

Review

Assessing the Impact of Pesticides on Earthworm Health: A Comprehensive Review

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Abstract | This review consolidates recent global research on pesticide production, usage patterns, and their ecological impacts on earthworms, emphasizing their role as bioindicators of soil health. The main objective is to identify key pesticide groups and biomarkers associated with adverse effects on earthworm physiology and ecology, filling existing knowledge gaps in understanding mechanisms of toxicity. We introduce a conceptual framework linking pesticide classes to specific biochemical markers and ecological consequences, highlighting how certain pesticide groups (such as neonicotinoids and organophosphates) pose significant risks. Our synthesis reveals that sub-lethal effects, including enzyme activity disruptions, DNA damage, and reproductive impairments, are crucial indicators of environmental health. These findings advance current understanding by pinpointing sensitive biomarkers and emphasizing the importance of sustainable pesticide management strategies to mitigate ecological harm and protect terrestrial ecosystems.

Novelty Statement | This study presents a comprehensive synthesis of recent research on the effects of various pesticides on earthworm health, highlighting novel insights into the mechanisms of toxicity and ecological implications that have not been systematically reviewed before.

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Introduction

The widespread use of synthetic pesticides has greatly increased the agricultural productivity by reducing losses resulted from presence of weeds, insects, and diseases (Wang *et al.*, 2012; El-Aswad *et al.*, 2019; Gowri and Thangaraj, 2020). However, this intensification has led to declining biodiversity in farm ecosystems (Lackmann *et al.*, 2023; Fouad and Abdel-Raheem, 2024).

Pesticide droplets often settle on both pests and non-target organisms, affecting a broad range of species sharing similar traits (Yatoo *et al.*, 2022). Despite efforts to limit their spread, pesticides drift, volatilize, runoff, and penetrate soil, impacting surrounding environments (Abd-Eldaim *et al.*, 2023; Fouad *et al.*, 2025a, b; Mostafa *et al.*, 2025). These pesticides (including insecticides, herbicides, fungicides, and others) target specific pests but can harm soil and environmental life at even trace levels, posing ecological and health risks (Chen *et al.*, 2018; Velki *et al.*, 2019; Abdelkhalek *et al.*, 2024; El-Aswad *et al.*, 2024a-c; Hussein *et al.*, 2024; Ibrahim *et al.*, 2024). Most pesticides contaminate air, water, and soil, with less than 0.1% reaching intended targets, leading to accumulation in

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ecosystems and entering the food chain. Recognizing soil's vital ecological role (supporting food production, nutrient cycling, carbon storage, and biodiversity) is increasingly important. Yet, agricultural and industrial activities intensify pressure on this finite resource, especially as pesticides contaminate soil directly or via runoff (Treder *et al.*, 2020; Fouad, 2023a-e; Shamsan *et al.*, 2023). Earthworms, key soil inhabitants, are particularly vulnerable they absorb pesticides through skin contact and ingestion, depending on chemical adsorption and soil conditions (Fouad *et al.*, 2023a-b; Abdul-Malik *et al.*, 2024; El-Ossaily *et al.*, 2024; Sebaiy *et al.*, 2024a, b). Moving up to 6.3 tons of soil per hectare annually, earthworms process organic and mineral matter, influencing soil health profoundly (Mosleh *et al.*, 2003a, b). Pesticides can harm earthworms by reducing cocoon production, juvenile density, and growth, and increasing mortality and tissue damage; they also induce genetic, biochemical, and physiological stress (Zhang *et al.*, 2014a). Despite developing some defenses, earthworms remain sensitive bioindicators of soil contamination ideal for ecotoxicological assessments due to their ease of study and consistent responses (Reddy and Rao, 2008). Their burrowing and casting activities enhance soil structure, nutrient cycling, and plant growth, making them vital for sustainable agroecosystems (Lackmann *et al.*, 2023). As key ecosystem engineers, earthworms support soil fertility and help process pollutants, with their abundance reflecting soil health and contamination levels (Tiwari *et al.*, 2016). Consequently, they are frequently used to evaluate the ecotoxic effects of pesticides, highlighting the importance of understanding and mitigating chemical impacts on soil biodiversity (Fouad *et al.*, 2024a-e).

Methodology

The methodology for assessing the impact of pesticides on earthworm health in a comprehensive review involves a systematic approach to gather, evaluate, and synthesize existing research. Initially, a thorough literature search is conducted across multiple scientific databases such as PubMed, Scopus, Web of Science, and Google Scholar to identify relevant studies spanning all years. Inclusion criteria are established to select peer-reviewed articles, experimental studies, field surveys, and meta-analyses that investigate various aspects of pesticide exposure and earthworm health. Data extraction involves collecting information on pesticide types, concentrations, application methods, duration of exposure, earthworm species studied, and health parameters assessed (e.g., survival rate, reproduction, behavioral changes, physiological and biochemical markers). The review critically analyzes experimental designs, methodologies, and results to identify patterns, correlations, and disparities across different studies. Additionally, the methodology may include a quality assessment of the selected studies to

ensure reliability and validity. Synthesis of data involves qualitative and quantitative approaches, such as meta-analysis if applicable, to derive overarching conclusions about the effects of pesticides on earthworms. Overall, this methodology aims to provide a comprehensive understanding of how pesticides influence earthworm health over time and across various environmental contexts. While numerous reviews have addressed the general effects of pesticides on soil organisms, many lack a focused synthesis on earthworms, particularly in relation to emerging pesticide formulations and application methods. This review uniquely consolidates recent data on the sub-lethal and long-term impacts of various pesticide classes on earthworm physiology, behavior, and reproductive success, highlighting inconsistencies and gaps in current methodologies.

Pesticide production and consumption in the world

In 2022, global pesticide active ingredient use reached 3.7 million tons up 4% from 2021, 13% over the past decade, and three times more than in 1990. Compared to the 1990s, pesticide use has increased by 48% for insecticides, 54% for fungicides and bactericides, and 121% for herbicides. During this period, herbicides' share of total pesticide use grew from 40–50%, while insecticides decreased from 26% to 22%, and fungicides and bactericides from 25% to 22% (see Figure 1). In 2022, pesticide exports totaled approximately 6.9 million tonnes (down 1% from 2021) but their value rose by 13% to USD 48.8 billion. Between 2013 and 2022, exports increased by 50%, and their value grew by 53%. The top five pesticide-consuming countries, from most to least, are China, the United States, Argentina, Thailand, and Brazil (FAO, 2024; Yatoo *et al.*, 2022).

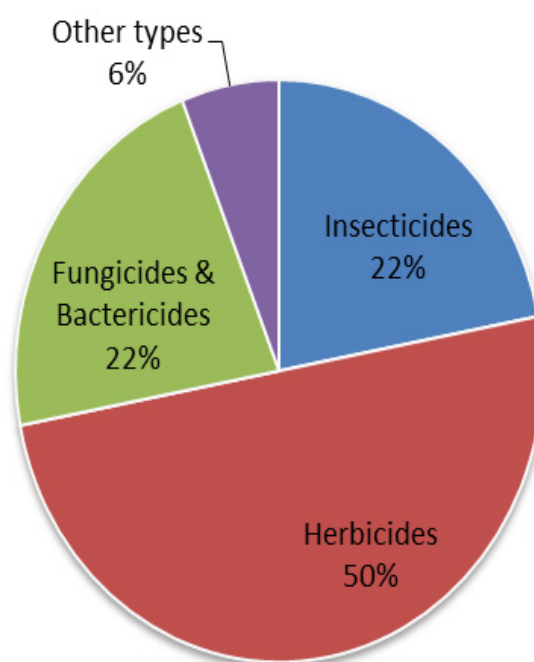


Figure 1: Worldwide pesticides consumption.

Ecological classification of earthworms

Over 7,000 earthworm species have been described globally, spread among 23 families and 700 genera. There are three categories into which earthworms fall depends on the area of soil that earthworms live in, as well as their feeding and burrowing habits. *Lumbricus castaneus* is an example of an epigeic species, which live and feed on soil surface. Anecic earthworms, such as *Lumbricus terrestris*, may emerge from their permanent vertical burrows in soil to feed onto surface litter. Endogeic species, such as *Allolobophora chlorotica*, feed on soil and dwell in transient horizontal burrows (Eisenhauer and Eisenhauer, 2020). Each category uniquely supports ecosystem services, with epigeic focusing on surface processes, anecic on vertical mixing and aeration, and endogeic on subsurface soil health. Together, they play vital roles in maintaining soil fertility and ecosystem productivity.

*LC₅₀ of pesticides on earthworms**Eisenia fetida*

Tables 1 and 2, the LC₅₀ values of different pesticides on *Eisenia foetida* are shown using filter paper contact technique and soil mixing technique. *E. fetida* exposed to atrazine (0.1–5 µg/cm²) had a mean 96-h LC₅₀ of 2.9 µg/cm². For cyanazine (0.5–10 µg/cm²), the LC₅₀ was 4.9 µg/cm², and for chlorpyrifos (1–15 µg/cm²), it was slightly higher at 8.3 µg/cm² (Lydy and Linck, 2003). LC₅₀ value of chlorpyrifos was 0.047 and 0.037 µg/cm² at 24 and 48 hours by Rao *et al.* (2003). The LC₅₀ of azodrin was 0.46 µg/cm² via paper contact after 48 hours, and 132 mg/kg in artificial soil after 14-d (Rao and Kavitha, 2004). Lindane was more toxic than deltamethrin to *E. fetida* over 14-d, with LC₅₀ values of 162.1 mg/kg and 432.9 mg/kg soil, respectively (Shi *et al.*, 2007). The LC₅₀ values for benomyl, carbendazim and lambda-cyhalothrin were 633, >1000, and 23.9 mg/kg into tropical artificial soil,

22, 5.8, and 99.8 mg/kg into artificial soil, 14.6, 4.1, and 139.9 mg/kg into loamy sand soil, respectively (Garcia *et al.*, 2008). The LC₅₀ values for profenofhos were 4.56 and 3.55 µg/cm² after 24 and 48 hours, respectively (Reddy and Rao, 2008). Wang *et al.* (2012) found that insect growth regulators were moderately toxic to worms (LC₅₀: 117.6–564.6 µg/cm² at 48 hours), pyrethroids were highly toxic (LC₅₀: 10.55–25.7 µg/cm²), and neonicotinoids were extremely toxic (LC₅₀: 0.0088–0.45 µg/cm²). Using an artificial soil bioassay, neonicotinoids exhibited greater acute toxicity than antibiotics, carbamates, insect growth regulators, organophosphates, and pyrethroids, which were the least hazardous. Terbutylazine herbicide was the most ecologically harmful to *E. fetida*, followed by galition (fenitrothion and malathion) insecticides, and metaldehyde limacid (Jovana *et al.*, 2014). The 96-h acute toxicity test in natural soil showed that carbendazim was more toxic than captan (Mandal *et al.*, 2015). The LC₅₀ of acetamiprid, imidacloprid, nitenpyram, thiacloprid and clothianidin was 2.69, 3.05, 4.34, 2.68 and 0.93 mg/kg artificial soil, respectively (Wang *et al.*, 2015a, b). It was found that carbendazim is highly toxic to *E. fetida*, with a 14-day LC₅₀ of 2 mg/kg. Dimethoate shows moderate toxicity (LC₅₀ of 28 mg/kg), whereas trichlorfon, tebuconazole, and prochloraz exhibit relatively low toxicity (Rico *et al.*, 2016). According to Chen *et al.* (2018), tribenuron-methyl exhibited low toxicity to *E. fetida* in studies using contact filter paper and artificial soil. Isoprocab increased mortality in a dose-dependent manner, with LC₅₀ values of 8.2 µg/cm² (48-h) on filter paper and 3.37 mg/kg (14-d) in soil (Gu *et al.*, 2021). In the artificial soil, the 14-day LC₅₀ value of flupyradifurone was 157.6502 mg/kg for juvenile *E. fetida* and 186.9773 mg/kg to adult *E. fetida* (Qiao *et al.*, 2022). Acetamiprid was 167.9-times more toxic for *E. fetida* than tetraconazo after a 7-day break. Likewise, acetamiprid's toxicity was 142.2 times greater than tetraconazo's over a 14-d interval (An *et al.*, 2025).

Table 1: LC₅₀ of pesticides to *Eisenia foetida* by filter paper contact (µg/cm²) technique.

Pesticide	Time (h)	LC ₅₀	Ref.
Chlorpyrifos, Atrazine, Cyanazine	96	8.3, 2.9, 4.9	Lydy and Linck, 2003
Chlorpyrifos	24 and 48	0.047 and 0.037	Rao <i>et al.</i> , 2003
Azodrin	48	0.46	Rao and Kavitha, 2004
Profenofos	24 and 48	4.56 and 3.55	Reddy and Rao, 2008
Acetamiprid, Clothianidin, Imidacloprid, Nitenpyram, Thiacloprid, Abamectin, Emamectin benzoate, Ivermectin, Buprofezin, Chlorfluazuron, Hexaflumuron, Tebufenozide, Cyhalothrin, Cypermethrin, Fenpropathrin, Lambda-cyhalthrin, Carbosulfan, Isoprocab, Metolcarb, Promecarb, Chlorpyrifos, Phoxim, Pyridaphenthion, Triazophos	48	0.0088, 0.28, 0.027, 0.22, 0.45, 23.08, 30.2, 4.40, 564.6, 536.2, 117.6, 508.1, 24.30, 10.63, 10.55, 25.7, 75.75, 3.64, 9.18, 10.58, 14.19, 54.65, 3.84, 14.21	Wang <i>et al.</i> , 2012
Tribenuron-methyl, Tebuconazole	24 and 48	377.9 and 9.4–135.6 and 5.7	Chen <i>et al.</i> , 2018
Isoprocab	24 and 48	32.04 and 8.20	Gu <i>et al.</i> , 2021

Table 2: LC₅₀ of pesticides to *Eisenia foetida* by soil mixing (mg/kg soil) technique.

Pesticide	Soil type	Time (d)	LC ₅₀	Ref.
Azodrin	Artificial	7 and 14	171 and 132	Rao and Kavitha, 2004
lindane, deltamethrin	Artificial	14	162.1, 432.9	Shi <i>et al.</i> , 2007
Benomyl, Carbendazim, Lambda-cy-halthrins	Tropical artificial	2	633, >1000, 23.9	Garcia <i>et al.</i> , 2008
	Artificial		22.0, 5.8, 99.8	
	Loamy sand		14.6, 4.1, 139.9	
Acetamiprid, Clothianidin, Imidacloprid, Nitenpyram, Thiocloprid, Abamectin, Emamectin benzoate, Ivermectin, Buprofezin, Chlorfluazuron, Hexaflumuron, Tebufenozide, Cyhalothrin, Cypermethrin, Fenpropathrin, Lambda-cyhalthrins, Carbosulfan, Isoprocarb, Metolcarb, Promecarb, Chlorpyrifos, Phoxim, Pyridaphenthion, Triazophos	Artificial	7 and 14	1.72 and 1.52 –7.44 and 6.06, 3.15 and 2.82, 4.42 and 3.91, 12.13 and 10.96, 31.50 and 27.86, 196.2 and 175.3, 68.11 and 56.34, 425.1 and 363.3, 406.5 and 381.8, 420.1 and 374.2, 434.8 and 386.7, 1530 and 1369, 1467 and 1272, 1532 and 1246, 1623 and 1370, 146.8 and 130.1, 69.4 and 60.8, 108.1 and 93.8, 31.23 and 28.43, 421.3 and 384.9, 1083 and 901.5, 273.3 and 243.7, 381.4 and 347.5	Wang <i>et al.</i> , 2012
Galition, Fenitrothion+Malathion, Terbutylazine, Metaldehyde	Artificial	14	368.25, 1.264, 3.66	Jovana <i>et al.</i> , 2014
Carbendazim, Captan	Natural	4	5.38, 10.41	Mandal <i>et al.</i> , 2015
Imidacloprid, Acetamiprid, Nitenpyram, Clothianidin, Thiocloprid	Artificial	14	3.05, 2.69, 4.34, 0.93, 2.68	Wang <i>et al.</i> , 2015a, b
Trichlorfon, Dimethoate, Carbendazim, Tebuconazole, Prochloraz	Artificial	14	122, 28, 2, 180, 261	Rico <i>et al.</i> , 2016
Tribenuron-methyl, Tebuconazole	Artificial	7 and 14	1007.3 and 746.3, 511.0 and 287.9	Chen <i>et al.</i> , 2018
Isoprocarb	Artificial	7 and 14	6.95 and 3.37	Gu <i>et al.</i> , 2021
Flupyradifurone	Artificial	14	186.9773 (adult) and 157.6502 (juveniles)	Qiao <i>et al.</i> , 2022
Acetamiprid, Tetraconazo	Artificial	7 and 14	1.96 and 1.54, 329 and 219	An <i>et al.</i> , 2025

Eisenia andrei

LC₅₀ values of various pesticides against *E. andrei* under different exposure regimes are shown in Table 3. The LC₅₀ values were 492-154, 23.4-13.5, and 7.10-38.9 mg/kg in artificial soil, while they were 481-106, 8.46-11.9, and 2.70-41.5 mg/kg in loamy sand soil for chlorpyrifos, carbofuran, and carbendazim at 20 and 26°C, respectively (De Silva *et al.*, 2009). The toxicity for mixture of chlorpyrifos, and cypermethrin (LC₅₀ = 35.06) was significantly higher than either of cypermethrin (LC₅₀ = 86.04), and chlorpyrifos (LC₅₀ = 116) individually onto *Eisenia fetida* Andrei (Zhou *et al.*, 2011). The LC₅₀ value is 25.34 mg/kg at 14-day for organophosphate azinphos-methyl on *E. Andrei* (Jordaan *et al.*, 2012). Quantified impact of imidacloprid, thiametoxam, fipronil, and captan (thiram + carboxin) on reproduction, survival, and behavior of *E. andrei*, with exception of imidacloprid (LC₅₀ = 53.25 mg/kg soil), none of pesticides tested caused mortality (Alves *et al.*, 2013). LC₅₀ values were 102.035, 10.149, 249.804, and 558.376 mg/kg dry soil after 1-d of treatment for thiocloprid, esfenvalerate, dimethenamid-p, and prosulfocarb, respectively (Lackmann *et al.*, 2023).

Aporrectodea caliginosa

Mosleh *et al.* (2003a) studied toxicity of cypermethrin,

profenofos, aldicarb, atrazine, metalaxyl and chlorfluazuron to earthworms, *A. caliginosa*. The result was that aldicarb (LC₅₀ = 0.68) was the most toxic to *A. caliginosa* in artificial soil after 28 days, followed in order by cypermethrin (LC₅₀ = 72.96), profenofos (LC₅₀ = 127), chlorfluazuron (LC₅₀ = 139.9), atrazine (LC₅₀ = 381.2), and metalaxyl (LC₅₀ = 518) (Table 4). The LC₅₀ value of acetamiprid was 0.199 and 0.009 after 5 and 10 day for *A. caliginosa* in alluvial soil (Abdel-Raheem *et al.*, 2023). The LC₅₀ values were (334.27 and 55.45 mg/kg) into clay soil, and (415.9 and 25 mg/kg) into sandy clay loam soil for fenitrothion after 5 and 10 days of exposure. LC₅₀ values were (0.93 and 0.41 mg/kg) into clay soil, and (55.28 and 10.65 mg/kg) into sandy clay loam soil after 5 and 10 day of exposure for thiobencarb (El-Aswad *et al.*, 2023a-b). Atrazine had higher intrinsic toxicity than metribuzin to earthworms (*A. caliginosa*), with LC₅₀ of 1.693 and 0.026 µg/cm² after 48 and 72 hours by filter paper contact test. LC₅₀ was reduced from 11.121 to 3.118 and 164.824 to 19.113 mg/kg into clay soil, from 32.221 to 17.33 and 324.141 to 41.028 mg/kg into clay soil: Sandy clay loam soil (1:1), and from 41.234 to 30.804 and 462.255 to 70.902 mg/kg into sandy clay loam soil of atrazine, and metribuzin after 5 and 10 days (Fouad *et al.*, 2023b).

Table 3: LC₅₀ of pesticides to *Eisenia andrei*, in different exposure techniques.

Pesticides	Exposure method	Time (d)	LC ₅₀	Ref.
Chlorpyrifos, Carbofuran, Carbendazim	In artificial soil (mg/kg dry soil) at 20 and 26 °C	28	492 and 154, 23.4 and 13.5, 7.10 and 38.9	De Silva <i>et al.</i> , 2009
	In loamy sand soil (mg/kg dry soil) at 20 and 26 °C		481 and 106, 8.46 and 11.9, 2.70 and 41.5	
Cypermethrin, Chlorpyrifos, Cypermethrin+Chlorpyrifos	In artificial soil (mg/kg dry soil)	14	86.04, 116.00, 35.06	Zhou <i>et al.</i> , 2011
Azinphos-methyl	In artificial soil (mg/kg dry soil)	14	25.34	Jordaan <i>et al.</i> , 2012
Imidacloprid, Thiametoxam, Fipronil, Carboxin+Thiram	In artificial soil (mg/kg dry soil)	14	25.53, >1000, >1000, >1000	Alves <i>et al.</i> , 2013
Difenoconazole+Fludioxonil, Fluopyram+Tebuconazole, Pyrimethanil, Thiram+Carboxin, Flutriafol+Thiabendazole, Fluroxypyr-meptyl, Thiamethoxam	On filter paper (µg/cm ²)	1, 2 and 3	1.4, 0.6 and 0.3, 5.9, 2.9 and 1.0, 40.9, 6.5 and 2.7, 31.8, 8.2 and 1.1, 74.3, 16.2 and 1.2, 19.7, 17.3 and 14.1, 724.0, 110.3 and 19.2	Velki <i>et al.</i> , 2019
Thiacloprid, Esfenvalerate, Dimethenamid-p, Prosulfocarb	In artificial soil (mg/kg dry soil)	2	102.035, 10.149, 249.804, 558.376	Lackmann <i>et al.</i> , 2023

Lumbricus terrestris

L. terrestris were exposed for commercial formulations of aldicarb and endosulfan at 2, 7, 15 days, with LC₅₀ were 12.29, 5.82 and 3.36 mg/kg for endosulfan, 3.09, 1.51 and 1.5 mg/kg for aldicarb (Mosleh *et al.*, 2003b). The LC₅₀ value was 26.804 and 7.001 mg/kg on earthworms in sandy loam soil after 21 and 42 days of glyphosate exposure (Table 4) (Stellin *et al.*, 2018).

Effect of pesticides on enzyme activities in earthworms

Acetylcholinesterase

Carbamate and organophosphorus pesticides primarily inhibit acetylcholinesterase (AChE). Using a standardized paper contact method, *E. fetida* shows a time-dependent decrease in AChE activity when exposed to azodrin and chlorpyrifos (Tiwari *et al.*, 2016). As chlorpyrifos and imidacloprid concentrations increased, AChE activity in *E. foetida* was progressively suppressed (Teng *et al.*, 2022). Azodrine also decreased AChE activity in a concentration-dependent manner (Rao and Kavitha, 2004). Diazinon reduced AChE activity in *E. fetida* body wall and gut (Zawisza-Raszka and Dolezych, 2013), with the lowest activity observed at 120 ng/cm² of temephos after 1 and 2 hours (Hackenberger *et al.*, 2008). Methiocarb significantly decreased AChE activity (Tiwari *et al.*, 2016), while ethyl-4-chlorophenyl, ethyl-4-bromophenyl carbamates, and propoxur caused weak inhibition (Iturbe-Requena *et al.*, 2019). Isoprocab also decreased AChE activity (Gu *et al.*, 2021), and compounds like carbendazim, trichlorfon, dimethoate, prochloraz, and tebuconazole significantly inhibited AChE in artificial soil (Rico *et al.*, 2016). Exposure to imidacloprid over 3 to 21 days reduced AChE activity in *E. fetida* by up to 46.7%, with inhibition rates of 28.9–46.7% (Wang *et al.*, 2015b). Flupyradifurone at 25 and 50 mg/kg maintained AChE inhibition over 28 days

(Qiao *et al.*, 2022). Conversely, atrazine exposure increased AChE activity in *E. fetida* (Lammertyn *et al.*, 2021). In *E. andrei*, carbaryl primarily inhibits AChE competitively (Gambi *et al.*, 2007). Organophosphates like diazinon and chlorpyrifos suppressed AChE by 72–87% within a day and remained suppressed in *A. caliginosa* for 14-d (Booth *et al.*, 1998). Parathion caused rapid AChE inhibition (~70%) within three days, reaching 80–90% after a week; similar patterns were observed in *A. chlorotica*, though at a slower pace (Rault *et al.*, 2008). AChE activity in *A. caliginosa* and *A. caliginosa* was inhibited after exposure to lambda-cyhalothrin, chlorpyrifos-ethyl, and fungicides like myclobutanil and folpet within three days (Reinecke and Reinecke, 2007; Schreck *et al.*, 2008). *L. terrestris* showed significant AChE inhibition as an indirect measure of chlorpyrifos bioavailability (Martínez Morcillo *et al.*, 2013). Conversely, *Dendrobaena veneta* exhibited increased AChE activity after a week of glyphosate exposure at 2.16 mg/kg soil (Hackenberger *et al.*, 2018). Chlorpyrifos and cypermethrin suppressed AChE in *Eudrilus eugeniae* in a dose and region-dependent manner, while glyphosate had no significant effect on AChE activity in *E. eugeniae*, *Libyodrilus violaceus*, and *Alma millsoni* (Owagboriaye *et al.*, 2020). Variations in AChE response are likely influenced by pesticide type and concentration, earthworm species, and soil properties (Yatoo *et al.*, 2022).

Glutathione-S-transferase

When glutathione is used, glutathione-S-transferase (GST) plays a vital role in detoxifying electrophilic chemicals (Yatoo *et al.*, 2022). Elevated GST levels can enhance defense against pesticides, making GST a potential biomarker for pollution monitoring (Tiwari *et al.*, 2016). Studies show that earthworms exposed to chemicals like glyphosate, QYR301, aldrin, endosulfan, and lindane exhibit significantly increased GST activity

(Owagboriaye *et al.*, 2020). Similarly, GST activity in

Table 4: LC₅₀ of pesticides to *Aporrectodea caliginosa* and *Lumbricus terrestris* in different exposure systems.

Earth-worms	Pesticides	Exposure method	Exposure time	LC ₅₀	Ref.
<i>A. caliginosa</i>	Aldicarb, Cypermethrin, Profenofos, Chlorfluazuron, Atrazine, Metalaxyl	In artificial soil (mg/kg dry soil)	28 days	0.68, 72.96, 127.00, 139.90, 381.20, 518.00	Mosleh <i>et al.</i> , 2003a
	Acetamiprid	In alluvial soil (µg/g soil)	5 and 10 days	0.199 and 0.009	Abdel-Raheem <i>et al.</i> , 2023
	Fenitrothion, Thiobencarb	On filter paper (µg/cm ²)	48 and 72 hours	83.16 and 24.67, 288.26 and 39.98	El-Aswad <i>et al.</i> , 2023a
		In clay soil (µg/g soil)	5 and 10 days	334.27 and 55.45, 0.93 and 0.41	
		In sandy clay loam soil (µg/g soil)		415.90 and 25.00, 55.28 and 10.65	
	Atrazine, Metribuzin	On filter paper (µg/cm ²)	24, 48 and 72 hours	43.007, 10.260 and 0.910, 65.620, 1.693 and 0.063	Fouad <i>et al.</i> , 2023b
		In clay soil (µg/g soil)	5 and 10 days	11.121 and 3.118, 164.824 and 19.113	
		In sandy clay loam soil (µg/g soil)		41.234 and 30.408, 462.255 and 70.902	
		In a mixture of clay soil and sandy clay loam soil (µg/g soil)		32.221 and 17.330, 324.141 and 41.028	
	Endosulfan, Aldicarb	In artificial soil (mg/kg dry soil)	2, 7 and 15 days	12.29, 5.82 and 3.36, 3.09, 1.51 and 1.50	Mosleh <i>et al.</i> , 2003b
<i>L. terrestris</i>	Glyphosate	In sandy loam soil (g/m ²)	21 and 42 days	26.804 and 7.001	Stellin <i>et al.</i> , 2018

E. fetida rises upon exposure to compounds such as flupyradifurone, azoxystrobin, fomesafen, sulfentrazone, and pyraclostrobin (Han *et al.*, 2014; Zhang *et al.*, 2014b; Li *et al.*, 2020; Qiao *et al.*, 2022). Long-term exposure to cyflumetofen also activates GST (Shi *et al.*, 2023). Conversely, some chemicals like cyantraniliprole initially decrease and later increase GST activity, while thifluzamide inhibits it (Qiao *et al.*, 2019; Yao *et al.*, 2020). Soil contamination with oxyfluorfen causes significant GST reductions in earthworms such as *Allolobophora molleri*, *E. fetida*, and *L. terrestris* (Tejada *et al.*, 2016). Similarly, exposure to lambda-cyhalothrin, chlorpyrifos-ethyl, and other pesticides over 14-d suppresses GST activity in *A. caliginosa nocturnus*, especially at higher concentrations (Schreck *et al.*, 2008).

Superoxide dismutase

Superoxide dismutase (SOD) is the primary antioxidant enzyme defending against reactive oxygen species. Owagboriaye *et al.* (2020) found that glyphosate-treated earthworms (*L. violaceus*, *E. eugeniae*, *A. millsoni*) exhibited significantly higher SOD activity than unexposed ones. Conversely, Liu *et al.* (2020) observed different SOD responses in *E. fetida* after trifloxystrobin exposure. Other studies reported significant variations in SOD activity

in *E. eugeniae* and *E. fetida* when exposed to nitenpiram, malathion, triflumezopyrim, and sulfoxaflor (Zhang *et al.*, 2020a-b; Jeyaprakasam *et al.*, 2021; Wen *et al.*, 2021; Zhang *et al.*, 2021). Exposure to azoxystrobin, QYR30, and flupyradifurone increased SOD activity in *E. fetida*, indicating detoxification and oxidative stress mitigation (Qiao *et al.*, 2022). SOD activity also rose with higher sulfentrazone levels, showing a dose-dependent effect (Li *et al.*, 2020). A maximum dose of spirotetramat (2.5 mg/kg) significantly elevated SOD activity during exposure (Zhang *et al.*, 2015). Conversely, atrazine and thifluzamide inhibited SOD activity, while increasing concentrations of isoprocarb reduced it (Song *et al.*, 2009; Gu *et al.*, 2021). Responses to cyantraniliprole varied, with initial decreases followed by increases in SOD activity (Qiao *et al.*, 2019).

Catalase

Catalase (CAT) converts hydrogen peroxide into oxygen and water, reducing its toxicity (Yatoo *et al.*, 2022). In *E. fetida*, CAT activity decreases at higher chlorpyrifos doses but remains unaffected at low levels (Zhu *et al.*, 2020). Tebuconazole (0.5, 5, 50 mg/kg soil) showed no significant impact on CAT activity (Zhang *et al.*, 2020a), and azoxystrobin did not increase CAT activity (Han *et al.*, 2014). Exposure to atrazine and isoprocarb reduced

CAT activity, while diazinon increased it (Gu *et al.*, 2021; Zawisza-Raszka and Dolezych, 2013). Triflumisopyrim initially elevated CAT activity, which normalized after 28 days (Wen *et al.*, 2021). Similarly, flupyradifurone increased CAT activity (Qiao *et al.*, 2022). Some fungicides, like spirotetramat, pyraclostrobin, thifluzamide, and QYR301, initially activated CAT but inhibited it over time (Zhang *et al.*, 2015; Yao *et al.*, 2020). Cyantraniliprole caused an initial decrease followed by an increase in CAT activity (Qiao *et al.*, 2019). During 7–21 days, sulfentrazone increased CAT activity at all concentrations, while at 7 and 28 days, there was no significant change (Li *et al.*, 2020). In *A. caliginosa* nocturnus, seven days of exposure to lambda-cyhalothrin, chlorpyrifos-ethyl, folpet, fosetyl-Al, myclobutanil, and metalaxyl decreased CAT activity (Schreck *et al.*, 2008).

Cellulase

Cellulase is a digestive enzyme involved in cellulose breakdown. Acute deltamethrin exposure decreased *E. fetida* cellulase activity, while lindane increased it (Shi *et al.*, 2007). Fomesafen at 500 mg/kg for 14 days markedly inhibited cellulase activity in *E. fetida* (Zhang *et al.*, 2014b), and high-dose spirotetramat also significantly reduced activity during exposure (Zhang *et al.*, 2015). Sublethal doses of dichlorovos and endosulfan significantly inhibited cellulase activity in *E. foetida* (Farrukh, 2017). Exposure to imidacloprid and acetamiprid increased cellulase activity, peaking at 14 days before returning to normal. Combined chlorpyrifos with these insecticides caused initial activation after one day, with levels normalizing after seven days (Teng *et al.*, 2022). Patnaik and Dash (1993) reported reduced cellulase activity in three earthworm species (*Drawida willsi*, *Lampito mauritii*, and *Octochaetona surensis*) after malathion exposure.

Effect of pesticides on histology in earthworms

Cellular and tissue damage in earthworms exposed to hazardous pesticides has been documented through histopathological studies. Imidacloprid induces dose and time dependent genotoxicity in coelomocytes of *Metaphire posthuma* and *E. eugeniae*, with midgut cells showing abnormal nuclei, reduced cytoplasm, and cellular breakdown as exposure increases (Dittbrenner *et al.*, 2011; Kaur *et al.*, 2023). Multiple neonicotinoids (including thiamethoxam, dinotefuran, acetamiprid, clothianidin, nitenpyram, and thiacloprid) damage midgut and epidermal tissues (Yan *et al.*, 2021; Elango *et al.*, 2023), with acetamiprid causing severe structural disintegration (Elango *et al.*, 2023). Acetamiprid also induces alterations in epidermal, muscle, and vascular tissues of *Aporrectodea giardi* (Berrouk *et al.*, 2021). Other pesticides, such as abamectin, cause posterior segment loss, fragmentation, and body thinning in *E. andrei* across all tested concentrations (Nunes *et al.*, 2016). Lindane exposure results in vacuolation and

muscle-epidermis separation, effects that intensify with higher doses of dimethoate and chlorpyrifos, leading to cellular destruction in *A. caliginosa* (Mukherjee and Parida, 2015; Abdel-Aziz *et al.*, 2023; Ahmed and Yahya, 2023). Dimethoate affects neurosecretory cell morphology and urosecretory processes in *Eudichogaste Kinneari* (Lakhani, 2015). Profenofos treatment causes body rupture, bleeding, glandular proliferation, and muscle disintegration in *E. fetida* (Reddy and Rao, 2008). Exposure to triazophos and deltamethrin damages the post-clitellar region of *E. eugeniae*, causing tissue rupture and necrosis (Singh *et al.*, 2019). Monocrotophos induces rupture, necrosis, and tissue fusion in earthworm body walls, while also negatively impacting intestinal histology and microbial populations in *L. mauritii* (Gowri and Thangaraj, 2020; Kavitha *et al.*, 2020). High concentrations of monocrotophos and glyphosate cause lesions, skin undulation, and deformities in *E. eugeniae*, with glyphosate damaging intestinal tissue in *Pheretima elongata* (Morowati, 2000; Samal *et al.*, 2019). Glyphosate exposure leads to cuticular degeneration, cytolysis, vacuolization, and muscle damage in *Nsukkadrilus mbae* (Stanley and Joy, 2014). Similarly, atrazine causes vacuolation, loss of nuclei, and tissue disorganization in *N. mbae* (Oluah *et al.*, 2010). Exposure to butachlor results in vacuolation and glandular enlargement in *Perionyx sansibaricus* and *E. fetida* (Muthukaruppan *et al.*, 2005; Gobi and Gunasekaran, 2010). Sulfentrazone damages the digestive and epidermal tissues of *E. fetida* after 21 days, with cellular disintegration observed (Li *et al.*, 2020). Triazole fungicides cause destruction of epidermal cells and disorganization of muscle layers, with higher concentrations inducing cytoplasmic staining and cell death (Gao *et al.*, 2013).

Genotoxic effects of pesticides on earthworms

E. fetida exhibits antioxidant responses to lipid peroxidation and DNA damage caused by subchronic exposure to acetamiprid and imidacloprid (Li *et al.*, 2018). Imidacloprid at 0.1–2 mg/kg soil can induce DNA damage after 21 days, as shown by sperm deformity assessments (Wang *et al.*, 2015b). Nitenpyram also causes notable DNA damage in *E. fetida*, with effects increasing dose-dependently (Zhang *et al.*, 2021). DNA damage metrics, such as tail moment, rise with higher cyantraniliprole concentrations and longer exposure (Xue *et al.*, 2023). Endosulfan and triflumezopyrim similarly induce DNA damage (Wen *et al.*, 2021), while deltamethrin + triazophos and profenofos + cypermethrin show moderate genotoxicity (Bhargavi *et al.*, 2020). Chlorpyrifos increases DNA damage and reactive oxygen species levels, leading to oxidative stress at low concentrations (Casabé *et al.*, 2007; Zhu *et al.*, 2020). Glyphosate formulations are cytotoxic and genotoxic both *in vivo* and *ex vivo* (Curieses *et al.*, 2018), but glyphosate alone shows no clear DNA harm risk in *Pheretima peguana* (Casabé *et al.*, 2007). Conversely,

paraquat is clastogenic and aneugenic (Muangphra *et al.*, 2014), and atrazine damages *E. fetida* DNA (Song *et al.*, 2009). Sulfentrazone and QYR301 induce oxidative stress, lipid peroxidation, and DNA damage in *E. fetida* (Li *et al.*, 2020). Short-term exposure to diuron (0–5 mg/kg) causes minimal oxidative stress and DNA damage (Wang *et al.*, 2023), while chloridazon, oxadiazon, bentazone, and pendimethalin increase DNA damage and chromosomal abnormalities in *Eisenia hortensis* (Ulukütük and Cigerci, 2020; Cigerci *et al.*, 2022). Azostrobin triggers oxidative stress and DNA damage in *E. fetida* (Han *et al.*, 2014), and carbendazim and metalaxyl-M cause significant DNA damage in *E. fetida* coelomocytes, with effects related to dose and exposure duration (Liu *et al.*, 2014; Huan *et al.*, 2016).

Effects of pesticides on growth of earthworms

Although glyphosate is relatively non-toxic to *Aporrectodea trapezoides*, *Aporrectodea rosea*, *A. caliginosa*, and *Aporrectodea longa* in field studies, it has been shown to reduce *A. caliginosa* growth in laboratory settings, decrease cocoon viability, and lower juvenile counts in the field. It also affects reproduction and development in *E. fetida* (Dalby *et al.*, 1995), leading to fewer juveniles. Similarly, glyphosate decreases cocoon viability and juvenile production in *E. fetida* (Casabé *et al.*, 2007). Laboratory tests reveal that 2,4-D and glyphosate significantly impact *E. fetida* reproduction and development, with treated soils lacking cocoons and juveniles (Correia and Moreira, 2010). These chemicals, along with carbendazim, dimethoate, and copper oxychloride, cause dose-dependent reductions in *E. fetida* growth (Yasmin and D'Souza, 2010). Both glyphosate and chlorpyrifos reduce *E. fetida andrei* feeding activity in field and lab conditions, though low chlorpyrifos concentrations do not affect reproduction (Casabé *et al.*, 2007). The weight of *E. fetida* is more sensitive than mortality to methamidophos and acetochlor exposure (Zhou *et al.*, 2006). Increased butachlor concentrations decrease *E. fetida* biomass and cocoon formation (Gobi and Gunasekaran, 2010), and inhibit *P. sansibaricus* growth and cocoon production (Muthukaruppan *et al.*, 2005). Lindane exposure reduces the biomass of *E. eugeniae* (Mukherjee and Parida, 2015). Pesticides such as chlorpyrifos, diazinon, aldicarb, profenofos, cypermethrin, chlorfluazuron, metalaxyl, and atrazine slow the growth of *A. caliginosa* (Booth *et al.*, 2000; Yasmin and D'Souza, 2010), with diazinon and chlorpyrifos notably reducing growth during exposure (Booth *et al.*, 2000). Endosulfan significantly decreases juvenile *A. trapezoides* weight within 35-days when applied at normal rates, while fenamiphos has similar effects only in the field. Malathion reduces *E. fetida* weight and sperm viability, with parathion also impairing *E. andrei* growth (Yasmin and D'Souza, 2010). Neonicotinoids such as imidacloprid, nitenpyram, acetamiprid, thiacloprid, and clothianidin are highly toxic

to *E. fetida*, significantly inhibiting fecundity (Wang *et al.*, 2015a,b), with high doses adversely affecting reproduction, growth, and physiology of *M. posthuma* and *E. eugeniae* (Kaur *et al.*, 2023). Acetamiprid induces mortality in *A. giardi* proportional to dose and exposure time (Berrouk *et al.*, 2021). Elevated abamectin levels decrease *E. andrei* reproductive efficiency (Nunes *et al.*, 2016), and cyantraniliprole exposure dramatically reduces *E. fetida* weights, likely due to loss of proteins, fats, and glycogen (Qiao *et al.*, 2019).

Effect of pesticides on the behavior of earthworms

These sublethal, organism-wide effects can occur even at low pesticide exposures, making behavioral changes highly sensitive and ecologically relevant (Lackmann *et al.*, 2023). Earthworms play a crucial role in soil structure and aeration; their soil preference or avoidance can indicate soil quality issues (Pereira *et al.*, 2010). Avoidance behavior is a well-established, sensitive indicator of soil pesticide contamination, as pesticides have been shown to influence earthworm avoidance responses. For instance, exposure to sulcotrione and penoxsulam affected avoidance in standardized soils (Marques *et al.*, 2009), while 2.5 and 5 mg/kg esfenvalerate increased avoidance (Lackmann *et al.*, 2023). Similarly, *E. fetida* and *E. andrei* avoided glyphosate-treated soils (Casabé *et al.*, 2007), though low chlorpyrifos concentrations showed no effect. Elevated doses of deltamethrin + triazophos or profenofos + cypermethrin altered earthworm behavior, causing body folding and reduced activity (Bhargavi *et al.*, 2020). Additionally, imidacloprid significantly affected burrow depth, length, and branching in *Aporrectodea nocturna* and *Allolobophora icterica*.

Conclusion

This review underscores the complex and multifaceted threats that pesticides pose to earthworm populations and, consequently, to soil health and ecosystem stability. Analysis of the existing literature reveals several consistent patterns: organophosphates and carbamates emerge as the most acutely toxic pesticide classes, exhibiting high mortality rates in earthworms at recommended agricultural doses, while certain neonicotinoids, though less immediately lethal, induce sub-lethal effects such as impaired reproduction and growth over time. Among various biomarkers, reductions in reproductive output, alterations in antioxidant enzyme activities (such as catalase and superoxide dismutase), and increased DNA damage measured via comet assays are the most sensitive indicators of pesticide-induced stress, signaling early and sustained biological impacts before mortality occurs. Pesticides can also induce significant histopathological alterations, including degeneration of the epidermis and gut epithelium, alongside genotoxic effects that

compromise cellular integrity and genetic stability, potentially impairing earthworm populations and their ecological functions. To address these issues, policymakers should implement strict regulations to limit or ban the use of the most toxic pesticide classes, promote integrated pest management strategies that prioritize biological control and biopesticides, mandate regular environmental monitoring using sensitive biomarkers for early detection, and educate farmers and stakeholders about the ecological costs of pesticide overuse and sustainable soil management practices. Future research should focus on longitudinal field studies to elucidate chronic and cumulative effects, standardize biomarker protocols for cross-study comparison, investigate potential bioaccumulation and transfer of pesticides through soil food webs, and develop earthworm-based bioassays as rapid, cost-effective soil health assessment tools. By synthesizing these patterns and translating them into targeted actions and research priorities, we can better safeguard earthworm populations, preserve vital ecosystem services, and promote sustainable agricultural practices for future generations.

Declarations

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IRB approval

The review was approved by the Department of Chemistry and Pesticide Technology.

Ethical statement

The study is a literature review on pesticides' effects on earthworms, involving no direct experimentation. It uses publicly available, peer-reviewed sources, properly cites all data, and adheres to ethical standards. No ethical approval was needed since it does not involve new experiments or fieldwork.

Consent for publication

The review contains no such material that may be unlawful, defamatory, or which would, if published, in any way whatsoever, violate the terms and conditions as laid down in the agreement.

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No Generative AI and AI-assisted technologies were used in the writing process.

Conflict of interest

The author has declared no conflict of interest.

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