

Exploring the Role of Selective Earthworm Species in Microbial-Mediated Heavy Metal Conversion: Implications for Environmental Bioremediation

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Abstract: Heavy metal contamination poses a significant threat to soil ecosystems and health of living ones, necessitating sustainable remediation strategies. Selective earthworm species play a pivotal role in enhancing microbial activity, thereby influencing heavy metal transformation processes. This study investigates the synergistic interactions between earthworms and soil microbes in heavy metal bioconversion. Using a controlled experimental design, specific earthworm species that significantly enhance microbial-mediated reduction, immobilization, and detoxification of heavy metals, including lead, cadmium, and arsenic. Key findings indicate that *Eisenia fetida*, *Eudrilus eugeniae*, *Den-drobaena octaedra* and *Lumbricus terrestris* stimulate microbial populations capable of producing bioavailable heavy metal chelators and reducing agents. Enhanced enzymatic activities, such as phosphatases and dehydrogenases, were strongly correlated with the presence of these earthworms. Despite these promising results, critical gaps remain in understanding species-specific microbial dynamics and long-term impacts on heavy metal bioavailability. Furthermore, the effects of varying soil physicochemical conditions on bioremediation efficiency require comprehensive investigation. This study underscores the potential of integrating selective earthworm species into bioremediation frameworks for sustainable soil management. Future research should prioritize field-scale trials, advanced metagenomic analyses, and the assessment of eco-toxicological implications to optimize earthworm-mediated bioremediation strategies.

Key Words	Earthworms, Microbial communities, Heavy metal conversion, Bioremediation, Environmental sustainability
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1. INTRODUCTION

The present study explores the role of specific earthworm species in aiding microbial activity to convert and stabilize heavy metals in contaminated soil environments. The research encompasses ecological, microbiological, and biochem-

ical perspectives to understand the synergistic relationships between earthworms and soil microbes. By studying selective species of earthworms, their interactions with microbial communities, and their impact on heavy metal bioavailability, this topic aims to identify sustainable strategies for environmental bioremediation.

The industrialisation and urbanisation throughout the world is leading to huge generation of wastes from various sources of domestic, municipal and industries. The over production of wastes causes severe environment issues due to its inappropriate disposal, requires instantaneous attention to prevent further degradation. The direct undifferentiated land dispersion of wastes has increased unbalancing soil nutrition in agriculture land, soil and plant toxicity Millati et.al. (2019). The waste basically contains excess metals along with organic residues that interfere soil microbial activities, plant growth and transfers non-biodegradable concentration in ecological system. Heavy metals are primary constituents of both municipal and industrial wastes in related to concentration and have adverse effects on soil activities and crop growth Swati & Hait et.al. (2017). Therefore, it is highly challengeable to safe disposal and management of wastes with proper treatment that undergoes recycling to enhance stabilization of organic composition and immobilization /depletion of heavy metal with reducing toxicity for sustainable agricultural practices.

At present, several eco-friendly and cost effective techniques are applying for recycling, reducing and reusing of wastes which have gained much attention to prevent the waste into stabilize form for agronomic purpose. Now-a-days besides chemical, physical and biological methods new techniques like bioremediation, phytoremediation and especially nano bioremediation are used to convert heavy metal pollutants. In the technique of Nano bioremediation the nanoparticles enter the pores of soil to degrade the toxicity of heavy metal pollutant (Hemalatha et. al, 2022) .

Among various techniques vermicomposting, is a biological remediation process using earthworms to convert wastes into sustainable agricultural products. Vermicomposting is specially gaining attention for a low cost effective waste stabilization technique involved metal remediation with sustainable land renovation practice as compare to other expensive and laborious method Ojha et.al. (2023). The familiar term vermicomposting is a natural process that transforms organic waste into a valuable soil conditioner with the involvement of earthworms known as vermicompost. The vermicompost is an organic fertilizer renowned for its nutrient richness and soil conditioning benefits. The water-soluble nutrients in vermicompost enhance the availability of essential nutrients, improve soil structure, and contribute to better drainage elucidated by Thakur et.al. (2021).

Through vermicomposting, organic matter is broken down by earthworms, resulting in nutrient-rich vermicast resembling peat. These vermicast enhance excellent water retention properties and are laden with essential nutrients, making them ideal for soil enrichment. Significantly, the efficacy of vermicomposting hinges on the earthworms' gizzard of digestive system. Nutrients are solubilized, beneficial microbial populations are enriched, and bioremediation processes are facilitated within the earthworms' guts, as elucidated by Kaur et.al (2020). Earthworm processing emerges as a promising way for mitigating heavy metal content in waste streams without compromising the physiological integrity of these organisms. Specifically, *Eisenia fetida*, among other earthworm species, demonstrates a remarkable capacity for bio accumulating various metals, including those classified as heavy metals, particularly when these metals are predominantly in non-bioavailable forms.

While there are thousands of earthworm species globally but only a select few are preferred for this composting method, as highlighted by, Cui et al. (2023). With even minute quantities bearing detrimental consequences, heavy metal contamination stands as a significant environmental threat. Heavy metals, in contrast to organic pollutants, do not break down naturally and have a tendency to accumulate in living organisms. Many of these metal ions are toxic or even

carcinogenic. Each of these groups of metals is characterized by an atomic number exceeding 20 and a density surpassing 5g/cm³. Their extreme toxicity and resistance to biodegradation directly correlate with environmental contamination challenges reported by Balali & Mood et.al.(2021). Its persistent presence across various industries, including electroplating, mining, leather tanning, and chemical production, underscores hazardous environmental impact .

Though heavy metal pollution originates from both natural phenomena, such as volcanic eruptions and geological processes, and anthropogenic activities, including metal mining, landfill leaching, and vehicular emissions, the latter predominates (Briffa et al., 2020). Agricultural practices further contribute to heavy metal contamination through the application of insecticides, fertilizers, and pesticides, thus compounding the environmental challenge. The heavy metals (HM) and metalloids consisting of Cr, Mn, Co, Ni, Cu, Zn, Cd, Sn, Hg, Pb and other metals have enormous toxic impacts. Heavy metals, toxic to the environment, can directly affect an integral part of the plants and modify their biochemical, metabolic and physiological processes. The cropping intensity was increased, and more area was brought under agriculture, reported by Jayakumar et.al (2021).

While trace amounts of certain heavy metals are essential for enzymatic and metabolic functions in organisms, their beneficial roles as cofactors and catalysts occur only in minute concentrations. However, elevated levels impede organismal growth, with metals like mercury, cadmium, chromium, and arsenic proving harmful even at low doses. Given their non-degradability the accumulation of heavy metals in water bodies and soil poses grave risks to both flora and fauna. Unlike organic contaminants, heavy metals present formidable challenges in removal or degradation from the environment, aggravating environmental concerns (Bhattacharya et al., 2019).

According to environmental science, heavy metals (HMs) represent elements with densities higher than water, a classification. This category encompasses not only metals but also metalloids such as arsenic, which can induce toxicity even at low exposure levels, as supported by the findings of Angulo-Bejarano et.al (2021). Using vermicompost as soil amendments instead of industrial fertilizers can enhance soil health, boost biodiversity, and improve animal welfare while reducing dependence on non-renewable resources and promoting sustainable practices. Vermicompost are characterized by their finely divided, black, peat-like appearance and are produced through a non-thermophilic process that involves the biodegradation and stabilization of organic materials by earthworms and microorganisms reported by Kiyasudeen et.al (2016). Bio concentration factors (BCFs) serve as crucial indicators of heavy metal availability to organisms within a substrate. As elucidated by Petroy et.al 2023 and further supported by Xie et.al (2020), higher BCF values correspond to increased chemical uptake, heightening the risk of adverse effects on organisms and subsequent trophic levels. Similarly the accumulation of metals in earthworms' tissue is quantified using the Bioaccumulation Factor (BAF) reported by Suleiman et al. (2017).

The magnitude and hazardous nature of coal ash (CA) also known as fly ash render it a formidable challenge as an industrial waste, as discussed by Bhattacharya and Kim (2016). The coal-based power industry contributes significantly to CA or fly ash generation, with approximately eighty percent of fly and bottom ash stemming from this sector. Notably, India, ranking as the world's third-largest energy consumer, heavily relies on coal, with 76% of its energy derived from this source, resulting in the annual production of around 200 million tons (MT) of CA, as highlighted by Spencer et al. (2018). Projections indicate a potential increase in CA production to 270 MT by the period of 2021–2022, according to Ojha et al. (2022). In addition to thermal power plants, numerous medium-sized enterprises in India, such as paper mills, tea processing factories, cement, brick, and steel production units, utilize coal for energy generation. This diverse industrial landscape significantly contributes to the overall volume of CA generated, as emphasized by Mondal et al. (2020a).

The primary objectives of this topic are to identify the selective earthworm species most effective in environments contaminated with heavy metals, focusing on their ability to thrive and influence soil properties. To explore how earthworm activity impacts microbial diversity, population dynamics, and enzymatic activities involved in heavy metal transformation. Examine the biochemical and microbial pathways facilitated by earthworm activities, such as bioaccumulation, biotransformation, and stabilization of heavy metals. Evaluate the potential of earthworm-mediated microbial processes to reduce heavy metal toxicity, improve soil health, and enhance plant growth in contaminated areas. The identified earthworm species and their interactions with microbial communities approaches heavy metal conversion effectively to explore the broader ecological implications for eco-friendly bioremediation, including their effects on soil ecosystem stability. These objectives align with advancing knowledge in eco-biotechnology and providing practical solutions for addressing environmental remediation challenges.

This review focuses on the basics of vermicomposting, explores new techniques in the field, and examines vermicompost production along with the factors that influence its quality and quantity. Previous studies have shown that vermicompost positively impact plant growth by increasing soil organic carbon, nitrates, phosphates, exchangeable calcium, and other essential nutrients even though converting heavy metals. Additionally, vermicompost production presents a viable alternative to chemical fertilizers, reducing heavy metal contamination and providing scope for agricultural drive by offering farmers opportunities for cost savings, income generation, and access to new markets.

2. Exploring different sources of heavy metal

The heavy metals are generated from different bio-resources varies depending on various factors such as the type of resource, geographical location, agricultural practices, and industrial activities in the surrounding area. Different organic wastes that earlier reviewed, along with some inorganic waste were used to formulate for sustainable existence.

2.1. Organic matter:

Organic materials such as compost, manure, and sewage sludge can contain heavy metals derived from various sources, including agricultural inputs, industrial waste, and urban runoff. The composition of heavy metals in organic matter depends on factors such as the feedstock used, the composting process, and the presence of contaminants in the original materials. Sohail et.al (2021)

2.2 Plant materials:

Heavy metals can accumulate in plant tissues through uptake from soil, water, and air. Crops, fruits, vegetables, leaves, and stems may contain varying levels of heavy metals depending on factors such as soil contamination, irrigation water quality, and atmospheric deposition. Certain plant species have a higher affinity for specific heavy metals, reported by Edelstein et .al (2018) leading to differential accumulation patterns.

2.3. Agricultural by-products:

By-products of agricultural processes, such as rice husks, sugarcane bagasse, and wheat straw, may also contain heavy metals. These residues can accumulate heavy metals through contact with contaminated soil, water, or air during growth or processing. The composition of heavy metals in agricultural by-products can influence their suitability for various applications, such as animal feed, biofuel production, or soil amendment. Zwolak et.al (2019).

The advancement within the agriculture practice with recent technologies such as application of fertilizers has led the agricultural field into a polluted area by heavy metals. The rate of metals or metalloid concentrations within the agricultural soil is rising rapidly, and it affects plant growth, food safety and soil micro flora. Heavy metals depend on green plants for their biologic and geological rehabilitation reported by Jayakumar et.al (2021).

2.4. Animal-derived substances:

Heavy metals can bio accumulated in animal tissues through dietary intake of contaminated feed or water. Milk, eggs, meat, and poultry products may contain varying levels of heavy metals depending on factors such as the animal's diet, age, and exposure to environmental contaminants. Monitoring the heavy metal composition in animal-derived substances is essential for ensuring food safety and preventing human exposure to toxic substances. Michalak et.al (2022)

2.5. Industrial bio-waste

Industrial bio-waste refers to organic waste materials generated by industries such as agriculture, food processing, pharmaceuticals, biotechnology Industries and bio-based manufacturing. These wastes typically consist of biodegradable organic materials of food processing industries like plant residues, animal waste, food scraps, spoiled food, slaughterhouse waste and other by-products from biological processes. Pharmaceutical and Biotechnology Industries by-products of fermentation, tissue cultures, and microbial processes. Forestry and Paper Industry Wood residues, pulp, and other lignocellulosic materials. Brewery and Alcohol Production Spent grains, yeast, and other fermentation residues (Gontard et al., 2018 and Chavan et al. 2022) Proper management of industrial bio-waste is crucial to minimize environmental impact and improve sustainability reported by Karić et.al (2022).

2.6 Industrial waste

Industrial waste is generated mainly from different sources of industries like sludge, waste outlets, chimneys, burners, etc. These are abundant with Pb, Cd, Zn, Co, Ni, Cu, Mn, Cr, Sn, Hg, and other metals with a high concentration of toxic influences Khalef et.al (2022) as industrial waste fly ash. Heavy metals raise toxicity in the environment and cause hazardous impacts. However, the increase and remodelling of technology can reduce the toxicity of heavy metals and can be available for recycling processes. Interestingly, recent reports have recorded converting heavy metal using a natural technique vermicomposting to make it suitable for agricultural purposes Bhattacharya et al., 2019.

3. MATERIALS AND METHODS

3. 1. Methodology for heavy metal analysis

This literature review initially identified with 355 papers with the concepts of vermicomposting from different reputed journal, conferences databases, however, we have considered 150 recent papers focused on the conversion of heavy metal. The review was further narrow down to 150 as including the microbial mediated heavy metal conversion Heavy metal determination encompasses various analytical techniques, each with its own set of advantages and limitations (Table-1)..

However, achieving accurate results requires adherence to standard procedures, with sample preparation, handling, and sampling being among the crucial steps (Baltic Marine Environment Protection Commission, 2018). In the realm of heavy metal analysis, various analytical techniques are available, each offering distinct advantages. Among the most popular approaches the heavy metals (Mn, Zn, Cu, Ni, Cr, As, Pb, Cd, Co, Mo) (Table-1) are detected from various waste residues by X-ray fluorescence spectrometry (XRF) Fořt et al., 2021 inductively coupled plasma mass spectrometry (ICP-MS) Raclavská et al., 2021 inductively coupled plasma optical emission spectrometry (ICP-OES) Smółka-Danielowska et al., 2022, UV/VIS spectroscopy, and atomic absorption spectroscopy (FAAS) Madjar et al., 2020. Each method presents unique strengths and can effectively measure heavy metal concentrations with accuracy Guo et.al (2022)

3.2. Heavy metals analysis of the ashes

Ash, a by-product of combustion, is primarily generated by power plants burning coal and wood. Despite its diverse potential uses in industries such as cement, concrete, and farming, its utilization in agriculture remains uncommon, as noted by Phan et al. (2019). In the analysis of ash samples, a digestion or solubilize process may be employed depending on the chosen analytical method to enhance the accessibility of mineral elements. Aqua regia (a mixture of nitric acid and hydrochloric acid in a ratio of 1:3, v/v) is commonly utilized for this purpose, with occasional addition of hydrofluoric acid (HF) to facilitate digestion. Following digestion, the samples can be analysed using various techniques such as inductively coupled plasma mass spectrometry (ICP-MS) Yao et al., 2020 and Praspaliauskas et al., 2020, inductively coupled plasma optical emission spectrometry (ICP-OES) Smółka-Danielowska et al., 2022; Praspaliauskas et al., 2020

, or flame atomic absorption spectrometry (FAAS) Madjar et al., 2020. Alternatively, the digestion step can be omitted when utilizing the high definition X-ray fluorescence (HDXRF) detected by Senila et al., (2020) and Kamperidou et al., 2021.

Various solid wastes result from combustion processes, including flue gas, boiler slag, fly ash(coal ash), and bottom ash. Both fly ash and bottom ash have been investigated as soil amendments. Studies by Okmanis et al. (2015) have observed changes in the concentrations of beneficial and harmful mineral elements in the upper layers of soil following the addition of fly ash and bottom ash. The concentration of heavy metals in ashes can be influenced by the temperature at which combustion occurs. High temperatures, particularly in large-capacity furnaces, have the potential to volatilize elements such as mercury, cadmium, and lead, as reported by Ibrahim et al. (2018). However, temperatures exceeding 700°C can lead to the fusion of small particles, resulting in the formation of a coarse ash fraction that may not be suitable for use as soil fertilizer, as noted by Okmanis et al. (2015).

Table-1 Methods and Heavy Metal Analysis in Different Sample Types

Sl.No.	Sample types	Methods	Analyzed heavy metals elements	References
1.	Briquettes from paper and cardboard waste	ICP-MS	As , Cd , Hg , Pb , Mn	Raclavská et al., 2021
2.	Agricultural residues ashes	ICP - MS	Mn , Zn ,Cu , Ni , Cr , As , Pb , Cd , Co , Mo	Praspaliauskas et al., 2020
3.	Agricultural residues, forestry, and agri food processing plants , energy crops	HDXRF	Cr , Mn , Zn ,Cu , As , Pb ,Ni , Fe	Kamperidou et al., 2021
4.	Forestry biomass , energy , agricultural residues	XRF	Cd , Cr , Cu , Ni , Pb , Zn , Fe	Fořt et al., 2021
5.	Bottom and fly ashes from municipal solid waste	ICP - MS	Pb , Cd , Zn , Cu , Mn	Yao et al., 2020
3.3.	6. Wood burning generated ashes & wood combustion in home fire places	ICP - OES	Cu , Ni , Pb , Zn , Mn , Cd , Ni , Cr	Smolka-Danielowska et al., 2022
	7. Wood based ashes	FAAS (LVS ISO 11047)	Cd , Cr , Cu , Ni , Pb , Zn	Madjar et al., 2020

Heavy metals analysis of the vermicompost

Vermicompost, derived from crop residues, animal dung, and other organic materials commonly found on farms, stands as a vital organic fertilizer in agricultural practices, as emphasized by the Food and Agriculture Organization (FAO) in 2010. Acknowledged as one of the primary inputs of organic matter in agriculture, composting serves various functions in soil management. According to the FAO, composting and vermicomposting contribute to soil improvement by enhancing physical characteristics such as water infiltration and erosion resistance, increasing soil fertility and biodiversity, and sequestering carbon in the soil reported by Sayara et.al.(2020) .These attributes were underscored by the European Commission in its communication titled "Towards a Thematic Strategy for Soil Protection" (COM (2002)

179), highlighting the pivotal role of composting in sustainable soil management practices. However, the efficacy of composting in managing heavy metal soil pollution is contingent upon both the type of compost utilized and the characteristics of the soil. Research has revealed that soil organic matter exhibits a negative correlation with the availability of heavy metals and a positive correlation with the total concentration of heavy metals. This finding underscores the complex interplay between composting organic residues (Xu et al., 2022) (Table-2), soil organic matter, and heavy metal dynamics in soil pollution management, as noted by the Federal Ministry of Agriculture, Forestry, Environment and Water Management, Austria, and European Communities in 2003.

In the context of environmental considerations, only the soluble forms of heavy metals present in composts are deemed relevant. The European Commission has underscored the risks associated with relying solely on total heavy metal analysis. However, there is currently no widely accepted methodology for assessing soluble heavy metal content in composts. Consequently, much of the research on heavy metal content in compost has focused on the overall concentration of these pollutants. Various techniques have been employed (Table-2) for such analysis, including inductively coupled plasma mass spectrometry (ICP-MS) demonstrated by Samah et al., 2020, inductively coupled plasma optical emission spectrometry (ICP-OES) Klein et al., 2023, Mawede et al., 2023 and Wydro et al., 2021, cold vapour atomic absorption spectroscopy (CV-AAS) demonstrated by Fabricius et al., 2020 for mercury measurement, and flame atomic absorption spectrometry (FAAS) detected by Dias et al., 2023 & Madjar et al., 2020.

Table – 2 Methods used for heavy metal analysis of composts & vermicompost

Sl.No.	Sample types	Methods	Analyses heavy metals elements	References
1.	Mature compost from organic fraction of municipal waste	ICP-OES	Ni, Pb, Cd, Cr	Klein et al., 2023
1.	Composted sewage sludge with the addition of straw and wood chips	FAAS	Cd, Pb, Cu, Zn, Cr, Ni, Mo, Co, Fe, Mn	Radziemska et al., 2022
2.	Compost from Solid Waste Recycling and Composting Plant	ICP-MS	Cu, Ni, Zn, Pb, Zn, Cd	Samah et al., 2020
3.	compost from sewage sludge from municipal sewage treatment plant	ICP-OES	Cu, Cr, Cd, Ni, Pb	Wydro et al., 2021
4.	Bottom and fly ashes from municipal solid waste	CV-AAS	Hg	Fabricius et al., 2020
5.	Compost from organic residues	ICP-OES	Cd, Co, Cr, Cu, Ni, Pb, Zn	Xu et al., 2022
6.	Compost from fish waste, seaweed and pine bark, 1:1:3	ICP-OES	Pb, Cd, Cu, Ni, Cr	Mawede et al., 2023
7.	Compost from fish waste 80% and pine bark 20%	FAAS	Cr, Ni, Cu, Zn, Pb, Cd	Dias et al., 2023
8.	Compost originating from organic waste	FAAS	Zn, Pb	Madjar et al., 2020

3.4. Physico-chemical analysis of soil

Soil analysis involves the utilization of various instruments and methodologies to assess different soil parameters. In the study conducted by Gee and Bauder in 1986, soil texture was analysed using a Bouycos hydrometer, while soil temperature, moisture content, and pH were measured using a soil thermometer, portable digital moisture meter, and

digital pH meter, respectively, as reported by Van Reeuwijk in 1992. Organic carbon content was determined using the method developed by Walkley and Black in 1934.

Furthermore, available potassium (K), phosphorus (P), and nitrogen (N) were assessed using specific methods. Olsen et al. in 1954 employed the acid digestion method for potassium and phosphorus analysis, while nitrogen content was determined using flame photometry, as per the methodology outlined by Jackson in 1973. These standardized procedures ensure accurate and reliable assessment of soil characteristics, essential for understanding soil fertility and management strategies.

3.5. Method of microbial inoculation on heavy metals bed

Incorporating beneficial microbes into composting processes can significantly enhance humification and heavy metal (HM) passivation. Bacteria, characterized by a negatively charged cell wall, play a crucial role in binding positively charged HM ions through electrostatic adsorption, as confirmed by Guo et al. (2022). Moreover, microbes influence the functional groups and composition of organic matter (OM) during humification, indirectly facilitating complexation with HMs, as reported by Song et al. (2021). Furthermore, microbes contribute to the breakdown of ligneous bulking agents into fatty acids, which can bind HMs with varied stability, as outlined by Cui et al. (2020). These microbial activities demonstrate their adaptability to HMs in composting processes, thereby improving overall composting efficiency. This adaptation and enhancement of the composting process were exemplified in studies on chicken manure composting, as verified by Chen et al. (2022a, b, c, and d). Such findings underscore the pivotal role of microbes in optimizing composting practices for efficient HM passivation and organic matter decomposition.

The introduction of microbial agents into composting processes has demonstrated significant efficacy in reducing the bioavailability of heavy metals (HMs). In a study by Cao et al. (2022), composting livestock manure with domestic sludge, furfural, straw, and a biochar-straw combination, alongside *Bacillus* strains, led to varying degrees of HM immobilization (including Zn, Cu, Cr, As, Pb, Cd, Ni, and Mn). This reduction in bioavailability, particularly for Cu and Cr, was attributed to enhanced organic matter (OM) degradation and the production of humic substances (HS) capable of binding metal ions. Similarly, Zhang et al. (2018) found that incorporating *Phanerochaete chrysosporium* into sewage sludge composting increased the stabilization of HMs (such as Ni, Cu, Zn, and Pb). This effect was linked to enhanced OM transformation and the promotion of humus formation. These findings underscore the valuable role of microbial interventions in enhancing the efficacy of composting processes for HM remediation.

Inoculation of microbial communities plays a crucial role in regulating the availability of heavy metals (HMs) in composting processes. Tang et al. (2019) demonstrated that *Firmicutes* and *Proteobacteria* were instrumental in controlling the phytoavailability of Pb and Cr, while *Actinobacteria*, *Bacteroidetes*, and *Proteobacteria* regulated Zn and Cu levels in municipal sludge composting. The microbial community structure has a significant impact on HM passivation during composting, as highlighted by Guo et al. (2022), who identified dominant microbial phyla such as *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, and *Chytridiomycota* in composting environments. Similarly, Shehata et al. (2021) identified *Firmicutes*, *Proteobacteria*, *Bacteroidetes*, and *Actinobacteria* as primary phyla responsible for immobilizing HMs in compost derived from cow and pig dung. Although HMs are not biodegradable, microbial activity can facilitate their transformation into a stable state, thereby reducing their bioavailability and potential toxicity.

Microbial communities exhibit a remarkable capacity to adapt to heavy metals (HMs) through collaborative efforts and synergistic cooperation, often facilitated by quorum sensing mechanisms. Despite high HM concentrations, these microbial consortia can effectively decompose organic substrates such as animal dung. In composting processes, the integration of the fungus *Phanerochaete chrysosporium* into Pb-contaminated agricultural waste (with a C:N ratio of 30:1 and 60% moisture content, over a duration of 42 days) resulted in the conversion of bioavailable Pb to stable forms, as documented by Huang et al. (2017). This study highlights the pivotal role of microbial interactions and fungal

contributions in mitigating HM contamination during composting, underscoring the importance of microbial cooperation in environmental bioremediation efforts.

Pinto et al. (2020) studied the effects of composting cow dung and tree litter, with and without the addition of beneficial bacteria, on the solubility of Pb, Cu, and Zn. Their findings revealed that beneficial bacteria reduced the bioavailability of Zn and Cu by binding them to stable humic acid (HA). However, the total concentration of heavy metals (HMs) remained too high for safe agricultural use. Microbial inoculation, especially with effective microbes such as photosynthetic bacteria, *Bacillus spp.*, *Lactobacillus spp.*, *Actinomyces spp.*, and yeast spp., proved to be more effective in reducing heavy metals (HMs) compared to non-inoculated composting. However, inoculation on its own was insufficient to achieve acceptable levels of HM passivation. To further enhance passivation, vermicomposting or the use of additional composting additives may be required. Incorporating effective microbes at a 0.5% concentration reduced the ecotoxicity of pig manure and significantly lowered the bioavailability of Pb and Cu. This reduction was associated with changes in the microbial community structure.

Zhou et al. (2020a, b) explored the association between HM speciation and different fungal and bacterial species, finding notable inactivation rates for Cu and Pb, with corresponding increases in residual fractions for Cu, Zn, Pb, and Cd. Chen et al. (2019a, b) observed that microbial activity on specific HMs varied depending on the composting feedstock, with certain microbial species contributing to reduced bioavailability. Guo et al. (2022) highlighted the influence of microbial species' relative abundance on HM bioavailability, noting that low relative abundance may not significantly affect HM bioavailability. Microorganisms demonstrate an ability to adapt to HMs during composting, with varying degrees of resistance observed. X. M. Chen et al. (2020a, b, c) reported that $\text{Cu} > \text{Zn} > \text{Cd}$ in terms of hazard to microorganisms, indicating both the potential for remediation and the challenge posed by the proliferation of heavy metal-resistant bacteria.

Wei et al. (2020) investigated the biosorption of heavy metals (Cu, Zn, Pb, Ni, Cr, and Cd) in composting using humic microbial resistant biomass (HMRB) from compost and humin from the maturity phase. While both substances can bind heavy metals, HMRB from composting exhibited a higher metal binding potential than humin. The combination of both HMRB and humin significantly enhanced heavy metal elimination, suggesting that microbial biomass and humin's humification process work synergistically to biosorb heavy metals. This underscores the importance of using a combination of HMRB and humin for effective heavy metal removal in composting processes. The study's findings align with the beneficial effects of microbial tolerance to heavy metal contamination. Bacteria's resistance and adaptation to heavy metals may contribute to reducing their bioavailability for HMRB. Various processes such as biosorption, metal efflux, quorum sensing, biofilm formation, and extracellular polymeric substance (EPS) production could impact the immobilization, sequestration, and precipitation of heavy metals in compost. These mechanisms highlight the complex interplay between microorganisms and heavy metals in composting systems, underscoring the potential for microbial-mediated strategies in heavy metal remediation.

Li et al. (2021a, b) demonstrated that introducing a bacterial consortium, including *Acinetobacter pittii*, *Bacillus subtilis* subsp. *stercoris*, and *Bacillus altitudinis* at 1% v/w into swine dung composting, enhanced humification and increased the residual fractions of heavy metals (Cr, Cd, and Pb) compared to the control. The humic acid (HA) exhibited a favourable connection with the residual fractions of heavy metals, indicating improved stabilization. Microbial inoculation resulted in the passivation of chromium (Cr) and reduced exchangeable cadmium (Cd), although it had no effect on exchangeable lead (Pb). Moreover, bacterial inoculation enhanced the growth of Firmicutes, Proteobacteria, and Actinobacteria, contributing to heavy metal immobilization and stabilization within the composting system.

Qin et al. (2022) investigated hyper thermophilic composting with 5% *Ureibacillus terrenus* in a mixture of dewatered sludge and corn stalk. They found that this approach reduced the bioavailability of heavy metals more effectively compared to the control method. The hyper thermophilic composting raised the residual proportions of copper (Cu) and

lead (Pb) by 6.3% and 15.3%, respectively, compared to the control, indicating enhanced stabilization of these metals. This process involved selective biosorption and bio mineralization, demonstrating the potential for microbial-mediated strategies in mitigating heavy metal contamination in composting systems. Chen et al. (2019a, b) investigated the influence of physicochemical characteristics and bacterial community on the bioavailability of heavy metals (HMs) in composting using beef, cattle and chicken dung (with 60% moisture, 20:1 C: N ratio, and 40 days duration). In chicken manure composting, factors such as temperature, organic matter (OM), pH, and moisture were identified as crucial in reducing HM bioavailability. Similarly, in beef manure composting, OM and temperature were found to be significant physicochemical elements affecting HM bioavailability. Throughout the thermophilic phase of chicken manure composting, the bioavailable concentration of cadmium (Cd) decreased, possibly due to temperature sensitivity or enhanced microbial activity at higher temperatures, leading to the release of metabolites. Quorum sensing mechanisms may induce the production of extracellular polymeric substances (EPS), which sequester HMs and reduce their bioavailability. Binding to OM can increase the residual proportion of HMs, while the degradation of OM promotes the production of humic substances (HS) that bind HMs. Additionally, high pH levels can limit the mobility of HMs, leading to precipitation and passivation.

Attributed reduced bioavailable Cd levels to high pH conditions. However, aging a 40-day animal manure compost for 50-365 days did not yield significant benefits in reducing HM bioavailability, as reported by Chen et al. (2019a, b). Shen et al. (2016) suggested that 20 days was the optimal composting duration for achieving passivation of HMs (such as copper (Cu) and lead (Pb)) during a 50-day composting period. These findings underscore the complex interplay between physicochemical factors, microbial communities, and composting duration in influencing HM bioavailability in composting systems.

4. Toxicity Conversion in Heavy Metals

Various techniques are enforced for efficiently reduction of toxic level in heavy metal generated from various sources for sustainable practices. Several processed techniques are reflected in formulating the toxicity of heavy metal, vermicomposting (Fig. 1) and (Fig. 2) is one of the bioprocessing method reviewed and focused for reduction of toxic content in heavy metal.

4.1 Role of earthworms on heavy metal conversion

In the domain of terrestrial ecology, the term "earthworms" encompasses a diverse array of over 1,800 species of worms classified under the class Oligochaeta within the phylum Annelida. Notably, members of the genus *Lumbricus*, among others, hold particular significance within this classification. These creatures, commonly known as angleworms, play pivotal roles in soil ecosystems and have garnered substantial attention in ecological research due to their ecological significance and widespread distribution. Ghosal et.al (2024) In ecological literature, earthworms are categorized into three primary eco-physiological groups based on their habitat preferences and behaviour such as epigeic earthworms, endogenic earthworms, Anecic earthworms, subsurface burrowing earthworms etc. Wang et.al. (2022). Earthworms serve as integral contributors to terrestrial ecosystem functioning and play a pivotal role in maintaining soil fertility.

In the 1990s, Nepal witnessed the introduction and subsequent widespread adoption of vermicomposting technology. *Eisenia fetida*, *Lumbricus rubellus*, *Perionyx excavatus*, *Lampiti mauritii*, and *Eudrilus eugeniae* emerged as the most commonly utilized earthworm species for this purpose. This transition towards vermicomposting reflected a departure from the conventional support on chemical pesticides and fertilizers for enhancing food production. Not only are these petrochemical-based inputs often unavailable, but they also pose significant environmental and health risks. In contrast, vermicompost emerged as a sustainable alternative, offering an exceptionally nutrient-rich organic fertilizer (Fig.1). When incorporated into soil, vermicompost enriches it with a high NPK (Nitrogen, Phosphorus, and Potassium) ratio,

as demonstrated by research such as Sande et al. (2024). This organic fertilizer not only sustains agricultural productivity but also promotes soil fertility, thereby contributing to long-term agricultural sustainability and environmental health. Utilizing earthworms in the vermicomposting process represents an effective method for composting organic waste. Earthworms play a crucial role in breaking down and homogenizing organic matter, thereby indirectly increasing the surface area available for microbial activity. The symbiotic relationship between earthworms and aerobic microorganisms accelerates the biodegradation of organic waste, facilitating the conversion process. Furthermore; the vermicast produced by earthworms contain a rich array of beneficial bacteria that have traversed their digestive tracts, as documented by Kaur et.al (2020).

The accumulation of metals in earthworms is quantified using the Bioaccumulation Factor (BAF), with heavy metal accumulation being influenced by various factors such as earthworm species (Fig.1), developmental stage (adults), overall metal concentration, pH, and organic carbon content, as discussed by Suleiman et al. (2017).

In their seminal work, provide a comprehensive examination of BCFs for inorganic compounds in earthworms, shedding light on the mechanisms underlying heavy metal uptake in these organisms. Among the key composting earthworm species, *Eisenia fetida*, *Lumbricus mauritii*, and *Perionyx excavatus*, investigations into the concentration of heavy metals in both their tissues and the resulting vermicompost have been pivotal reported by Singh et.al (2018). These studies aim to ascertain whether vermicomposting can effectively reduce heavy metal concentrations in the final product. The focal point of such investigations lies in evaluating the concentrations of heavy metals, including lead (Pb), cadmium (Cd), copper (Cu), and zinc (Zn), in both earthworms and vermicompost at the conclusion of experiments reported by Pegu et.al (2024). Utilizing BCFs, researchers, as exemplified by Xie et.al (2020), calculate the amount of metal accumulated by composting earthworms, providing insights into the efficacy of vermicomposting as a means of heavy metal remediation.

Allison et al. (2020) (Table – 3) reported that the *Pheretima alexandri* sp earthworms in associaton with *Pseudomonas oxalaticus* aid to deposit oxalate for soil fertility comprises various components essential for their physiological functions. Notably, they contain 76% protein, with specific amino acids suc as glutamic acid, tyrosine, lysine, and hydroxyproline present in significant proportions have tendency to reduce the heavy metal effects.. Raval et.al (2023) reported the conversion of heavy metal of various concentration fly ash by using *Eisenia fetida* Ozturk et al. (2020) (Table – 3) in vermicomposting bed that influence the morphological and cytological characteristics of *Ricinus Cummunis L*. In another report Satapathy et.al (2021) achieved in conversion of heavy metal content of various concentration fly ash by using *Eudrilus eugeniae* that effect on growth of *Oryza sativa*.

Suleiman et al. (2017) further elucidated that, compared to *Eisenia fetida* and *Eisenia Andrei*, *Dendrobaena veneta* exhibits lower tolerance to the harmful effects of heavy metals in composted sewage sludge. This finding underscores the differential responses of earthworm species to metal contamination. Additionally, despite the inability of earthworms to regulate metal intake, they are known to display dietary selection behaviours, suggesting potential avenues for mitigating metal uptake.



Fig.1 Schematic Representation of Vermicomposting Process and Its Environmental and Agricultural Benefit (Vukovic, 2021)

4.2. Effects of Vermicomposting on Heavy Metal Dynamics

Vermicompost, an organic amendment abundant in nutrients, is derived from the collaborative efforts of earthworms and microorganisms in decomposing organic waste. While microorganisms predominantly drive the decomposition process, earthworms play a vital role in vermicomposting. Through the aeration and fragmentation of organic matter, earthworms indirectly enhance microbial biomass and activity. This augmentation in microbial activity results in an expanded surface area accessible to microorganisms, thereby influencing the composition and structure of microbial communities involved in the vermicomposting process, as discussed by Devi et.al (2022).

Directly, Wong et al. (2020) and Selvi, et.al. (2023) (Table – 3) explained by Vermicompost influences plant growth by producing hormones and enzymes that regulate various physiological processes essential for plant development. Indirectly, it contributes to plant health by mitigating the impacts of pests, nematodes, and plant diseases, thereby reducing yield losses. These dual mechanisms underscore the multifaceted role of vermicompost in promoting soil fertility and supporting sustainable agriculture practices, as elucidated by Oyege et.al. (2023).

Vermicompost and its by-products offer sustainable and eco-friendly solutions for improving production and managing pests in grain crops such as maize, wheat, barley, rice, and pearl millet. These organic amendments significantly enhance soil quality, increase nutrient availability, and boost crop productivity reported by Edwards et al. (2022) (Table -3). Vermicompost, characterized by its nutrient-rich, peat-like composition boasting a low C: N ratio and high porosity, serves as a potent soil amendment with diverse benefits for plant growth and development Satapathy et al. (2021).

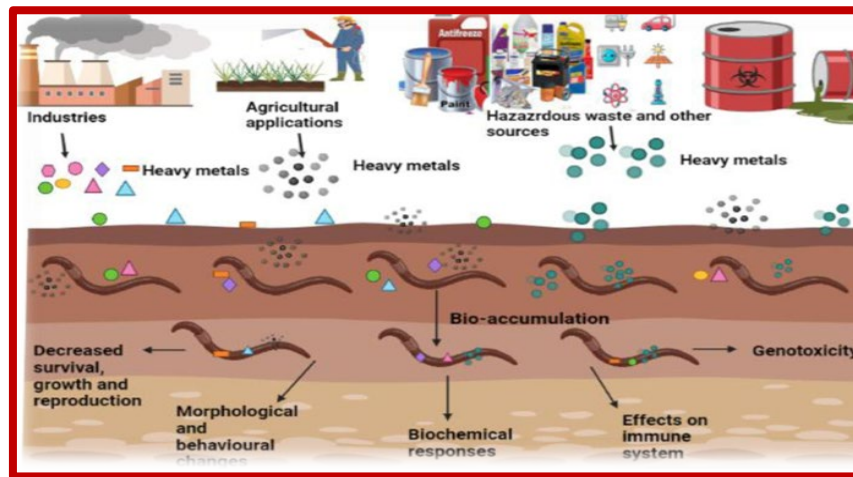


Fig. 2 Emission of Heavy Metals from Various Sources and role of Earthworms on it (Renu,2023)

4.4 Microbial Influence on heavy metal during vermicomposting

Heavy metals exert detrimental effects on microorganisms by binding to proteins and disrupting enzymatic function, thereby impairing microbial viability. Notably, the oligodynamic nature of heavy metals confers potent antibacterial activity against *Staphylococcus aureus* even at low concentrations, as reported on *Eisenia fetida* by Eghomwanre, et al. (2016) and Chowdhury et al. (2023) (Table -3). This characteristic underscores the significant ecological implications of heavy metal contamination in soil environments. The rod-shaped bacterium *C. metallidurans* thrives in heavy metal-enriched soils, highlighting its adaptation to environments rich in these toxic substances. Furthermore, certain minerals present in soil undergo decomposition over time, *Microscolex dubius* associated with bacteria *R. meliloti* L5-30R leading to fix nitrogen with increase root nodulation Suresh et al. (2022) (Table-3) and release of hydrogen and hazardous heavy metals into the atmosphere, as documented by Fashola, M.O., et al. (2016).

The proliferation of bacteria experiences a decline with increasing concentrations of heavy metals, reflecting their toxic impact on microbial growth. Furthermore, research has elucidated the diverse defense mechanisms adopted by different microbial species to counter the toxicity of specific heavy metals and suppress the activity of plant pathogen Pathma et al. (2019) (Table-3) . Consequently, the effects of each heavy metal vary significantly depending on the microbial species under investigation, as documented by Ameen et.al (2021). This discrepancy in lead's impact on microbial growth may be attributed to bacteria's ability to mitigate lead toxicity by depositing it as a phosphate salt using membrane transport pumps and intra- and extracellular binding mechanisms, These findings corroborate the conclusions drawn by Longhi et.al(2022). And other researchers, indicating that while lead may not pose a significant hazard to microbes compared to other heavy metals, its toxic effects on microbial growth should not be overlooked.

Microbial activity plays a pivotal role in the breakdown of organic matter (OM) during composting processes, essential for humification and the overall composting efficacy. Moreover, microbes exhibit the capacity to sorb, transform, or attenuate heavy metals (HMs), thereby reducing their bioavailability and toxicity within composting environments Guo et al. (2022) elucidate that the negatively charged bacterial cell wall facilitates electrostatic binding with positively charged metal ions, thereby enhancing metal absorption. This phenomenon, known as biosorption, involves various mechanisms including adsorption, chelation, ion exchange, complexation, precipitation, or bioaccumulation. The interaction between HMs and bacterial cell walls is influenced by both the characteristics of the cell wall and the surrounding environment. Understanding these microbial traits and their interaction with heavy metals is crucial for optimizing composting processes and mitigating heavy metal contamination in composted materials Aryal et.al. (2021). The phenomenon of biosorption extends to dead microbial cells, allowing for heavy metal (HM) binding irrespective of cellular metabolic activity. In contrast, bioaccumulation involves the intrusion of HMs through the cell wall into the

cytoplasm, where they may undergo localization, precipitation, complexation, or transformation processes. Extracellular polymeric substances (EPS) play a crucial role in enhancing HM biosorption by bacteria, owing to their absorbent properties. EPS, composed mainly of proteins and polysaccharides, feature functional groups such as phenolic, phosphoryl, carboxyl, sulfhydryl, and hydroxyl, capable of chelating HMs, as highlighted by Cui et al. (2021b).

Additionally, EPS comprises phospholipids, lipoproteins, lipopolysaccharides (LPS), lipids, humic acid (HA), nucleic acids, and uranic acid. LPS, particularly in gram-negative bacteria, serves as a potent chelator, binding HMs and interacting with membrane proteins and functional groups on peptidoglycan. In gram-positive bacteria, LPS, phospholipids, and lipoproteins on the cell wall may exert a similar effect, potentially leading to stronger HM interactions. Microbial enzymes play a vital role in detoxifying and converting HMs, subsequently facilitating their elimination via efflux pumps. Through contact with microbial metabolites and extracellular enzymes, HMs can undergo conversion into less harmful chemicals as reported by Cui et al. (2021b). This comprehensive understanding of microbial-mediated processes in HM interaction underscores their potential for environmental remediation and waste management strategies.

Bacterial enzymes, including reductases, play a crucial role in the transformation of heavy metals (HMs) into less harmful forms, either directly or indirectly. Additionally, Singh et al. (2020) examined by microbes possess the capability to bio mineralize HMs, converting them into stable mineral forms such as Fe-Mn oxides, phosphates, and carbonates. Research conducted by Leśniańska et al. (2022) revealed that composting processes can elevate zinc (Zn) levels in Fe-Mn oxides. Furthermore, earthworms contribute significantly to HM mitigation by absorbing and converting heavy metals within their gut and intestines, leading to passivation and reduced pollution, as documented by Swati and Hait et al. (2017). Earthworms also play a vital role in enhancing compost humification and microbial activity, thereby facilitating HM passivation. Moreover, earthworms can effectively reduce HM levels in compost through bioaccumulation, thereby minimizing HM contamination.

This review paper highlights the profound impact of heavy metal toxicity on microbial physiology and underscores the need for further research to elucidate the underlying mechanisms governing microbial responses to heavy metal stress.

Table: 3 Earthworm Species, Associated Bacteria, and Their Beneficial Traits

SL NO	Earthworm Species	Associated Bacteria	Beneficial Traits	References
1	<i>Pheretima</i> sp., <i>Pheretima alexandri</i>	<i>Pseudomonas oxalaticus</i>	Oxalate degradation	Allison et al. (2020)
2	Unspecified	<i>Rhizobium trifolii</i>	Nitrogen fixation and growth of leguminous plants	Edwards et al. (2022)
3	<i>Lumbricus rubellus</i>	<i>R. japonicum</i> , <i>P. putida</i>	Plant growth promotion	Wong et al. (2020)
4	<i>L. terrestris</i>	<i>Bradyrhizobium japonicum</i>	Improved distribution of nodules on soybean roots	Selvi et al. (2023)
5	<i>Aporrectodea trapezoids</i> , <i>A. rosea</i>	<i>P. corrugata</i> 214QR	Suppresses <i>Gaeumannomyces graminis</i> in wheat	Pathma et al. (2019)

SL NO	Earthworm Species	Associated Bacteria	Beneficial Traits	References
6	<i>A.trapezoids</i> , <i>Microscolex dubius</i>	<i>R. meliloti</i> L5-30R	Increased root nodulation and nitrogen fixation in legumes	Suresh et al. (2022)
7	<i>Eisenia foetida</i>	<i>Bacillus</i> spp., <i>B. megaterium</i> , <i>B. faecalis</i> DSM2570, <i>Staphylococcus</i> , <i>B. pumilus</i> , <i>B. subtilis</i>	Antimicrobial activity against <i>Staphylococcus aureus</i>	Chowdhury et al. (2023)
8	<i>L. terrestris</i>	Fluorescent pseudomonads	Suppresses <i>Fusarium oxysporum</i> f. sp. <i>asparagi</i> and other pathogens in various crops	Elmeret al. (2016)
9	<i>Eudrilus</i> sp.	Filamentous actinomycetes, free-living nitrogen-fixers (<i>Azospirillum</i> , <i>Azotobacter</i> , etc.), phosphate solubilizers	Plant growth promotion through nitrification, phosphate solubilization, and disease suppression	Singh et al. (2020)
10	<i>E. foetida</i>	<i>Proteobacteria</i> , <i>Bacteroidetes</i> , <i>Verrucomicrobia</i> , <i>Actinobacteria</i> , <i>Firmicutes</i>	Anti-fungal activity against <i>Colletotrichum coccodes</i> , <i>R. solani</i> , <i>P. ultimum</i> , <i>P. capsici</i> , <i>F. moliniiforme</i>	Ozturk et al. (2020)
11	Unspecified	<i>Eiseniicola composti</i> YC06271T	Antagonistic activity against <i>F. moniliforme</i>	Selvi et al. (2023)

Table 4 - Effects of Heavy Metals on Different Earthworm Species: A Summary of Toxicological Studies

SL NO	Earthworm Species	Heavy metals	Effects	References
1	<i>Eudrilus eugeniae</i>	Pb and Ni	Ni and Pb have dose-dependent detrimental effects on earthworm survival, growth and reproduction	Renu et al. (2020)
2	<i>Eisenia fetida</i>	Pb	No effect on survival but growth was inhibited	Zaltauskaite et al. (2020)
3	<i>Eisenia fetida</i>	Cd	No mortality	Liu et al. (2020)

4	<i>Dendrobaena octaedra</i>	Ni, Pb and Hg	Significant decline in survival and reproduction	Bindesbol et al. (2009)
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Eudrilus eugeniae is highly sensitive to heavy metals such as lead (Pb) and nickel (Ni). Renu et al. (2020) reported dose-dependent toxic effects of these metals, resulting in significant reductions in survival, growth, and reproduction. *Eisenia fetida* exhibits a degree of tolerance to Pb contamination, with no observed effects on survival. However, growth is significantly inhibited, indicating sub-lethal toxicity. Zaltauskaite et al. (2020) demonstrated that while this species can withstand Pb exposure, its reduced growth highlights potential ecological risks. For cadmium (Cd) exposure, *Eisenia fetida* shows resilience, with no recorded mortality under tested conditions. Liu et al. (2020) emphasized the species' potential as a bioindicator for Cd contamination, as it tolerates Cd while still reflecting sub-lethal impacts on soil health. *Dendrobaena octaedra* is particularly sensitive to heavy metals such as Ni, Pb, and mercury (Hg). Bindesbol et al. (2009) observed significant declines in survival and reproduction, highlighting the species' vulnerability and its potential use as a bioindicator in assessing soil contamination and ecosystem health.

4.5 Transform of heavy metals contents during vermicomposting

During the vermicomposting process, two key processes, namely bioaccumulation by earthworms and volume reduction due to organic decomposition, influence heavy metal concentrations. As organic matter undergoes mineralization and breakdown, heavy metal levels may concentrate and escalate as a result the weight and volume reduce during vermicomposting. Research conducted by Mekersi et al. (2024) supports this phenomenon, highlighting the potential for heavy metal accumulation during vermicomposting processes (Fig.3). This review paper emphasizes the importance of understanding the dynamics of heavy metal concentrations in vermicompost and the need for effective management strategies to mitigate heavy metal pollution while harnessing the benefits of vermicomposting for organic waste management and soil improvement.

In contrast to conventional composting, vermicomposting introduces a dynamic interaction between microorganisms and earthworms, significantly complicating the dynamics of heavy metal evolution within the system (Fig.3).. The inclusion of earthworms inherently elevates the complexity of heavy metal dynamics during vermicomposting. Firstly; the observed increase in heavy metal concentrations within earthworm bodies' and reduction of toxicity in the content of heavy metal by increasing nitrogen, phosphorous, potassium Satapathy et.al.,(2021).

Moreover, the interaction between earthworms and microorganisms during mineralization and humification processes also influences heavy metal transformation during vermicomposting. Changes in pH, electro-conductivity, organic matter content, and humic acid levels affect the mobility and speciation of heavy metals. Overall, the accumulation of heavy metals by earthworms and alterations in Physico-chemical characteristics of substrates play pivotal roles in heavy metal transformation during vermicomposting, ultimately reducing their mobility and availability.

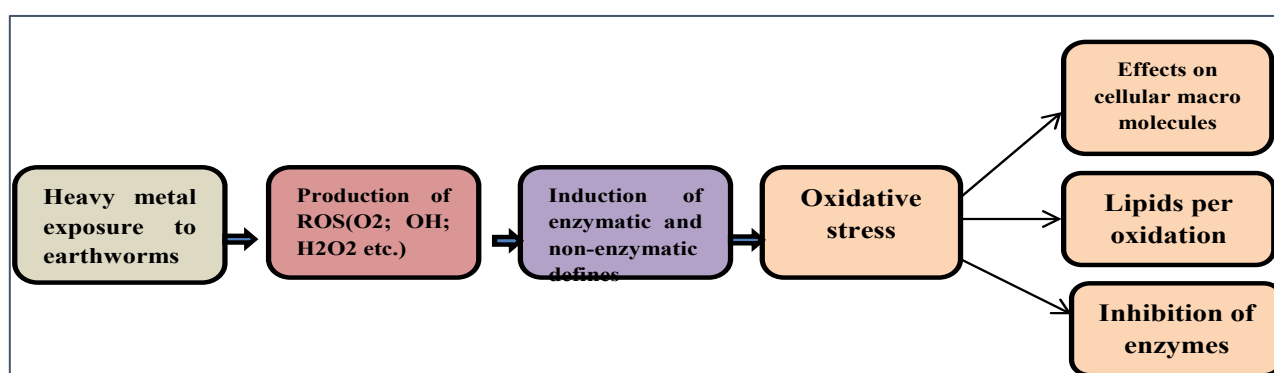


Fig. 3 Biochemical Responses of Earthworms on Heavy Metal conversion (Renu,2023)

5. Role of converted heavy metal into vermicompost

Vermicompost, a product of earthworm-mediated decomposition of organic matter, holds immense potential as a soil conditioner. This paper explores the diverse ways in which Vermicompost enhances soil texture, aeration, water retention capacity, and overall biological, physical, and chemical qualities. Oyege et.al (2023).

5.1. Soil Texture Enhancement: Vermicompost contributes to the improvement of soil texture by fostering soil aggregation and reducing compaction. The organic compounds secreted by earthworms promote the formation of stable soil aggregates, soil porosity facilitating better root penetration and nutrient distribution (Fig. 4). Aeration Improvement the presence of earthworms in Vermicompost aids in soil aeration by burrowing, creating channels that enhance gas exchange and microbial activity. Improved soil aeration supports root respiration, nutrient cycling, and overall soil health. Vuković et.al (2021).

5.2. Enhanced Water Retention Capacity: Vermicompost increases soil water retention capacity by enhancing soil structure and organic matter content. Humic substances and polysaccharides present in Vermicompost act as natural hydrogels, improving soil moisture retention and mitigating drought stress (Fig. 4).. Vermicompost enriches soil with diverse microbial communities, including beneficial bacteria, fungi, and actinomycetes. These microorganisms play crucial roles in nutrient cycling, disease suppression, and plant growth promotion, thereby enhancing soil fertility and ecosystem resilience. In addition to soil structure improvement, Vermicompost reduces soil erosion, compaction, and surface crusting. These physical enhancements contribute to better soil tilth, facilitating sustainable land management practices and promoting soil health. Khan, et.al (2024).

5.3. Chemical Properties: Vermicompost enriches soil with essential nutrients such as nitrogen, phosphorous and pottasium, organic matter as organic carbon, and bioactive compounds, enhancing soil fertility and plant nutrition. The presence of humic substances improves nutrient retention and availability, supporting optimal nutrient uptake by plant. Aransiola et.al (2024).

Vermicompost serves as a versatile soil conditioner, offering a multitude of benefits for soil health and agricultural productivity. By improving soil texture, aeration, water retention capacity, and fostering biological, physical, and chemical qualities, Vermicompost plays a crucial role in sustainable soil management practices. Its widespread adoption can contribute significantly to mitigating environmental degradation and promoting resilient agricultural ecosystems. Oyege et.al (2023).

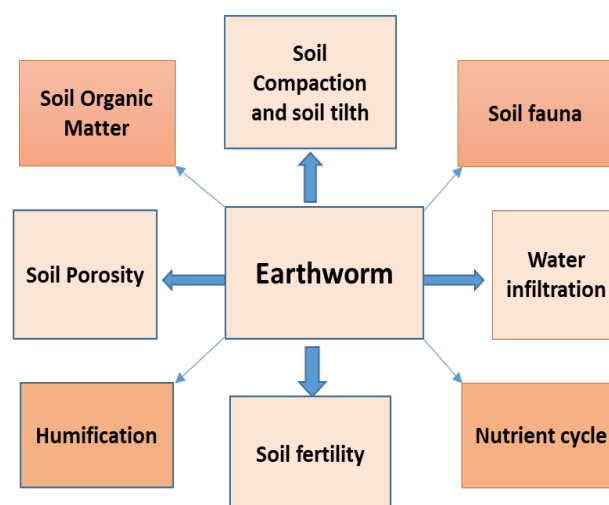


Fig. 4 Impact of Earthworms on Soil Property Enhancement

6. Impact of earthworms on soil properties containing heavy metals

Earthworms are integral to soil ecosystems, playing a crucial role in improving soil structure through their burrowing activities and influencing various soil properties essential for enhancing soil richness and primary production (Hallam and Hodson, 2020; Kumar et al., 2020). Their profound impact on soil's chemical and physical qualities, including nutrient cycling, soil aeration, moisture regulation, and overall structure, underscores their significance in ecosystem functioning (Fig.4) and (Fig. 5)

The burrowing activities of earthworms are instrumental in forming macro pores in soil, which enhance soil aeration, water infiltration, and root penetration, ultimately fostering nutrient uptake by plants and supporting primary production. Moreover, earthworms play a crucial role in nutrient cycling by consuming organic matter and releasing nutrient-rich casts, thereby facilitating decomposition and nutrient redistribution in soil ecosystems. Their activities also influence soil properties such as organic carbon content, pH levels, and the mobility of heavy metals, which have implications for plant growth and environmental quality. In addition to their role in nutrient cycling and soil structuring, earthworms contribute to trash decomposition, accelerating the breakdown of organic matter and facilitating nutrient release. Vásquez et.al (2023).

Heavy metals pose a significant threat to earthworm populations, with implications for soil fertility and ecosystem functioning. Liu et al. (2020) investigated the effects of cadmium (Cd) on leaf litter breakdown and soil fertility, highlighting the indirect impact of heavy metals on soil qualities through their effects on earthworm activity. The inhibition of leaf litter breakdown and reduction in soil fertility attributed to Cd exposure underscore the importance of understanding the interactions between heavy metals, earthworms, and soil properties. Cd not only affects leaf litter breakdown but also influences the physical and chemical qualities of soil through earthworm activities, as demonstrated by Liu et al. (2020).

7. Mechanisms of heavy metals passivation in compost

7.1. Improved humification

During composting, heavy metals interact with humic substances, leading to the formation of organo-metal complexes. These complexes are crucial in immobilizing heavy metals and reducing their bioavailability in composted materials. Studies by De Souza et al. (2019) and Song et al. (2021) have provided insights into the mechanisms of organo-metal complex formation during composting. Microbial-derived fatty acids, produced during composting, play a significant role in binding heavy metals. These fatty acids interact with heavy metals by breaking down lignocellulose molecules, thereby reducing their mobility and toxicity in composted materials. Research by Cui et al. (2020) has elucidated the mechanisms underlying heavy metal binding by microbial-derived fatty acids. Microbial processes are essential for heavy metal passivation in composting, influencing the fate and behaviour of heavy metals in composted materials. Understanding these processes is critical for optimizing composting practices and enhancing heavy metal passivation. Further research is needed to explore the intricate interactions between microbes, organic matter, and heavy metals in composting systems.

Humification influences the physicochemical properties of compost, which subsequently affects the redistribution and speciation of heavy metals. The high pH of compost promotes the passivation of heavy metals (HMs) and their transformation into residual fractions, as HMs become less mobile at higher or alkaline pH levels, according to Vandecasteele et al. (2023), Shen et al. (2016), and Guo et al. (2022). Humic acid (HA) has been shown to have a positive correlation with the passivation of heavy metals (Cu, Cd, Zn, and Pb), as reported by Li et al. (2021a, b), and

Pinto et al. (2020). Additionally, various humic substances (HS) have demonstrated a relationship with heavy metal passivation, as noted by Zhang et al. (2018) and Cao et al. (2022). Total phosphorus (TP) is also positively correlated with the passivation of certain heavy metals, including Pb, Zn, and Cd, as reported by Guo et al. (2022) and Xu et al. (2022). Fulvic acid (FA), humus, and organic matter (OM) have significant effects on certain heavy metals, such as Cu, Zn, Cd, and Pb, as reported by Pinto et al. (2020), and Li et al. (2019a, b, c).

Through electrostatic interaction, CEC enhances HM charcoal sorption and influences HM speciation by (Jain et al., 2019; Liu et al., 2017). Phosphate enhances the conversion of heavy metals (HMs) into stable compounds, primarily zinc, copper, and lead. Kulikowska and Gusiatin (2019) reported that Fulvic acid (FA), which is abundant in compost feedstock, is transformed into more stable humic acid (HA). The HA contains active functional groups, such as hydroxyl, hydroxyl quinone, carboxyl, and quinone, which enhance the adsorption and complexation of heavy metals (HMs). HM passivation results from a decrease in the C: N ratio combined with the breakdown of OM. Positively charged metal ions can be bound by negatively charged OM. The high temperatures during the thermophilic phase of composting boost microbial activity, which accelerates humification and promotes the passivation of heavy metals (HMs). Therefore, temperature plays a critical role in compost humification.



Fig. 5 Enhancing Humification: The Role of Composting Additives in Reducing Heavy Metal Bioavailability (Ejileugha, 2024)

7.2. Mechanisms of thermophilic phase

The thermophilic phase of composting is essential as it drives microbial activity, which plays a key role in humification (Fig.6). This phase is strongly influenced by high nitrogen (N) levels and low carbon (C) content. To ensure the timely onset of the thermophilic phase (Fig.6), it's important to maintain an appropriate C:N ratio and adequate porosity at the start of composting, since high carbon levels and low porosity can delay its initiation, as reported by Wu et al. (2017). The correct amount of bulking agents is added to create favourable conditions for composting. Key factors that influence the success of composting and the quality of the final product include the peak temperature during the thermophilic phase, the duration of this phase, and the time required to reach it. These factors are critical as they regulate microbial activity, shape the microbial community, affect compost maturity, determine the overall composting duration, and help eliminate harmful bacteria. Research by Xu et al. (2022) has also shown that leaching of heavy metals like Cd, Pb, Ni, and Cr can reduce their overall concentration after composting (Fig. 5).. Heavy metal (HM) leaching is most pronounced at elevated temperatures. During this phase, the high rate of microbial activity leads to increased production of microbial metabolites and enzymatic activities, which can interact directly or indirectly with HM ions, either reducing them or

generating compounds with varying levels of stability (Fig. 6). Microbes and their by-products can produce exopolymeric substances (EPS), metabolites, and enzymes, as well as utilize mechanisms like efflux pumps and cell wall interactions to sequester, immobilize, inactivate, complex, chelate, absorb, or precipitate heavy metals.

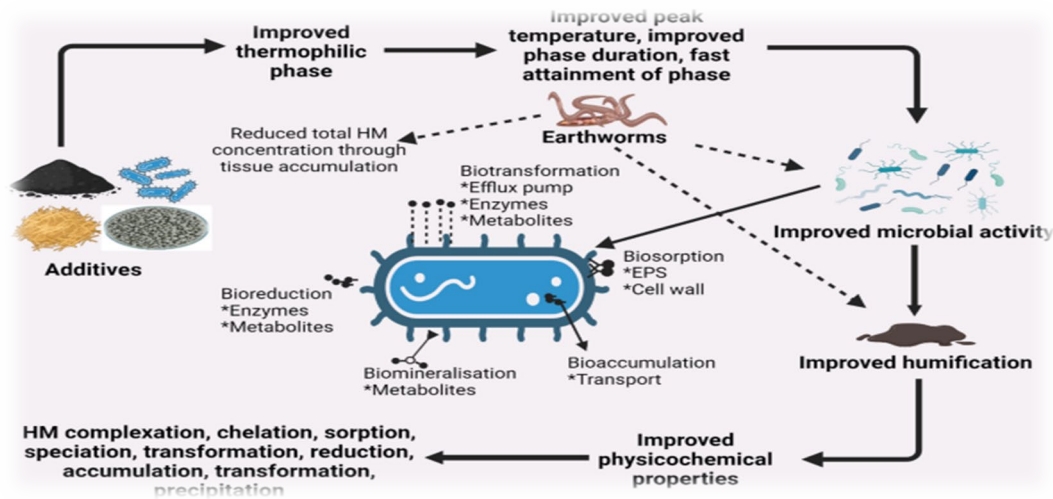


Fig.6. Role of Additives in Composting: Promoting Heavy Metal Passivation and Bioavailability Reduction (Ejileugha, 2024)

8. CONCLUSIONS

In conclusion, the exploration of selective earthworm species in microbial-mediated heavy metal conversion highlights a promising avenue for advancing environmental bioremediation. This review emphasizes that certain earthworm species, due to their unique interactions with soil microbiota, play a pivotal role in enhancing the transformation and detoxification of heavy metals. These earthworms facilitate microbial activity through their feeding habits, burrowing, and the enhancement of soil structure, which in turn improves the efficiency of microbial processes responsible for heavy metal conversion. The implications of these results are significant for bioremediation strategies. By harnessing specific earthworm species that positively influence microbial communities involved in heavy metal detoxification. In future we can develop more targeted and effective bioremediation approaches such as industrial waste alongwith organic wastes. This could lead to the restoration of contaminated soils and sediments more efficiently, reducing environmental and health risks associated with heavy metal pollution. Future research should focus on identifying and characterizing the specific microbial strains and metabolic pathways involved in these processes, as well as evaluating the long-term stability and efficacy of earthworm-mediated bioremediation integrating and interacting with microbes in diverse environmental settings. However, this research predominantly focus on the mechanism of conversion of heavy metal with the activation of earthworm through gut analysis ,microbial culture and later with antimicrobial property and molecular analysis to be carried out from vermicompost. Additionally, integrating these findings with practical applications, such as the design of engineered bioremediation systems, could further enhance the utility of earthworms in sustainable environmental management.

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