Biological Forum – An International Journal **15(3): 778-783(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

An Overview on Earthworm and Soil Toxicology Studies

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(Received: 15 January 2023; Revised: 13 February 2023; Accepted: 22 February 2023; Published: 20 March 2023)
(Published by Research Trend)

ABSTRACT: Since the late 1960s, the study of soil ecotoxicology has gained popularity. There are numerous studies on the effects of various classes of pesticides on aquatic life and humans. Although there is a dearth of data in this area of ecotoxicology, adverse effects of pesticides on soil invertebrate fauna have also been studied over time. Earthworms have recently become recognized as useful model organisms in the study of soil ecotoxicology. With earthworms, it is possible to study the effects of different pesticides, contaminants in the soil, and their residues. When exposed to dangerous chemical substances, they exhibit changes in morphology, behavior, fecundity, and biochemical components. Although the toxicity of metal contaminated soils has been assessed with various bioassays, more information is needed about the biochemical responses, which may help to elucidate the mechanisms involved in metal toxicity. Previously reported that the earthworm, *Eisenia fetida*, accumulates cadmium in its seminal vesicles. The bio-accumulative ability of earthworms is well known, and thus the earthworm could be a useful living organism for the bio-monitoring of soil pollution. Here, different aspects point of view of use of earthworms in study of soil toxicology have been summarized.

Keywords: Earthworm, Ecosystem, Soil toxicity, Biomonitoring.

INTRODUCTION

Due to human activity, the environment has been exposed to contamination brought on by numerous toxic agents. Deactivated industries, contaminant leakage, pesticide use, and improper solid and liquid waste disposal are the main causes of environmental impact, such as soil, surface water, and groundwater contamination. The primary goal of environmental policies around the world is acknowledged to be soil protection and the protection of its inherent communities (Filser *et al.*, 2008).

While plant cultivation for food production is the most obvious use of soil, it also plays a role in sustainability maintenance. In addition to filtering and directing rainwater to underground aquifers, soil holds onto water and controls water resources. In addition to being the habitat for a wide range of organisms including bacteria, fungi, viruses, nematodes, insects, and worms, among others, it also plays the role of recycling raw materials and nutrients. The food that living things eat is made in the soil (Pretty and Bharucha 2014).

In addition to being the base for the majority of man-made foods, the soil also contains a lot of pollutants. Pesticides, soil amendments, chemical and organic fertilizers, and other substances are frequently responsible for polluting the air and water. Soil is a vital part of the Earth's natural resources and a crucial part of the environmental chemical cycles. The most valuable resource in the society is the quality of the soil and climate, which promote productivity (Brulsema, 2018; Drobnik *et al.*, 2018).

Ecotoxicological tests are internationally acknowledged as complementary tools to chemically analyze soil contamination. The behavior and toxicity of soil components or elements shouldn't only be evaluated using chemical criteria. The inclusion of biological parameters in these investigations should be of the utmost importance because chemical analyses separately applied to the compounds may not show their behavior in the environment (Wang *et al.*, 2018).

Terrestrial ecotoxicology aims to learn more about how chemicals are released into the environment and affect the organisms that live there. By observing the lethal, morphological, behavioral, physiological, cytogenetic, and biochemical effects on organisms exposed to these pollutants, it is essential to understand how hazardous the use of chemicals, alone or in mixtures, as well as where its effects are observed (Wang *et al.*, 2018; da Silva Souza *et al.*, 2014).

The macro-, meso-, and microfauna of soil contains a wide variety of organisms, including bacteria, fungi, and species that are referred to as bioindicators. These organisms identify various types of modification before environmental changes worsen and signal changes in the environment at an early stage. They also identify the types of pollution that can harm a particular ecosystem.

Additionally, they can support monitoring and decision-making for better, less disruptive soil management. Earthworms, nematodes, enchytraeids, Collembola, and other species are examples of standardized bioindicator species found all over the world. The use of bioindicators in monitoring programs enables the early detection of environmental changes or the evaluation of the efficacy of environmental quality improvement measures (da Silva Souza *et al.,* 2014).

More and more details are becoming available regarding the poisonous effects of chemical pesticides on fauna that lives in soil. The pesticide residues left behind in the soil are washed away when water from agricultural fields is discharged into groundwater systems and rivers. These chemical pesticide residues are also exposed in this way to aquatic life and humans. Although there is a dearth of data in this area of ecotoxicology, adverse effects of pesticides on soil invertebrate fauna have also been studied over time. In the recent times, earthworms have emerged as good model organisms in the field of soil ecotoxicology. Effect of various pesticides/soil contaminants and their residues can be effectively studied with earthworms. They show changes in morphology, behavior, fecundity, biochemical constituents when exposed to harmful chemical substances. Here, different aspects point of view of use of earthworms in study of soil ecotoxicology have been described in this review.

PHYSICAL AND CHEMICAL INFLUENCES ON SOIL TOXICITY

The aforementioned physical and chemical parameters can markedly affect the bioavailability of contaminants to soil dwelling or other exposed organisms. Bioavailability is defined as the physicochemical access that a toxicant has to the biological processes of an organism.[9] The less the bioavailability of a toxicant, the less its toxic effect on an organism. For example, the partitioning of metals to soil may reduce their availability for mobilization and uptake by microbes, plants, and animals. Metals may become toxic to soil dwelling organisms when significant levels of free metal ions dissolved in soil porewater are available for uptake by microbes or plants. Numerous physical and chemical factors, including soil pH, organic matter, and chemical form of the element in the environment affect the potential for metal ionization and availability. In addition, animals also can be exposed to soil-bound contaminants by ingestion (Allen, 2002). Toxicity tests are a useful means to assess the bioavailability of chemicals in soil.

SOIL CONTAMINATION

One of the most significant environmental problems in the world is soil contamination (Filser *et al.,* 2008). Since ancient times, there has been concern over the unintentional use of the soil for industrial waste disposal, waste disposal, and agricultural activities. Such inappropriate discharge results in significant environmental harm in addition to a large quantity of contaminated soils that are unusable for construction sites or for farming (Petrik *et al.,* 2018).

The need to control weeds and pests in agriculture has increased the use of pesticides. Many agricultural products contain potentially polluting substances in addition to their active toxic ingredients, including trace elements and emulsifying surfactants (Liu *et al.,* 2018; Xing *et al.,* 2018). Sewage sludge, industrial waste (which is made up of urban waste), and agricultural waste are all being used more frequently for recycling or disposal. These residues have high contents of organic matter and mineral elements capable of improving the chemical, physical, and biological properties of the soil. However, these residues may contain trace elements, pathogens, and many other substances that cause environmental damages (Xing *et al.,* 2018; Melo *et al.,* 2018).

When toxic chemicals from chemical classes like inorganic ions (metals), organic solvents, radioactive materials, pharmaceuticals, polycyclic aromatic hydrocarbons (PAHs), and pesticides (herbicide, insecticide, and fungicide) are present, it is referred to as "soil contamination (Oga *et al.,* 2014). Inorganic contaminants are referred to as "toxic elements" as per the International Union of Pure and Applied Chemistry (IUPAC). They include metal and metalloid substances formerly referred to as "heavy metals." This nomenclature includes substances that are biologically necessary to living things in low concentrations. However, these essential elements also cause imbalance and exhibit some toxicity when they are found in concentrations that are higher than the necessary ones or above the advised limits.

Processes associated with the chemical properties of substances in the soil and to environmental compartments control the displacement of water, soil, and air contaminants as well as the interface between various compartments. Due to its heterogeneity, soil is one of the environment's most complicated matrices. There are many different particle sizes and ecophysiological traits present in different types of soil (Sparks, 1995).

Water diffuses contaminants into the lithosphere by passing through spaces between soil particles. Contaminant displacement speed is influenced by soil properties like humidity, clay type, specific area, cation exchange capacity, pH, redox potential, temperature, porosity, and permeability as well as molecular weight and the concentration of the contaminant gradient. Inherent characteristics of soil contamination include the cumulative nature and low mobility of pollutants. However, because these factors facilitate the solubility of toxic elements in the environment, this mobility increases in soil recording relatively low pH or significant amounts of sand at the expense of clay. As a result, the soil is more vulnerable to water leaching into subterranean sheets and other bodies of water. Therefore, whether a specific substance is persistent and whether it is potentially hazardous to soil compartment depends on interactions between the chemical structure of contaminants, soil properties, and entry mode in the environment (Sparks, 1995; Baver *et al.,* 1972).

Due to the characteristics of the soil and the crucial role that its biota plays in maintaining physical-chemical equilibrium, the soil must be viewed as a living entity. The soil becomes contaminated as a result of unfavorable changes in the existing equilibria, which create circumstances that make life in the assessed area difficult or impossible. Additionally, they cause environmental harm that may not be repaired for millennia.

EARTHWORMS

Earthworms are highly liable to changes in ecological factors, particularly those essential to the soil, and earthworm performance can, therefore, reflect soil contamination. Most of the world's cereal crops grow from seeds treated with insecticides and fungicides (Bruhl *et al.*, 2011). Pesticides and herbicides are now an essential component of agricultural technology and production in the modern world (Stephan *et al.*, 2011; Pimentel *et al.*, 2011). Today's food contains a mixture of prohibited and regulated chemicals like DDT, benzene hexachloride (BHC), aldrin, dieldrin, lindane, and others that cause disease and functional disorder (Das *et al.*, 2003). Pesticide use in Indian agriculture increased from 90 g (a.i.)/ha when the Green Revolution began in 1965–1966 to about 467 g (a.i.)/ha by the end of the 20th century (Peshin and Zhang 2014). Pesticide use per unit area is the highest on cotton (*Gossypium* sp.), followed by vegetables and rice (*Oryza sativa* L.) with insecticides constituting 56% of the total pesticide utilized (Peshin, 2014).

The faecal pellets or castings released by the earthworms nourish the soil with many important nutrients. They cause bio enrichment of the soil, by increasing the nutrient content of the soil, especially, nitrogen, phosphorus, and potassium. Earthworms play important role in cycling of nitrogen through their pellets/casts, particularly in shifting agriculture (Bhadauria and Ramakrishnan 1996). Earthworms are responsible for pedogenesis (soil formation), humus consumption as well as its formation, they also ingest smaller pebbles, as rocks are often encountered in their gizzard, by doing so they aid in the weathering of rocks. Ingestion of tiny rocks is done to assist in the digestive process. Due to their repeated tilling and worm casting habit, earthworms also play a key role in the differentiation of the soil strata. Earthworms thus are involved in the biogenic transfer of sediment material altering the stratigraphy and chemical profile of the soil which in turn causes change in the sediment size and porosity of the soil, this process is known as bioturbation. The soil structure is determined by the arrangement of sediment particles and by porosity. Earthworms are involved in pedogenesis by causing bioturbation of the soil (Cunha, 2016), and they do this by both compacting and decompacting. All of these are significant processes for maintenance of soil health and thus play an enormous role in soil ecology and agronomy (Fig. 1) (Feller *et al.*, 2003).

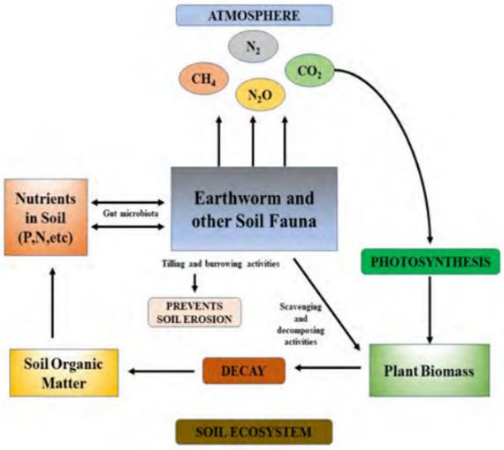

Fig. 1. Showing influence of earthworms in the ecosystem.

Earthworms reside at different depths in the soil, according to the strata of the soil they are part of, they are classified into epigeic worms (reside in the top layer of soil, basically foraging on the dead and decaying plant matter, these worms show coloration in the body as they are constantly exposed to the sun. These worms feed on the dead and decaying leaf litter and play important role in both consumption and formation of humus), endogeic worms (reside in deeper layers of soil, they make horizontal tunnels and actively aerate the soil, as they rarely venture out of the tunnels, thus remain unexposed to the sun, the body is light in colour, their casting and tunneling habit, helps in growth of plant roots) and anecic worms (they are long worms which build vertical tunnels and they do venture out of their tunnels at night and forage on the surface, they are dorsally coloured and ventrally light, they release their casting on the surface of the soil and also line the tunnels with it, aiding to the fertility of the soil). The process of bioturbation and pedogenesis varies with the ecophysiological categories of earthworms found in a particular soil. As the epigeic worms largely remain on the surface and in the leaf litter, they are unable to dig deep into the soil, their role in creating porosity and moving sediment particles is low. The endogeics on the other hand are deep soil dwelling worms, thus they create many galleries and deposit their casts in them. The anecics build huge burrow system and do not fill them as they release the casts on soil surface. The combined effect of rain, vegetation, and earthworm casting cause compaction and decompaction of soil which aids in pedogenesis (Lavelle, 1988).

A. Earthworm and Soil Toxicity Studies

Eisenia fetida, also referred to as redworm, brandling worm, or tiger worm, is the kind of earthworm that is advised for use in studies on soil toxicity because it thrives in decaying plant matter and manure. It is possible to identify any soil contamination that has taken place and determine the effects of the contamination by examining the tissues of these worms. This will enable the situation to be remedied in the most appropriate and effective way possible to avoid negative outcomes.

For many years, earthworm-based soil monitoring initiatives have regularly used the accumulation of pollutants in earthworms and their effects on mortality rate, cocoon production, and growth in earthworms (Brulsema, 2018). Although helpful, these suggested endpoints can be challenging to apply to real-world problems and do not offer information about the molecular causes of toxicity. To fully comprehend the effects of toxicants and their toxic mechanisms, it is necessary to measure specific responses to pollutants, such as oxidative stress or specifically expressed proteins, in addition to effects on survival, growth, development, and reproduction. This can be accomplished by studying the corresponding molecular biomarkers and biological responses. Furthermore, the standard parameters may not be suitable for assessing the effects of short-term or low-dose exposure to contaminants.

It would be helpful to describe the extent of their toxic effects on the environment, even at low levels, and the timeframe over which the toxicity becomes apparent, given the rising prevalence of heavy metal soil pollution in the soil. Metabolomics biomarkers at the molecular level are required; a more sensitive technique that could make this possible is now available.

Earthworms come in direct contact with many pesticides and their residues when they are released in the soil ecosystem. Exposure to pesticides can impact earthworms in various ways

(a) At individual level, they can affect the physiology or expression of genes. They can cause mortality, reduce the fecundity rate, hamper the enzymatic processes.

(b) At population level, they can alter the population density by causing behavioral changes and altering the life history traits.

(c) At community level, due to altering of the population densities of different earthworm populations, overall biomass can be affected.

Sub lethal toxicity effects of pesticides and other soil contaminants can also be studied using earthworms. *Eisenia fetida* is the most commonly used earthworm for composting and also makes a good model for study of soil ecotoxicology due to its good reproductive (Ali, 2018). Other earthworms common in soil ecotoxicology research are *Perionyx excavatus*, *Apporoctodea caliginosa*, *Lumbricus terrestris* (De Silva *et al.,* 2009).

B. Role of Earthworm on Soil Contaminants

To assess the effect of a contaminant on soil biota earthworms make a good model organism. OECD guidelines no. 207 for acute toxicity tests on earthworm are specified. For the study of avoidance behavior filter paper test is used. For long term exposure and fecundity and behavioral studies at sub lethal concentrations, the artificial soil test is done. Significant loss in weight in *Eisenia fetida* treated with sub lethal dose of methamidophos (organophosphate) was observed and effects were dose dependent and loss of the body weight is attributed to the release of body fluids under stressful conditions (Espinoza-Navarro *et al.*, 2013), and similar results were obtained when *Eisenia fetida* was exposed to parathion, sperm count and spermatic viability was also found to be negatively affected (Bustos-Obregón and Espinazo-Navarro, 2005). Exposure to different concentrations ranging from 19 to 76 mg of dichlorvos caused significant decline in growth rate of earthworms and affected the cocoon production, viable juvenile which emerged from the cocoon over hatching also declined (Farrukh and Ayesha 2011). Reduction in weight was also observed in *Eisenia fetida* exposed to a fungicide carbendazim, herbicide glyphosate and organophosphate dimethoate. Studies have been showing similar results in other earthworm species apart from *Eisenia fetida*. Endosulfan, an organophosphate pesticide which is banned worldwide, it is a chemical which has been linked to cause serious deformities in humans also, for earthworm *Apporoctodea trapezoides* it had shown significantly reduced weight at sublethal dose in juveniles also cocoon production was inhibited. A significant effect on reduction in growth and overall weight of earthworms were observed and some of the pesticides (Metlaxyl, atrazine) though they are not toxic to earthworms but still caused significant reduction in the weight of the worms, this directs the attention towards the fact that some pesticide metabolites persist in the environment much longer as compared to other pesticides and thus they can more harmful effects on the soil fauna in the long run. In our study with Aluminum phosphide (ALP) powder residue and an organophosphate, Dichlorvos (DDVP), it was observed that even at sub lethal concentrations as low as 1/3 LC_{50} value of ALP and DDVP, the cocoon production declined to less than 0.5 cocoons/worm/week which means that many worms were not even laying cocoons. The cocoon production is a parameter that is studied to assess the reproductive health of the worms. Under healthy conditions, an average of 2-5 cocoons per week is produced by the worms (Venter and Reinecke 1988). Decline in average cocoon production can point to stressful conditions the worms may be exposed to. Testing of cocoon hatchability is another parameter for testing reproductive health of the worms. Cocoons which are unhealthy maybe compressed, discolored, or would not hatch into juveniles. Healthy cocoons on the other hand hatch into a tiny juvenile worm. For *Eisenia fetida*, cocoon hatches in 9-12 days and multiple juveniles come out of the cocoon. Survivability of the juveniles is another criterion which can be studied. This reduction in fecundity is an important observation which implies that soil biota community structure can significantly change due to many contaminants and their residues which persist in soil. They may not directly cause mortality, but affect the population size by affecting reproduction.

C. Morphology and Behavioral Studies

Effects on behavior and morphology due to chronic and acute exposure to pesticides have been studied in varied earthworm species. In a study on the toxic effects of an organophosphate called profenofos on the earthworm *Eisenia fetida*, it was discovered that the worms exhibited coiling and curling, excessive body lifting indicative of stress brought on by the exposure, slow movement, and the appearance of lesions on the body over time. All over the body, coelomic fluid was released from dorsal pores, and prolonged exposure caused constriction and the development of swelling near the clitellar region (Reddy and Rao 2008). Both the herbicide paraquat and the organophosphate dichlorvos had similar behavioral effects, including skin discoloration, sluggish movement, a significant impact on the worms' tunneling behavior, and an increased propensity to coil (Ogunwole *et al.*, 2018). Lampito mauritii, after exposure to monocrotophos, showed curling behavior and random movement. At exposure to increasing concentrations of pesticide chlorpyrifos, earthworm *Eisenia fetida* showed degradation of the softer tissues of the prostomium, there were several cracks in the cuticular lining and with increasing concentration autotomy of the hinder segments was observed. At lower concentrations of azodrin, excessive secretion of mucous from the body was observed apart from coiling and sluggish movements but at higher concentrations there was extrusion of coelomic fluid which caused development of bloody lesions and culminated in autolysis of the hinder part of the body. The ALP powder residue at sub lethal dose was observed that worms showed excessive coiling and coelomic fluid extrusion (Yadav *et al.*, 2019).

D. Biochemical Studies

Estimation of acetylcholinesterase (AChE) activity is considered an important parameter for studying toxicity in earthworms related to heavy metals and pesticides, especially organophosphates. Earthworm, *Eisenia fetida* reared in an artificial soil treated with azodrin was tested for AChE activity. It was found that the inhibition of AChE happened in a concentration dependent manner, in the initial days of exposure at low concentrations the inhibition of the enzyme reached maximum 31 percent at the highest concentration of azodrin, but after long term exposure to azodrin concentrations for period of 14 days, the inhibition was up to 90 percent (Rao and Kavitha 2004). Change in the protein, carbohydrates, lipid, phenolic content are also good parameters for estimation of effects caused by different pesticides and contaminants on earthworms.

E. Oxidative Stress Studies

Oxidative stress induced by many pesticides have been studied in different species of earthworm. Many bioassays are available to assess the stress. The level of reactive oxygen species (ROS) can be assessed and along with it many enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), glutathione reductase (GST), malondialdehyde (MDA) etc. In one study the effect of clothianidin (a very novel and effective neonicotinoid pesticide), was studied on earthworm *Eisenia fetida.* With increasing concentration of the pesticides, the ROS levels also increased in earthworm, superoxide dismutase is the enzyme which removes the free radical O_2^- and causes formation of H_2O_2 and O_2. Increased in the activity of the enzymes points towards the presence of excess of free radical O_2^-. Increased levels of SOD were observed with clothianidin, which indicates the resistance shown by the worms against the harmful ROS. Over a long-term exposure, it was found that SOD levels decreased which indicate that scavenging capacity of the enzyme is only up to a certain level and after that the enzyme activity declines. Catalase and peroxidase enzymes are found in the peroxisomes and they are responsible for eliminating H_2O_2 from the cell. With increasing concentration of clothianidin, both catalase and peroxidase enzyme concentration increase. But the enzyme GST, which is a detoxifying enzyme shows a trend towards decrease in concentration as the enzyme exposure concentration increased, the enzyme also decreased the concentration of the clothianidin in earthworm. Increased level of ROS also has damaging effect on the lipid and protein content and also cause DNA damage (Liu *et al.*, 2017).

CONCLUSION AND FUTURE SCOPE

Understanding the long-term effects of various environmental pollutants and chemicals present in the soil ecosystem is the ultimate goal of soil toxicological studies. It also necessitates a deeper understanding of the mechanisms by which different chemicals and their residues are neutralized in biological systems, how they affect various metabolic pathways, and how they can affect soil-dwelling organisms at sub-lethal concentrations. While earthworms can be an important part of these studies, more research in the field of soil ecotoxicology should focus on extending the range of test organisms to include numerous ecologically significant soil fauna. The effects of various chemicals on multiple generations can also be investigated, which may reveal details about how the soil fauna adapts and changes under challenging circumstances. Furthermore, based on the information provided by earthworms, it is unclear how the degree of soil pollution should be assessed. Although numerous studies have been carried out, to our knowledge no standardized methodology has been established. It will take more research to develop a more comprehensive bio-monitoring technique that evaluates soil pollution using earthworms.

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How to cite this article: Akhila A. and Keshamma E. (2023). An Overview on Earthworm and Soil Toxicology Studies. *Biological Forum – An International Journal, 15*(3): 778-783.