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REVIEW PAPER

Decoding next-generation heavy metal bioremediation via species-specific Earthworm and its gut microbiome interactions: insights from molecular responses, multi-omics, synthetic biology, and artificial intelligence

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Abstract Heavy metal (HM) contamination represents a persistent global threat, demanding bioremediation strategies that are both mechanistically robust and ecologically sustainable. This review provides a next-generation perspective on vermiremediation by integrating species-level physiology, gut microbiome functionality, molecular detoxification pathways, synthetic biology innovations, multi-omics insights, and artificial intelligence (AI)-driven modeling into a unified framework. A central novelty of this work lies in the detailed elucidation of earthworm-microbe consortia and their synergistic contributions to metal sequestration, transformation, and detoxification-moving beyond traditional organism-centric views toward eco-engineered host-symbiont systems. We synthesize species-specific bioaccumulation patterns, toxicological responses, and detoxification mechanisms, supported by enrichment kinetic models. At the molecular scale, we highlight antioxidant defense pathways involving catalase, glutathione-S-transferase, and superoxide dismutase, alongside oxidative stress signaling, macromolecular damage, and thresholds that differentiate adaptive resilience from system failure. Advancements in synthetic biology includes gene editing, pathway reconstruction, and designer symbiotic microbes which are examined as emerging tools to enhance gut microbial functionality and engineer targeted metal-binding pathways. Multi-omics approaches provide a systems-level view of detoxification networks, revealing previously uncharacterized genes, enzymes, and metabolic signatures associated with HM tolerance and early biomarkers of sub-lethal stress. The incorporation of AI-based models introduces a data-driven dimension, enabling accurate prediction of remediation outcomes and optimization of vermiremediation strategies. Overall, this review advances vermiremediation from an empirical practice to a programmable, systems-biotechnology platform for sustainable HM bioremediation.

Keywords AI modelling · Earthworm-microbiome consortia · Heavy metal detoxification · Multi-omics and synthetic biology · Species-specific bioaccumulation

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Introduction

Heavy metal (HM) contamination has emerged as a critical global environmental and public health crisis,

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affecting soil, water, air, and biota across all continents. Unlike organic pollutants that degrade over time, metals persist indefinitely, cycling between soil, water, and biota, thereby posing long-term risks to ecosystems and human health (Hussain et al. 2023; Kharmawphlang et al. 2024a). Globally, industrialization, urbanization, and intensive agriculture have accelerated HM emissions into the environment. Major anthropogenic sources include mining, metal smelting, electroplating, tanneries, fossil fuel combustion, waste incineration, and excessive use of fertilizers and pesticides (Sabina et al. 2025a). Toxic HMs such as aluminum (Al), arsenic (As), lead (Pb), cadmium (Cd), cobalt (Co), chromium (Cr), mercury (Hg), selenium (Se), silver (Ag), and gold (Au) have no known beneficial biological functions and can be harmful even at very low concentrations. Their high reactivity allows them to disrupt cellular homeostasis by inactivating metal-sensitive enzymes and interfering with metabolic pathways, ultimately leading to cellular dysfunction and death (Hussain et al. 2023). In contrast, non-toxic HMs like iron (Fe), copper (Cu), nickel (Ni), magnesium (Mg), manganese (Mn), and zinc (Zn) play essential roles in physiological and metabolic processes but become toxic when present in excessive amounts (Sabina et al. 2025b). Importantly, all HMs, regardless of category, pose potential risks to living organisms when their concentrations exceed biological tolerance limits or when exposure is prolonged, as they accumulate in soft tissues and can trigger adverse biochemical and physiological effects (Hussain et al. 2023).

Severe contamination has been documented worldwide. Across Asia, HM pollution has reached critical levels in soils, groundwater, and drinking water, particularly in China, Bangladesh, Vietnam, Thailand, Nepal, and India (Chen et al. 2025a, b). China alone introduces nearly 0.9 million tons of HMs annually into waste streams, while in India, HM-contaminated groundwater spans more than 34,000 km^2 and threatens the health of nearly 30 million people (Kharmawphlang et al. 2024a). Globally, the United Nations Environment Programme (2021) estimates over 10 million contaminated sites, with HMs dominating roughly 50–60% of them (Hussain et al. 2023). In many developing nations, unregulated industrial discharge and informal e-waste recycling amplify the problem, particularly in peri-urban agricultural regions where crops are grown on polluted soils. At United States (U.S.) Superfund sites, soil Pb, Cd, and As concentrations (400, 15, and 30 mg/kg) meet or exceed EPA safety thresholds, especially for Cd and As (Chen et al. 2025a, b). They disrupt nutrient cycling, inhibit microbial activity, impair enzymatic processes, and progressively degrade soil fertility (Kharmawphlang et al. 2024a). Through leaching and runoff, metals contaminate groundwater and surface waters, eventually entering food chains via plants, livestock, and fish. Bioaccumulation and biomagnification elevate metal concentrations at higher trophic levels, intensifying toxicological effects in humans and wildlife (Hussain et al. 2023). In soils, HMs severely disrupt microbial communities by damaging cells and enzymes, reducing microbial biomass, and impairing key processes such as nutrient cycling, nitrogen transformations, and organic matter decomposition, while also disturbing beneficial plant-microbe interactions and altering soil pH, structure, porosity, and water-holding capacity (Angon et al. 2024). Excess metals destabilize soil aggregates, increase compaction and erosion, and alter chemical properties by affecting pH, redox reactions, nutrient balance, and organic matter decomposition (Adnan et al. 2025). Plants readily accumulate HMs in edible tissues, leading to reduced photosynthesis, inhibited enzymatic activity, oxidative stress, nutrient imbalances, stunted growth, and lower crop yields, thereby introducing metals into the food chain (Angon et al. 2024). Elevated HM levels have been widely reported in developing nations, where contaminated vegetables, grains, fish, and even livestock products contribute to chronic human exposure linked to renal, hepatic, neurological, cardiovascular, and developmental disorders, as well as carcinogenic effects (Adnan et al. 2025).

The human health burden of HMs exposure is profound and multifaceted. Chronic ingestion of contaminated food and water leads to severe neurological, renal, hepatic, cardiovascular, and developmental disorders (Angon et al. 2024). Each metal exhibits distinct environmental behavior. For instance, Pb persists in soils and is associated with cognitive impairment in children and hypertension in adults. Studies conducted by the National Health and Nutrition Examination Survey (NHANES) have identified alarmingly excessive Pb levels (greater than 10 mg/dL) in significant portions of the United States child population (Mitra et al. 2022). This is particularly

concerning because children and infants are far more vulnerable to even low-level Pb exposure than adults. Numerous published studies confirm the devastating adverse effects associated with elevated Pb blood levels in children, including decreased IQ scores, retarded neurobehavioral development, speech and language handicaps, and various impairments in cognitive development (Fatima and Singh 2023a, b). Cd, on the other hand, accumulates in crops and induces kidney damage, bone disorders, and developmental toxicity. Arsenic contaminates groundwater and causes skin lesions and internal cancers. Arsenic contamination, particularly prevalent in South and Southeast Asia, affects more than 200 million people worldwide, leading to skin lesions, cancer, and cardiovascular disease (Mitra et al. 2022). Across several regions globally, including China, Thailand, West Bengal, Bangladesh, Inner Mongolia, Hungary, Mexico, Chile, Argentina, and Finland, extensive epidemiological studies have documented that large populations exposed to high levels of As in their drinking water suffer from a wide range of severe health problems (Chen et al. 2025a, b). These As-related conditions include neurologic and neurobehavioral disorders, hematologic disorders, cardiovascular and peripheral vascular disease, and developmental anomalies (Adnan et al. 2025). Cr(VI), the most toxic Cr species, causes respiratory, gastrointestinal, and carcinogenic effects. Similarly, Hg exposure through fish consumption remains a pressing global concern, especially in coastal and artisanal mining regions (Chen et al. 2021). Even in developing countries, it is estimated that up to 10–15 million miners are still suffering from chronic Hg intoxication. From an ecological perspective, HMs disrupt biodiversity and ecosystem services. Their bioavailability, persistence, and multiple exposure pathways underscore the urgent need for environmental monitoring and effective remediation strategies. Soil invertebrates, plants, and microbes suffer from oxidative stress and enzymatic inhibition, leading to reduced soil fertility, lower crop productivity, and altered trophic interactions (Angon et al. 2024). This degradation of soil health threatens food security, particularly in low-income regions reliant on contaminated farmlands. Addressing the global burden of HMs contamination requires an integrated approach that combines pollution prevention, remediation technologies, circular-economy strategies, and policy enforcement (Kharmawphlang et al. 2024a). Conventional physicochemical remediation methods, including soil washing, vitrification, and chemical immobilization, are frequently bypassed because they are often prohibitively expensive, environmentally disruptive, and impractical for large-scale applications (Adnan et al. 2025). This has prompted the scientific community to pivot toward utilizing biological and nature-based solutions. These innovative approaches rely on harnessing the natural metabolic and ecological capabilities of living organisms to restore polluted environments effectively. Within this domain, earthworms have gained prominence as key ecosystem engineers, offering significant potential for both detoxifying HM-contaminated soils and concurrently enhancing soil structure and fertility (Hussain and Sarma 2025).

Earthworms are crucial components of terrestrial ecosystems due to their significant biomass and activity, contributing positively to the physical, chemical, and biological properties of the soil (Sabina et al. 2025c). Earthworms are widely employed in soil ecotoxicology studies and are essential for soil functioning, as they ingest organic matter, decompose it, and blend it with soil to create water-stable aggregates development (Fatima and Singh 2023a, b). Metals such as Cd, Cu, Hg, Zn, and Pb are frequently bioaccumulated in earthworm tissues, making them excellent biological indicators for monitoring HM soil pollution (Yadav et al. 2023). However, prolonged exposure to HMs can negatively impact their survival, growth, reproduction, cocoon production, behavior, and overall density, changes in antioxidant enzyme activities and cytokinesis, and damage to nephridia and DNA (Liang et al. 2011). Declining earthworm populations due to soil contamination, therefore, threaten soil quality and sustainable agricultural practices, particularly in regions like India (Sabina et al. 2025c).

While numerous studies have described HM toxicity in earthworms, the authors note a lack of a comprehensive review summarizing all aspects of this toxicity, the possible mechanisms involved, and the ensuing environmental consequences. This review addresses a critical gap in understanding the comparative physiological analysis of HM uptake, bioaccumulation, and detoxification pathways across diverse earthworm species. Earthworm species differ fundamentally in their feeding habits and ecological roles, which directly dictate how they interact with HMs in

the soil (Yadav et al. 2023). Uptake of HMs by earthworms is governed by a constellation of physiological traits such as dermal permeability, feeding mode and selectivity, gut transit time, presence and activity of metal transporters, and sequestration tissues such as chloragogenous cells (Xiao et al. 2022). These traits determine whether metals are absorbed, stored, transformed, or excreted, and they modulate both acute toxicity and long-term accumulation. Understanding physiology is therefore essential to predict species-specific performance as bioindicators, immobilizers, or vectors of contaminants. Different ecological categories of earthworms, such as epigeic, endogeic, and anecic (eg, *Eisenia fetida*, *Aporrectodea caliginosa* and *Lumbricus rubellus*), exhibit distinct physiological adaptations influencing metal absorption through the epidermis and alimentary canal, and their sequestration in chloragogenous tissues or binding with metallothioneins (MTs) (Kilpi-Koski et al. 2020). Most studies report single-species outcomes without mechanistic cross-comparison. A comparative review that organizes uptake pathways by physiological trait and links those traits to observed bioaccumulation patterns converts a scattered empirical literature into a predictive taxonomy. This makes it possible to recommend species for specific contamination scenarios and to parameterize mechanistic uptake models with biologically informed priors.

Remediation is a process that occurs over time, making instantaneous bioaccumulation factor (BAF) values insufficient for assessing long-term risk and cleanup duration. Kinetic models provide crucial parameters for translating experimental uptake data into transferable parameters (uptake rate m_1, elimination rate m_2, assimilation efficiency, and half-lives) (Xiao et al. 2022). These parameters enable prediction of accumulation over time, forecast of steady-state burdens, and estimation of time-to-recovery after remediation measures. By integrating one- and two-compartment kinetic models, this review highlights species-specific variations in uptake, elimination, and equilibrium phases of metal accumulation, offering a mechanistic understanding of exposure-response dynamics under various soil conditions.

The review also advances the field by focusing on the earthworm gut microbiota as an integral component of HM detoxification. The gut microbiome is the proximate catalytic environment for many transformations that determine metal speciation: reduction/oxidation, methylation/demethylation, biosorption, and biomineralization (Gudeta et al. 2023). Gut communities differ from bulk soil communities in composition, functional potential, and resilience, and they respond dynamically to metal exposure, diet, and host physiology (Liu et al. 2020a, b, c). Understanding which taxa and functions are reliably present or induced in earthworm guts under contamination is essential for predicting in vivo transformations and post-egestion soil outcomes. The composition and dynamics of gut-associated microbiomes, including metal-resistant genera such as *Bacillus*, *Pseudomonas*, and *Arthrobacter,* are discussed in relation to their metabolic potential for metal solubilization, chelation, and redox transformations (Gudeta et al. 2023). The novelty here lies in the detailed elucidation of earthworm-microbe consortia, where synergistic interactions enhance the overall efficiency of metal detoxification and transformation processes, leading to eco-engineered solutions for bioremediation.

At the molecular level, this review synthesizes findings on biochemical and enzymatic responses to metal stress, emphasizing antioxidant defense mechanisms involving catalase (CAT), glutathione-S-transferase (GST), and superoxide dismutase (SOD). It explores the molecular signaling pathways associated with oxidative stress, protein carbonylation, and lipid peroxidation, thereby linking biochemical markers to environmental metal toxicity. HMs disrupt proteins, lipids, and nucleic acids and raise reactive oxygen species, eliciting antioxidant responses (glutathione systems, SOD, CAT) and repair pathways. These molecular responses both signal stress and mediate tolerance or failure of detoxification mechanisms (Yadav et al. 2023). Profiling molecular responses helps distinguish adaptive upregulation from overwhelmed systems that presage population decline.

In addition, the incorporation of synthetic biology and multi-omics integration offers a novel frontier in vermitechnology. Through gene editing, pathway reconstruction, and modular genetic circuit design, synthetic biology allows the creation of engineered microbial strains capable of superior metal binding, redox transformation, biosorption, or biomineralization, as well as heightened production of chelators, siderophores, and detoxifying enzymes (Somayaji et al. 2022). In the context of earthworms, synthetic biology can be used to enhance gut microbial consortia, engineer symbiotic microbes with targeted

metal-processing functions, or introduce designer metabolic pathways that strengthen the worms' natural sequestration mechanisms. These tools also enable biosensing circuits for real-time detection and reporting of metal stress within worm tissues or microbial populations. Overall, synthetic biology brings unprecedented precision, tunability, and innovation to vermiremediation systems, creating engineered host-microbe partnerships with significantly improved detoxification efficiency and expanding the scope of earthworm-based bioremediation into a programmable, high-performance biotechnology platform. On the other hand, multi-omics approaches offer a transformative and novel framework for understanding HM detoxification in earthworms and their associated microbes by providing a holistic, systems-level view of the molecular, cellular, and metabolic processes involved (Phurailatpam et al. 2022). Unlike traditional single-endpoint assays, integrated genomics, transcriptomics, proteomics, metabolomics, and metagenomics reveal previously unknown detoxification genes, metal transporters, stress-response pathways, and microbial functional traits that drive metal tolerance. These approaches uncover how earthworms and their gut microbiota jointly regulate metal sequestration, redox transformations, and subcellular partitioning, while also identifying metabolites and microbial enzymes that directly influence detoxification efficiency (Raza et al. 2024). Multi-omics further enables early detection of sub-lethal metal stress through sensitive biomarker discovery and supports predictive modeling of species-specific remediation capacities. Together, these insights shift vermiremediation from an empirical practice to a mechanistic and potentially engineerable system, highlighting the novelty and power of multi-omics in advancing ecological and biotechnological applications. Furthermore, this review introduces the emerging role of AI-driven models in predicting bioremediation outcomes. Machine learning algorithms can analyse large datasets on metal concentration, soil physicochemical properties, and biological responses to forecast remediation efficiency and optimize treatment strategies (Zhang et al. 2021). This integration of artificial intelligence with ecological and biological data represents a forward-thinking approach toward data-driven environmental management. Overall, this review uniquely integrates species-level physiology, kinetic modeling, gut microbiome functionality, molecular biomarkers, synthetic biology, multi-omics, and AI-driven analytics to construct a comprehensive, mechanistic, and future-oriented framework for advancing earthworm-mediated HM detoxification and sustainable bioremediation.

Ecophysiological determinants of heavy metal uptake in Earthworms

Earthworms are globally distributed soil organisms that make up a major portion of below-ground biomass and thrive best under moderate temperature, neutral pH, and high moisture conditions (Sabina et al. 2025a). They vary widely in abundance and diversity, comprising about 4,000 species grouped into epigeic, anecic, and endogeic ecotypes, each contributing uniquely to soil processes such as organic matter decomposition, nutrient cycling, and soil structure improvement (Kharmawphlang et al. 2026). Earthworms enhance soil fertility through burrowing, casting, and promoting carbon and nitrogen stabilization, thereby benefiting crop productivity (Kharmawphlang et al. 2026). They also play key roles in pollution mitigation, including HM remediation, vermicomposting, wastewater treatment, and pathogen suppression (Sabina et al. 2025b). However, increasing HM concentrations severely impacts their survival and behavior, making them reliable bio-indicators of soil contamination. Epigeic species like *E. fetida* and *Eudrilus eugeniae* are widely used in vermicomposting and bioremediation, with research demonstrating their capacity to accumulate and reduce toxic metals by altering soil properties and interacting with plants and microbes (Kharmawphlang et al. 2024b). The uptake occurs primarily through oral ingestion of metal-laden soil particles and secondarily via cutaneous absorption from pore-water, although the efficiency of these pathways can vary depending on the metal and environmental factors (Srut et al. 2019). Oral intake of food and soil particles is generally the dominant route for bioaccumulation. Despite the prevalence of dietary uptake, numerous studies confirm that HMs can be absorbed through the skin of earthworms via the soil porewater (Xiao et al. 2022). Empirical work has established a positive correlation between the concentration of free metal ions in the soil solution and the metal content in the tissue of the earthworm. *E. fetida* showed elevated Cd accumulation as the concentration of free Cd^{2+} in the soil

solution increased. Sophisticated experimental protocols have definitively demonstrated this phenomenon, including mouth-sealing surgical procedures and filter paper exposure tests (Xiao et al. 2022). A key experiment by Vijver et al. (2003) involved sealing the mouth of *L. rubellus* with medical glue, which resulted in a significant decrease in Zn accumulation, confirming the importance of oral intake, but still leaving constant uptake levels for other metals like Cd, Cu, and Pb that were absorbed cutaneously. Additionally, Homa et al. (2005) showed that just three days of dermal exposure via HM-soaked filter paper increased metal concentrations in adult *E. fetida*, with accumulation increasing in the order Zn<Cu<Pb<Cd. Furthermore, it has been hypothesized that a damaged integument, or outer layer, could enhance dermal uptake by increasing ion diffusion through enlarged intercellular spaces, although this possibility lacks extensive research (Xiao et al. 2022).

Earthworms from all eco-physiological groups consume diverse materials, including soil microorganisms, detritus, and soil organic matter (Gudeta et al. 2023). The endogeic earthworms are particularly relevant to dietary uptake as they consume large amounts of soil concurrently with burrowing and feeding (Wang et al. 2018b; Srut et al. 2019). Studies indicate high soil ingestion rates for these geophagous species. For instance, geophagous worms consume between 0.2 and 6.7 g of dry soil per gram of earthworm daily, with some endogeic species, like *Pontoscolex corethurus*, showing ingestion rates as high as 3.2–26.0 g per gram of earthworm per day. This direct ingestion of metal-enriched soil particles and organic material is critical in evaluating HM uptake (Wang et al. 2018b). Intriguingly, some studies suggest that slight HM contamination may cause worms to increase their feeding rate, a compensatory behaviour that could aid in detoxification but might also increase total metal accumulation (Xiao et al. 2022). The intricate physiology of the earthworm gut significantly influences the fate and mobility of metals (Srut et al. 2019). A study by Duarte et al. (2012) mentioned that the secretion of calcium and carbonate in the gut of *E. fetida* has been shown to alter the fractionation of Cu and Zn from pig manure during digestion. Furthermore, the secretion of Ca from the intestine is effective in substantially reducing the mobility of Pb and Mn in both the body of the earthworm and the surrounding soil.

Species-specific bioaccumulation dynamics, toxicological impacts, and detoxification mechanisms of heavy metals in Earthworms

Earthworms exhibit strong capacities for bioaccumulation and biotransformation of HMs and organic contaminants, enabling them to function as effective agents for soil remediation, as shown in Fig. 1 (Xiao et al. 2022). HM uptake follows kinetic phases, including rapid initial accumulation, stabilization at a steady state, and rapid elimination once exposure ceases, as demonstrated for metals like Cr and Cu in *E. andrei* (Kilpi-Koski et al. 2020). Accumulation efficiency varies with both ecological category and physiology (Table 1). Endogeic worms generally accumulate more nonessential metals than epigeic or anecic species due to their ingestion of HM-rich mineral soil during burrowing, with studies reporting higher Zn, Cd, Pb, and Cu in *Aporrectodea caliginosa* compared to *Lumbricus rubellus* and greater metal loads in *Metaphire posthuma* than *Lampito mauritii* (Xiao et al. 2022). Species-specific traits such as alimentary tract structure, assimilation efficiency, and excretion patterns further govern accumulation, with more elaborate typhlosoles in endogeic *Aporrectodea* spp. enabling greater Cd and Pb uptake than *E. fetida* (Gupta 2020). Differences in detoxification strategies, including subcellular partitioning and targeted sequestration of metals in tissues like the chloragogenous tail segment, allow species such as *Pheretima posthuma* to safely store accumulated elements, including Se, for long periods (Yue et al. 2021). These interspecific variations underline the importance of comparative studies to identify how ecology, feeding behavior, and detoxification mechanisms shape species-specific HM burdens and their suitability as bioindicators or vermiremediators. Bioaccumulation factors (BAFs) generally increase with rising concentrations of bioavailable metals, such as DTPA-extractable fractions (Wang et al. 2018b). Studies show species-specific trends, with *E. fetida* often exhibiting BAFs in the order Cd > Ca > Cu > Mg > Pb > Sr > Zn and a higher affinity for certain chemical forms, such as selenium compared to selenite (Ecimovic et al. 2018). Native species in Hunan Province, including *Amynthas homochaetus, A. pecteniferus, and A. heterochaetus,* exhibited relatively low BAFs in soils where metal levels followed Cd > Zn > Cu > Pb (Wang et al. 2018b).

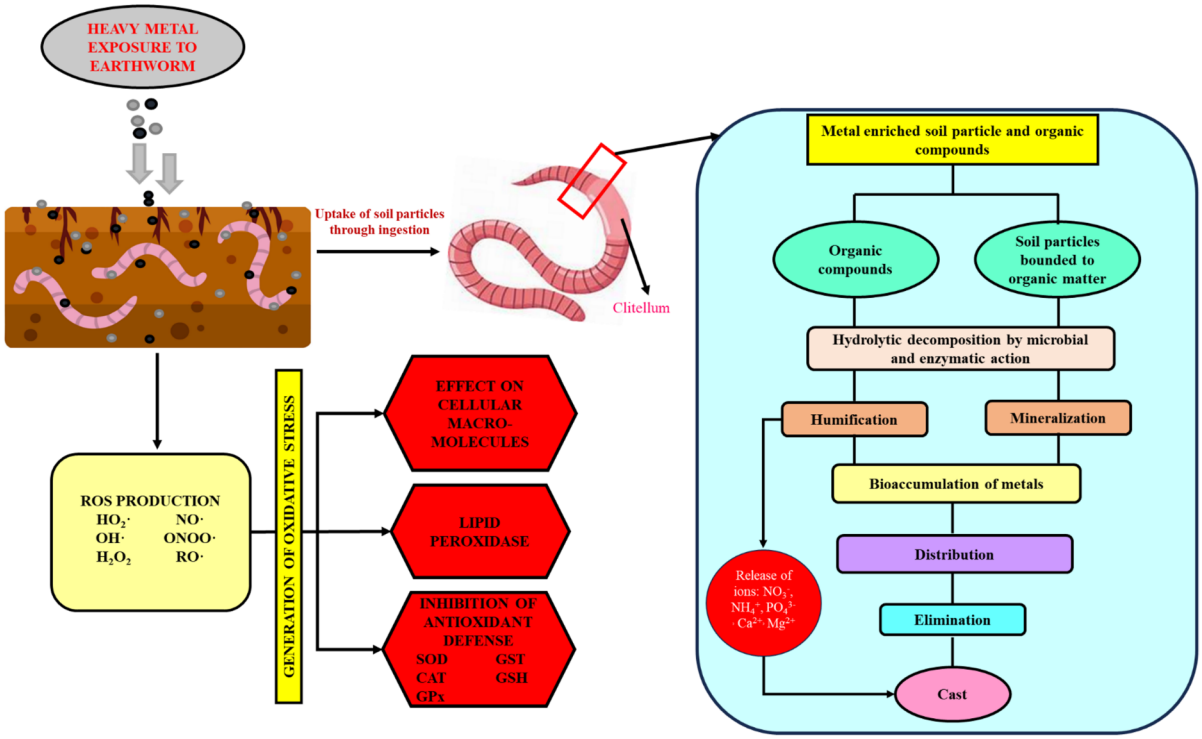

Fig. 1 Mechanisms of heavy metal uptake, oxidative stress responses, and vermicomposting-mediated detoxification and redistribution by earthworms

HM contamination severely affects earthworm health, reducing growth, reproduction, and population stability as shown in Table 2. Field studies show declining biomass, survival, and diversity near industrial pollution sources, while laboratory findings reveal that metals like Pb cause mortality, sperm deformities, and impaired fertilization (Yadav et al. 2023). Exposure to multiple metals can slow down hatching and result in cocoons that fail to produce viable offspring, while Cd exposure also delays the maturation of juveniles (Andleeb et al. 2024). Moreover, even when cocoon production remains unaffected, a decline in juvenile numbers indicates reduced offspring viability (Yadav et al. 2023). Dose-dependent toxicity of Pb and Ni significantly decreases survival, growth, and cocoon production in *E. eugeniae* (Gupta 2020). Notably, *E. eugeniae* and *L. mauritii* show strong tolerance in municipal solid waste systems, with *E. eugeniae* exhibiting a 2.9-fold weight increase, highlighting its suitability for HM-tolerant vermiremediation (Kharmawphlang et al. 2024b).

Earthworms have evolved sophisticated detoxification mechanisms involving internal sequestration, gut-mediated chemical transformation, and microbial symbiosis to tolerate high concentrations in contaminated soil (Yadav et al. 2023). The primary internal detoxification mechanism is sequestration within chloragogenous tissues, which line the coelomic surfaces and are enclosed within the typhlosole fold of the posterior alimentary canal, and the intestinal epithelial cells, where metals bind to MTs, cysteine-rich proteins that buffer essential metals and safely immobilize toxic ones (Hussain et al. 2021). This binding acts as a cellular metal buffer, essential for two key functions, including storing biologically necessary metals and safely sequestering toxic ones (Ogunlaja et al. 2020). Metals are removed from the body either by excretion via the posterior alimentary canal or directly by coelomocyte extrusion through the dorsal pores (Yadav et al. 2023). Consequently, MTs

Table 1 Effectiveness of earthworm-assisted remediation in heavy metal detoxification (Nishad et al. 2024)

Sl. No	Substrate(s)	Earthworm species	Metals detoxified	Remediation efficiency	Reference
1	Buffalo dung mixed with kitchen waste	*Eisenia fetida*	Co, Cr, Pb, Ni, Cd, As	Metal reduction achieved: Co (2.47%), Cr (0.40%), Pb (64.12%), Ni (8.02%), Cd (0.34%), As (2.10%)	Bhartiya and Singh (2011)
2	Sewage sludge combined with spent mushroom compost	*Lumbricus rubellus*	Cr, Cu, Zn, Cd, Pb	Major removal of Cd, Cr, and Pb (90–98.7%); slight increase observed in Zn and Cu	Azizi et al. (2013)
3	Soil	*Metaphire californica*, *Amynthas homochaetus*, *Amynthas pectiniferus*, *Amynthas heterochaetus*	Cd, Zn, Cu, Pb	Earthworms displayed elevated metal uptake in polluted areas; bioaccumulation followed the order Cd > Zn > Cu > Pb	Wang et al. (2018a)
4	Cotton textile sludge blended with cow dung	*Eudrilus eugeniae*	Cr, Zn, Cd, Pb	Total heavy metal content decreased by approximately 50–60%	Paul et al. (2020)
5	Soil	*Eudrilus eugeniae* and *Lumbricus terrestris*	Cd, Cr, Pb, Ni, Zn	*L. terrestris* showed greater removal of Cd, Zn, and Ni, while *E. eugeniae* was more efficient in eliminating Cr and Pb	Iheme et al. (2021)
6	Soil	*Aporrectodea rosea*, *Eisenia veneta*, *Allolobophora chlorotica*	Cu, Zn, Pb	Internal metal levels varied with tissue composition and possibly morphological features of the skin–muscular sac	Kokhia et al. (2022)
7	Soil	*Eisenia fetida*	Cu, Zn, Mn, Cr, Ni	Soil treated with *E. fetida* had reduced concentrations of Cu (17.4%), Zn (19.45%), Mn (9.44%), Cr (23.8%), and Ni (9.6%)	Borah and Deka (2023)
8	Animal dung mixed with soil and vermicompost	*Lampito mauritii*	Co, Cr, Pb	*L. mauritii* decreased Pb by 30.91%, Cr by 9.40%, and Co by 43.75%	Borah and Deka (2024)
9	Municipal solid waste enriched with animal dung	*Lampito mauritii*	Co, Cr, Pb, Ni, Cd	*L. mauritii* reduced Co by 2.40%, Cr by 0.71%, Pb by 14.38%, Ni by 11.91%, and Cd by 1.11%	Fatima and Singh (2023b)

Table 1 (continued)

Sl. No	Substrate(s)	Earthworm species	Metals detoxified	Remediation efficiency	Reference
10	Municipal solid waste enriched with cow dung	*Eisenia fetida, Eudrilus eugeniae* and Lampito mauritii	Pb, Cd, Cr, Zn	*Eudrilus eugeniae* achieved 88.3% Cd, 93.9% Cr and 95.1% Pb reduction, *Lampito mauritii* showed 70.01% Cd, 77.5% Cr and 96.04% Pb reduction, while Zn removal was consistently high (75.9–86.1%) across all three earthworm species	Kharmawphlang et al. (2024a, b)
11	Sewage sludge mixed with sugar-cane trash	*Eisenia fetida*	Mn, Fe, Cu, Zn, Pb	Notable decline in metal levels: Cu decreased by 4.98–30.5%, Fe by 5.08–12.64%, Mn by 3.31–18.0%, Zn by 2.52–15.90%, and Pb by 2.38–20.0%	Nishad et al. (2024)
12	Sewage sludge	*Eisenia fetida, Eudrilus eugeniae, Perionyx excavatus*	Mn, Cu, Zn, Pb	Heavy metal concentrations reduced to levels below regulatory limits	Nishad et al. (2024)

are critical for HM detoxification, maintaining metal homeostasis, protecting tissues from oxidative damage, and facilitating the transfer of essential metals (Hussain et al. 2021). Studies have shown that MT expression is strongly detected not only in the chloragogenous tissue but also in the apical cytoplasm of intestinal cells, nephridia, secretory epithelia of the calciferous gland, and coelomocytes (Ogunlaja et al. 2020). Coelomocytes are known to express MTs and are crucial participants in the cellular stress response (Homa et al. 2015). Earthworms express two main MT isoforms, MT-1 and MT-2, both characterized by a high cysteine content (Yadav et al. 2023). Experimental results confirm a dose- and time-dependent increase in concentration of MT in the coelomic fluid of *L. terrestris* exposed to Cu and Cd and in *E. fetida* exposed to Pb (Chao et al. 2016), reflecting the protective surge. Further research on *L. rubellus* wMT-2 demonstrated that the Cd-bound form is structurally more stable than the Zn-bound form, suggesting a specialized biochemical role in protection (Kowald et al. 2016). The induction of MTs and associated defense mechanisms is significant across species, with Cd exposure notably elevating transcription in species like *M. posthuma* and *Polypheretima elongata* in a dose- and time-dependent manner, confirming the role of MT-2 in cellular protection and accumulation for potential bioremediation (Liang et al. 2011).

MT expression is a primary detoxification mechanism it does not fully explain all HM accumulation in earthworms. Another crucial non-MT component is phytochelatins (PCs), which are short, glutathione-derived peptides historically known in plants, but whose synthesizing enzyme, phytochelatin synthase (encoded by the *pcs* gene), is also found in earthworm species like *E. fetida* (Homa et al. 2015). In *E. fetida*, PCs act as HM-induced stress biomarkers with a high affinity for metals, particularly Cd^{2+} (Homa et al. 2015). Other non-MT proteins, like a large metal-induced protein (MIP), which is rich in glutamic contributes significantly to sequestration. This protein was found to accumulate ingested Cd in the intestinal region before its deposition in the chloragogenous tissue, demonstrating an alternative detoxification pathway (Hussain et al. 2021). Furthermore, the complete defense response involves the concurrent induction of other stress proteins, such as heat shock proteins (HSP72) in Cd-exposed *Allolobophora chlorotica,* and the differential expression of MT, annetocin, and

Table 2 Impact of heavy metal exposure on earthworm ecology (Yadav et al. 2023)

Heavy metals	Earthworm species	Exposure duration	Tested concentrations	Evaluated parameters	Key findings	Reference
Cu, Cr, Pb, Zn, Cd	*Allolobophora parva*, *E. fetida*	45 days	500–2500 mg/kg	Survival and cocoon formation	Higher concentrations produced strong decreases in survival and cocoon production	Mo et al. (2012)
Ni, Cu, Zn, Cd	*E. fetida*	90 days	Cu (40 mg/kg), Ni (61 mg/kg), Cd (85 mg/kg), Zn (100 mg/kg)	Survival, cocoon production, and body mass	All metals considerably suppressed survival, cocoon generation, and worm weight	Dominguez-Crespo et al. (2012)
Cu	*E. fetida*	28 days	25, 50, 100, 200 mg/kg	Cocoon formation and biomass	Copper exposure resulted in decreased cocoon numbers and lower body weights	Mo et al. (2012)
Pb, Ni	*E. eugeniae*	90 days	0.02–0.06 ppm	Survival, cocoon output, body length and mass	Ni and Pb showed dose-dependent adverse effects on growth, survival, and reproductive traits	Renu et al. (2020)
Pb	*E. fetida*	4 weeks	40–2500 mg/kg	Growth and survival	Lead did not affect survival but significantly inhibited growth	Zaltauskaite et al. (2020)
Cd	*E. fetida*	48 h	0–300 mg/L	Survival	No mortality occurred at 0–50 mg/L; limited mortality at 100–150 mg/L; substantial mortality observed at 200–300 mg/L	Liu et al. (2020a, b, c)
Cd, Pb	*E. fetida*	28 days	Pb (20–2500 µg/g), Cd (1–100 µg/g)	Growth, reproduction, and survival	Increased Pb levels resulted in higher mortality; Cd did not cause significant mortality. Both metals reduced biomass and cocoon output	Xiao et al. (2022)
Cd, Hg	*Eisenia fetida*	8 months	2–10 µg/g dry soil	Reproductive output and body weight	Both Cd and Hg negatively influenced reproduction; Cd did not alter body mass, while Hg exposure resulted in reduced biomass	Xiao et al. (2022)

Table 2 (continued)

Heavy metals	Earthworm species	Exposure duration	Tested concentrations	Evaluated parameters	Key findings	Reference
Cd, Pb	*E. fetida*	14 weeks	Cd (1–500 mg/g), Pb (20–2500 mg/g)	Survival, growth, maturation, cocoon production	Both metals impaired survival, caused abnormal development and juvenile weight loss, delayed sexual maturity, and lowered cocoon production	Yadav et al. (2023)
Ni, Pb, Hg	*Dendrobaena octaedra*	6 weeks	Ni (100–500 mg/kg), Hg (20–160 mg/kg), Pb (200–1200 mg/kg)	Survival and reproductive performance	All metals caused marked declines in survival and reproductive success	Yadav et al. (2023)

antimicrobial peptide genes in *E. fetida* (Mo et al. 2012). Beyond internal protein pathways, some species, like *E. eugeniae* and *L. mauritii*, employ a distinct detoxification strategy in vermicomposting by facilitating humic composite-mediated chelation for the reduction of potentially toxic HMs (Pb, Cr, Cd, and Zn) rather than relying on conventional high bioaccumulation (Kharmawphlang et al. 2024b). This combination of intracellular sequestration, protein induction, and external chemical modification, followed by partial elimination via excretion (Xiao et al. 2022), confirms the species-specific complexity that makes certain earthworms essential for vermiremediation technology. Hence, comparative studies on the detoxification pathways of HMs by different earthworm species reveal crucial variations in their reliance on mechanisms like MT-based sequestration, non-MT protein binding (e.g., phytochelatins), and humic composite-facilitated chelation, which ultimately governs their tolerance and effectiveness in vermiremediation.

Heavy metals enrichment kinetic models

Based on a mechanistic understanding of metal bioaccumulation by earthworm various models have been developed to stimulate HMs enrichment in earthworms; of which the one-compartment and two-compartment models are the most commonly used. Only the uptake and elimination phases were included in the one-compartment kinetic model (Xiao et al. 2022). In contrast, the two-compartment kinetic model is more complex and accounts for all scenarios involving the dynamics of HMs in earthworms, including uptake, assimilation, storage, and removal.

One-compartment kinetic model

Uptake phase:

$$C_t = C_b + \frac{k_1}{k_2} \times C_s \times \left(1 + e^{-k_{2\times t}}\right) \quad (1)$$

Elimination phase:

$$C_t = C_b + \frac{k_1}{k_2} \times C_s \times \left(e^{-k_{2\times(t-d)}} + e^{-k_{2\times t}}\right) \quad (2)$$

where C_t is the element content in earthworms (μg g^{-1} d.w.) at time t (d), C_b is the background element

content in earthworms (μg g^{-1} d.w.), C_s is the element content in soils (μg g^{-1} dry soil), k_1 and k_2 are the rate constants for HMs uptake (g soil g^{-1} worm d^{-1}) and elimination (d^{-1}), respectively. Additionally, d is the day that earthworms transformed into non-contaminated soil. Moreover, the ratio of k_1/k_2 indicates the affinity of metal to earthworms, and the values of $\ln(2)/k_2$ indicate the period required to eliminate half of the accumulated metal under clean soil conditions.

Two-compartment kinetic model

Uptake phase:

$$C_w = C_{gut} + C_1 + C_2 \tag{3}$$

$$C_1(t) = \frac{k_1 \times C_e}{k_2 + k_i} \times \left[1 - e^{-(k_2+k_i)\times t}\right] \tag{4}$$

$$C_2(t) = \frac{k_i \times k_1 \times C_e}{(k_2 + k_i)^2} \times \left[\left(k_2 + k_1\right) \times t + e^{-(k_2+k_i)\times t} - 1\right] \tag{5}$$

Elimination phase:

$$C_1(t) = C_t(d) \times e^{-(k_2+k_i)\times(t-d)} \tag{6}$$

$$C_2(t) = \frac{k_i \times C_1(d)}{(k_2 + k_i)^2} \times \left[1 - e^{-(k_2+k_i)\times(t-d)}\right] + C_2(d) \tag{7}$$

where, C_{gut} is the concentration of elements in the gut after 4 h of exposure, $C_1(t)$ and $C_2(t)$ are the internal element contents (μg g^{-1} dry weight) in compartment 1 (loosely-bound metal compartment) and compartment 2 (storage compartment) at time t (d), and C_e is the HMs content in soils. C_1 (d) and C_2 (d) are element contents in different compartments in earthworms after d days. Additionally, k_1, k_2, and k_i are rate constants for metal(loid) uptake (g soil g^{-1} worm d^{-1}), elements elimination (g soil g^{-1} worm d^{-1}), and the elements transfer from loosely-bound HMs compartment to the storage compartment (d^{-1}), respectively (Xiao et al. 2022).

Some earthworm species show more complex patterns, including different elimination rates during uptake and depuration, or transient overshoot peaks in body metal concentrations during the early exposure phase (Yue et al. 2024). These patterns require multi-phase or modified kinetic models. For instance, overshoot dynamics in *E. andrei* exposed to Zn were better captured using a specialized one-compartment variant (Smith et al. 2010). Kinetic models have also been extended into broader frameworks. The OMEGA model integrates allometric scaling and biochemical transport processes, allowing estimation of k_1 and k_2 across species and soil types (Ardestani et al. 2014). Furthermore, toxicokinetics has been combined with biotic ligand models (TK-BLM) and toxicodynamic models (TK-TD) to link accumulated metal burdens to biological effects (Ardestani et al. 2014).

Many earthworms tightly regulate essential metals. Zn levels often remain constant across a range of soil concentrations in species such as *E. andrei*, *E. fetida*, *L. rubellus*, *Dendrobaena veneta*, and *A. tuberculata* (Smith et al. 2010). Even so, Zn often shows rapid initial uptake (high k_1) followed by fast elimination (high k_2), leading to equilibrium within a few days. For example, *A. tuberculata* displayed $k_1 \approx 0.08$ g soil g^{-1} day^{-1} and $k_2 \approx 0.06$ day^{-1}, while *E. andrei* showed an overshoot peak within 24 h before declining to a regulated level (Smith et al. 2010). Biphasic Cu excretion patterns in *D. veneta* and *A. tuberculata* required two-compartment models to describe storage and turnover (Marinussen et al. 1997). Cd accumulation in earthworms is typically variable, with low uptake rates and very low elimination rates. For *A. tuberculata*, k_1 was only 0.011–0.014 g soil g^{-1} day^{-1} and k_2 as low as 0.0028–0.0076 day^{-1} (Gonzalez-Alcaraz et al. 2018). In *E. andrei*, Cd accumulated steadily over time, with higher k_1 values (0.04–0.76 g soil g^{-1} day^{-1}) but still low elimination constants (0.0063–0.094 day^{-1}), reflecting species-specific differences (Peijnenburg et al. 1999). Pb typically shows linear uptake and variable elimination. *E. fetida* exhibited very low uptake (k_1 = 0.00006–0.01 g soil g^{-1} day^{-1}) and moderate elimination (k_2 = 0.02–1.15 day^{-1}) depending on soil characteristics (Spurgeon and Hopkin 1999). *E. andrei* showed faster Pb elimination, with k_2 values up to 2.1 day^{-1} in field soils (Peijnenburg et al. 1999). Extremely rapid Pb excretion was seen in *D. veneta*, where k_2 reached 1.71 day^{-1} (Marinussen et al. 1997).

Cd associated with MTs in acclimated *E. andrei* accumulated linearly and required longer to reach equilibrium (Ardestani et al. 2014). In *E. andrei*, Zn showed consistently rapid kinetics under all climate treatments, while Cd uptake and elimination varied

significantly, with higher k_1 and k_2 under warm, moist conditions (Gonzalez-Alcaraz et al. 2018). Co-contaminants such as ciprofloxacin altered Cd toxicokinetics by increasing uptake flux and elimination rate, and shifting Cd distribution among subcellular pools (Wen et al. 2011). Arsenic showed very slow uptake and elimination in *E. fetida*, failing to reach equilibrium in 70 days, and its kinetics were influenced by soil properties affecting As mobility (Lee et al. 2013). Hg uptake models indicated that k_1 increases in soils with lower organic matter and higher pH, while k_2 remained relatively stable (Huu Nguyen et al. 2019). Selenium kinetics in *E. fetida* followed first-order one-compartment modelling, with tissue-specific differences in k_1 and k_2, and higher kinetic BAF values than steady-state estimates (Yue et al. 2024). Kinetic modelling helps determine whether earthworms reach steady-state metal burdens, how long toxicity tests should last, and whether depuration effectively removes accumulated metals (Ardestani et al. 2014). However, results from one soil do not always predict kinetics in another, due to strong soil-specific effects on metal bioavailability (Ardestani et al. 2014). Despite the theoretical stability of kinetic rate constants, extrapolation across soils should be approached for an accurate understanding of HM remediation.

Inter-species variability of earthworm gut microbiomes in heavy metal sequestration and transformation

Composition and dynamics of gut-associated microbiomes

The earthworm gut acts as a specialized microecological niche, hosting a dynamic bacterial community crucial for regulating the distribution and detoxification of HMs in soil (Xiao et al. 2022; Gudeta et al. 2023). Characterized by an anoxic, neutral, and organic matter–rich environment, the gut supports microbial activity far exceeding that of bulk soil (Srut et al. 2019; Liu et al. 2020a, b, c). These conditions promote microbially driven redox transformations that alter metal speciation and ultimately govern their toxicity. A body of research supports this concept, highlighting the direct or indirect influence of these microbial factors on metal-specific distribution and the detoxification processes carried out by the earthworms (Srut et al. 2019). The complex microbial ecosystem within the intestines, characterized by interactions such as mutualism and competition, is a key component of interconnected ecological networks.

The alimentary canal of an earthworm begins at the mouth and extends to the anus, consisting of various specialized regions such as the muscular pharynx, oesophagus, crop, gizzard, foregut, midgut, and hindgut, along with associated digestive glands (Xiao et al. 2022; Gudeta et al. 2023). The gut contains mucus rich in proteins, polysaccharides, organic and mineral matter, amino acids, and a diverse community of microbial symbionts, including bacteria, protozoa, and microfungi (Liu et al. 2020a, b, c). Elevated levels of organic carbon, nitrogen, and moisture within the gut create favorable conditions for microbial activation, spore germination, and enzymatic activity (Gudeta et al. 2023). Numerous digestive enzymes such as amylase, cellulase, protease, lipase, chitinase, and urease have been detected in the gut, with cellulase and mannose activity particularly attributed to gut-associated microbes (Chowdhury et al. 2024). As the ingested material passes through the gut, it is mixed with digestive enzymes and resident microbes and subsequently excreted as partially digested casts. These casts serve as sites of further microbial decomposition and nutrient stabilization, marking the maturation phase of composting (Chowdhury et al. 2024). The relationship between earthworms and microbes is complex; while some microorganisms form beneficial symbioses, others are digested as part of the diet of the worm. Studies have shown that species like *Fusarium oxysporum, Alternaria solani*, and certain microfungi are digested by earthworms such as *Drawida calebi, L. terrestris*, and *E. fetida*. Similarly, microbial populations, including *Bacillus cereus var. mycoides, Escherichia coli,* and *Serratia marcescens,* are significantly reduced or eliminated during gut passage (Liu et al. 2020a, b, c).

Functional traits: metal solubilization, chelation, and redox transformations

Earthworm gut microbes are highly adept at resisting and eliminating HMs (Table 3). They achieve this through biodegradation, absorption, biotransformation, enzymatic usage, exopolysaccharide production, synthesizing metallothionein, and bioaccumulation,

Table 3 List of microbial taxa inhabiting earthworm guts that contribute to resilience against heavy metal toxicity (Gudeta et al. 2023)

Microbial taxon	Heavy metals assessed	Earthworm host	Source/environmental context	Reference
Achromobacter	Cd, Pb, Cr	*Eisenia. fetida*	Cow-dung based vermicompost	Wang et al. (2017)
Acinetobacter	Cd, Pb, Cr	*E. fetida*	Vermicomposted cattle manure	Wang et al. (2017)
Agrobacterium	Cd, Pb, Cr	*E. fetida*	Vermicompost derived from cow dung	Wang et al. (2017)
Arthrobacter	Cd, Pb, Cr	*E. fetida*	Cow-dung vermicompost	Wang et al. (2017)
Bacillus (general)	Cd, Pb, Cr	*E. fetida*	Vermicompost of bovine manure	Wang et al. (2017)
Burkholderia	Cd, Pb, Cr	*E. fetida*	Cow-dung vermicompost	Wang et al. (2017)
Exiguobacterium	Cd, Pb, Cr	*E. fetida*	Earthworm-processed cow-dung substrates	Wang et al. (2017)
Luteimonas	Cd, Pb, Cr	*E. fetida*	Vermicompost from cattle manure	Wang et al. (2017)
Microbacterium	Cd, Pb, Cr	*E. fetida*	Bovine-manure-based vermicomposting system	Wang et al. (2017)
Paracoccus	Cd, Pb, Cr	*E. fetida*	Cow-dung vermicompost	Wang et al. (2017)
Sphingomonas	Cd, Pb, Cr	*E. fetida*	Vermicompost derived from cow manure	Wang et al. (2017)
Bacillus licheniformis KX 657843	Cu, Zn	*Metaphire posthuma*	Laboratory culture	Biswas et al. (2018a)
Bacillus megaterium strain MF589715	Cu, Zn	*M. posthuma*	Laboratory culture	Biswas et al. (2018b)
Staphylococcus haemolyticus strain MF589716	Cu, Zn	*M. posthuma*	Laboratory culture	Biswas et al. (2018b)
B. licheniformia strain MF589720	Cu, Zn	*M. posthuma*	Laboratory culture	Biswas et al. (2018b)
Bacillus safensis (MF 589718)	Cr, Zn	*M. posthuma*	Metal-polluted aqueous solution	Banerjee et al. (2019)
Bacillus flexus (MF 589717)	Cr, Cu	*M. posthuma*	Metal-polluted aqueous solution	Banerjee et al. (2019)
Staphylococcus haemolyticus (MF 589719)	Cr, Cu, Zn	*M. posthuma*	Metal-polluted aqueous solution	Banerjee et al. (2019)
Paenibacillus	Cd	*E. fetida*	Polluted anthropogenic environment	Srut et al. (2019)
Flavobacterium	Cd	*E. fetida*	Anthropogenically impacted soils	Srut et al. (2019)
Pseudomonas spp.	Cd, Ni, Cu, Zn	*E. fetida*	Human-impacted polluted sites	Srut et al. (2019)
Trichoderma brevicompactum QYCD-6	Cd, Pb, Cr, Zn	*Pheretima tschiliensis*	Metal-polluted aquatic environment	Zhang et al. (2020)
Bacillus (soil isolate)	Cd	*E. fetida*	Experimentally contaminated soil	Xiao et al. (2022)
Bacillus xiamenensis	Pb, Ni, Cd	*E. fetida*	Laboratory culture	Wagh et al. (2022)

thus helping the earthworm overcome anthropogenic pollutants (Roy et al. 2024). Specific studies have illuminated these mechanisms, for instance, enriching the earthworm gut microbiota was shown to reduce the extrusion of both As(V) and As(III) (Wang et al. 2019). Furthermore, the presence of bacterial

genera such as *Flavobacterium, Paenibacillus, and Pseudomonas* has been analysed and confirmed to enable earthworms to combat the toxicity of metals like Cd. According to Biswas et al. (2018a), *Bacillus licheniformis* strain KX 657843 was discovered to be resistant to Cu^{2+} and Zn^{2+} after being extracted from the gut of *M. posthuma* worms. Further analysis showed that the extracellular polymeric materials of KX 657843 not only allowed the strain to survive in high concentrations of Cu^{2+} and $Zn^{2+,}$ but also reacted with these ions to reduce their levels. Biswas et al. (2018b) isolated three additional bacteria, *Bacillus megaterium* strain MF589715, *Staphylococcus haemolyticus* strain MF589716, and *B. licheniformis* strain MF589720 from the gut of *M. posthuma* that can detoxify HMs. The three strains, which can solubilize phosphate, can reduce the toxicity of Cu^{2+} and Zn^{2+} by forming a compound with HM ions. Sun et al. (2020) investigated the gut bacterial response of *E. fetida* and *L. mauritii* to exposure to Zn^{2+}, As^{2+}, and Mn^{2+}. The study found that the gut bacteria produced MTs, which greatly reduced the toxicity of the ions through complexation and absorption. The presence of these strains in the worm gut contributed to the decrease in HM toxicity through bioaccumulation, biotransformation, and other processes (Sun et al. 2020). In addition, in a separate investigation of the effects of As on the gut microbiota in the earthworm *M. sieboldi*, where high-throughput sequencing was used to identify 16 genes related to As biotransformation (Wang et al. 2019). *Bacillus safensis* (MF 589718), *Bacillus flexus* (MF 589717), and *S. haemolyticus* (MF 589719) were successfully isolated and identified from *M. posthuma* (Banerjee et al. 2019). The *Bacillus* strains were found to be significantly more effective than the *Staphylococcus* strain in removing toxic Cr(VI), Cu(II), and Zn(II) from aqueous solutions. *B. safensis* and *B. flexus* exhibited comparable capabilities in eliminating Cr(VI). Notably, *B. safensis* was superior in extracting Zn(II), whereas *B. flexus* showed greater proficiency in removing Cu(II). The gut isolates successfully solubilized phosphate even in toxic HM-contaminated conditions. The gut microbiota of the earthworm *A hupeiensis* holds significant potential for the in-situ remediation of Cd-polluted soil (Yang et al. 2022). The primary pathways that *C. freundii* utilizes to manage Cd stress include the regulation of ABC transporters, bacterial chemotaxis, cell motility, oxidative phosphorylation, and the two-component system, suggesting a widespread presence of the corresponding bacterial community in the worm gut (Xu et al. 2024). Exposure to HMs can significantly alter the gut microbiota, which in turn impacts the availability and toxicity of HMs to the earthworm (Yang et al. 2022). Changes in the composition and abundance of gut microbes, such as the decrease of *Mycobacterium* under Cd stress, can serve as a biological indicator of soil contamination (Sun et al. 2020). High-throughput sequencing has revealed that the earthworm gut community, which is distinct from the soil community, with a higher relative abundance of *Proteobacteria* (45.7%) and *Bacteroidetes* (18.8%), is more sensitive to HM contamination than the soil community (Liu et al. 2020a, b, c). Accumulation of HMs surprisingly also encourages the growth of plant growth-promoting bacteria (PGPB) within the digestive tract of earthworms. These PGPB produce compounds like aminocyclopropane-1-carboxylic acid (ACC) deaminase and 1-indole-3-acetic acid (IAA), which are valuable for the phytoremediation of HMs (Liu et al. 2020a, b, c). Generally, it has been observed that the environmental thresholds for bacterial generalists in the earthworm gut are higher than those for bacterial specialists. The biosorption capacity of *Pseudomonas* sp., for instance, has been tested against multiple metals, with certain strains demonstrating a high capacity to accumulate them (Srut et al. 2019). This approach, alongside the discovery of tolerant fungal species like *Trichoderma brevicompactum* QYCD-6 from earthworms, offers a promising path for sustainable soil decontamination (Zhang et al. 2020). Comparative studies are vital for elucidating how the functional traits of gut microbes differ across various earthworm species, enabling us to understand the species-specific role of the gut microbiome in the overall detoxification efficiency and fate of HMs in contaminated soil.

Species-specific molecular and biochemical mechanisms in Earthworm-mediated heavy metal detoxification

Cellular macromolecules

Heavy metals can easily enter the bodies of earthworms and disturb their normal functions, setting off a chain of biochemical reactions that act as early

warning signs of soil contamination (You et al. 2024). Once inside the cells, these metals mainly harm proteins, the molecules that carry out most of the cell's work. Many proteins depend on special chemical groups called thiols (-SH groups) to keep their proper shape and to work correctly. HMs like Cd, Pb, Ni, and Cu attach strongly to these thiol groups, which causes proteins to lose their structure and stop functioning (Wang et al. 2025). Some proteins need specific metal ions like Zn, Cu, Fe, or Mg to function. HMs can replace these essential ions, which disables the protein (Yadav et al. 2023). HMs can oxidize certain parts of proteins, making them break apart or clump together (You et al. 2024). These disruptions cause cells to produce large amounts of reactive oxygen species (ROS), which push the cells into oxidative stress. This type of stress damages DNA, proteins, and lipids all at once (Wang et al. 2025). This internal damage shows up clearly in biochemical studies of earthworms. For example, in *E. eugeniae*, exposure to Pb and Ni greatly reduced carbohydrates, lipids, and proteins, showing that the worms were losing energy and important structural molecules under stress (Yadav et al. 2023). In *E. andrei*, even low levels of Cu caused increasing DNA damage over time (Mincarelli et al. 2019).

Earthworms may show both protective and harmful changes depending on metal concentration. In *E. fetida*, low levels of Cd caused protein levels to rise, but at high Cd levels, protein content fell sharply because the cells were severely damaged (Yadav et al. 2023). Pb, however, reduced protein content even at low doses. Sometimes an increase in proteins indicates adaptation. Earthworms (*E. eugeniae*) living near sawmills showed extra protein bands, suggesting that they produced more stress-related or detoxification proteins to cope with long-term exposure, but in many cases, the response is negative; exposure to Hg and Co greatly reduced proteins, lipids, and carbohydrates in several species, showing that HMs were draining energy reserves (Jatwani et al. 2016). More advanced techniques confirm these changes. FTIR studies on *E. fetida* showed clear shifts in the structure of proteins, lipids, and other biomolecules after HM exposure, proving that metal buildup directly changes cellular chemistry (Muthukaruppan 2015). Earthworms also activate genes involved in immunity. In *E. andrei*, Cu exposure increased the expression of a Toll-like receptor (TLR) and an antimicrobial peptide called fetidin (FET), showing that the immune system participates directly in responding to metal stress (Mincarelli et al. 2019).

Enzymatic defense mechanisms and oxidative stress pathways under heavy metal exposure

The enzymatic response of earthworms to HM stress represents a tightly coordinated physiological defense designed to preserve cellular homeostasis under toxic conditions. Species such as *E. fetida* and *L. terrestris*, widely recognized as ecosystem engineers and bioindicators, exhibit characteristic biochemical alterations that serve as sensitive markers of soil contamination (Wang et al. 2025). HM exposure initiates a chain of intracellular disruptions, beginning with the inactivation of sulfhydryl (SH)-containing molecules such as glutathione (GSH), a major antioxidant responsible for maintaining cellular redox balance (Yadav et al. 2022). As metals penetrate cell membranes, their ionic properties promote the direct or indirect generation of reactive oxygen species (ROS), particularly hydroxyl (·OH) and perhydroxyl (HO_2) radicals, which attack lipids, nucleic acids, and proteins (Wang et al. 2025). Elevated ROS levels have been widely documented in *E. fetida* and other earthworm species exposed to Cd, Cu, and Zn (Yan et al. 2021).

One of the most destructive consequences of ROS accumulation is the initiation of lipid peroxidation (LPO), a chain reaction where radicals target polyunsaturated fatty acids in cellular membranes. This oxidative degradation alters membrane fluidity and permeability, leading to cellular dysfunction (Wang et al. 2025). The resulting accumulation of malondialdehyde (MDA), the primary LPO by-product, serves as a measurable biomarker of membrane damage and overall oxidative stress (Yan et al. 2021). High HM concentrations have also been shown to directly inhibit enzymatic proteins (Yadav et al. 2023). The interplay between LPO and enzyme impairment is evident in species such as *Eutyphoeus waltoni* and *E. fetida*, where prolonged exposure to metals led to elevated LPO levels and a marked reduction in detoxification enzyme activity, signaling a breakdown of the organism's defensive capacity (Maity et al. 2018). Similar outcomes have been consistently reported in other species,

reinforcing LPO as a central mechanism of HM-induced toxicity (Aguzie et al. 2021). To counter rising ROS, earthworms activate an antioxidant machinery involving superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), peroxidase (POD), and glutathione-S-transferase (GST), as shown in Table 4 (Fig. 1). This ensemble forms the primary biochemical defense against oxidative injury (Maity et al. 2018). Mechanistically, SOD converts superoxide radicals to hydrogen peroxide (H_2O_2), which is subsequently decomposed by CAT and POD into water and oxygen (Wang et al. 2025). In *E. fetida*, *Perionyx excavatus*, and *P. posthuma*, these enzymes typically exhibit an early dose- and time-dependent elevation under moderate HM exposure, reflecting an adaptive response to oxidative challenge (You et al. 2024; Yadav et al. 2022). GST plays an additional role by conjugating GSH with toxic intermediates, enhancing their solubility and facilitating excretion (Yadav et al. 2022). However, this biphasic defense has limits. Under prolonged or high-intensity HM exposure, antioxidant activities in species such as *M. posthuma* decrease sharply, accompanied by increased pro-oxidant levels, clear evidence of severe oxidative stress, and enzyme system exhaustion (Ray et al. 2019). Similar declines in SOD, CAT, and GST have been reported across species under chronic contamination (Yadav et al. 2022; You et al. 2024). Beyond oxidative pathways, HMs impair broader physiological functions. In *P. excavatus*, metals like Zn, Cu, and Hg suppress key metabolic enzymes, including lactate dehydrogenase (LDH) and succinate dehydrogenase (SDH), indicating disruption of cellular respiration and energy metabolism (Yan et al. 2021). Neurological disruption is also evident through altered acetylcholinesterase (AChE) activity in several species, reinforcing its value as a biomarker of neurotoxicity under environmental contamination (Li et al. 2020). Despite these vulnerabilities, some earthworms exhibit notable adaptive capacity. *E. eugeniae*, for example, may show enhanced or synergistic enzyme activity under certain conditions, modulating metal speciation and contributing to soil detoxification. Such resilience underscores the mechanistic potential of earthworms in vermiremediation and the restoration of soil biochemical integrity (Borah and Deka 2023).

System biology and multi-omics integration

Integrating multi-omics for heavy metals detoxification

Bioremediation involving microorganisms efficiently eliminates pollutants such as HMs, leveraging local microbial populations. Effective contamination management is contingent upon factors including microbial composition, the nature of the pollutant, and environmental conditions (Phurailatpam et al. 2022). Advanced omics and molecular techniques, including metagenomics, transcriptomics, proteomics, and

Table 4 Alterations in earthworm enzymes triggered by heavy metal exposure (Yadav et al. 2023)

Earthworm species	Heavy metals	Enzymes affected	Reference
Aporrectodea tuberculata	Cu, Zn	CYP1A monooxygenase and GST	Yadav et al. (2023)
Eisenia fetida	Cd, Pb, Cu, Cr, Mn, Zn, Al, Fe, Ni	Cellulase, SOD, CAT, GST, Acid phosphatase (AP), POD, AChE, GR	Li et al. (2020); Liu et al. (2020a, b, c); Wang et al. (2020)
Eisenia andrei	Se	Carboxylesterase, GST, GR, CAT, and SOD	Ecimovic et al. (2018)
Alma millsoni	Cd, Pb, Cr, Cu, and Zn	SOD, POD, GST, and GSH	Dedeke et al. (2018)
Nsukkadrilus mbae	Pb and Cd	CAT, GPX, and SOD	Aguzie et al. (2021)
Perionyx excavatus	Zn, Cu, Hg	SOD, CAT, GPx, POD, GST, LDH, SDH, AChE	Yadav et al. (2022)
Metaphire posthuma	Not specified	SOD, CAT, GST	Ray et al. (2019)
Eudrilus eugeniae	Not specified	SOD, CAT, GST, others	Borah and Deka (2023)
Libyodrilus violaceus	Cd, Pb, Cr, Cu, and Zn	SOD, POD, GST, and GSH	Dedeke et al. (2018)

metabolomics, have been exhaustively studied to elucidate the efficacy of microbial pollution mitigation.

Metagenomic insights into community structure and functional genes associated with heavy metal remediation

Metagenomics enables the direct analysis of DNA extracted from environmental samples, allowing comprehensive characterization of entire microbial communities, including the vast majority of non-culturable microorganisms, through sequence-based and function-based approaches (Sharma et al. 2021). This culture-independent methodology has become a powerful tool for discovering novel microbes, identifying gene clusters encoding pollutant-degrading enzymes, and tracking microbial community shifts in contaminated ecosystems (Sharma et al. 2021). The technological advancements have been instrumental in uncovering HM resistance mechanisms, as demonstrated by shotgun metagenomic analyses of Cd-contaminated agricultural soils, which revealed a diverse resistome enriched in P-type ATPases, efflux systems (*czcA, czcD, czrA*), and resistance genes for Co, Ni, Cu, Fe, and Hg (Salam et al. 2020). Across contaminated environments, metal-tolerant microbiomes are consistently dominated by phyla such as Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes, Chloroflexi, Acidobacteria, Spirochetes, and Patesibacteria (Sharma et al. 2021). Metagenomic investigations of earthworm gut microbiota have provided valuable insights into metal biotransformation processes. For instance, studies on *Metaphire sieboldi* exposed to As-contaminated soils revealed enrichment of As redox and efflux genes, with limited representation of methylation and demethylation pathways. The gut microbial community, dominated by Actinobacteria, Firmicutes, and Proteobacteria, differed markedly from surrounding soil, indicating that As exposure selectively reshapes gut microbiota toward arsenate reduction and arsenite extrusion (Wang et al. 2019). Similarly, 16S rRNA amplicon sequencing of the gut microbiota of *Perionyx excavatus*, *Eudrilus eugeniae*, and *Pontoscolex elongata* showed dominance of Proteobacteria, followed by Actinobacteria, Firmicutes, and Bacteroidetes, with functional predictions indicating enrichment of aerobic and anaerobic taxa, including ammonia oxidizers, sulphate reducers, nitrite reducers, and dehalogenators, underscoring the role of earthworm gut microbiomes in nutrient cycling and biodegradation (Thakur et al. 2021). Metagenomic annotation of the unculturable gut microbiota of *Metaphire nanaoensis* from Pb-contaminated paddy fields further revealed a diverse bacterial assemblage dominated by Prevotella, along with Firmicutes and Actinobacteria, highlighting the adaptive potential of gut microbiota under HM stress (Bora and Devi 2024). Beyond taxonomic and resistance profiling, metagenomic screening has enabled the discovery of enzymes with high bioremediation potential, including laccases, dioxygenases, phenol-degrading enzymes, and esterases, from polluted soils, wastewater, activated sludge, oil-contaminated waters, and compost systems (Sharma et al. 2021). These findings demonstrate that continued advancements in sequencing technologies and expanding metagenomic databases have transformed environmental biotechnology. By revealing the functional potential, adaptive strategies, and enzymatic capabilities of complex microbial communities, metagenomics is driving the development of next-generation bioremediation strategies and more informed ecosystem management approaches.

Transcriptomic profiling of heavy metal-responsive gene expression

High-throughput RNA sequencing has transformed our ability to understand how organisms respond to environmental stress. By rapidly generating complete gene expression profiles, RNA-Seq allows researchers to uncover both known and previously unrecognized protein-coding and non-coding transcripts (Chai et al. 2020). This technology offers a panoramic view of transcriptional activity, enabling the identification of stress-responsive genes, metabolic shifts, and biomarkers across a wide range of organisms (Chai et al. 2020).

In earthworms, RNA-Seq has revealed the intricate molecular defense strategies mobilized under HM exposure. When *E. fetida* is subjected to increasing Cd concentrations, its transcriptome shows sweeping changes in genes responsible for enzyme function, metabolism, oxidative stress management, regeneration, and programmed cell death. Notably, metallothionein-2 (*MT2*), phytochelatin synthase 1a (*PCS1a*), CuZn-superoxide dismutase (*CuZn-SOD*), and TP53-regulated inhibitor of apoptosis 1-like

(*TRIA1-like*) are strongly upregulated, while genes involved in cellulose breakdown and apoptosis are suppressed. This coordinated shift indicates that *E. fetida* activates multiple protective layers, including metal binding, antioxidant defense, and damage repair, to buffer Cd toxicity, underscoring its value as a bioindicator of Cd-polluted soils (Chai et al. 2020). A complementary study on *E. fetida* further showed that Cd destabilizes cytoskeletal structures, disrupts DNA replication, and impairs signaling pathways. Even low Cd levels triggered pronounced disturbances in cellular metabolism, pointing to a broad, organism-wide stress response (You et al. 2024). Similar mechanistic patterns emerge under Cu stress. Long-term Cu exposure in *E. fetida* led to the downregulation of energy-metabolism genes lactate dehydrogenase (*LDH*), glycogen synthase (*GYS*), ATPase H^+ Transporting V0 Subunit a (*ATP6N*), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), cytochrome c oxidase copper chaperone 17 (*COX17*), detoxification genes UDP-glucuronosyltransferase (*UGT)*, cytochrome P450 family 2 subfamily U member 1 (*CYP2U1*), cytochrome P450 family 1 subfamily A member 1 (*CYP1A1*), and an immune-related gene chitinase (E3.2.1.14). At the same time, antioxidant genes glutamate-cysteine ligase catalytic subunit (*GCLC*), and hematopoietic prostaglandin D synthase (*HPGDS*) were activated, reflecting a shift toward oxidative stress mitigation when other physiological systems become compromised (Yu et al. 2022). Another Cu-focused transcriptome analysis found upregulation of heat shock protein 70 (*Hsp70*), cathepsin L (*CTSL*), glutathione-S-transferase (*GST*), ChaC glutathione specific γ-glutamylcyclotransferase (*CHAC*), and glutamate-cysteine ligase catalytic subunit (*GCLC*), highlighting glutathione metabolism as a central pathway in Cu detoxification (Zhang et al. 2023). Similar defensive signatures are seen in *E. andrei,* where exposure to polymetallic soils induces genes such as *cat, gst, mt, p21*, and topoisomerase, all linked to oxidative stress defense, metal sequestration, and DNA damage repair (Hattab et al. 2023). Together, these earthworm studies show that HM stress prompts a characteristic transcriptional architecture: suppression of routine metabolic functions and simultaneous enhancement of antioxidant, repair, and detoxification pathways.

Beyond earthworms, transcriptomics also illuminates microbial responses to metal toxicity. Because it sits at the intersection of genomics, proteomics, and cellular physiology, transcriptomics reveals how microorganisms reprogram their gene networks to survive environmental pressures. DNA microarrays and RNA-Seq have shown, for example, that oxygen deprivation in anaerobic *Bacillus subtilis* alters expression of more than 100 genes, while exposure to o-xylene in *Rhodococcus opacus* R7 triggers a massive 542-gene shift affecting stress tolerance, osmoregulation, and central metabolism. Comparative transcriptomics across kingdoms further supports the idea of a conserved HM defense strategy. In *E. coli, Saccharomyces cerevisiae, and Chlamydomonas reinhardtii*, Cd stress induces species-specific changes yet consistently regulates four shared detoxification pathways: antioxidant defenses, sulfur metabolism, cell wall restructuring, and metal transport. qPCR validation of 43 genes and the identification of 12 protein homologues confirmed the conservation of these core mechanisms across bacteria, fungi, and algae (Chen et al. 2021).

Metal-specific responses are also clear in bacteria. In *B. cereus* ATCC14579, silver nitrate exposure upregulated genes associated with chaperone activity, nutrient acquisition, DNA replication, and membrane synthesis, while suppressing chemotaxis genes, effectively reducing motility to conserve energy under stress. Fourteen small non-coding RNAs were also induced, suggesting regulatory fine-tuning under Ag stress (Hattab et al. 2023). Likewise, *Bacillus velezensis* LB002 responded to high Cd^{2+} levels by upregulating genes for chemotaxis, siderophore synthesis, metal transport, and metabolic enzymes argininosuccinate lyase (*argH*), ornithine carbamoyltransferase (*argF*), urocanate hydratase (*hutU*), histidine ammonia-lyase (*hutH*), lipoamide dehydrogenase (*lpdA*), and aconitate hydratase A (*acnA*), promoting Cd mineralization and fixation and significantly increasing survival (Huang et al. 2022). RT-qPCR validation confirmed the transcriptional basis of these detoxification mechanisms. By illuminating conserved detoxification pathways across bacteria, fungi, algae, and earthworms, transcriptomics provides a unifying blueprint of HM resilience, one that holds immense promise for advancing biomonitoring and sustainable remediation technologies.

Proteomic mapping of enzymatic and structural proteins mediating heavy metal detoxification

Proteomics provides critical insights into the molecular pathways underlying HM stress in earthworms and microorganisms. Rather than capturing isolated biochemical events, proteomics reveals entire networks of proteins that organisms mobilize to survive, adapt, and actively transform contaminated environments. In earthworms, proteomics has illuminated how *E. fetida* reprograms its cellular machinery when challenged by toxic metals. Under Cd exposure, the species exhibited 143 differentially expressed proteins (DEPs), including canonical stress responders such as *HSP60*, caspase-8, and SOD, along with proteins governing energy metabolism, metal binding, cytoskeletal organization, and immune processes. This suite of changes portrays a coordinated defensive shift in which the worm simultaneously enhances detoxification, restructures metabolism, and stabilizes damaged proteins to withstand Cd-induced oxidative and structural stress (Wang et al. 2010). A parallel investigation into As^{3+} exposed *E. fetida* (5–80 mg kg^{-1}) deepened this picture, identifying 24 DEPs through 2-D electrophoresis (2-DE) and Matrix-assisted laser desorption/ionization-time of flight/time of flight mass spectrometry (MALDI-TOF/TOF-MS), with 79.2% showing marked upregulation, including a dramatic 53.8-fold increase in one protein spot. Functional clustering placed these proteins within metabolic regulation, transport, and stress pathways, and network analysis revealed serum albumin (ALB) as a central interaction hub linking six other DEPs (Wang et al. 2022). Together, these findings narrate a highly integrated proteomic defense programme that enables earthworms to maintain homeostasis while actively sequestering or mitigating HM toxicity.

Microbial proteomics offers an equally compelling narrative. Unlike genomic tools that show what could occur, proteomics shows what is occurring inside cells as they encounter pollutants. Using techniques such as 2-DE, liquid chromatography-mass spectrometry (LC-MS), and LC-MS/MS, researchers have been able to track shifts in protein abundance that directly reflect microbial efforts to detoxify contaminants. Advances such as immobilised pH gradient strips have improved resolution and reproducibility, allowing clearer detection of protein shifts in organisms experiencing HM stress (Huibo et al. 2023). LC-MS approaches, traditionally used in aquatic contaminant detection, now also map the metabolic intermediates generated when microbes degrade pesticides, surfactants, pharmaceuticals, and other pollutants (Lata et al. 2023).

At the ecosystem level, proteins within soil itself function as sensitive biosensors of contamination. In soils near a smelter, HMs significantly suppressed protein concentration, microbial biomass carbon, acid phosphatase, and dehydrogenase activity, with proteins showing the steepest decline. Denaturing gradient gel electrophoresis (DGGE) further revealed a reduction of microbial diversity and altered community structure, while a distinct low-molecular-weight protein appeared exclusively in contaminated soils, hinting at its potential as a biomarker for HM impact (Zhang et al. 2013). At the species level, bacterial proteomes paint a vivid picture of stress adaptation. *Klebsiella pneumoniae* isolated from a contaminated river demonstrated enhanced tolerance to Pb and Co, driven by differential expression of DNA gyrase A and l-isoaspartate protein carboxymethyltransferase type II, proteins linked to DNA stability and protein repair (Bar et al. 2007). Cd-resistant strains *Pseudomonas putida* SB32 and *P. monteilli* SB35 showed 24 and 21 DEPs, respectively. Key stress-related proteins, such as the ATP-dependent Clp protease and 20S proteasome subunits A and B in *P. putida* and nitroreductase in *P. monteilli*, highlight the centrality of proteostasis, protein turnover, and detoxifying enzymes in survival under Cd pressure (Jain and Bhat 2013). *Pseudomonas fluorescens* displayed similarly metal-specific proteomic signatures: *spoVG* and enolase were upregulated under Pb and Co stress, a hypothetical protein was downregulated under Co, and Co uniquely induced xylosyltransferase, ORF 18 phage phi KZ, OMP H1, and EF-Tu, signalling fine-tuned proteomic remodeling in response to individual metals (Sharma et al. 2006). Proteomic data from 2-DE and shotgun proteomics of *Streptomyces* sp. MC1 exposed to Cr(VI) revealed pronounced activation of molecular defenses, including chaperones, DNA repair enzymes, and metabolic proteins. The strong induction of dehydrogenases implicated in redox reactions suggests an active enzymatic reduction of Cr(VI) to its less toxic Cr(III) form, an adaptation that underpins the organism's remarkable resistance and detoxification capability (Bonilla et al. 2020). These proteomic studies reveal a unifying narrative whether

in earthworms or microbes, HM exposure triggers a complex, multi-layered reorganization of cellular systems. Detoxification, protein repair, metal sequestration, and metabolic rewiring operate not as isolated responses but as a tightly integrated survival strategy.

Metabolomic signatures and mechanistic pathway disruptions under heavy metal stress

Metabolomics has rapidly evolved with advances in analytical chemistry and interdisciplinary integration, enabling precise evaluation of toxicological responses to HMs (Liu et al. 2022). By profiling shifts in endogenous metabolites, metabolomics connects metal exposure to downstream metabolic disruption, revealing how altered gene expression and enzyme activity ultimately reconfigure an organism's biochemical landscape (Zhang et al. 2023). Because endogenous metabolite composition is tightly coupled to physiological state, metabolomics provides a sensitive window into HM-induced stress pathways and internal damage (Liu et al. 2022).

Cd is one of the most prevalent environmental HMs due to industrial and agricultural inputs, has been repeatedly shown through metabolomics to disturb core metabolic pathways in exposed organisms (Bhowmik et al. 2025). Similar broad toxic impacts are also documented for Pb, which destabilizes ecosystem balance, and for Hg, which infiltrates biota through multiple exposure routes (Bhowmik et al. 2025). Using ^{1}H NMR metabolomics, Tang et al. (2018) demonstrated that low-dose Hg exposure in earthworms profoundly perturbed osmotic regulation and amino acid-energy metabolism. Significant decreases in glutamine and 2-hexyl-5-ethyl-3-furansulfonate, combined with increases in glycine, alanine, glutamate, scyllo-inositol, t-methylhistidine, and myoinositol, indicate osmotic imbalance and disrupted nutrient fluxes. Cr toxicity elicits similarly multifaceted metabolic consequences. High-dose Cr exposure induced ROS generation and oxidative DNA damage in *E. fetida* coelomocytes (Sforzini et al. 2017). When *E. fetida* was exposed to Cr-contaminated soil (Cr-CS) at environmentally relevant concentrations, 2D NMR metabolomics revealed disturbances in osmoregulation, energy production, and nucleic acid metabolism. Betaine, scyllo-inositol, dimethylglycine, and 2-hexyl-5-ethyl-3-furansulfonate emerged as sensitive biomarkers for Cr-induced stress.

Integrative omics further strengthens mechanistic resolution. Combined metabolomic and transcriptomic analysis showed that Cu exposure disrupts amino acid, lipid, and carbohydrate metabolism in *E. fetida* (Zhang et al. 2023). Long-term Cu exposure also downregulated genes central to energy metabolism (*LDH, GYS, ATP6N, GAPDH, COX17*), immunity (*E3.2.1.14*), and detoxification (*UGT, CYP2U1, CYP1A1*), while suppressing metabolites such as glucose-1-phosphate, glucose-6-phosphate, and TCA intermediates (fumaric acid, allantoic acid, malate, malic acid). In contrast, antioxidant-related genes (*GCLC, HPGDS*) and digestive/immune metabolites (e.g., trehalose-6-phosphate) were upregulated, highlighting compensatory defense mechanisms (Yu et al. 2022). In *A. hupeiensis*, ^{1}H NMR profiles from low and high-HM-contaminated soils showed shifts in pathways related to energy metabolism, amino acid biosynthesis, glycerophospholipid metabolism, inositol phosphate metabolism, and glutathione metabolism, marking broad cellular stress (Liu et al. 2023).

Metabolomics has also clarified microbial HM response. Under Pb stress, *Lactiplantibacillus plantarum* CCFM8661 showed substantial increases in proline, arginine, glutamic acid, and mannitol, reflecting adjustments in amino acid metabolism and osmotic balance. These metabolic shifts, supported by proteomic data, underpin the superior membrane stability and stress tolerance of the Pb-resistant CCFM8661 relative to the Pb-sensitive strain CCFM578 (Chen et al. 2025a, b). HMs induce profound metabolic rewiring across earthworms and microorganisms, altering osmotic balance, energy flux, amino acid metabolism, and redox homeostasis. By resolving these biochemical signatures with high precision, metabolomics not only identifies early biomarkers of HM toxicity but also exposes the metabolic strategies that underpin resilience, resistance, and bioremediation potential.

Synthetic biology and engineered microbes for metal detoxification

The redesign and repurposing of naturally existing biological components for practical applications lie at the core of synthetic biology. Naturally evolved mechanisms that enable organisms to tolerate and

remediate HMs provide critical insights for engineering efficient systems to address HM toxicity and environmental pollution (Tang et al. 2024). Organisms inhabiting metal-contaminated environments have developed a vast array of adaptive biological components and regulatory mechanisms which allow them to survive and function under such stressful conditions. These biological systems form the foundation for developing engineered solutions for HM bioremediation (Somayaji et al. 2022).

In bacterial systems, operons serve as coordinated genetic regulatory units in which genes encoding functionally related proteins are organized together under shared regulatory control. Operon-based mechanisms are among the key strategies employed by bacteria for HM resistance and remediation. Several metal-specific operons have been identified, including the *mer* operon for Hg, *ars* operon for As, *czc* operon for Co, Zn, and Cd, and operons associated with Pb resistance (Chauhan et al. 2023). Upon exposure to HMs, regulatory proteins linked to promoter regions bind to specific metal ions, facilitating the recruitment of RNA polymerase to initiate transcription. As a result, genes responsible for efflux systems, enzymatic conversion, and various resistance mechanisms are expressed (Somayaji et al. 2022). Among these, efflux systems represent one of the most common strategies for reducing intracellular metal concentrations and enhancing bacterial survival in toxic environments.

Another critical component of HM detoxification across biological systems is metallothioneins (MTs), which are present in bacteria, fungi, plants, and other eukaryotes (Tang et al. 2024). Exposure to elevated concentrations of metals such as Zn, Cd, Hg, Cu, Co, Cr, and Ni leads to a significant increase in the expression of MT-related genes, including *smtA* (Chauhan et al. 2023). Notably, MTs found in ciliates exhibit unique properties, including larger molecular size and higher cysteine content, enabling them to bind a greater number of metal ions compared to conventional MTs. Other mechanisms contributing to Pb resistance include the production of siderophores and the formation of insoluble phosphate complexes (Somayaji et al. 2022). Among the most widely studied applications of synthetic biology in this context is the development of microbial biosensors for HM detection. Metals such as Hg, As, Pb, Cu, and Cd are among the most prevalent environmental pollutants, contributing to severe ecological damage and human health risks. To detect these metals effectively, researchers have engineered biosensors that combine metal-responsive regulatory elements with reporter systems such as bioluminescent, fluorescent, or colorimetric outputs (Fig. 2). These systems are designed to provide high sensitivity, specificity, and cost-effectiveness across a range of environmental conditions. For Hg detection, several innovative genetic circuits have been developed. For instance, the Pmer/merR-lucGR system induces luciferin-mediated bioluminescence, achieving detection ranges of 100 nM–10 µM in *Escherichia coli* and 100 nM–1 µM in *Pseudomonas fluorescens* (Somayaji et al. 2022). Another approach utilized a pmerRBPmerlux circuit in a bioluminescent immobilized *E. coli* MC106 strain, combined with a rhamnolipid biosurfactant to enhance Hg release from soil into aqueous phases, thereby improving detection efficiency (Tang et al. 2024). Alternative strategies include gas-based biosensing systems, where the MerR protein binds Hg^{2+} ions and activates the expression of ethylene-forming enzymes (EFE), producing detectable gaseous signals for rapid on-site monitoring (Chauhan et al. 2023). Additionally, both whole-cell and cell-free biosensor systems incorporating *merR* with fluorescent (EmGFP) and luminescent (LucFF) reporters have demonstrated detection limits as low as 1 ppb (Gupta et al. 2019). Notably, cell-free systems offer improved adaptability to environmental fluctuations such as pH variations and metal-induced quenching effects.

Significant advancements have also been made in As detection using synthetic biology approaches. To overcome the limitations of low sensitivity in earlier biosensors, researchers have engineered systems by modifying untranslated regions and introducing auxiliary binding sites for the ArsR regulator, thereby enhancing signal-to-noise ratios (Kumawat et al. 2025). Further improvements have been achieved through modular cascaded signal amplification strategies and the development of ArsR mutants via error-prone PCR and high-throughput screening (Somayaji et al. 2022). Cell-free biosensors have been developed using engineered ArsR variants, achieving detection limits as low as 3.65 µg/L, which fall within the permissible limits defined by the WHO (Chauhan et al. 2023). Additional biosensor designs, such as Pars/arsR-phiYFP systems, exhibit time- and dose-dependent fluorescence responses to both arsenite

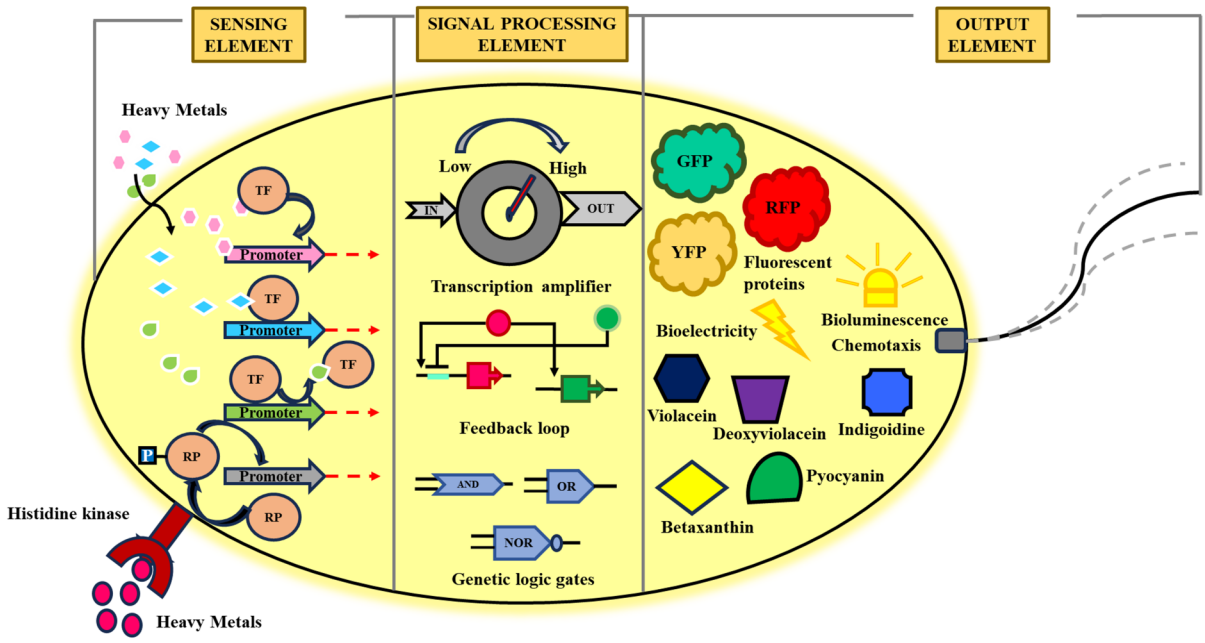

Fig. 2 Schematic representation of synthetic bioremediation bacteria engineered for heavy metal detection, illustrating metal-responsive regulatory proteins and transcription factors driving reporter gene expression. *GFP* green fluorescent protein, *RFP* red fluorescent protein, *RP* regulatory protein, *TF* heavy metal-responsive transcription factor, *YFP* yellow fluorescent protein) (extracted from Thai et al. 2023)

and arsenate (Hu et al. 2010). Other systems, including Pars/arsR-lucGR, luxCDABE/arsR/luxAB, and arsR/crtI constructs, provide diverse detection outputs ranging from bioluminescence reduction to visible color changes (Kumawat et al. 2025). Pb detection has similarly benefited from synthetic biology innovations, although challenges related to sensitivity and specificity remain. Biosensors based on promoter-pbrR-GFP constructs have demonstrated high sensitivity across multiple bacterial hosts, including *Enterobacter*, *Pseudomonas*, and *Shewanella* (Guo et al. 2021). Other systems, such as pGL3-luc/pbr constructs, exhibit high specificity for Pb even in the presence of competing metal ions (Nourmohammadi et al. 2020). However, some luminescent biosensors developed using *Bacillus subtilis* and *Staphylococcus aureus* show limited specificity (Somayaji et al. 2022). In *Alcaligenes eutrophus*, Pb detection (~ 331 µg/mL) also showed high specificity, although culture conditions strongly influenced sensitivity (Nourmohammadi et al. 2020). Cd biosensing has seen notable progress through the development of advanced genetic circuits. Multi-signal biosensors based on engineered cad operons have demonstrated strong responsiveness within defined concentration ranges (Guo et al. 2021). Dual-reporter systems incorporating CadR and CadC regulators, coupled with fluorescent proteins such as mCherry and eGFP, enable quantitative detection based on fluorescence intensity (Hui et al. 2021). The GFP-based *E. coli* DH5α (pVLCD1) biosensor, regulated by cadC and its native promoter, reached extremely low detection thresholds of 0.1 nmol/L for Cd(II) and Sb(III), and 10 nmol/L for Pb(II), and successfully quantified metal bioavailability in soil (Chauhan et al. 2023). Further improvements have been achieved using toggle-switch CadR-lacI-gfp circuits and amplification modules, which reduce detection limits and enhance specificity. A larger screen of 30 Cd circuits identified *P. putida* KT2440 WCB KT-5-R (CadR-mCherry) as the most robust strain, especially when modified with a T7RNAP positive-feedback module, which significantly improved Cd tolerance and achieved a detection limit of 0.01 µM (Jia et al. 2021). In addition, an innovative Cu biosensor was created by integrating engineered *E. coli* with a microbial fuel cell (MFC). Cu^{2+} activates the PcusC promoter, inducing OprF porin and ribB expression, which increases riboflavin

secretion and proportionally enhances the MFC voltage. These systems demonstrate linear relationships between metal concentration and electrical output, highlighting their potential for real-time monitoring (Zhou et al. 2021).

Beyond detection, synthetic biology has enabled significant advancements in bioremediation strategies, particularly in biosorption and bioaccumulation. Biosorption refers to the passive binding of metal ions to biological surfaces through mechanisms such as electrostatic interactions, complexation, chelation, and ion exchange (Somayaji et al. 2022). Surface display technologies have further improved biosorption efficiency by increasing the availability of binding sites. However, biosorption is influenced by environmental factors such as pH and ionic strength, and biosorbents often suffer from limited lifespan due to biomass degradation and fouling of binding sites (Kumawat et al. 2025). Bioaccumulation, on the other hand, requires living cells and is therefore subject to challenges such as maintaining appropriate growth conditions, ensuring sufficient nutrient availability, and preserving cell viability (Somayaji et al. 2022). Despite these limitations, synthetic biology approaches have successfully enhanced metal uptake and tolerance in engineered microorganisms.

Initial efforts in genetic engineering of biosorbents involved the expression of eukaryotic MTs in bacterial systems. For example, intracellular expression of human MTs in *E. coli* resulted in a 3- to 5-fold increase in metal accumulation (Somayaji et al. 2022). Similarly, surface expression of MTs fused to membrane proteins significantly enhanced Cd-binding capacity (Tang et al. 2024). Additional studies have demonstrated that co-expression of MT with metal transporters further improves bioaccumulation efficiency (Kumawat et al. 2025). Surface-displayed phytochelatin analogs have also been shown to increase Cd and Hg accumulation by up to 12-fold and 20-fold, respectively (Kumawat et al. 2025). Studies have identified glutathione synthesis as a key limiting factor in phytochelatin production, highlighting the importance of pathway optimization. Other strategies, including precipitation, enzymatic transformation, and phosphate-mediated immobilization, have also been explored for metal removal. Recent research has focused on integrating detection and remediation into multifunctional systems (Chauhan et al. 2023). Biosensors play a crucial role in this context by enabling the initial detection of metal contamination in environmental samples. Engineered microorganisms capable of both sensing and removing metals offer a sustainable and efficient approach to environmental remediation. For example, engineered *Ralstonia metallidurans* expressing surface-bound MTs have been used to immobilize Cd in soil, thereby protecting plants from metal toxicity (Wuana and Okieimen 2011). Similarly, engineered *Escherichia coli* strains producing extracellular protein nanofibers have been shown to form biofilms that enhance Hg adsorption and reduce intracellular toxicity (Tay et al. 2017). For As, *Staphylococcus epidermidis* embedded on nylon mesh was engineered with dual circuits enabling both fluorescent detection and ion removal (De Santy 2016). Cd remediation has been advanced with *E. coli* M4 expressing MT and a Cd transport system, doubling Cd^{2+} uptake across pH 4–8 (Deng et al. 2007). More extensive engineering of *E. coli* with TcPCS1, *gshA, gshB, TcHMA3,* and *cysE* revealed that enhancing GSH synthesis, specifically the *gshB* step, is the rate-limiting bottleneck for phytochelatin-based Cd sequestration (Chauhan et al. 2023).

Despite these advances, several challenges remain in the practical application of synthetic biology for HM remediation. Many engineered systems rely on plasmid-based constructs, which are prone to instability and loss over time, limiting their long-term effectiveness (Giachino et al. 2021). Additionally, the metabolic burden associated with engineered pathways can negatively impact host cell growth and viability. Environmental variability further complicates system performance, while issues related to biosafety, regulatory approval, and ethical considerations pose significant barriers to field deployment (Kumawat et al. 2025). Overall, while synthetic biology offers powerful tools for addressing HM pollution, further research is needed to improve system stability, scalability, and environmental compatibility. Continued advancements in genetic engineering, systems biology, and regulatory frameworks will be essential for translating these technologies from laboratory settings to real-world applications.

AI-driven models for predicting bioremediation outcomes

Artificial intelligence (AI), a core discipline of computer science, is designed to emulate human cognitive functions such as learning, pattern recognition, and decision-making. Within this field, machine learning (ML) enables systems to improve predictive performance through experience rather than explicit programming, relying on statistical and computational methods that detect complex patterns in multidimensional datasets (Ondrasek et al. 2025). Unlike conventional statistical models, ML approaches are assumption-free, highly adaptive, and ideal for decoding nonlinear relationships in environmental data.

Over the past few years, a number of in-situ and ex-situ approaches for soil remediation have been developed to repair, rehabilitate, or remove the unwanted HM-polluted soil (Angon et al. 2024). The bioremediation techniques of contaminated soil employ a variety of working procedures and specify distinct advantages and implementation limits. There are considerable differences in the effectiveness of various techniques and their associated costs when used in actual field situations (Kharmawphlang et al. 2024a, b). Conventional methods for soil trace metal analysis are highly sensitive but are often costly, laborious, and time-consuming as they require sample digestion and extraction. This necessitates the urgent development of AI-based prediction models for rapid and inexpensive HM detection in soil. Moreover, AI can predict the effectiveness of metal immobilization across diverse environmental settings by identifying suitable amendments (e.g., biochar, bioash) and environmental parameters that maximize stabilization efficiency (Ondrasek et al. 2025).

AI and ML are essential to controlling or managing soil contamination through prediction, clustering, data-centric analysis, and soil quality evaluation. Predictive models may be created using ML, which can analyse and learn from massive, complex, and multidimensional datasets (Fig. 3). Among the numerous ML frameworks, artificial neural networks (ANNs) are particularly versatile due to their ability

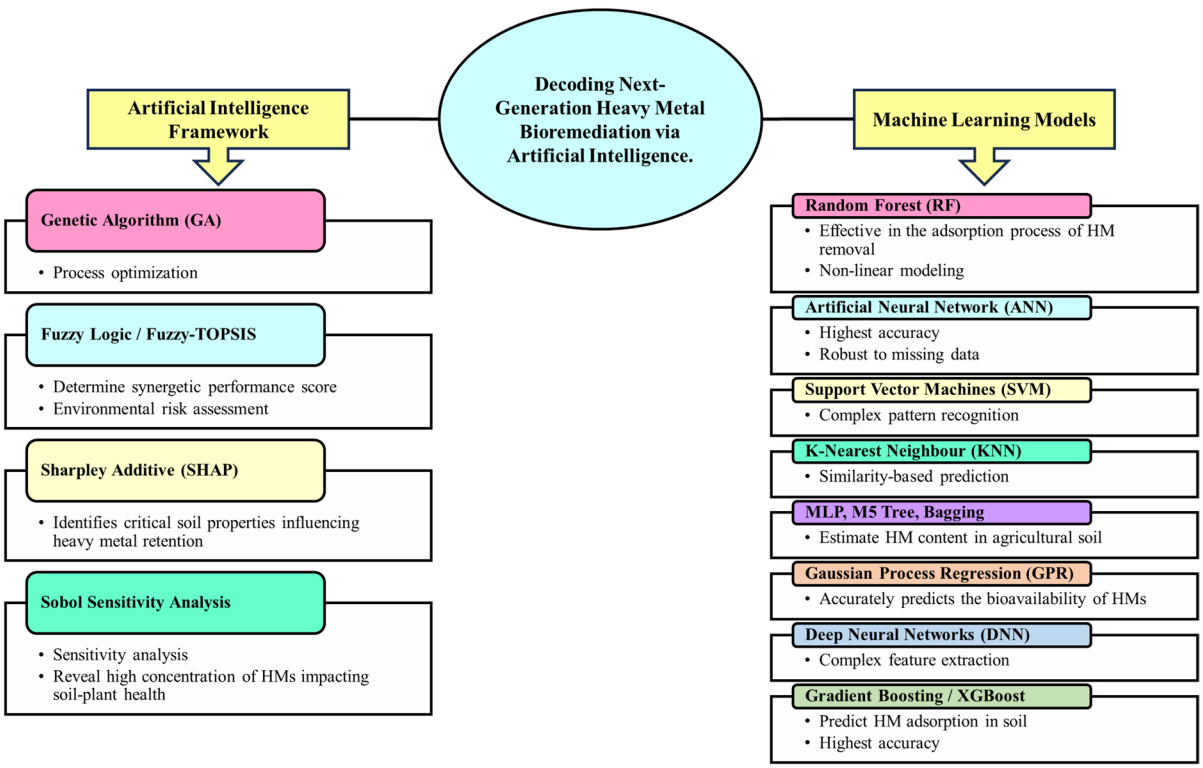

Fig. 3 Overview of commonly used machine learning algorithms, illustrating major supervised and unsupervised techniques employed for data modeling, classification, regression, and pattern recognition

to capture both linear and nonlinear relationships in complex datasets. Other frequently applied ML algorithms include support vector machines (SVMs), random forests (RF), fuzzy logic systems, and k-nearest neighbours (KNN). ANN, SVM, and RF have emerged as powerful tools for analyzing complex multivariate relationships, extending their use to predict adsorption and the immobilization efficiency of HMs in amended soils. Although initially considered black-box models, advances in interpretable ML models and methods like global sensitivity analysis (GSA) are now allowing researchers to analyse the contribution of various input factors. This capability is vital for designing effective soil remediation strategies, as it can significantly reduce the manpower and resources required for future projects (Palansooriya et al. 2022). Recently, the ANN model was utilized and reported to be an efficient tool for determining the effectiveness of the adsorption process for the removal of As(V) and Pb(II) in industrial wastewater removal. In both cases, the trained ANN model predicted with better efficiencies the optimum parameters for maximum As(V) and Pb(II) removal (Ibrahim et al. 2024). ML models, such as gradient boosting decision trees, have been developed to predict HM adsorption in soils, utilizing extensive datasets to improve accuracy (Yang et al. 2021). Techniques like the Shapley additive explanation method help identify critical soil properties influencing HM retention, which can inform earthworm behavior and effectiveness in bioremediation (Yang et al. 2021). However, some studies have successfully used ML, like stochastic gradient boosting (SGB) and random forest (RF), multilayer perceptron neural network (MLP), the M5 model tree (M5), and a bagging approach (BM5P) (Sihag et al. 2019), to estimate metal content in agricultural soils. Furthermore, Trifi et al. (2022) compared ANN, RF, and SVM to predict Zn, Pb, Fe, Mn, Cu, and Cd concentrations in Tunisian soil samples and found the RF model performed best. Additionally, Shi et al. (2022) successfully simulated Cu, Zn, and As content in eastern China's coastal agricultural soils using RF, attributing contamination largely to human and agricultural activities. Furthermore, Sergeev et al. (2019) combined spatial autocorrelation with ANNs to accurately predict Cu, Mn, Ni content, showing improved accuracy over a standalone ANN model, and Chu et al. (2021) noted the strong predictive accuracy and generalization ability of ANNs for Cu, Sn, Zn, Pb across different soil types, underscoring capability of ML to model complex, non-linear relationships (Zhang et al. 2024). A recent study was conducted to compare the performance and stability of various ML models, specifically ANN, deep neural network (DNN), RF, KNN, and adaptive boosting (AB) in estimating Cu, Fe, Mn and Zn contents in soils from intensely cultivated paddy fields (Tasan et al. 2024). The researchers used soil physicochemical parameters like EC, pH, Na, K and soil depth as covariates to enhance accuracy. Model performance was evaluated using the coefficient of determination (R^2), mean absolute error (MAE) and root mean square error (RMSE). The findings revealed that the RF model was the most accurate for Cu, Fe, and Mn, while the ANN model was most accurate for Zn. In terms of stability, the AB model performed best for Cu and the ANN model was the most stable for Fe, Mn, and Zn, despite the observation of overfitting in the RF model. Sensitivity analysis showed that EC, pH, and N were the most significant factors impacting the accumulation of Zn, Cu, Mn, and Fe. The overall better performance of the ANN models for some metals was attributed to their superior ability to model the complex non-linear relationships (Tasan et al. 2024). AI models are trained on experimental and field data to forecast the final effectiveness of a remediation strategy. ML models, such as RF and SVR, are used to predict the immobilization efficiency of HMs when soil is amended with materials like biochar. A study by Sun et al. (2022) used ANN and RF models to predict remediation efficiency of Pb, Cd, Cu, As, and Zn based on biochar characteristics, soil properties, incubation conditions, and the initial metal state. Both ANN and RF models outperformed linear models ($R^2 > 0.84$), with the RF model demonstrating higher tolerance for missing data. Sensitivity analysis identified the type of HM, the pH value of biochar, and the dosage and remediation time as the most influential factors on remediation success. Advanced ML techniques like Gaussian process regression (GPR) are used to accurately predict the bioavailability of HMs like As, Cd, Cu, Cr, and Zn in soil or compost, which is crucial for risk assessment and management. AI allows for the dynamic adjustment of bioremediation parameters to maximize microbial efficiency. AI algorithms like genetic algorithms and ANNs analyse parameters such as pH, temperature, nutrient levels, and microbial activity to determine the optimal

conditions for the degradation or immobilization of HMs. Integrated AI systems continuously collect sensor data from contaminated sites, enabling real-time monitoring and predictive modeling to forecast remediation outcomes and allow for proactive adjustments.

In the realm of vermiremediation, AI models are crucial for assessing toxicity, predicting risk, and optimizing the biological clean-up process. A study assessing the use of vermicomposted tannery waste as a soil amendment utilized several AI and ML tools to analyse the environmental impact (Chakraborty et al. 2024). Fuzzy-TOPSIS was used to determine the synergetic performance score and environmental risks of various soil treatments. ANN sensitivity assay was employed to predict health risks associated with different metal fractions and their bioavailable forms. Sobol sensitivity analysis (SSA) was applied to identify the most sensitive factors, revealing that high concentrations of potential metals Ni, Pb, Cr, and Cu adversely impacted soil-plant health, and that microbial biomass carbon and acid phosphatase activity were the most sensitive factors affected by metal concentrations (Chakraborty et al. 2024). The ANN assay specifically revealed that the exchangeable fraction of Cr was the primary factor contributing to toxicity in the tannery waste treatments. This ability of AI to pinpoint the most toxic metal fraction and link it to biological indicators (like enzymatic activity) allows for targeted intervention and confirms the potential of vermicomposting to decrease the bioavailability of harmful metals safely. The close alignment between the actual and RF predicted removal efficiencies validated the model's reliability in handling the complex, non-linear interactions governing the adsorption process, which is analogous to the complexity of the biological detoxification and HM removal mechanisms carried out by earthworms in soil. Recent studies further demonstrate the integration of AI with earthworm-mediated systems. For instance, AI tools such as artificial neural networks (ANN) and Sobol sensitivity analysis (SSA) have been used to evaluate vermicomposted steel slag systems, where models identified treatment-specific toxicity risks and highlighted that elevated bioavailable HMs negatively affect soil microbial and enzymatic activities, while also confirming the effectiveness of vermicomposting in reducing these risks (Jha et al. 2024). Furthermore, advanced AI-driven modelling integrating ANN, SSA, and multiple machine learning algorithms (RF, SVM, KNN, XGBoost, and MARS) has demonstrated high predictive accuracy for HM detoxification in earthworm-based systems by capturing complex nonlinear interactions among soil properties, microbial dynamics, and earthworm stocking density. These approaches also enabled the development of multi-parameter quality indices and identified optimal earthworm densities for maximum metal immobilization, emphasizing that remediation efficiency is governed by intricate system interactions rather than simple linear relationships (Kharmawphlang et al. 2026). Collectively, these findings highlight the growing role of AI in enhancing the precision, scalability, and effectiveness of vermiremediation strategies. The close alignment between predicted and observed outcomes validates the robustness of AI models in handling the complexity of biological detoxification processes. Predicting the efficiency of HM removal by earthworms requires models capable of capturing nonlinear relationships between initial metal concentration, earthworm activity (e.g., growth, feeding rate), and final metal bioavailability. ANNs are particularly well-suited for this purpose, as they can learn relationships between input variables (soil type, metal concentration, pH, earthworm biomass) and outputs (metal removal rate), making them powerful tools for designing and scaling up vermiremediation projects. Consequently, AI-assisted approaches can guide the selection of suitable earthworm species, optimize operational parameters, and significantly reduce the time and cost associated with field-scale remediation trials.

Challenges and future perspectives in advanced technology-driven vermiremediation systems

The application of advanced technology-driven strategies for HM remediation using earthworm-based systems faces several critical challenges. A primary constraint is the selection of appropriate earthworm species, as interspecific variability in metal tolerance, bioaccumulation capacity, and survival under contaminated conditions is not comprehensively understood across different soil types and contamination profiles. Maintaining stable habitat conditions, particularly pH, moisture, temperature, and organic matter under field conditions, remains difficult, often resulting in reduced biological activity

and inconsistent remediation efficiency. Furthermore, the investigation of earthworm gut microbial communities is complicated by their highly dynamic and environment-sensitive nature, coupled with a lack of standardized frameworks for functional characterization. Quantification of bioaccumulation, metal transformation, and detoxification pathways is also limited by spatial temporal variability and insufficient long-term datasets. The integration of advanced approaches such as multi-omics, kinetic modeling, systems biology, and artificial intelligence is further constrained by high operational costs, technical complexity, data integration challenges, and the limited generalizability of predictive models across heterogeneous contaminated sites.

To overcome these limitations, the development of integrated and standardized frameworks is essential for advancing HM remediation efficiency. The establishment of trait-based, species-specific databases and decision-support tools can facilitate the rational selection of earthworm species tailored to specific metal contaminants and site conditions. Habitat engineering strategies, including the use of soil amendments and controlled environmental buffering, can help maintain optimal conditions and enhance remediation performance. Standardization of multi-omics pipelines, coupled with advances in microbiome research, will enable deeper insights into gut-mediated metal transformation mechanisms. The generation of large-scale, high-quality datasets through long-term and multi-site studies is critical for improving the robustness of kinetic models and AI-based predictions. Additionally, the integration of systems biology with interpretable and scalable AI frameworks can enhance predictive accuracy while ensuring practical applicability. Collectively, these approaches will enable the development of efficient, site-adaptable, and sustainable technology-driven systems for HM remediation.

Conclusion

The persistent global threat posed by HM contamination necessitates a decisive paradigm shift in environmental management, moving beyond costly and environmentally invasive physicochemical techniques toward integrated, nature-based solutions. This review provides the essential mechanistic and technological blueprint for this transition, establishing a unified framework for earthworm-mediated remediation. Our analysis moves past simple empirical observations by detailing the intricate comparative physiology of earthworms, which underpins the development of a predictive taxonomy for species selection. We demonstrated how the integration of kinetic models is crucial for translating instantaneous bioaccumulation factors into transferable parameters, enabling the robust prediction of long-term cleanup durations and steady-state burdens. The true novelty and future potential of vermiremediation lie in the convergence of advanced biological and computational tools. We have established the gut microbiome of earthworms as a pivotal bioreactor, highlighting the catalytic role of microbe-host consortia in metal speciation and detoxification. Furthermore, the synthesis of multi-omics data is key to decoding cellular stress responses and identifying molecular targets for resilience, enabling precision bioremediation strategies. This biological depth is synergistically amplified by the incorporation of AI-driven predictive modeling, offering the necessary computational power to analyse complex environmental variables and optimize treatment protocols in a data-driven manner. By coupling ecological efficacy with economic feasibility and technological innovation, this systems-level approach linking ecology, molecular physiology, microbiology, and artificial intelligence ensures that earthworm-mediated solutions transition from niche remediation techniques into a regenerative, economically attractive alternative for securing global soil health and supporting UN Sustainable Development Goals.

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References

Adnan M, Zhao P, Xiao B, Ali MU, Xiao P (2025) Heavy metal pollution and source analysis of soils around abandoned Pb/Zn smelting sites: environmental risks and fractionation analysis. Environ Technol Innov 38:104084. https://doi.org/10.1016/j.eti.2025.104084

Aguzie IO, Enekwe KD, Emekekwue IJ, Asogwa CN, Onyishi GC, Oluah NS, Ekeh FN, Nwani CD (2021) Behavioral

and oxidative stress responses of earthworm, *Nsukkadrilus mbae* (Segun 1976), exposed to lead and cadmium: a preliminary investigation. Soil Sediment Contam 30:569–589. https://doi.org/10.1080/15320383.2021.1873912

Andleeb S, Naseer A, Liaqat I, Sirajuddin M, Utami M, Alarifi S, Ahamed A, Chang SW, Ravindran B (2024) Assessment of growth, reproduction, and vermi-remediation potentials of *Eisenia fetida* on heavy metal exposure. Environ Geochem Health 46:290. https://doi.org/10.1007/s10653-024-02055-7

Angon PB, Islam MS, Das A, Anjum N, Poudel A, Suchi SA (2024) Sources, effects and present perspectives of heavy metals contamination: soil, plants and human food chain. Heliyon. https://doi.org/10.1016/j.heliyon.2024.e28357

Ardestani MM, van Straalen NM, van Gestel CA (2014) Uptake and elimination kinetics of metals in soil invertebrates: a review. Environ Pollut 193:277–295. https://doi.org/10.1016/j.envpol.2014.06.026

Azizi AB, Lim MPM, Noor ZM, Abdullah N (2013) Vermi removal of heavy metal in sewage sludge by utilising *Lumbricus rubellus*. Ecotoxicol Environ Saf 90:13–20. https://doi.org/10.1016/j.ecoenv.2012.12.006

Banerjee A, Biswas JK, Pant D, Sarkar B, Chaudhuri P, Ra M, Meers E (2019) Enteric bacteria from the earthworm (Metaphire posthuma) promote plant growth and remediate toxic trace elements. J Environ Manag 250:109530. https://doi.org/10.1016/j.jenvman.2019.109530

Bar C, Patil R, Doshi J, Kulkarni MJ, Gade WN (2007) Characterization of the proteins of bacterial strain isolated from contaminated site involved in heavy metal resistance-a proteomic approach. J Biotechnol 128:444–451. https://doi.org/10.1016/j.jbiotec.2006.11.010

Bhartiya DK, Singh K (2011) Accumulation of heavy metals by *Eisenia foetida* from different animal dung and kitchen wastes during vermicomposting. Int J Life Sci Technol 4:47

Bhowmik S, Kumar S, Raj A, Adom D, Saxena R (2025) Metabolomics in understanding and mitigating metal toxicity. Microbial metabolomics: recent developments, challenges and future opportunities. Springer Nature Singapore, Singapore, pp 347–375. https://doi.org/10.1007/978-981-96-4824-5_15

Biswas JK, Banerjee A, Rai M, Naidu R, Biswas B, Vithanage M, Dash MC, Sarkar SK, Meers E (2018a) Potential application of selected metal resistant phosphate solubilizing bacteria isolated from the gut of earthworm (*Metaphire posthuma*) in plant growth promotion. Geoderma 330:117–124. https://doi.org/10.1016/j.geoderma.2018.05.034

Biswas JK, Banerjee A, Rai MK, Rinklebe J, Shaheen SM, Sarkar SK, Dash MC, Kaviraj A, Langer U, Song H, Vithanage M (2018b) Exploring potential applications of a novel extracellular polymeric substance synthesizing bacterium (*Bacillus licheniformis*) isolated from gut contents of earthworm (*Metaphire posthuma*) in environmental remediation. Biodegradation 29:323–337. https://doi.org/10.1007/s10532-018-9835-z

Bonilla JO, Callegari EA, Estevé MC, Villegas LB (2020) Intracellular proteomic analysis of *Streptomyces sp.* MC1 when exposed to Cr (VI) by gel-based and gel-free methods. Curr Microbiol 77:62–70. https://doi.org/10.1007/s00284-019-01790-w

Bora P, Devi NN (2024) Bioremediation of heavy metal lead (Pb) by enteric bacteria and metagenomic analysis of an endogenic earthworm *Metaphire nanaoensis* found in the semi-urban paddy fields of Kamrup District, Assam. Water Air Soil Pollut 235:367. https://doi.org/10.1007/s11270-024-07170-w

Borah G, Deka H (2023) Vermiremediation of heavy metals (HMs)-contaminated agricultural land: synergistic changes in soil enzyme activities and earthworm's growth parameters. Environ Sci Pollut Res 30:115266–115278. https://doi.org/10.1007/s11356-023-30500-0

Borah G, Deka H (2024) Synergistic interactions in enzyme activities during vermiremediation of heavy metals (HMs) polluted soil: elucidating the impact of *Eudrilus eugeniae*. Soil Sediment Contam 33:887–904. https://doi.org/10.1080/15320383.2023.2273348

Chai L, Yang Y, Yang H, Zhao Y, Wang H (2020) Transcriptome analysis of genes expressed in the earthworm *Eisenia fetida* in response to cadmium exposure. Chemosphere 240:124902. https://doi.org/10.1016/j.chemosphere.2019.124902

Chakraborty P, Ghosh S, Banerjee S, Bhattacharya S, Bhattacharyya P (2024) Evaluating the efficacy of vermicomposted products in rain-fed wetland rice and predicting potential hazards from metal-contaminated tannery sludge using novel machine learning tactic. Chemosphere 358:142272. https://doi.org/10.1016/j.chemosphere.2024.142272

Chao G, Jingbo X, Ji L, Zhengtao L (2016) Biological responses in the earthworm *Eisenia fetida* exposed to soils near a typical lead acid battery plant. Soil Sediment Contam 25:573–585. https://doi.org/10.1080/15320383.2016.1184619

Chauhan P, Panthi N, Mazumdar I, Hussain N (2023) Bioaccumulation and detoxification of metals through genetically engineered microorganism. Green technologies for industrial waste remediation. Springer Nature Switzerland, Cham, pp 147–187

Chen M, Wang L, Zheng X, Cohen M, Li X (2021) Cross-kingdom comparative transcriptomics reveals conserved genetic modules in response to cadmium stress. mSystems 6:e01189-21. https://doi.org/10.1128/mSystems.01189-21

Chen F, Yu L, Pan J, Zhang C, Zhang C, Zhao J, Arjan N, Chen W, Tian F, Zhai Q (2025a) The mechanisms of lead resistance in *Lactiplantibacillus plantarum*: insights from proteomics and metabolomics analyses. Chemosphere 378:144399. https://doi.org/10.1016/j.chemosphere.2025.144399

Chen Z, Kama R, Cao Y, Liu Z, Qiu J, Yang X, Li H (2025b) The potential of earthworms and arbuscular mycorrhizal fungi to enhance phytoremediation in heavy metal-contaminated soils: a review. Mycorrhiza 35:1–14. https://doi.org/10.1007/s00572-025-01207-6

Chowdhury R, Hussain N, Mukherjee S, Barman S, Mandal H, Raul PK, Bhattacharya SS (2024) Bioethanol production from lignocellulosic waste without pre-treatment employing vermicompost and earthworm gut-isolated bacteria: insights on waste-to-wealth conversion efficiency

towards cleaner lifestyle. Waste Biomass Valor 15:5573–5587. https://doi.org/10.1007/s12649-024-02548-7

Chu Y, Liu S, Cai G, Bian H (2021) Artificial neural network prediction models of heavy metal polluted soil resistivity. Eur J Environ Civ Eng 25:1570–1590. https://doi.org/10.1080/19648189.2019.1585962

Dedeke GA, Iwuchukwu PO, Aladesida AA, Afolabi TA, Ayanda IO (2018) Impact of heavy metal bioaccumulation on antioxidant activities and DNA profile in two earthworm species and freshwater prawn from Ogun River. Sci Total Environ 624:576–585. https://doi.org/10.1016/j.scitotenv.2017.12.037

Deng X, Yi XE, Liu G (2007) Cadmium removal from aqueous solution by gene-modified *Escherichia coli* JM109. J Hazard Mater 139:340–344. https://doi.org/10.1016/j.jhazmat.2006.06.043

Dominguez-Crespo MA, Sanchez-Hernandez ZE, Torres-Huerta AM, Negrete MDLLX, Conde-Barajas E, Flores-Vela A (2012) Effect of the heavy metals Cu, Ni, Cd and Zn on the growth and reproduction of epigeic earthworms (*E. fetida*) during the vermistabilization of municipal sewage sludge. Water Air Soil Pollut 223:915–931. https://doi.org/10.1007/s11270-011-0913-7

Duarte AP, Melo VF, Brown GG, Pauletti V (2012) Changes in the forms of lead and manganese in soils by passage through the gut of the tropical endogeic earthworm (*Pontoscolex corethrurus)*. Eur J Soil Biol 53:32–39. https://doi.org/10.1016/j.ejsobi.2012.08.004

Ecimovic S, Velki M, Vukovic R, Camagajevac IS, Petek A, Bosnjakovic R, Grgic M, Engelmann P, Bodo K, Filipovic-Marijic V, Ivankovic D (2018) Acute toxicity of selenate and selenite and their impacts on oxidative status, efflux pump activity, cellular and genetic parameters in earthworm *Eisenia andrei*. Chemosphere 212:307–318. https://doi.org/10.1016/j.chemosphere.2018.08.095

Fatima N, Singh K (2023a) Accumulation of heavy metals in soil: sources, toxicity, health impacts, and remediation by earthworms. Eur J Biol Res 13:129–143. https://doi.org/10.5281/zenodo.8206486

Fatima N, Singh KP (2023b) Cobalt, chromium and lead heavy metals accumulation from animal dung, soil and rice grain through vermic-activity by *Lampito mauritii* Kinberg. Int J Zool Appl Biosci 8:5–18. https://doi.org/10.55126/ijzab.2023.v08.i03.002

Giachino A, Focarelli F, Marles-Wright J, Waldron KJ (2021) Synthetic biology approaches to copper remediation: bioleaching, accumulation and recycling. FEMS Microbiol Ecol 97:249. https://doi.org/10.1093/femsec/fiaa249

González-Alcaraz MN, Loureiro S, van Gestel CA (2018) Toxicokinetics of Zn and Cd in the earthworm *Eisenia andrei* exposed to metal-contaminated soils under different combinations of air temperature and soil moisture content. Chemosphere 197:26–32. https://doi.org/10.1016/j.chemosphere.2018.01.019

Gudeta K, Kumar V, Bhagat A, Julka JM, Bhat SA, Ameen F, Qadri H, Singh S, Amarowicz R (2023) Ecological adaptation of earthworms for coping with plant polyphenols, heavy metals, and microplastics in the soil: a review. Heliyon. https://doi.org/10.1016/j.heliyon.2023.e14572

Guo Y, Hui CY, Zhang NX, Liu L, Li H, Zheng HJ (2021) Development of cadmium multiple-signal biosensing and bioadsorption systems based on artificial cad operons. Front Bioeng Biotechnol 9:585617. https://doi.org/10.3389/fbioe.2021.585617

Gupta RK (2020) Behavioral and morphological changes as early markers of heavy metals induced toxicity in *Eudrilus eugeniae*. J Exp Zool India 23:241

Gupta S, Sarkar S, Katranidis A, Bhattacharya J (2019) Development of a cell-free optical biosensor for detection of a broad range of mercury contaminants in water: a plasmid DNA-based approach. ACS Omega 4:9480–9487. https://doi.org/10.1021/acsomega.9b00205

Hattab S, Boughattas I, Cappello T, Zitouni N, Touil G, Romdhani I, Livet A, Bousserrhine N, Banni M (2023) Heavy metal accumulation, biochemical and transcriptomic biomarkers in earthworms *Eisenia andrei* exposed to industrially contaminated soils from south-eastern Tunisia (Gabes Governorate). Sci Total Environ 887:163950. https://doi.org/10.1016/j.scitotenv.2023.163950

Homa J, Olchawa E, Stürzenbaum SR, John Morgan A, Plytycz B (2005) Early- phase immunodetection of metallothionein and heat shock proteins in extruded earthworm coelomocytes after dermal exposure to metal ions. Environ Pollut 135:275–280. https://doi.org/10.1016/j.envpol.2004.10.019

Homa J, Rorat A, Kruk J, Cocquerelle C, Plytycz B, Vandenbulcke F (2015) Dermal exposure of *Eisenia andrei* earthworms: effects of heavy metals on metallothionein and phytochelatin synthase gene expressions in coelomocytes. Environ Toxicol Chem 34:1397–1404. https://doi.org/10.1002/etc.2944

Hu Q, Li L, Wang Y, Zhao W, Qi H, Zhuang G (2010) Construction of WCB-11: a novel phiYFP arsenic-resistant whole-cell biosensor. J Environ Sci 22:1469–1474. https://doi.org/10.1016/S1001-0742(09)60277-1

Huang S, Liu R, Sun M, Li X, Guan Y, Lian B (2022) Transcriptome expression analysis of the gene regulation mechanism of bacterial mineralization tolerance to high concentrations of Cd^{2+}. Sci Total Environ 806:150911. https://doi.org/10.1016/j.scitotenv.2021.150911

Hui CY, Guo Y, Wu J, Liu L, Yang XQ, Guo X, Xie Y, Yi J (2021) Detection of bioavailable cadmium by double-color fluorescence based on a dual-sensing bioreporter system. Front Microbiol 12:696195. https://doi.org/10.3389/fmicb.2021.696195

Huibo Z, Yong Z, Rui L, Guorui L, Jianjun D, Qi W, Xiaotian L, Mingda Y, Yanpeng W, Zhiyan W, Fenglan H (2023) Analysis of the mechanism of *Ricinus communis L.* tolerance to Cd metal based on proteomics and metabolomics. PLoS ONE 18:e0272750. https://doi.org/10.1371/journal.pone.0272750

Hussain N, Sarma S (2025) Nano-fertilizers and their potential application in agriculture. Nanoparticles synthesis by soil microbes. Academic Press, pp 271–288

Hussain N, Chatterjee SK, Maiti TK, Goswami L, Das S, Deb U, Bhattacharya SS (2021) Metal induced non-metallothionein protein in earthworm: a new pathway for cadmium detoxification in chloragogenous tissue. J Hazard Mater 401:123357. https://doi.org/10.1016/j.jhazmat.2020.123357

Hussain N, Goswami L, Bhattacharya SS (2023) Microbial detoxification of contaminated land. Bio-inspired land remediation. Springer, Cham, pp 189–219

Huu Nguyen V, Yee SK, Hong Y, Moon DH, Han S (2019) Predicting mercury bioavailability in soil for earthworm *Eisenia fetida* using the diffusive gradients in thin films technique. Environ Sci Pollut Res 26:19549–19559. https://doi.org/10.1007/s11356-019-05180-4

Ibrahim M, Haider A, Lim JW, Mainali B, Aslam M, Kumar M, Shahid MK (2024) Artificial neural network modeling for the prediction, estimation, and treatment of diverse wastewaters: a comprehensive review and future perspective. Chemosphere 362:142860. https://doi.org/10.1016/j.chemosphere.2024.142860

Iheme PO, Ajayi AT, Ayo-Komolafe KO, Njoku KL (2021) Vermiremediation potentials of *Lumbricus terrestris* and *Eudrilus euginae* in heavy metal contaminated soil from mechanic, welder workshop and metallic dumpsite. Niger J Biotechnol 38:118–133. https://doi.org/10.4314/njb.v38i2.13

Jain S, Bhatt A (2013) Proteomic analysis of diversified extremophilic strains of Pseudomonas in the presence of cadmium. Agric Res 2:354–359. https://doi.org/10.1007/s40003-013-0083-5.

Jatwani C, Gupta RK, Rai R, Bansal N (2016) Effects of Hg/Co toxicity in soil on biomolecules of earthworm, *Eisenia fetida*. Procedia Environ Sci 35:450–455. https://doi.org/10.1016/j.proenv.2016.07.028

Jha S, Banerjee S, Ghosh S, Verma A, Bhattacharyya P (2024) Appraisal of heavy metal risk hazards of *Eisenia fetida*-mediated steel slag vermicompost on *Oryza sativa L.*: insights from agro-scale inspection and machine learning analytics. Agriculture 14:2020

Jia X, Liu T, Ma Y, Wu K (2021) Construction of cadmium whole-cell biosensors and circuit amplification. Appl Microbiol Biotechnol 105:5689–5699. https://doi.org/10.1007/s00253-021-11403-x

Kharmawphlang IM, Deka H, Hussain N (2024a) Unravelling the detoxification trail of potential toxic heavy metals: an insight into heavy metal auditing and ecological health upon valorisation by *Lampito mauritii* and *Eudrilus eugeniae*. Environ Sci Pollut Res 31:64007–64022. https://doi.org/10.1007/s11356-024-35417-w

Kharmawphlang IM, Paul J, Doloi S, Hussain N (2024b) Waste to energy technologies: a sustainable key approach towards circular economy and energy recycling. Trash or treasure: entrepreneurial opportunities in waste management. Springer Nature Switzerland, Cham, pp 143–181. https://doi.org/10.1007/978-3-031-55131-4_6

Kharmawphlang IM, Saha A, Beirapawngia G, Ghosh S, Deuri D, Hussain N (2026) Does earthworm stocking density act as an ecological lever to modulate microbial communities, phospho-lipid fatty acid signatures, and mineralization-humification dynamics? Bioresour Technol Rep. https://doi.org/10.1016/j.biteb.2026.102545

Kilpi-Koski J, Penttinen OP, Väisänen AO, van Gestel CA (2020) Toxicity of binary mixtures of Cu, Cr and As to the earthworm *Eisenia andrei*. Ecotoxicology 29:900–911. https://doi.org/10.1007/s10646-020-02240-1

Kokhia M, Lortkipanidze M, Gorgadze O, Kuchava M, Nebieridze D (2022) Earthworms (Oligochaeta: *Lumbricidae*) and heavy metals: content and bioaccumulation in the body. J Agric Sci. https://doi.org/10.15159/jas.22.20

Kowald GR, Stürzenbaum SR, Blindauer CA (2016) Earthworm *Lumbricus rubellus* MT-2: metal binding and protein folding of a true cadmium-MT. Int J Mol Sci 17:65. https://doi.org/10.3390/ijms17010065

Kumawat R, Jha S, Joshi D, Rawat A (2025) Synthetic biology; an advancement in heavy metal detection and detoxification.

Lata S, Sharma S, Kaur S (2023) OMICS approaches in mitigating metal toxicity in comparison to conventional techniques used in cadmium bioremediation. Water Air Soil Pollut 234:148. https://doi.org/10.1007/s11270-023-06145-7

Lee BT, Lee SW, Kim KR, Kim KW (2013) Bioaccumulation and the soil factors affecting the uptake of arsenic in earthworm, *Eisenia fetida*. Environ Sci Pollut Res 20:8326–8333. https://doi.org/10.1007/s11356-013-2087-x

Li X, Wang M, Jiang R, Zheng L, Chen W (2020) Evaluation of joint toxicity of heavy metals and herbicide mixtures in soils to earthworms (*Eisenia fetida*). J Environ Sci 94:137–146. https://doi.org/10.1016/j.jes.2020.03.055

Liang SH, Chen SC, Chen CY, Kao CM, Yang JI, Shieh BS, Chen JH, Chen CC (2011) Cadmium-induced earthworm metallothionein-2 is associated with metal accumulation and counteracts oxidative stress. Pedobiologia (Jena) 54:333–340. https://doi.org/10.1016/j.pedobi.2011.07.011

Liu C, Duan C, Meng X, Yue M, Zhang H, Wang P, Xiao Y, Hou Z, Wang Y, Pan Y (2020a) Cadmium pollution alters earthworm activity and thus leaf-litter decomposition and soil properties. Environ Pollut 267:115410. https://doi.org/10.1016/j.envpol.2020.115410

Liu P, Yang Y, Li M (2020b) Responses of soil and earthworm gut bacterial communities to heavy metal contamination. Environ Pollut 265:114921. https://doi.org/10.1016/j.envpol.2020.114921

Liu X, Xiao R, Li R, Ali A, Zhang Z (2020c) Bioremediation of Cd-contaminated soil by earthworms (*Eisenia fetida*): enhancement with EDTA and bean dregs. Environ Pollut 266:115191. https://doi.org/10.1016/j.envpol.2020.115191

Liu L, Wu Q, Miao X, Fan T, Meng Z, Chen X, Zhu W (2022) Study on toxicity effects of environmental pollutants based on metabolomics: a review. Chemosphere 286:131815. https://doi.org/10.1016/j.chemosphere.2021.131815

Liu Y, Chen M, Mu X, Wang X, Zhang M, Yin Y, Wang K (2023) Responses and detoxification mechanisms of earthworm *Amynthas hupeiensis* to metal contaminated soils of North China. Environ Pollut 327:121584. https://doi.org/10.1016/j.envpol.2023.121584

Maity S, Banerjee R, Goswami P, Chakrabarti M, Mukherjee A (2018) Oxidative stress responses of two different ecophysiological species of earthworms (*Eutyphoeus waltoni* and *Eisenia fetida*) exposed to Cd-contaminated soil. Chemosphere 203:307–317. https://doi.org/10.1016/j.chemosphere.2018.03.189

Marinussen MPJC, van der Zee SEATM, de Haan FAM, Bouwman LM, Hefting MM (1997) Heavy metal (copper, lead, and zinc) accumulation and excretion by earthworm, *Dendrobaena veneta*. J Environ Qual 26:278–284

Mincarelli L, Tiano L, Craft J, Marcheggiani F, Vischetti C (2019) Evaluation of gene expression of different molecular biomarkers of stress response as an effect of copper exposure on the earthworm *Eisenia andrei*. Ecotoxicology 28:938–948. https://doi.org/10.1007/s10646-019-02093-3

Mitra S, Chakraborty AJ, Tareq AM, Emran TB, Nainu F, Khusro A, Idris AM, Khandaker MU, Osman H, Alhumaydhi FA, Simal-Gandara J (2022) Impact of heavy metals on the environment and human health: novel therapeutic insights to counter the toxicity. J King Saud Univ Sci 34:101865. https://doi.org/10.1016/j.jksus.2022.101865

Mo X, Qiao Y, Sun Z, Sun X, Li Y (2012) Molecular toxicity of earthworms induced by cadmium contaminated soil and biomarkers screening. J Environ Sci 24:1504–1510. https://doi.org/10.1016/S1001-0742(11)60957-1

Muthukaruppan G (2015) Heavy metal induced biomolecule and genotoxic changes in earthworm *Eisenia fetida*. Invertebr Surviv J 12:237–245

Nishat F, Singh A, Singh PK, Pandey AK, Singh K (2024) Heavy metals in the environment: bioremediation technique utilizing earthworms for remediating heavy metal contaminated soil. Int J Zool Invest 10:387–409. https://doi.org/10.33745/ijzi.2024.v10i02.037

Nourmohammadi E, Hosseinkhani S, Nedaeinia R, Khoshdel-Sarkarizi H, Nedaeinia M, Ranjbar M, Ebrahimi N, Farjami Z, Nourmohammadi M, Mahmoudi A, Goli M (2020) Construction of a sensitive and specific lead biosensor using a genetically engineered bacterial system with a luciferase gene reporter controlled by pbr and cadA promoters. Biomed Eng Online 19:79. https://doi.org/10.1186/s12938-020-00816-w

Ogunlaja A, Sharma V, Lin J (2020) Ex-situ induction of metallothionein gene in *Lybiodrilus violaceous* post cadmium and zinc exposure. Gene Rep 20:100701. https://doi.org/10.1016/j.genrep.2020.100701

Ondrasek G, Shepherd J, Rathod S, Dharavath R, Rashid MI, Brtnicky M, Shahid MS, Horvatinec J, Rengel Z (2025) Metal contamination–a global environmental issue: sources, implications & advances in mitigation. RSC Adv 15:3904–3927. https://doi.org/10.1039/D4RA04639K

Palansooriya KN, Li J, Dissanayake PD, Suvarna M, Li L, Yuan X, Sarkar B, Tsang DC, Rinklebe J, Wang X, Ok YS (2022) Prediction of soil heavy metal immobilization by biochar using machine learning. Environ Sci Technol 56:4187–4198. https://doi.org/10.1021/acs.est.1c08302

Paul S, Goswami L, Pegu R, Bhattacharya SS (2020) Vermiremediation of cotton textile sludge by *Eudrilus eugeniae*: insight into metal budgeting, chromium speciation, and humic substance interactions. Bioresour Technol 314:123753. https://doi.org/10.1016/j.biortech.2020.123753

Peijnenburg WJGM, Baerselman R, de Groot AC, Jager T, Posthuma L, van Veen RP (1999) Relating environmental availability to bioavailability: soil type-dependent metal accumulation in the Oligochaete *Eisenia andrei*. Ecotoxicol Environ Saf 44:294–310

Phurailatpam L, Dalal VK, Singh N, Mishra S (2022) Heavy metal stress alleviation through omics analysis of soil and plant microbiome. Front Sustain Food Syst 5:817932. https://doi.org/10.3389/fsufs.2021.817932

Ray S, Gautam A, Ray A, Das S, Ray M (2019) Analysis of oxidative stress and cellular aggregation in the coelomocytes of earthworms collected from metal contaminated sites of industrial and agricultural soils of West Bengal, India. Environ Sci Pollut Res 26:22625–22640. https://doi.org/10.1007/s11356-019-05438-x

Raza A, Salehi H, Bashir S, Tabassum J, Jamla M, Charagh S, Barmukh R, Mir RA, Bhat BA, Javed MA, Guan DX (2024) Transcriptomics, proteomics, and metabolomics interventions prompt crop improvement against metal (loid) toxicity. Plant Cell Rep 43:80. https://doi.org/10.1007/s00299-024-03153-7

Renu R, Kumar R, Kaur T (2020) Eco-toxic effects of lead and nickel on survivability, reproduction and growth of earthworm (*Eudrilus eugeniae*). J Entomol Zool Stud 8:1351–1355. https://doi.org/10.22271/j.ento.2020.v8.i5s.7691

Roy A, Jha AK, Kumar A, Bhattacharya T, Chakraborty S, Raval NP, Kumar M (2024) Heavy metal pollution in indoor dust of residential, commercial, and industrial areas: a review of evolutionary trends. Air Qual Atmos Health 17:891–918. https://doi.org/10.1007/s11869-023-01478-y

Sabina R, Dey R, Ghosh S, Bhattacharya P, Bhattacharya SS, Hussain N (2025a) Unlocking the potential of *Eudrilus eugeniae* in mitigating the pollution risk of pesticides and heavy metals: fostering machine learning tactics to optimize environmental health. Sci Total Environ 971:179039. https://doi.org/10.1016/j.scitotenv.2025.179039

Sabina R, Paul J, Hussain N (2025b) Chlorpyrifos-primed microbial consortia reinforce earthworm gut homeostasis: a metagenomics-validated and targeted approach to accelerate detoxification and prevent dysbiosis. Biores Technol Rep. https://doi.org/10.1016/j.biteb.2025.102407

Sabina R, Paul J, Sharma S, Hussain N (2025c) Synthetic nitrogen fertilizer pollution: global concerns and sustainable mitigating approaches. Agricultural nutrient pollution and climate change: challenges and opportunities. Springer Nature Switzerland, Cham, pp 57–101. https://doi.org/10.1007/978-3-031-80912-5_3

Salam LB, Obayori OS, Ilori MO, Amund OO (2020) Effects of cadmium perturbation on the microbial community structure and heavy metal resistome of a tropical agricultural soil. Bioresour Bioprocess 7:25. https://doi.org/10.1186/s40643-020-00314-w

Sergeev AP, Buevich AG, Baglaeva EM, Shichkin AV (2019) Combining spatial autocorrelation with machine learning increases prediction accuracy of soil heavy metals. CATENA 174:425–435. https://doi.org/10.1016/j.catena.2018.11.037

Sforzini S, Moore MN, Mou Z, Boeri M, Banni M, Viarengo A (2017) Mode of action of Cr (VI) in immunocytes of earthworms: implications for animal health. Ecotoxicol Environ Saf 138:298–308. https://doi.org/10.1016/j.ecoenv.2017.01.013

Sharma S, Sundaram CS, Luthra PM, Singh Y, Sirdeshmukh R, Gade WN (2006) Role of proteins in resistance mechanism of *Pseudomonas fluorescens* against heavy metal

induced stress with proteomics approach. J Biotechnol 126:374–382. https://doi.org/10.1016/j.jbiotec.2006.04.032

Sharma P, Kumar S, Pandey A (2021) Bioremediated techniques for remediation of metal pollutants using metagenomics approaches: a review. J Environ Chem Eng 9:105684. https://doi.org/10.1016/j.jece.2021.105684

Shi T, Zhang J, Shen W, Wang J, Li X (2022) Machine learning can identify the sources of heavy metals in agricultural soil: a case study in northern Guangdong Province, China. Ecotoxicol Environ Saf 245:114107. https://doi.org/10.1016/j.ecoenv.2022.114107

Sihag P, Keshavarzi A, Kumar V (2019) Comparison of different approaches for modeling of heavy metal estimations. SN Appl Sci 1:780. https://doi.org/10.1007/s42452-019-0816-6

Smith BA, Egeler P, Gilberg D, Hendershot W, Stephenson GL (2010) Uptake and elimination of cadmium and zinc by *Eisenia andrei* during exposure to low concentrations in artificial soil. Arch Environ Contam Toxicol 59:264–273

Somayaji A, Sarkar S, Balasubramaniam S, Raval R (2022) Synthetic biology techniques to tackle heavy metal pollution and poisoning. Synth Syst Biotechnol 7:841–846. https://doi.org/10.1016/j.synbio.2022.04.007

Spurgeon DJ, Hopkin SP (1999) Comparisons of metal accumulation and excretion kinetics in earthworms (*Eisenia fetida*) exposed to contaminated field and laboratory soils. Appl Soil Ecol 11:227–243

Srut M, Menke S, Höckner M, Sommer S (2019) Earthworms and cadmium–heavy metal resistant gut bacteria as indicators for heavy metal pollution in soils? Ecotoxicol Environ Saf 171:843–853. https://doi.org/10.1016/j.ecoenv.2018.12.102

Sun M, Chao H, Zheng X, Deng S, Ye M, Hu F (2020) Ecological role of earthworm intestinal bacteria in terrestrial environments: a review. Sci Total Environ 740:140008. https://doi.org/10.1016/j.scitotenv.2020.140008

Sun Y, Zhang Y, Lu L, Wu Y, Zhang Y, Kamran MA, Chen B (2022) The application of machine learning methods for prediction of metal immobilization remediation by biochar amendment in soil. Sci Total Environ 829:154668. https://doi.org/10.1016/j.scitotenv.2022.154668

Tang RG, Ding CF, Dang F, Ma YB, Wang JS, Zhang TL, Wang XX (2018) NMR-based metabolic toxicity of low-level Hg exposure to earthworms. Environ Pollut 239:428–437. https://doi.org/10.1016/j.envpol.2018.04.027

Tang H, Xiang G, Xiao W, Yang Z, Zhao B (2024) Microbial mediated remediation of heavy metals toxicity: mechanisms and future prospects. Front Plant Sci 15:1420408. https://doi.org/10.3389/fpls.2024.1420408

Tasan M, Demir Y, Tasan S, Öztürk E (2024) Comparative analysis of different machine learning algorithms for predicting trace metal concentrations in soils under intensive paddy cultivation. Comput Electron Agric 219:108772. https://doi.org/10.1016/j.compag.2024.108772

Tay PKR, Nguyen PQ, Joshi NS (2017) A synthetic circuit for mercury bioremediation using self-assembling functional amyloids. ACS Synth Biol 6:1841–1850. https://doi.org/10.1021/acssynbio.7b00137

Thai TD, Lim W, Na D (2023) Synthetic bacteria for the detection and bioremediation of heavy metals. Front Bioeng Biotechnol 11:1178680. https://doi.org/10.3389/fbioe.2023.1178680

Thakur SS, Lone AR, Tiwari N, Jain SK, Yadav S (2021) Metagenomic exploration of bacterial community structure of earthworms' gut. J Pure Appl Microbiol 15:1156–1172. https://doi.org/10.22207/JPAM.15.3.05

Trifi M, Gasmi A, Carbone C, Majzlan J, Nasri N, Dermech M, Charef A, Elfil H (2022) Machine learning-based prediction of toxic metals concentration in an acid mine drainage environment, northern Tunisia. Environ Sci Pollut Res 29:87490–87508. https://doi.org/10.1007/s11356-022-21890-8

DeSanty KM (2016) Using biofilms for the detection and bioremediation of arsenic.

Vijver MG, Vink JPM, Miermans CJH, van Gestel CAM (2003) Oral sealing using glue: a new method to distinguish between intestinal and dermal uptake of metals in earthworms. Soil Biol Biochem 35:125–132. https://doi.org/10.1016/S0038-0717(02)00245-6

Wagh MS, Osborne WJ, Sivarajan S (2022) *Bacillus xiamenensis* and earthworm *Eisenia fetida* in bioremoval of lead, nickel and cadmium: a combined bioremediation approach. Appl Soil Ecol 176:104459. https://doi.org/10.1016/j.apsoil.2022.104459

Wang X, Chang L, Sun Z, Zhang Y, Yao L (2010) Analysis of earthworm *Eisenia fetida* proteomes during cadmium exposure: an ecotoxicoproteomics approach. Proteomics 10:4476–4490. https://doi.org/10.1002/pmic.201000209

Wang Y, Han W, Wang X, Chen H, Zhu F, Wang X, Lei C (2017) Speciation of heavy metals and bacteria in cow dung after vermicomposting by the earthworm, Eisenia fetida. Biores Technol 245:411–418. https://doi.org/10.1016/j.biortech.2017.08.118

Wang K, Qiao Y, Zhang H, Yue S, Li H, Ji X, Liu L (2018a) Bioaccumulation of heavy metals in earthworms from field contaminated soil in a subtropical area of China. Ecotoxicol Environ Saf 148:876–883. https://doi.org/10.1016/j.ecoenv.2017.11.058

Wang K, Qiao Y, Zhang H, Yue S, Li H, Ji X, Liu L (2018b) Influence of metal- contamination on distribution in subcellular fractions of the earthworm (*Metaphire californica*) from Hunan Province, China. J Environ Sci 73:127–137. https://doi.org/10.1016/j.jes.2018.01.021

Wang HT, Zhu D, Li G, Zheng F, Ding J, O'Connor PJ, Zhu YG, Xue XM (2019) Effects of arsenic on gut microbiota and its biotransformation genes in earthworm *Metaphire sieboldi*. Environ Sci Technol 53:3841–3849. https://doi.org/10.1021/acs.est.8b06695

Wang G, Xia X, Yang J, Tariq M, Zhao J, Zhang M, Huang K, Lin K, Zhang W (2020) Exploring the bioavailability of nickel in a soil system: physiological and histopathological toxicity study to the earthworms (*Eisenia fetida*). J Hazard Mater 383:121169. https://doi.org/10.1016/j.jhazmat.2019.121169

Wang HT, Ma L, Zhu D, Ding J, Li G, Jin BJ, Shao YH, Zhang WX, Song MY, Fu SL (2022) Responses of earthworm *Metaphire vulgaris* gut microbiota to arsenic and nanoplastics contamination. Sci Total Environ 806:150279. https://doi.org/10.1016/j.scitotenv.2021.150279

Wang X, Cheng N, Chu Z, Dong Z, Shen L, Shen B (2025) Effects of rice husk biochar-aided vermicomposting of sewage sludge on heavy metals distribution in earthworm subcellular fractions. Environ Technol 46:5417–5430. https://doi.org/10.1080/09593330.2025.2538824

Wen B, Huang R, Wang P, Zhou Y, Shan XQ, Zhang S (2011) Effect of complexation on the accumulation and elimination kinetics of cadmium and ciprofloxacin in the earthworm *Eisenia fetida*. Environ Sci Technol 45:4339–4345. https://doi.org/10.1021/es104034g

Wuana RA, Okieimen FE (2011) Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. Int Sch Res Not 2011:402647. https://doi.org/10.5402/2011/402647

Xiao R, Ali A, Xu Y, Abdelrahman H, Li R, Lin Y, Bolan N, Shaheen SM, Rinklebe J, Zhang Z (2022) Earthworms as candidates for remediation of potentially toxic elements contaminated soils and mitigating the environmental and human health risks: a review. Environ Int 158:106924. https://doi.org/10.1016/j.envint.2021.106924

Xu B, Chu T, Zhang R, Yang R, Zhu M, Guo F, Zan S (2024) Earthworm gut bacteria facilitate cadmium immobilization through the formation of CdS nanoparticles. Chemosphere 361:142453. https://doi.org/10.1016/j.chemosphere.2024.142453

Yadav R, Gupta RK, Kumar R, Kaur T, Moun R (2022) Biochemical response of earthworm, *Eisenia fetida* to heavy metals toxicity. J Appl Nat Sci 14:990

Yadav R, Kumar R, Gupta RK, Kaur T, Kour A, Kaur S, Rajput A (2023) Heavy metal toxicity in earthworms and its environmental implications: a review. Environ Adv 12:100374. https://doi.org/10.1016/j.envadv.2023.100374

Yan X, Wang J, Zhu L, Wang J, Li S, Kim YM (2021) Oxidative stress, growth inhibition, and DNA damage in earthworms induced by the combined pollution of typical neonicotinoid insecticides and heavy metals. Sci Total Environ 754:141873. https://doi.org/10.1016/j.scitotenv.2020.141873

Yang H, Huang K, Zhang K, Weng Q, Zhang H, Wang F (2021) Predicting heavy metal adsorption on soil with machine learning and mapping global distribution of soil adsorption capacities. Environ Sci Technol 55:14316–14328. https://doi.org/10.1021/acs.est.1c02479

Yang R, Luo L, Zhao N, Guo F, Zhu M, Zan S, Yu T, Han FX, Huang J (2022) Indigenous earthworms and gut bacteria are superior to chemical amendments in the remediation of cadmium-contaminated seleniferous soils. Ecotoxicol Environ Saf 245:114122. https://doi.org/10.1016/j.ecoenv.2022.114122

You R, Li H, Li X, Luo L, Wang P, Xia H, Zhou Y (2024) Ecotoxicological impacts of cadmium on soil microorganisms and earthworms *Eisenia foetida*: from gene regulation to physiological processes. Front Environ Sci 12:1479500. https://doi.org/10.3389/fenvs.2024.1479500

Yu W, Zhang Y, Sang W (2022) Integration of transcriptomic and metabolomic reveals metabolic pathway alteration in earthworms (*Eisenia fetida*) under copper exposure. Comp Biochem Physiol C Toxicol Pharmacol 260:109400. https://doi.org/10.1016/j.cbpc.2022.109400

Yue SZ, Huang CD, Wang RP, Qiao YH (2021) Selenium toxicity, bioaccumulation, and distribution in earthworms (*Eisenia fetida*) exposed to different substrates. Ecotoxicol Environ Saf 217:112250

Yue S, Wang R, Huang C, Qiao Y, Shen Z, Wei Y, Li Z (2024) Toxicokinetics of selenate in earthworm sub-tissues and potential bio-accessibility assessment of earthworm-derived selenium. Ecotoxicol Environ Saf 281:116643. https://doi.org/10.1016/j.ecoenv.2024.116643

Zaltauskaite J, Kniuipyte I, Kugelyte R (2020) Lead impact on the earthworm *Eisenia fetida* and earthworm recovery after exposure. Water Air Soil Pollut 231:1–8. https://doi.org/10.1007/s11270-020-4428-y

Zhang X, Li F, Liu T, Xu C, Duan D, Peng C, Zhu S, Shi J (2013) The variations in the soil enzyme activity, protein expression, microbial biomass, and community structure of soil contaminated by heavy metals. Int Sch Res Not 2013:803150. https://doi.org/10.1155/2013/803150

Zhang D, Yin C, Abbas N, Mao Z, Zhang Y (2020) Multiple heavy metal tolerance and removal by an earthworm gut fungus *Trichoderma brevicompactum* QYCD-6. Sci Rep 10:6940. https://doi.org/10.1038/s41598-020-63813-y

Zhang H, Yin A, Yang X, Fan M, Shao S, Wu J, Wu P, Zhang M, Gao C (2021) Use of machine-learning and receptor models for prediction and source apportionment of heavy metals in coastal reclaimed soils. Ecol Indic 122:107233. https://doi.org/10.1016/j.ecolind.2020.107233

Zhang Y, Zhao J, Sa N, Huang C, Yu W, Ma T, Yang H, Ma F, Sun S, Tang C, Sang W (2023) Multi-omics analysis reveals copper-induced growth inhibition mechanisms of earthworm (*Eisenia fetida*). Environ Pollut 318:120862. https://doi.org/10.1016/j.envpol.2022.120862

Zhang F, Zhou S, Jia Z, Xie X, Xu M, Wu S (2024) A framework integrating affinity propagation algorithm and spatial bivariate analysis for enhanced identification and localisation of soil heavy metals pollution sources. Environ Geochem Health 46:455. https://doi.org/10.1007/s10653-024-02246-2

Zhou T, Li R, Zhang S, Zhao S, Sharma M, Kulshrestha S, Khan A, Kakade A, Han H, Niu Y, Li X (2021) A copper-specific microbial fuel cell biosensor based on riboflavin biosynthesis of engineered *Escherichia coli*. Biotechnol Bioeng 118:210–222. https://doi.org/10.1002/bit.27563