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Morphological, Biochemical and Molecular Responses of Earthworms to Environmental Contaminants

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A B S T R A C T

Earthworms are keystone soil organisms and serve as sensitive bio-indicators due to their direct interaction with pollutants. Exposure to heavy metals induces oxidative stress, alters antioxidant enzyme activity and damages gut tissues. Herbicides like diuron trigger ROS accumulation, histopathological damage in intestinal epithelium and DNA fragmentation. Antibiotics such as oxytetracycline impair antioxidant defences, modify metabolic pathways, and reduce coelomocyte viability. Micro-plastics, both biodegradable and non-biodegradable, disrupt biomass, gut micro-biota, and enzymatic activity in different earthworm species. Exposure to petroleum hydrocarbons affects glycolysis, TCA cycle, and amino acid metabolism, demonstrating metabolic disruption. Combined pollutant exposures, such as neonicotinoids with heavy metals, intensify oxidative damage, AChE inhibition, and genotoxicity.

Introduction

Earthworms (families Lumbricidae and Megascolecidae) are recognized as important soil invertebrates due to their indispensable role in regulating soil structure. They are necessary for nutrient turnover and maintaining microbial community dynamics in soil. Their burrowing activity and continuous ingestion and egestion of soil influences soil porosity, aeration and water retention. Thus, they create a favourable habitat for both plants and microorganisms.

Beyond their capacity to manage ecosystem, earthworms also act as crucial mediators of organic matter decomposition. They also facilitate the conversion of complex residues into bioavailable nutrients that reinforce terrestrial productivity.

As they are close to and in persistent contact with soil and its substrates, they are highly sensitive to xenobiotics and environmental contaminants, ranging from heavy metals, pesticides, pollutants such as micro-plastics and pharmaceuticals. This ecological sensitivity makes them valuable bio-indicators of soil quality and environmental pollution. Bioassays based on earthworms can be utilised to understand soil ecotoxicology. It can help to assess acute toxicity as well as can be used to evaluate sub-lethal responses such as oxidative stress, enzymatic modulation, and reproductive impairments (Shukla *et al.,* 2011). Recent studies emphasise the ability of earthworms to bio-accumulate and bio-magnify contaminants and thus they serve as a consolidative measure of pollutant bioavailability within terrestrial ecosystems.

As global concerns about soil degradation and chemical pollution increase, earthworms can be utilised for assessing environmental stressors as the studies are cost-effective, ecologically valid, and ethically acceptable.

Morphological Relevance

Heavy metals, such as cadmium (Cd) and lead (Pb), are major toxicants for earthworms. Cd exposure induces epidermal lesions, pigmentation anomalies, and segmental swelling (Lapinski and Rosciszewska, 2008; Hirano and Tamae, 2010). Lead exposure disrupts calcium and zinc metabolism, causing degeneration of coelomocytes and altered membrane stability (Yadav *et al.,* 2023).

Pesticides impose physiological stress; they mostly go through the skin and the body wall of earthworms. They cause reduction in biomass, disturb clitellum development, cocoon production and post-hatching development of young individuals and lower the earthworm population in soil (Soda and Arimoro *et al.,* 2025). Chlorpyrifos impairs gut epithelial cells and nutrient absorption, Carbendazim alters enzyme activity in *Eisenia fetida*, initially stimulating and later inhibiting acetylcholinesterase (Wang *et al.,* 2024).

Pesticides can disrupt gut microbial diversity, affecting symbiotic bacterial communities (Astaykina *et al.,* 2022). Antibiotics, such as oxytetracycline, cause swelling of body segments and intestinal tissue damage in earthworms (Liu *et al.,* 2022).

Antibiotic contamination in the soil disrupts gut microbiota and impairs enzymatic activity (Feng *et al.,* 2018). Residual antibiotics reshape the gut biome and promote antibiotic-resistant bacteria (Yatoo *et al.,* 2022).

Microplastics alone have the capability to reduce growth and reproductive output (Zhang *et al.,* 2022). Microplastics ingested particles reduce body weight, survival, and reproduction, while obstructing gut function (Amaya-Vías *et al.,* 2025; Klimasz and Grobelak, 2025). Microplastics can also enhance the bioavailability of heavy metals, aggravating toxicity (Xin Bao *et al.,* 2025). Even aged polyethylene particles can act as carriers for pesticides like azoxystrobin, amplifying combined toxicity (Thakur *et al.,* 2025).

Biochemical and Molecular Relevance

Pollutants trigger oxidative stress in earthworms. This is indicated by reactive oxygen species (ROS) accumulation and upregulation of antioxidant enzymes like SOD, CAT, and GPx (Qiao *et al.,* 2022). Pesticides such as carbendazim and chlorpyrifos significantly reduce AChE activity in *Eisenia fetida*, highlighting sub-lethal neurotoxicity (Wang *et al.,* 2024). At the molecular level, pollutants induce DNA damage, alter gene expression, and trigger epigenetic modifications (Dong *et al.,* 2021).

Damages occurring in the nervous system cause neurotoxic effects including acetylcholinesterase (AChE) inhibition, impairing locomotion and feeding. Neurotoxic effects result in impaired locomotion and changes in the feeding behaviour (Shukla *et al.,* 2011).

Fungicides, heavy metals and antibiotics can cause methylation changes and transcriptional dysregulation affecting detoxification pathways in earthworms. Metabolomic parameters shift in response to pollutants, cause disruption in cellular metabolism and creates stress response pathways (Brown *et al.,* 2009; Xia *et al.,* 2023).

They can cause severe DNA damage, altered gene expression, and epigenetic modifications even in low doses. Stress resistance of earthworms may vary depending on their diet and environmental contaminants present in soil (Wen *et al.,* 2023; Li *et al.,* 2025).

Morphological Changes in Earthworms under Pollutant Stress

External Morphological Alterations

Epidermal Lesions and Pigmentation Changes

Exposure to cadmium and lead, induces epidermal ulceration, roughness, and pigmentation loss in *Eisenia fetida*. These metals interfere with melanin synthesis and disrupt epidermal cell membranes, leading to oxidative stress and cellular necrosis (Lapinski and Rosciszewska, 2008).

Chlorpyrifos exposure similarly disrupts cuticle integrity, causing localized tissue degeneration, thinning of the epidermal layer, and impaired moulting processes. Chronic pesticide exposure may also affect the secretion of coelomic fluid proteins, reducing the worm's defence against microbial pathogens.

Polyethylene and PET particles induce segmental swelling, cuticular abrasions, and irregularities on their body surface. Ingestion of microplastics can physically obstruct the gut, triggering coelomic swelling. This indirectly causes epidermal stress. Studies further demonstrate that microplastics act as vectors for adsorbed pollutants and damage internal tissues. These stressors impair locomotion, feeding efficiency, and reproductive output. (Zhang *et al.,* 2022).

Clitellum Deformities and Reproductive Impairment

Exposure to diuron significantly reduces clitellum size and decreases cocoon production in earthworms, indicating disruption of reproduction (Wang *et al.,* 2023).

Petroleum hydrocarbons can cause atrophy of reproductive organs as they are found to bioaccumulate in lipid-rich tissues, cause oxidative stress, and interfere with steroid hormone pathways, leading to impaired gametogenesis (Liu *et al.,* 2023).

Antibiotics such as oxytetracycline disrupt the development of reproductive tissues, altering germ cell proliferation and reducing reproductive output (Lirui *et al.,* 2024)

Swelling, Body Weight Reduction, and Growth Impairment

Toxic metals in soil including heavy metals induce segmental swelling and reduce overall body weight due to disruption of ion balance, osmotic stress, and damage to gut epithelium (Vaidya, 2023).

Microplastics reduce growth rates and biomass by physically obstructing the digestive tract and impairing nutrient absorption. Antibiotic exposure exacerbates nutrient assimilation defects as gut microbial communities get altered or destroyed and this disrupts enzymatic activity. The breakdown and uptake of essential nutrients is further impaired. Combined exposure to these stressors can amplify oxidative stress and metabolic disruptions, decreasing growth and they compromise overall physiological fitness (Amaya-Vías *et al.,* 2025; Klimasz and Grobelak, 2025).

Internal Morphological Alterations

Histopathological Damage

Exposure to heavy metals thins the intestinal epithelium and induces necrosis in *Eisenia fetida*, compromising nutrient absorption and disrupting overall metabolic function.

Pesticides provoke mitochondrial swelling, vacuolation, and epithelial degeneration, causing impaired cellular energy metabolism and oxidative stress in gut tissues (Wang *et al.,* 2024).

Microplastic ingestion disrupts gut lining abrasion, inflammation, and alters gut microbial composition. Physical and chemical distresses weaken digestion, nutrient assimilation and coelomic tissue health highlighting the sensitivity of earthworm gastrointestinal systems to environmental contaminants (Guo *et al.,* 2023).

Reproductive System Degeneration

Hydrocarbons exposure results in a reduction of seminal vesicle size and oocyte density in earthworms due to their bioaccumulation in lipid-rich tissues.

They cause oxidative damage and disruption of steroidogenic pathways leading to compromised gametogenesis. Reproductive disturbances can significantly reduce reproductive success. Antibiotics alter protein composition and also reduce antimicrobial and enzymatic activity. This affects immune defence and nutrient transport (Zhou *et al.,* 2024).

Coelomic Cavity and Immune Cell Alterations

Heavy metals and pesticides reduce coelomocyte counts. They interfere with signaling pathways, impair phagocytic activity in earthworms and affects innate immune defence. They also increase susceptibility to pathogens. These immunotoxic effects occur due to cellular stress. Coelomic disturbances can significantly reduce population viability (Xia *et al.,* 2023).

Biochemical and Molecular Responses

Oxidative Stress

Environmental contaminants and soil pollutants induce oxidative challenges like presence of reactive oxygen species (ROS), antioxidant enzymes, lipid peroxidation and membrane damage. These pollutant-induced oxidative and stress responses damage cellular homeostasis, metabolism, and overall fitness (Vaidya, 2023; Wang *et al.,* 2024).

Neurotoxicity

Pesticide exposure inhibits acetylcholinesterase (AChE) activity in earthworms, disrupting cholinergic signaling and impairing locomotion, burrowing, and feeding behaviour due to carbaryl exposure (Gambi *et al.,* 2007). They can additionally induce oxidative stress in neuronal tissues, aggravating behavioural responses. Petroleum hydrocarbons disrupt neurotransmitter balance, cause altered nerve impulse transmission, disrupting coordination as well as foraging efficiency.

All these neurotoxic stresses create danger for survival (Wang *et al.,* 2024).

Metabolic and Enzymatic Alterations

Petroleum and hydrocarbons disrupt glycolysis, the tricarboxylic acid (TCA) cycle, and amino acid metabolism, leading to reduced energy production and compromised physiological performance.

These alterations affect growth, reproduction and overall fitness (Liu *et al.,* 2025).

Immune and Coelomocyte Responses

Heavy metals and neonicotinoids reduce coelomocyte density and impair phagocytic activity. This weakens innate immune defences and pathogen clearance (Vaidya, 2023). Neonicotinoid-induced oxidative stress further diminishes coelomocyte functionality and cytokine signalling (Zhou *et al.,* 2024).

DNA Damage and Molecular Responses

Soil pollutants induce DNA strand breaks and oxidative lesions in earthworm tissues, reflecting compromised genomic integrity and increased cellular stress (Wang *et al.,* 2023). Treatment with antibiotics influences immune gene transcription, affecting coelomocyte-mediated defence and antimicrobial peptide production (Zhou *et al.,* 2024).

In conclusion, earthworms are critical bio-indicators for monitoring soil pollution due to their sensitivity to chemical, pharmaceutical, and microplastic contaminants. Their constant soil contact and ingestion of organic matter make them highly susceptible to pollutants, allowing for early detection of ecosystem disturbances. The major markers to identify their distress include morphological and biochemical markers.

Morphological markers like epidermal lesions describe heavy metal-induced stress and indicate tissue necrosis and cuticular damage. Clitellum deformities are observed under pesticide exposure affecting fertility. Segmental swelling, roughened body surface, and reproductive organ degeneration appear due to antibiotic exposure, impairing gametogenesis and coelomic fluid function. Microplastics induce surface irregularities and interfere with nutrient assimilation, compounding morphological stress.

Biochemical markers can be used as an early warning of soil pollution. Oxidative stress is evident through ROS accumulation, lipid peroxidation, and upregulation of antioxidant enzymes and lead to cellular damage. Neurotoxicity impairs locomotion and feeding efficiency, and affects organic matter turnover. Metabolic disruption alters glycolysis, TCA cycle, and amino acid metabolism. Microplastic exposure alters gene expression related to antioxidant defence, detoxification, and immunity.

As Earthworms provide an all-inclusive assessment tool for soil ecosystem health therefore, they serve as sensitive early-warning indicators of pollutant risk and thus they can be used for understanding risk assessment and management of contaminated soils. Future research should emphasize long-term, field-based studies, standardization of morphological, biochemical, and molecular biomarkers, and evaluation of pollutant mixtures at environmentally relevant concentrations to maintain a healthy soil environment. Understanding interactions among pollutants, microbiota alterations, and metabolic pathways is essential for predicting soil ecosystem resilience and designing mitigation strategies.

Table.1 Key Morphological, Biochemical, and Molecular Changes Observed in Earthworms Under Different Pollutant Stresses

S. No.	Pollutant Type	Morphological Changes	Biochemical Changes	Molecular/Cellular Changes
1.	Heavy metals (Cd, Pb)	Epidermal lesions, segmental swelling, clitellum deformities	Increase in ROS, SOD, CAT, Lipid peroxidation	DNA damage, coelomocyte reduction
2.	Pesticides (Diuron, Chlorpyrifos)	Clitellum deformities, gut tissue degeneration	Increase in ROS, MDA, AChE inhibition	Mitochondrial swelling, gene expression change
3.	Antibiotics (Oxytetracycline)	Body segment swelling, surface roughness	Decreased GPx, impaired coelomic fluid metabolism	Altered immune gene transcription
4.	Microplastic (PE,PET,PBAT)	Segmental swelling, surface roughness, reduce growth	Increases in ROS,HSP70/HSP90 upregulation, enzymatic disruption	Gut microbiota dysbioses, gene modulation
5.	Petroleum hydrocarbons	Degeneration of reproductive organs, reduce growth, gut histology	Disruption of glycolysis, TCA cycle, amino acid metabolism	Altered detoxification enzyme expressions
6.	Combined pollutants	Severe epidermal lesions, reproductive organ atrophy	Increase in ROS, AChE inhibition, lipid peroxidation	Enhanced DNA damage, coelomocyte depletion
7.	Pharmaceuticals Microplastics	Growth inhibition, segmental deformities	Enzymatic disruption, oxidative stress	Metabolic dysregulation, gut microbiota imbalance

Author Contributions

Eshita Pandey (Corresponding Author): Conceived the original idea, wrote the manuscript, formal analysis, reviewing and validation; Anjali: Wrote the manuscript, formal analysis, reviewing, and editing.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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