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EFFECT OF HEAVY METAL TOXICITY (LEAD AND CADMIUM) ON THE GROWTH, SURVIVAL AND REPRODUCTION OF EARTHWORM (*Eisenia fetida*)

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ABSTRACT

Earthworms serve as vital indicators of soil health and are integral in maintaining soil fertility through their burrowing activities, nutrient cycling, and decomposition of organic matter. Their ecological functions can be severely compromised by environmental stresses, notably heavy metal contamination, including lead (Pb) and cadmium (Cd). The goal of the present study was to evaluate the impact of heavy metals on various qualities of earthworms as well as their accumulative ability for heavy metals like Pb and Cd. Earthworms were collected from different agricultural fields and raised under laboratory conditions at the University of Agriculture Faisalabad, PARS campus, for 28 days. For this purpose, four treatment groups were created. The T1 control group was not exposed to any metal. The T2, T3, and T4 groups were given Pb at concentrations of 1.5 mg/kg, 2.0 mg/kg, and 2.5 mg/kg of soil, respectively, and Cd at concentrations of 0.1 mg/kg, 0.2 mg/kg, and 0.3 mg/kg of soil, respectively. The earthworms were kept in trays and soil for one week prior to the trial and were fed with normal feed. Total weight, length, biomass, cocoon production, and population dynamics were measured. In terms of growth, the average growth value significantly dropped in T3 to 1.108 with a standard deviation of ±0.061774, indicating high variability. Regarding the survival rate, the average survival rate significantly decreases in T3 to 5.5 with ±2.95804, indicating a very wide range of values. For cocoon production in T3, the average number of cocoons produced reached 12.25 ±4.710. Results recorded and statistically analyzed using one-way ANOVA showed the significant increase in value of ($P > 0.05$) in growth. The ($P < 0.05$) which is below the significant level in survival. The reproduction showed that ($P > 0.05$) which is greater than significant value.

Keywords: Effect of Heavy Metal Toxicity (Lead And Cadmium), Growth, Survival, Reproduction of Earthworm

INTRODUCTION

Earthworms are often referred to as "ecosystem engineers" due to their profound impact on soil health and ecosystem dynamics. These invertebrates play several crucial roles in maintaining and enhancing soil fertility, structure, and biological activity. Their ecological importance can be broadly categorised into soil structure and aeration, nutrient cycling, organic matter decomposition, and their role as bioindicators of soil health (Paul B Tchounwou *et al.,* 2012).the unfavourable soil condition can be a significant factor which results in the failure of earthworm activities. In addition to providing nutrients, the organic matter may also help earthworm conditions by reducing fluctuations in moisture and temperature (Blouin *et al.,* 2013).

Earthworms, the most well-known macrofauna of the soil, are formidable bioturbators that live under the surface. With the exception of Antarctica, earthworms are present on every continent (Khayatzadeh & Abbasi, 2010). Because of their widespread invasiveness, non-native earthworm populations have recently attracted the attention of scientists once again. In the formerly glaciated forests of North America, where it is now established that earthworms were eradicated during the last glaciation but have since been reintroduced by farming, fishing, and other human activities, introductions and ecological impacts of non-native earthworms have been thoroughly studied (Craven *et al.,* 2017).

Earthworms are considered bioengineers. Through their burrowing activities, nutritional recycling, and organic matter breakdown, they serve a critical role in preserving soil fertility and health (Yadav *et al.,* 2023). Many environmental pressures, such as heavy metal poisoning can seriously impair their health and ecological functions. Among the heavy metals of concern lead (Pb) and cadmium (Cd) have drawn a lot of attention because of their pervasive presence in the environment and their harmful effects on living things (van Capelle *et al.,* 2016).

The constant infiltration of heavy metals into the soils via various industrial and agricultural processes, soil heavy metal contamination is a significant environmental issue (Wu *et al.*,2012). When heavy metal concentrations build up in soils, they can cause serious environmental problems due to their mobility, non-degradability, and capacity for bioaccumulation (Xu *et al.,* 2021). Higher concentrations of heavy metals not only pollute the soil but also change its structure by influencing the soil organisms. By breaking down organic waste, they significantly contribute to the formation of soil and are a vital source of food for numerous species (Datta *et al.,* 2016).

The significant role earthworms play in soil ecosystems, the effects of lead (Pb) and cadmium (Cd) on earthworms are an important field of research in ecotoxicology. Common environmental contaminants like lead and cadmium can have a big impact on earthworm physiology, survival, and behaviour. Even while earthworms like *Eisenia fetida* can survive in environments contaminated with lead, research indicates that exposure to lead can stunt growth, lower rates of reproduction, and cause biochemical and physiological stress (Abdel-Aty *et al.,* 2013). In a similar vein, exposure to cadmium can cause oxidative stress, cellular damage, and decreased metabolic activity in earthworms, all of which have a negative impact on the health and capacity to condition soil. These heavy metals are also bio accumulated by earthworms, which may have an impact on how they are used in soil bioremediation (Ahmad *et al.,* 2021).

Growth is an important and easily accessible trait that encompasses a variety of physiological and biochemical impacts. Individual energy budgets are altered because exposed species need to expend more energy for metabolism, detoxification, or sequestration and excretion of the contaminants (Aigner *et al.,* 2023). This increased energy requirement results in decreased growth. The development and reproduction of earthworms were negatively correlated, which implies that if the earthworms did not expand, they were unable to produce cocoons (Burgos *et al.,* 2005).

The earthworm reproduction test is widely used because it can identify the effects of toxins at low doses and is more sensitive than conventional acute toxicity assays. Additionally, a large number of specialists agree that this test is valuable for forecasting the impact on soil ecosystems (Huang *et al.,* 2023). Reproduction has a significant impact on population dynamics, so it is important to take this into account when assessing the dangers of ecotoxicological compounds (Kim *et al.,* 2019). Reproduction rates have been demonstrated to be sensitive as an endpoint in numerous investigations on the toxicity of various metals and chemicals, including hydrocarbons, PCBs, and pesticides (Hubálek *et al.,* 2007).

Heavy metals, especially lead (Pb) and cadmium (Cd), have a significant and harmful impact on earthworm growth. It has been demonstrated that exposure to these metals slows the growth of certain earthworm species. Due to reduced nutritional absorption and metabolic changes, lead intoxication can cause lower body weight and development retardation (Ajayan *et al.,* 2011). exposure to cadmium results in cellular structural damage and oxidative stress, which further inhibits growth. these metals also disrupt the processes of growth and reproduction, which lowers overall fitness and causes population declines in contaminated soils (Abdel-Aty *et al.,* 2013).

Materials And Methods

Earthworms will be collected from the agriculture land using hand-sorting or soil extraction methods. The location of the current research work will be department of zoology, wild life and fisheries in the University of Agriculture, Faisalabad community college PARS campus. The collected earthworm species (*Eisenia fetida*) will be identified using standard taxonomic keys.

Depending on the particular needs of the study or experiment being carried out, the length of time earthworms must be acclimated in a lab environment can change. The kind of soil substrate that is used in the acclimation plastic containers might also change based on the objectives of the study. In order to establish an appropriate environment for the earthworms, common substrates include a mixture of soil, organic materials (such as compost or leaf litter) and other substances. Depending on the kind of earthworms being investigated and the environmental conditions they are used to in their natural home, different temperatures and humidity levels may be needed. Generally speaking, temperature between 15°C and 25°C

EXPERIMENTAL DESIGN

The experiment will be followed by the preparing the soil and trays.

Treatments will be included for test groups T_0, T_1, T_2 and T_3. The T_1, T_2 and T_3 will be given the LC50 of Pb1.5mg/kg, 2.0mg/kg and2.5mg/kg of the soil and LC50 of Cd0.1mg/kg, 0.2mg/kg and0.3mg/kg of soil.

Control groups will not receive heavy metal exposure.

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Summary of different doses of Lead and Cadmium

Treatment groups	Lead	Cadmium
T_0(Control)	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

HEAVY METAL EXPOSURE

Lead and cadmium exposure will be achieved by adding lead and cadmium compounds, to the soil substrate at predetermined concentrations. Soil pH and moisture content will be adjusted to ensure uniform metal availability across treatments. Earthworms will be exposed to heavy metals for 28 days through direct contact with contaminated soil.

MEASUREMENT OF GROWTH PARAMETERS

Earthworm growth parameters including body length, body weight and biomass will be measured before and after heavy metal exposure. Body length will be measured using a digital caliper and body weight will be determined using a precision balance. Biomass will be The growth parameters of earthworms

ASSESSMENT OF SURVIVAL

Earthworm survival was monitored daily during the exposure period. Dead earthworms had identified based on lack of movement and lack of response to stimuli. Survival data recorded and used to calculate survival rates for each treatment group.

Assessing the survival rate of earthworms is essential for understanding their role in soil ecosystems, their responses to environmental stressors, and the impact of agricultural practices. This section details the materials and methodology required to measure the survival of earthworms accurately, ensuring the reliability and reproducibility of the results.

EVALUATION OF REPRODUCTIVE OUTPUT

Reproductive output assessed by counting the number of cocoons produced by earthworms in each treatment group. Cocoons were collected periodically throughout the exposure period and incubated under controlled conditions to quantify hatchling emergence. The number of hatchlings per cocoon were recorded as a measure of reproductive success.

Measured the reproductive rate of earthworms is crucial for understanding their population dynamics, ecological impact, and responses to environmental changes.

RESULTS AND DISCUSSION

Survival rates of earthworms are also negatively impacted by lead and cadmium exposure. These heavy metals can cause oxidative stress by generating reactive oxygen species (ROS) that damage cellular components, including lipids, proteins, and DNA. This oxidative damage can result in increased mortality rates among earthworm populations. Moreover, chronic exposure to sub-lethal concentrations of lead and cadmium can weaken the immune

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system of earthworms, making them more susceptible to diseases and environmental stressors (André *et al.,* 2010).

Reproductive functions of earthworms are particularly sensitive to heavy metal contamination. Lead and cadmium can disrupt the hormonal regulation of reproductive processes, leading to reduced fertility and fecundity. For instance, these metals can impair the development and maturation of reproductive organs, resulting in fewer and less viable cocoons. Additionally, lead and cadmium exposure can decrease the number of offspring produced and affect their survival and development. Such reproductive impairments can have long-term consequences for earthworm populations, ultimately affecting soil health and ecosystem functioning (Semenov *et al.,* 2003).

GROWTH

Growth parameters were observed in different treatments such as t0, t1, t2and t3 which explained in tables below**.**

Table 1: The table presents the growth measurements of earthworm in four weeks

Week 1	9±0.707107
Week 2	8±1.224745
Week 3	7±0.816497
Week 4	5.5±2.95804

This combined table 1 showed that The average growth value for Week 1 is 9.Standard Deviation (±0.707): Indicates that the growth values were quite consistent, with only a small variation around the mean. The average growth value for Week 2 decreased to 8 Standard Deviation (±1.225): The data showed a bit more variability compared to Week 1, suggesting some fluctuations in growth.The average growth value further decreased to 7 in week 3. Standard Deviation (±0.816): The variation was relatively small, similar to Week 1, indicating fairly consistent measurements around the mean.The average growth value significantly dropped to 5.5.Standard Deviation (±2.958)in week 4 The high standard deviation suggests that the growth measurements were highly variable, indicating a wide range of growth values.

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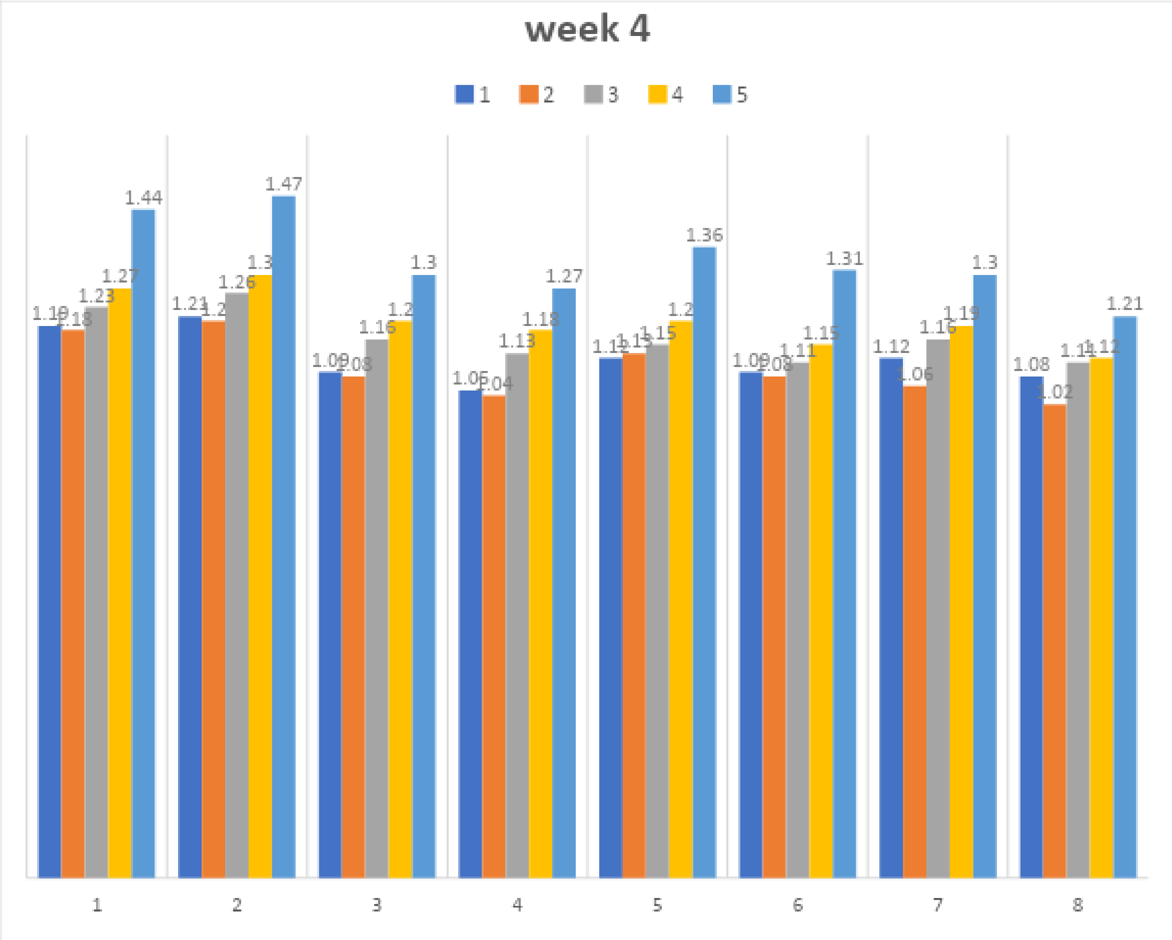

The graph 1 showed the initial and final weights of five groups (1 to 5) over several weeks In Week 1, initial weights ranged from 119 g to 121 g. By the end of the week, final weights increased to approximately 127-144 g.In Week 2, initial weights ranged from 109 g to 112 g, with final weights increasing slightly to about 113-137 g.In Week 3, initial weights ranged from 105 g to 112 g, and final weights increased to 112-136 g.In Week 4, initial weights ranged from 108 g to 112 g, with final weights slightly increasing to around 121-131 overall, the chart indicates consistent weight gain across all groups each week, with minor variability. This suggests that the different treatments had a similar impact on the growth of the subject

SURVIVAL:

Survival parameters were observed in different treatments such as t0, t1, t2and t3.

The table presents the rate of survival over a period of four weeks

Week 1	0.75±0.829156
Week 2	3±1.870829
Week 3	7.5±2.95804
Week 4	12.25±4.710361

The table 2 indicated the average survival rate for Week 1 is 0.75.Standard Deviation (±0.829): Indicates that the survival rates were quite variable, with some measurements deviating significantly from the mean. The average survival rate for Week 2 increased to 3.Standard Deviation (±1.871): The data showed greater variability compared to Week 1, suggesting more fluctuations in survival rate.The average survival rate further increased to 7.5 in week 3. Standard Deviation (±2.958): The variation was even higher, indicating a wider range of survival rates around the mean. The average survival rate significantly increased to 12.25 in week 4 and Standard Deviation is (±4.710): The high standard deviation suggests that the survival rates were highly variable, indicating a very wide range of values.

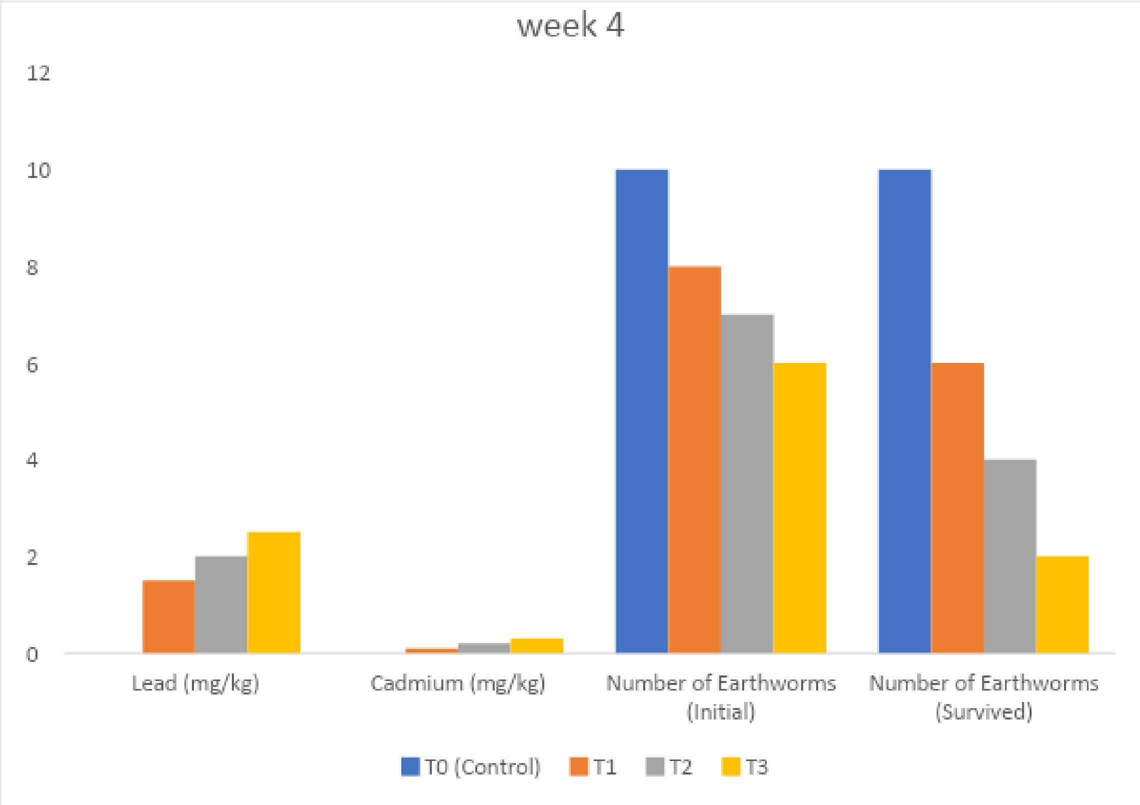

Figure 2: Graph of survival in week 4

The chart 2 displayed the initial and final survival rate of four groups over several weeks. In the control group, the earthworms were not exposed to any lead or cadmium. The initial number of earthworms in this group was 10, and all 10 earthworms survived until the end of the study. The T1 group The initial number of earthworms in this group was 8, and 6 earthworms survived until the end of the study. The T2 group was The initial number of earthworms in this group was 7, and 4 earthworms survived. The T3 group was. The initial number of earthworms in this group was 6, and only 2 earthworms survived until the end of the study.

Average number of cocoons produced in each week

Week 1	0.75±0.829156
Week 2	3±1.870829

Week 3	7.5±2.95804
Week 4	12.25±4.710361

The table 3 showed the average number of cocoons produced is 0.75 with a standard deviation of ±0.829. Week 2: The average number of cocoons produced increases to 3 with a standard deviation of ±1.871.Week 3: The average number of cocoons produced further increases to 7.5 with a standard deviation of ±2.958.Week 4: The average number of cocoons produced reaches 12.25 with a standard deviation of ±4.710.

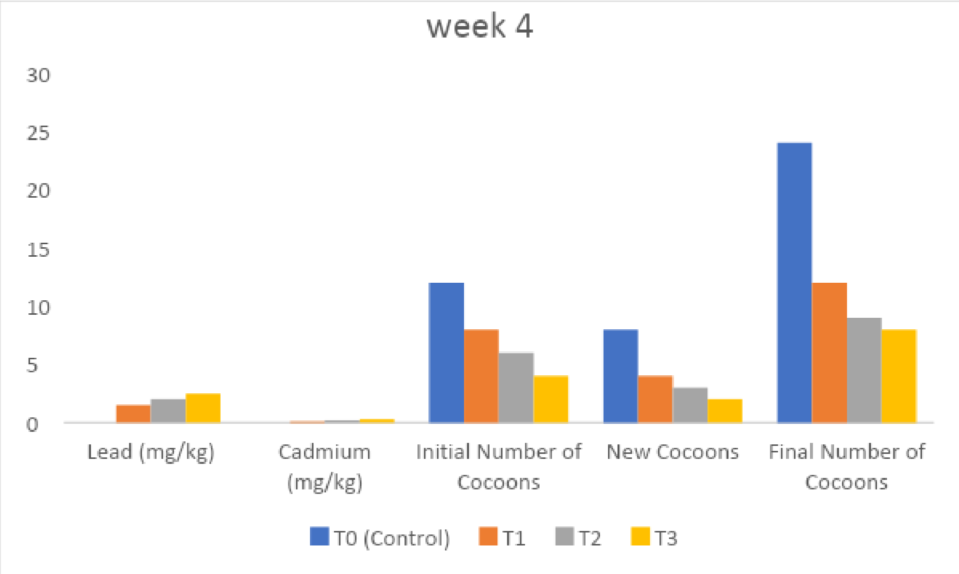

Figure 3: Graph of number of cocoons in week 4

The graph 4.3 displayed data on lead and cadmium concentrations and the initial, new, and final number of cocoons for four groups: T0 (Control), T1, T2, and T3. Lead Exposure: T0 (Control) shows no lead exposure, while T1, T2, and T3 are exposed to increasing levels of lead: 1.5 mg/kg, 2.0 mg/kg, and 2.5 mg/kg, respectively. Cadmium Exposure: T0 (Control) shows no cadmium exposure, while T1, T2, and T3 are exposed to increasing levels of cadmium: 0.1 mg/kg, 0.2 mg/kg, and 0.3 mg/kg, respectively.

DISCUSSION

Earthworms are one of the most important parts of an ecosystem and are capable of breaking down complicated compounds into simpler ones, earthworms are crucial to the health of soil. The different anthropogenic activities have contributed significantly to the eco-complex chemicals. The presence of heavy metals in soil has a direct impact on the creatures that live there. Since animals that do not live in dirt eat animals that have heavy metal accumulation in their bodies, the contamination caused by heavy metals affects the entire food chain. A common criterion for assessing soil pollution is the presence of heavy metals. Earthworms are fatally affected by heavy metals (Paul B Tchounwou *et al.*, 2012) . Because earthworms are biological indicators, they are frequently utilized in research to determine ecological risk. This is because the concentration of heavy metals in soil and the bodies of earthworms that live there have a positive correlation.

The presence of different concentration of lead and cadmium had different effects on the cellular activities and growth of earthworm. The weight of earthworm was negatively affected when treated with lead of about 2.5mg/kg. this might be due to disruption in protein synthesis and enzymatic activities crucial for growth and development(Raiesi *et al.,* 2020) suggested that the growth decreased by 6% due to presence of lead in soil . Cadmium binds to and inhibits key enzymes involved in metabolic processes, which hampers nutrient assimilation and energy production essential for growth. (Huerta Lwanga *et al.,* 2016) suggested that the growth of earthworm was decreased by 7% due to presence of cadmium in soil. Reported that exposure to cadmium at concentrations of 50, 100, and 200 mg/kg in soil led to a significant reduction in the biomass of earthworms (*Eisenia andrei*). Higher concentrations resulted in more pronounced biomass loss, suggesting that cadmium and lead accumulation in earthworm tissues adversely affects their growth.

The survival rates observed in this study clearly indicate that both lead (Pb) and cadmium (Cd) are toxic to earthworms at the tested concentrations. The survival rate in control group (T0) was higher. In contrast, the highest concentration of cadmium in group (T3) showing the most significant decline, at the end only 2 earthworms survived. This finding suggested that the concentrations of 2.5 mg/kg Pb and 0.3 mg/kg Cd were sufficiently high to cause mortality, confirming their toxic nature to earthworms. The reason might be that lead and cadmium can interfere with calcium metabolism, which is essential for various cellular functions, including muscle contraction and cellular signalling. This disruption can impair survival. A study conducted by (Jacob *et al.,* 2018) demonstrated that the combined exposure to lead and cadmium caused greater oxidative stress and biochemical disruptions in earthworms which can decrease their survival rate. survival rate of *Lumbricus rubellus* decreased significantly when exposed to soils contaminated with Pb (100, 500, 1000 mg/kg) and Cd (1, 5, 10 mg/kg). Higher concentrations led to higher mortality, indicating the acute toxicity of these metals .According to a study by (Raeeszadeh *et al.,* 2023), earthworms exposed to a mixture of heavy metals, including lead and cadmium, showed elevated levels of oxidative stress markers and increased mortality.

The present study demonstrated that the earthworms exposed to 2.5 mg/kg lead had lowest survival rate and highest mortality. The reason might be that the accumulation of lead in earthworm can cause oxidative stress and increases the production of reactive oxidative species in their body which can block various cellular activities that leads to death of cells and individual. Similar results were supposed by (Fryzova *et al.,* 2018) stated that the accumulation of lead in earthworm can cause damage to their cells and led to death of earthworms. The earthworms from T3 were treated with 0.3 mg/kg cadmium showed highest mortality rate. The reason might be that long term exposure to cadmium can reduce activities of antioxidant enzymes which can cause death of organism. Similar results were suggested by (Jakimska *et al.,* 2011) stated that cadmium can increase the risk of death of earthworm by declining reproduction and antioxidant enzyme activity. Reproductive metrics, including cocoons production were also adversely affected by heavy metal exposure. The control group (T0) produced the highest number of cocoons indicating healthy reproductive activity. However, as the concentration of Pb and Cd increased, the number of cocoons and juveniles produced significantly decreased. This study showed that the maximum number of new cocoons are produced in T0 with no exposure to heavy metal which is 8 and minimum numbers of cocoons are produced in T3 which is treated by the 2.5 mg/kg Pb and 0.3 mg/kg Cd the number of new cocoons produced in T3 were two. Which showed that number of cocoons are decreased in T3 as compared to the control group. Its reason might be that heavy metals can reduce the number of cocoons produced by earthworms. This can be due to the direct toxic effects on the reproductive organs. Heavy metals induce physiological stress in earthworms, leading to increased energy expenditure on detoxification processes rather than reproduction. The cocoon creation rate (range 29–65%) was considerably ($p < 0.05$) suppressed by the other concentrations, and no production in the soil was seen. The EC50 value of 349 μgPb g–1 was obtained based on a regression study that revealed a substantial decrease in the cocoon production rate with increasing lead concentration in soil (R2 = 0.65, p = 0.0001). The EC50 value in our investigation was noticeably lower than that found in prior investigations (Heikens *et al.,* 2001). High lead toxicity to earthworm reproduction could be because the lead's bioavailability was quite high and the lead was out of balance with the soil.

In present study when earthworms are treated by 0.3mg/kg of cadmium it showed that numbers of cocoons produced in this group was decreased as compared to the control group earthworms which was not treated by any type of heavy metal it is due to the Because cadmium affects the quality of sperm and eggs, it can decrease earthworm fertility by reducing the likelihood of successful fertilization and producing fewer viable offspring., Additionally, worms' ability to reproduce was hindered by cadmium.

The cocoon production rate fluctuated with no correlation between soil cadmium levels and cocoon production. However, cocoons exposed to cadmium were suppressed by 18-65% compared to the control group. The reproduction-related NOEC and EC50 varied significantly between studies, with some reporting higher NOECs than others. Our results show that whereas growth weight and cocoon formation are more sensitive endpoints, death is the least sensitive. Metal sensitivity was greater in reproduction.

Growth was less susceptible to most agents (pesticides, metals) than reproduction. Our research confirmed that the earthworm reproduction test had a higher sensitivity than the acute earthworm mortality test.

SUMMARY

This study aimed to investigate the effects of lead (Pb) and cadmium (Cd) on the survival, growth, and reproduction of earthworms, which are crucial indicators of soil health. Earthworms play a vital role in soil structure and fertility through their activities, and understanding the impact of heavy metal contamination on them is essential for environmental management.

The observed toxic effects can be attributed to several mechanisms. Lead and cadmium may accumulate in earthworm tissues, causing cellular and systemic toxicity. Both metals can induce oxidative stress by generating reactive oxygen species, leading to cellular damage. Cadmium, in particular, can disrupt calcium metabolism, which is critical for various biological functions. These disruptions can impair normal physiological functions, resulting in decreased survival and reproductive success.

The experiment was designed with four treatment groups: T0 control group with no heavy metal exposure, and three treatment groups T1, T2, T3) with increasing concentrations of Pb (1.5, 2.0, and 2.5 mg/kg) and Cd (0.1, 0.2, and 0.3 mg/kg). The earthworms were monitored over a period of28 days to assess survival rate and cocoon production. Soil was prepared and evenly distributed into trays, and each treatment group received its respective concentrations of Pb and Cd.

i. The exposure of lead and cadmium cause significant changes in the weight of earthworm. Earthworms showed maximum growth in T0 group which is (1.288±0.097858)

ii. Earthworms showed the minimum growth in T3(1.108±0.061774) with the significant value of P greater than (0.05). Exposure of lead and cadmium also cause significant changes on earthworm survival.

iii. T0 showed the maximum survival rate which was 100%.

iv. Earthworms showed the minimum survival rate in T3 of week4 which was (12.25±4.710361). its p value less than 0.005 which is below the significant level.

v. Lead and cadmium also affected the reproduction of earthworms. Numbers of cocoons decreased during the heavy metal exposure. Maximum number of cocoons were observed in T0 group.

vi. Numbers of earthworms decreased in T3. The number of cocoons was decreased to 2 which shows that number of cocoons reduced by effect of heavy metal its p value is 0.078 which is greater than significant value.

In conclusion, this study highlights the significant negative effects of lead and cadmium on the survival, growth, and reproduction of earthworms. Addressing heavy metal contamination in soils is crucial to protect soil health and ensure the sustainability of terrestrial ecosystems. Effective remediation strategies and regulatory measures can help mitigate the impact of heavy metals and promote a healthier environment for all organisms.

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