/kg	50.67 ± 0.84	157.67 ± 2.72	0.0062 ± 0.0020 *	0.30 ± 0.32 *	3.3 ± 0.64 *
500 mg/kg	45.67 ± 0.21	237.67 ± 3.90	0.0033 ± 0.0010 *	-	3.3 ± 0.62 *
1000 mg/kg	36.10 ± 3.90	273.30 ± 4.57 *	-	-	3.3 ± 1.27 *

Note: * The difference is significant compared with the value of the control variant with $p \leq 0.05$.

The addition of 100 mg/kg MoO_3 NPs to the soil caused an increase in the total number of microorganisms by more than two times the background sample.

When introducing MoO_3 NPs into the soil at a concentration of 50 mg/kg, the number of amylolytic bacteria decreased by 49.1%. On the contrary, a further increase in concentration stimulated the development of microorganisms, and the maximum value was noted for the variant of the experiment at a MoO_3 NP concentration of 100 mg/kg.

Increasing the concentration of MoO_3 NPs in the soil caused a linear decrease in the number of cellulolytic microorganisms ($r = -0.5$, $p \leq 0.05$) and fungi ($r = -0.82$, $p \leq 0.05$). Concentrations of MoO_3 NPs of 500 mg/kg and 1000 mg/kg caused complete suppression of fungal and cellulolytic microflora, respectively.

Bacteria of the genus *Azotobacter* showed high sensitivity to the introduction of molybdenum oxide. A concentration of nanoparticles of 50 mg/kg caused a complete inhibition of bacterial growth, and with an increase in concentration to 100 mg/kg, the value of the number of lumps of fouling increased to 13.3% and with increasing concentration of the active substance decreased to 3.3%.

3.6. Activity of Soil Enzymes When Introducing MoO_3 Nanoparticles into Soil

NPs of molybdenum oxide with an increase in concentration in the range of 50–1000 mg kg decreased the activity of urease by 32–36.7% relative to the controls.

The lowest activity of catalase (10.6 mL of O_2 per 1 g of soil per 1 min) is observed when the concentration of MoO_3 NPs is 1000 mg/kg. The activity of invertase against the background of the introduction of MoO_3 NPs is reduced by more than 33.8% (Figure 6).

Catalase ($r = -0.84$, $p \leq 0.05$) and invertase ($r = -0.72$, $p \leq 0.05$) activity showed a negative linear dependence on NP concentration of MoO_3.

The activity of PPO in the soils of the model experiment did not have a pronounced dependence on NP concentration and varied from 7.7 to 7.2 mg of 1,4-Benzoquinone per 1 g of soil within 30 min. On the contrary, PO activity increased after the introduction of MoO_3 NPs, which was confirmed by a high correlation coefficient ($r = -0.99$, with $p \leq 0.05$) (Figure 7). The maximum value of the indicator is noted at a concentration of 1000 mg/kg and amounted to 16.8 mg of 1,4-Benzoquinone per 1 g of soil within 30 min.

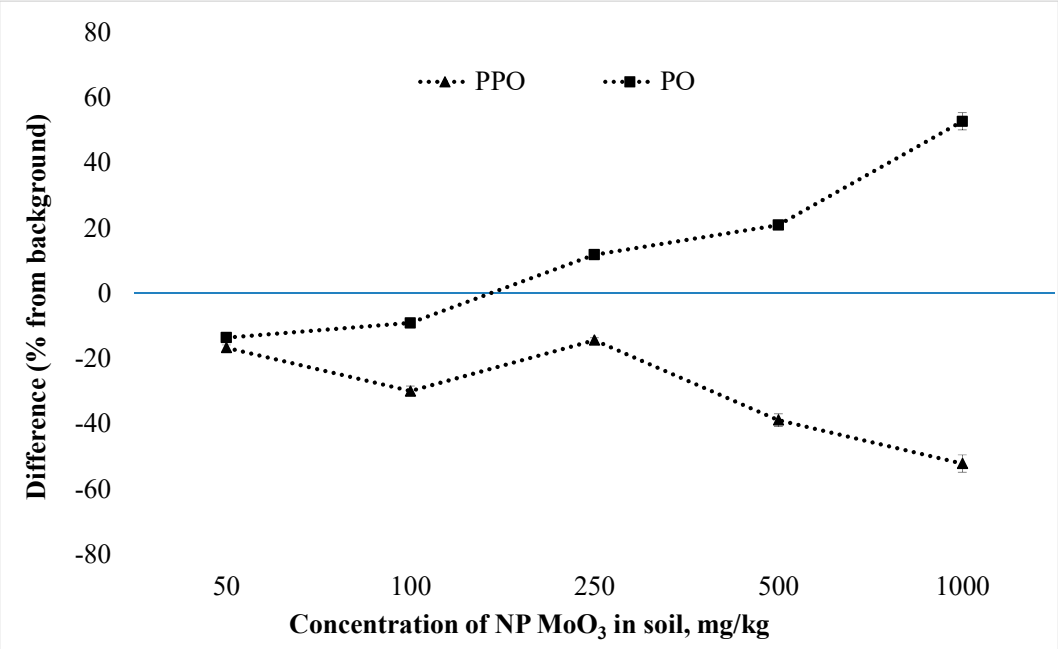

Figure 6. Activity of soil exoenzymes.

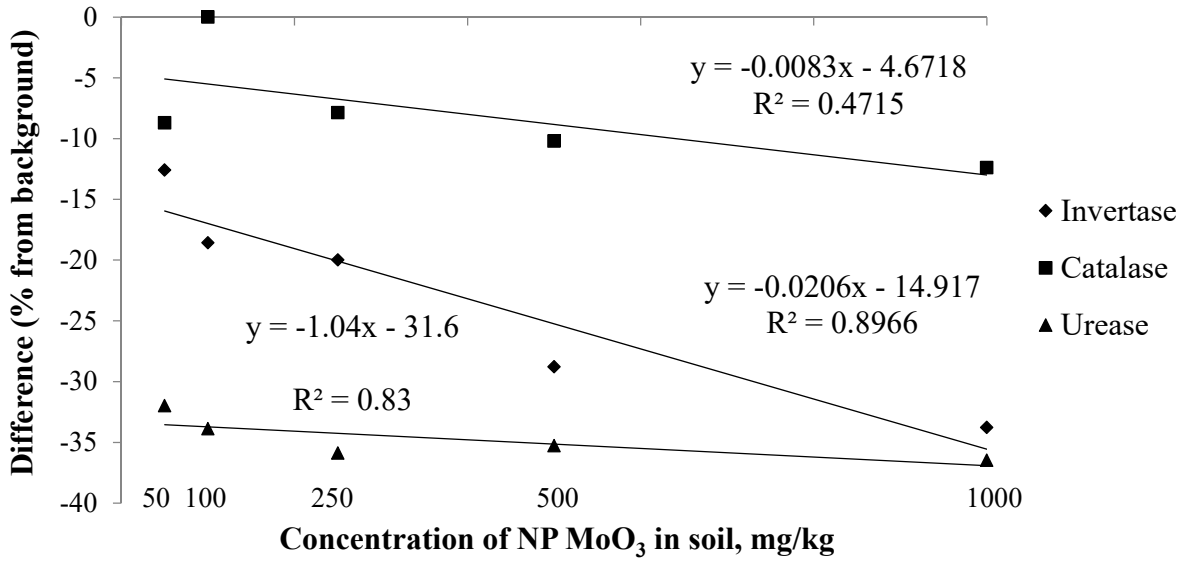

Figure 7. Activity of soil exoenzymes.

4. Discussion

The release of various nanoparticles into the environment is associated with their use in industry and agriculture, but the consequences of their entry into the soil are still unclear [25,26].

Of particular importance is studying the complex effect of numerous NPs on ecosystem components and elucidating their toxicity. Therefore, to describe the effects of nanoform elements, in particular, we evaluated the effect of molybdenum nanoparticles on the microbiological and animal components of the soil cenosis. We also evaluated the effect of molybdenum nanoparticles on the activity of soil exoenzymes as agents of global geochemical cycles.

Testing of the OECD animal model included a survival test, but the concentrations chosen for the experiment did not reach the LD50 or LD100 dose. Indicators of the body

weight, protein content, and antioxidant status of *E. fetida* showed the greatest information. The experiment measuring the body mass showed the effect of the duration of nanoparticle exposure on *E. fetida*, which led to a loss in body weight by the 28th day of the experiment. The protein decrease was not proportional to the NP concentration, which is not necessarily an unexpected finding. The absence of a directly proportional decrease in the physiological and biochemical parameters of animals due to soil contamination was similar to the results obtained for another nanooxide [17].

It should be noted that the indicator of SOD activity and the content of malondialdehyde in the worms showed higher values for 28 days of exposure compared to the 14-day period; all of the experimental variants were significantly lower than the controls, and the parameter depended almost linearly on the concentration of MoO_3 NPs. It was expected this is due to the high adaptive abilities of *E. fetida*. But as indicators of the assessment of nanoparticle toxicity on the worms, catalase activity and protein content were most sensitive. Similar studies have demonstrated the dependence of the protein status of worms on the dose of pollutant. These studies link this phenomenon to a stabilized decrease in protein content at maximum doses with their active inclusion of proteins in processes of detoxification and inactivation of genes responsible for protein synthesis [27].

The functional significance of metals in the environment depends on the biological activity of the soil and metal. It is suggested that the effect of metals is determined, among other things, by the concentration of their ions in the soil and there is evidence that the degree of absorption of molybdenum depends on its concentration in the soil [28]. We determined the effect of the concentration of MoO_3 oxide nanoparticles on the bioaccumulation of metals under constant soil conditions; the results are consistent with the data obtained by Gomes et al. (2016) [29], Topuz et al. (2017) [30], and Makama et al. (2016) [31]. An increase in the concentration of MoO_3 NPs led to an increase in the concentration of molybdenum in the soil, while the maximum accumulation of molybdenum in the *E. fetida* was observed at a concentration of 250 mg/kg.

Bacteria of the genus *Azotobacter* showed high sensitivity to the introduction of MoO_3 NPs. The concentration of nanoparticles of 50 mg/kg caused a complete suppression of bacterial growth, while an increase in concentration to 100 mg/kg increased the amount of fouling lumps to 13.3%.

The route of entry of metals into the body of a worm includes sorption through direct skin contact and direct absorption. The stabilization of absorption, according to Sivakumar et al. (2015) [32], is associated with the limiting rate of molybdenum ions in the soil and the aggregation of nanoparticles [33,34], all of which lead to a decrease in element availability and intake by *E. fetida*.

The number of adaptation mechanisms of the worm and the peculiarities of the transformations of nanoparticles in natural soils make it possible to use protein status, body mass, and catalase activity as markers of MoO_3 NPs entering the environment.

The absence of a pronounced reaction from worms on exposure to nanomaterials in traditional endpoints (mortality and loss of fertility) is described by works [35,36], confirming more informative indicators of antioxidant status as more reliable biomarkers. MoO_3 NPs in any concentration caused oxidative stress, as evidenced by the decrease in activities of catalase, malondialdehyde, and superoxide dismutase in the first 14 days of the experiment. Detoxification mechanisms, due to neutralization by absorption and binding to protein agents, reduced oxidative stress, and only CAT had lower values compared to the controls. At the same time, the positive excess of net soil index at a dose of 1000 mg/kg MoO_3 NPs suggests that the toxic effect is overcome due to the aggregation of nanoparticles in the soil, and a decrease in their activity due to interacting with soil components, for example, humic acids. Similar results were obtained in studies [37,38].

The antimicrobial effect of nanomaterials has been widely studied in relation to the bacteria involved in different stages of the nitrogen cycle. A significant reduction in the number of microorganisms when introducing molybdenum oxide nanoforms was observed for fungi, bacteria participating in different stages of the nitrogen cycle, and cellulolytic

microorganisms. For the intestinal microbiocenosis of the worms, a significant stimulation of bacteria of the genus *Azotobacter* at a dose of 50 mg/kg MoO_3 NPs was noted. This is due to the active entry of molybdenum ions into the worm's body and stimulation of the body, with fixative of molecular nitrogen through the activation of the enzymes responsible for nitrogen fixation (Table 2) [39].

In soil, the absence of the worm's body wall as barrier led to a stronger decrease in the number of microorganisms, among which ammonifying bacteria, fungi, cellulose-depleting bacteria, and *Azotobacter* showed the greatest inhibition. These were the most sensitive indicators of soil microbiological activity to the introduction of MoO_3 NPs. Features of soil microflora suppression, when exposed to nanomaterials, were demonstrated in studies [40,41]. These studies showed the suppression of CuO NPs microorganisms involved in the carbon and nitrogen cycles, as well as fungal microflora (Table 3).

The actinomycetes isolated in the CAA were stimulated due to the selective toxicity of MoO_3 NPs with respect to microbial groups of the soil, as described in the research of Gorczyca et al. (2018) [42], who investigated the resistance of *Pseudomonas* and *Bacillus* in wheat rhizoplane to titanium nanooxide.

The mechanisms behind the effect of NPs on microbial cells are associated with the direct interaction of the bacterial cell wall with the agents and their effect on the cell membrane due to accumulation of reactive oxygen species [43,44].

Soil enzymes are involved in the decomposition of plant, animal, and microbial residues, and they participate in the global circulation of elements. They are distinguished by exceptionally high activity, strict specificity of action, and their large dependence on various environmental conditions. Special sensitivity of enzymes is manifested in relation to metal ions that deactivate protein molecules, and this feature is of great importance for their use in bioindication [45].

A general trend in decreasing activity of invertase and urease in soils is described by linear approximation equations. One exception was the indicator of catalase activity. This enzyme is characterized by a slight increase in the dose of 100 mg/kg MoO_3 NPs, which may be due to both an increase in the number of individual groups of microorganisms (ammonifiers), and the intense death of oligotrophic, cellulolytic, and fungal flora [46].

An increase in the activity of enzymes participating in the C cycle (peroxidase) is also associated with mass cell death and the release of readily available organic matter fractions into the soil solution. Carbon and nitrogen cycle enzymes are sensitive markers to the effects of nano-pollutants [47].

The experimental conditions are limited to only one ambient temperature and one soil pH. This is a limitation of the study. This may be one of the factors influencing our results. Due to this, the direction of our research can be expanded considering the present limitations.

5. Conclusions

Evaluation of the effect of molybdenum nanooxide on animal and microbiological components of soil demonstrated the presence of a negative effect with respect to the number of indicators. The specificity of nano-pollutants and the soil environment, one of the most complex mediums, even under model experimental conditions, explains the absence of a number of classical recording parameters (i.e., the survival rate of *E. fetida*, and the number of individual groups of bacteria). The high adaptive potential of the worm itself also contributed to a decrease in oxidative stress on the 28th day of the experiment, which can be indirectly assumed by the restoration of the SOD level. However, such indicators as protein content, body weight, and CAT activity were the most sensitive and promising in assessing the effects of soil contamination by MoO_3 NPs.

The antimicrobial activity of NPs has been demonstrated by a number of studies, allowing one to conclude that MoO_3 NPs suppress the number of groups of microorganisms carrying out individual parts of the C and N cycles. The overall decrease in the number of microorganisms and the suppression of soil oxidase and hydrolase activity raise particular

concerns in assessing the potential environmental risks of nano-pollutants entering the surrounding environment amid the growing market demand for new materials. It is difficult to predict the behaviour of nanoparticles in soils due to the complexity of their chemical properties; the dynamics of physicochemical parameters makes it necessary to further study the effects of nanoparticles. An important stage of these studies should be identifying the mobility of nanoparticles in soils, in a gradient of soil parameters, and the impact on the biota and its ability to perform global geochemical functions.

The prospects of this study are that in future work it will be advisable to expand the list of nanoparticles studied, as well as to test the fraction of dissolved ions in soils and study the possible aggregation mechanisms of nanoparticles when interacting with soil particles. It should be noted that the importance of determining soluble forms of Mo is necessary for assessing the toxicity of pollutants in soil systems.

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