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Heavy Metals in the Environment: Bioremediation Technique Utilizing Earthworms for Remediating Heavy Metal-Contaminated Soil

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Abstract: The environment and human health are greatly impacted by heavy metals, and there is an increasing global concern about these potentially dangerous compounds. Heavy metal contamination can have a substantial effect on how the soil ecosystem functions. For this, feasible, effective, and advantageous restorative methods are required. "Ecosystem engineers," as earthworms are called, may alter and enhance the quality of soil. Earthworms might be a valuable biological indicator of soil pollution since they can bioaccumulate significant levels of metals in their tissues. Earthworms are utilized in a novel field of bioremediation research called vermiremediation to purify soils that have been polluted by organic matters. Earthworms have a potent metabolic system, and their propensity to valorize and detoxify heavy metals is mostly due to the gut flora and chloragocyte cells within them. Remediation with earthworms is seen as sustainable, efficient, and friendly to the environment. This review provides in-depth information on earthworms' capacity as possible species for bioremediation and detoxification of heavy metal-polluted soil, addressing problems connected to both human health and the environment.

Keywords: Bioremediation, Environmental pollution, Earthworms, Heavy metals, Human health, Soil contamination, Vermiremediation

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Introduction

The rapid industrialization of the world and the extraction of natural resources for metal mining and manufacturing have resulted in contamination by heavy metals, which is having a severe adverse impact. One of the most serious consequences facing the modern world is the pollution of air, land, and water by heavy metals (Raghunandan *et al*., 2018; Abo-Alkasem *et al*., 2023). According to

Tchounwou *et al*. (2012), these are naturally occurring elements with a high atomic weight and a density that is at least five times that of water. There may be a relationship between heaviness and toxicity because metalloids like arsenic (As), which may be harmful at low exposure levels, are included in the category of "heavy metals" (Duffus, 2002; Abd Elnabi *et al*., 2023). Several heavy metals are recognized to be detrimental to the ecosystem and physiologically necessary, such as arsenic (As), copper (Cu), zinc (Zn), lead (Pb), cadmium (Cd), nickel (Ni), chromium (Cr), and mercury (Hg) (Jacob *et al*., 2018). Over half of the world's land has been contaminated by heavy metals, an issue that has been well-acknowledged. Over 10 million sites have been impacted (Li *et al*., 2022; Luo *et al*., 2023).

Researchers have a challenge in fully detoxifying metals because heavy metals are complex to eradicate from the soil. The world continues to experience the potentially hazardous effects of heavy metal pollution, despite the efforts made to cope with the problem. Due to the numerous negative consequences that heavy metal pollution has on both human health and the health of organisms that exist in the soil, it is still a serious issue. Heavy metals have been released into the environment from both natural and man-made sources (Ray *et al*., 2019; Yadav *et al*., 2023). The production of electronics, burning fossil fuels, smelters, mining activities, and industrial wastes are examples of anthropogenic sources of heavy metal contamination in soil (Khan *et al*., 2021; Li *et al*., 2024). Since heavy metals are resistant to biological or thermal degradation, they are persistent environmental contaminants that significantly affect the physicochemical characteristics of soil and are challenging to eliminate from the soil. When these heavy metal levels are exceeded, they have toxicological consequences for plants, animals, and soil microorganisms, which significantly reduce the microbial activity and activity of enzymes in the soil (Chen *et al*., 2014; Xie *et al*., 2016; Kuźniar *et al*., 2018; Tang *et al*., 2024).

Heavy metals are accumulated by plants through their roots and foliage; the kind of plant and its natural protective mechanisms against toxicity play a major role in this process of accumulation. In plants contaminated with heavy metals, photosynthetic activity is significantly reduced, which leads to a lowered biomass yield (Oves *et al*., 2016). Additionally, some heavy metals can accumulate and impact physiological and biochemical functions such as the cell cycle, respiration, metabolism, transcription, translation, and transcription. These impacts can be hazardous to crops. Food is the main route by which heavy metals bioaccumulate at significant levels within the environment's ecosystem. Heavy metals can also enter the body through the air, water, and other systems of the body (Yadav *et al*., 2017). Regular exposure to heavy metals can harm immunological response and growth suppression, which can provide a direct or secondary risk to human health. Furthermore, heavy metals can enter the body through the skin or air and are inherently carcinogenic and mutagenic. In severe cases, these effects can include immune system dysfunction, development, growth retardation, and concerns with cardiovascular, respiratory, and reproductive functions (Silva *et al*., 2005; Liu *et al*., 2013; Yousaf *et al*., 2016). Today, there is a major global risk to food safety from heavy metal contamination. Managing heavy metal-contaminated soil is not an easy operation (Wang *et al*., 2024).

Particular heavy metals, such as iron (Fe), chromium (Cr), nickel (Ni), manganese (Mn), zinc (Zn), magnesium (Mg), and copper (Cu), have important metabolic functions at low concentrations but can be detrimental at higher doses (Siddiquee *et al*., 2015; Fatima and Singh, 2023a). Due to this, depending on the level and duration of exposure, all heavy metals tend to be harmful to living organisms (Ali *et al*., 2019). In terms of biomass and action, earthworms constitute important features of the majority of temperate terrestrial ecosystems. They enhance the soil's physical, chemical, and biological properties (Shi *et al*., 2017). In addition to having ingested vast amounts of soil, they have also lowered the exterior barriers, keeping them from approaching the soil. By turning mineral nutrients into compounds that plants may utilize, earthworms improve soil fertility through the consumption, excretion, and combination of organic waste during the vermicomposting activity (Rehman *et al*., 2022; Rehman *et al*., 2023). In soil eco-toxicological research, earthworms have therefore been extensively utilized (Singh and Fatima, 2022). According to Singh and Fatima (2019), earthworms are valuable biological indicators for identifying heavy metal pollution in soil because heavy metals like Cd, Cu, Hg, Zn, and Pb have detrimental effects on them and are mostly bioaccumulated in their tissues. Their significance as biological markers of soil pollution has been acknowledged, since they contribute important information about risks to the environment and a correlation exists between the concentration of certain chemicals in the soil and their bodies (Nannoni *et al*., 2011; Lévèque *et al*., 2013). *Eisenia fetida* is the most commonly used species in ecotoxicological investigations and has the potential to be a valuable bio-indicator for determining the level of soil contamination (Hirano and Tamae, 2011). In addition to being utilized as soil contamination indicators, earthworms are becoming increasingly prevalent in metal detoxification applications (Benedetti *et al*., 2015; Gu *et al*., 2017).

According to Raghunandan *et al*. (2014, 2018), heavy metal pollution can have disastrous consequences that can be reduced by implementing modern technologies like bioremediation. By adding the biological source to the contaminated soil *in situ*, the bioremediation procedure can be carried out *in situ*; alternatively, the contaminated area may be relocated and treated *ex-situ*. Therefore, treating heavy metal pollution in ecologically appropriate ways is important. In this review, bioremediation techniques like vermiremediation have been addressed. The following topics are specifically covered in this section: (i) heavy metal bioavailability and potential adverse effects on soil, plants, and human health; and (ii) the efficiency of earthworms in heavy metal remediation.

Heavy Metals Bioavailability:

The specific bioavailability, responsiveness, transportation, and mechanism of uptake of heavy metals by plants and other soil microflora must be ascertained, even though the overall amounts of heavy metals in the soil do not indicate anything unique about their availability and mobility (Luo *et al*., 2011; Wu *et al*., 2012). Based on the available information, soil metals have been divided into five main geochemical forms: exchangeable, linked to the carbonate phase, linked to iron and manganese oxides, linked to organic materials, and residual metal. These soil metals exhibit a wide range of mobility, biological availability, and chemical activity, most likely as a result of their propensity to react to generate organic molecules, including low-molecular organic acids, carbohydrates, and enzymes generated by microbes (Patel *et al*., 2008). Heavy metal bioavailability and accumulation in soil are influenced by several variables. These include soil type and texture, physicochemical characteristics of the soil, photosynthetic capacity of plants and their genotypes, interactions between microbes and plants in the soil, and agronomic techniques including crop rotation, irrigation, and fertilizer application (Chopin *et al*., 2008; Oves *et al*., 2016).

Sources of Heavy Metal in the Environment:

The heavy metals have naturally been in the crust since the beginning of the Earth. According to Gautam *et al*. (2016) and Briffa *et al*. (2020), there is an increase in metallic compounds in both terrestrial and aquatic habitats due to the exponential expansion in the utilization of heavy metals. There are two types of heavy metal sources: anthropogenic and natural (Fatima and Singh, 2023a). Both natural and human-caused sources are linked to the dispersion of heavy metals in soils. Some examples of natural sources are the geological split of parent rock components and volcanic eruptions. Anthropogenic factors,

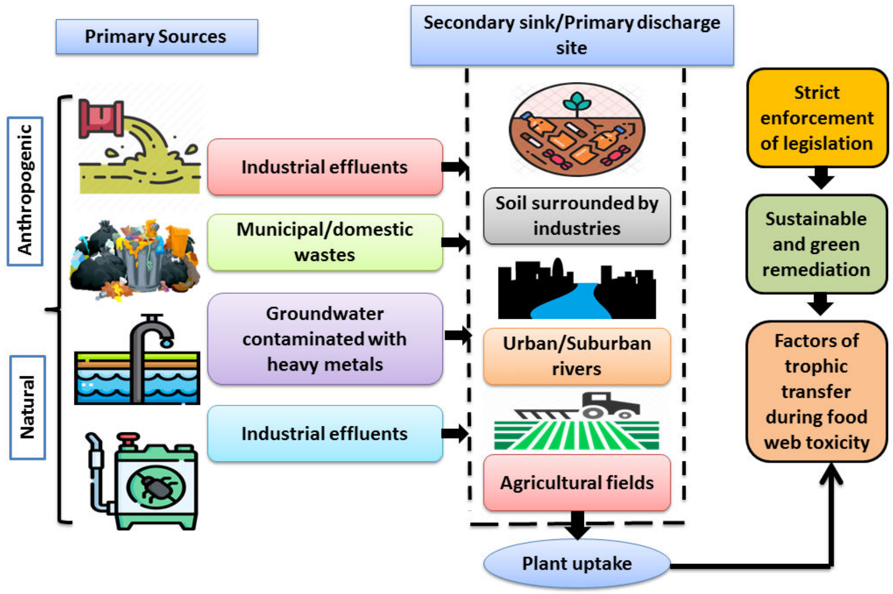

Fig. 1: Different sources of heavy metals in the environment.

including the widespread use of pesticides and fertilizers (both organic and inorganic), irrigation of wastewater, sewage sludge supplementation, raised atmospheric deposition by factories, and the combustion of fossil fuels, have all contributed to higher concentrations of inorganic pollutants in soils (Cai *et al*., 2009). Depending on where they come from, fungicides, phosphate fertilizers, and inorganic fertilizers might include different amounts of heavy metals like Pb, Cd, Cr, Ni, and Zn. The following reasons constitute these heavy metals potentially harmful in soil environments: (i) they are produced at rates faster by human activity than by natural processes; (ii) they migrate from mines to different environmental regions where direct exposure is more probable; (iii) the level of these metals in discarded products is relatively high when compared to the obtaining environment; and (iv) the chemical form of metal in the acquiring environmental system may make it readily accessible for biological absorption (D'Amore *et al*., 2005). The environmental sources of heavy metals are illustrated in Figure 1.

Effect of Heavy Metal Toxicity on Soil:

Chromium (Cr), iron (Fe), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), cadmium (Cd), and lead (Pb) accumulation are the main consequences of heavy metal (HM) environmental damage in soil. Certain heavy metals (HMs), including magnesium (Mg), iron (Fe), zinc (Zn), and copper (Cu), have positive effects on human health and should be included in diets. To maintain their concentrations within safe and allowable ranges, nevertheless, is necessary. On the other hand, certain heavy metals (HMs) such as lead (Pb), cadmium (Cd), mercury (Hg), and arsenic (As) have negative impacts on human health even at low concentrations and have no biological function (Uddin *et al*., 2021). Heavy metal (HM) contaminated soil is regarded as a serious environmental issue with the potential to seriously impact the ecosystem. Because of the widespread industrialization of the world, heavy metals (HMs) have caused dangerously high levels of soil pollution, which have lowered soil fertility and changed the nature and diversity of microbial flora. The concentration of clay, organic matter, pH levels, and soil biochemistry are all significantly impacted by heavy metals like chromium, copper, arsenic, and cadmium. Microbial development has been discovered to be negatively impacted by the buildup of certain heavy metals in the soil (Gülser and Erdoğan, 2007). The disruption of actinomycetes, fungus, and bacterial communities is the main effect of exposure to heavy metals. Because of their chemical propensity for soil structure, many heavy metals affect not just microbes but also soil enzymes (Karaca *et al*., 2010). The diversity and metabolic activities of soil microbes are vital for maintaining soil composition, recycling vital nutrients for plants, controlling plant pests and diseases, and neutralizing toxic substances. Studying the behavior of soil microorganisms in habitats exposed to heavy metal pollution for an extended period is crucial (Wang *et al*., 2007). The principal obstacles associated with the cumulative toxicity of heavy metals are the restricted availability of vital nutrients and the acidity of the soil.

Garnier *et al*. (2006) reported that Cr (VI) can be extremely dangerous due to its strong oxidizing agent properties. Higher intensities have been demonstrated to negatively impact cell metabolism and change the overall composition of microbial populations (Huang *et al*., 2009). Cadmium (Cd) building in the soil is becoming a more concerning heavy metal due to the industry's rapid development, the rise of capitalism, and contemporary agricultural technologies (Alengebawy *et al*., 2021). The pH and quantity of organic matter in the soil are frequently the primary factors affected by the accumulation of cadmium. A change in the composition of the soil was detected by an increase in Cd bioavailability when soil pH decreased. An (2004) demonstrated that cadmium (Cd) exhibits high mobility in soil, resulting in significant toxicity that impacts essential microorganisms, inhibits microbial activities, depletes organic matter in the soil, and alters its physicochemical characteristics. According to Qi *et al*. (2018), lead (Pb) has been classified as a hazardous heavy metal pollutant because of its high toxicity. Extended exposure to low Pb concentrations raises the hazardous levels. Pb's geogenic contribution, which reduces soil microbial activity, is the main cause of Pb pollution in soil. Pb has many different impacts on soil, some of which include decreased fertility, diversity of microorganisms, and soil minerals (Dotaniya *et al*., 2020). Many studies have been done in different geographical areas to investigate the impact of lead (Pb) on agricultural soil. The relationship between Pb absorption, retention, and soil properties was reported by Vega *et al*. (2010). The accumulation of lead has been found to have an impact on the characteristics of several basic soil elements, such as pH levels, organic matter content, cation exchange capacity, and texture, all of which are important drivers of Pb mobility and bioavailability in soil.

Effect of Heavy Metal Toxicity on Plants:

The plant generally needs specific nutrients to develop. Higher quantities of heavy metals cause serious damage to numerous metabolic functions, which ultimately leads to plant death. Plants grown in a municipal solid waste dump and its surroundings are contaminated with heavy metals, which may adversely affect the food chain (Vongdala *et al*., 2019). According to Zhuang *et al*. (2009), heavy metals can contaminate plants directly by accumulation from the atmosphere or by absorption through the roots of plants growing in contaminated soil. Plants are first affected by heavy metals in the rhizosphere, where root exudates and metalliferous minerals and compounds interact (Alengebawy *et al*., 2021). When exposed to heavy metals, plants undergo oxidative stress, which damages cells and throws off their ionic balance (Nadal *et al*., 2004). To mitigate the deleterious consequences of exposure to heavy metals and their accumulation, plants have evolved detoxification mechanisms, mostly centered on subcellular partitioning and chelation (Schumacher *et al*., 2004). Excessive lead may cause problems with some cellular enzymes in plants, altering normal metabolic processes and perhaps preventing plants from photo-synthesizing. Olowoyo (2015) states that disrupted biosynthetic pathways, oxidative stress, and the destruction of DNA are often caused by excessive levels of heavy metals. Toxic levels of heavy metals also impact the root system and limit photosynthesis, stomata functions, enzyme functioning, plant growth, and the accumulation of

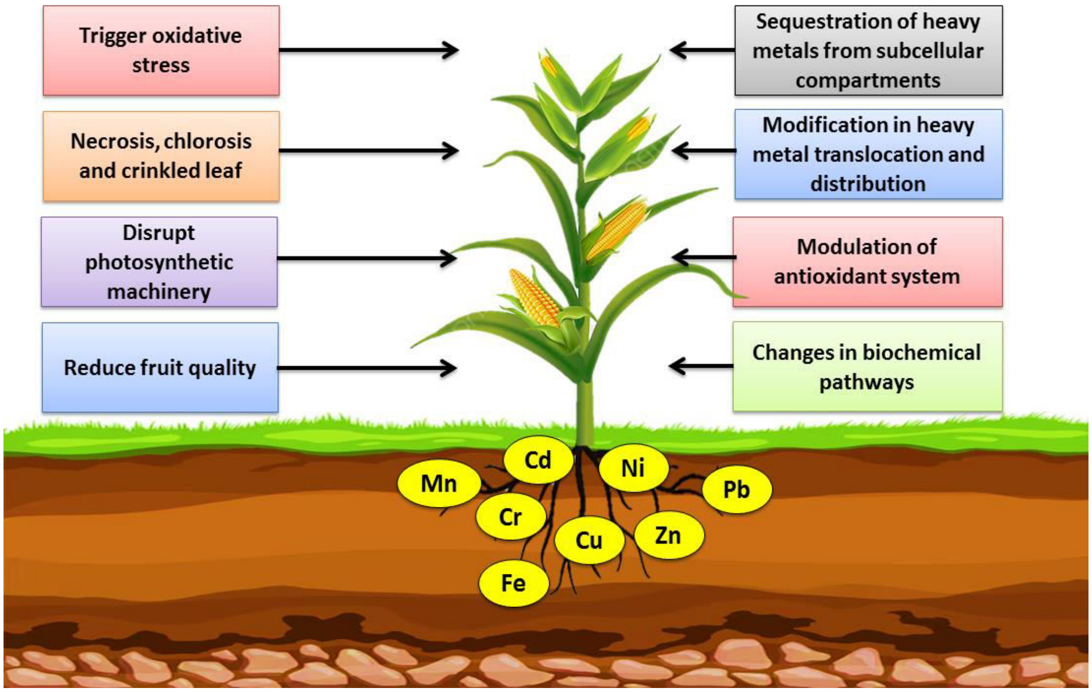

Fig. 2: Hazardous heavy metals' effects on plant development.

other nutrients (Fig. 2)(Abrahams, 2002).

Depending on factors including mobility, bioavailability, and concentration, plant roots absorb mainly cadmium (Cd) ions; the residual amount can be absorbed straight from the atmosphere (Ismael *et al*., 2019). Furthermore, after passing through extracellular routes, such as calcium (Ca) pathways, Cd accumulates in the roots, shoots, and edible sections of plants (Zhou *et al*., 2019). Feng *et al*. (2010) report that a range of physiological and biochemical changes occur in plants exposed to elevated levels of Cd. Additional negative effects of elevated Cd in plants might include inhibition of some processes (mineral transportation, photosynthetic apparatus, and nutrient uptake). Furthermore, it can stop plant roots from absorbing iron (Schützendübel *et al*., 2002; Jibril *et al*., 2017). According to Hayat *et al*. (2018), Cd harms a variety of plant phenotypes, including lowering plant weight and length of roots and shoots, cytotoxicity (e.g., decreasing chlorophyll concentration and limiting photosynthetic performance), and metabolic activities (e.g., chlorosis and cell mortality). Lipid peroxidation, which exacerbates cell membrane damage, occurs in plants subjected to oxidative stress caused by chromium (Cr). Growth declines as a result of photosynthetic pigment degradation brought on by oxidative stress caused by chromium. Excessive levels of chromium can cause the ultrastructure of the chloroplast to be impacted, which will disrupt photosynthetic activity (Asati *et al*., 2016). A surplus of Ni^{+2} in the soil causes several physiological alterations in plants, particularly rice, as well as a range of toxic symptoms, such as chlorosis and necrosis (Zornoza *et al*., 1999; Rahman *et al*., 2005). Plants cultivated on soil with high Ni^{+2} content showed disorders in cell membrane functions and nutrient balance impairment. The most frequent harmful effects of heavy metals on plants are enumerated in Table 1 as an overview of the previously indicated unfavorable consequences.

Heavy Metal Toxicity's Effect on Human Health:

Heavy metal contamination, even at low concentrations, has attracted significant attention

Table 1: The Toxic Forms of Heavy Metals and Their Impact on Plants

S. No.	Heavy Metals	Toxicity Form	Toxic Effects on Plants	References
1.	Cd	Cd^{2+}	Reduce the permeability of the stem, prevent seed germination, and shorten the length and biomass of the roots	Oumenskou *et al*. (2018); Jibril *et al*. (2017); Vardhan *et al*. (2019); Loi *et al*. (2018)
2.	Pb	Pb^{2+}	Stunted foliage, decreased protein concentration, decreased chlorophyll content, and destruction of DNA	Masindi and Muedi (2018); Lan *et al*. (2020); Zhou *et al*. (2018)
3.	Cu	Cu salts	Deformation of the roots, reduction in shoot length, decrease in polypeptides, and alteration in lipid content	Caetano *et al*. (2016); Frenk *et al*. (2013); Panagos *et al*. (2018)
4.	Zn	Zn^{2+}	Interveinal chlorosis, blocking of element passage, and alterations in enzyme function	Ciarkowska *et al*. (2016); Guan *et al*. (2018); Plum *et al*. (2010)
5.	Ni	Ni^{2+}	The nutritional balance of plants growing in high Ni^{2+} soil was impaired, and this led to a disruption in the activities of the cell membranes.	Asati *et al*. (2016)
6.	Hg	Hg^{2+}	In plants, toxic concentrations of Hg^{2+} can cause visual damage as well as physiological issues.	Zhou *et al*. (2007)
7.	Cr	Cr(VI)	Apart from modifying the process of germination, Cr can also have detrimental impacts on the development of roots, stems, and leaves in plants	Shanker *et al*. (2005)
8.	Mn	Mn^{2+}	Manganese (Mn) toxicity can result in oxidative stress and reduced photosynthesis, which can create wrinkles, necrotic dark spots on older leaves, and interveinal chlorosis in immature leaves	Li *et al*. (2019)
9.	As	As(III) and As(V)	Arsenic poisoning causes a reduction in the number of leaves on plants, chlorosis, necrosis leaf senescence, and defoliation; physiological consequences include a restriction on the growth of shoots and roots, a blockage of stomatal conductance and consumption of nutrients, the breakdown of chlorophyll, and a limitation on biomass and yield generation	Abbas *et al*. (2018)
10.	Co	Co^{2+}	Higher concentrations of cobalt (Co) are harmful to plants. Excessive levels of cobalt can lead to an iron shortage in plants as well as pale-colored leaves, discolored veins, and leaf loss	Xiu *et al*. (2021)

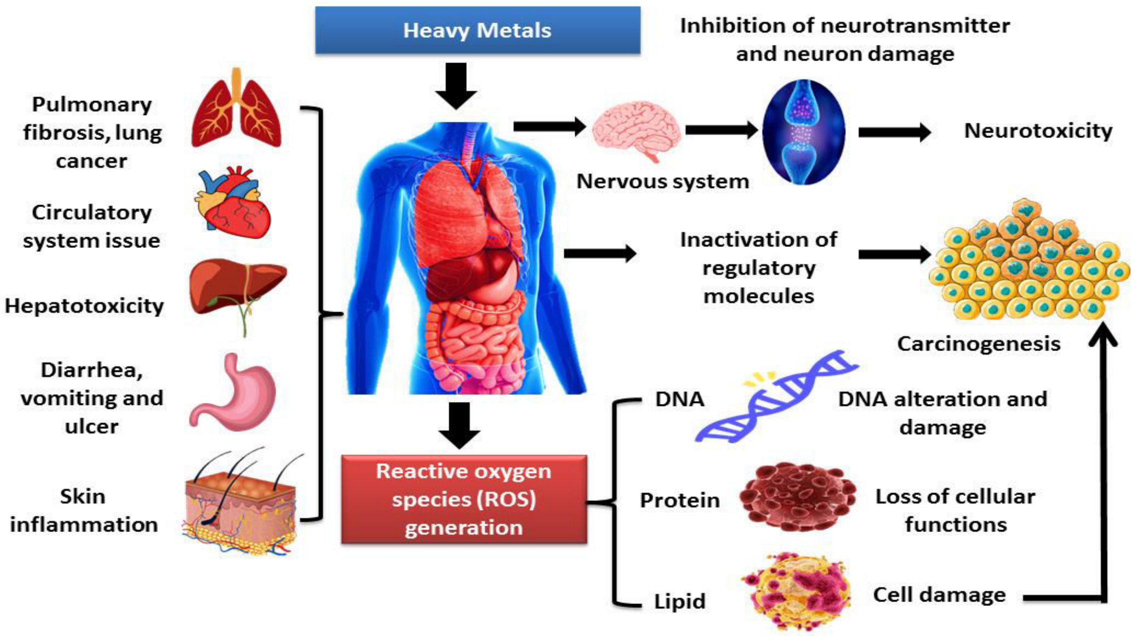

Fig. 3: Health effects due to exposure to toxic metals.

due to its detrimental effects, protracted buildup, and bio-magnification capabilities. Heavy metals are thought to be one of the most dangerous pollutants among the many layers of contaminants present in soil and the environment (Jiang *et al*., 2019; Xiao *et al*., 2019). Because heavy metals are present in the environment, there is a greater chance that they will be consumed by living things and end up building up in different body organs like the kidneys, liver, bones, and many more (Fig. 3). The neurological, skeletal, endocrine, immune, liver, kidney and circulatory systems are among the many organs that heavy metals may damage and accumulate in (Lamas *et al*., 2016; Ma *et al*., 2016; Yadav *et al*., 2024). Around the world, breathing in or consuming food or drink can expose people to heavy metals. Both being close to a location where these metals have been unlawfully released into the environment and working in or near industries that employ these metals and their compounds put workers at high risk. Hunting and fishing activities that are part of a subsistence lifestyle may potentially include increased risks of exposure and health consequences. The challenges posed by emissions of ecologically dangerous metals have grown significantly as a result of the widespread use of modern applications for a wide range of metals in industry and daily life (Wang *et al*., 2020).

Chunhabundit (2016) states that proximal convoluted tubule damage, which is linked to mitochondrial dysfunction, can result from cadmium (Cd) exposure and induce kidney injury. There have been reports of cadmium's teratogenic, mutagenic, hyperglycemic, anemia-inducing, carcinogenic, and decreased immune-potential effects (Rehman and Anjum, 2010). Chromium (Cr) comes in two forms: trivalent (Cr III) and hexavalent (Cr VI), the latter of which is more hazardous to human health. The majority of allergic responses, including skin rashes, nasal irritation, and bleeding, impact those who work in the leather and textile sectors (Karadede *et al*., 2004). The body develops genetic abnormalities when chromium concentrations are too high (Kherici-Bousnoubra *et al*., 2009). A wide range of detrimental effects on body systems are caused by lead (Pb). The majority of symptoms are non-specific; examples include declining adult cognitive function, miscarriages in women, infertility in men, and behavioral abnormalities in youngsters. Among the other typical symptoms are anemia, renal failure, hypertension, and stomach colic (Wani *et al*., 2015). Malik and Zeb (2009) stated that lead causes changes to heme production, neurotoxicity, and nephrotoxicity complications. In addition to vesicular eczema and nasal and lung cancer, being exposed to nickel (Ni) can cause complications with the liver, kidney, spleen, brain, and tissues. Moreover, it disrupts the skeleton's ability to incorporate calcium, which damages parakeratosis, and disrupts iron resorption, which results in anemia (Aliasgharpour, 2020). Table 2 gives an overview of the heavy metal pollutants' effects on human health as well as their sources of contamination.

Bioremediation: A Solution to Heavy Metal Contamination:

Bioremediation refers to a range of natural processes that utilize soil fauna (vermiremediation), microbes (bioaugmentation), and plants (phytoremediation) to biodegrade and mitigate contamination of the soil (da Silva *et al*., 2020; Shen *et al*., 2022). The amalgamation of all those endeavors is what converts the pollutants into harmless substances, thereby restoring the environment's fundamental balance (Halder, 2014). Earthworms' biotic and abiotic interactions, life cycle, burrowing, and feeding activities are used in the process of vermiremediation, which was first defined by Rodriguez-Campos *et al*. (2014). This process transforms, degrades, or removes pollutants from the soil's ecosystem (Dada *et al*., 2021). Their role in the terrestrial ecosystem is essential because earthworms positively affect the biological, physical, and chemical characteristics of soil (Yatoo *et al*., 2022). Because of their excavating and soil-ingestion behaviors, earthworms are essential to the dynamics of organic matter because they can modify the architecture of the pore system and redistribute components within the soil profile (Àlvarez *et al*., 2023). When it comes to soil remediation, earthworms may eliminate pollutants directly through digestion and absorption as well as indirectly through stimulating the growth of helpful microbes (Cao *et al*., 2015). As a result, there are several different mechanisms involved in vermiremediation: vermiextraction, which is the technique by which earthworms remove pollutants from the substrate by dermal and gastrointestinal absorption and accumulate them in their tissues (Shi *et al*., 2020); vermitransformation, which is the process by which organic pollutants are altered as they pass through the earthworm's gut by the microorganisms and enzymes that inhabit it (Shi *et al*., 2014; Dada *et al*., 2021); drilodegradation occurs in the 2 mm thick zone surrounding earthworms' burrow walls, known as the drilosphere (Schlatter *et al*., 2019).

Earthworm Species Suitable as Bioremediating Agents for Heavy Metal Contamination:

Given their significance as indicators of food production and environmental services, earthworm ecotoxicological study is especially essential. More precisely, the bioaccumulation of heavy metals in the soil may cause changes in the physiologies of earthworms, which might have negative effects on the ecosystem and ultimately human health. Earthworms are in continuous contact with soil particles due to their porous skin and digestive systems (Roubalová *et al*., 2015). Sensitivity to soil contaminants is high due to the sensory tubercles covering their bodies and the chemoreceptors in the front and posterior regions (Langdon *et al*., 2003; Cui *et al*., 2022; Kumar and Singh, 2022). Due to their diverse lifestyles and dietary preferences, earthworms' geographical range might affect how susceptible they are to soil pollution (Huang *et al*., 2021). Suthar *et al*. (2008) reported that metal accumulation patterns vary among species of earthworms based on their ecological type. The main factors which determine

Table 2: Heavy metal sources and their effects on human health

S. No.	Heavy Metals	Health Effects	Sources	References
1.	Ag	Skin discoloration and breathing issues	Mining of copper, copper-nickel, lead, and lead-zinc ores	Obasi and Akuddinobi (2020); Hiew *et al*. (2018)
2.	As	Cancer, skin disorders, and abdominal discomfort	Arsenic mining, smelting coal combustion, industrial production	Hiew *et al*. (2018); Liu *et al*. (2018)
3.	Cd	Damage to the kidneys, bones, endocrine system, carcinogenic, and mutagenic	Surface waters and precipitation in industrialized and metropolitan areas, weathering or erosion of bedrock, seepage from contaminated areas, and landfills	Hiew *et al*. (2018); Liu *et al*. (2018); Lim *et al*. (2018)
4.	Cr	Higher risk of cancer, hair loss, headaches, diarrhea, and nausea	Tanneries, pulp, and rubber manufacturing processes	Hiew *et al*. (2018); Liu *et al*. (2018); Lim *et al*. (2018)
5.	Cu	Liver cirrhosis, persistent anemia, sleeplessness, and harm to the brain and kidneys	Mining of copper, metal smelting, mineral processing, steel manufacturing, and plastics manufacturing	Liu *et al*. (2018); Lim *et al*. (2018); Burakov *et al*. (2018)
6.	Hg	Neurological, cardiovascular, and autoimmune illnesses, as well as injuries to the brain and lung	Industrial activity, electronic industry, mining	Obasi and Akuddinobi (2020); Hiew *et al*. (2018); Liu *et al*. (2018)
7.	Ni	Chronic asthma, pulmonary, nasal, and throat cancer, hair loss, and allergic skin conditions	Dust carried by the wind, leftover and fuel oils, the burning of municipal garbage, and the extraction and refinement of nickel	Hiew *et al*. (2018); Liu *et al*. (2018); Hanfi *et al*. (2020)
8.	Pb	Cognitive decline, delayed growth, and a higher chance of heart disease	Road transportation, lead battery wastewater, the glass and ceramic industries, lead chemical wastewater, electronic waste, and the mining industry	Hiew *et al*. (2018); Liu *et al*. (2018); Lim *et al*. (2018)
9.	Zn	Tiredness, anemia, dizziness, and weakened immunity	Industrial and agricultural activities	Hiew *et al*. (2018); Liu *et al*. (2018)

the patterns of earthworm metal accumulation include feeding habits, ecological classification, niche design, and the horizontal transport of toxicants in soil strata. They claimed that differences in the burrowing behaviors of endogeic and anecic earthworms might affect the patterns of metal bioaccumulation (Suthar *et al*., 2008; Leveque *et al*., 2013). Lukkari and Haimi (2005) suggest that because epigeic earthworms (*Eisenia fetida*) seem to be able to withstand larger concentrations of heavy metals than endogeic earthworms (*Aporrectodea tuberculata*), they are more suited to particularly control tissue heavy metal concentrations.

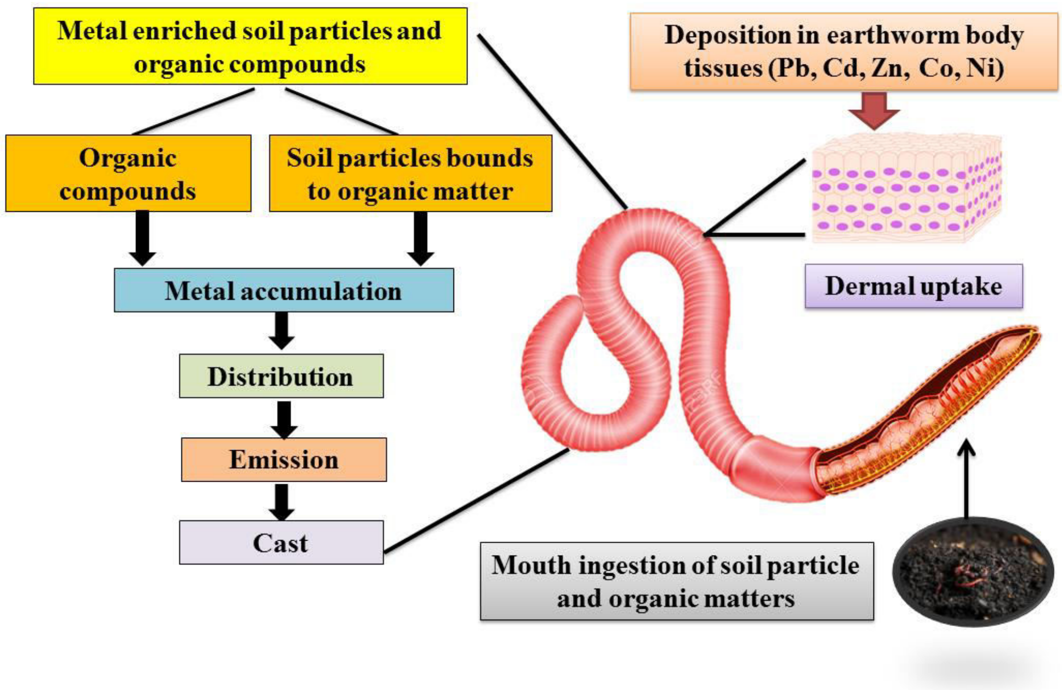

Fig. 4: Remediation of heavy metals by earthworms.

Through consumption and direct skin contact, earthworms mostly accumulate heavy metals found in soil (Fig. 4) (Wijaywardena *et al*., 2017). According to Xiao *et al*. (2022), several factors affect the quantity of heavy metals acquired by earthworms, such as the species of earthworm, the physiological and chemical composition of the soil, the degree of pollution, and other environmental conditions, including soil moisture and temperature. Heavy metal deposition in the intestinal region has been documented, with particular emphasis on the chloragogenous tissue that envelops the coelomic surface of the gut (Stürzenbaum *et al*., 2004). Panday *et al*. (2016) concluded that after 28 days of exposure, the earthworms (*Metaphire posthuma*) exhibited Cd and Pb values that were considerably greater than those in the control group. Numerous studies have demonstrated that earthworms bioaccumulate heavy metals (Chao *et al*., 2016; Cao *et al*., 2017; Zhao *et al*., 2018; Maity *et al*., 2018; Yuvaraj *et al*., 2020; Žaltauskaitė *et al*., 2020; Fatima and Singh, 2023a). Pattnaik and Reddy (2011) removed elements such as Cd, Pb, zinc (Zn), and manganese (Mn) from urban waste by employing three different kinds of earthworms. The metal contents in earthworm tissue showed consistent, substantial elevations from the beginning to the completion of the trial. After the 60-day study period, *Eudrilus eugeniae*, *Eisenia fetida*, and *Perionyx excavatus*, respectively, accumulated declines in Pb and Zn of up to 32% and 37%, 45% and 44%, and 51% and 56%. Dada *et al*. (2016) used the tropical wetland earthworm *Libyodrilus violaceus* to remediate soils during a 12-week period that had been contaminated with Zn, Pb, and Cd *ex-situ* in individual and combination quantities. Owing to the presence of *Libyodrilus violaceus*, the metal pollutants in the soil exhibited significant reductions. Similarly, Cheng-Kim *et al*. (2016) used *Lumbricus rubellus* for ninety days to clean up soil samples that were contaminated with Cu, Mn, Pb, Fe, Cr, Ni, Zn, and As in Malaysia. According to Ireland (1979) investigation, Pb, and

Cd accumulate primarily in the chloragogen cells of *Lumbricus terrestris* and *Dendrobaena rubida*, where they are stored as Pb- and Cd-metallothioneins.

Earthworms have developed many defensive systems to stave off the harmful effects of heavy metal contamination. According to Kamitani and Kaneko (2007), their tissues, especially the posterior alimentary canal, can be controlled, detoxified, and removed excesses of heavy metals. According to Stürzenbaum *et al*. (2004), the majority of earthworm heavy metals are absorbed by and bind to metallothioneins (MTs), which are induced metalloproteins. Toxic and physiologically required metals are stored and sequestered in cells' metal buffer, which is a component of MTs' production of cysteine thiolate ligands (Foster and Robinson, 2011). The functions of metallothioneins (MTs) include tissue protection against oxidative stress, metal homeostasis, heavy metal detoxification, and the transfer of vital metals (Capdevila *et al*., 2012; Wang *et al*., 2014). They are naturally capable of efficiently sequestering and compartmentalizing heavy metals through an MT-mediated mechanism (Stürzenbaum *et al*., 2004).

Vermicomposting Used to Remove Metal Pollutants:

For the bioremediation of polluted soils, vermicompost provides desirable characteristics as a substrate. The three elements that comprise the bioremediation potential are the high content of organic matter, the variety and quantity of microorganisms, and the existence of exoenzymes that detoxify contaminants. Though its chemical features suggest that vermicompost will be an excellent sorbent substrate for metals, limiting their bioavailability and toxicity in soil, very little research has examined the potential applications of vermicompost in the remediation of soil polluted by metals. Zhu *et al*. (2017) have suggested that vermicompost is a viable method for the effective removal of hazardous metals from soil. Several kinetic tests, comprising adsorption and desorption, were conducted using different Pb^{+2} and Cd^{+2} solutions to assess the metal binding capabilities of cow dung and the vermicompost generated from this waste product. Vermicompost serves as a useful metal adsorbent. In a lab experiment including soil polluted with metals, Hoehne *et al*. (2016) examined vermicompost's capacity to restrain Cd, Cr, and Pb. Soobhany *et al*. (2015) confirmed the vermiaccumulation of additional heavy metals with the following decline in bioaccumulation factors (BCFs): Cd > Ni > Cu > Co > Cr > Zn. According to Suleiman *et al*. (2017), three species of earthworms- *Eisenia andrei*, *Eisenia fetida*, and *Dendrobaena veneta*- have been found to accumulate heavy metals. The order of BCFs was as follows: Cd > Co > Cu > Zn > Ni > Pb > Cr. The *Eisenia* species of earthworms had the greatest capacity to vermiaccumulate heavy metals among all the species examined. *Eisenia fetida* vermicomposting can reduce overall heavy metal concentrations and toxicity while notably changing the amount and type of pathogens (Wang *et al*., 2017; Fatima and Singh, 2023a). Vermicomposting is a biological process that breaks down organic waste in an aerobic condition with the help of earthworms and bacteria. The majority of organic molecules have the potential to be transformed into nitrogen, phosphorus, and potassium-rich byproducts (Fu *et al*., 2015; Lv *et al*., 2016). Earthworms such as *Eisenia fetida*, *Lampito mauritii*, and *Eisenia andrei* are commonly used in vermicomposting procedures to decompose organic waste. They are also used in ecological, toxicological, and genetic research studies.

Earthworms' Uptake, Bioaccumulation, Distribution, and Excretion of Heavy Metals:

When earthworms acquire heavy metals, they endure oxidative stress, higher levels of free radicals, and eventually become equally toxic and carcinogenic as other living things (Wang and Cui, 2016; Wallace and Djordjevic, 2020; Yan *et al*., 2021). Nevertheless, to resist heavy metals in soils and a specific concentration of potentially poisonous elements (PTEs), earthworms evolved species-specific detoxification processes (such as the formation of antioxidants, metallothionein proteins, and the enzyme cytochrome P450) (Hussain *et al*., 2021; Yuvaraj *et al*., 2021). According to studies by Hobbelen *et al*. (2006) and Šrut *et al*. (2019), earthworms can absorb soil particles and/or organic soil components that have significant amounts of heavy metals in their intestines, or they can accumulate heavy metals from soils via their skin through pore water in the soil. Nevertheless, oral ingestion of soil particles and organic matter—especially in soils with low PTE concentrations in soil solution or porewater—is most likely more crucial for the buildup of heavy metals in earthworms than cutaneous absorption (Becquer *et al*., 2005; Hobbelen *et al*., 2006; Lanno *et al*., 2019). When it comes to PTE bioaccumulation in earthworms, food absorption is frequently the more common pathway (Becquer *et al*., 2005; Hobbelen *et al*., 2006; Lanno *et al*., 2019). However, the concentration of accessible PTEs in soils has a significant influence on each pathway's involvement, which is concentration-dependent (Beaumelle *et al*., 2015). Numerous studies have documented the cutaneous absorption of PTEs by earthworms via porewater-mediated methods (Hobbelen *et al*., 2006; Chen *et al*., 2018; Wang *et al*., 2018a). *Eisenia fetida* Cd content rose in proportion to the quantity of free Cd^{2+} present in the soil solution, according to Li *et al*. (2009a). Presumably, Saxe *et al*. (2001) found that over 70% of Zn in earthworms was absorbed dermally and that dermal exposure is the only way that Cd, Cu, and Pb are deposited in earthworms. Furthermore, the cutaneous pathway carried more than 96% of the mercury in earthworms (Hobbelen *et al*., 2006).

Distinct ecophysiological types of earthworms ingest a range of materials, including soil microorganisms, organic compounds present in surface soil, and soil organic matter (humus or waste products) (Bonkowski *et al*., 2000). Regardless of the food source or worm genus, earthworms, especially endogeic worm species, eat soil particles by swallowing and burrowing (Wang *et al*., 2018b; Šrut *et al*., 2019). PTE accumulation is often higher in earthworms that ingest soil with high PTE concentrations (Wang *et al*., 2018b; Zhang *et al*., 2020). Before reaching the intestine, food and contaminants go through the mouth, throat, oesophagus, stomach, and gizzard after being consumed (Li *et al*., 2009a, b; Marichal *et al*., 2017). PTEs are extracted from soils by a process known as complexation, and they dissolve in the intestines with the food as it is being digested (Marichal *et al*., 2012). The PTEs decompose, are absorbed by the villi, and then make it possible for the bloodstream to absorb them. Earthworm intestines feature a complex digestive mechanism, which will affect PTE redistribution and fractionation (Sizmur *et al*., 2011; Pass *et al*., 2015). After absorption, PTEs are progressively removed from the body by mucus, urine, or gastric discharge. Earthworms' diverse subcellular fractions are where they are mostly distributed and enriched (Vijver *et al*., 2004; Ardestani *et al*., 2014). The bioaccumulation patterns of essential and nonessential PTEs, however, differ significantly (Vijver *et al*., 2004; Ardestani *et al*., 2014). To be more accurate, the primary mechanism controlling the accumulation of nonessential PTEs in earthworms is "active excretion from the PTEs excess pool of internal storage without elimination," whereas the main mechanism for essential PTEs is "regulated uptake and excrete" (Sizmur and Hodson, 2009; Ardestani *et al*., 2014). Gonźalez-Alcaraz and van Guestel (2016) reported that after 1-3 days of exposure, the earthworms' Cd content steadily rose to 12.6–18.5 μg g^{-1}, whereas their As and Zn concentrations stabilized. Different heavy metals remedied by different earthworm species are presented in Table 3.

Future Prospects:

Vermiremediation is an effective way of cleaning up heavy metal-contaminated soil, especially in mild to moderate cases of contamination (Xiao *et al*., 2022). It may be used alone or in combination with other bioremediation techniques. To fully understand the underlying mechanisms of the

Table 3: Vermiremediation's Effectiveness in Detoxifying Heavy Metals

S. No.	Substrates	Earthworm Species	Metals Detoxified	Effectiveness of Remediation	References
1.	Sewage sludge + Sugarcane trash	*Eisenia fetida*	Mn, Fe, Cu, Zn, Pb	Reduction of heavy metal concentration: Cu (4.98-30.5%), Fe (5.08-12.64%), Mn (3.31-18.0%), Zn (2.52-15.90%), Pb (2.38-20.0%)	Suthar (2009)
2.	Cotton textile sludge + Cow dung	*Eudrilus eugeniae*	Cr, Zn, Cd, Pb	The concentration of heavy metals was lowered by 50–60%	Paul *et al*. (2020)
3.	Sewage sludge + spent mushroom compost	*Lumbricus rubellus*	Cr, Cu, Zn, Cd, Pb	Cd, Cr, and Pb were removed about 90-98.7%, but Zn and Cu amounts slightly rose	Azizi *et al*. (2013)
4.	Sewage sludge	*Eisenia fetida, Eudrilus eugeniae* and *Perionyx excavatus*	Mn, Cu, Zn, Pb	The level of heavy metals has been lowered below the permitted limits	Khwairakpam and Bhargava (2009)
5.	Buffalo Dung + Kitchen Waste	*Eisenia fetida*	Co, Cr, Pb, Ni, Cd, As	When buffalo dung and kitchen wastes were combined, *Eisenia fetida* decreased Co by 2.47%, Cr by 0.40%, Pb by 64.12%, Ni by 8.02%, Cd by 0.34, and As by 2.10%	Bhartiya and Singh (2011)
6.	Soil	*Metaphire californica*, *Amynthas homochaetus*, *Amynthas pecteniferus*, and *Amynthas heterochaetus*	Cd, Zn, Cu, Pb	Metal concentrations taken up by the examined earthworms showed increased plasticity in contaminated locations, with BAF values ranging from Cd > Zn > Cu > Pb	Wang *et al*. (2018c)
7.	Soil	*Aporrectodea rosea*, *Eisenia veneta,* and *Allolobophora chlorotica*	Cu, Zn, Pb	Lead, zinc, and copper levels in earthworm bodies are influenced by the composition of their tissues and perhaps by the shape of the skin-muscular sac	Kokhia *et al*. (2022)
8.	Soil	*Eudrilus eugeniae* and *Lumbricus terrestris*	Cd, Cr, Pb, Ni, Zn	Compared to *Eudrilus eugeniae*, *Lumbricus terrestris* has a greater tendency to remove Cd, Zn, and Ni from the soil, whereas *Eudrilus eugeniae* is better at removing Cr and Pb	Iheme *et al*. (2021)
9.	Soil	*Eisenia fetida*	Cu, Zn, Mn, Cr, Ni	Cu, Zn, Mn, Cr, and Ni concentrations in soil treated with *Eisenia*	Borah and Deka (2023a)

				fetida were found to have decreased by 17.4%, 19.45%, 9.44%, 23.8%, and 9.6%, respectively, after the research studies	
10.	Soil	*Eudrilus eugeniae*	Cu, Zn, Mn, Cr, Ni	During 90 days of experimental testing, *Eudrilus eugeniae* could reduce the Cu, Zn, Mn, Cr, and Ni concentrations in the soil by 17.56–26.30%	Borah and Deka (2023b)
11.	Animal dung + Soil + Vermicompost	*Lampito mauritii*	Co, Cr, Pb	*Lampito mauritii* decreased the amount of Pb by approximately 30.91%, Cr by 9.40%, and Co by 43.75%	Fatima and Singh (2023b)
12.	Municipal solid waste + Animal Dung	*Lampito mauritii*	Co, Cr, Pb, Ni, Cd	*Lampito mauritii* reduced Co concentration by 2.40%, Cr by 0.71%, Pb by 14.38%, Ni by 11.91%, and Cd by about 1.11%	Fatima and Singh (2023c)

absorption, accumulation, and transportation of heavy metals in earthworms, as well as their detoxifying action, further study is required, as there are enough long-term and field studies in this area. In the future, this knowledge might greatly improve the use of vermiremediation treatments. As of yet, there are few relevant field-scale investigations on earthworms, despite their potential as efficient agents for cleaning up heavy metal-contaminated soil. The study indicated above has yielded promising results regarding the cleanup of mild to moderately contaminated soil through the use of vermiremediation.

Conclusion

As a result of the high expenditures and environmental damage connected with conventional physicochemical cleanup approaches, the instances mentioned above show a change in attention towards biologically *in situ* alternatives. When it comes to mild to severe heavy metal contamination, using earthworms to remediate contaminated soil is beneficial. Earthworms serve as valuable ecological bioindicators in the restoration of soil contaminated with heavy metals. Their ability to bioaccumulate heavy metals in their tissues enhances metal absorption and boosts their tolerance to metal toxicity. Consequently, the characteristics of earthworms offer significant benefits for evaluating pollution, emphasizing soil quality, and implementing the vermiremediation process.

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