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Earthworms as Biological Tools for Assessing Soil Pollutants

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ABSTRACT

Soil contamination has increased significantly over the last few decades due to anthropogenic activities such as industrial waste disposal, agricultural practices, urbanization, construction, mining and drilling, waste dumping, and lack of regulation and enforcement. These activities cause soil contamination with pollutants such as heavy metals, pesticides, industrial chemicals, and pathogens. These pollutants disrupt the food chain, contaminate crops and water, and threaten living beings and the environment. To effectively assess soil pollution, it is essential to establish non-hazardous threshold technologies for determining the quantity of pollutants in the soil. A bio-monitoring approach that focuses on metal toxicity and its availability in the soil, as well as its impact on unidentified metabolites, is appropriate. As a major component of soil biota, earthworms play a crucial role in organic matter decomposition and soil reconstruction in terrestrial ecosystems. Several studies have indicated that earthworms can serve as biological tools for monitoring soil pollutants and exhibit stress responses to contaminants. Their density and biomass are significantly affected by local soil pollution because they are directly exposed to the soil environment. This review article summarizes various research studies on how earthworm species function as bioindicators or biomonitors and their response to different soil pollutants. Further research is required to identify the species-specific responses of earthworms and to understand their biochemical, anatomical, physiological, and genetic changes in particular pollutant-contaminated soils. These efforts are aimed at helping us maintain soil health for sustainability.

Keywords Bioindicators, Biomonitors, Earthworm, Food chain, Pollutant, Soil biota, Sustainability.

INTRODUCTION

Soil health is essential for global ecosystems and sustaining life on Earth. It provides a wide range of ecosystem services, including food production, climate regulation, water management, energy provision, and the support of diverse life forms. Additionally, soil plays a crucial role in nutrient recycling, groundwater replenishment, maintaining soil fertility, decomposing organic matter, and forming fossil fuels (Yadav *et al.* 2021). The significance of soil health extends beyond agriculture to encompass broader environmental, economic, and social dimensions. Healthy soils support diverse and productive ecosystems by providing a stable substrate for plant roots and a rich reservoir of nutrients, organic matter, and microorganisms. Soil plays a crucial role in regulating

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water availability and quality, as well as in mitigating the impacts of floods, droughts, and erosion. Additionally, soils act as a carbon sink, storing significant amounts of carbon and helping to alleviate climate change by sequestering atmospheric carbon dioxide. However, soil health is increasingly threatened by various anthropogenic activities with soil pollution being a significant concern.

Soil pollution is a significant and growing threat to human health. According to Münzel *et al.* (2023), soil can become contaminated by heavy metals, organic chemicals such as pesticides, biological pathogens, and micro/nano plastic particles. This pollution reduces the soil's ability to produce food, leading to the contamination of food crops and associated health risks. Various sources contribute to soil pollution, such as industrial discharge, agricultural runoff, improper waste disposal, and urbanization. The impact of soil pollution can cause food contamination, water resources contamination, loss of biodiversity, and also adverse impacts on human health through the ingestion of contaminated food and water. Additionally, soil degradation can increase the vulnerability of ecosystems to climate change, soil erosion, desertification, and loss of arable land. One of the major contributors to soil pollution in India is the improper disposal of industrial and municipal waste, as studied by Titirmare *et al.* (2023). Inadequate treatment and disposal of hazardous waste can release toxic substances into the soil, posing a risk to human health and the environment.

A bio-monitoring method would be appropriate to evaluate metal toxicity, because of its sensitivity and availability for unknown metabolites. Organisms such as fish, snails, and plants have been employed as bio-monitors (Harnly *et al.*1997, Citterio *et al.* 2002). Earthworms, ecosystem engineers of the underground, provide a unique opportunity to monitor soil pollution and environmental degradation. They are considered bioindicators of soil health and pollution due to their sensitivity to environmental changes and intimate relationship with the soil ecosystem (Paoletti 1999). Earthworm populations reflect the general health and fertility of soils. Higher earthworm abundance and diversity typically signify healthier soil conditions with good organic matter content and nutrient availability. As reported by Kumar *et al.* (2021) various climatic and edaphic factors are responsible for the diversity, distribution, and abundance of earthworms in different habitats. Declines in earthworm populations or changes in species composition may indicate soil degradation, pollution, or habitat disturbance. Verma *et al.* (2021) observed that earthworms are highly sensitive to changes in soil properties, including moisture content, pH level, temperature, and organic matter and pollutants. Their diverse behaviors and ecological functions make them effective indicators of overall soil ecosystem health and quality. Earthworms respond differently to various soil pollutants, such as heavy metals, pesticides, and organic contaminants. Recently, considerable attention has been paid to the potential of earthworms, alone or combined with other soil organisms and soil amendments, to remediate potentially toxic elements contaminated soils (Ran *et al.* 2022). As biological indicators, they can provide a sensitive and integrated measure of soil health, reflecting the cumulative effects of pollutants and ecosystem stressors. Römbke *et al.* (2005) reported that earthworms are suitable for use in soil classification and assessment approaches. Some earthworm species can accumulate certain pollutants in their bodies, making them valuable for monitoring soil contamination levels. Earthworms' bio-accumulative ability is well known and thus could be a living organism for bio-monitoring soil pollution (Hirano and Tamae 2011). They play crucial roles in human welfare as waste decomposers, bio-fertilizer manufacturers, land reclaimer, protein producers, food and vitamin sources, natural detoxicant, as pollution bio-indicators, bait for fishes, industrial raw materials, and above all drug sources (Hajam *et al.* 2023). They complement traditional chemical analyses by providing insights into the biological effects and ecological implications of soil contamination.

Earthworm monitoring programs have the potential to improve land management practices, pollution remediation efforts, and environmental policy decisions aimed at safeguarding soil ecosystems and human health. In this context, earthworms can play a major role in indicating the health of the soil and providing crucial insights into the hidden world beneath our feet. Such biological tool provides cost effective, non-invasive, and ecologically relevant methods for assessing soil pollution, complementing traditional chemical analyses and providing a more comprehensive understanding of ecosystem health. By using biological resources we can develop ecosystem-based approaches for pollution monitoring.

Ecological role of earthworms

Earthworms are one of the most significant soil animals; they have the potential to maintain the fertility of the soil and thus play a key role in sustainability. Fig. 1 shows the contribution of earthworms to the ecosystem (Liu *et al.* 2019). They are also known as farmers' friends, ecological engineers, biological indicators, intestines of the earth, and plowmen of the field (Akhila and Entoori 2022). Earthworms are essential for soil ecosystems as they significantly impact soil structure, plant growth, and nutrient cycling and indirectly influence microbial communities. Their burrowing activities enhance soil structure, aeration, water infiltration, and drainage, which benefits plant growth. Earthworms improve the soil's biological, chemical, and physical properties and serve as soil conditioners (Ahmed and Al-Mutairi 2022).

Additionally, they play a crucial role in decomposing organic matter, thereby increasing soil nutrient availability. However, future land management strategies should consider the importance of soil organisms, including earthworms, in delivering ecosystem services, as they are an important resource to protect (Ponge 2015). This contribution to multiple ecosystem services is vital for sustainable agriculture. Overall, earthworms are key drivers of soil ecosystem processes, contributing to soil fertility, biodiversity, and resilience.

Soil pollutants and their impact

Soil contamination is the result of human activity, high use of pesticides, untreated industrial effluent, and agricultural runoff, including the entry of industrial wastes into soil through atmospheric deposition or application of agrochemicals and untreated municipal waste to the land (Thakur and Paika 2024). Common soil pollutants encompass a wide range of substances originating from natural and human-made sources, which play a role in the distribution of heavy metals in the pedoecosystem (Table 1). Heavy metals such as lead, cadmium, mercury, and arsenic often accumulate in soils due to industrial processes, mining activities, agriculture practices, and atmospheric deposition. These heavy metals pose severe threats to human health and ecosystems (Li *et al.* 2023).

Commonly used in agriculture pesticides-in-

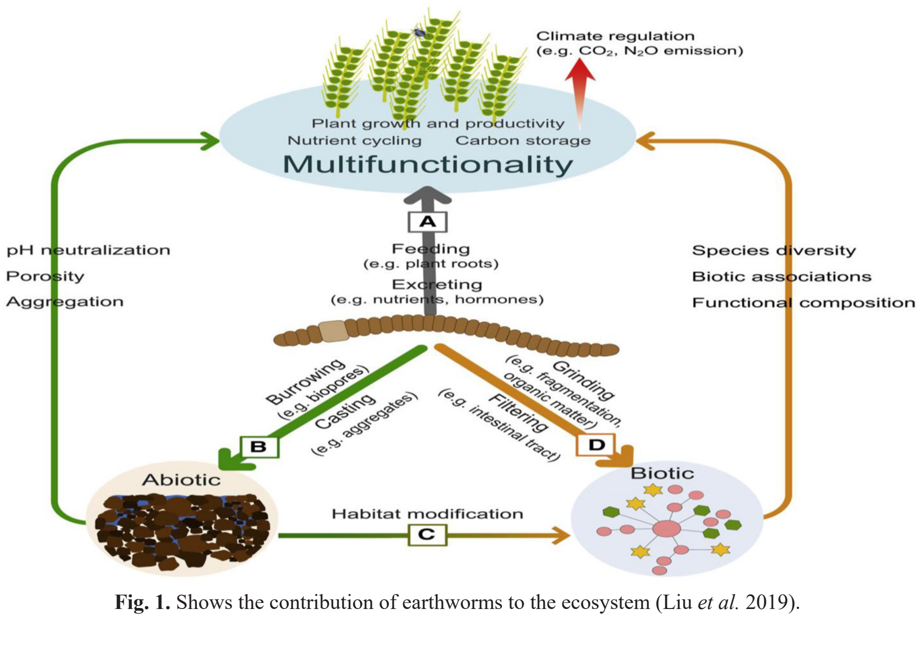

Fig. 1. Shows the contribution of earthworms to the ecosystem (Liu *et al.* 2019).

Table 1. A list of natural and anthropogenic sources which play a role in the distribution of heavy metals in the pedoecosystem.

Sl. No.	Heavy metals	Natural sources	Anthropogenic sources	References
1	As	Volcanic eruption, Hydrothermal activity, Forest fires, Dust storms, As rich minerals	Metal mining and smelting, coal mining and burning of As rich coals, pesticide, timber industry, pyrotechnics	Owen *et al.* (2024)
2	Cd	Zn and Pb minerals, Phosphate rocks	Mining waste, electroplating, metal industry, automobile exhaust, phosphate mineral fertilizer	Johnson and Lee (2019)
3	Cu	Sulphides, Oxides, Carbonates	Domestic and industrial waste, mining waste, animal manures, metal industry, Cu based fungicides, car breaks	Martin and Thompson (2021)
4	Hg	Mercury sulphide ores, volcanoes, forest fires, ocean emissions	Artisanal and small scale gold mining, chemical industry, fossil fuel combustion, nonferrous metals production	Nicola *et al.* (2009)
5	Cr	Cr minerals	Steel industry, electroplating, industrial sewage	Adams *et al.* (2017)
6	Pb	Pb minerals	Battery manufacturing facilities, private and industrial waste, Rifle ranges and military facilities, leaded paints, insecticides, aerial emissions from petrol	Garcia *et al.* (2018)
7	Ni	Ni minerals	Metal works, battery plants, electronics, industrial waste, surgical instruments, steel alloys	Li and Xu (2019)
8	Zn	Zn minerals	Battery plants, metal industry, phosphate fertilizers, rubber industry	Yang and Zhao (2020)

cluding herbicides, insecticides, and fungicides-can linger in soils, and poses significant hazards to the environment and public health (Ahmad *et al.* 2024). Chemical pesticides can be carcinogenic, cytotoxic, and mutagenic to human health (Fang *et al.* 2020). Arzate-Cárdenas *et al.* (2024) explored that organic compounds such as hydrocarbons, polychlorinated biphenyls, phenolic compounds, polycyclic aromatic compounds, toluene, polypropylene, methoxybenzene, oil spills, and plastic have a significant impact on human health humans and can lead to cancer, immune system suppression, chronic disease, and affect cognitive and neurobehavioral functions. Urban runoff, inappropriate garbage disposal, and industrial effluents can all contaminate the soil.

Swartjes (2011) categorized soil pollutants according to IUPAC, as illustrated in the Fig. 2. A broad category of uncontrolled pollutants that are becoming more prevalent in the environment are known as emerging contaminants (ECs). The sources of soil contaminants are diverse; they enter and accumulate in soil through various pathways. All these contaminants, including pharmaceuticals, personal care products, endocrine disruptors, and industrial chemicals, can enter the environment through variety of routes and persist, accumulating in the food chain and posing risks to ecosystems and human health (Xingyu li *et al.* 2024).

Earthworms as bioindicators

Earthworms serve as effective bioindicators of soil health and pollution due to their sensitivity to environmental changes and their direct exposure to contaminants. They are useful for determining soil toxicity because they show behavioral, physiological, and biochemical responses to soil pollutants, such as heavy metals and pesticides (Bhowmik *et al.* 2022, Dawood *et al.* 2017). Their role extends beyond mere indicators; earthworms also contribute to bioremediation processes by enhancing soil aeration, nutrient availability and microbial activity, which aids in the degradation of contaminants like biodiesel and pesticides (Immich *et al.* 2023). Dewi *et al.* (2023) observed that earthworm abundance correlates significantly with soil quality indices, indicating their potential as reliable indicators for soil health assessments. Furthermore, their ability to process and detoxify pollutants through gut enzymes

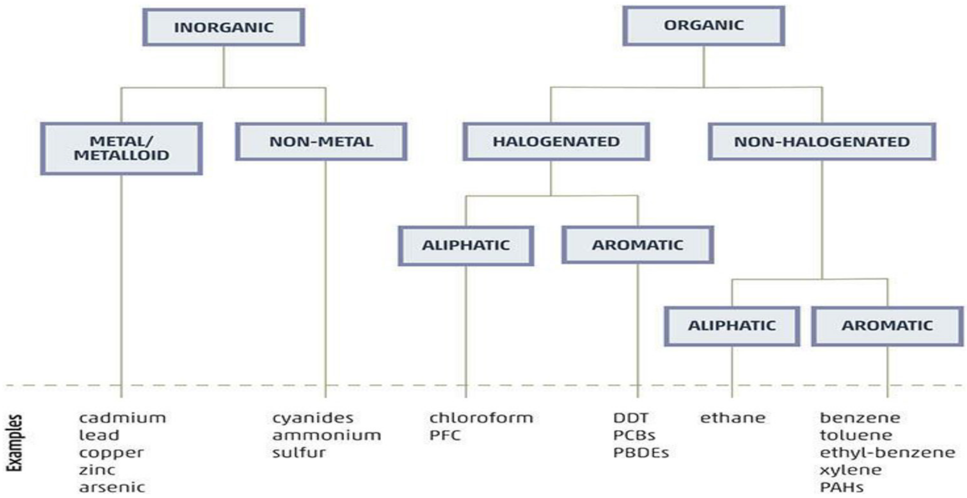

Fig. 2. According to IUPAC major soil pollutants systematically categorized by Swartjes (2011).

and their influence on microbial communities underscores their importance in maintaining soil ecosystem functions (Mishra *et al.* 2022, Dawood *et al.* 2017).

Earthworms exhibit several physiological and behavioral adaptations that make them effective bioindicators of environmental pollution. Their sensitivity to various contaminants, including heavy metals and organic pollutants, allows them to reflect soil health and toxicity levels accurately (Singh and Fatima 2022, Bhowmik *et al.* 2022). According to Kujawska and Wójcik-Oliveira (2019) earthworms have the physiological capacity to bioaccumulate pollutants, including heavy metals, in their tissues. This allows for a constant ratio between soil and organism concentrations, which facilitates the assessment of pollution bioavailability. Earthworms' behaviorally responses to soil toxicity include altered feeding and movement patterns, which further suggest environmental stressors (Bhowmik *et al.* 2022).

Earthworms serve as effective bioindicators of environmental contaminants due to their sensitivity to various pollutants, including emerging contaminants, leachate, pesticides, and heavy metals. According to studies (Junior *et al.* 2024), earthworms exposed to leachate from dumpsites, like *Eisenia andrei*, show notable oxidative stress responses, including behavioral changes, cytotoxicity, and changes in antioxidant systems. Additionally, a meta-analysis revealed that earthworms and enchytraeids have similar sensitivity to chemical stressors, particularly pesticides and heavy metals, highlighting their function in determining the health of soil (Serbource *et al.* 2024). Furthermore, the chemical sensitivity of different earthworm species varies based on habitat and ecological traits, with findings suggesting that soil pH influences their response to contaminants like imidacloprid and copper (Duque *et al*. 2023).

All things considered, earthworms' diverse responses to pollutants highlight their potential for application in bioremediation and ecotoxicological evaluations (Houida *et al.* 2024, Singh and Fatima 2022). The investigations of heavy metal bioaccumulation included a variety of earthworm species (Table 2). Because of their critical role in soil ecosystems and sensitivity to environmental changes, earthworms are often used as bioindicators to monitor ecosystem health and soil contamination. Earthworms are helpful markers. Particularly in no-tillage agroecosystems, earthworm populations are a definite measure of soil health in agricultural systems. The strong relationship between earthworm populations and soil quality is demonstrated by the close correlation between species richness and abundance and key soil properties such as pH, phosphorus, carbon, and clay content (Bartz *et al.* 2024). Given everything considered, earthworms

Table 2. Species of earthworms used for the heavy metals bioaccumulation studies.

Sl. No.	Earthworm species	Heavy metals	References
1	*Eisenia fetida*	Cu, Zn, Pb, Cd	(Li *et al*. 2010)
2	*Eisenia andrei*	Hg	(Roux *et al*. 2016)
3	*Lumbricus terrestris*	Cu, Fe, Zn, Pb,Cd	(Latifi *et al*. 2020, Adie *et al*. 2022)
4	*Alma nilotica*	Hg, Co	(Nfor *et al*. 2022)
5	*Libyodrilus violaceous, Eudrilus eugeniae Alma millsoni*	Cu, Zn, Pb, Cd, Mn	(Dedeke *et al*. 2016)
6	*Metaphire californica, Amynthus homochaetus, Amynthus pecteniferus, Amynthus heterochaetus*	Cd, Zn, Cu, Pb	(Wang *et al*. 2018)
7	*Metaphire posthuma, Lampito mauritii*	Zn, Fe, Pb, Mn, Cu	(Suthar *et al*. 2008)
8	*Lumbricus rubellus, Aporrectodea caliginosa*	Cu, Cd	(Hobbelen *et al*. 2006)
9	*Allolobophora parva, Eisenia fetida*	Cu, Cd, Pb, Zn, Cr	(Garg *et al*. 2009)
10	*Lumbricus castaneus*	Cd, Zn	(Audusseau *et al*. 2020)

provide a useful, cost-effective, and reliable tool of monitoring pollution and soil health. Because they offer crucial information on the sustainability and ecological balance of soil ecosystems, they are a vital tool for environment monitoring and management (Serbource *et al.* 2024).

Numerous soil contaminants, including pesticides, heavy metals, and nanoparticles, can be detected by earthworm. To assess the impact of glyphosate and zinc, earthworm species like *Eudrilus eugeniae* and *Eisenia fetida* were employed. Changes in survival, reproduction, and biochemical markers, such as acetylcholinesterase (AChE) activity, which show neurotoxicity and oxidative stress, were reported by Singh *et al.* (2024). Earthworm abundance in forest ecosystems reflects variations in soil quality across various land covers and correlates with soil quality indexes (Dewi *et al.* 2023). All of these studies show how effective earthworms are as bioindicators by emphasizing their roles in detecting and minimizing soil contamination through bioaccumulation and bioremediation processes.

Methods for assessing soil pollution using earthworms

Earthworms exhibit significant physiological responses to soil pollutants, which serve as indicators of soil contamination levels. Various investigations reveal that exposure to heavy metals like cadmium, arsenic, and chromium leads to increased oxidative stress and biochemical alterations in earthworms, such as elevated activities of antioxidant enzymes (SOD, CAT, POD) and malondialdehyde (MDA) levels, reflecting cellular damage and adaptive responses to toxicity (Qiao *et al.* 2024, Yadav *et al.* 2022). Additionally, earthworms exposed to antimony show altered behavior, such as avoiding contaminated soil which suggest that ecological processes disrupted at high level contamination (Su *et al.* 2023). The health of earthworm is also affected by emerging contaminants, as their gut microbiome play a significant role in detoxification processes (Houida *et al.* 2024). Collectively, these physiological responses, including growth inhibition and reproductive impairment, underscore the potential of earthworms as bioindicators for evaluating soil pollution and its ecological consequences (Bhowmik *et al.* 2022). The sensitivity of earthworms to various pollutants and their integral role in soil health have led to their recognition as effective biomarkers for assessing soil contamination levels.

Studies have shown that earthworms behave differently when exposed to contaminant substances like zinc, leachate from dumpsites, and antimony. This indicates their potential to reflect soil toxicity levels (Singh *et al.* 2024, Junior *et al.* 2024, Su *et al.* 2023). A notable avoidance behavior and decreased reproductive success in earthworms were observed when exposed to zinc chloride (Singh *et al.* 2024). Furthermore, the Biomarker Response Index (BRI) has been used to quantify the effects of leachate exposure, revealing a spectrum of biological responses that correlate with contamination levels (Junior *et al.* 2024). These findings support the use of earthworms in ecotoxicological assessments, providing a reliable means to monitor soil health and contamination (Singh and Fatima 2022, Bhowmik *et al.* 2022). Common assessing methods of soil pollutants using earthworms are shown in Fig. 3.

Biochemical markers such as metallothioneins, acetylcholinesterase activity, and heat shock proteins are important physiological indicators of soil pollutant exposure in earthworms. These marker shows the organism's stress response to pollutants like heavy metals and pesticides (Bhowmik *et al.* 2022, Dawood *et al.* 2017, Kammenga *et al.* 2000). These indicators vary across different soil types due to factors such as soil composition, organic matter content, and the specific contaminants present, which influence the bioavailability and uptake of pollutants by earthworms (Dawood *et al.* 2017, Sanchez-Hernandez 2006). For instance, earthworms in contaminated sandy soils may exhibit different biomarker responses compared to those in clay-rich soils, as the latter can retain pollutants more effectively (Georgescu and Weber 2009, Sanchez-Hernandez 2006).

Analytical methods

***QuEChERS extraction and LC-MS*:** This method identifies pollutants in earthworms that are transported by wastewater by combining QuEChERS extraction with liquid chromatography-mass spectrometry. It provides a sensitive and accurate way to monitor soil contamination from with treated wastewater irrigation (Nicola *et al.* 2024).

***Vermiremediation or bioremediation*:** Earthworms support bioremediation processes by boosting soil microbial activity and directly decomposing organic and inorganic pollutants, such as pesticides (Tagliabue *et al.* 2023, Mishra *et al.* 2022).

***Burrowing behavior as a behavioral indicator*:** Earthworms display avoidance behavior in response to heavy metal contamination, preferring unpolluted soils over polluted ones. This behavior is evident in studies where earthworms avoided soils with high levels of metallic trace elements (MTEs) such as lead, zinc, and copper, indicating their sensitivity to these pollutants (Marion 2023).

***Utilizing biochemical markers for biomonitoring*:** Biomarkers like metallothioneins and acetylcholinesterase activity in earthworms are used to detect xenobiotic exposure and provide early warnings of soil contamination (Dawood *et al.* 2017).

***Bioaccumulation of particular pollutants*:** Earthworms storage and excretion system allows them to control the amount of arsenic in their bodies. For example, *Metaphire guillelmi* exhibits a significant capacity for arsenic efflux, which helps in managing arsenic stress (Xiayun *et al.* 2024). In earthworms,

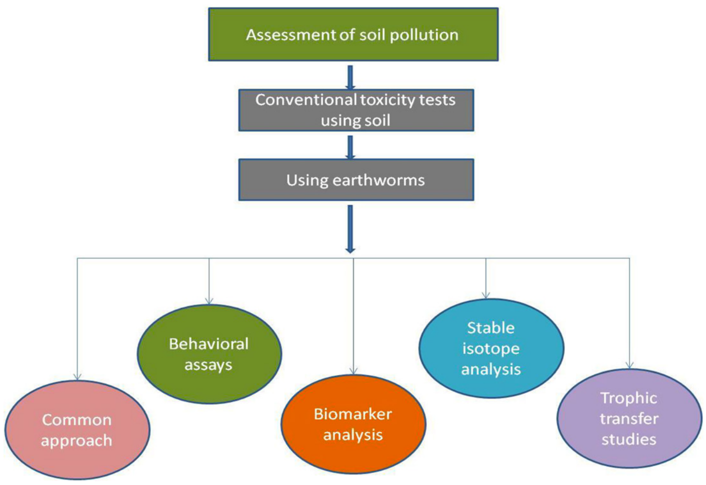

Fig. 3. Showing different methods of the assessment of soil pollution using earthworms.

inorganic arsenic is biotransformed into less hazardous organic forms, which are subsequently excreted. According to Wang and Cui (2016), this procedure is essential for detoxifying and lowering arsenic toxicity.

***Coelomocyte extraction as a cellular biomarker*:** Coelomocytes are extracted from earthworms using techniques such as fiber needle extraction and ultrasound protocols, which enable cellular and molecular-level evaluation of soil pollution (Mao 2011).

By offering both direct and indirect indicators of soil health and contamination, these approaches highlight the diverse roles earthworms play in evaluating soil pollution.

Challenges and future directions

Recent research indicates that there are some disadvantages and possible uses for employing earthworms as a method to assess and remove soil contamination. There are several challenges in using earthworms as trustworthy bioindicators because of their diverse responses to different contaminants. For example, earthworm species exhibit varying levels of susceptibility and bioaccumulation to different metals like zinc. $ZnCl_2$ it is more hazardous than ZnO-NPs. According to Singh *et al.* (2024), it is most likely due to the ionic forms of zinc rather than its size. Additionally, earthworms' gut microbiome has a significant impact on how they respond to emerging contaminants (ECs). However, the exact relationship between earthworm reactions and their gut microbiota remains unclear (Houida *et al.* 2024). In different vermicompartments, earthworm-associated microbial populations react differently to pollutants like pyrene and cadmium. This implies that earthworm-associated microbiomes are sensitive indicators of soil contamination (Li *et al.* 2023).

Furthermore, earthworm performance in bioremediation processes like vermicomposting is influenced by soil texture and organic matter concentration, which affect metal mobility and pollutant degradation rates (Das *et al.* 2024, Chao *et al.* 2022). Additionally, soil texture and organic matter content, which impact metal mobility and pollutant degradation rates, have an impact on how well the earthworms perform bioremediation, such as vermicomposting (Das *et al.* 2024, Chao *et al.* 2022). Despite these obstacles, the use of earthworms in soil pollution control shows promise for the future. A more sophisticated method of assessing soil risk is provided by the development of sophisticated testing techniques, such as the earthworm cocoon test in conjunction with toxicokinetic-toxicodynamic modeling. According to Rakel *et al.* (2024), this approach offers comprehensive data on growth and reproduction under pollution exposure. Furthermore, earthworms may play a variety of roles in soil remediation due to their capacity to break down complex contaminants such as pesticides, heavy metals, and microplastics through their enzymatic activities and gut-associated microbes (Gudeta *et al.* 2023, Mishra *et al.* 2022). Future studies should concentrate on figuring out how earthworms and their microbiomes interact, improving vermiremediation methods, and investigating how earthworms have ecologically adapted to different types of pollution. Thus, understanding their use in managing soil contamination will be improved (Tagliabue *et al.* 2023, Zhao *et al.* 2022).

CONCLUSION

Earthworms emerge as valuable bioindicators of soil health and pollution, offering insights into ecosystem dynamics, pollutant fate, and ecological resilience. Despite facing challenges in standardization and biomarker identification, their sensitivity and ubiquity make them indispensable tools for monitoring soil quality and ascertaining environmental management practices. Moving forward, interdisciplinary research efforts and technological advancements offer the potential to improve the reliability and scope of earthworm-based assessments. By prioritizing the integration of earthworm monitoring into soil management strategies and policy frameworks, we can promote sustainable stewardship of soil resources, safeguard ecosystem integrity, and enhance human well-being in a rapidly changing world.

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