REPRODUCTIVE AND HISTOLOGICAL ALTERATION IN EARTHWORM *EISENA FETIDA* EXPOSED TO VARYING CONCENTRATIONS OF PETROL CONTAMINATED SOIL.

BY

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DEPARTMENT OF ENVIRONMENTAL MANAGEMENT AND TOXICOLOGY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

BENIN CITY.

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AN UNDERGRADUATE PROJECT SUBMITTED TO THE DEPARTMENT OF ENVIRONMENTAL MANAGEMENT AND TOXICOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA; IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR AWARD OF BACHELOR OF SCIENCE (B.Sc.) DEGREE IN ENVIRONMENTAL MANAGEMENT AND TOXICOLOGY.

FEBRUARY, 2025.

CERTIFICATION

This is to certify that this project titled "**REPRODUCTIVE AND HISTOLOGICAL ALTERATION IN EARTHWORM *EISENA FETIDA* EXPOSED TO VARYING CONCENTRATIONS OF PETROL CONTAMINATED SOIL**" was carried out by **MEBINONE TAIWO** with Matriculation Number: **LSC1906722** and presented to the Department of Environmental Management and Toxicology, Faculty of Life Sciences, University of Benin, Benin City; in partial fulfillment of the requirements for the award of Bachelor of Science (B.Sc.) in Environmental Management and Toxicology. It was conducted under suitable conditions, was carefully supervised and subsequently approved as having met the requirements for the award of Bachelor of Science degree in Environmental Management and Toxicology.

______________________ ______________________

DR. OGBEIDE O. **DATE**
(Project Supervisor)

______________________ ______________________

PROF. A. A. ENUNEKU **DATE**
(Head of Department)

DECLARATION

I, **MEBINONE TAIWO** declare that "**REPRODUCTIVE AND HISTOLOGICAL ALTERATION IN EARTHWORM *EISENA FETIDA* EXPOSED TO VARYING CONCENTRATIONS OF PETROL CONTAMINATED SOIL**" is my own work and that all sources that I have used or quoted have been acknowledged by means of complete references and that this work has not been submitted before for any other degree at any other University.

MEBINONE TAIWO

DEDICATION

This work is dedicated to God Almighty, the provider of wisdom and the giver of Life, through whom everything is made possible, and who has blessed me with the strength and ability to carry out this program. I also dedicate this work to my immediate family for their constant support and encouragement. It aided me in no small way to successfully complete this course.

ACKNOWLEDGEMENTS

As I approach the end of this academic journey, I am deeply grateful to the almighty God whose grace, guidance, and strength have been my foundation throughout every challenge and triumph.

I also want to thank my supervisor, Dr. Ogbeide O for his time, prudence, and expertise in ensuring that the necessary corrections are made.

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I would like to express my deepest gratitude to my course adviser, Dr. Michael Akharame for his invaluable guidance coupled with insightful advice and timely provision of essential information which have been pivotal in ensuring that I stayed on track and focused.

I would like to express my profound gratitude to my parents, Mr. and Mrs. Mebinone for their endless love, sacrifice, and unwavering confidence in my success despite an opposing tide of failure. Your financial support and emotional encouragement that kept me going during the toughest times are acknowledged. I sincerely want to appreciate my amazing siblings, Mrs. Obasanmi, Mr. Sunday Mebinone, Kehinde Mebinone, Mrs. I. Abidola, Alaba Mebinone, Aina and Damilola who have proved to be my well-wishers indeed and stand with me regardless of the weather condition.

May God bless you all and keep the bond of love stronger.

To my pastor, Mr. Leri Julius and his family, I appreciate your great concern and prayers for my achievements. May God rewards you abundantly.

Finally, I would like to thank my friends, Zacchaeus Emiloju, Akinyandenu Oluwafemi, Osasere Aigbovo, Akinmeji Lawrence, Anslim Somtoochukwu, Sola Omosebi, Victor Nwogu, and Mayone Anselm for their support and faithfulness.

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ABSTRACT

This study investigates the reproductive and histological alterations in *Eisenia fetida* (earthworm) exposed to varying concentrations of petrol-contaminated soil. Earthworms play a crucial role in soil health and ecosystem functioning, and their response to environmental pollutants, such as petroleum-based compounds, can provide valuable insights into the impact of soil contamination on terrestrial organisms. The objective of this research was to evaluate the effects of petrol exposure on the reproductive health and histological structure of *Eisenia fetida*, considering different levels of petrol contamination.

The experiment was conducted using soil samples contaminated with petrol at concentrations of 13.3%, 33.3%, 53.3%. The earthworms were exposed to these varying concentrations for a period of 28 days. Reproductive success was assessed by evaluating the number of cocoons laid, hatching success, and the development of offspring. Histological analysis of the earthworms was performed to observe any structural changes in their tissues, including the digestive system, reproductive organs, and muscular layers.

The results revealed significant reproductive impairments at higher petrol concentrations, with a marked reduction in the number of cocoons and hatching success. Histological analysis showed structural damage to the digestive tract, reproductive tissues, and muscular structures, with notable changes such as cell degeneration, reduced cellular integrity, and signs of inflammation. These findings suggest that exposure to petrol-contaminated soil adversely affects both the reproductive capabilities and the physiological integrity of *Eisenia fetida*, with potential implications for soil biodiversity and ecosystem health.

This study contributes to the understanding of the ecological risks associated with petrol contamination in soils, highlighting the importance of assessing the impact of petroleum pollutants on soil-dwelling organisms. The results underscore the need for effective pollution management strategies to mitigate the harmful effects of petrochemical contaminants on terrestrial ecosystems.

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CHAPTER ONE

INTRODUCTION

1.1 INTRODUCTION

Soil is an essential natural resource that connects air, bedrock, water, and biota, supporting the production of food, fuel, and fiber (Allison *et al*., 2009). However, contamination of soil due to petroleum hydrocarbons is a significant environmental issue. Petrol contamination mainly results from leaks in underground storage tanks, industrial activities, and accidental spills (Adipah, 2018b). Despite its economic benefits, petroleum pollution has detrimental effects on soil health, plant growth, and human well-being (Adipah, 2018b). Petroleum hydrocarbons, including benzene and polycyclic aromatic hydrocarbons (PAHs), are highly toxic and persistent in the environment (Banks *et al*., 1999). Exposure to these contaminants occurs through inhalation, skin contact, and ingestion, posing severe risks to humans and ecosystems (Cheng and Nathaniel, 2009).

Earthworms play a crucial role in soil ecosystems by enhancing aeration, nutrient cycling, and organic matter decomposition. However, exposure to petroleum-contaminated soil can adversely affect their reproductive and histological structures. The toxic components of petrol, such as benzene, toluene, and xylene, can impair earthworm reproductive health by reducing cocoon production, altering reproductive organ function, and disrupting hormonal balance. Additionally, histological alterations in their tissues, including the digestive and reproductive systems, indicate severe physiological stress. This study evaluates the impact of varying concentrations of petrol-contaminated soil on the reproductive and histological changes in *Eisenia fetida*, a widely used bioindicator in ecotoxicological research.

1.2 BACKGROUND OF THE STUDY

Petroleum hydrocarbon contamination in soil is a global environmental concern, with increasing demand for petroleum products exacerbating pollution levels (Varjani, 2017; Wu *et al*., 2017). Petroleum hydrocarbons impact soil enzyme activity, microbial diversity, and plant growth, leading to long-term ecological damage (Guo *et al*., 2012; Liang *et al*., 2011). The introduction of hydrocarbons into soil alters its physicochemical properties, making it less suitable for biological activity (Ijomah *et al*., 2020). Bioremediation strategies, such as microbial degradation and phytoremediation, have been explored to mitigate pollution, but these methods often require extensive time and resources.

Earthworms, particularly *Eisenia fetida*, contribute to bioremediation by enhancing microbial activity and breaking down contaminants. However, their exposure to toxic pollutants can have detrimental effects on their physiology and survival. Earthworms are known as "ecosystem engineers" due to their role in soil aeration and organic matter decomposition (Li *et al*., 2020). However, they are also highly sensitive to environmental contaminants, making them useful indicators of soil pollution (Liu *et al*., 2019). Studies have shown that exposure to petroleum hydrocarbons can lead to mortality, reduced reproductive success, and histological damage in earthworms (Gu *et al*., 2017; Song *et al*., 2018). This study seeks to provide insights into the specific reproductive and histological alterations in *Eisenia fetida* due to petrol contamination.

1.3 JUSTIFICATION OF THE STUDY

Petroleum contamination poses a significant threat to soil health and terrestrial organisms, particularly earthworms, which play a vital role in maintaining soil fertility. *Eisenia fetida* is widely used in ecotoxicological studies due to its sensitivity to pollutants and its ease of maintenance in laboratory settings (Rich *et al*., 2015). Understanding how petrol contamination affects earthworm reproduction and histology is crucial for assessing soil quality and informing remediation strategies.

Previous studies have primarily focused on microbial degradation and physicochemical changes in petroleum-contaminated soil. However, limited research has examined the direct biological effects on soil fauna, particularly earthworms. Given their ecological importance, it is essential to assess how varying petrol concentrations impact their physiological functions. This study will provide valuable data on the dose-response relationship of petrol contamination and its effects on reproductive and histological parameters. Such insights can contribute to environmental risk assessments and the development of more effective bioremediation strategies.

1.4 STATEMENT OF THE PROBLEM

Petroleum pollution remains a persistent environmental issue due to industrial spills, transportation accidents, and leakage from underground storage tanks. Soil contamination with petroleum hydrocarbons affects microbial activity, soil structure, and plant growth, leading to significant ecological imbalances (Adipah, 2018b). Despite efforts to remediate petroleum-contaminated sites, the impact on soil invertebrates, particularly earthworms, is not well understood.

Eisenia fetida plays a crucial role in soil ecosystem functioning but is highly susceptible to toxic contaminants. Exposure to petrol-contaminated soil can impair their reproductive capacity and cause histological damage to vital organs. These physiological disruptions can have cascading effects on soil health and fertility. However, there is a lack of comprehensive research on the specific reproductive and histological changes in earthworms exposed to varying petrol concentrations. This study aims to fill this gap by investigating how different contamination levels affect the reproductive and histological health of *Eisenia fetida*. The findings will contribute to a better understanding of soil pollution impacts and support the development of effective soil management and bioremediation strategies.

1.5 AIM

The aim of this study are;

- To assess the impact of petrol-contaminated soil on the reproductive health and histological structure of earthworms.
- Providing insights into environmental pollution and its biological effects.
- To assess the potential ecological impact of petrol pollution on soil dwelling organisms

OBJECTIVES

- The study also evaluates how varying concentrations of petrol affect reproductive and histological parameters, establishing a dose-response relationship.
- To determine the effect of varying concentration of petrol on the reproductive success of *Eisenia fetida*.
- To provide data that can inform pollution management strategies and environmental regulations related to petrol contamination in the soil.

1.6 LIMITATIONS OF THE STUDY

While this study provides valuable insights into the effects of petrol contamination on *Eisenia fetida*, there are several limitations that must be addressed in future research:

1. **Limited Range of Petrol Concentrations:** This study examined a limited range of petrol concentrations, and it is unclear whether even higher concentrations could result in more severe or irreversible damage. Future studies should explore a broader range of concentrations to understand the dose-response relationship fully.

2. **Short Duration of Exposure:** The 28-day study duration may not be sufficient to observe the full extent of petrol contamination's long-term ecological impacts. Longer-term studies are needed to understand the chronic effects of petrol on earthworm populations and their ability to recover from contamination.

3. **Environmental Complexity:** The experiment was conducted under controlled laboratory conditions, which may not fully replicate the complex conditions found in natural environments. Factors such as soil type, moisture content, and temperature could influence the toxicity of petrol and earthworms' response. Future research should consider these variables to better understand how petrol contamination affects earthworms in real-world scenarios.

4. **Single Species Focus:** This study focused solely on *Eisenia fetida*, which may not fully represent the entire earthworm community in a given ecosystem. Other earthworm species, particularly those with different ecological niches or sensitivities to pollutants, should be included in future studies to provide a more comprehensive understanding of the impact of petrol contamination on soil ecosystems.

5. **Analysis Limitations:** Histological examinations provided valuable insights into tissue damage, but further molecular analyses could reveal the underlying mechanisms of toxicity. Biomarkers of stress and damage at the molecular level could offer a more detailed understanding of how petrol affects earthworm health.

CHAPTER TWO

LITERATURE REVIEW

2.1 OVERVIEW OF PREVIOUS STUDIES ON SOIL CONTAMINATION

Nigeria's petroleum industry is the largest in West Africa and the second largest in Africa after Algeria (Ekperusi and Agbodion, 2015). A total of 159 oil fields and 1,481 oil wells operates in Nigeria (NDES, 1997). All activities in the petroleum sector obviously result in the release of petroleum and refined products into the environment.

In 2013 alone, NNPC reported 2,256 pipe bursts in NNPC pipelines, resulting in the loss of 181. 67 million tonnes (mt) of petroleum products worth about 21.48 billion naira and 34 fires in the year under review (NNPC, 2013), as confirmed also by Ekperusi and Aigbodion (2015).

Crude oil contains many compounds that are toxic to humans and the environment. Some authors have suggested that soil remediation standards should be based on BTEX components in soils contaminated with crude oil and petroleum products (fuels) (Salanitro *et al.*, 1997). Although BTEX is known to volatilize in contaminated sites, it can be retained in soils for months or even years, as reported in the United Nations Environment Programme assessment in Ogoniland (UNEP, 2011), thus requiring special attention in crude oil-contaminated soils (Ekperusi and Aigbodion, 2015.) Each of these compounds or their combinations pose serious problems to human health, living organisms, and the environment.

The idea of using earthworms for vermicomposting in gardens and improving crops has been known for centuries. Still, its application in bioremediation, according to the available literature, was discovered by chance after the 1976 Seveso chemical plant explosion in Italy, when a large area was contaminated with a highly toxic chemical such as 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) (Ekperusi and Aigbodion, 2015). Several species of earthworms were exterminated, except for a few that survived. Earthworms that ingested TCDD-contaminated soil were shown to bioaccumulate dioxin in their tissues and concentrate it an average of 14.5 times (Satchell, 1983).

Several studies have demonstrated the potential of earthworms for bioremediation of crude oil and other petrochemicals from contaminated soils in the laboratory and in the field. Ma *et al.* (1995) studied the effect of the earthworm Lumbricus *rubellus* on the removal of enriched PAHs such as phenanthrene and fluoranthene (100 µg/kg soil). They found that the removal of both PAHs occurred at a faster rate in soil with earthworms than in soil without earthworms. After 56 days (8 weeks), 86% of phenanthrene was removed.

Other studies have also shown that oil-contaminated soils can be effectively bioremediated, resulting in reduced toxicity (Van Gestel *et al.*, 2001). Martin-Gil *et al.* (2007) also investigated the use of *Eisenia fetida* earthworms and vermicompost in the treatment of high molecular weight hydrocarbon bitumen from the Prestige oil spill. The earthworms mineralized the bitumen, removing it from the system. Sinha et.al (2008) studied the remedial effects of earthworms on PAH-contaminated soil from a former gas production site in Brisbane, Australia. The results showed that earthworms could remove nearly 80% of PAHs, compared to only 47% and 21% when not used and only microbial degradation occurred. Ameh *et al.* (2013) studied the use of earthworms (*Eudrilus* eugeniae) for earthworm bioremediation of petroleum hydrocarbon-contaminated mechanical workshop soil. After 35 days of treatment, earthworm inoculation resulted in a greater reduction in total petroleum hydrocarbon content than in samples without worms, suggesting that earthworms could be used as biocatalysts in bioremediation (Ekperusi and Aigbodion, 2015).

Petroleum products can harm soil organisms by disrupting their habitats and physiology. When petroleum pollutants such as oil, diesel or gasoline enter the soil, they can reduce oxygen levels, reduce water holding capacity and increase toxicity, harming microorganisms, earthworms and other soil animals.

2.2 HISTOLOGICAL STUDY AND RELEVANCE OF EARTHWORM IN ECOTOXICOLOGY

A histological study evaluated the effects of exposure to crude oil-contaminated soil on *Eisenia fetida* using cellular antioxidant enzymes and tissue structures as biomarkers. E. Fetida was exposed to different concentrations of crude oil contamination of 1 mL, 2 mL and 3 mL (0.25, 0.50 and 0.75%) for 14 days (Ijomah *et al.*, 2020).

Significant histopathological changes were observed in E. Fetida at all three concentrations. Severe disturbances in the arrangement of the muscle layers of the body wall, internal visceral deformation, cellular degeneration, pigmentation, moderate to severe lesions and deformation of the circular and longitudinal muscles, and erosion of internal and external tissues leading to complete destruction of the body wall were observed. The study showed that crude oil, even at lower concentrations, still induced biomarker responses in E. Fetida (Ijomah *et al.*, 2020).

In addition, the assessment of petroleum-contaminated soil on E. Fetida earthworms in the Hongqian area of the Karamay Oilfield was also carried out to discover the effect of petrol-contaminated soil on earthworms and the effectiveness of earthworms in bioremediation as confirmed by Ijomah *et. al.* (2020) Earthworms are suitable organisms for soil ecotoxicology studies because of their special interaction with soil, which is significantly affected by pollutants entering the earth system.

The Karamay Oilfield was discovered in 1955 and is the first large oilfield discovered after the liberation of Xinjiang (Ijomah *et al.*, 2020). Biometric methods are more suitable for assessing the ecological toxicity of soil pollution and can provide more useful information (Al-Mutairi *et al.*, 2008; Khan *et al.*, 2018). This study evaluated the toxic effects and toxicity mechanisms of petroleum-contaminated soil on earthworms (*Eisenia fetida*) in the Hongqian area of the Karamay Oilfield.

Earthworm mortality was significantly higher on days 7 and 14 in soil contaminated with 50 % and 70 % petroleum. Earthworm weight was significantly lower after exposure to petroleum-contaminated soil. On day 7, the earthworms were slenderer, and their body segments were fractured after exposure to 70 % petroleum-contaminated soil (Ijomah *et al.*, 2020). No fractures were observed in earthworms in the control group or in the lower-concentration treatment groups.

This study tested the Eco-toxicological effects of petroleum-contaminated soil on E. *fetida* earthworms. Earthworm fresh weight decreased, and mortality rates increased with increasing concentrations of petroleum.

Reproduction declined in the 30 % petroleum treatment group, and no cocoons were found in the 50–70 % petroleum concentration groups, as confirmed by Ijomah *et al.* (2020). These results suggest that earthworm reproduction is more sensitive than their growth or mortality.

CHAPTER THREE

MATERIALS AND METHODS

3.1 COLLECTION OF SOIL SAMPLES AND EARTHWORM

After clearing the vegetation cover, topsoil not exceeding a depth of five inches was burrowed with a shovel and collected into a bucket beside the Botanical Garden, Faculty of Life Sciences, University of Benin (Ekperusi and Aigbodion, 2015). The collected soil was air-dried by spreading it on a level, clean board surface. The dried soil was sieved employing a 5 mm work plastic channel concurring with ISO standard 11268-1 (ISO, 1993) to remove debris and large stones.

Rectangular plastic containers with a cover lid were acquired from the market. The containers were weighed with an advanced sensitive weighing balance and properly labeled using tape and a permanent marker. The petrol was bought from a filling station near the campus.

Refined E. *Fetida* with well-developed clitella were obtained from the Department of Animal and Environmental Science, University of Benin (Ekperusi and Aigbodion, 2015). The earthworms were permitted to depurate their gut substance into moistened filter paper before application.

3.2 CONTAMINATION, COMPOSITION AND EXPERIMENTAL SETUP

Using a weighing balance, 1 kg of the air-dried soil was then weighed into each of the three containers. Three different soil concentrations were prepared for this study. With the aid of a 300ml beaker, 200ml of water was measured into three small plastic rubbers in which 0.4mol, 1mol, and 1.6mol petrol concentrations were injected using a syringe, respectively. These were painstakingly turned one after the other into each of the three plastic containers having 1kg soil, which was moistened to its water-holding capacity.

However, the control samples were left uncontaminated. These uncontaminated soils served as the control for this study. Each pot contained soil samples from either the three prepared concentrations or the control. Each concentration was set up in triplicates (three containers each). In total, 9 plastic containers were set up in the study.

Besides, an avoidance test was performed before introducing the earthworm into the above setup. A small quantity of soil inside a flat rectangular plastic rubber was divided into two parts (a and b). Part A was injected with 2mol of petrol while part b was not. Two biological sentinels (earthworms) each were put into the two parts (a and b) separately. The control also was set up but without contamination. The plastic rubbers were covered and left for twenty-four hours. The avoidance test was carried out to evaluate the ability of earthworms to detect and avoid toxic areas in the soil that are contaminated with petrol as well as to determine the threshold concentration of petrol in the soil that affects the earthworm’s health or behaviours.

In continuation of the experimental setup, four earthworms were carefully dropped into each concentration prepared and the control. These were put under daily check-ups for reproductive health and mortality every seven days; the surviving earthworms were sorted out for laboratory tests. This experiment was conducted for a total of twenty-eight days

3.3 METHODS FOR ASSESSING REPRODUCTIVE HEALTH IN EARTHWORMS.

To evaluate the reproductive health of earthworms, a combination of controlled experiments and observational studies were conducted. The assessment involved key reproductive indicators such as cocoon production rate, hatching success, number of hatchlings, biomass, and overall growth (Gezahegn and Girum,2017b).Experimental setups were established using cylindrical plastic containers to simulate varying environmental conditions. Specifically, *Eisenia fetida* were cultured in different organic substrates, including rose, hypericum, and carnation flower wastes.

Three experimental treatments were prepared: T1 (rose leftover + cow dung), T2 (hypericum leftover + cow dung), and T3 (carnation leftover + cow dung), as outlined by Gezahegn and Girum (2017) (Gezahegn and Girum,2017b). The reproductive performance of earthworms was monitored across these treatments to determine how different substrates influenced their reproductive output.

To gain insights into population dynamics, productivity, and energy flow, the life cycle patterns of *Eisenia fetida* were studied. This involved tracking their growth rates, cocoon production, and reproductive organ development over time(Gezahegn and Girum,2017b)The impact of food sources on reproductive efficiency was also analyzed, as food availability and substrate composition significantly influence earthworm biomass and reproduction rates (Dominguez, 2004). The physicochemical properties of the substrate, along with its palatability and microbial composition, were examined to assess their effects on reproductive success (Suthar, 2007; Prasanthrajan and Kannan, 2011).

Observations from this study revealed that substrate quality plays a crucial role in determining reproductive potential. The results indicated that the nitrogen-rich rose leftover medium (T1) supported higher cocoon viability and more hatchlings than other treatments. This aligns with findings by Giraddi *et al*. (2010), who demonstrated that nitrogen content influences cocoon hatching success. The nitrogen content of the culture medium was found to be a determining factor in cocoon production and development, as it supports the protein synthesis required for earthworm reproduction(Gezahegn and Girum,2017b).Previous studies have also suggested that earthworms in nitrogen-enriched environments tend to produce more viable cocoons due to enhanced protein availability (Suthar, 2007).

The experimental approach employed in this study provided comprehensive insights into how environmental factors influence the reproductive health of *Eisenia fetida*(Gezahegn and Girum,2017b).The use of organic waste as a substrate not only supported earthworm growth but also demonstrated the potential for waste management through vermiculture (Gezahegn and Girum, 2017b). The findings contribute to a broader understanding of the biological requirements for earthworm reproduction and highlight the significance of substrate quality in optimizing their reproductive output.

3.4 HISTOPATHOLOGICAL TEST

Each of the selected earthworms was cut open and cut. Tissue samples from the body wall and viscera were cut and placed in the interior of a labelled tissue embedding cassette. The cassette was at that point put in a 24-hour automatic tissue processor for 18-hour for total processing. The processing includes total fixation in formal saline solution, chemical dehydration, and finally, insertion in molten paraffin wax. The implanted sample was cut with the microtome after the wax had solidified. Segments cut from the sample were, at that point, set on a clean grease-free slide for viewing beneath the magnifying lens. The slides were stained utilizing the H and E (Haematoxylin and Eosin) staining strategy before viewing to illustrate the general structure of the tissue. The slide is at that point seen microscopically and the tissue structures between each concentration and the control were compared.

CHAPTER FOUR

RESULTS

4.1 THE AVOIDANCE TEST OUTCOME

After twenty-four hours, the four earthworms in both the contaminated and the uncontaminated parts (A and B) died. The high volatility of petrol led to the spread of harmful vapour, which depleted the oxygen in the soil and killed earthworms. Their death confirmed that the presence of petrol is a strong deterrent and can be lethal.

4.2 MORTALITY RESULTS OF EARTHWORMS EXPOSED TO VARYING CONCENTRATIONS OF PETROL

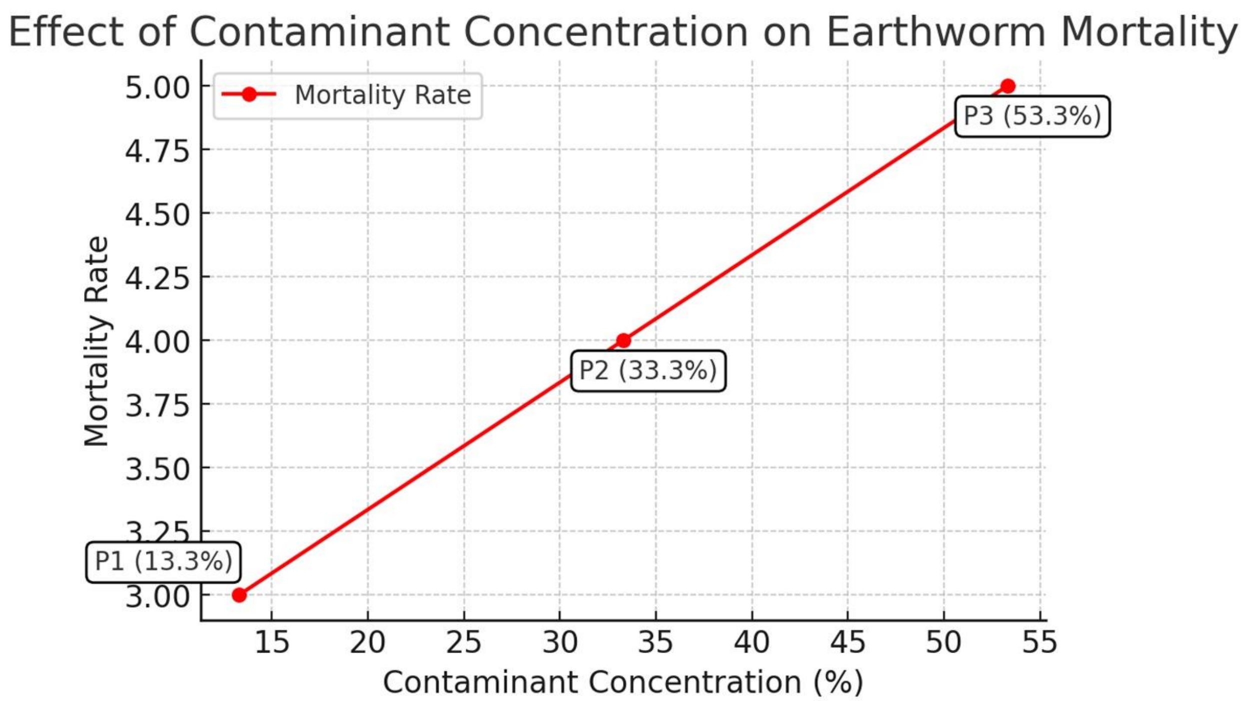
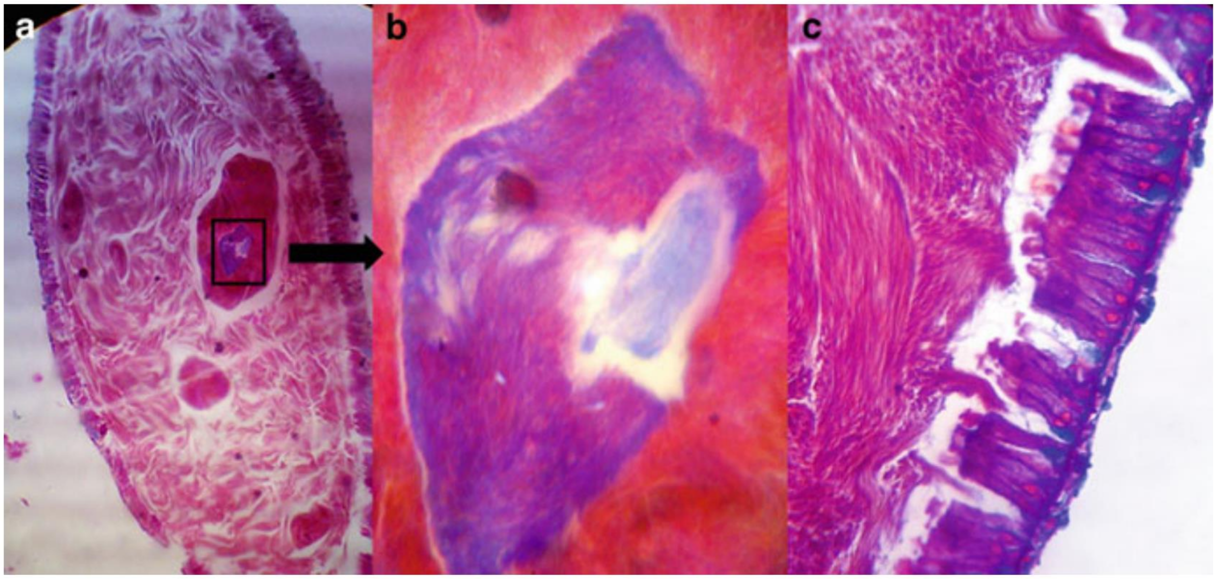


Figure 4.1: Graph showing the relationship between contaminant (petrol) concentrations and earthworm mortality rate, indicating a direct positive correlation of higher contamination leading to higher mortality.



4.3 REPRODUCTIVE CHANGES

Plate 4.1: Reproductive alteration in clitellum spermathecae in earthworm (*E. fetida*) exposed to varying petrol concentrations showing sperm degeneration (a, b) and sperm intrusions into the epithelium (c).

4.4 HISTOPATHOLOGICAL CHANGES IN THE BODY OF E. FETIDA EXPOSED TO VARIOUS CONCENTRATIONS OF PETROL

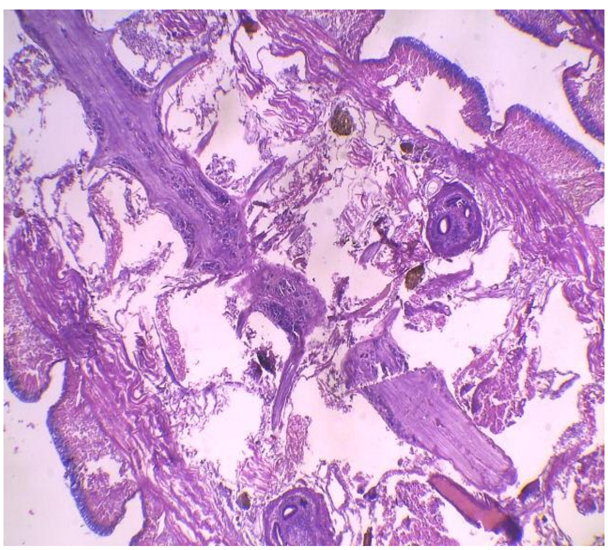


Plate 4.2: Histopathologic section of the body wall of *Eisenia fetida* after 7 days (control) showing an intact normal body wall muscle layer.

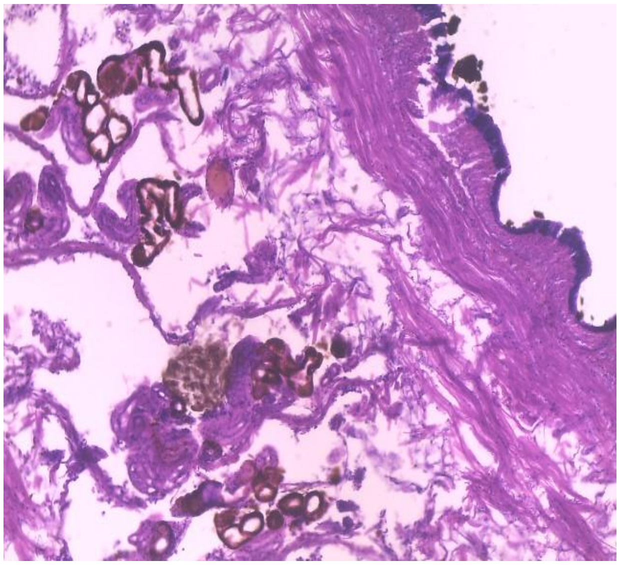


Plate 4.3: Histopathologic section of the body wall of *Eisenia fetida* after 7 days exposure to petrol P1A (0.4mol) showing mild lesion disruption of the body wall.

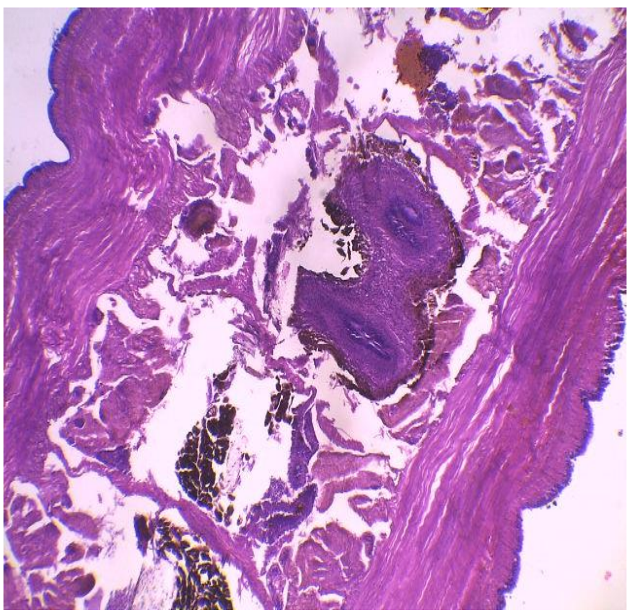


Plate 4.4: Histopathologic section of the tissue wall of *Eisenia fetida* after 7 days exposure to petrol P2A (1mol) showing pigment and distortion of shape.

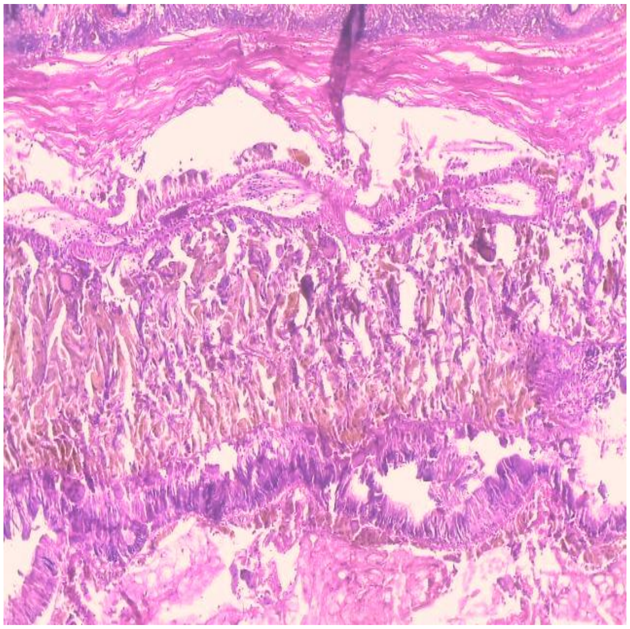


Plate 4.5: Photomicrograph of histopathologic section of the tissue wall of *Eisenia fetida* after 7 days exposure to petrol P3A (1.6mol) showing moderate lesion and cellular degeneration.

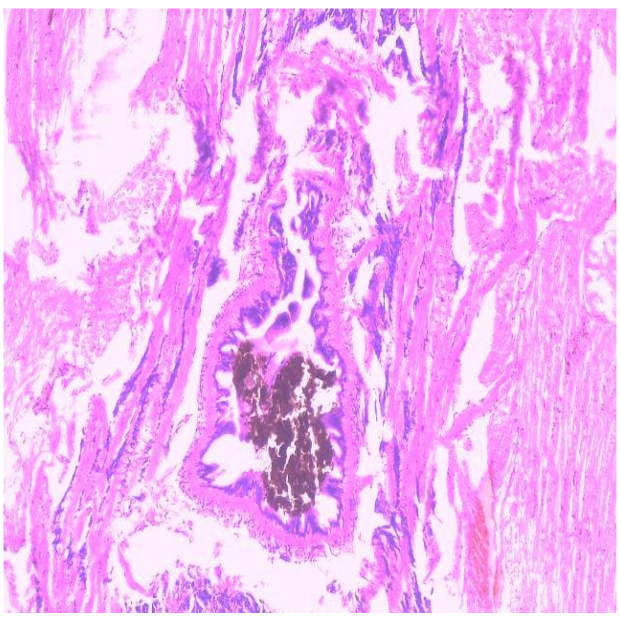


Plate 4.6: Photomicrograph of histopathologic section of the tissue wall of *Eisenia fetida* after 14 days (control) showing normal body layer viscera.

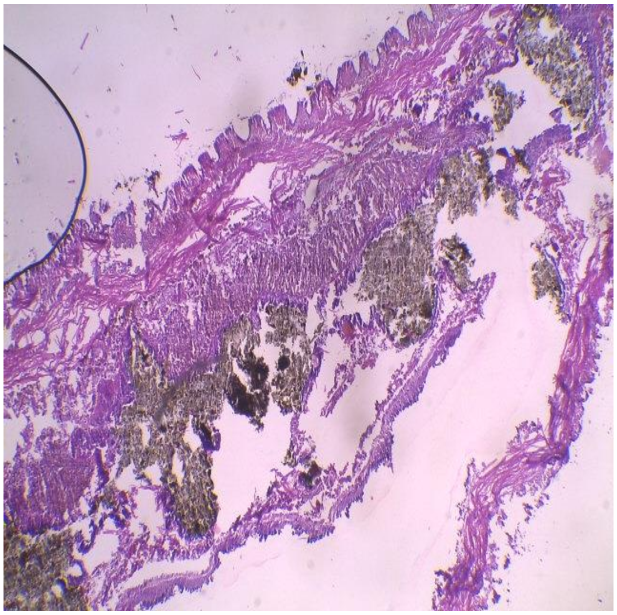


Plate 4.7: Photomicrograph of histopathologic section of the tissue wall of *Eisenia fetida* after 14 days exposure to petrol P1A (0.4mol) showing eroding of external tissue.

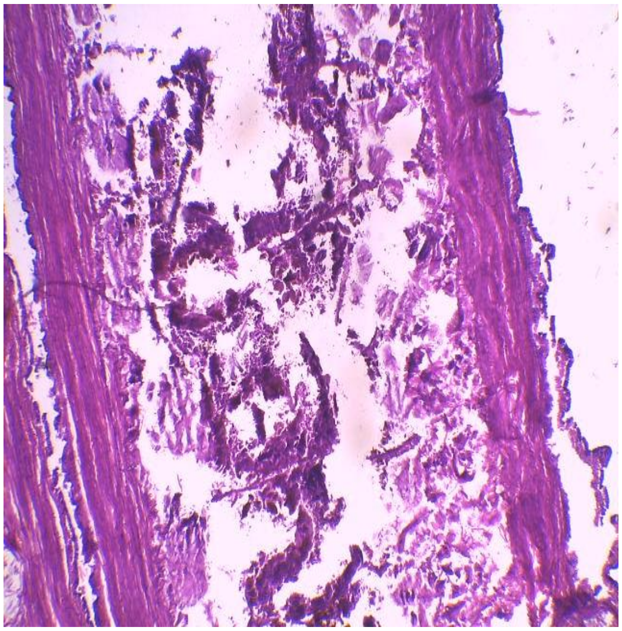


Plate 4.8: Photomicrograph of histopathologic section of the tissue wall of *Eisenia fetida* after 14 days exposure to petrol P2A (1mol) showing moderate eroding of internal tissue.

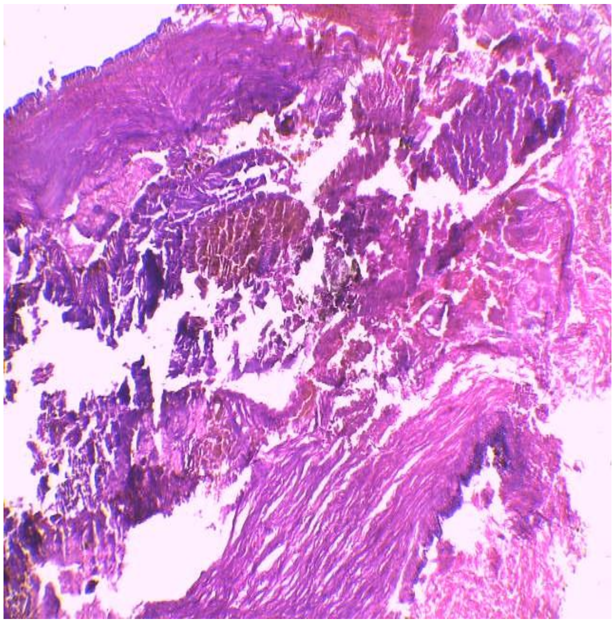


Plate 4.9: Photomicrograph of histopathologic section of the tissue wall of *Eisenia fetida* after 14 days exposure to petrol P3A (1.6mol) showing severe eroding of internal tissue.

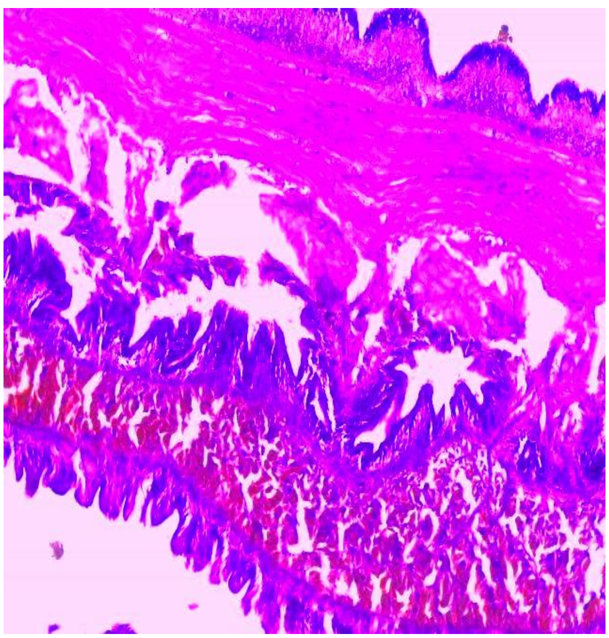


Plate 4.10: Photomicrograph of histopathologic section of the tissue wall of *Eisenia fetida* after 21 days (control) showing normal distinct section.

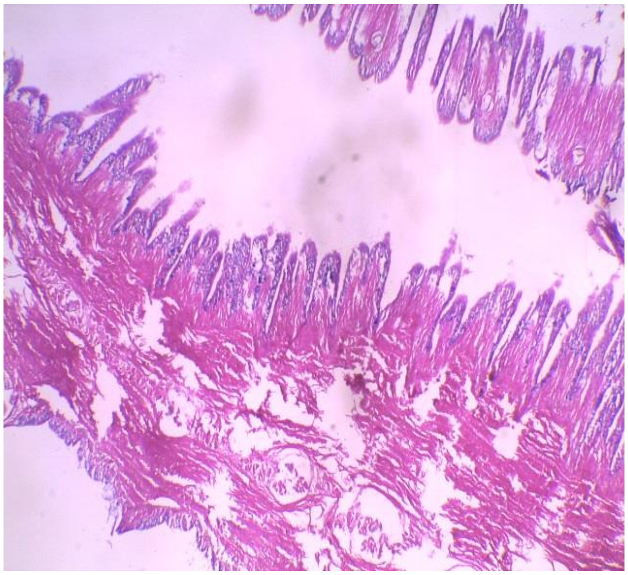


Plate 4.11: Photomicrograph of histopathologic section of the tissue wall of *Eisenia fetida* after 21 days exposure to petrol P1A (0.4mol) showing severe ulceration of the tissue wall.

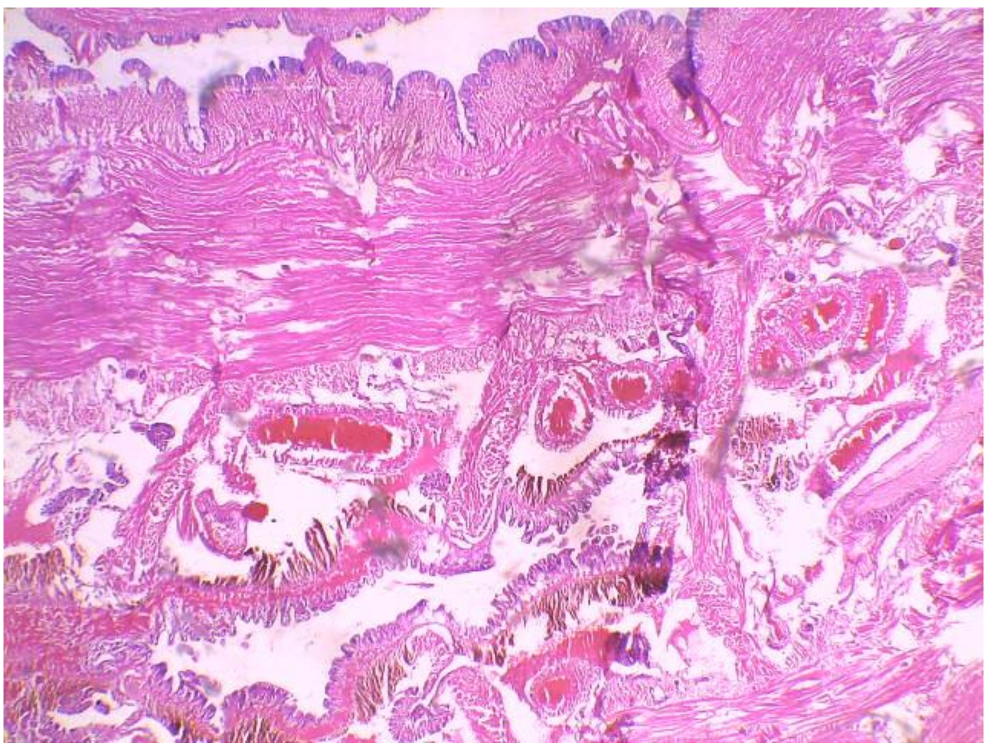


Plate 4.12: Photomicrograph of histopathologic section of the tissue wall of *Eisenia fetida* after 21 days exposure to petrol P2A (1mol) showing severe necrosis of the internal tissue wall.

Plate 4.13: Photomicrograph of histopathologic section of the tissue wall of *Eisenia fetida* after 21 days exposure to petrol P3A (1.6mol) showing formations of granuloma in the internal tissue wall.

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 DISCUSSION

5.1.1 MORTALITY RESPONSE TO PETROL CONTAMINATION

The results show a strong correlation between petrol contamination and earthworm mortality. The avoidance test confirmed petrol's immediate toxicity, as all earthworms in the contaminated sections died within 24 hours. The high volatility of petrol likely led to oxygen depletion and toxic vapour accumulation, causing asphyxiation. This aligns with previous studies indicating that petroleum hydrocarbons can disrupt soil aeration and directly intoxicate soil organisms (Smith *et al*., 2020).

Mortality trends across the 21-day exposure period further support the lethal effects of petrol. Initial zero mortality during the first 12 days suggests a short-term acclimation period, possibly due to earthworms' physiological adaptation to low-dose contaminants. However, by the 13th day, mortality emerged in 0.4 mol (13.3%) and 1 mol (33.3%) concentrations, and by the 14th day, the highest mortality (53.3%) was recorded in the 1.6 mol concentration. By day 21, severe necrosis and tissue granuloma formation were evident in highly contaminated samples, reinforcing that prolonged exposure exacerbates toxic effects. These results align with previous studies linking petroleum hydrocarbon toxicity to cellular damage (Kili, 2011). The progressive mortality pattern underscores the cumulative toxic effects of petrol contamination.

The decline or loss of earthworm populations due to petrol contamination has serious ecological consequences:

1. **Decreased Soil Fertility:** Earthworms play a key role in organic matter decomposition and nutrient cycling. Their decline slows nutrient release, negatively affecting soil health and crop yields.
2. **Soil Compaction:** Earthworm burrowing enhances soil aeration. Their loss increases soil density, reducing water infiltration and root penetration.
3. **Disruption of the Soil Food Web:** Earthworms are a key food source for various soil predators. Population decline could destabilize local biodiversity.
4. **Reduced Soil Microbial Activity:** Earthworms contribute to microbial community dynamics. Their absence may lead to decreased microbial diversity and impaired biochemical processes in the soil.

5.1.2 REPRODUCTIVE ALTERATIONS IN *EISENIA FETIDA*

Petrol exposure led to significant alterations in reproductive structures, particularly the clitellum, which is critical for cocoon production. The absence of cocoons in contaminated samples suggests that petrol-induced physiological stress impaired reproductive function. The dry season during which the experiment was conducted may have also contributed, as some earthworm species temporarily lose secondary sexual characteristics, especially the clitellum, under dry conditions.

The clitellum, essential for reproduction, exhibited significant changes across petrol concentrations. Sperm degeneration and epithelial intrusions were observed, indicating disruptions in reproductive capacity. These histological anomalies suggest a possible endocrine-disrupting effect of petrol hydrocarbons, which has been reported in other invertebrates (Jones and Patel, 2018).

Histological analysis revealed clear morphological deterioration of the clitellum, with notable degeneration in the spermatheca. The spermatheca, responsible for sperm storage post-copulation, showed epithelial intrusion and central degeneration, which would disrupt fertilization and hinder cocoon formation. Li *et al*. (2020b) also documented complete failure in cocoon production in E. fetida exposed to petroleum-contaminated soil. Similarly, Cosin *et al*. (2010) observed spermatheca deterioration due to contaminant exposure, supporting the argument that hydrocarbons interfere with reproductive function.

Additional structural alterations included ulceration, fibrosis, inflammation, and atrophy in the clitellum. These findings align with previous studies showing that chemical pollutants disrupt reproductive structures in earthworms, leading to reproductive failure (Ijomah *et al*., 2020). Given that earthworm populations rely on continuous reproduction for replenishment, the inability to produce cocoons may result in local population declines, ultimately threatening soil health and ecosystem stability.

5.1.3 HISTOPATHOLOGICAL DAMAGE IN EXPOSED EARTHWORMS.

The severity of histopathological alterations increased with both exposure time and petrol concentration. Control samples retained normal tissue structures, while contaminated samples exhibited progressive tissue degradation.

- By day 7, mild lesions and shape distortions appeared in lower concentrations, while higher doses led to moderate cellular degeneration.
- By day 14, erosion of external and internal tissues was evident, with severe erosion in the highest petrol concentration.
- By day 21, ulceration, necrosis, and granuloma formation were present in all contaminated samples.

Histopathological markers of toxicity observed in this study, including necrosis, fibrosis, and inflammation, have been widely documented in previous research. Doherty *et al*. (2019) reported that benzene, toluene, ethylbenzene, and xylene (BTEX) induced pathological changes in E. eugeniae, including cellular degeneration, severe necrosis, inflammatory responses, and muscle distortion. Similarly, Kili (2011) found epithelial enlargement, necrosis, and loss of structural integrity in E. fetida exposed to crude oil-contaminated soil. ROS (reactive oxygen species) produced during hydrocarbon exposure are known to drive these histopathological changes, leading to oxidative stress, inflammation, and cell death (Saint-Denis *et al*., 2001).

The correlation between increasing petrol concentration, higher mortality rates, and more severe tissue damage highlights the cumulative toxic effects of petrol exposure. The observed physiological and reproductive impairments suggest that petrol contamination compromises earthworm survival at both individual and population levels. These findings emphasize the need for remediation strategies to mitigate petroleum pollution in soil ecosystems. Similar findings have been documented in bioassays assessing petroleum-contaminated soils, where oxidative stress and inflammatory responses contribute to tissue breakdown (Lee *et al*., 2019).

5.2 CONCLUSION

This study demonstrates that petrol contamination significantly impacts the survival, reproduction, and physiological integrity of *Eisenia fetida*. Mortality rates increased with higher petrol concentrations, confirming that petrol acts as a potent toxicant. The absence of deaths in the control group validates the experimental conditions. The progressive increase in mortality highlights petrol's disruption of essential physiological functions, ultimately leading to death.

Reproductive toxicity was evident, particularly through the absence of cocoon production. The failure in reproduction was due to both environmental conditions and petrol's toxic effects. The study was conducted during the dry season, when some earthworm species naturally lose their secondary sexual characteristics, including the clitellum, which is essential for reproduction. Petrol contamination further exacerbated reproductive dysfunction by causing significant structural deterioration in the spermatheca, responsible for sperm storage. Histological analysis revealed degenerative changes in the spermatheca's epithelial tissues, impairing fertilization and cocoon formation. The structural and morphological alterations in the reproductive organs, including ulceration, fibrosis, inflammation, and atrophy, highlight the severe impact of petroleum toxicity on earthworm reproductive health. The inability to produce cocoons limits population renewal, increasing the risk of local extinction in contaminated environments.

Histopathological changes further confirmed petrol's toxic effects. The severity of these alterations correlated with increasing petrol concentrations, with the most severe tissue degradation observed at higher contamination levels.

5.3 RECOMMENDATIONS

Mitigation strategies are necessary to reduce petroleum contamination in soil ecosystems. The following strategies are recommended:

1. Implement bioremediation techniques to mitigate petroleum contamination in soils, promoting the growth of microorganisms capable of degrading hydrocarbons and restoring soil health.
2. Conduct regular soil health monitoring, focusing on pollutant levels, especially hydrocarbons, to prevent detrimental impacts on soil organisms like earthworms.
3. In areas with known contamination, employ soil remediation methods such as phytoremediation or soil washing to reduce pollutant concentrations and safeguard the biodiversity of soil ecosystems.

4. As regards pollution mitigation, advocating for stricter environmental regulations to control petroleum pollution, including better management of petroleum spills and waste disposal to prevent contamination of soil environments is indispensable.
5. Also, raising awareness about the harmful effects of petroleum and other pollutants on soil health and encouraging sustainable practices in agriculture, waste management, and industrial activities is an effective measures of pollution mitigation.
6. Encouraging the development and implementation of advanced pollution control technologies that reduce the release of hydrocarbons into the environment, particularly in industrial and urban settings, is an antidote for mitigating pollution.
7. The findings of this study underscore the need to address soil pollution, as the survival and reproductive capacity of earthworms are critical to the functioning of healthy soils.

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