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Impact of Heavy Metals (Zinc and Copper) on the Survival Rate and Reproductive Behaviour of Earthworm (*Eisenia Fetida*)

Amina Zafar[1], Amara Akhtar[1], Ayesha Noreen[1]

[1]Department of Zoology, Wildlife and Fisheries, University of Agriculture Faisalabad, Pakistan.

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Correspondence to: Amina Zafar, Department of Zoology, Wildlife and Fisheries, University of Agriculture Faisalabad, Pakistan.
Email: az02245768@gmail.com.

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ABSTRACT

Earthworms play a crucial role in maintaining soil health by enhancing fertility through their burrowing, nutrient recycling and organic matter decomposition. Their ecological functions disrupted by environmental stresses particularly heavy metal contamination from elements like Zinc (Zn) and Copper (Cu). This study aimed to assess the impact of heavy metals on earthworms and their ability to accumulate Zn and Cu. Earthworms were collected from different agricultural sites and maintained under laboratory conditions for a period of 28 days. Four experimental groups were established. The control group (T0) received no exposure to heavy metals, whereas the treatment groups (T1, T2, and T3) were subjected to increasing concentrations of Zinc (Zn) at 1.5 mg/kg, 2.0 mg/kg, and 2.5 mg/kg and Copper (Cu) at 0.1 mg/kg, 0.2 mg/kg, and 0.3 mg/kg, respectively. Prior to the experiment, all earthworms underwent a one-week acclimatization period in soil trays and were provided with a standard feed. Key parameters assessed included total body weight, length, biomass, cocoon production and population changes. A significant reduction in growth was observed in group T3 (1.098 ± 0.030) reflecting high variability when compared with the other treatments. Survival rates were lowest in T3, where only 5 earthworms survived, while the highest survival was recorded in the control group (T0), where all 15 earthworms remained alive. Cocoon production also declined in T3, with only 2 cocoons produced compared to 8 in the control group. Data were statistically analyzed using one-way ANOVA. Growth and survival rates showed significant declines ($P < 0.05$), indicating that exposure to higher metal concentrations adversely affected these parameters. However, reproductive output showed no statistically significant difference ($P > 0.05$).

INTRODUCTION

Earthworms often referred to as "ecosystem engineers" play a crucial role in maintaining soil health and promoting ecosystem processes. Their activities enhance soil structure, fertility and biological activity making them integral to nutrient cycling, organic matter decomposition and soil health bioindication (Tchounwou *et al.*, 2012). As exceptional bioturbators earthworms contribute significantly to soil ecosystems worldwide with the exception of Antarctica (Khayatzadeh and Abbasi, 2010). However the widespread introduction of non-native earthworm species particularly in glaciated regions of North America has raised concerns about their ecological impact leading to significant transformations in soil morphology, nutrient availability, carbon cycling and biodiversity (Wackett *et al.*, 2018).

Beyond their natural contributions earthworms play a pivotal role in soil engineering and sustainable agriculture. The development of techno-sols which integrate organic and mineral waste for agronomic purposes is significantly influenced by earthworm activity, improving soil aggregation, water retention and plant yield (Blouin *et al.*, 2013). Additionally, vermiculture the cultivation of earthworms for organic waste decomposition has emerged as an eco-friendly practice that enhances soil fertility reduces landfill waste and promotes environmental sustainability (Dominguez and Edwards, 2011).

Earthworms are highly sensitive to soil contaminants particularly heavy metals such as zinc (Zn) and copper (Cu) which accumulate in soils due to industrial and agricultural activities. These metals interfere with earthworm physiology reproduction and survival, disrupting their essential roles in soil (Ahmad *et al.*, 2021). Exposure to heavy metals results in bioaccumulation, oxidative stress, enzyme inhibition and physiological damage posing a threat to soil health and biodiversity (Latha and Basha, 2016). As bioindicators earthworms provide critical insights into soil contamination levels emphasizing the need for effective soil remediation strategies (Zheng *et al.*, 2013b).

Heavy metal contamination has profound effects on earthworm growth, survival and reproduction. Chronic exposure to toxic metals alters metabolic processes reduces cocoon production and impairs juvenile development leading to population declines in contaminated soils (Spurgeon *et al.*, 2003). Furthermore, disruptions in enzymatic activity and cellular damage hinder their ability to contribute to soil health reducing their ecological effectiveness. Understanding these impacts is crucial for assessing soil quality and developing strategies to mitigate contamination risks (Lock and Janssen, 2003).

Zinc and copper toxicity pose serious threats to earthworm populations by disrupting their physiological and reproductive functions. Exposure to elevated concentrations of these heavy metals leads to oxidative stress resulting in cellular damage and metabolic imbalances (Bolyen *et al.*, 2019). Earthworms subjected to high levels of Zn and Cu exhibit reduced survival rates due to impaired feeding, energy depletion and enzyme inhibition. The reproductive output declines significantly as cocoon production decreases and juvenile development is hindered (Nahmani *et al.*, 2007). These adverse effects highlight the pressing need for monitoring heavy metal contamination in soil ecosystems and implementing effective remediation techniques to mitigate toxicity risks.

MATERIALS AND METHODS

Earthworms play a crucial role in soil ecosystems contributing to organic matter decomposition and nutrient cycling. In this study *Eisenia Fetida*, commonly known as the red wiggler was selected due to its widespread use in ecotoxicological studies and its known sensitivity to heavy metal contamination.

Sampling of Earthworms and Soil

The earthworms were collected from agricultural fields in Faisalabad where the soil was free use another suitable word instead of free of heavy metal contamination. A total of 60 mature earthworms with a well-developed clitellum were collected from the top 10 cm of moist soil using a damp towel method and were immediately transferred to the laboratory in the same soil from which they were collected.

Test Animals and Acclimatization

The collected earthworms were acclimatized for one week in the laboratory under controlled conditions in plastic containers filled with soil and cow dung. The temperature was maintained at 25°C to create a suitable environment for acclimatization. The earthworms were weighed and their lengths were measured prior to the experiment. Moisture levels in the soil were maintained by adding an adequate amount of water. Before introducing them into the experimental setup, earthworms were washed thoroughly with deionized water to remove any adhering soil particles.

Soil Collection and Preparation

Soil samples were collected from different fields at a depth of 10 cm. The soil was sieved to remove plant material, litter and debris. A standard artificial soil composition was used consisting of 70% sand, 20% kaolin clay and 10% sphagnum peat. The soil pH was adjusted to 6.0 ± 0.5 using calcium carbonate and moisture content was maintained at 60% of the soil's water-holding capacity. A total of 1.5 kg of soil was placed into each of four plastic containers (4 L capacity). The containers were sterilized with distilled water and dried before use, following the method described by Rathi (2011). Each container was filled with 3 kg of prepared soil mixed thoroughly with 500 g of cow dung as an organic nutrient source. The soil was allowed to stabilize for 24 hours before the introduction of earthworms. pH and temperature were measured at the beginning and end of the experiment to evaluate potential variations influencing heavy metal accumulation.

Experimental Design and Treatments

Four treatment groups were established:

Table 1

Different Treatments Applied in Experimental Beds

Treatment groups	Zinc	Copper
T_0(Control group)	No heavy metal	No heavy metal
T_1	1.5mg/kg	0.1m/kg
T_2	2.0mg/kg	0.2mg/kg
T_3	2.5mg/kg	0.3mg/kg

The specified concentrations of zinc (Zn) and copper (Cu) were mixed thoroughly into the soil of each treatment group to ensure homogeneous distribution.

Earthworm Introduction and Maintenance

Fifteen earthworms were introduced into each experimental container. They were maintained under controlled conditions with temperature and moisture levels optimized for their survival and growth. The survival rate, growth and reproductive parameters were monitored at regular intervals.

Assessment of Survival

Earthworm survival was recorded daily. Mortality was determined by the absence of movement and response to tactile stimuli. Dead earthworms were removed and recorded and survival rates were calculated as follows:

Reproductive Output Assessment

Reproductive performance was evaluated by monitoring cocoon production in each treatment group. Cocoons were collected on a weekly basis and incubated under controlled environmental conditions to ensure proper development. Upon hatching, the number of juveniles emerging from each cocoon was recorded. The average number of hatchlings per cocoon was used as an indicator of reproductive success.

Statistical Analysis

All collected data were analyzed using one-way ANOVA to determine significant differences between treatment groups. Results were expressed as mean ± standard deviation (SD), with statistical significance set at $p < 0.05$.

RESULTS

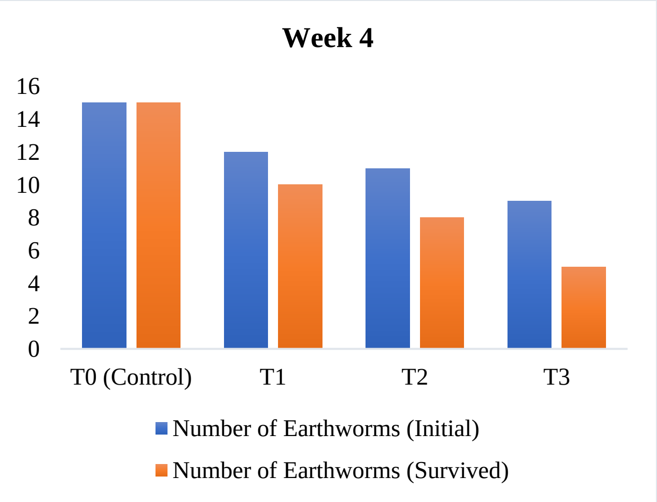
Survival Rate

Table 2

Week	Mean Survival ± SD
Week 1	13.75 ± 0.629
Week 2	12.75 ± 0.85
Week 3	11.75 ± 1.25
Week 4	9.5 ± 2.10

This table summarizes the earthworm survival rates across different treatment groups (T_0, T_1, T_2, and T_3) over a four-week period. The data shows a clear dose-dependent decline in survival as the concentration of zinc and copper increases with the highest mortality observed in the T_3 group. The results from the statistical analysis confirm that these differences are not due to chance supporting the hypothesis that heavy metal exposure adversely affects earthworm survival. These findings contribute to the understanding of the ecological impacts of zinc and copper contamination in soil ecosystems, particularly in relation to soil organisms such as earthworms.

Figure 1



This graphical representation of survival rates over the fourth-week period further illustrates this trend showing a significant decline in survival in the higher exposure T_2 and T_3 groups.

Reproduction

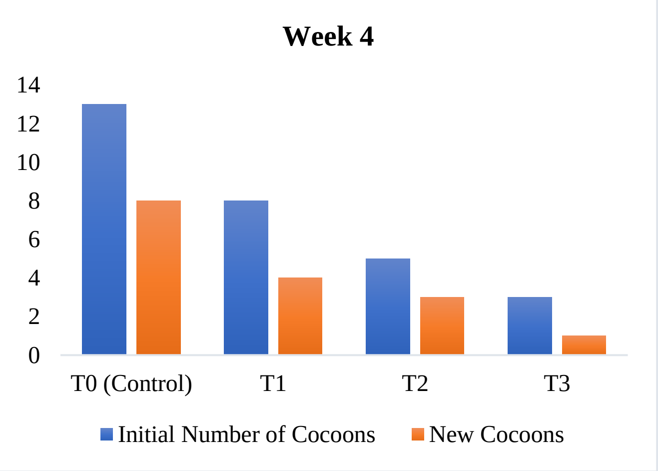
Table 1

Reproduction Parameters of Earthworms Under Zinc and Copper Exposure

Week	Mean Initial Cocoons ± SD	Mean New Cocoons ± SD	Mean Final Cocoons ± SD
1	0 ± 0	1 ± 0.707	1 ± 0.707
2	0.75 ± 0.829	2.75 ± 0.85	3.75 ± 1.54
3	3.75 ± 1.54	3.5 ± 0.64	7.25 ± 2.17
4	7.25 ± 2.17	4.25 ± 1.31	11.5 ± 3.47

The data demonstrates a progressive increase in cocoon production over the four-week period with a notable trend of reduced reproduction in groups exposed to higher levels of zinc and copper. In the control group (T_0) where earthworms were not exposed to heavy metals, the number of cocoons increased substantially over time. Conversely groups exposed to higher concentrations of zinc and copper (T_1, T_2 and T_3) exhibited lower cocoon production indicating a dose-dependent decline in reproductive output. Here is the graphical representation of cocoon production in 4_{th} week.

Figure 2



This graphical representation of cocoon production in the fourth week further illustrates this trend, showing a significant disparity between the control group and the exposed groups. The decline in reproductive success in the higher exposure groups suggests that heavy metal contamination negatively influences the reproductive potential of earthworms.

DISCUSSION

Earthworms play an essential role in maintaining soil health and ecosystem stability by enhancing organic matter decomposition, improving soil aeration and promoting nutrient cycling (Blouin *et al.*, 2019). Their burrowing activities enhance soil structure and fertility, making them crucial for sustainable agriculture and environmental balance. However, their sensitivity to environmental contaminants particularly heavy metals raises concerns about the ecological impacts of pollution on soil biodiversity and functionality (Jayamurali *et al.*, 2021).

Zinc (Zn) and copper (Cu) are essential micronutrients required for various physiological processes in organisms but excessive concentrations can lead to toxicity and disrupt biological functions. These heavy metals enter the soil through industrial waste, agricultural runoff and atmospheric deposition leading to their accumulation in soil ecosystems (Massányi *et al.*, 2020). When present in excessive amounts, Zn and Cu interfere with enzymatic activity, metabolic regulation and cellular functions thereby affecting the survival and reproduction of earthworms (Ullah *et al.*, 2021). These negatively impact microbial communities, reducing the availability of essential nutrients required for earthworm growth and reproduction

The observed decline in earthworm survival rate in this study indicated that higher concentrations of Zn and Cu are toxic to earthworms. Survival rate in T_0 is higher than the treatment groups, only 5 earthworms survived in T_3. The findings suggested that the concentrations of 2.5 mg/kg of zinc or 0.3 mg/kg of copper were sufficiently high to cause mortality. The reason might be that high Zn and Cu exposure is attributed to heavy metal-induced oxidative stress and metabolic disruptions. (Zheng *et al.*, 2013a) studied that excessive metal accumulation in earthworm tissues disrupts enzymatic pathways leading to cellular damage reduced energy production and impaired physiological functions. (Abdel-Aty *et al.*, 2013) demonstrated that high concentrations of Zn and Cu inhibit the activity of metallothionein which are responsible for metal detoxification leading to an overload of free metal ions that induce toxicity and cellular apoptosis. (Basha and Latha, 2016) explained that the dose-dependent decrease in survival rates observed in this study consistent with findings from previous research indicating that prolonged exposure to Zn and Cu leads to increased mortality in earthworm populations.

The present study demonstrated that earthworm exposed to 2.5 mg/kg of zinc or 0.3 mg/kg of copper negatively influenced cocoon production and hatchling success. T_0 with no heavy metal exposure produced the maximum of 8 cocoons while T_3 (2.5 mg/kg Zn and 0.3 mg/kg Cu) had the minimum of 2. This decline suggests that heavy metals reduce cocoon production in earthworms. The fact might be that zinc (Zn) and copper (Cu) negatively affect earthworm reproduction primarily through oxidative stress, endocrine disruption and direct cytotoxic effects. The accumulation of these metals in earthworm tissues leads to the generation of reactive oxygen species (ROS), causing oxidative damage to lipids, proteins and nucleic acids. This oxidative stress impairs the normal functioning of reproductive organs particularly the clitellum which plays a vital role in cocoon production. (Smical *et al.*, 2008) reported that Zn and Cu disrupt reproductive physiology by impairing sperm viability, egg fertilization and embryonic development. (Vinodhini and Narayanan, 2008) found similar results i.e., no discernible link between soil copper amounts and cocoon formation rate. The reproduction-related reported NOEC and EC_{50} differ significantly between research. (Van Capelle *et al.*, 2017) suggested that these metals interfere with hormonal regulation and cellular processes essential for reproduction.

This study provides valuable insights into the impact of Zn and Cu contamination on earthworm survival and reproduction further research is needed to better understand the underlying physiological mechanisms.

CONCLUSION

This study highlights the detrimental effects of Zn and Cu contamination on earthworm survival and reproduction. Higher concentrations of these metals led to significant declines in survival rates, while reproductive output also exhibited a decreasing trend. The findings underscore the importance of monitoring heavy metal pollution in agricultural soils and implementing soil remediation techniques to mitigate its ecological impact.

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