Research Article

Bioremediation Potential of Indigenous Earthworms in Radionuclide-Contaminated Soils of the Absheron Peninsula

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Abstract

The current investigation illustrates the impact of radionuclide-contaminated soils from the Surakhani oil and gas processing plant and the Ramana iodine plant territories of the Absheron peninsula on the survival, feeding, and excretion functions of earthworms, as well as the antioxidant enzyme activity of the soils. Earthworms are highly sensitive to high radioactivity and are well-suited for assessing radioactive soils as bioindicators, as evidenced by model experiments. However, at lower contamination levels, earthworms accumulated radionuclides and contributed to their immobilisation in coprolites, potentially reducing their bioavailability. Contamination resulted in a significant increase ($p < 0.05$) in catalase activity, suggesting the presence of oxidative stress responses. These discoveries underscore the dual function of earthworms as highly sensitive bioindicators and prospective agents for the bioremediation of radionuclide-contaminated soils.

Keywords: Absheron Peninsula, bioindicators, bioremediation, catalase activity, earthworms, soil contamination, radionuclides

1. Introduction

Environmentally safe utilization of originally different organic wastes, as well as the remediation of anthropogenically contaminated soils, is the most actual problem nowadays. Mainly, the transformation of radionuclides from organic residues to the mineral fraction of soil and consequently into plants decreases only after deep ploughing of soils. In more than 1480 kKi/km2 contaminated territories, ploughing is lethal for saprophyte microflora of soil and results in a decrease in biomass of some kinds of invertebrates in soils, and finally sharp pathological deformation of soils, which results in the acceleration of mineralization of isotopes [1]. For the fertility of soils, first, it is important to rehabilitate the soil organisms, microorganisms, and plants. During millions of years, earthworms have played a special part in the formation of the fertile layer of soil [2]. In normal terrestrial conditions, lumbricides make up 80% of the biomass of invertebrates in pedoecosystems [3], [4]. Hence, the International Commission of Radiological Protection requested the determination of the effect of radiation on non-human biota for the radioecological safety of the environment (ICRP 2003) and suggested earthworms as reference organisms for radiation protection of the environment [5], [6], [7], [8].

As a result of many years of oil production, large territories of the Absheron peninsula were contaminated with oil and sewage waters. Due to the leakage of residues of oil production, some radionuclides are transported to sewage waters, then to artificial canals and lakes, and radionuclides accumulated to soil and increase the radiation background of nearby areas. As well as some territories of the Absheron peninsula were directly contaminated with radionuclides and heavy metals due to residues of iodine production [9].

The study evaluates the response of native earthworms (*Nicodrilus caliginosus trapezoides (Savigny)*) to radionuclide-contaminated soils from Surakhani and Ramana regions. The effects on survival, feeding, excretion, and soil catalase activity were assessed under controlled laboratory conditions [10], [11].

2. Materials and Methods

Earthworms (*Nicodrilus caliginosus trapezoides (Savigny)*) have been collected from the grey-brown soils of the natural cenosis of the Absheron peninsula and prepared according to OECD standards [5]. Because of the high radioactivity, the soils of the territory Surakhani oil production plant were diluted in a 1:1 ratio, and the soil from the territory of Ramana iodine production plant in 1%, 2,5%, 5%, 10% concentrations with grey-brown soils. Earthworms with plant residues (for feeding) for 4 weeks by keeping 60-70% of humidity in the container, at room temperature, in laboratory conditions, have been cultivated in plastic dishes (with 300 ml volume) with pores for aeration. During the experiment for keeping a normal humidity level, the soils were 3 times watered with 20ml of distilled water. Radioactive background was measured with a radiometer-dosimeter (Inspector 1000, MKS AT 1125), and the activity of radionuclides was measured with a gamma spectrometer (HP Ge Gamma Spectrometer). Earthworms (*N. caliginosus trapezoides*) were collected from grey-brown soils of natural ecosystems of the Absheron Peninsula and prepared according to OECD Guideline 207 for earthworm acute toxicity testing. Due to high contamination levels, Surakhani soils were diluted at a 1:1 ratio with uncontaminated grey-brown soils. Ramana soils were tested at concentrations of 1%, 2.5%, 5%, and 10%.

Earthworms were maintained for four weeks in plastic containers (300 mL) with aeration pores under laboratory conditions at room temperature and 60-70% soil moisture. Plant residues were provided as a food source. Soil moisture was maintained by adding 20 mL of distilled water three times during the experiment.

The radioactive background was measured using a radiometer-dosimeter (Inspector 1000, MKS AT 1125), while radionuclide activity was determined using a gamma spectrometer (HPGe Gamma Spectrometer).

3. Statistical Analysis

Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to evaluate differences between control and treatment groups. Data are presented as mean ± standard deviation (SD). Differences were considered statistically significant at $p < 0.05$.

4. Results

4.1. Survival of Earthworms

The effect of different concentrations of radioactive soils on the survival rate of earthworms is shown in Figure 1. It is obvious from that, with the increasing of radioactive concentration of soils, the survival rate decreases. In Surakhani soil samples, the final survival rate of earthworms was 13.30% less than in control (grey-brown soils) samples, whereas in 1% contaminated Ramana soils, this index decreased 5 times.

Survival decreased significantly with increasing radionuclide concentration ($p < 0.05$). High-dose Ramana soils (≥2.5%) resulted in acute mortality within 72 hours. A statistically significant decrease in survival was observed with increasing radionuclide concentration ($p < 0.05$) (Figure 1).

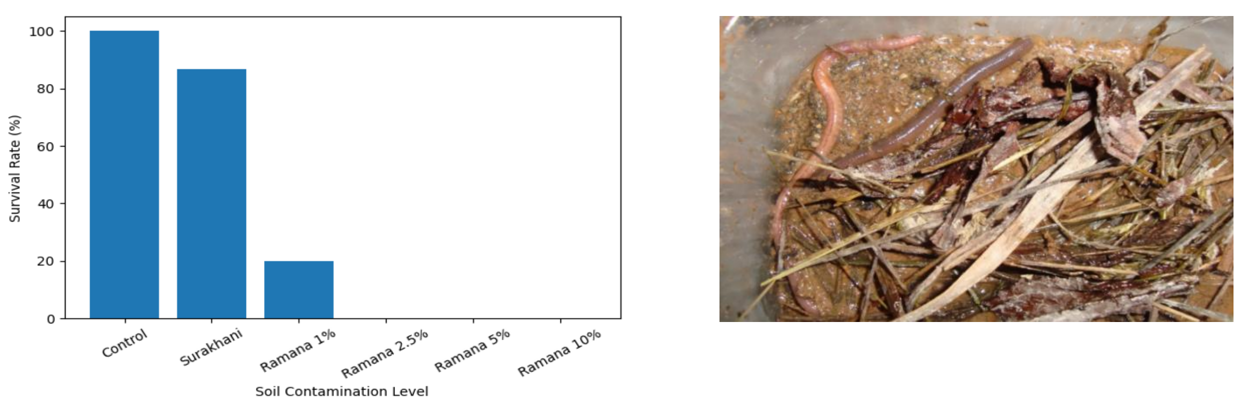
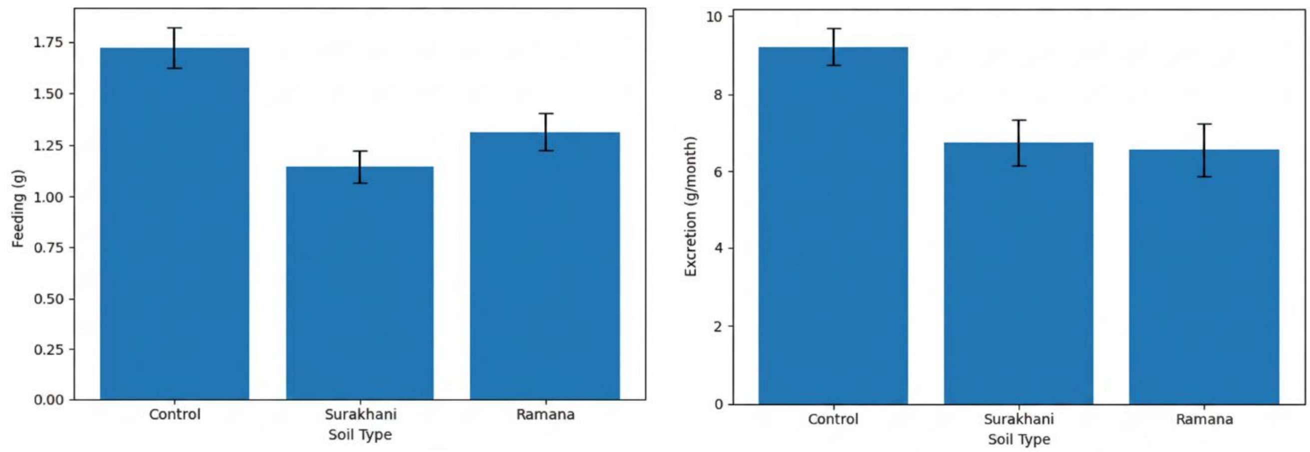


Figure 1. Survival rate of experimental earthworms.

4.2. Feeding and Excretion Activity

The physiological analyses of earthworms in radionuclide-contaminated soils have shown that, with the increasing of radioactive concentration of radionuclides, the feeding and excretion of earthworms have been contrarily decreased (Figure 2, a & b). Thus, in control, non-contaminated soils, the feeding activity was 1.709g of initial 2g, and excretion activity was 9.2g per month; in Surakhani soils, these data was 34% less feeding activity than in control, and excretion activity was about 27 % less. For Ramana soils, it appeared as 24% less feeding and 29% less excretion than in control, but for 1% contaminated Ramana soils, which was 20% less feeding and 89% less excretion activity than in control samples for 2.5% contaminated soils.



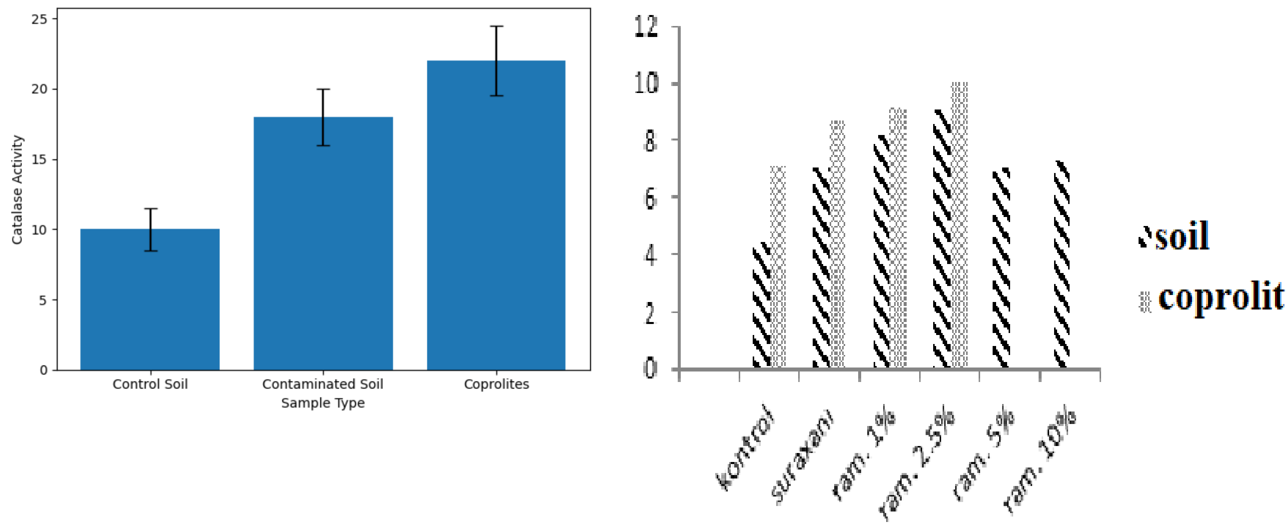
(a) **(b)**

Figure 2. (a) Feeding activity (Mean ±SD) and (b) Excretion activity (Mean ±SD).

Excretion activity was more sensitive to contamination than feeding, indicating early physiological disruption.

4.3. Catalase Activity

At the end of the month, catalase activity was measured in the earthworm excreta and in the rest of the experimental soils. It was established that, following the radioactive effects of radionuclides on earthworms and soil biota, catalase activity increased in a direct proportion to radionuclide concentration (Figure 3, a & b). It was determined that in coprolites (excrements of earthworms), the quantity of catalase was a little bit more than in the remaining soils of the samples. This interesting difference is due to the action of saprotrophic bacteria, which symbiotically live in the gut of earthworms, so their action under the radioactive soils that pass through the intestines of earthworms also increases the catalase activity.



(a) (b)

Figure 3. (a) Catalase activity (Mean ±SD) and (b) The quantity of catalase in experimental soils and coprolites of earthworms (cm^3 O_2/g 2min.

The observed increase in catalase activity reflects enhanced oxidative stress due to radionuclide exposure.

4.4. Radionuclide Accumulation

One of the significant points of experiments is the accumulation of radionuclides by earthworms (Table 1). It was determined that earthworms, by taking the soil through their gut, absorb more than 50% of radionuclides, absorbing them in coprolites as stable chelate complexes. So, radionuclides isolated from soil are becoming non-toxic for plants. Earthworms significantly altered radionuclide distribution between soil and coprolites ($p < 0.05$).

Table 1. Quantity of radionuclides before and after experiments in model soil samples from Surakhani and Ramana regions (Bq/kg).

Samples	Radionuclide compounds of soils					
	^{137}Cs	^{40}K	^{226}Ra	^{228}Ra	^{235}U	^{238}U
Control Grey brown	-	-	16± 2	15± 2	2.5±0.3	123±12
Surakhani soils						
Initial soils	10,7±0,8	133,5±5,5	1004 ± 5	14,7 ± 1,1	-	-
Remained soils	-	389±33	732±6	22±2	-	-
Coprolite	-	561± 33	709±7.7	8.7± 0.4	-	-
Ramana soils						
Initial soils	-	-	35345± 98	436 ± 27	622 ± 15	30400±800
Remained soils						
1%	-	-	560±56	23±2.3	11±1.7	542±53
2,5%	-	-	1055±103	22.7±3	10.7±2	512±49
5%	-	-	2170±223	33±3	28±2.5	1351±107
10%	-	-	4140±413	69±6.7	44.7±4.7	2177±220
Coprolites						
1%	-	-	200 ± 10	25 ± 3	30 ± 5	1460± 100
2,5%	-	-	100 ± 10	6.0 ± 0.7	60 ± 7	2900±150
5%	-	-	780 ± 30	40 ± 10	140 ± 15	6880 ±150
10%	-	-	-	-	-	-

5. Discussion

The results of this investigation are in accordance with earlier studies showing that soil invertebrates are extremely vulnerable to radiation-induced stress. A well-established mechanism under radiation stress is the activation of antioxidant defence systems in response to elevated levels of reactive oxygen species (ROS), as shown by the observed increase in catalase activity. Similar enzymatic reactions have been reported in contaminated environments, emphasising the significance of oxidative stress as a major toxicity mechanism. Coprolites' increased enzymatic activity raises the possibility that gut-associated bacteria could improve detoxification procedures.

The detrimental effects of radioactive exposure on soil invertebrates are confirmed by the reported decrease in survival and physiological activity. Studies on radiation-induced stress in soil ecosystems, where oxidative damage is a major factor, have revealed similar results.

Additionally, earthworms' capacity to store radionuclides and transfer them into coprolites suggests a possible natural detoxifying process. This process may assist the use of radionuclides in bioremediation techniques by lowering their bioavailability and limiting their transfer into plants and higher trophic levels. This is consistent with other research showing how earthworms may immobilise contaminants and heavy metals. Nevertheless, the efficacy of this mechanism seems to be concentration-dependent, as acute mortality was observed in the presence of elevated contamination levels.

The results suggest that earthworms have the potential to be used in sustainable soil remediation strategies, particularly in moderately contaminated environments.

6. Conclusions

This research supports the concept that soil invertebrates are exceedingly susceptible to radiation-induced stress, as elevated radionuclide exposure results in substantial toxicity, including diminished physiological activity and survival. The observed increase in catalase activity suggests that earthworms initiate antioxidant defence mechanisms to combat oxidative damage (ROS), which is a consequence of this stress.

Key discoveries concerning detoxification mechanisms indicate that earthworms accumulate radionuclides and recirculate them as coprolites (excrement). This implies a natural immobilisation mechanism that diminishes the bioavailability of these contaminants. The significant function of gut-associated microbiota in detoxification processes is indicated by the increased enzymatic activity of these coprolites.

Nevertheless, the efficacy of this mechanism is contingent upon the concentration, as acute mortality was observed in the presence of elevated contamination levels. Consequently, earthworms are most effectively employed as a sustainable, long-term bioremediation strategy (vermediation) in moderately contaminated environments, as opposed to severely contaminated sites.

Earthworms are very sensitive bioindicators of radiation pollution. Under conditions of radiation stress, a significant decrease in their physiological functions is observed. The increase in catalase activity confirms the occurrence of oxidative stress mechanisms. At the same time, worms have the ability to accumulate radionuclides and immobilize them to a certain extent. These properties make them possible to use as effective bioremediation on agents in low and moderate levels of contamination.

Author Contributions

Aygun S. Suleymanova contributed to the experimental investigation and the writing of the manuscript. Constantin Daniel Negut performed the gamma spectrometric measurements and reviewed the manuscript.

Conflict of Interest

The authors declare no conflicts of interest.

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