Journal of Basics and Applied Sciences Research (JOBASR)
ISSN (print): 3026-9091, ISSN (online): 1597-9962
Volume 3(4) July 2025
DOI: https://dx.doi.org/10.4314/jobasr.v3i4.10

EFFECTS OF PESTICIDES ON EARTHWORM AS AN ECOSYSTEM PROVIDER

Linda Soda Baga[1*] & Francis Ofurum Arimoro[2]

[1&2]Department of Animal Biology, Federal University of Technology, P.M.B. 65, Minna, Niger State

[*]Corresponding Author Email: lindasodab@gmail.com

ABSTRACT

Pesticides, which are typically harmful to non-target soil organisms, particularly earthworms, which are essential for assessing soil fertility, are used extensively in agricultural expansion to solve the issue of food security for the growing population. Agrochemicals used carelessly can cause poor survival rates, lower growth and reproduction rates, disrupt enzymatic activity, damage some tissues and ultimately a decrease in the overall biomass of earthworms yet very little is now known about the harmful effects of pesticides on these earthworms. Earthworms can come into contact with these pesticides through their skin or by feeding on contaminated soil litter. These toxins mostly go through the skin and the body wall of earthworms. Even according to agricultural recommendations for dosage and rate of application, insecticides such as Pyrethroid, Neonicotinoids and Organophosphates are extremely toxic to earthworms. The data that is currently available on how pesticides affect various earthworm species suggests that the pesticides caused changes in the way certain important enzymes functioned. These enzymes may be useful markers of the toxicity of pesticides to earthworms. Pesticides have a harmful impact on earthworm survival and weight gain, according to studies on growth and survival metrics. This results in decreased biomass production, cocoon creation, and post-hatching development of young individuals. As the concentration of pesticides increases, the biomass of earthworms decreases. Proven that pesticides may drastically lower the earthworm population in soil, this information provided may assist farmers and policymakers in developing and implementing improved farming techniques that minimize excessive pesticide contamination of the soil.

Keywords:
Enzyme,
pesticides,
oligochaetes,
soil,
earthworm

INTRODUCTION

Earthworms are terrestrial invertebrates that are members of the Order *Oligochaeta*, Class *Chaetopoda*, and Phylum *Annelida* (sometimes referred to as segmented worms). Many tropical soils and the majority of temperate soils are home to earthworms. They are separated into about 700 genera, over 7,000 species, and 23 families. They are likewise bilaterally symmetrical, segmented invertebrates that can grow up to 30 cm in length and exhibit indeterminate growth after sexual maturity. A mouth, a crop, a gizzard, an intestine, and an anus make up their digestive tract. Several pairs of hearts and blood arteries make up the circulatory system (5 in Lombricids). Their respiratory exchanges take place through their skin since they lack lungs. They are hermaphrodites, they reproduce by copulation between two individuals at the level of an external gland (the clitellum) producing the cocoons where the eggs are laid.

The tiny, lemon-shaped cocoons are typically found close to the ground's surface, although they can also be found deeper when dry circumstances prevail. Depending on the species and the habitat, there are differences in the incubation period before hatching. Earthworms that have just hatched lack pigmentation, but within a few days, they acquire the color of an adult. Under favourable conditions, they achieve maturity in several weeks. One sign of maturity is the clitellum. They eat the bacteria and fungi that grow on dead and decaying organic debris, and they are important decomposers of this material. They break down organic materials and play a significant role in recycling the nutrients they contain. They are detritivores, which are creatures that consume decomposing and dead organic debris.

How to cite this article: Linda Soda Baga and Francis Ofurum Arimoro (2025). Effects Of Pesticides On Earthworm As An Ecosystem Provider. *Journal of Basics and Applied Sciences Research*, *3*(4), 73-89. https://dx.doi.org/10.4314/jobasr.v3i4.10

Earthworms are often known as “the intestines of earth and the restoring agents of soil fertility” (Shipley, 1970). Earthworms are an essential biological component of soil and are also important in regulating the dynamics of soil matter and structure. They are important macro invertebrates of soil fauna and make up around 80% of the biomass of terrestrial invertebrates. Earthworms are essential for soil structure, microbial community activity, nutrient immobilization, nitrogen and organic matter mineralization, soil permeability, and soil characteristics (pH, organic matter, nitrogen, and granulometry), claim Kumar and Kumawat (2018). They contribute to the formation and removal of soil fragments as well as the movement of organic materials. They may have an impact on the pH, organic matter, nitrogen, and other characteristics of the soil. The soil is altered chemically and microbiologically as it passes through the earthworms' digestive tracts (Lemtiri *et al*., 2014). Due to their ease of collection, identification, and breeding, as well as their high sensitivity to environmental pollution, earthworms are excellent sentinel species for ecotoxicological studies of pesticides in terrestrial ecosystems (Owagboriaye *et al*. 2020, Wang *et al*. 2012, Chen *et al*. 2018).

Herbicides, fungicides, nematocides, rodenticides, and substances that destroy bacteria and viruses are all considered pesticides. The United States Environmental Protection Agency defines pesticides as "any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest; any substance or mixture of substances intended for use as a plant regulator, defoliant, or desiccant, or any nitrogen stabilizer" (USEPA, 2015c). According to Lanno *et al*. (2004), Nahmani *et al*. (2007), Kellogg *et al*. (2002). Pesticides are chemicals or combinations of chemicals used to stop, eliminate, deter, or lessen pests. Because they prevent pests from harming farm crops, they are utilized as crop protection. Pesticides are generally defined as chemical or biological substances (such as bacteria, viruses, antibiotics, or disinfectants) that, by their actions, deter, kill, or incapacitate pests (Miller, 2004). Any organism (plant or animal) that spreads disease, causes devastation, or is otherwise an annoyance is considered a pest. Pesticide use that is not controlled has had catastrophic effects on the environment. Overuse of pesticides has raised major issues for biodiversity and human health (Agrawal *et al*., 2010). An estimated 5.2 billion pounds of pesticides are used each year worldwide. In order to manage cockroaches, mosquitoes, rats, fleas, ticks, and other harmful insects, they are employed in homes as sprays, poisons, and powders. Agricultural fields are not the only places they are used. Inappropriate use of pesticides to boost agricultural productivity has damaged soil fauna, especially earthworms, which are affected either directly or indirectly as non-targeted organisms. The improper use of agrochemicals can lead to damage at the genetic and tissue levels, interfere with enzymatic processes, reduce the rate of survival, growth, and reproduction, and ultimately reduce the overall biomass of earthworms. When compared to other terrestrial invertebrates, earthworms are among the greatest bio-indicators of the ecosystem's relative health. There are several ways that pesticide residues get into the soil, including through industrial and agricultural processes. They can then find their way into the soil and water, reaching the habitat of earthworms, where they may harm the earthworms and reduce or even completely eradicate their population. The usage of pesticides continues to rise in spite of their negative environmental effects. The manufacturing and use of pesticides receive more attention than the negative consequences they have. There is still limited information regarding the connection between pesticide use and earthworm mortality and reduction, and little is understood about how pesticides affect earthworms. Around the world, earthworms are used as indicators for chemical evaluation and as standard test organisms in soil toxicity testing. However, little is currently known about the detrimental effects of pesticides on these organisms, despite the fact that they have been widely used to assess environmental damage from heavy metal pollution (Rodríguez-Castellanos & Sanchez-Hernandez, 2007). Pesticides may adversely affect earthworms by coming into contact with their skin or through feeding on contaminated soil litter. The earthworm mostly absorbs these chemicals through its skin and body wall. Earthworm skin is believed to be a primary conduit for the absorption of toxicants and comes into direct contact with polluted soils, according to research (Saxe *et al*. 2001, Jager *et al*. 2003, Vijver *et al*. 2003). The earthworm's epidermis and cuticle, which also contain ions, serve as its main barriers against the environment and either permit or prohibit the passage of xenobiotics into the body (Clauss, 2001).

MATERIALS AND METHODS

EARTHWORMS’ ECOLOGICAL GROUPS AND PESTICIDES EXPOSURE

Bouche (1977) and Lee (1985) divided earthworms into three main ecological groups. This classification was created based on the soil horizons—litter, topsoil, and subsoil—where earthworms were most frequently discovered. The following are the three main groups and how they are exposed to pesticides (Rashi and Satpal 2019);

Epigeic: The term "epigenetic" describes earthworm species that live on the soil's surface. They consume litter as food. Usually located in the upper 10 to 15 cm of soil, they eat the organic waste that has broken down in the litter. Because they consume litter, the species in this category are exposed to a lot of pesticides while ingesting litter. Examples includes; *Eudrilus eugenaie, Lumbricus festivus, Lumbricus friendi, Eisenia fetida, Allolobophora parva*, and *Dendrodrilus rubidsus.*

Anecic: Anecic are earthworms that dwell in topsoil and consume the soil-mixed leaf litter. They are often larger and more pigmented, and they mostly dig vertical burrows in the ground. They have strong muscles and are very active burrowers; some species can reach massive sizes of 10 to 110 cm. They dig long, subvertical tunnels that are between one and six meters long, and they mostly consume surface trash at night. They consequently consume more dirt and come into contact with pesticides when they ingest contaminated soil. Examples are; *Aporrectodea longa, Lumbricus terrestris, Lumbricus friendi, Lampito mauritii,* and *Aporrectodea nocturna.*

Endogeic: Compared to epigeic or anecic species, these earthworms take in more soil and feed on humus. They prefer to build horizontal burrows in the ground and are larger, ranging in size from 1 to 20 cm. They consume organic material that has been combined with minerals in the soil, which has already been contaminated by pesticides. Examples are; *Aporrectodea rosea, Aporrectodea caliginosa,* and *Metaphire posthuman.*

IMPORTANCE OF EARTHWORMS

Known as "ecosystem engineers," earthworms are a vital biological component of soil that greatly enhances its fertility and structure (Jouquet *et al*. 2006, R¨ombke *et al*. 2005). Because of their ecological significance, ease of collection, identification, and breeding, as well as their vulnerability to environmental contamination, they have been chosen as suitable model organisms for ecotoxicological studies of pesticide residues in terrestrial ecosystems (Owagboriaye *et al*. 2020). *Eisenia fetida* and *Eisenia andrei* have been widely used as standard test organisms for assessing pesticide risk, and methodologies have been widely established to assess their sensitivity to chemical contamination (Rico *et al*. 2016). These testing techniques have mostly been used to evaluate biomass changes, acute effects, and lethal effects for a wide range of pesticides (Wang *et al*. 2012). Additionally, due to their capabilities, earthworms are used as "vermiremediators" to promote the degradation of pesticide-contaminated soil through their physical and metabolic processes as well as by promoting the growth, enrichment, and stimulation of microbial activity (Morillo & Villaverde 2017). Additionally, they mix soil layers, aerate the soil, and infiltrate water (Pelosi *et al*., 2014). Because earthworms have the ability to alter the dynamics of organic materials and reduce pesticide mobility, there is a chance that their runoff and leaching will be lessened (Sanchez-Hernandez *et al*., 2019). According to Lin *et al*. (2019), While soil-bounded and accessible atrazine decreased more slowly than when earthworms were present, humus-fixed atrazine increased over time when they were not present. By promoting the natural microorganisms that mineralize the pesticide or adding gut atrazine-degrading plants to the soil, they aid in atrazine remediation without building up the herbicide. While there have been a number of research on earthworms' function in atrazine degradation, the most of them have concentrated on the removal of the herbicide, the characteristics of either the microbial communities or the soil (Lin *et al*. 2018, Morillo & Villaverde 2017, Neuwirthov´a *et al*. 2019). Assessing earthworm's well-being becomes crucial whether they are employed as sentinels for evaluating risks or to remediate fields. Other importance of earthworms are;

Food: In addition to eating them, early humans employed earthworms as bait for fishing and hunting. Maori in New Zealand, Aborigenes in Australia, and Native Americans all ate earthworms. Native Americans dried, smoked, and preserved earthworms for winter consumption after soaking them in water or giving them special feed to wash off intestinal debris and make them more palatable. Earthworms were acknowledged for their ethnomedical properties in China between 200 B.C. and 200 A.D., and the Divine Farmer's Materia Medica Classic made reference to them (Shen 2010). They can be cooked, fried, roasted, tossed, added to meatloaf, and used in a variety of international cuisines.

Baits: Earthworms were previously utilized as fishing and hunting baits in early populations. Charles Darwin was the first to record earthworms' significance for the decomposition of organic materials, and by the 18th century, they were being used as feedstock for domestic animals. In the United States, particularly in California, the industrial production of earthworms for use as fishing baits began in the 20th century.

Vermicomposting: Although the practice of using earthworms as vermicomposters started in the late 1930s, it never gained traction since these businesses were pyramid schemes. In the 1970s, there was a resurgence of interest in the United States, Germany, and the United Kingdom. The process of vermicomposting involves the biological degradation and stabilization of organic waste by earthworms and microorganisms (Abad and Shafiqi 2024). The earthworms accelerate the pace of mineralization, break up organic waste, and increase microbial activity. Waste is transformed into finer-structured, humus-like materials by all of these processes. When clay soil is mixed with vermicompost, the earth becomes loosened. In the end, this enhances air entry and, consequently, soil porosity (Lavelle & Spain 2001). Because the mucus connected to the cast is hydroscopic, vermicast increases the soil's ability to retain water. Because of its improved absorption ability, this also keeps soil from becoming waterlogged. The organic carbon in vermicompost releases nutrients that are good for plants gradually and consistently. While native species like *Perionyx excavatus* have demonstrated efficacy in composting earthworms in tropical or subtropical environments, epigeic earthworms such as *Eisenia fetida*, *Lumbricus rubellus, Eudrilus eugeniae,* and *Perionyx excavatus* are utilized for vermicomposting. The physical, chemical, and biological qualities of soil can all be enhanced by vermicompost. It has been shown to have a positive impact on all crops and encourage plant growth. These days, earthworms are mostly used to make vermicompost and to supply fish and farm animals with protein. Unlike other composting methods, vermicomposting does not emit unpleasant odours, which is one of its benefits. Earthworms are capable of breaking down a variety of waste materials from industrial, animal, and human activities.

Growth enhancer of plants: Earthworms are a great way to prepare the ground for plant growth. They also have positive impacts on soil, which boosts agricultural productivity and plant growth (Lalitha et al., 2000). Earthworms release auxins and cytokinins that are beneficial to plant growth.

Biomarker: A biomarker is defined as a "biochemical, cellular, physiological or behavioural variations that can be measured in tissue or body fluid samples or at the level of whole organisms, to provide evidence of exposure and/or effects from one or more contaminants" (Depledge 1994). At lower levels, such as biochemical, cellular, and physiological consequences, pollutants have an impact more quickly than at higher ones, such as ecological effects. This suggests a more sensitive early warning system for toxicological consequences in populations of earthworms. As an early indicator of negative ecological consequences, earthworm biomarkers are helpful instruments for monitoring and evaluating soil (Sanchez-Hernandez 2006, Rodriguez-Castellanos & Sanchez-Hernandez 2007). Earthworms are now useful biomarkers for determining how pollutants affect soil organisms. The biomarker technique is used to detect soil pollution.

Stimulation of microbial activity; Even though they consume microorganisms, earthworms' excrement or castings contain far more microbes than the organic material they consume. As organic matter passes through their intestines, it is broken up and inoculated with microorganisms, which facilitates the cycling of nutrients from organic materials and their transformation into forms that plants can readily absorb.

Mix and aggregate soil; As they consume organic materials and mineral particles, earthworms create waste in the form of casts, which are a type of soil aggregate. According to Charles Darwin's estimates, earthworms have the ability to move vast volumes of soil from the lower strata to the surface and carry organic items down into deeper soil layers. In ten to twenty years, earthworms can flip over the top six inches (15 cm) of soil, and they consume a lot of soil in their digestive tracts.

Increase infiltration: Earthworms increase the porosity of the soil as they move through it. Some creatures burrow underground tunnels that are rather deep. These burrows can remain in place for a long time after the tenant has died and can be very important for soil drainage, particularly when there is a lot of rainfall. Additionally, the burrows lessen surface water erosion.

Enhance water-holding capacity: Earthworms can greatly improve soil water-holding capacity by breaking down organic matter and boosting soil aggregation and porosity.

Create root growth channels: Deep-burrowing earthworms create these channels, which are lined with easily accessible nutrients and facilitate roots' ability to reach deep into the soil.

Bury and shred plant residue; As earthworms take surface debris into their tunnels plant and agricultural residue are eventually buried by cast material produced on the surface.

RESULTS AND DISCUSSION

EFFECTS OF PESTICIDES ON EARTHWORM'S BIOMASS

Earthworm populations can decrease as a result of pesticides, particularly insecticides, which can kill them directly. The concentration of the pesticide and the length of exposure tend to affect the mortality rate of earthworms exposed to it. Reinecke and Reinecke (2007a) observed the effects of organophosphate insecticides on earthworms in orchards in the Western Cape, South Africa. In contrast to nearby uncultivated fields (152 per m2) that were farther away from the orchards, earthworm concentrations in the orchards were extremely low (22 per m2). An investigation was conducted into the possibility of an impact of organophosphate pesticides on earthworms. Prior to the spraying season, background soil concentrations of chlorpyrifos were low (0.2–2.7 mg/kg), but they remained constant for up to six months beyond the last spraying event. Rainfall caused the pesticide to be carried by runoff to non-target areas. Background concentrations of azinphos methyl were higher than those of chlorpyrifos (1.6–9.8 mg/kg) but not detectable 2 weeks after the spraying event. The majority of azinphos methyl was carried to adjacent locations by wind (spray drift). The effects of chlorpyrifos on earthworms were demonstrated by microcosm research that measured changes in biomass and inhibition of cholinesterase. It was determined that both intermittent (azinphos methyl) and chronic (chlorpyrifos) exposure to the pesticides had a negative impact on earthworms. The biomarker response and biomass change of earthworms exposed to chlorpyrifos in microcosms were also studied by Reinecke and Reinecke (2007b). To do this, earthworms of the species *Aporrectodea caliginosa* were added to microcosms that were filled with soil from the same locations. In a controlled laboratory setting, they administered varying amounts of chlorpyrifos to microcosms. These quantities were picked because they were within the soils' background ranges. Over the course of five weeks, they routinely measured the earthworms' biomass and noted any earthworms in a state of estivation. In order to perform biomarker studies, earthworms were removed out of the microcosms every week for cholinesterase (ChE) inhibition assays and two weeks after the exposures began for a neutral red retention determination. They observed that earthworms exposed to the highest concentration of pesticide (8.0 mg/kg) had the most noticeable loss of biomass. Among earthworms exposed to higher exposure concentrations, esctivation was higher. ChE inhibition rose with time and higher exposure concentrations, despite the lack of a clear dose-related response. They showed a clear dose-related response with exposure concentration for the neutral red retention trial. Immediately following the second chlorpyrifos application, there was a link between biomass change and ChE inhibition. To prove that *Metaphire posthuma* is a sensitive test model for ecotoxicological investigations, Saxena *et al*. (2014) conducted an experiment. They evaluated the acute toxicity of carbaryl, carbofuran, cypermethrin, and fenvalerate on *Eisenia fetida* and *Metaphire posthuma* using bioassays for soil toxicity and contact filter paper toxicity. Of all the substances they looked at, they found that carbofuran was the most dangerous to both earthworm species. After comparing the toxicity data of these compounds for the two earthworm species, they found that *M. posthuma* was more sensitive than *E. fetida*. Acute toxicity studies showed that for *M. posthuma*, the order of pesticide toxicity was carbofuran > cypermethrin > carbaryl > fenvalerate, while for *E. fetida*, the order was carbofuran > carbaryl > fenvalerate. They observed specific morphological changes in the organisms exposed to these substances in all test methods, with *M. posthuma* exhibiting these changes more pronouncedly at lower concentrations than *E. fetida*. They concluded that for ecotoxicity studies, *M. posthuma* is a more sensitive and trustworthy model than *E. fetida*.

Xiao *et al*. (2004) examined the effects of urea and acetochlor, both separately and together, on populations of *Eisenia fetida* earthworms in phaiozem. From low concentration to high concentration, they discovered that acetochlor exhibited increased toxicity. When the concentration of acetochlor was raised from 164 to 730 mg/kg, the earthworm mortality rate after a 6-day exposure changed from 0 to 86.7%, and the weight change rate varied from 7.86 to 30.43%. They found that when the amount of urea was less than 500 mg/kg, it had positive and beneficial effects on earthworms; but, when the concentration was greater than 1000 mg/kg, it became extremely poisonous. When the concentration of urea exceeded 1500 mg/kg, the mortality rate of earthworms exposed to it reached 100%. The two agrochemicals had antagonistic effects on earthworms when the urea concentration was less than 500 mg/kg; when the urea concentration was greater than 500 mg/kg, the harmful effects of excessive urea and acetochlor on earthworms were synergistic. Regardless, the health of soil ecosystems is severely harmed by excessive urea treatment. The effects of different doses of lindane and deltamethrin on mortality, growth, and cellulase activity in earthworms (*Eisenia fetida*) cultivated in artificial soil during typical acute (14 days) and sub-chronic (42 days) exposure durations were compared in laboratory tests by Shi *et al*. (2007). They observed that the toxicity order for earthworm mortality from the 14-day exposure was lindane > deltamethrin, with median lethal concentrations (LC 50) of 162.1 and 432.9 mg/kg, respectively. Garcia-Torres *et al*. (2014) examined how *Eisenia fetida*'s cocoon viability, biomass, fecundity, and adult mortality were affected by soil exposure to glyphosate. By day seven of exposure, they discovered that 71% of *E. fetida* died at the maximum glyphosate dose (50,000 mg/kg). *E. fetida* biomass did not change between the control and 5,000 mg/kg dose at day 14, and only after 7 days of exposure to 50,000 mg/kg did it exhibit a notable reduction in weight. When the glyphosate dosage reached 5,000 mg/kg and higher, they noticed negative impacts on adult fecundity and cocoon viability.

EFFECTS OF PESTICIDES ON EARTHWORM'S WEIGHT

Earthworm weight decreases as a result of pesticide exposure, which is a major sign of physiological stress. This weight loss is linked to a number of toxic symptoms, such as sluggish movements, swelling, and coiling. The weight of earthworms can be negatively impacted by pesticide exposure. According to (Zhou *et al*., 2006), the earthworms' weight was a more sensitive indicator of the harmful impacts of methamidophos and acetochlor than their mortality. *Eisenia fetida* was treated with the organophosphate insecticide malathion by Espinoza-Navarro and Bustos-Obreg'on (2004). Weight loss has also been reported for organochlorine pesticides intoxication and for the effects of fungicides and herbicides in *Eisenia fetida* and *Lumbricus terrestris* (Helling *et al*., 2000). According to Choo and Baker (1998), when endosulfan was applied to soil at a normal application rate in both the field and the laboratory, it drastically decreased the weight of juvenile *Aporrectodea trapezoides* within 5 weeks, whereas fenamiphos did the same in the field alone. When applied at a 10× usual rate, fenamiphos and methiocarb both decreased earthworm weight in the lab. In relation to the level of intoxication and exposure duration, weight loss seems to be a useful marker of physiological stress (Frampton *et al*., 2006). Another symptom that is present in all earthworms treated with Parathion is coiling, which is associated with weight loss and is thought to be the result of a change in muscle function brought on by organophosphoric pesticides. This may help to explain the earthworms' difficulty moving around and their relative incapacity to feed themselves. According to (Xiao *et al*., 2006), growth can be considered as a sensitive parameter to assess acetochlor's toxicity to earthworms. The effects of copper oxychloride were examined in a controlled environment by Helling *et al*. (2000), while Yasmin & D'Souza (2007) examined the effects of carbendazim, glyphosate, and dimethoate *on Eisenia fetida* and discovered a notable, dose-dependent decrease in earthworm growth. *Eisenia andrei* growth was impacted by parathion. (Booth *et al*., 2000a) examined the effects of two organophosphates, chlorpyrifos and diazinon while (Mosleh *et al*., 2003a) examined the toxicity of aldicarb, cypermethrin, profenofos, chlorfluazuron, atrazine, and metalaxyl in the earthworm *Aporrectodea caliginosa* and found that all pesticide-treated worms exhibited a decreased growth rate. (Mosleh *et al*., 2003b) examined how endosulfan and aldicarb affected *Lumbricus terrestris* and proposed growth rate as a key biomarker for endosulfan and aldicarb contamination. After eight weeks, earthworms exposed to 5 mg/kg of chlorpyrifos showed negative growth effects, according to an assessment by Zhou *et al*. (2011). According to some research, earthworm growth seemed to be more negatively impacted during the juvenile stage as opposed to the adult stage (Shiping *et al*., 2008, De Silva *et al*., 2009).

EFFECTS PESTICIDES ON EARTHWORM'S REPRODUCTION

The formation of cocoons and hatchlings, the survivability of the earthworms produced, and sexual maturation are among the many reproductive characteristics that have been studied in earthworms exposed to different pesticides. According to (De Silva *et al*., 2009), cocoon hatchability was most sensitive to pentachlorophenol, parathion, and carbendazim, copper oxychloride, whilst cocoon production was the most sensitive to paraquat, fentin, benomyl, phenmedipham, carbaryl, copper oxychloride, and dieldrin. The impact of commercial parathion exposure on reproductive parameters, including sperm and cocoon production and genotoxicity on *Eisenia fetida* male germ cells, was investigated by Bustos-Obreg ´on and Goicochea (2002). They found that changes in reproductive parameters were noticeable in terms of the quantity of sperm, cocoons, and earthworms produced. According to Xiao *et al.* (2006), the number of juveniles per cocoon can be considered a sensitive metric to assess the toxicity of acetochlor on earthworms. Choo and Baker (1998) also discovered that methiocarb at 10×usual rate and endosulfan and fenamiphos at normal treatment rates inhibited cocoon development in *Aporrectodea trapezoides*. When Espinoza-Navarro and Bustos-Obreg´on (2004) applied the organophosphate pesticide malathion to *Eisenia fetida*, they discovered that it modified the DNA structure of spermatogonia, changed cell proliferation, and reduced spermatic viability in spermatheca. Malathion may have an impact on sperm count, but its metabolites may also have an impact on sperm quality. Sperm count also appears to be a highly sensitive marker. According to some experts, pesticides have a dose-dependent effect on earthworm reproduction, having greater impact at higher chemical concentrations. These effects include decreased cocoon production, a longer incubation period, and a lower mean and maximum number of hatchlings per cocoon. Even at the lowest test concentration of 0.125 mg/kg, Gupta and Saxena (2003) discovered anomalies in the sperm heads of earthworms, *Metaphire posthuma*, when they investigated the impact of carbaryl, an N-methyl carbamate insecticide, on their reproductive characteristics. Abnormalities of the wavy head were seen at 0.125 mg/kg carbaryl, while the sperm heads turned amorphous and the head nucleus became granules that were deposited inside the wavy head at 0.25 mg/kg and 0.5 mg/kg. (Xiao *et al*., 2006) proved that at field doses of 5–10 mg/kg−1, acetochlor had no lasting influence on *Eisenia fetida* reproduction. Acetochlor (20–80 mg/kg) showed sublethal toxicity to *Eisenia fetida* at higher concentrations. After eight weeks, earthworms exposed to 5 mg/kg of chlorpyrifos showed a negative impact on fertility, according to an assessment by Zhou *et al*. (2006). Cypermethrin seemed to have a more significant impact on earthworm reproduction in the juvenile stage than at the adult stage, according to Shiping *et al*. (2008). When 20 mg/kg of cypermethrin was applied, it had a major negative impact on earthworm reproduction. Coiling, which is observed in earthworms treated with parathion, also hinders reproduction because it makes it more difficult for the earthworms to locate their mate and causes abnormal mating posture during copulation. Despite a noticeable impact on sperm production during parathion treatment, as discussed by Bustos-Obreg´on and Goicochea (2002), a significant number of spermatozoa are observed in intoxicated earthworms because ejection of sperm appears to be hindered as well. Espinoza-Navarro and Bustos-Obregon (2004) claimed that malathion also has a direct cytotoxic effect that causes the sperm count to change, with increase of metachromasia of the chromatin of the spermatozoa and the tail to coil.

EFFECTS OF PESTICIDES ON EARTHWORM'S ENZYMES

Several types of enzymes are referred to be biomarkers because they are essential for neurocholinergic transport and cell stability, which prevents the harmful effects of chemicals (Sanchez-Hernandez, 2006, Novais *et al*., 2011, Mekhalia *et al*., 2016). Dimethoate is an organophosphate insecticide that has harmful effects on Eisenia kinneari's protein profile, cellular enzyme system, and testicular histomorphology (Lakhani *et al*., 2012, Dutta & Dutta 2016). Acetylcholinesterase is a crucial enzyme that is involved in the nervous system's transport process. According to Rault *et al*. (2007) and Lionetto *et al*. (2012), acetylcholinesterase (AChE) is believed to be the primary cholinesterase in earthworms. It is an important enzyme that is essential to the nervous system's transmission process. It is an important enzyme that is essential to the nervous system's transmission process. The neurotransmission that takes place at cholinergic synapses is caused by acetylcholine hydrolyzing into choline and acetate. Generally, two pesticides—organophosphorus and carbamate—can inhibit AChE activity. By covalently phosphorylating the serine debris inward the active site group, organophosphorus mostly inhibited the activity of acetyl cholinesterase (Tiwari *et al*., 2016). AChE activity has been shown to be strongly inhibited when *Eisenia fetida* is exposed to the carbamate insecticide methiocarb (Calisi *et al*., 2009).

By consuming glutathione, the cytosolic enzyme glutathione-S-transferase (GST) is essential for the detoxification and biotransformation of several electrophilic chemicals. According to Lionetto *et al*. (2012), pesticide exposure may result in changes in enzyme activity that represent metabolic disruptions and cell damage in particular tissues. According to Oruc *et al*. (2004), an increased amount of GST may provide greater defence against the harmful effects of pesticides and can therefore be utilized as a biomarker for pollution monitoring. Through isomerization processes, glutathione-dependent peroxidase activity, or enzymatic glutathione conjugation, GSTs neutralize a variety of pesticides and endogenous metabolic by-products (Hayes *et al*., 2005). Earthworm GST is sensitive to heavy metals and pesticide exposure, according to reports from many researchers (Aly & Schroder 2008, Maity *et al*., 2008, Lukkari *et al*., 2004, Saint-Denis *et al*., 2001, Booth *et al*., 2000b). With evidence of tissue-specific isoforms, activity, location, the capacity to detoxify cellularly harmful compounds, and a possible reaction to pollution, the earthworm *Lumbricus rubellus*, have a variety of GSTs that are linked to those from other species, such as nematodes and humans (LaCourse, 2009). Earthworms cellulase activity decreases when exposed to deltamethrin, but increases when exposed to lindane. These findings show that lindane has an inducing effect on earthworm biochemical metabolism while deltamethrin has a detrimental effect (Shi *et al*., 2007). The earthworms' posterior area of the stomach contains a high activity of cellulase, which aids in the digestion of complex plant polysaccharides and the production of energy. Assays of the enzyme amylase, cellulase, invertase and pectinase from the gut of *E. eugeniae* revealed that their production was reduced in the fipronil mixed with soil as compared to that of control (Salokhe *et al.*, 2014). The complex organic compounds in soil are detoxified by the carboxylesterases (CbEs), which are serine hydrolases. Earthworms secrete the CbE in the lumen of their stomach. Chlorpyrifos (OP) has been demonstrated to dramatically reduce the CbE activity in *Lumbricus terrestris* (Martinez *et al*., 2013).

EFFECTS OF PESTICIDES ON HISTOPATHOLOGY OF EARTHWORMS

Since histopathology offers valuable information about the growth, damage, and disarray of tissues, it is one of the most effective methods for assessing the effects of industrial pollutants, organic wastes, agricultural pesticides, etc., at the tissue level of an organism. Histopathological investigations may indicate a harmful impact of organisms brought on by past or ongoing exposure to harmful substances. It has been demonstrated that pesticides in soil have an impact on earthworms at the tissue level. Nutrient absorption is significantly influenced by the gut wall's epithelial cell layer. Earthworms exhibit cellular expansion and the loss of chromatin components in their nuclei with exposure to various chemicals and pesticides (Muthukaruppan *et al*. 2005). Morowati (2000) examined the histopathology of earthworms (*Pheretima elongata*) exposed to glyphosate in the field. He found that the worms' intestinal epithelial cell structure was lost, there was no cell regeneration, and there was a complete loss of chromatin from the first to the third week of exposure. However, in the fourth week of exposure, there was a noticeable cell regeneration. Muthukaruppan *et al*. (2005) investigated the sublethal toxicity of the herbicide butachlor on the intestine of the *Perionyxex cavatus* earthworm. They found that the earthworm's intestine had glandular cell enlargement after being exposed to the herbicide. They also noted that changes in the intestinal region may have a significant impact on food intake, which may in turn indirectly limit the earthworm's ability to reproduce. The impact of the organophosphorus insecticide profenofos on the histological alterations in the body wall of the earthworm *Eisenia fetida* has been studied by Reddy & Rao (2008). According to Bansiwal and Rai (2010), a sublethal dosage of the organophosphate insecticide malathion caused noticeable pathological alterations in the body wall, including a perforated cuticle and a deformation of the longitudinal muscle cells' structure. According to Oluah *et al*. (2016), the earthworm *Nsukkadeilus mbae* showed damage to its chloragogenous layer, epithelial tissues, glandular expansion of the epithelial tissues, significant vacuolations, and pylenotic cells following atrazine exposure. The muscular and intestinal epithelium of tropical earthworms *G tuberosus* and *E eugeniae* may sustain major harm from high pesticide concentrations (Nayak *et al*. 2018, Samal *et al*. 2019a).

AVOIDANCE RESPONSE OF EARTHWORMS TO PESTICIDE EXPOSURE.

Using *Eisenia fetida*, the earthworm avoidance test was created in the United States (Yeardley *et al*. 1996) and further developed by Hund-Rinke and Wiechering (2001) and Hund-*Rinke et al*. (2003). Since then, numerous studies have been carried out to observe the effects of different chemical classes on earthworms. The avoidance test is quick and simple to complete. It has a reputation for being sensitive to a variety of substances. Because avoidance studies using model soil organisms are sensitive, economical, and ecologically relevant, they are utilized as early screening methods in ecological risk assessment of contaminated areas (Natal-da-Luz *et al*. 2008). This test's basic idea is to expose the earthworms to both the control soil and the pesticide-spiked soil sample at the same time. A reference soil and a test soil are placed independently in each side of a box for the test. Following the two-day test period, the earthworms' position is established (ISO 2008). A species' propensity to steer clear of a particular study soil in favor of the contaminant-free control soil is used as an avoidance test to regulate soil quality and the impact of certain chemicals on earthworm species' behavior (ISO 2011). These tests are based on the idea that chemicals in soil can be absorbed by earthworms and are grouped into distinct factions based on the kind of soil and the degree of contamination. Because of their sensory tubercles on the surface body and chemoreceptors on their anterior segments, earthworms are able to detect a wide variety of pollutants (Reinecke *et al*. 2002). After seven days of exposure, significant avoidance responses were seen for all comparisons except the C0–C1 combination in a study by Natal-da-Luz *et al*. (2008) on avoidance tests using carbendazim. Using cypermethrin-treated soils, De Sousa *et al*. (2011) found that the earthworms' avoidance response was demonstrated by the fact that the number of earthworms in the control condition (C0) was noticeably higher than in the treated conditions. Using methomyl concentrations, Pereira *et al*. (2010) found that earthworms avoided the spiked soil in every test, and that the avoidance rate rose with increasing toxicant concentrations after 48 hours of exposure.

CONCLUSION

A few attempts have been made to provide a thorough review of the toxicity of pesticides to one of the non-target taxa, namely earthworms. Excessive use of chemicals is not only harmful to the soil quality but also affects the diversity of earthworms, and if discretion in the use of these pesticides and concrete implementation are not enforced, earthworms may soon be extinct. There is clear evidence from this review that the population of earthworms and other non-target soil biota are influenced by the use of pesticides, and the impact is extensive and causes an undesired shift in the community.

By boosting agricultural output and reducing infectious diseases, pesticides were utilized to improve human life and survival, but their negative effects on the environment and human health were disregarded. A decrease in earthworms and other helpful soil microbes was one of the negative consequences. One of the vital soil species, earthworms, is particularly vulnerable to pesticide exposure. The current review documents earthworms' vulnerability to pesticides. This review concludes that the biological parameters of earthworms can be negatively affected by the usage of pesticides. Following the application of pesticides at varying concentrations, there is a precise impact on biomass and cocoon generation. With minimal soil disturbance and the ability to be adjusted for the maximum activity of earthworms in the soil for healthy and productive soil, enough organic manures should be added in place of chemical fertilizers to lessen the effects of pesticides. Given the significance of earthworms and the need to minimize or cut back on pesticide use to maintain biodiversity and the environment, farmers should be educated about the beneficial role earthworms play in the soil.

Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding

Not applicable.

Acknowledgements

We extend our gratitude to academic and non-academic staff of the department of Animal Biology, Federal University of Technology Minna, Nigeria..

REFERENCE

Abad Q and Shafiqi (2024). Vermicompost: Significance and Benefits for Agriculture. *Journal for Research in Applied Sciences and Biotechnology* 3:10.55544/jrasb.3.2.36.202-207.

Agrawal, A., Pandey, R. S & Sharma, B (2010). Water pollution with special reference to pesticide contamination in India. *Journal of Water Resource Protection* 2: 432–448.

Aly, MAS & Schröder, P (2008). Effect of herbicides on glutathione S-transferases in the earthworm, *Eisenia fetida*. *Environmental Science and Pollution Research*, *15*(2), 143-149.

Bansiwal, K & Rai, N (2010). Assessment of malathion toxicity in certain organs of earthworm, *Eisenia fetida*. *Bioscan*, *5*(3), 473-476.

Booth, L, Heppelthwaite, V & Mcglinchy, A (2000b). The effect of environmental parameters on growth, cholinesterase activity and glutathione S-transferase activity in the earthworm (*Apporectodea caliginosa*). *Biomarkers*, *5*(1), 46-55.

Booth, LH, Heppelthwaite, V. J & O'halloran, K (2000a). Growth development and fecundity of the earthworm *Aporrectodea caliginosa* after exposure to two organophosphates. *New Zealand Plant Protection*, *53*, 221-225.
Bouché, M.B (1977). Strategies lombriciennes. *Ecological Bulletins*, 122-132.
Bustos-Obregón, E & Goicochea, R. I (2002). Pesticide soil contamination mainly affects earthworm male reproductive parameters. *Asian journal of andrology*, *4*(3), 195-200.

Calisi, A, Lionetto, M. G & Schettino, T (2009), Pollutant-induced alterations of granulocyte morphology in the earthworm *Eisenia fetida*. *Ecotoxicology and Environmental Safety*, *72*(5), 1369-1377.

Chen, J, Saleem, M, Wang, C, Liang, W & Zhang, Q (2018). Individual and combined effects of herbicide tribenuron-methyl and fungicide tebuconazole on soil *earthworm Eisenia fetida*. *Scientific reports*, *8*(1), 1-9.

Chen, J, Saleem, M, Wang, C, Liang, W & Zhang, Q (2018). Individual and combined effects of herbicide tribenuron-methyl and fungicide tebuconazole on soil earthworm *Eisenia fetida*. *Scientific reports*, *8*(1), 1-9.

Choo, L.P.D & Baker, G.H..1(998). Influence of four commonly used pesticides on the survival, growth, and reproduction of the earthworm *Aporrectodea trapezoides* (Lumbricidae). *Australian Journal of Agricultural Research*, *49*(8), 1297-1303.

Clauss, W.G, (2001). Epithelial transport and osmoregulation in annelids. *Canadian journal of zoology*, *79*(2), 192-203.

De Silva, P.M.C & van Gestel, C.A (2009). Comparative sensitivity of *Eisenia andrei* and *Perionyx excavatus* in earthworm avoidance tests using two soil types in the tropics. *Chemosphere*, *77*(11), 1609-1613.

De Sousa, A. P. A., & De Andréa, M. M. (2011). Earthworm (*Eisenia andrei*) avoidance of soils treated with cypermethrin. *Sensors*, 11(12), 11056-11063.

Depledge, M.H. (1994). The rational basis for the use of biomarkers as ecotoxicological tools. *In Nondestructive Biomarkers in Vertebrates*, M.C., Fossi, C., Leonzio, (Eds.), pp. 271– 295, Lewis Publisher, ISBN 978-0873716482 Boca Raton, USA

Dutta, A & Dutta, H (2016). Some insights on the effect of pesticides on earthworms. *International Research Journal of Environment Sciences*, *5*(4), 61-66.

Espinoza-Navarro, O & Bustos-Obregón, E (2004). Sublethal doses of malathion alter male reproductive parameters of *Eisenia fetida*. *International Journal of Morphology*, *22*(4), 297-302.

Frampton, G.K, Jänsch, S, Scott-Fordsmand, J.J, Römbke, J & Van den Brink, P.J (2006). Effects of pesticides on soil invertebrates in laboratory studies: a review and analysis using species sensitivity distributions. *Environmental Toxicology and Chemistry: An International Journal*, *25*(9), 2480-2489.

García-Torres, T, Giuffré, L, Romaniuk, R, Ríos, R.P & Pagano, E.A (2014). Exposure assessment to glyphosate of two species of annelids. *Bulletin of environmental contamination and toxicology*, *93*(2), 209-214.

Gupta, S.K & Saxena, P.N (2003). Carbaryl-induced behavioural and reproductive abnormalities in the earthworm *Metaphire posthuma*: a sensitive model. *Alternatives to laboratory Animals*, *31*(6), 587-593.

Hayes, J.D, Flanagan, J.U & Jowsey, I.R (2005). Glutathione transferases. *Annual review of pharmacology and toxicology*, *45*(1), 51-88.

Helling, B, Reinecke, S.A & Reinecke, A.J (2000). Effects of the fungicide copper oxychloride on the growth and reproduction of *Eisenia fetida* (Oligochaeta). *Ecotoxicology and environmental safety*, *46*(1), 108-116.

Hund-Rinke, K & Wiechering, H (2001). Earthworm avoidance test for soil assessments. *Journal of Soils and Sediments*, *1*(1), 15-20.

Hund-Rinke, K, Achazi, R, Römbke, J & Warnecke, D (2003). Avoidance test with *Eisenia fetida* as indicator for the habitat function of soils: Results of a laboratory comparison test. *Journal of soils and Sediments*, *3*(1), 7-12.

International Organization for Standardization ISO (2008). Soil quality-Avoidance test for determining the quality of soils and effects of chemicals on behaviour - Part 1: Test with earthworms (*Eisenia fetida* and *Eisenia andrei*). ISO 17512–1, *International Organization for Standardization*, Geneva, Switzerland.

International Organization for Standardization ISO (2011). Soil quality-Avoidance test for determining the quality of soils and effects of chemicals on behaviour - Part 2: Test with collembolans (*Folsomia candida*). ISO 17512–2, *International Organization for Standardization*, Geneva, Switzerland.

Jager, T, Fleuren, R.H, Hogendoorn, E.A & De Korte, G (2003). Elucidating the routes of exposure for organic chemicals in the earthworm, *Eisenia andrei* (Oligochaeta). *Environmental science & technology*, *37*(15), 3399-3404.

Jouquet, P, Dauber, J, Lagerlöf, J, Lavelle, P & Lepage, M (2006). Soil invertebrates as ecosystem engineers: intended and accidental effects on soil and feedback loops. *Applied soil ecology*, *32*(2), 153-164.

Kellogg, R.L, Nehring, R.F, Grube, A, Goss, D.W & Plotkin, S (2002). Environmental indicators of pesticide leaching and runoff from farm fields. In *Agricultural productivity* (pp. 213-256). Springer, Boston, MA.

Kumar, K & Kumawat, P (2018). A review on the effect of herbicides on the earthworms. *International Journal of Zoology Studies*, *3*(2), 120-125.

LaCourse, E.J, Hernandez-Viadel, M., Jefferies, J.R., Svendsen, C, Spurgeon, D.J., Barrett, J & Brophy, P.M. (2009). Glutathione transferase (GST) as a candidate molecular-based biomarker for soil toxin exposure in the earthworm *Lumbricus rubellus*. *Environmental Pollution*, *157*(8-9), 2459-2469.

Lakhani, L, Khatri, A & Choudhary, P (2012). Effect of dimethoate on testicular histomorphology of the earthworm *Eudichogaster kinneari* (Stephenson). *International Research Journal of Biological Sciences*, *1*(4), 77-80.

Lalitha, R, Fathima, K and Ismail, S.A (2000). Impact of biopesticides and microbial fertilizers on productivity and growth of *Abelmoschus esculentus*. *Vasundhara The Earth*., 1 & 2: 4-9.

Lanno, R, Wells, J, Conder, J, Bradham, K & Basta, N (2004). The bioavailability of chemicals in soil for earthworms. *Ecotoxicology and environmental safety*, *57*(1), 39-47.

Lavelle, P & Spain, A.V (2001). Soil ecology. *Soil ecology*.

Lee, K.E (1985), Earthworms: their ecology and relationships with soils and land use. *Academic Press Inc.*

Lemtiri, A, Colinet, G, Alabi, T, Cluzeau, D, Zirbes, L, Haubruge, É & Francis, F (2014). Impacts of earthworms on soil components and dynamics. A review. *Biotechnologie, Agronomie, Société et Environnement*, *18*.

Lin, Z., Zhen, Z., Liang, Y., Li, J., Yang, J., Zhong, L & Zhang, D (2019). Changes in atrazine speciation and the degradation pathway in red soil during the vermiremediation process. *Journal of hazardous materials*, *364*, 710-719.

Lin, Z., Zhen, Z., Ren, L., Yang, J., Luo, C., Zhong, L & Zhang, D (2018). Effects of two ecological earthworm species on atrazine degradation performance and bacterial community structure in red soil. *Chemosphere*, *196*, 467-475.

Lionetto, M.G., Calisi, A & Schettino, T (2012). Earthworm biomarkers as tools for soil pollution assessment. *Soil health and land use management*, *16*, 305-331.

Lukkari, T, Taavitsainen, M, Soimasuo, M, Oikari, A & Haimi, J (2004). Biomarker responses of the earthworm *Aporrectodea tuberculata* to copper and zinc exposure: differences between populations with and without earlier metal exposure. *Environmental Pollution*, *129*(3), 377-386.

Maity, S, Roy, S, Chaudhury, S & Bhattacharya, S (2008). Antioxidant responses of the earthworm *Lampito mauritii* exposed to Pb and Zn contaminated soil. *Environmental Pollution*, *151*(1), 1-7.

Martínez Morcillo, S, Yela, J.L, Capowiez, Y, Mazzia, C, Rault, M & Sanchez-Hernandez, JC (2013). Avoidance behaviour response and esterase inhibition in the earthworm, *Lumbricus terrestris*, after exposure to chlorpyrifos. *Ecotoxicology*, *22*(4), 597-607.

Mekahlia, M.N, Tine, S, Menasria, T, Amieur, H & Salhi, H (2016). In vitro biomarker responses of earthworm *Lumbricus terrestris* exposed to herbicide sekator and phosphate fertilizer. *Water, Air, & Soil Pollution*, *227*(1), 1-8.

Miller, G.T (2004). Sustaining the earth, Thompson learning. *Inc. Pacific Grove, California*, *9*, 211-216.

Morillo, E & Villaverde, J (2017). Advanced technologies for the remediation of pesticide-contaminated soils. *Science of the Total Environment*, *586*, 576-597.

Morowati, M (2000). Histochemical and histopathological study of the intestine of the earthworm (*Pheretima elongata*) exposed to a field dose of the herbicide glyphosate. *Environmentalist*, *20*(2), 105-111.

Mosleh, Y.Y., Ismail, S.M., Ahmed, M.T & Ahmed, Y.M (2003a). Comparative toxicity and biochemical responses of certain pesticides to the mature earthworm *Aporrectodea caliginosa* under laboratory conditions. *Environmental Toxicology: An International Journal*, *18*(5), 338-346.

Mosleh, Y.Y., Paris-Palacios, S, Couderchet, M & Vernet, G (2003b). Acute and sublethal effects of two insecticides on earthworms (*Lumbricus terrestris* L.) under laboratory conditions. *Environmental Toxicology: An International Journal*, *18*(1), 1-8.

Muthukaruppan, G, Janardhanan, S & Vijayalakshmi, G (2005). Sublethal Toxicity of the Herbicide Butachlor on the Earthworm *Perionyx sansibaricus* and its Histological Changes (5 pp). *Journal of Soils and Sediments*, *5*(2), 82-86.

Nahmani, J, Hodson, M.E & Black, S (2007). A review of studies performed to assess metal uptake by earthworms. *Environmental pollution*, *145*(2), 402-424.

Natal-da-Luz, T., Amorim, M.J., Römbke, J & Sousa, J.P (2008), Avoidance tests with earthworms and springtails: defining the minimum exposure time to observe a significant response. *Ecotoxicology and environmental safety*, *71*(2), 545-551.

Nayak, S., Mishra, C.S.K., Guru, B.C & Samal, S (2018). Histological anomalies and alterations in enzyme activities of the earthworm *Glyphidrillus tuberosus* exposed to high concentrations of phosphogypsum. *Environmental monitoring and assessment*, *190*(9), 1-7.

Neuwirthová, N., Trojan, M., Svobodová, M., Vašíčková, J., Šimek, Z., Hofman, J & Bielská, L (2019). Pesticide residues remaining in soils from previous growing season (s)-Can they accumulate in non-target organisms and contaminate the food web?. *Science of the Total Environment*, *646*, 1056-1062.

Novais, S.C., Gomes, S.I., Gravato, C., Guilhermino, L., De Coen, W., Soares, A.M & Amorim, M.J (2011). Reproduction and biochemical responses in *Enchytraeus albidus* (Oligochaeta) to zinc or cadmium exposures. *Environmental Pollution*, *159*(7), 1836-1843.

Oluah, N.S., Obiezue, R.N., Ochulor, A.J & Onuoha, E (2016). Toxicity and histopathological effect of atrazine (Herbicide) on the earthworm *Nsukkadrilus mbae* under laboratory conditions. *Animal Research International*, *7*, 1287-1293.

Oruc, E.O., Sevgiler, Y & Uner, N (2004). Tissue-specific oxidative stress responses in fish exposed to 2, 4-D and azinphosmethyl. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *137*(1), 43-51.

Owagboriaye, F., Dedeke, G., Bamidele, J., Aladesida, A., Isibor, P., Feyisola, R & Adeleke, M (2020). Biochemical response and vermiremediation assessment of three earthworm species (*Alma millsoni*, *Eudrilus eugeniae* and *Libyodrilus violaceus*) in soil contaminated with a glyphosate-based herbicide. *Ecological Indicators*, *108*, 105678.

Pelosi, C., Barot, S., Capowiez, Y., Hedde, M & Vandenbulcke, F (2014). Pesticides and earthworms. A review. *Agronomy for Sustainable Development*, *34*(1), 199-228.

Pereira, J.L., Antunes, S.C., Ferreira, A.C., Goncalves, F & Pereira, R (2010). Avoidance behaviour of earthworms under exposure to pesticides: is it always chemosensorial?. *Journal of Environmental Science and Health Part B*, *45*(3), 229-232.

Rashi Miglani & Satpal Singh Bisht (2019). World of earthworms with pesticides and insecticides. *Interdisciplinary Toxicology*. Volume 12(2): 71–82.

Rault, M., Mazzia, C & Capowiez, Y (2007). Tissue distribution and characterization of cholinesterase activity in six earthworm species. *Comparative Biochemistry and Physiology Part B:*

Reddy, N.C & Rao, J.V (2008). Biological response of earthworm, *Eisenia fetida* (Savigny) to an organophosphorous pesticide, profenofos. *Ecotoxicology and Environmental Safety*, *71*(2), 574-582.

Reinecke, A.J., Maboeta, M.S, Vermeulen, L.A & Reinecke, S.A (2002). Assessment of lead nitrate and mancozeb toxicity in earthworms using the avoidance response. *Bulletin of Environmental Contamination and Toxicology*, *68*(6), 779-786.

Reinecke, S.A & Reinecke, A.J (2007a). The impact of organophosphate pesticides in orchards on earthworms in the Western Cape, South Africa. *Ecotoxicology and environmental safety*, *66*(2), 244-251.

Reinecke, S.A & Reinecke, A.J (2007b). Biomarker response and biomass change of earthworms exposed to chlorpyrifos in microcosms. *Ecotoxicology and Environmental Safety*, *66*(1), 92-101.

Rico, A., Sabater, C & Castillo, M.Á (2016). Lethal and sub-lethal effects of five pesticides used in rice farming on the earthworm *Eisenia fetida*. *Ecotoxicology and Environmental Safety*, *127*, 222-229.

Rodríguez-Castellanos, L & Sanchez-Hernandez, J.C (2007). Earthworm biomarkers of pesticide contamination: current status and perspectives. *Journal of Pesticide Science*, 0710050002-0710050002.

Römbke, J., Jänsch, S., & Didden, W. (2005). The use of earthworms in ecological soil classification and assessment concepts. *Ecotoxicology and Environmental Safety*, *62*(2), 249-265.

Saint-Denis, M., Narbonne, J., Arnaud, C & Ribera, D (2001). Biochemical responses of the earthworm *Eisenia andrei* exposed to contaminated artificial soil: effects of lead acetate. *Soil Biology and Biochemistry*, *33*(3), 395-404.

Salokhe, S.G., Sonawane, H.V & Deshpande, S.G (2014). Laboratory evaluation of fipronil on biological, parameters, gut microflora and physiology of *Eudrilus eugeniae*. *International Journal of Scientific and Research Publications*, *4*, 1-6.

Samal, S., Mishra, C.S.K. & Sahoo, S. (2019). Setal-epidermal, muscular and enzymatic anomalies induced by certain agrochemicals in the earthworm *Eudrilus eugeniae* (Kinberg). *Environmental Science and Pollution Research*, *26*(8), 8039-8049.

Sanchez-Hernandez, J.C (2006). Earthworm biomarkers in ecological risk assessment. *Reviews of environmental contamination and toxicology*, 85-126.

Sanchez-Hernandez, J.C, Cares, X.A, Pérez, M.A & Del Pino, J.N (2019). Biochar increases pesticide-detoxifying carboxylesterases along earthworm burrows. *Science of the Total Environment*, *667*, 761-768.

Saxe, J.K., Impellitteri, C.A., Peijnenburg, W.J & Allen, H.E (2001). Novel model describing trace metal concentrations in the earthworm, *Eisenia andrei*. *Environmental science & technology*, *35*(22), 4522-4529.

Saxena, P.N., Gupta, S.K. & Murthy, R.C (2014). Comparative toxicity of carbaryl, carbofuran, cypermethrin and fenvalerate in *Metaphire posthuma* and *Eisenia fetida*—a possible mechanism. *Ecotoxicology and environmental safety*, *100*, 218-225.

Shen, Y (2010). Earthworms in Traditional Chinese Medicine: (Oligochaeta: Lumbricidae, Megascolecidae). *Zoology in the Middle East*, *51*(sup2), 171-173.

Shi, Y., Shi, Y., Wang, X., Lu, Y & Yan, S (2007). Comparative effects of lindane and deltamethrin on mortality, growth, and cellulase activity in earthworms (*Eisenia fetida*). *Pesticide biochemistry and physiology*, *89*(1), 31-38.

Shiping, Zhou., Changqun, Duan., Xuehua, Wang., Michelle, W.H.G., Zefen, Y.U. & Hui, F.U. (2008). Assessing cypermethrin-contaminated soil with three different earthworm test methods. *Journal of Environmental Sciences*, *20*(11), 1381-1385.

Shipley, A.E. (1970). In: The Cambridge Natural History. (Harmer, S. F. and Shipley, A. E. eds.). Codicote, England.

Tiwari, R.K., Singh, S., Pandey, R.S. & Sharma, B. (2016). Enzymes of earthworm as indicators of pesticide pollution in soil. *Advances in Enzyme Research*, *4*(04), 113.

United State Environmental Protection Agency (USEPA,2015). What is a Pesticide?, EPA Available at: https://www.epa.gov/minimum-risk-pesticides/what-pesticide.

Vijver, M.G., Vink, J.P., Miermans, C.J. & van Gestel, C.A(. 2003). Oral sealing using glue: a new method to distinguish between intestinal and dermal uptake of metals in earthworms. *Soil Biology and Biochemistry*, *35*(1), 125-132.

Wang, Y., Wu, S., Chen, L., Wu, C., Yu, R., Wang, Q. & Zhao, X. (2012). Toxicity assessment of 45 pesticides to the epigeic earthworm *Eisenia fetida*. *Chemosphere*, *88*(4), 484-491.

Xiao, H., Zhou, Q.X. & Liang, J.D. (2004). Single and joint effects of acetochlor and urea on earthworm *Esenia fetida* populations in phaiozem. *Environmental Geochemistry and Health*, *26*(2), 277-283.

Xiao, N., Jing, B., Ge, F. & Liu, X. (2006). The fate of herbicide acetochlor and its toxicity to *Eisenia fetida* under laboratory conditions. *Chemosphere*, *62*(8), 1366-1373.

Yasmin, S. & D'Souza, D. (2007). Effect of pesticides on the reproductive output *of Eisenia fetida*. *Bulletin of environmental contamination and toxicology*, *79*(5), 529-532.

Yeardley Jr R.B, Gast, L.C. & Lazorchak, J.M. (1996). The potential of an earthworm avoidance test for evaluation of hazardous waste sites. *Environmental Toxicology and Chemistry: An International Journal*, *15*(9), 1532-1537.

Zhou, Q.X., Zhang, Q.R. & Liang, J.D. (2006). Toxic effects of acetochlor and methamidophos on earthworm *Eisenia fetida* in phaiozem, northeast China. *Journal of Environmental Sciences*, *18*(4), 741-745.

Zhou, S., Duan, C., Michelle, W.H.G., Yang, F. & Wang, X. (2011), Individual and combined toxic effects of cypermethrin and chlorpyrifos on earthworm. *Journal of Environmental Sciences*, *23*(4), 676-680.