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Chronic Toxicity of Cadmium Nitrate on the Earthworm *Eisenia fetida*: Effects on Survival, Growth Dynamics and Morphological Alterations

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ABSTRACT

The present study evaluated the chronic toxicity of cadmium nitrate [$Cd(NO_3)_2 \cdot 4H_2O$] to *Eisenia fetida* under laboratory conditions. Adult earthworms were exposed for seven weeks to eight cadmium concentrations (30–1200 µg g^{-1} vermifeed). Mortality, mean body weight of surviving individuals, and morphological changes were recorded at weekly intervals. Mortality data were analyzed using generalized linear models with a binomial distribution and probit link function to estimate time-dependent LC_{50} values. Two-way factorial ANOVA and quadratic regression were used to analyze the body weight data. Cadmium toxicity increased significantly with both concentration and exposure duration. no Mortality was observed in the control and 30 µg g^{-1} treatments. Complete mortality occurred at 1200 µg g^{-1} after three weeks and at 900 µg g^{-1} after seven weeks. The estimated LC_{50} decreased progressively from 906 to 354 µg g^{-1} over the exposure period, accounting for 61% increase in toxicity with chronic exposure. The mean body weight of surviving earthworms was significantly influenced ($P < 0.001$) by cadmium concentration, exposure duration, and their interaction. The growth phase was progressively shortened with increasing cadmium concentration as revealed by quadratic regression. Chronic exposure also induced concentration-dependent morphological abnormalities, including loss of normal body tapering, localized swelling, body constrictions, curvature, fragmentation, and rupture of the body wall. These findings demonstrate that prolonged cadmium exposure progressively compromises the survival, growth, and morphology of *E. fetida*, highlighting its suitability as a sensitive bioindicator for chronic soil ecotoxicity assessment.

Keywords Cadmium nitrate, *Eisenia fetida*, Heavy metals, LC_{50}, Soil ecotoxicology.

INTRODUCTION

Due to the non-biodegradable nature, persistence and adverse effects on soil ecosystems, heavy metal contamination has become a major environmental concern. Heavy metals such as cadmium (Cd), lead (Pb), mercury (Hg), chromium (Cr), and arsenic (As) enter the soil through both natural processes and hu-

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man activities, including mining, industrial discharge, sewage sludge application, wastewater irrigation, and intensive use of agrochemicals (Alloway 2012). Once these heavy metals accumulate in the soil, their relatively low concentrations can also cause toxic effects on soil organisms.

In soil ecosystems, microorganisms and earthworms constitute the most sensitive components. Heavy metal contamination decreases soil fertility and affects ecosystem functioning by disrupting microbial metabolism, inhibiting key enzymes involved in nutrient cycling, and reducing microbial biomass and diversity (Giller *et al*. 1998; Khan *et al*. 2010). Important processes such as organic matter decomposition, nitrogen fixation, and phosphorus solubilization are negatively affected by the changes in microbial communities.

Cadmium is one of the most toxic heavy metals due to its high mobility, bioavailability, and persistence in the environment (Ali *et al*. 2019). Mining activities, industrial emissions, sewage sludge application, wastewater irrigation, and long-term use of phosphate fertilizers containing cadmium impurities have resulted in substantial cadmium contamination of agricultural soils (Alloway 2012; Shahid *et al*. 2017). Cadmium can also enter terrestrial food webs, posing a serious threat to organisms occupying higher trophic levels (Nahmani *et al*. 2007). Unlike many organic pollutants, it is non-biodegradable and can remain in soils for several years. It gradually accumulates and enters the food chains through crop uptake (WHO 2011; Haider *et al.* 2021). Cadmium nitrate [$Cd(NO_3)_2.xH2O$], is a highly soluble form of cadmium used in the manufacturing of pigments, electronics, specialty glass, porcelain, photography, and electroplating. Due to its high solubility, it has high mobility and bioavailability, consequently more chances of uptake by soil organisms (Alloway 2012). Exposure to cadmium nitrate has been shown to decrease microbial biomass and diversity, leading to the dominance of cadmium-tolerant microbial populations, suppressing beneficial microbes involved in nitrogen fixation and organic matter decomposition (Khan *et al.* 2010).

Earthworms are considered critically important in terrestrial ecosystems due to their role in organic matter decomposition, nutrient cycling, soil aeration, and aggregate formation. They are susceptible to heavy metal contamination due to their close association with soil and continuous soil ingestion. Therefore, earthworms are widely used as bioindicators of soil quality and environmental pollution.

Heavy metal exposure can result in several physiological and biochemical changes in earthworms, including reduced growth, impaired reproduction, increased mortality (Spurgeon and Hopkin 1999a), reduced abundance, and species diversity (Spurgeon and Hopkin 1999b).

Heavy metals-induced toxicity in earthworms results from multiple physiological, biochemical, and molecular mechanisms. The exposure to heavy metals can induce lysosomal membrane destabilization (Hønsi *et al.* 2003), alter enzyme activity and gene expression (Li *et al.* 2009; Spurgeon *et al.* 2006), and generate oxidative stress through the production of reactive oxygen species that damage lipids, proteins, and DNA (Fourie *et al.* 2007; Wu *et al.* 2012). Cadmium is severely toxic because it accumulates in chloragogenous tissues, gut epithelium, and body wall tissues involved in metabolism and detoxification (Liu *et al*. 2018). The toxic effects of cadmium are reflected as reduced growth, impaired reproduction, decreased cocoon production and hatchability, and increased mortality (Neuhauser *et al.* 1985; Spurgeon *et al*. 1994; Reinecke *et al.* 2001; Davies *et al.* 2003).

Eisenia fetida is one of the most widely used model organisms in ecotoxicological studies of soil due to its ecological relevance, ease of culture, and sensitivity to environmental contaminants. Earlier studies have demonstrated that cadmium exposure adversely affects growth, survival, feeding activity, reproductive ability, and physiological fitness in *E. fetida*, with toxicity increasing with concentration and duration of exposure (Davies *et al.* 2003; Neuhauser *et al.* 1985; Zhou *et al.* 2014; Žaltauskaitė and Sodienė 2010). However, information on the chronic effects of cadmium nitrate on biomass dynamics and survival under prolonged exposure remains limited.

Therefore, the present study was undertaken

to evaluate the toxic effects of cadmium nitrate [$Cd(NO_3)_2$] on *Eisenia fetida* under laboratory conditions. The specific objectives were to assess changes in biomass and survival following exposure to different concentrations of cadmium nitrate and to generate insights into the ecological implications of heavy metal contamination for sustainable soil management.

MATERIALS AND METHODS

Collection and acclimatization of earthworms

Healthy adult *Eisenia fetida* possessing a well-developed clitellum were collected from a vermiculture unit located at Washere, District Ahilyanagar, Maharashtra, India. The species was identified using the OPAL Earthworm Identification Key (Earthworm Identification Guide, n.d.) based on morphological characteristics including body length (>2 cm), stripy body pattern, and the presence of a well-developed clitellum similar in color to the rest of the body.

Earthworms were acclimatized for seven days in a vermifeed substrate prepared from partially decomposed organic matter supplemented with well-decomposed cow dung (10:1, w/w). During acclimatization, earthworms were maintained at 25 ± 2°C under natural photoperiod with substrate moisture maintained at approximately 60–70% by periodic addition of distilled water.

Preparation of experimental substrate

The experimental substrate consisted of 1 kg of partially decomposed organic matter thoroughly mixed with 100 g of well-decomposed cowdung. Prior to use, the substrate was pre-composted for approximately 15–20 days to stabilize microbial activity. Plastic troughs were prepared by placing a 1-cm layer of sieved soil at the base, followed by the prepared vermifeed substrate.

Experimental design

Cadmium nitrate tetrahydrate [$Cd(NO_3)_2 \cdot 4H_2O$] was used as the test chemical. Nine experimental treatments consisting of a control and eight cadmium concentrations (30, 150, 300, 450, 600, 750, 900 and 1200 $\mu g\ g^{-1}$ vermifeed) were established.

The required quantity of cadmium nitrate for each treatment was dissolved in distilled water and thoroughly mixed into the substrate to ensure uniform distribution. Twenty-five healthy adult earthworms were introduced into each experimental trough. Each treatment was maintained in triplicate, resulting in an initial population of 75 earthworms per treatment. Experimental units were maintained under identical environmental conditions throughout the seven-week exposure period.

Assessment of survival, body weight and morphological changes

Earthworm survival was recorded weekly throughout the experiment. Dead individuals were removed immediately after observation, and cumulative mortality was calculated relative to the initial population.

The fresh body weight of all surviving earthworms was determined weekly using a digital analytical balance. To eliminate the influence of mortality on biomass estimates, growth was expressed as the mean body weight per surviving earthworm for each treatment.

Representative external morphological abnormalities observed during chronic cadmium exposure were photographed and documented. Morphological observations included alterations in body shape, tapering of the anterior and posterior ends, swelling, constrictions, body curvature, fragmentation and rupture of the body wall.

Statistical analysis

Survival and LC_{50} estimation

Weekly mortality data were analyzed using Generalized Linear Models (GENLIN) implemented in IBM SPSS Statistics Version 32. Mortality was modelled assuming a binomial error distribution with a probit link function. Separate models were fitted for each exposure week to estimate time-dependent LC_{50} values together with their 95% confidence intervals. Model

adequacy was evaluated using Pearson χ^2/degrees of freedom and Deviance/degrees of freedom statistics.

Analysis of body weight

Changes in the mean body weight of surviving earthworms were analyzed using two-way factorial analysis of variance (ANOVA) with cadmium concentration and exposure duration as fixed factors. Type III sums of squares were used to evaluate the main effects and their interaction. Effect sizes were expressed as partial eta squared (partial η^2). Multiple comparisons among treatment means were performed using the Bonferroni adjustment, and statistical significance was accepted at $P < 0.05$.

Growth trajectory analysis

Temporal changes in the mean body weight of surviving earthworms were further evaluated by fitting quadratic regression models separately for each cadmium concentration. The fitted equations and coefficients of determination (R^2) were used to describe changes in growth trajectories under increasing cadmium stress.

RESULTS

Survival and Mortality Pattern of *Eisenia fetida* During Chronic Cadmium Nitrate Exposure

No mortality was observed during the first week (Week 0 to week 1) of cadmium nitrate exposure for all the concentrations, including at the highest concentration (1200 µg g^{-1} vermifeed). After one week of exposure, first mortality was recorded. The mortality increased with the increase in cadmium nitrate concentration and duration of exposure (Table 1).

The mortality was negligible at lower concentrations (30 and 150 µg g^{-1}). The cumulative mortality did not exceed 9.3% by the end of the experiment. Mortality increased gradually for the Intermediate concentrations (300–750 µg g^{-1}). Rapid mortality was observed at higher concentrations (900 and 1200 µg g^{-1}). Complete mortality occurred at 1200 µg g^{-1} after three weeks of exposure and at 900 µg g^{-1} after seven weeks of exposure. A clear concentration- and time-dependent toxic response was demonstrated as seen through the progressive decline in survival with increasing concentration, (Table 1, Fig. 1).

Concentration–Response Relationship and Time-Dependent LC_{50}

To evaluate the effect of cadmium concentration on mortality, week-wise mortality data were modelled using generalized linear models (GENLIN) with a binomial error distribution and a probit link function. A highly significant increase in mortality with increase in the concentration was observed from the first week. The cadmium concentration significantly affected the mortality of earthworms throughout the experimental period, as evident by the likelihood ratio chi-square values ranging from 243.337 to 492.331 (P

Table 1. Weekly survival and cumulative mortality (%) of *Eisenia fetida* exposed to cadmium nitrate.

Cd (µg g^{-1} vermifeed)	Week 0	1	2	3	4	5	6	7
Control	75 (0.0)	75 (0.0)	75 (0.0)	75 (0.0)	75 (0.0)	75 (0.0)	75 (0.0)	75 (0.0)
30	75 (0.0)	75 (0.0)	75 (0.0)	75 (0.0)	75 (0.0)	75 (0.0)	75 (0.0)	75 (0.0)
150	75 (0.0)	75 (0.0)	75 (0.0)	75 (0.0)	75 (0.0)	75 (0.0)	71 (5.3)	68 (9.3)
300	75 (0.0)	75 (0.0)	75 (0.0)	75 (0.0)	72 (4.0)	69 (8.0)	61 (18.7)	59 (21.3)
450	75 (0.0)	70 (6.7)	64 (14.7)	51 (32.0)	45 (40.0)	39 (48.0)	28 (62.7)	24 (68.0)
600	75 (0.0)	69 (8.0)	58 (22.7)	39 (48.0)	27 (64.0)	18 (76.0)	11 (85.3)	8 (89.3)
750	75 (0.0)	54 (28.0)	42 (44.0)	32 (57.3)	21 (72.0)	12 (84.0)	9 (88.0)	3 (96.0)
900	75 (0.0)	42 (44.0)	36 (52.0)	27 (64.0)	13 (82.7)	8 (89.3)	4 (94.7)	0 (100)
1200	75 (0.0)	13 (82.7)	6 (92.0)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)

Values represent the number of surviving earthworms. Values in parentheses indicate cumulative mortality (%) relative to the initial population of 75 earthworms per treatment. Week 0 represents the pre-exposure (baseline) observations, while Weeks 1–7 correspond to one to seven weeks of cadmium nitrate exposure.

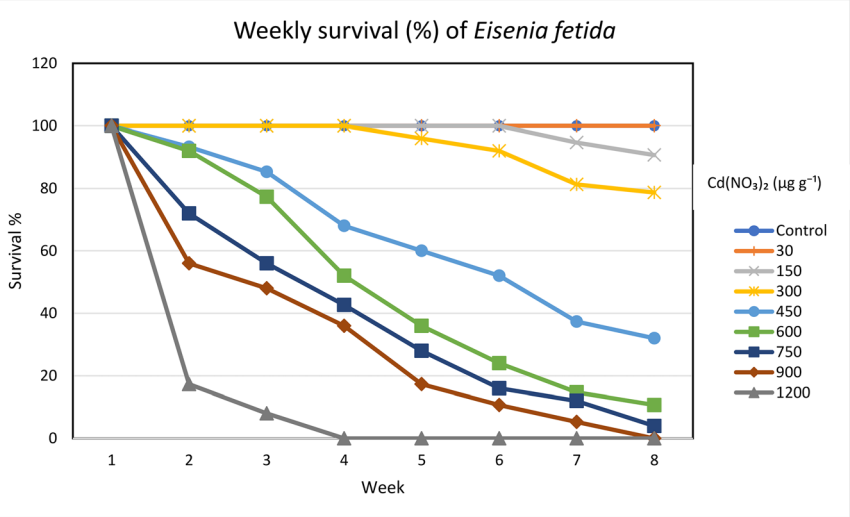


Fig. 1. Weekly survival (%) of *Eisenia fetida* exposed to different concentrations of cadmium nitrate in vermifeed over a seven-week exposure period. Survival remained at 100% in the control and 30 µg g^{-1} treatments throughout the experiment. Progressive reductions in survival were observed with increasing cadmium concentration and exposure duration, with complete mortality occurring at 1200 µg g^{-1} after three weeks of exposure and at 900 µg g^{-1} after seven weeks of exposure.

< 0.001). The Wald chi-square statistics ranged from 104.622 to 181.004 ($P < 0.001$), further confirming the lethal effect of different cadmium concentrations.

The LC_{50} values progressively decreased with increasing exposure duration. The estimated LC_{50} declined from 906 µg $Cd(NO_3)_2$ g^{-1} vermifeed after one week of exposure to 789 µg g^{-1} after two weeks, 645 µg g^{-1} after three weeks, 547 µg g^{-1} after four weeks, 485 µg g^{-1} after five weeks, 396 µg g^{-1} after six weeks and 354 µg g^{-1} after seven weeks of exposure (Table 2). Corresponding 95% confidence intervals demonstrated progressively improved precision of LC_{50} estimation as mortality increased.

Goodness-of-fit statistics indicated satisfactory agreement between the fitted probit models and the observed mortality data. Pearson χ^2/df values ranged

Table 2. Time-dependent LC_{50} estimates of cadmium nitrate for *Eisenia fetida* following chronic exposure.

Exposure duration (weeks)	Likelihood Ratio χ^2	Wald χ^2	LC_{50} (µg g^{-1} vermifeed)	95% Confidence Interval	Pearson χ^2/df	Deviance/df
1	243.337	104.622	906	843–973	4.53	4.08
2	310.210	123.454	789	743–837	1.89	1.97
3	363.813	137.689	645	606–684	4.24	5.47
4	431.675	153.476	547	514–581	1.74	2.10
5	478.779	158.668	485	455–516	1.18	1.28
6	459.369	180.209	396	367–427	1.58	1.64
7	492.331	181.004	354	329–382	3.26	3.06

1. LC_{50} values were estimated using generalized linear models (GENLIN) with a binomial error distribution and a probit link function.
2. Likelihood Ratio χ^2 and Wald χ^2 tests were significant for all exposure periods ($P < 0.001$), indicating a significant concentration-dependent mortality response.
3. Pearson χ^2/df and Deviance/df values close to 1 indicate good agreement between observed and predicted mortality.

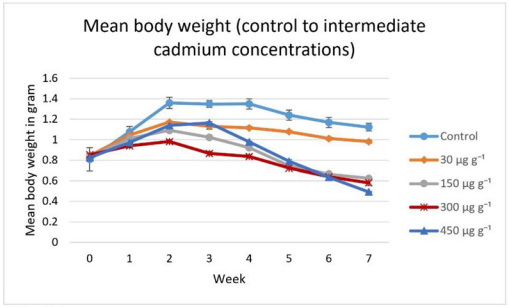


Fig.2A

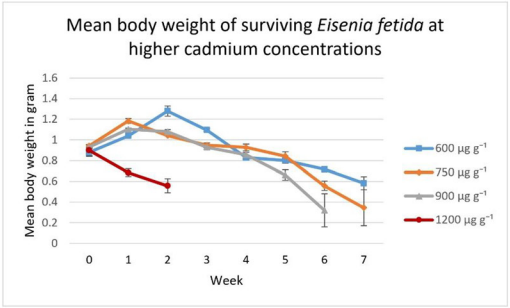


Fig.2B

Fig. 2A. Mean body weight of surviving *Eisenia fetida* exposed to control, 30, 150, 300 and 450 µg g^{-1} vermifeed during seven weeks of chronic exposure. Values represent treatment means.

Fig. 2B. Mean body weight of surviving *Eisenia fetida* exposed to 600, 750, 900 and 1200 µg g^{-1} vermifeed during seven weeks of chronic exposure. Missing values indicate that no surviving earthworms remained because of complete mortality.

from 1.18 to 4.53, while Deviance/df values ranged from 1.28 to 5.47. The best model fit was obtained after five weeks of exposure (Pearson χ^2/df = 1.18), whereas the first and third weeks exhibited moderate overdispersion but remained acceptable for LC_{50} estimation.

Overall, the LC_{50} declined by approximately 61% between one and seven weeks of exposure, indicating a progressive increase in cadmium toxicity with prolonged exposure.

Changes in Mean Body Weight of Surviving Earthworms

The mean body weight of surviving *Eisenia fetida* was found to vary significantly with both cadmium concentration and exposure duration (Table 3). During the first two weeks, the earthworms maintained under control conditions, and those treated to lower cadmium concentrations (30 and 150 µg g^{-1}) exhibited growth, attaining the maximum body weights after approximately two weeks of exposure. The mean body weight gradually declined after this initial period.

Intermediate concentrations (300–600 µg g^{-1}) also caused an initial increase in body weight; however, the duration and magnitude of growth were progressively reduced with increasing cadmium concentration. The growth phase was notably shortened at higher concentrations (750–1200 µg g^{-1}), followed by rapid reductions in body weight. At 1200 µg g^{-1}, body weight declined sharply before complete mortality occurred. (Fig. 2A and 2B).

The reduction in mean body weight became increasingly pronounced with increasing cadmium

Table 3. Change in mean body weight with Cd concentration and exposure.

Cd (µg g^{-1} vermifeed)	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
Control	0.81 ± 0.11	1.08 ± 0.05	1.36 ± 0.06	1.35 ± 0.04	1.35 ± 0.05	1.24 ± 0.05	1.17 ± 0.05	1.12 ± 0.04
30	0.84 ± 0.01	1.04 ± 0.02	1.17 ± 0.01	1.13 ± 0.03	1.11 ± 0.01	1.08 ± 0.01	1.01 ± 0.01	0.98 ± 0.02
150	0.83 ± 0.01	1.01 ± 0.01	1.09 ± 0.02	1.02 ± 0.00	0.92 ± 0.00	0.72 ± 0.02	0.66 ± 0.00	0.62 ± 0.00
300	0.85 ± 0.01	0.94 ± 0.02	0.98 ± 0.02	0.87 ± 0.02	0.84 ± 0.02	0.72 ± 0.00	0.64 ± 0.01	0.58 ± 0.03
450	0.82 ± 0.02	0.97 ± 0.01	1.14 ± 0.00	1.17 ± 0.01	0.98 ± 0.01	0.79 ± 0.02	0.63 ± 0.02	0.49 ± 0.01
600	0.88 ± 0.04	1.04 ± 0.01	1.28 ± 0.05	1.10 ± 0.02	0.83 ± 0.02	0.80 ± 0.02	0.72 ± 0.02	0.58 ± 0.06
750	0.94 ± 0.01	1.18 ± 0.02	1.04 ± 0.00	0.95 ± 0.02	0.93 ± 0.03	0.84 ± 0.04	0.56 ± 0.04	0.35 ± 0.17
900	0.93 ± 0.01	1.10 ± 0.01	1.08 ± 0.02	0.93 ± 0.02	0.86 ± 0.02	0.66 ± 0.05	0.32 ± 0.16	NE*
1200	0.90 ± 0.05	0.68 ± 0.04	0.56 ± 0.07	NE*	NE*	NE*	NE*	NE*

*NE- Mean weight could not be estimated due to complete mortality.

Table 4. Two-way factorial ANOVA showing the effects of cadmium concentration and exposure duration on the mean body weight of surviving *Eisenia fetida*.

Source	df	SS	MS	F	P	Partial η^2
Cadmium concentration	8	12.158	1.520	304.145	<0.001	0.944
Exposure duration	7	6.574	0.939	187.936	<0.001	0.901
Concentration × Exposure duration	56	5.448	0.097	19.467	<0.001	0.883
Error	144	0.720	0.005	–	–	–

1. ANOVA was performed using Type III sums of squares.
2. Dependent variable: Mean body weight per surviving earthworm (g).
3. The fitted model explained 97.1% of the total variation in mean body weight ($R^2 = 0.971$; Adjusted $R^2 = 0.957$).

concentration and exposure duration, indicating progressive growth impairment in surviving earthworms.

Effects of Cadmium Concentration and Exposure Duration on Mean Body Weight

Two-way factorial analysis of variance (ANOVA) revealed that the mean body weight of surviving *Eisenia fetida* was affected significantly by cadmium concentration, exposure duration, and their interaction (Table 4).

The effect of cadmium concentration on mean body weight was found to be highly significant ($F_{8,144} = 304.145$, $P < 0.001$, partial $\eta^2 = 0.944$). This indicates that the increase in cadmium concentration caused progressively greater reductions in the body weight. Mean body weight was also affected significantly by the duration of exposure ($F_{7,144} = 187.936$, $P < 0.001$, partial $\eta^2 = 0.901$). Moreover, the interaction between cadmium concentration and exposure duration was also highly significant ($F_{56,144} = 19.467$, $P < 0.001$, partial $\eta^2 = 0.883$), indicating

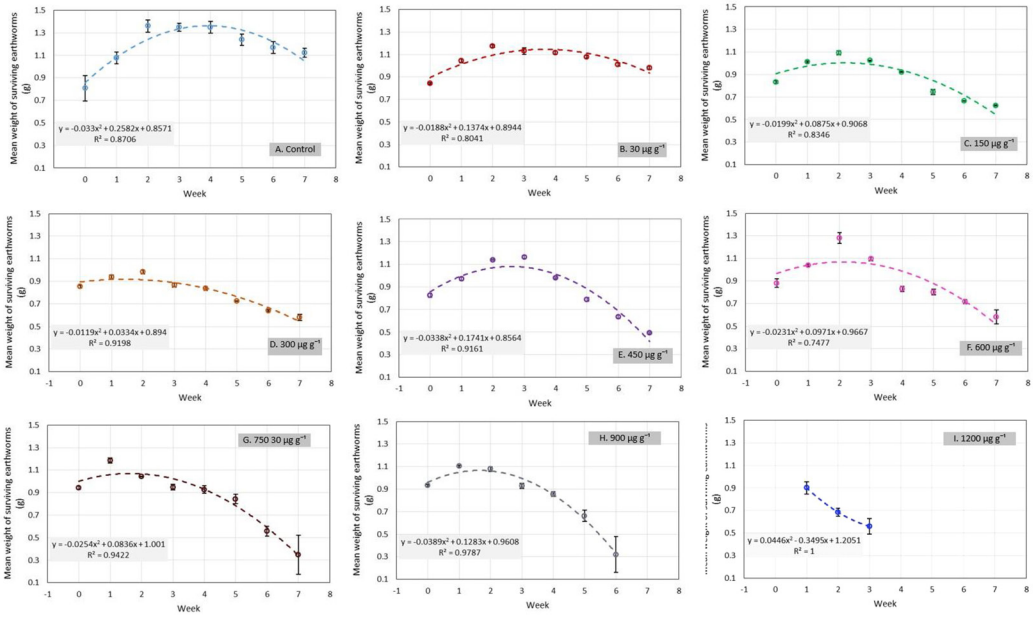


Fig. 3. Quadratic regression analysis of temporal changes in the mean body weight of surviving Eisenia fetida under different cadmium nitrate concentrations (**A.** Control, **B.** 30 µg g^{-1}, **C.** 150 µg g^{-1}, **D.** 300 µg g^{-1}, **E.** 450 µg g^{-1}, **F.** 600 µg g^{-1}, **G.** 750 µg g^{-1}, **H.** 900 µg g^{-1}, **I.** 1200 µg g^{-1}).

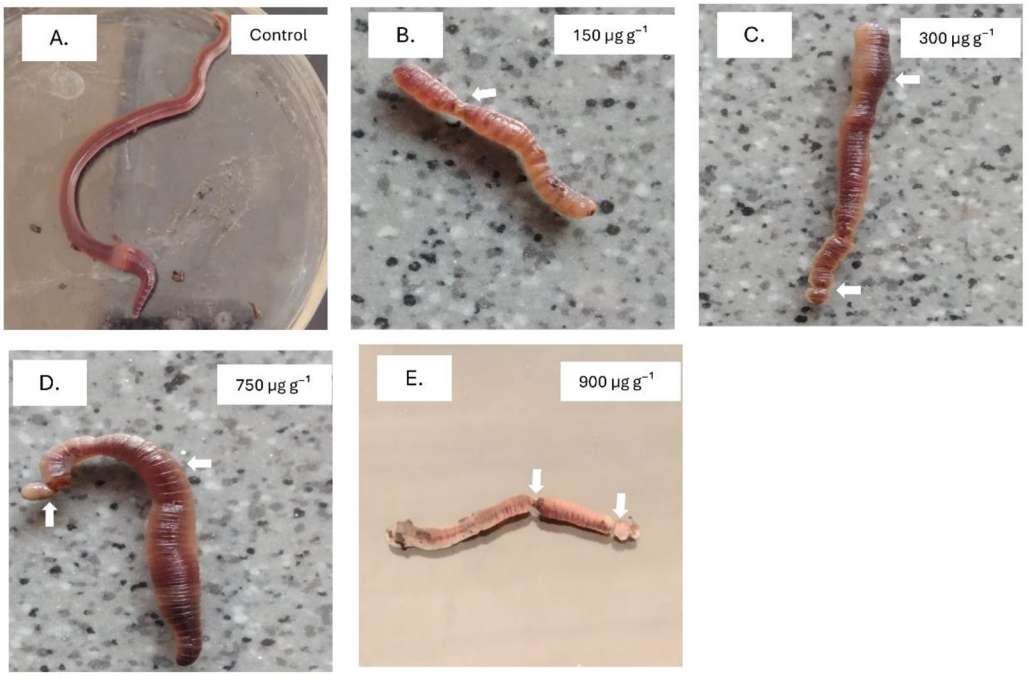


Fig. 4. External morphological abnormalities observed in *Eisenia fetida* following chronic exposure to cadmium nitrate. **(A)** Healthy control with normal cylindrical body form, well-developed clitellum, characteristic tapering of the anterior and posterior ends, uniform pigmentation and regular segmentation. **(B)** 150 µg g^{-1} vermifeed exposure showing loss of normal tapering at the anterior and posterior ends, localized swelling and the onset of body constriction. **(C)** 300 µg g^{-1} vermifeed exposure exhibiting pronounced anterior swelling together with multiple constricted regions, resulting in an irregular body contour. **(D)** 750 µg g^{-1} vermifeed exposure showing severe body curvature, localized swelling, loss of normal tapering at the anterior end and marked body constriction. **(E)** 900 µg g^{-1} vermifeed exposure exhibiting fragmentation and rupture of the body wall with severe external deformation.

that the effect of cadmium on body weight depended on the duration of exposure.

The ANOVA model explained 97.1% of the total variation in mean body weight ($R^2 = 0.971$; adjusted $R^2 = 0.957$), indicating an excellent fit to the observed data. Bonferroni-adjusted pairwise comparisons indicated that the control group differed significantly from all cadmium-treated groups ($P < 0.001$). No significant differences were observed among the intermediate concentrations of 150, 300, 450, 600 and 750 µg g^{-1} for several pairwise comparisons ($P > 0.05$), whereas comparisons involving 900 and 1200 µg g^{-1} were generally significant ($P < 0.001$), indicating progressively greater growth inhibition at higher cadmium concentrations.

Growth Trajectories of Surviving Earthworms

The changes in the mean body weight of surviving *Eisenia fetida* with respect to time across all treatments are described by Quadratic regression analysis effectively (Fig. 3). An initial increase in body weight during the early weeks of exposure was observed in the control and lower cadmium treatments. Maximum growth was seen around the second week, after which body weight declined gradually. A rapid decline in the body weight was observed in the earthworms exposed to 750 µg g^{-1} and higher concentrations, indicating notable growth suppression and progressive physiological impairment. The fitted quadratic models closely followed the observed growth patterns, as reflected by the consistently high coefficients of determination (R^2).

Morphological alterations following cadmium exposure

Several external morphological abnormalities result-

ing due to chronic exposure to cadmium nitrate were noticed. The severity of the abnormalities appeared to increase with increasing concentration of cadmium nitrate (Fig. 4). The characteristic features of *Eisenia fetida* such as cylindrical body shape, distinct clitellum, uniform pigmentation and gradually tapering anterior and posterior ends were retained in control group (Fig. 4A). However, individuals exposed to cadmium nitrate developed several external deformities affecting body shape and structural integrity.

At 150 µg g^{-1}, initial signs of toxicity were visible in the form of loss in the normal tapering of the anterior and posterior ends, localized swelling and the initial development of body constrictions (Fig. 4B). Earthworms exposed to 300 µg g^{-1} exhibited more pronounced anterior swelling together with multiple constricted regions, producing an irregular body contour (Fig. 4C). At 750 µg g^{-1}, pronounced body curvature was observed along with localized swelling, and increased constriction of the body wall (Fig. 4D). The most severe morphological damage occurred at 900 µg g^{-1}, where fragmentation and rupture of the body wall resulted in extensive external deformation (Fig. 4E).

DISCUSSION

Sinkakarimi *et al.* (2020), who also studies toxic effects of cadmium through cadmium nitrate, demonstrated that *E. fetida* is comparatively less susceptible to cadmium toxicity than *Aporrectodea trapezoides* and *A. rosea* under similar exposure conditions, suggesting that higher cadmium concentrations may be required to produce measurable toxic responses in *E. fetida*. This characteristic explains why relatively high concentrations were included in the present study. The selected concentration range successfully captured the complete progression from negligible mortality to complete lethality, allowing both sublethal and lethal responses to be evaluated within the same experimental design.

Chronic cadmium exposure caused progressive mortality

The present study clearly shows the toxic effect of cadmium nitrate on *Eisenia fetida* with respect to the concentration and duration of exposure. The earthworms survived the lower concentrations and initial exposure period for moderately high concentrations. However, the mortality increased after concentrations of 450 µg g^{-1}. The rapid loss of individuals at 900 and 1200 µg g^{-1} indicates that the earthworms could not tolerate the prolonged exposure after tolerating initially tolerable stress ultimately leading to mortality. This pattern is consistent with earlier studies showing that cadmium and other metals adversely affect survival, growth and reproduction in *Eisenia fetida* under laboratory and contaminated-soil conditions (Spurgeon *et al.* 1994; Nahmani *et al.* 2007; Žaltauskaitė and Sodienė 2014). At the same time, the findings also support the observation by Wu *et al.* (2020) that the earthworms show high tolerance to Cd owing to the its gut microbiota and physiological adjustment. The recent study by Tariq *et al.* (2024) also that cadmium exposure under laboratory conditions causes significant reductions in survival of *E. fetida.* This further supports the concentration-dependent mortality pattern observed in the present study.

The current study differs from the majority of past findings in two significant ways, despite the fact that the overall mortality pattern is in good agreement with earlier studies. First, instead of using the OECD artificial soil that is frequently used in laboratory toxicity experiments, cadmium was given as cadmium nitrate in an exposure medium based on vermifeed. Second, survival was tracked every week for eight weeks, making it possible to track the mortality development. Earthworms kept in OECD artificial soil, including the control group, lost body weight during the experiment, according to Spurgeon *et al.* (1994). They concluded that the standard test substrate lacked a sufficient nutritional component and recommended that animal manure be used in the test medium in future toxicity studies. In contrast, the vermifeed used in the present study contained decomposed organic matter supplemented with cow dung, providing a nutritionally favorable substrate in which control earthworms exhibited continuous growth during the initial weeks of the experiment. Consequently, the mortality observed in cadmium-treated groups is less likely to have been influenced by nutritional stress and more likely reflects the direct toxic effects of cadmium nitrate.

One of the key findings of the present study was the continuous decrease in LC_{50} as the duration of exposure increased. The LC_{50} decreased from 906 µg g^{-1} vermifeed after one week to 354 µg g^{-1} after seven weeks. This represents a reduction of approximately 61% demonstrating progressive and cumulative cadmium toxicity. Previous investigations have similarly emphasized that chronic exposure produces considerably greater toxic effects than short-term exposure because cadmium accumulates within tissues and progressively disrupts physiological processes, including energy metabolism, growth and reproduction (Spurgeon *et al.* 1994; Spurgeon *et al.* 2004). Consequently, chronic LC_{50} values provide a more ecologically relevant estimate of cadmium toxicity than acute toxicity tests. The current investigation shows that *Eisenia fetida* displays morphological changes, growth inhibition, and progressive, concentration-dependent mortality throughout an exceptionally broad range of cadmium doses up to 1200 mg/kg. These results expand the proven usefulness of *E. fetida* as a sentinel organism for assessing chronic cadmium toxicity in highly contaminated soils by showing that it is responsive even in highly contaminated environments.

One significant finding of the current study is that, even at the highest cadmium concentrations, there was no death during the first phase of exposure. This result suggests that the ecological risks related to cadmium exposure may be significantly underestimated based solely on short-term survival. The mortality rate increased over the next few weeks as earthworms that survived the initial phases of exposure were unable to sustain long-term vitality. Žaltauskaitė and Sodienė (2014) arrived at similar conclusions, showing that long-term exposure to cadmium had significantly more negative effects than short-term exposure and emphasizing that long-term exposure should be taken into account when evaluating ecological risks related to heavy metal contamination.

Although the present study did not quantify biochemical or molecular responses, this interpretation is supported by recent mechanistic studies demonstrating that cadmium exposure induces oxidative stress, disturbs cytoskeletal organisation, interferes with DNA replication and alters the expression of genes associated with cellular defence and energy metabolism in *Eisenia foetida* (You *et al.* 2024). Similarly, Li *et al.* (2009) reported significant biochemical alterations and DNA damage following cadmium exposure, indicating that mortality represents the final outcome of progressive physiological impairment rather than an isolated toxic event.

Progressive decline in LC_{50} demonstrates cumulative cadmium toxicity

One of the most significant findings of the present investigation was the progressive decline in LC_{50} with increasing exposure duration. The estimated LC_{50} decreased continuously from Week 1 to Week 7, indicating that earthworms became increasingly susceptible to cadmium nitrate as exposure progressed. This trend clearly demonstrates that toxicity cannot be adequately described by a single LC_{50} value because the lethal concentration required to kill 50% of the population changes substantially with exposure duration. The results therefore emphasise that cadmium toxicity is cumulative and that prolonged exposure greatly enhances its biological effects.

The decline in LC_{50} observed in the present study closely agrees with the findings of Žaltauskaitė and Sodienė (2014), who also reported a significant decrease in LC_{50} between Weeks 3 and 7 of cadmium exposure, concluding that chronic exposure to the same contaminant concentration produces considerably greater adverse effects than short-term exposure. The close agreement between the two studies is particularly noteworthy because they differed in several experimental aspects, including developmental stage and exposure substrate. While Žaltauskaitė and Sodienė (2014) investigated juvenile earthworms maintained in artificial soil, the present investigation employed mature *E. fetida* maintained in vermifeed. Despite these methodological differences, both studies independently demonstrate that exposure duration is a major determinant of cadmium toxicity.

Earlier investigations by Spurgeon *et al.* (1994) determined LC_{50} values following 14- and 56-day exposures but did not estimate LC_{50} at successive intervals throughout the experiment. Consequently, although those authors clearly demonstrated increased toxicity with prolonged exposure, the temporal

progression of toxicity could not be quantified. In contrast, the weekly LC_{50} estimates generated in the present study provide a continuous description of changing toxicity throughout the exposure period, revealing how susceptibility increased progressively with time rather than abruptly at the end of the experiment. This approach offers a more detailed understanding of chronic toxicity and may therefore provide a more informative framework for ecological risk assessment than reliance on a single endpoint LC_{50} value. Acute toxicity tests conducted over relatively short durations may underestimate the hazards posed by persistent contaminants such as cadmium because organisms that survive the initial exposure may subsequently succumb to cumulative physiological damage. Weekly LC_{50} estimation therefore provides valuable information on the temporal dynamics of toxicity and may improve predictions of long-term ecological risk. Given that cadmium is highly persistent and remains bioavailable in contaminated soils for prolonged periods, incorporating time-dependent toxicity assessments into future ecotoxicological studies would provide a more realistic evaluation of its environmental impact than reliance on conventional endpoint measurements alone.

Cadmium inhibited growth and altered biomass dynamics of surviving earthworms

In addition to increasing mortality, chronic cadmium exposure markedly affected the growth of surviving *Eisenia fetida*. The mean body weight of surviving earthworms was significantly influenced by cadmium concentration, exposure duration and the interaction between these two factors, demonstrating that growth inhibition became progressively more severe as exposure continued. Unlike mortality, which represents the final outcome of toxicity, changes in body weight provide a sensitive sublethal endpoint that reflects the physiological condition of surviving individuals. The progressive decline in survivor biomass observed in the present study therefore indicates that cadmium impaired physiological performance well before death occurred.

An interesting pattern emerged during the early phase of exposure. Earthworms in the control and lower cadmium treatments exhibited an initial increase in body weight during the first two to three weeks before a gradual decline occurred. Similar transient increases in biomass have been reported previously in chronic toxicity studies involving *E. fetida*. Spurgeon *et al.* (1994) observed an initial increase in fresh weight during the first two weeks of exposure, which they attributed partly to continued feeding and ingestion of soil particles. However, they also reported that earthworms maintained in the OECD artificial soil progressively lost weight thereafter, including individuals in the control treatment. The authors suggested that the artificial substrate provided inadequate nutritional support for long-term maintenance and recommended supplementation with animal manure in future toxicity studies. This observation is particularly relevant to the present investigation because the experimental substrate consisted of vermifeed enriched with decomposed organic matter and cowdung, thereby providing a substantially richer nutritional environment. Consequently, the sustained increase in body weight observed in the control group during the early weeks is more likely to represent normal somatic growth rather than a transient increase caused by soil ingestion alone. This distinction strengthens the interpretation that the subsequent reduction in body weight in cadmium-treated earthworms primarily resulted from cadmium toxicity rather than nutritional limitation.

The inhibitory effect of cadmium on body weight observed in the present study agrees closely with previous investigations. Žaltauskaitė and Sodienė (2014) demonstrated that prolonged cadmium exposure significantly reduced fresh body weight of juvenile *E. fetida*, with weight loss becoming increasingly pronounced at higher concentrations. Similarly, Lapinski and Rosciszewska (2008) reported substantial reductions in body mass of *E. fetida* following chronic exposure to cadmium and mercury, indicating that biomass reduction is a common response to heavy metal stress. Nahmani *et al.* (2007) likewise observed significant decreases in growth of earthworms exposed to metal-contaminated field soils, although the magnitude of the response depended upon the bioavailability of individual metals and the physicochemical characteristics of the soil. Collectively, these studies support the conclusion that body weight is a sensitive indicator of chronic metal toxicity and often

responds before severe mortality becomes evident.

The present study differs from earlier investigations in an important methodological aspect. Rather than evaluating total biomass or final body weight, growth was assessed as the mean body weight of surviving earthworms throughout the exposure period. This approach eliminates the confounding influence of mortality on biomass estimates. A decline in total biomass may simply reflect the death of individuals, whereas a reduction in the mean weight of survivors indicates that the surviving earthworms themselves experienced impaired growth. Consequently, the observed decrease in survivor body weight provides direct evidence that cadmium adversely affected physiological performance even in individuals that remained alive throughout the experiment.

Several physiological mechanisms may account for the observed reduction in body weight. Cadmium exposure has been shown to suppress feeding activity, reduce nutrient assimilation efficiency and increase the metabolic costs associated with detoxification and cellular repair. Energy that would normally be allocated to growth is instead diverted towards maintenance of homeostasis, synthesis of stress proteins and repair of damaged cellular components. In addition, cadmium-induced oxidative stress can impair mitochondrial function and reduce ATP production, thereby limiting the energy available for tissue growth. Recent transcriptomic analyses by You *et al.* (2024) demonstrated that cadmium exposure altered genes associated with carbohydrate metabolism, amino acid metabolism, oxidative phosphorylation and cytoskeletal organization, providing molecular evidence that disturbances in energy metabolism contribute to growth inhibition. Similar conclusions have been reached by Li *et al.* (2009), who demonstrated significant biochemical alterations and DNA damage in cadmium-exposed *E. fetida*, suggesting that reduced growth represents a consequence of cumulative physiological impairment rather than a simple reduction in food consumption.

The highly significant interaction between cadmium concentration and exposure duration observed in the present study further indicates that growth inhibition was dynamic rather than static. Cadmium did not reduce body weight by a constant amount throughout the experiment; instead, its adverse effects intensified progressively with prolonged exposure. This finding agrees with the concept of cumulative toxicity proposed by Spurgeon *et al.* (1994) and reinforced by Žaltauskaitė and Sodienė (2014), who demonstrated that chronic exposure substantially increased the severity of cadmium-induced effects. Together with the progressive decline in LC_{50} and survival, the body-weight data clearly demonstrate that prolonged cadmium exposure results in a gradual deterioration of physiological condition, ultimately culminating in mortality.

The quadratic regression models generated in the present study provide an additional perspective on the growth response of *E. fetida* under cadmium stress. Under control conditions and lower cadmium concentrations, body weight followed the expected biological pattern of initial growth followed by a gradual decline as earthworms approached the later stages of the experiment. Increasing cadmium concentrations progressively shortened the growth phase, reduced the maximum body weight attained and accelerated the subsequent decline. Although few previous studies have modelled earthworm growth using regression analyses, the fitted curves effectively illustrate how cadmium altered the temporal growth trajectory rather than merely reducing final body weight. Such modelling provides a more comprehensive representation of chronic toxicity and may prove useful for predicting long-term biological responses under continuous exposure.

Progressive morphological abnormalities reflect increasing structural deterioration

In addition to the quantitative reductions in survival and body weight, chronic cadmium exposure induced distinct external morphological abnormalities in *Eisenia fetida*. The severity of these alterations increased progressively with cadmium concentration, indicating a gradual deterioration of body structure during prolonged exposure. Earthworms in the control treatment retained the normal cylindrical body shape, characteristic reddish pigmentation, distinct clitellum and gradually tapering anterior and posterior ends throughout the experiment. In contrast, cadmi-

um-exposed earthworms exhibited a characteristic sequence of external abnormalities beginning with loss of normal tapering, followed by localized swelling, constriction of the body wall, abnormal curvature and, at the highest concentrations, fragmentation and rupture of the body wall.

Loss of the normal tapering of the anterior and posterior ends represented one of the earliest visible changes observed in the present study. This abnormality was accompanied by localized swelling and constricted body regions, suggesting progressive disruption of normal tissue architecture. With increasing cadmium concentration, these alterations became increasingly severe, resulting in irregular body contours and pronounced body curvature. The highest concentration produced fragmentation and rupture of the body wall, indicating extensive structural deterioration associated with advanced toxicity.

Comparable external abnormalities have been reported previously in cadmium-exposed earthworms, although the sequence of changes has rarely been documented in detail. Rodriguez *et al.* (2013) observed body coiling, excessive mucus secretion, reduced locomotory activity and histopathological damage in *E. foetida* exposed to cadmium, concluding that these abnormalities reflected progressive tissue injury. Similarly, Houda and Asma (2025) described external deformities and behavioral abnormalities following cadmium exposure, including altered body shape and reduced mobility. The present observations extend these earlier findings by documenting a concentration-associated progression of external abnormalities from mild alterations in body form to severe structural damage.

Although only external morphology was examined in the present study, the observed deformities are consistent with the known cellular effects of cadmium toxicity. Cadmium has been shown to accumulate predominantly within chloragogenous tissues and intestinal epithelium, where it disrupts membrane integrity, induces oxidative stress and interferes with cytoskeletal organization. These cellular disturbances may weaken muscular coordination and connective tissue integrity, thereby contributing to swelling, constrictions and abnormal body curvature. Transcriptomic studies have further demonstrated that cadmium alters genes involved in cytoskeletal organization and extracellular matrix function, providing a mechanistic basis for the structural abnormalities observed externally (You *et al.* 2024). Nevertheless, the precise histopathological changes underlying the external deformities observed in the present investigation remain to be determined.

One noteworthy aspect of the present study is that the external abnormalities were documented together with survival, body weight and temporal growth analyses. Rather than representing isolated observations, the morphological changes closely paralleled the progressive decline in physiological performance recorded throughout the experiment. Mild deformities appeared at concentrations where body weight had already begun to decline but survival remained relatively high, whereas severe structural damage occurred only at concentrations associated with marked mortality and substantial reductions in survivor biomass. This temporal association suggests that external morphology may serve as a practical visual indicator of progressive cadmium toxicity during chronic exposure.

Although qualitative assessment of external morphology cannot replace histopathological examination, it provides valuable supplementary information regarding organismal health and may represent an inexpensive screening tool for identifying sublethal toxicity under laboratory and vermiculture conditions. Future investigations combining external morphology with histopathology, ultrastructural analysis, antioxidant enzyme activity, metallothionein expression and tissue cadmium accumulation would provide a more comprehensive understanding of the mechanisms responsible for cadmium-induced structural damage in earthworms.

CONCLUSION

The present study demonstrates that chronic exposure to cadmium nitrate exerts pronounced concentration- and time-dependent toxic effects on *Eisenia fetida*, affecting survival, growth, and external morphology. Progressive mortality, a continuous decline in LC_{50} values with increasing exposure duration, significant

reductions in the mean body weight of surviving earthworms, and the development of characteristic external deformities collectively indicate that cadmium toxicity intensifies with prolonged exposure. These findings emphasize that evaluating toxicity solely on the basis of short-term survival may substantially underestimate the ecological risks associated with persistent heavy metal contamination.

Unlike many previous studies that relied primarily on endpoint measurements, the present investigation monitored survival and biomass at weekly intervals over seven weeks, allowing the temporal progression of toxicity to be characterized in detail. The weekly estimation of LC_{50} demonstrated a marked increase in cadmium toxicity over time, while quadratic regression modelling effectively described changes in growth trajectories under different exposure concentrations. The documentation of concentration-dependent external morphological abnormalities further complemented the quantitative analyses by providing visible evidence of progressive physiological deterioration.

The use of a nutritionally enriched vermifeed substrate also enabled the effects of cadmium nitrate to be evaluated under conditions more representative of organic matter-rich soils and vermicomposting systems than conventional artificial soil tests. Consequently, the observed reductions in growth and survival can be attributed primarily to cadmium toxicity rather than nutritional limitations.

Overall, the findings reaffirm the suitability of *E. fetida* as a sensitive bioindicator for monitoring heavy metal contamination in terrestrial ecosystems and highlight the importance of incorporating long-term exposure assessments into ecological risk evaluation. The integrated approach adopted in this study, combining survival analysis, time-dependent LC_{50} estimation, survivor biomass, growth trajectory modelling and morphological assessment, provides a comprehensive framework for evaluating chronic metal toxicity in earthworms.

This study demonstrates that chronic cadmium toxicity cannot be adequately assessed using a single endpoint. Integrating time-dependent LC_{50} estimation, survivor biomass, growth trajectory modelling and morphological assessment provides a more comprehensive and ecologically relevant framework for evaluating heavy metal toxicity in earthworms.

Future studies should complement these observations with histopathological, biochemical, molecular and bioaccumulation analyses to elucidate the mechanisms underlying cadmium-induced toxicity. Such multidisciplinary investigations would improve our understanding of chronic heavy metal stress and contribute to the development of more ecologically relevant soil quality assessment and management strategies.

REFERENCES

Ali, H., Khan, E., & Ilahi, I. (2019). Environmental chemistry and ecotoxicology of hazardous heavy metals: Environmental persistence, toxicity and bioaccumulation. *Journal of Chemistry*, 2019 (1), 6730305.

Alloway, B. J. (2012). Bioavailability of elements in soil. In *Essentials of Medical Geology*: *Revised Edition* (pp, 351—373). Dordrecht: Springer Netherlands.

Davies, N. A., Hodson, M. E., & Black, S. (2003). Is the OECD acute worm toxicity test environmentally relevant ? The effect of mineral form on calculated lead toxicity. *Environmental Pollution*, 121 (1), 49—54.

Earthworm identification guide. (n.d.). https://www.imperial.ac.uk/media/imperial-college/research-centres-and-groups/opal/SOIL-4pp-chart.pdf .

Fourie, F., Reinecke, S. A., & Reinecke, A. J. (2007). The determination of earthworm species sensitivity differences to cadmium genotoxicity using the comet assay. *Ecotoxicology and Environmental Safety*, 67 (3), 361—368.

Giller, K. E., Witter, E., & Mcgrath, S. P. (1998). Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils: A review. *Soil Biology and Biochemistry*, 30 (10-11), 1389—1414.

Haider, F. U., Liqun, C., Coulter, J. A., Cheema, S. A., Wu, J., Zhang, R., ... & Farooq, M. (2021). Cadmium toxicity in plants: Impacts and remediation strategies. *Ecotoxicology and Environmental Safety*, 211, 111887.

Hønsi, T. G., Stubberud, H. E., Andersen, S., & Stenersen, J. (2003). Lysosomal fragility in earthworms (*Eisenia veneta*) exposed to heavy metal contaminated soils from two abandoned pyrite ore mines in Southern Norway. *Water, Air and Soil Pollution*, 142 (1), 27—37.

Houda, B., & Asma, N. (2025). Evaluation of the effects of heavy metal (cadmium nitrates and mercuric chloride) on growth, survival and morphology of the earthworm Aporrectodea Trapezoides (Dugés 1828). *Brazilian Journal of Animal and Environmental Research*, 8 (1), e76484.

Khan, S., El-Latif Hesham, A., Qiao, M., Rehman, S., & He, J. Z. (2010). Effects of Cd and Pb on soil microbial commu-

nity structure and activities. *Environmental Science and Pollution Research*, 17 (2), 288—296.

Lapinski, S., & Rosciszewska, M. (2008). The impact of cadmium and mercury contamination on reproduction and body mass of earthworms. *Plant Soil and Environment*, 54 (2), 61.

Li, M., Liu, Z., Xu, Y., Cui, Y., Li, D., & Kong, Z. (2009). Comparative effects of Cd and Pb on biochemical response and DNA damage in the earthworm *Eisenia fetida* (Annelida, Oligochaeta). Chemosphere, 74 (5), 621—625.

Liu, H., Li, M., Zhou, J., Zhou, D., & Wang, Y. (2018). Effects of soil properties and aging process on the acute toxicity of cadmium to earthworm *Eisenia fetida*. *Environmental Science and Pollution Research*, 25 (4), 3708—3717.

Nahmani, J., Hodson, M. E., & Black, S. (2007). A review of studies performed to assess metal uptake by earthworms. *Environmental Pollution*, 145 (2), 402—424.

Neuhauser, E. F., Loehr, R. C., Milligan, D. L., & Malecki, M. R. (1985). Toxicity of metals to the earthworm *Eisenia fetida*. *Biology and Fertility of Soils*, 1 (3), 149—152.

Reinecke, A. J., Reinecke, S. A., & Maboeta, M. S. (2001). Cocoon production and viability as endpoints in toxicity testing of heavy metals with three earthworm species. *Pedobiologia*, 45(1), 61—68.

Rodriguez, M. G., Rivera, B. H., Ventura-Juárez, J., & Muñoz-Ortega, M. H. (2013). Cadmium toxicity evaluation in the earthworm *Eisenia foetida*: Behavior and histopathological effects. *Trends in Comparative Biochemistry & Physiology*, 17, 81—92, S0304423810002578.

Shahid, M., Dumat, C., Khalid, S., Niazi, N. K., & Antunes, P. M. C. (2017). Cadmium bioavailability, uptake, toxicity and detoxification in soil–plant system. *Reviews of Environmental Contamination and Toxicology*, 241, 73—137.

Sinkakarimi, M. H., Solgi, E., & Colagar, A. H. (2020). Interspecific differences in toxicological response and subcellular partitioning of cadmium and lead in three earthworm species. Chemosphere, 238, 124595.

Spurgeon, D. J., & Hopkin, S. P. (1999a). Life-history patterns in reference and metal-exposed earthworm populations. *Ecotoxicology*, 8 (2), 133—141.

Spurgeon, D. J., & Hopkin, S. P. (1999b). Seasonal variation in the abundance, biomass and biodiversity of earthworms in soils contaminated with metal emissions from a primary smelting works. *Journal of Applied Ecology*, 36 (1), 173—183.

Spurgeon, D. J., Hopkin, S. P., & Jones, D. T. (1994). Effects of cadmium, copper, lead and zinc on growth, reproduction and survival of the earthworm *Eisenia fetida* (Savigny): Assessing the environmental impact of point-source metal contamination in terrestrial ecosystems. *Environmental Pollution*, 84 (2), 123—130.

Spurgeon, D. J., Lofts, S., Hankard, P. K., Toal, M., McLellan, D., Fishwick, S., & Svendsen, C. (2006). Effect of pH on metal speciation and resulting metal uptake and toxicity for earthworms. *Environmental Toxicology and Chemistry*, 25 (3), 788—796.

Spurgeon, D. J., Stürzenbaum, S. R., Svendsen, C., Hankard, P. K., Morgan, A. J., Weeks, J. M., & Kille, P. (2004). Toxicological, cellular and gene expression responses in earthworms exposed to copper and cadmium. *Comparative Biochemistry and Physiology* Part C: *Toxicology & Pharmacology*, 138 (1), 11—21.

Tariq, M., Zafar, A., & Akhtar, A. (2024). Effect of heavy metal toxicity (lead and cadmium) on the growth, survival and reproduction of earthworm (*Eisenia fetida*). Remittances Review, 9 (3), 1465—1477.

World Health Organization. (2011). Guidelines for drinking-water quality (4th edn.). World Health Organization.

Wu, B., Liu, Z., Xu, Y., Li, D., & Li, M. (2012). Combined toxicity of cadmium and lead on the earthworm *Eisenia fetida* (Annelida, Oligochaeta). *Ecotoxicology and Environmental Safety*, 81, 122—126.

Wu, Y., Chen, C., Wang, G., Xiong, B., Zhou, W., Xue, F., ... & Liu, Z. (2020). Mechanism underlying earthworm on the remediation of cadmium-contaminated soil. *Science of the Total Environment*, 728, 138904.

You, R., Li, H., Li, X., Luo, L., Wang, P., Xia, H., & Zhou, Y. (2024). Ecotoxicological impacts of cadmium on soil microorganisms and earthworms *Eisenia foetida*: From gene-regulation to physiological processes. *Frontiers in Environmental Science,* 12, 1479500.

Žaltauskaitė, J., & Sodienė, I. (2010). Effects of total cadmium and lead concentrations in soil on the growth, reproduction and survival of earthworm *Eisenia fetida*. *Ekologija*, 56 (1-2), 10—16.

Žaltauskaitė, J., & Sodienė, I. (2014). Effects of cadmium and lead on the life-cycle parameters of juvenile earthworm *Eisenia fetida*. *Ecotoxicology and Environmental Safety*, 103, 9—16.

Zhou, C. F., Wang, Y. J., Sun, R. J., Liu, C., Fan, G. P., Qin, W. X., ... & Zhou, D. M. (2014). Inhibition effect of glyphosate on the acute and subacute toxicity of cadmium to earthworm *Eisenia fetida*. *Environmental Toxicology and Chemistry*, 33 (10), 2351—2357.