Toxicological Assessment of Lead Nitrate on *Lumbricus terrestris*: Behavioral and Biochemical Implications

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Abstract

During the study, individuals of the earthworm *Lumbricus terrestris* were exposed to various levels of lead (II) nitrate at different times. The soil and earthworm samples were collected from local orchards within the Shaqlawa district. Soil samples were tested for physical and chemical characteristics, including pH, Electrical conductivity (EC), soil moisture content, organic matter, and texture. The studied organisms were exposed to variable concentrations of lead nitrate, ranging from 0 to 5000 ppm. Also, the lead concentrations in the soil and the earthworm body were measured. Earthworms collected from the experimental media were tested for biochemical markers, including acetylcholinesterase (AChE), glutathione S-Transferase (GST), catalase (CAT), and malondialdehyde (MDA). After 96 hours, the lethal concentration (LC_{50}) was estimated to be 4723.45 ppm. The findings on behavior revealed that lead altered morphology, and the behavior of treated individuals was compared to that of the controls. Lead concentration increased within the worm's body as the soil lead level decreased. The value of AChE was inversely related to lead concentration. However, GST, CAT, and MDA increased with lead exposure.

Keywords: Lead nitrate, Earthworm, LC_{50}, Behavioral and Biochemical

پوختە

لە کاتی تۆیژینەوەکەدا، تاکەکانی کرمی زەوی Lumbricus terrestris لە کاتە جیاوازەکاندا بەرکەوتەی ئاستی جیاوازی نتراتی رصاص (II)بوون. نمونەی خاک و کرمی زەوی لە باخچە ناوخۆییەکانی ناو قەزای شەقلاوە کۆکرایەوە. نمونەکانی خاک تاقیکرانەوە بۆ تایبەتمەندییە فیزیایی و کیمیاییەکان، لەوانەpH ، ڕێبەرایەتی کارەبایی (EC)، ڕێژەی شێی خاک، مادە ئۆرگانییەکان و پێکهاتە. زیندەوەرە لێکۆڵینەوەکراوەکان بەرکەوتەی چڕیی گۆڕاوی نتراتی رصاصبوون، کە لە نێوان ٠ بۆ ٥٠٠٠ ppm بوو. هەروەها چڕیی سرکە لە خاک و جەستەی کرمی زەویدا پێوانە کرا. کرمی زەوی کە لە میدیای تاقیکارییەوە کۆکرابوونەوە تاقیکرانەوە بۆ نیشاندەری بایۆکیمیایی، لەوانە ئەسیتیلکۆلین ئیستراز(AChE) ، گلوتاتیون-S ترانسفێراس(GST) ، کاتالاز(CAT) ، و مالۆندیالدەهید .(MDA)

دوای ٩٦ کاتژمێر، چڕیی کوشندە (LC50) بە ٤٧٢٣.٤٥ ppm خەملێندرا. دۆزینەوەکان لەسەر ڕەفتار دەریانخست کە سرکە مۆرفۆلۆژیای گۆڕیوە، و ڕەفتاری کەسانی چارەسەرکراو بەراورد کران بە ڕەفتاری کۆنترۆڵەکان. چڕیی رصاص لەناو جەستەی کرمەکەدا زیاد بوو لەگەڵ کەمبوونەوەی ئاستی رصاص لە خاکدا. بەهای AChE پەیوەندی پێچەوانەی هەبوو بە چڕیی رصاص بەڵام لەگەڵ بەرکەوتنی رصاص GST و CAT و MDA زیادیان کرد.

وشەی سەرەکی: نتراتی رصاص، کرمی زەوی، LC50، ڕەفتار و بایۆکیمیایی

ملخص

خلال الدراسة، عُرّضت أفراد من دودة الأرض Lumbricus terrestris لمستويات متفاوتة من نترات الرصاص (II) في أوقات مختلفة. جُمعت عينات التربة وديدان الأرض من بساتين محلية ضمن قضاء شقلاوة. وخضعت عينات التربة لاختبارات فيزيائية وكيميائية، بما في ذلك الرقم الهيدروجيني (pH)، والتوصيل الكهربائي (EC)، ومحتوى الرطوبة في التربة والمادة العضوية، والقوام. تعرّضت الكائنات المدروسة لتركيزات متفاوتة من نترات الرصاص، تراوحت بين ٠ و٥٠٠٠ جزء في المليون. كما قيست تركيزات الرصاص في التربة وجسم دودة الأرض. وُجدت علامات بيوكيميائية في ديدان الأرض التي جُمعت من الأوساط التجريبية، بما في ذلك إنزيم أستيل كولين إستراز (AChE)، وإنزيم الجلوتاثيون S-ترانسفيراز (GST)، وإنزيم الكاتالاز (CAT)، وإنزيم مالونديالدهيد (MDA). بعد اتساعة، قُدّر التركيز المميت (LC50) بـ ٤٧٢٣.٤٥جزء في المليون. كشفت نتائج السلوك أن الرصاص غيّر مورفولوجيا الدودة، وكان سلوك الأفراد المُعالَجين مُشابهًا لسلوك المجموعة الضابطة. ازداد تركيز الرصاص داخل جسم الدودة مع انخفاض مستوى الرصاص في التربة. ارتبطت قيمة إنزيم الأسيتيل كولينستريز عكسيًا بتركيز الرصاص. ومع ذلك، ازدادت قيم GST وCAT وMDA مع التعرض للرصاص.

الكلمات المفتاحية: نترات الرصاص، دودة الأرض، LC50، السلوكيات والكيمياء الحيوية

Introduction

Agrochemicals have been widely employed to protect crops from pests and diseases, as well as to control diseases in living organisms. Due to their selective nature, these agrochemicals have the potential to damage non-target organisms (Guzzetti, Sureda, Tejada, & Faggio, 2018). Life is directly impacted by lead (II) nitrate (Pb (NO□) □), a common environmental contaminant. Oligochaetes appear to influence soil structure and the distribution of contaminants within the soil profile through their burrowing, feeding, and casting behaviors. Comprising 70% of the soil macrofauna, they contribute to mineralization and organic matter breakdown, increase nutrient availability, transmit energy, and encourage pedogenesis —all of which assist in modifying soil profiles. Due to their prevalence in agricultural systems, earthworms are susceptible to the non-target effects of pollutants produced by chemical spills, stormwater, draining, and/or direct or indirect evaporation (Datta, Singh, Singh, & Singh, 2016; Pelosi, Barot, Capowiez, Hedde, & Vandenbulcke, 2014). Earthworms exposed to agrochemicals show detrimental physiological changes, including decreased reproduction, weight loss, avoidance behavior, decreased overall survival, and enzyme inhibition (Gu, Yuan, Cai, Wang, & Lv, 2021).

The crucial ecotoxicological tools for detecting the impacts of toxicants on organisms are biomarkers, because they can detect changes in metabolic parameters in vivo and the onset of oxidative stress. Chronic or sublethal toxicological investigations use a more pragmatic approach, exposing organisms to low and realistic doses of toxicants, while the half-lethal concentration (LC_{50}), the endpoint of fatality, is the most often utilized preliminary assessment of toxic effects (Deng *et al.*, 2021; Liu *et al.*, 2024; Solé, 2020).

Antioxidant enzymes are the most common indicators used to monitor pollution levels. One of the first compounds to be used as a biomarker for lipid peroxidation was malondialdehyde (MDA). One of the stress indicators is the alteration of membrane phospholipids through lipid peroxidation, a crucial stage in oxidative stress. A typical toxicity signal is oxidative stress brought on by chemicals (Hao & Liu, 2019). The acetylcholinesterase (AchE) enzyme hydrolyzes acetylcholine, which forms choline and acetic acid. The enzyme regulates ionic currents in excitable membranes and is necessary for nerve conduction at the neuromuscular junction. The inhibition of AChE is intimately related to the mechanism of agrochemicals' detrimental effects (Gagné, 2014; Nihal Suhail Hanna & Yahya Ahmed Shekha, 2024). In the cellular antioxidant defense system, catalase (CAT) is the primary enzyme responsible for detoxifying O□ and H□O□. CAT activity in the hepatopancreas of individuals exposed to heavy metals was significantly decreased. An important stage II enzyme with several functions, glutathione S-transferase (GST) aids in the detoxification of xenobiotic substances and lowers oxidative stress (Nihal S Hanna & Yahya A Shekha, 2024; Jiang *et al.*, 2019). The present research aimed to use behavioral and specific enzyme activities to assess the toxicity of lead nitrate on *L. terrestris* species collected from a nearby orchard.

Materials and Methods

Earthworm Collection and Acclimatization

The earthworm *L. terrestris* was used to conduct a toxicity test. The specimens of earthworms were collected by hand from the topsoil at a depth of approximately 15-20 cm in a local orchard located at 44° 34' 51.02" E longitude and 36° 39' 43.96" N latitude, 933 m above sea level, within the Shaqlawa district. The earthworms were then placed in a pot with moist soil until they were transported to the laboratory. The earthworms were first stored in a soil sample that was sieved through a 1 mm mesh in a 1-liter glass container and acclimated for 7 days at a room temperature of 25±2 °C with a dark: light cycle of 9:15 hours and humidity of 70–80%. For the toxicity test, adults with a fully developed clitellum region that weighed between 250 and 500 mg were selected. The species was taxonomically identified using the standard taxonomic keys (Jorge Escudero, Lagerlöf, Martínez Debat, & Pérez, 2019; Sims & Gerard, 2023).

Molecular Study

After morphological identification, three earthworm specimens were chosen for genetic study. Using the GeneAll® ExgeneTM for Clinic Cell SV small kit (Songpa-gu, Seoul, Korea), genomic DNA was isolated from the clitellum region. A NanoDrop 1000 spectrophotometer was used to determine the content and purity of the recovered DNA, and agarose gel electrophoresis was employed to verify its integrity (Bakr, Abdul-Rahman, & Hamasalih, 2021). The primer pair LCO1490 (forward) and HC02198 (reverse), obtained from Macrogen (Korea) and first described by (Folmer, Black, Hoeh, Lutz, & Vrijenhoek, 1994) was used to amplify the COI gene. 25 µL of

2× AMPLIQON master mix (Denmark), 1.0 µL of each primer (10 pmol), 3 µL of DNA template, and 50 µL of PCR-grade water were used to conduct PCR reactions in a 50 µL reaction. Initially, denaturation occurred at 94°C for 5 minutes, followed by 40 cycles of denaturation at 94°C for 50 seconds, annealing at 50°C for 45 seconds, and extension at 72°C for 50 seconds, with a final extension at 72°C for 7 minutes. This constituted the PCR conditions. Good amplifications were delivered to Macrogen (South Korea) for sequencing after PCR products were confirmed on a 0.8% agarose gel using 1× TAE buffer (Hamasalih & Abdulrahman, 2020). The Molecular Evolutionary Genetics Analysis (MEGA) program was employed to examine the obtained sequences.

Table 1. COI Primer Sequences

Direction	Name	Sequence
Forward	LCO1490	5'-GGTCAACAAATCATAAAGATATTGG-3'
Reverse	HC02198	5'-TAAACTTCAGGGTGACCAAAAAATCA-3'

Soil Sampling and Analysis

The soil samples used in the experiment were collected from a local orchard as untreated natural soil. The site from which the soil was collected had no prior history of agrochemical application for at least twenty years. Soil samples were collected in polyethylene containers, and analyses were performed for several physical and chemical parameters, including pH, EC, soil moisture content, soil organic matter, and soil texture (Richards, 1954; Ryan, Estefan, & Rashid, 2001).

Chemicals and Toxicity Test

During the test, six separate groups of 30 earthworms were exposed to different concentrations of lead (II) nitrate, including 0, 1000, 2000, 3000, 4000, and 5000 ppm, for various exposure durations of 24, 48, 72, and 96 hrs. After exposure, the entire bodies of the earthworms were collected for biochemical analysis. The lethal concentration 50 (LC_{50}) was measured using probit analysis. The mortality is indicated by visual observation. Additionally, the behavior of individuals in the control group was compared with that of the exposed groups (Aguzie *et al.*, 2021).

Lead Estimations

The lead level was determined in the experimental medium during the test. For lead analysis, 1 g of the oven-dried soil sample was weighed; after that, 15 ml of HCl and 5 ml of HNO□ were added, and the mixture was heated until the solution became transparent. After cooling and filtering the solution, it was diluted to 50 ml and analyzed using an atomic absorption spectrophotometer (Maurya, Kesharwani, & Mishra, 2018).

The lead levels were also estimated within the bodies of exposed individuals during all test periods. The whole body of the exposed worm was oven-dried at 70°C for 48 hrs. After drying the crushed worm body, 1 g was taken and mixed with 5 ml of concentrated HNO□ (70%) and 1 ml of concentrated HCl for 1 hour, and then heated gradually to 200°C. The final volume was adjusted to 25 mL with distilled water and cooled to room temperature. The lead concentration was determined using an atomic absorption spectrophotometer (Haswell, Mendham, Butler, & Smith, 1988).

Biochemical Markers

The whole body of the worm was washed with saline solution. The whole body was homogenized using a hand-held glass homogenizer with an extract solution. The mixture was centrifuged at 8000 rpm for 10 minutes at 4°C. The supernatant was stored at -80°C until analysis (Alnahdi, Ramadan, Farid, & Ayaz, 2018; Hanna, Khudhur, & Shekha, 2024). The concentrations of AChE, CAT activity, GST, and MDA were determined using commercial assay kits.

The statistical analysis

Version 25 of SPSS was used for data analysis. Analysis of variance (ANOVA) is one way to treat biochemical test data. To detect the significant variations between groups, Duncan's post hoc test was used, and a p-value of 0.01 was taken into consideration. Every measurement was three replications.

Results and Discussion

Earthworms' reactions to heavy metal toxicity may be quickly and accurately predicted by certain enzymes in the organism. The activity of these enzymes in earthworms is adversely affected by the presence of heavy metals (Łaszczyca *et al.*, 2004; Zheng, Liu, Li, Cui, & Li, 2013).

Molecular identification of earthworms using pan- or specific primer amplification was coupled with phenotypic assessment. CDS nucleotide sequencing provided information for molecular samples, as well as their isolation, identification, and precise characterization. Earthworm DNA sequencing included nucleotide sequences from *L. terrestris* GenBank accession numbers (OR122656, OR122657, and OR122658).

The following were the findings of the physicochemical properties of the soil: pH 7.9; moisture content 70.57%; organic matter 2.88%; electrical conductivity (EC) 890.00 µS/cm. The soil texture is categorized as clay loam and is composed of a combination of sand (27.2%), silt (41.1%), and clay (31.7%). Earthworm populations may thrive in clay loam, according to (Lapied, Nahmani, & Rousseau, 2009).

The acute toxicity of lead (II) nitrate to the exposed groups of earthworms significantly induced stress at various concentrations. Consequently, morphological and behavioral responses of treated individuals were compared to those of controls, and the behavioral alterations were noted (Table 2) following exposure for 24, 48, 72, and 96 hours. According to the results, tracking morphological and behavioral symptom responses could be a helpful method for identifying ecotoxicity brought on by exposure to harmful compounds in the environment. Similar results were reported by (Aguzie *et al.*, 2021) about the impact of heavy metal exposure on earthworm behavior. Over and above that, the metal-induced mortality was high enough to calculate an LC50 for *L. terrestris* at 96 hr. The LC50 value was 4723.45 ppm. For instance, the toxicological effects of heavy metals on earthworms have been studied by (Davies, Hodson, & Black, 2002, 2003), who have also calculated the LC50 values of the metals.

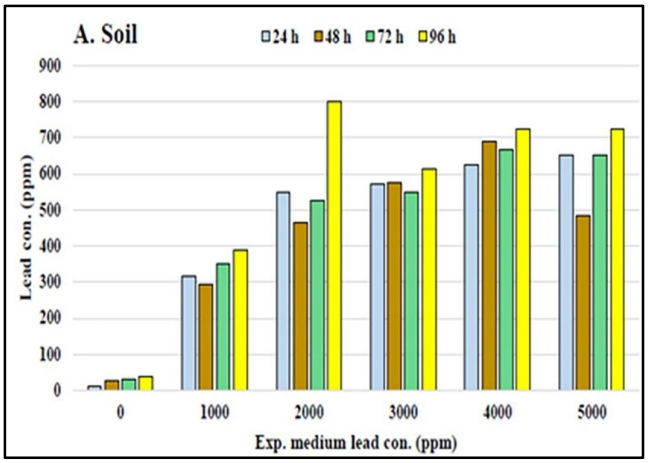
Table 2. Comparison between the morphology and behaviors of the control and exposed groups

Response	Behavior & Morphological Symptoms				
	Control	24 h	48 h	72 h	96 h
Escape attempts	not exhibit	rapid response	tolerate toxicity	tolerate toxicity	tolerate toxicity
Burrowing & Movement	very quick	quick	slowed down	very slow down	not observed

Preclitellar swelling & Segmental bulging	not observed	not observed	initiated	increased	quantitatively increased

Soil lead content is affected by pH, soil organic matter, and the form of lead present. Earthworms absorb lead in bioavailable forms, ingesting soil particles while burrowing. Earthworm individuals absorb lead directly through their digestive systems and permeable skin. Bamgbose et al. (2000) discovered a significant relationship between earthworm populations and soil's lead concentrations. Figure (1A) illustrates lead concentration changes over 24, 48, 72, and 96 hours in experimental soil. Concentrations of lead varied significantly. The level of control lead was 12.130 ppm at 24 hours, while in the soil treated with 2000 ppm lead (II) nitrate, it reached 800.376 ppm during 96 hours. Statistically significant ($p \leq 0.01$) variations were observed in lead levels. Earthworms can accumulate lead, making them useful bioindicators. This emphasizes the possibility of lead transmission across the food chain and has consequences for environmental risk assessments (Latifi, Musa, & Musa, 2020).

In addition to experimental media, the lead levels were determined within the earthworm bodies. From Figure 1B, the concentrations of lead changed with exposure conditions. The minimum value (10.473 ppm) was observed in the bodies of individuals in the control group after 24 hours; however, the maximum value (271.260 ppm) was detected in individuals exposed to 3000 ppm lead (II) nitrate after 48 hours of the test. A significant difference ($p \leq 0.01$) was found among treatments. These results indicated that the lead accumulation within the worm body is influenced by the surrounding concentration and time of exposure. Their growth, reproduction, and survival are similarly impacted by lead exposure; avoidance and mucus secretion are examples of physiological reactions that may be defensive measures.



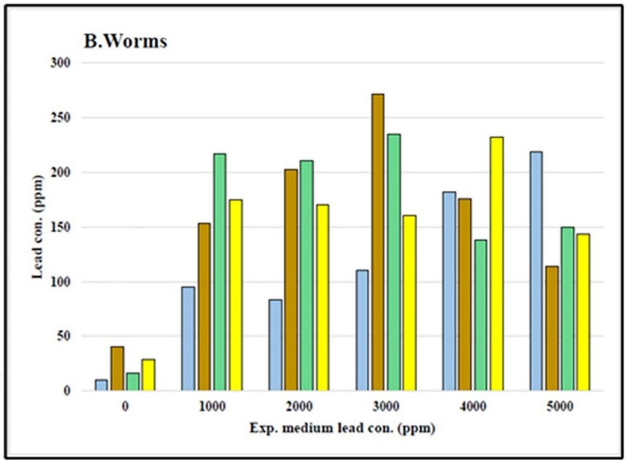


Fig. 1. Concentration of lead in the A- experimental media, B- body of the worms during test periods.

In earthworms, specific enzymes accurately indicate their response to heavy metal toxicity. The

effect of concentration and time of exposure to lead (II) nitrate on biochemical markers, including AChE, GST, CAT, and MDA, was significantly different ($P \leq 0.01$). The biomarker AChE significantly influences the breakdown of acetylcholine and provides an indicator for identifying the toxicity of xenobiotics in the environment (Cravo *et al.*, 2009; Nihal S Hanna & Yahya A Shekha, 2024). The concentrations of AChE levels in individuals treated with different concentrations of lead are illustrated in Table 3. The lowest level of lead was 55.25±2.69 U/mg protein detected in individuals exposed to 5000 ppm after 96 hours of the test; however, the highest level was 127.75±0.47 U/mg protein observed in individuals of the control group. AChE level significantly decreased during the test ($p \leq 0.01$); this is due to inhibition of enzyme function by lead (II) nitrate. (Calisi, Zaccarelli, Lionetto, & Schettino, 2013; Hanna *et al.*, 2024) reach the same results while exposing invertebrates to agrochemicals.

Table 3. Mean±SE of AChE (U/mg protein) in *L. terrestris* exposed to lead (II) nitrate.

Concentration (ppm)	24 h	48 h	72 h	96 h
0	123.27±0.57^{b}	125.59±0.31^{ab}	126.27±1.38^{a}	127.75±0.47^{a}
1000	108.96±0.07^{a}	98.69±0.17^{b}	96.30±1.01^{c}	88.80±0.17^{d}
2000	97.33±0.32^{a}	90.99±0.97^{b}	82.29±0.69^{c}	76.68±1.06^{d}
3000	95.14±0.05^{a}	87.55±0.31^{b}	69.05±0.06^{c}	56.12±0.14^{d}
4000	79.26±0.38^{a}	71.13±0.15^{b}	60.65±0.81^{d}	68.77±0.35^{c}
5000	64.56±0.23^{b}	72.05±0.06^{a}	59.36±0.28^{c}	55.25±2.69^{c}

Note: concentrations in each row that have different letters exhibit significant differences.

A significant increase ($p \leq 0.01$) in GST during the experiment times is shown in Table 4. The minimum level of GST was 4.30±0.17 U/mg protein in individuals of the control group, while the maximum value was 17.84±0.04 U/mg protein in individuals exposed to 5000 ppm lead(II) nitrate. When individuals are exposed to xenobiotics, GST increases, which helps with detoxification by binding toxic intermediates to glutathione. The synthesis of metal-binding proteins by free radicals, which aid in neutralizing and sequestering metals to improve survival, may be the cause of the higher GST levels in the following experiment. (Jeyanthi, Paul, Selvi, & Karmegam, 2016) recorded similar findings.

Table 4. Mean±SE of GST (U/mg protein) in *L. terrestris* exposed to lead (II) nitrate.

Concentration (ppm)	24 h	48 h	72 h	96 h
0	4.51±0.03^{ab}	4.97±0.30^{a}	4.30±0.17^{b}	5.04±0.01^{a}
1000	5.77±0.05^{d}	6.84±0.11^{c}	7.34±0.18^{b}	8.88±0.05^{a}
2000	7.93±0.02^{b}	8.31±0.04^{b}	7.79±0.62^{b}	9.49±0.18^{a}
3000	8.28±0.02^{c}	9.43±0.06^{b}	9.25±0.22^{b}	10.77±0.10^{a}
4000	10.66±0.21^{c}	12.86±0.32^{b}	14.76±0.04^{a}	12.78±0.01^{b}
5000	13.41±0.04^{d}	15.33±0.15^{c}	16.64±0.33^{b}	17.84±0.04^{a}

Note: concentrations in each row that have different letters exhibit significant differences.

In response to the lead (II) nitrate concentrations and times of exposure, the individuals of *L. terrestris* showed variation in the level of CAT activity (Table 5). The greatest CAT activity concentration was 29.48±0.28 U/mg protein, which was measured at 5000 ppm after 24 hours of the test, whereas the lowest value was 10.21±0.07 U/mg protein at 1000 ppm, also after 24 hours of the test. The 1000 ppm and 5000 ppm treatments showed significant differences ($p \leq 0.01$) throughout all periods. According to (Hu, Zhang, Li, Lin, & Ji, 2016; Kono & Fridovich, 1982), oxidative stress makes CAT activity decline, as was seen after two days of exposure.

Table 5. Mean±SE of CAT activity (U/mg protein) in *L. terrestris* exposed to lead (II) nitrate.

Concentration (ppm)	24 h	48 h	72 h	96 h
0	12.51±0.12^{b}	13.62±0.06^{a}	12.42±0.02^{b}	13.40±0.16^{a}
1000	10.21±0.07^{d}	14.76±0.05^{c}	15.72±0.08^{b}	20.15±0.03^{a}
2000	18.55±0.33^{a}	18.95±0.01^{a}	14.52±0.09^{c}	17.28±0.03^{b}
3000	17.34±0.02^{b}	20.26±0.04^{a}	17.41±0.01^{b}	14.37±0.02^{c}
4000	28.10±0.06^{a}	21.55±0.10^{b}	21.72±0.15^{b}	15.38±0.24^{c}
5000	29.48±0.28^{a}	24.21±0.17^{b}	15.88±0.06^{c}	14.56±0.19^{d}

Note: concentrations in each row that have different letters exhibit significant differences.

In comparison to the individuals of the control group, there was a significant ($p \leq 0.01$) decline in the levels of MDA (Table 6). the minimum value of MDA was 1.12±0.01 nmol/mg protein was observed in control group after 24 hours of the test; however, the maximum value was 8.26±0.09 nmol/mg protein was recorded in the individuals treated with 5000 ppm lead (II) nitrate at the same time. the consistently raised MDA level in all exposed groups indicates oxidative stress and the accumulation of lipid peroxidation products due to increased reactive oxygen species. These findings are consistent with those of (Hu *et al.*, 2016)

Table 6. Mean±SE of MDA levels (nmol/mg protein) in *L. terrestris* exposed to lead (II) nitrate.

Concentration (ppm)	24 h	48 h	72 h	96 h
0	1.12±0.01^{c}	1.24±0.01^{b}	1.23±0.01^{b}	1.29±0.01^{a}
1000	1.92±0.12^{b}	2.15±0.03^{b}	2.23±0.12^{b}	3.39±0.13^{a}
2000	3.43±0.19^{b}	4.68±0.12^{a}	3.65±0.11^{b}	4.32±0.22^{a}
3000	3.93±0.25^{c}	5.87±0.05^{a}	5.59±0.09^{a}	4.92±0.03^{b}
4000	4.81±0.13^{d}	6.27±0.03^{b}	6.77±0.12^{a}	5.14±0.04^{c}
5000	8.26±0.09^{a}	7.38±0.03^{c}	6.95±0.03^{d}	7.74±0.08^{b}

Note: concentrations in each row that have different letters exhibit significant differences.

Conclusions

During the study, the earthworm *L. terrestris* was exposed to various concentrations of lead (II) nitrate. The study on the uptake of lead by earthworms in experimental media revealed that the lead level in soil decreased, whereas it increased within the test organism's body, thus leading to behavioral alterations. Biomarker levels were changed with changing metal concentrations and exposure times. Experimental findings suggest that lipid peroxidation is triggered by a series of biochemical reactions involving antioxidant enzymes in response to exposure to lead concentrations. During the exposure period, enzyme activity was suppressed, which could have been related to oxidative stress. It was discovered that the concentration of lead had an inverse relationship with the value of AChE. However, when the concentration of lead exposure rose, so did the levels of all other biomarkers, such as GST, CAT, and MDA.

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Conflicts of Interest

The authors declare that there are no conflicts of interest

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