



THE EFFICIENCY OF EARTHWORMS AS A BIOMARKER FOR ENVIRONMENTAL POLLUTION

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Abstract: Pollution from various sources is currently regarded as one of the most severe issues everywhere. However, certain biological indicators can detect a variety of contaminants, and each one is used to identify a specific pollutant or a group of pollutants. The use of molecular and biochemical biomarkers is one of the ecotoxicological approaches in assessing pollutant bioavailability and sublethal effects. One of the most rapidly growing fields in ecotoxicological research is biomarkers in soil invertebrates, such as earthworms. Due to the widespread use of biocides and fertilizers in agriculture, industrial operations, urban trash, and air deposition, soil contamination has risen in recent decades. Earthworms serve as an excellent bio indicator for monitoring soil pollution. There are a variety of biomarkers of toxic substances in earthworm ecotoxicology. The present review is focused on the use of earthworms as a biomarker to analyze the impact of toxicological substances.

Keywords: Biomarkers, Bio indicators, Earthworms, Ecotoxicology, Environmental pollution.

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INTRODUCTION

Environmental pollution has become a critical public health concern since it is a leading cause of health risk and is associated with a variety of serious diseases all over the world (Briggs, 2003; Balwan and Saba, 2021). Chemical fertilizer application is one of the most widely used practices in today's developing intensive agriculture. However, the long-term use of chemical fertilizers has resulted in several unintended effects. Soil is an important

component of mineral, organic, and faunal substances. The soil fauna, which are the primary consumers and decomposers of the soil ecosystem, play a major role in the management of soil quality, *i.e.* the fertility and functioning of tropical ecosystems (Handrix, 2000). Soil pollution reduces soil fertility, alters soil structure, disrupts the balance of flora and fauna in the soil, contaminates crops, and pollutes groundwater, causing serious damage to living beings.



Anatomy, physiology, and biochemistry responses of an organism that indicate susceptibility or consequences of a source of stress are referred to as biomarkers. Biomarkers are used to analyze the impact of various pollutants on the terrestrial environment as a sensitive, 'early warning' technique. Biomarkers that are sensitive to both pollutant bioavailability and early biological reactions are good biomarkers. The identification of genuine biomarkers and the construction of process-level links between biomarkers and higher-level responses are critical to biomarkers efficacy in assisting ecological risk assessment (Adams, 2003). To examine the biological health of the soil, many soil macrofauna has been used as bioindicators.

Earthworms belong to class Oligochaeta of phylum Annelida. Annelids are the first true coelomates appeared during course of evolution (Verma and Prakash, 2020). The earthworms act as a significant biological component of soil and are often referred to be 'ecosystem engineers', as they help to improve the soil structure and fertility (Römbke *et al.*, 2005; Jouquet *et al.*, 2006). The earthworms are having antioxidant activity, and used as ecological indicator species for risk evaluation or field remediation, assessing their health (Deswal *et al.*, 2020). The earthworms are the friends of farmers as they increase the soil fertility (Verma, 2017) but their population is declined due to high surface temperature, absence of ground cover, pollution and dry soils (Wakale and Kulkarni, 2021). For toxicity, testing earthworms are considered as the most important terrestrial model organisms.

Earthworms serve as a significant indicator for potential pollutants harming the ecosystem in ecotoxicology risk assessments, and they also serve as an early warning system in monitoring changes caused by pollution (Zaghloul *et al.*, 2020). The international authorities and scientific community are becoming more concerned about soil pollution monitoring and evaluation as a result of increasing concerns about chemical contamination of soil. The standard method of evaluating soil pollution,

which involves the analyzing pollutant concentrations in the soil and comparing them to specific threshold values, does not reveal whether contaminants have harmful effects on the biota or not.

When compared to other terrestrial invertebrates, earthworms have a set of ecological and physiological characteristics which make them excellent bioindicators of soil pollution (Lanno *et al.*, 2004). Biomarkers are used to determine the exposure level and sublethal effects of pollutants in the ecological risk assessment of pollution (Rodriguez-Castellanos and Sanchez-Hernandez, 2007).

EARTHWORMS AS BIOINDICATOR ORGANISMS

Pollution of the terrestrial ecosystem is a major environmental issue that affects people all over the world. A domain soil organism is an earthworm. To evaluate soil contaminations in acute toxicity, test species have been suggested. Pollution has a substantial effect on earthworm density and biomass. Earthworms act as a suitable bioindicator species for monitoring soil pollution. Soil pollutants are directly exposed to earthworms via their epidermis and alimentary surfaces. They can accumulate a wide range of organic as well as inorganic pollutants from the soil (Morrison *et al.*, 2000).

Earthworms are abundant invertebrate species in soil and are widely used in the field as bio indicators of soil quality. Earthworm species such as *Eisenia fetida* are considered as the most common species for estimating pollution levels in the environment and also represent as standard test organism used in terrestrial ecotoxicology studies. Earthworms can accumulate heavy metals such as Pb, Cu, Cd, and Hg in their bodies without affecting them, and hence can act as a bio indicator in dumpsite ecosystems (Bamgbose *et al.*, 2005). Earthworms are exposed to high level of chemicals in their terrestrial environment affect their growth, reproduction, and behaviors (Ali and Naaz, 2013). Due to its rapid life cycle and efficiency of laboratory culture, *Eisenia fetida* is the primary testing organism used in terrestrial ecotoxicology (Lionetto *et al.*, 2012).

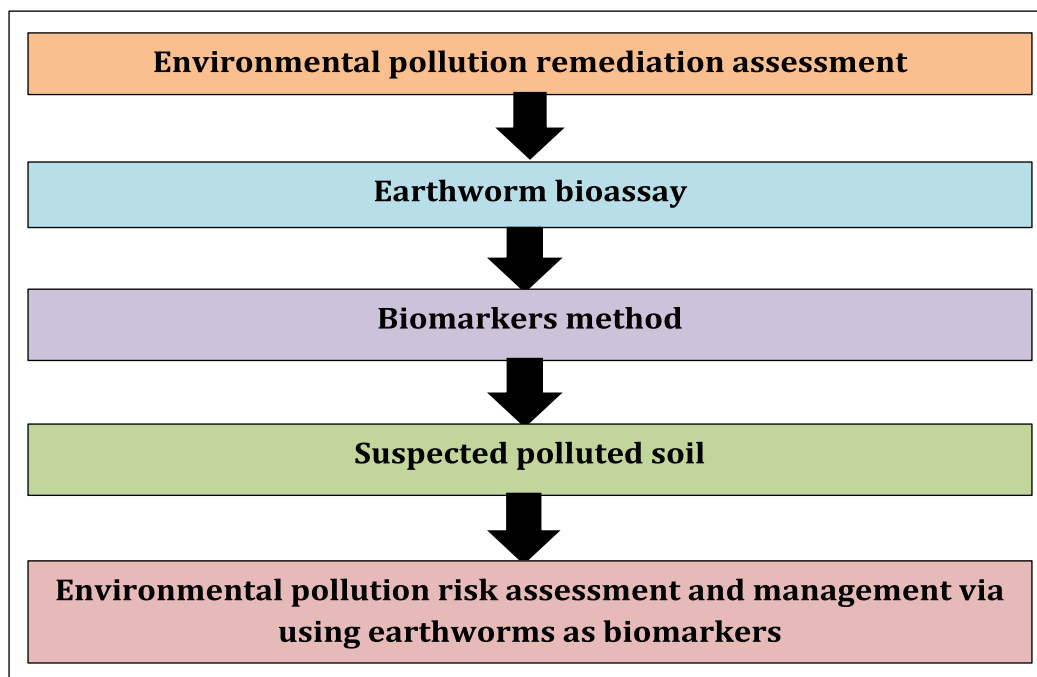


Figure 1: Uses of earthworms biomarkers in pollution management.

BIOMARKERS

Biomarkers are biochemical, cellular or molecular alterations or physiological changes in an organism's cells, body fluid, tissues, or organs that indicate xenobiotic exposure or effect (Lam and Gray, 2003). Biomarkers are usually classified into two broad categories such as biomarkers of exposure, and biomarkers of effect (Chambers *et al.*, 2002). Biomarkers can be utilized as early warning indicators of biological effects as well as quantitative assessments of chemical exposures and biologically effective concentrations (NRC, 1987; Depledge *et al.*, 1995).

The limitations of the traditional approach to environmental toxicology are one of the main reasons for the present interest in biomarkers (Scott-Fordsmand and Weeks, 2000). By exclusively assessing the bioactive portion of contaminants, biomarkers address the issue of toxicity. Biomarkers are biological agents that are used to indicate the condition of a biological system. Weber *et al.* (2012) reported that the biomarker is a biological agent whose concentration indicates whether a disease is present, severe, or progressing. Environmental biomarker responses can be used to measure the influence of chemicals, such as pesticides, and therefore can be utilized as rapid and sensitive indicators of organism harm (Mazzia *et al.*, 2018).

BIOMARKERS IN EARTHWORMS

Earthworms can be found in a wide range of soil types and horizons, so they are divided into three ecological groups: epigeic species that live on the surface and in the litter (e.g., *Eisenia fetida*), endogeic species that live in the organic horizons, and dig horizontal burrows (e.g., *Aporrectodea caliginosa*), and anecic species living in vertical and deep burrows and consuming excessive amounts of soil (e.g., *Lumbricus terrestris*) (Rodriguez-Castellanos and Sanchez-Hernandez, 2007). The biochemical biomarkers are used for the analysis of pollutant toxicity, metabolization, and detoxification in earthworms, which can then be used for the identification and assessment of contaminants that influence environmental changes (Denoyelle *et al.*, 2007; Reinecke and Reinecke, 2007; Gastaldi *et al.*, 2007).

The use of earthworm biomarkers to assess the effect of pollutants on soil organisms is becoming more and more significant gradually (Fig. 1). The acetylcholinesterase, metallothionein, biotransformation enzymes, and antioxidant defenses are among the most commonly utilized biomarkers because of their important roles in neurocholinergic transmission and cell homeostasis, which protects cells from chemical toxicity (Novais *et al.*, 2011).

As a biomarker of chemical pollution, earthworms provide society with a forecasting tool of ecosystem quality (Saint *et al.*, 1998; Muthukaruppan *et al.*, 2005). Based on their responses and behavioral characteristics, there have been established techniques for earthworm acute and sublethal chemical testing on polluted soils (OECD, 1984; van Gestel, 2012). The majority of earthworm biomarker research focuses on the impacts of specific heavy metals (e.g., Cu, Cd, Zn, or Pb), and there is some research on biomarker responses to organic pollutant exposure. In earthworms, a wide range of substances has been investigated for biomarker reactions, although each biomarker has not been subjected to a rigorous test regime. In addition, the investigation of new, powerful biomarkers in earthworms for assessing the impact of soil contaminants is progressing rapidly.

ENZYMES AS BIOMARKERS IN EARTHWORM

Enzymes of various types are utilized as biomarkers because of their vital involvement in neurocholinergic transmission and cell homeostasis, which protects cells from chemical toxicity (Novais *et al.*, 2011; Mekhalia *et al.*, 2016). Although enzymatic responses to atrazine in earthworms have been considered effective biomarkers of imminent damage and an early warning tool, the number of studies assessing enzymatic responses to atrazine in earthworms is quite low (Lammertyn *et al.*, 2021). AChE is involved in neurotransmission and muscle contraction (Hackenberger *et al.*, 2018; Wang *et al.*, 2015), and when earthworms were exposed to organophosphorus (OP) and carbamate chemicals, it was specifically evaluated. Lactate dehydrogenase (LDH), an intracellular enzyme involved in anaerobic glycolysis, has also been suggested as an enzymatic biomarker of stress exposure in earthworms (Owagboriaye *et al.*, 2020). Inhibition of AChE in earthworms is now considered as an early warning of a pesticide's harmful effect (Booth and O'Halloran, 2001).

Liu *et al.* (2010) reported that several studies indicate that the antioxidant enzymes can be stressed by either organic or inorganic contaminants, implying that they could be used as general biomarkers for detecting the effects of pollutants in terrestrial ecosystems at early stages and low concentrations. However, antioxidant

enzymes dose and time-dependent responses to pollutant exposure can be complicated, and a better understanding of their behavior under stress is required for their use in monitoring and evaluating programs. The earthworm, *Lumbricus rubellus*, has a variety of Glutathione-S-Transferase (GST), a cytosolic enzyme that is similar to those found in other taxa such as nematodes and humans, including tissue-specific isoforms, activity location, the ability to detoxify cellular toxicity products, and a potential reaction to pollution (LaCourse, 2009).

CELLULAR BIOMARKERS ON COELOMOCYTES OF EARTHWORMS

Several research studies have investigated biomarkers for subcellular changes, and they present a positive association with analyzed parameters at higher physiological levels, indicating early warning signs of environmental pollution (Maleri *et al.*, 2008; Yadav, 2016). Different contaminants can alter the integrity and functionality of earthworm coelomocytes, and their reactions can be used as biomarkers for sublethal contamination exposure (Correia *et al.*, 2021). The lysosomal membrane is the most investigated coelomocyte modification. Chemical exposure and biological consequences are measured using stability as a biomarker (Svendsen *et al.*, 1996; Maboeta *et al.*, 2002; Svendsen *et al.*, 2004). Transcriptomic investigations into earthworm coelomocytes have recently been used to identify genes whose expression varies with metal exposure (Brulle *et al.*, 2010). From an ecotoxicological perspective, earthworm's coelomic fluid has a lot of potential for the development of new cellular biomarkers. Lysosomal fragility caused by heavy metal toxicity has been identified as a possible biomarker in earthworms (Weeks and Svendsen, 1996).

BIOMARKERS OF PESTICIDE EXPOSURE

Earthworms are utilized as 'vermiremediators' to amend pesticide-contaminated soil because of their capacity to assist in the decomposition of toxic substances through metabolic and physical activities, as well as contribute to the enrichment, proliferation, and stimulation of microbial action (Morillo and Villaverde, 2017). As earthworms can change the dynamics of organic matter and

reduce pesticide mobility, the potential to reduce pesticide runoff and leaching arises (Sanchez-Hernandez *et al.*, 2019). Pesticides have negative impacts on earthworms at numerous levels of an organization, according to research, including changes in behavior, altered metabolism, and enzyme functioning, increased mortality, decreased fertility, and inhibited growth and reproduction (Pelosi *et al.*, 2013).

BIOMARKERS OF TOXIC METAL EXPOSURE

There is different toxic metal pollution that is widely dispersed in the environment and easily detected in all types of soil. When earthworms are exposed to contaminated soils, toxins accumulate in their bodies and are transferred to birds, small mammals, and other soil organisms through the terrestrial food chain (Spurgeon and Hopkin, 1996; Cotter-Howells *et al.*, 2005; Nahmani *et al.*, 2007). Earthworms such as *Lumbricus terrestris*, *Lumbricus rubellus*, *Eisenia fetida* and *Eisenia andrei* have been used in terrestrial ecotoxicology studies around the world to detect environmental pollution (OECD, 1984). Earthworms can bind heavy metals in a variety of ways. The use of earthworms as metal soil pollution biomonitors has resulted in the identification of metallothioneins (MTs) in these organisms.

CONTAMINANTS OF EMERGING CONCERNS INDUCE METALLOTHIONEIN IN EARTHWORMS

Metallothioneins (MTs) are cysteine-rich metal-binding proteins with a low molecular weight. MTs induction is a widely used biomarker in earthworms and is used as an initial biomarker of heavy metal exposure in soil monitoring. The key functions of metallothionein include trace metals homeostasis (Zn, Cu, Mn, Fe, etc.), oxidative stress protection and xenobiotic metal detoxification, metal ion transport, redox pool maintenance, and radical scavenging (Wu *et al.*, 2015; Chidinma *et al.*, 2016). The impact of metallothioneins and other biomarkers on pollution tolerance and ecosystem management is extensively recognized (Aemere *et al.*, 2020).

EARTHWORM CASTS GENERATION AS A NOVEL TOXICITY BIOMARKER

Casts are excreted by earthworms. These can be

laid in the soil to help with soil mixing, or they can be placed on top of the soil. Earthworms that dig vertical burrows typically deposit surface casts. The cast is incredibly productive, as they contain digested soil and plant matter, as well as microbial agents that continue to change nutrients. They normally include a lot of phosphate, nitrogen, and potassium (Yeates, 2008). The resulting casts display very complex microscale architecture because the stomach route leads to a fine-scale mixing of mineral and organic soil elements, as well as earthworm-derived mucus and bacteria (Vidal *et al.*, 2019). The quantity of soil consumed by the earthworms is indicated by cast production, which can be linked to the quantity ingested (feeding behavior). Cast production is simple, quick to measure, and environmentally beneficial. Capowiez *et al.* (2010) concluded that cast production appears to be a promising (i.e., ecologically relevant) indicator of ecotoxicity assessments.

CONCLUSION

Earthworm indicators are becoming more prominent for evaluating the effects of pollutants on soil organisms. DNA alterations, activation of metal-binding proteins (MTs and MBP), depression of AChE activity and other enzymatic reactions, energy reserve responses, immunological responses, behavioral abnormalities, and metal exposure (MT induction) have all been evaluated in earthworms as possible biomarkers. As a result, an improved understanding of the chemical components, modes of action, and means of degradation of pollutants in the soil are required to reduce the harmful effects on soil fauna and organisms in the food web.

CONFLICT OF INTEREST

The authors declare no conflict of interest regarding the publication.

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