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*Review Article*

# Effects of Pesticides on the Growth and Reproduction of Earthworm: A Review

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Scientific literature addressing the influence of pesticides on the growth and reproduction of earthworm is reviewed. Earthworms are considered as important bioindicators of chemical toxicity in the soil ecosystem. Studies on this aspect are important because earthworms are the common prey of many terrestrial vertebrate species such as birds and small mammals, and thus they play a key role in the biomagnification process of several soil pollutants. Majority of the studies have used mortality as an endpoint rather than subtler endpoints such as reproductive output. It is now emphasized that, whereas higher concentrations of a pollutant can easily be assessed with the acute (mortality) test, contaminated soils with lower (sublethal) pollutant concentrations require more sensitive test methods such as reproduction test in their risk assessment.

## 1. Introduction

A greater proportion (>80%) of biomass of terrestrial invertebrates is represented by earthworms which play an important role in structuring and increasing the nutrient content of the soil. Therefore, they can be suitable bioindicators of chemical contamination of the soil in terrestrial ecosystems providing an early warning of deterioration in soil quality [1–3]. This is important for protecting the health of natural environments and is of increasing interest in the context of protecting human health [4] as well as other terrestrial vertebrates which prey upon earthworms [5]. The suitability of earthworms as bioindicators in soil toxicity is largely due to the fact that they ingest large quantity of the decomposed litter, manure, and other organic matter deposited on soil, helping to convert it into rich topsoil [6, 7]. Moreover, studies have shown that earthworm skin is a significant route of contaminant uptake [8] and thus investigation of earthworm biomarkers in the ecological risk assessment (ERA) can be helpful [9] .

*Eisenia fetida* is the standard test organism used in terrestrial ecotoxicology, because it can be easily bred on a variety of organic wastes with short generation times [10–13]. Its susceptibility to chemicals resembles that of true soil organisms. Sensitivity tests of multiple earthworm species have revealed that *Eisenia fetida* is comparatively less sensitive [14–16]. Although, earthworm species vary in their tolerance, reports have shown a decline in earthworm populations in response to large amounts of organic chemical deposition [17].

Mortality has been the most frequently used parameter to evaluate the chemical toxicity in earthworms [18–20]. It is postulated, however, that survival is less sensitive from an ecotoxicological point of view [21] and acute mortality tests would not provide the most sensitive risk estimate for earthworms in the majority (95%) of cases [22]. Amorim et al. [23] tested with herbicide Phenmedipham and found reproduction to be a more sensitive endpoint than mortality in *Enchytraeus albidus* and *Enchytraeus luxuriosus*. It is suggested that the chronic test, aiming at sublethal effects, is more sensitive and is a more realistic approach for the prediction of environmental effects because in the field, the exposure concentrations of pesticides are usually quite low [24]. Moreover, the lethal effect of a chemical is not a necessary consequence in intoxication and sublethal effects may be produced. According to Riepert et al. [25] the acute earthworm test is part of the basic test set, but the earthworm reproduction test is considered ecologically more relevant. Therefore, growth and reproduction have been recommended as useful sub lethal criteria [26, 27]. This article reviews in short the available scientific literature on the effects of pesticides on the key biological processes, that is growth and reproduction of earthworms.

## 2. Sublethal Toxicity Testing Method

The earthworm reproduction test with *Eisenia fetida/Eisenia andrei* aims to assess the impact of soil contaminants on sublethal parameters in earthworms. Endpoints include reproductive parameters (cocoon production per adult per week, juveniles hatching per adult per week and cocoon viability) and weight change of adults. During the test, adult mature worms are exposed to different concentrations of a substance (pollutant) in a standard test soil; when field soils are used, homogenised and air-dried soil samples are sieved and added to the test chamber and brought to a given moisture content. Ten acclimatized individuals are added to each vessel containing 500 g dry weight of the selected soil. Growth effects and mortality are determined after four weeks and effects on reproduction are assessed after eight weeks of exposure. The assay has been used to measure the effects of a wide range of chemicals such as metals [28] and pesticides [29]. In addition use of a suitable control soil is essential. This test is standardized at the international level, being recognized and promoted by international organizations (OECD—Organization of Economical Cooperation and Development, and ISO—International Organization of Standardization), aiming to elaborate international guidelines on environment quality assessment.

## 3. Effects on Growth

A number of studies have been conducted on the standard worm *Eisenia fetida/andrei.* Some of the responses of earthworms to sublethal concentrations of pesticides is shown in Table 1. Zhou et al. [30] have reported that the weight of the earthworms was a more sensitive index compared to the mortality in indicating toxic effects of acetochlor and methamidophos. Espinoza-Navarro and Bustos-Obregón [31] treated *Eisenia fetida* with organophosphate insecticide malathion and Bustos-Obregón and Goicochea [3] explored the effect of exposure to commercial parathion on *Eisenia fetida;* both observed decrease in the body weight of treated worms . Weight loss has also been reported for organochlorine pesticides intoxication [18, 32, 33] and for the effects of fungicides and herbicides in *Eisenia fetida* and *Lumbricus terrestris* [34–36]. Choo and Baker [37] found endosulfan did significantly reduce the weight of juvenile *Aporrectodea trapezoides* within 5 weeks when applied to soil at normal application rate in both the field and laboratory while fenamiphos did so at normal application rate in the field only. Both fenamiphos and methiocarb reduced earthworm weight in the laboratory when applied at 10× normal rate. Weight loss appears to be a valuable indicator of physiological stress, related to the degree of intoxication and time of exposure [22, 38]. Coiling, another symptom seen in 100% of the Parathion treated worms, is related with weight loss and is regarded as the consequence of alteration in muscular function elicited by organophosphoric pesticides which may explain the difficulties for locomotion of the intoxicated worms and their relative inability to feed themselves [3].

Negative impact of pesticides on earthworm growth has been reported by various researchers. Xiao et al. [39] suggested that growth can be regarded as sensitive parameters to evaluate the toxicity of acetochlor on earthworms. Helling et al. [36] tested in laboratory the effect of copper oxychloride, while Yasmin and D'Souza [40] investigated the impact of carbendazim, glyphosate and dimethoate on *Eisenia fetida* and found a significant reduction in the earthworm growth in a dose-dependent manner. According to Van Gestel et al. [27] parathion affects the growth of *Eisenia andrei*. Booth et al. [41] studied the effect of two organophosphates, chlorpyrifos and diazinon, while Mosleh et al. [42] investigated the toxicity of aldicarb, cypermethrin, profenofos, chlorfluazuron, atrazine, and metalaxyl in the earthworm *Aporrectodea caliginosa* and observed a reduction in growth rate in all pesticide-treated worms. Mosleh et al. [43, 44] studied the effects of endosulfan and aldicarb on *Lumbricus terrestris* and have suggested growth rate as important biomarkers for contamination by endosulfan and aldicarb. Zhou et al. [45] assessed and found chlorpyrifos had adverse effect on growth in earthworm exposed to 5 mg/kg chlorpyrifos after eight weeks. Some studies have shown that growth of earthworms appeared to be more severely affected at juvenile stage than at adult stage [46, 47].

## 4. Effects on Reproduction

Numerous reproductive parameters have been studied in earthworms exposed to various xenobiotics: cocoon and hatchling production, viability of the worms produced [18, 20, 48–54], and sexual maturation [50]. Cocoon production was found to be the most sensitive parameter for paraquat, fentin, benomyl, phenmedipham, carbaryl, copper oxychloride, dieldrin [36, 55–57], while cocoon hatchability was most sensitive for pentachlorophenol, parathion and carbendazim, copper oxychloride [36, 55, 56]. Bustos-Obregón and Goicochea [3] explored the effect of exposure to commercial parathion on the reproductive parameters such as sperm and cocoon production and genotoxicity on male germ cells of *Eisenia fetida* and reported that alterations in reproductive parameters were conspicuous in regard to the number of sperm, cocoons, and worms born. Numbers of juveniles per cocoon can be regarded as sensitive parameters to evaluate the toxicity of acetochlor on earthworms as reported by Xiao et al. [39]. Choo and Baker [37] also found that cocoon production in *Aporrectodea trapezoides* was inhibited by endosulfan and fenamiphos at normal application rates and methiocarb at 10× normal rate.

Table 1: Laboratory experiments on responses of earthworm to sublethal concentrations of pesticides.

| Pesticide | Concentration of pesticide/exposure | Test conditions | Species | Responses | Reference |
|---|---|---|---|---|---|
| Copper oxychloride (pure) | 8.92, 15.92, 39.47, 108.72, 346.85 mg Cu/ kg substrate<br>56 days | Substrate = Dried, ground, finely sieved cattle manure<br>pH = 7.1 ± 0.2–6.1 ± 0.3<br>Moisture = 77.6 ± 0.7–78.8 ± 1.1<br>Temp = 25°C | *Eisenia fetida* (Freshly hatched earthworms) | Earthworm growth and cocoon production were significantly reduced | [36] |
| Malathion (pure) | 80.150, 300, 600 mg/kg soil<br>1, 5, 15, 30 days | Soil like substrate<br>pH = 6.5,<br>Temp = 21-22°C,<br>Moisture = 50%, | *Eisenia fetida* (Adults) | Significant reduction in body weight decreased spermatic viability | [31] |
| Acetochlor | 5, 10, 20, 40 and 80 mg/kg soil<br>7, 15, 30, 45 and 60 days (growth)<br>28 days (reproduction) | OECD artificial soil<br>pH = 6.5,<br>Moisture = 50%<br>Temp = 20 ± 1°C | *Eisenia fetida* (Adults) | At higher concentrations of acetochlor (20–80 mg/kg growth and numbers of juveniles per cocoon were affected significantly | [39] |
| Chlorpyrifos (pure) | 5, 20, 40, 60, 80 mg/kg soil<br>4 and 8 weeks | OECD artificial soil<br>pH = 6.0 ± 0.5.<br>Moisture = 50%<br>Temp = 20°C | *Eisenia fetida* (Adults) | Adverse impact on growth and reproduction | [45] |
| Cypermethrin (pure) | 5, 10, 20, 40, 60 mg/kg soil<br>4 and 8 weeks | OECD artificial soil<br>pH = 6.0 ± 0.5,<br>Moisture = 50%<br>Temp = 20°C | *Eisenia fetida* (Juveniles) | Significant reduction in cocoon production<br>Juveniles more sensitive than adults | [47] |
| Benomyl (pure) | 0.32, 1.0, 3.2, 10, 32 mg/kg soil<br>56 days | OECD artificial soil<br>Temp = 20 ± 2°C<br>pH = 6.1<br>Moisture = 56%<br>LUFA 2.2<br>pH = 6.1<br>Temp = 20 ± 2°C<br>Moisture = 50%<br>Tropical artificial soil<br>Temp = 28 ± 2°C<br>pH = 6.6<br>Moisture = 47%<br>Tropical natural soil, Brazil<br>pH = 3.9<br>Temp = 28 ± 2°C<br>Moisture = 40% | *Eisenia fetida* (Adults) | Toxicity of benomyl was lower in tropical than temperate artificial soils<br>No reproduction in tropical natural soil due to low pH | [24] |

Table 1: Continued.

| Pesticide | Concentration of pesticide/exposure | Test conditions | Species | Responses | Reference |
|---|---|---|---|---|---|
| Chlorpyrifos (pure)<br>Carbofuran (pure)<br>carbendazim, formulated as Derosal (AgrEvo, 360 g/L) | 1, 3, 10, 30, 100, 300, 900 mg/kg soil<br>0.5, 1, 2, 4, 8, 16, 32 mg/kg soil<br>0.1, 0.3, 1, 3, 10, 30, 90 mg/kg soil<br>28 days, 56 days | OECD artificial soil<br>Moisture = 50%<br>Temp = 20 ± 2∘C<br>pH = 6<br>LUFA 2.2 soil<br>Moisture = 50%<br>Temp= 20 ± 2∘C<br>pH = 5.9–6.1<br>OECD artificial soil<br>Moisture = 50%<br>Temp = 26 ± 2°C<br>pH = 6<br>Natural soil<br>Moisture = 45%<br>Temp = 26 ± 2°C<br>pH = 6.2 | *Eisenia Andrei* (Adults) | Toxicity of chlorpyrifos and carbofuran on growth and reproduction in artificial soil was higher at 26°C. in the natural soil<br>carbendazim toxicity was lower at 26°C in both the soil types | [48] |
| Carbaryl (pure)<br>Dieldrin (pure) | 0, 25, 50, 100, 150, 200, 250, 500 mg/kg soil<br>0, 25, 50, 100, 150, 200, 250, 500 mg/kg soil<br>4, 6, 8 weeks | Horse manure, sand and deionized water<br>Moisture = 75 ± 5%<br>Temp = 25°C | *Eisenia fetida* (Juveniles) | Inhibition of growth and cocoon production | [49] |
| Paraquat (pure),<br>Parathion (pure)<br>Fentin (pure)<br>Benomyl (pure)<br>Pentachlorophenol (pure)<br>Carbendazim (formulated as Derosal 60%)<br>Phenmedipham (formulated as Betanal 16.2%) | 20, 45, 100, 200, 450, 1000 mg/kg soil<br>10, 18, 32, 56, 100, 180 mg/kg soil<br>0.32, 1, 3.2, 10, 32 mg/kg soil<br>0.1, 0.32, 1,3.2 mg/kg soil<br>5, 10, 20, 40, 60 mg/kg soil<br>0.6, 1.92, 6 mg/kg soil<br>1.62, 5.18, 16.2, 51.8, 162 mg/kg soil | OECD artificial soil<br>pH = 6.0–7.3,<br>Temp = 20 ± 5°C<br>Moisture = 35% | *Eisenia Andrei* (Adults) | Reduction in growth rate<br>reduction in number of juveniles produced per worm | [27] |
| Dieldrin (pure) | 10, 30, 50, 100 mg/kg<br>Every 15 days, 90 days | Washed cow manure<br>Moisture = 60%<br>Temp = 20°C | *Eisenia fetida* (Juveniles) | Growth was retarded even at agricultural doze of 5 kg/ha<br>Clitellum development retarded, influencing reproduction | [50] |
| Diazinon (formulated as Basudin 600 EW),<br>Chlorpyrifos (formulated as Lorsban 40 EC) | High = 60 mg/kg; Low = 12 mg/kg<br>High = 28 mg/kg; Low = 4 mg/kg | Natural soil<br>pH = 6.5–7,<br>Temp = 20°C<br>Moisture = 20–25% | *Aporrectodea caliginosa* (Adults and juveniles) | Significant effect on growth of juveniles and adults<br>cocoon production significantly reduced | [46] |

Table 1: Continued.

| Pesticide | Concentration of pesticide/exposure | Test conditions | Species | Responses | Reference |
|---|---|---|---|---|---|
| Endosulfan formulated as END 35 (endosulfan concentration of 350 g/L)<br>Aldicarb formulated as Temik 10 G | Different concentrations used; LC10 and LC25 for aldicarb; LC10, LC25, and LC50 for endosulfan<br>2,7 and 15 days | Natural soil<br>Temp = 14 ± 1°C,<br>R.H = 70–90%<br>pH = 8.16 | *Lumbricus terrestris* (Adults) | Loss in weight<br>Reduction in the growth rate<br>Aldicarb was more toxic than endosulfan | [44] |
| Aldicarb (formulated as aldicarb; granular mix, 10% active ingredient)<br>Cypermethrin (formulated as Cypermethrin emulsifiable concentrate 5%)<br>Profenofos (formulated as Curacron, 50% EC)<br>Chlorfluazuron (formulated as Atabron emulsifiable concentrate 50%)<br>Atrazine (formulated as Gesaprim®, 80% WP)<br>Metalaxyl Mn-Zn (formulated as Ridomil, 72% WP) | Different concentrations used<br>1, 2, 3, and 4 weeks | Artificial soil<br>Temp = 23 ± 1°C<br>R.H = 70–90% | *Aporrectodea caliginosa* (Adults) | Reduction in growth rate chlorfluazuron, atrazine, and metalaxyl caused the highest reduction in worm growth rate. | [42] |
| Chlorpyrifos (pure)<br>Chlorpyrifos formulated as Judo 40 EC<br>Carbofuran (pure)<br>Carbofuran formulated as Curater (3% a.i.G)<br>Mancozeb (pure) | 1, 3, 10, 30, 100, 300 and 900 mg/kg soil<br>0.5, 1, 2, 4, 8, 16 and 32 mg/kg soil<br>1, 3, 10, 30, 100, 300, 900 and 1200 mg/kg soil | OECD artificial soil<br>Moisture = 50%<br>Temp = 26 ± 2°C<br>pH = 6.5–6.8 | *Perionyx excavates* (Adults) | Toxicity decreased in the order of carbofuran > chlorpyrifos > mancozeb for both the pure compounds and the formulations<br>Chlorpyrifos, carbofuran and mancozeb are more toxic to P. excavatus than to the standard test species E. andrei at temperatures representative of tropical conditions<br>Formulated compounds depressed earthworm reproduction more than the pure compounds. | [51] |

Espinoza-Navarro and Bustos-Obregón [31] treated *Eisenia fetida* with organophosphate insecticide malathion and found that malathion decreased the spermatic viability in spermatheca, altering the cell proliferation and modifying the DNA structure of spermatogonia. Sperm count also seems to be a very sensitive marker [42, 50], malathion could affect the sperm count, but in addition, its metabolites could affect sperm quality [58].

Several scientists have reported that pesticides influence the reproduction (cocoon production, a reduced mean and maximum number of hatchlings per cocoon, and a longer incubation time) of worms in a dose-dependent manner, with greater impact at higher concentration of chemical [35, 40, 41, 56]. Gupta and Saxena [56] studied the effects of carbaryl, an N-methyl carbamate insecticide, on the reproductive profiles of the earthworm, *Metaphire posthuma* and found sperm head abnormalities even at the lowest test concentration of 0.125 mg/kg. Wavy head abnormalities were observed at 0.125 mg/kg carbaryl, whereas at 0.25 mg/kg and 0.5 mg/kg, the sperm heads became amorphous and the head nucleus was turned into granules deposited within the wavy head.

Xiao et al. [39] showed that acetochlor had no long-term effect on the reproduction of *Eisenia fetida* at field dose (5–10 mg/kg$^{-1}$). At higher concentrations, acetochlor (20–80 mg/kg) revealed sublethal toxicity to *Eisenia fetida*. Zhou et al. [45] assessed and found chlorpyrifos had adverse effect on fecundity in earthworm exposed to 5 mg/kg chlorpyrifos after eight weeks. According to Zhou et al. [47] reproduction of earthworms appeared to be more severely affected by cypermethrin at juvenile stage than at adult stage. Application of 20 mg/kg, cypermethrin caused significant toxic effects in reproduction of worms.

Coiling, seen in the parathion treated worms, interferes with the reproduction too since worms find their partner less easily and copulation is abnormal in terms of mating posture. Ejection of sperm seems also to be hindered and therefore a large number of spermatozoa are found in intoxicated worms in spite of a clear effect on sperm production under parathion treatment as discussed by Bustos-Obregón and Goicochea [3]. According to Espinoza-Navarro and Bustos-Obregon [58] malathion also has a direct cytotoxic effect causing coiling of the tail, with increase of metachromasia of the chromatin of the spermatozoa and altering the sperm count

## 5. Confounding Variables

The results of earthworm ecotoxicological tests may be confounded with different properties of soils such as organic matter, water holding capacity, pH, cation exchange capacity, Carbon/Nitrogen ratio, and clay content and its interaction with chemical substances and different species of earthworm chosen as test species [23]. Soil pH may affect the survival of adults and thus production of juveniles [23, 59]. Low reproduction of earthworm was seen in finely sieved soil as compared to sandy soil [23] indicating that porosity of soil may influence earthworm mobility and gaseous exchange, thus affecting its life cycle. Further, the effects of a pesticide can differ strongly when tested under tropical and temperate conditions [24]. This may be because the physicochemical variables affecting the biotic processes as well as the fate of pesticides in the tropics are different from those in temperate regions [60, 61]. The high temperature and humidity, found in the tropics, seem to favor degradation and volatilization of the chemical in the soil [62, 63]. On the other hand, humid and warmer conditions might enhance the toxicity of some pesticides by increasing the penetration through the skin of animals, and these might be taken up more quickly by tropical biota [64].

Furthermore, information on the side effects of pesticides in the tropics is scarce [65] and a risk assessment based on temperate data could be less appropriate for tropical conditions. Some of the studies have been conducted in this direction, for example, Garcia [66] attempted to compare the toxicity of selected pesticides on different strains of *Eisenia fetida* in temperate and tropical conditions, whereas, Helling et al. [36], Römbke et al. [24], and Garcia et al. [67] applied standardized protocols to determine pesticide effects to soil invertebrates under tropical conditions. De Silva et al. [48] found that sublethal effects (reproduction and growth) varied inconsistently with temperature and soil types. All these researchers suggested that toxicity of pesticides in tropics cannot be predicted from data generated under temperate conditions, even within the same species [48]. Furthermore, it is suggested that tropical risk assessment may be more realistic when conducted on ecologically relevant earthworm species, rather than standard *Eisenia* sp [51]. De Silva [68] suggests that *Eisenia* being temperate compost worms is less ecologically relevant and *Perionyx excavatus* may be used as standard test species for tropical soils.

An important aim in earthworm ecotoxicology is to be able to predict the effects of harmful chemicals in the field on the basis of laboratory experiments. Holmstrup [69] estimated the *in situ* cocoon production in grassland of two earthworm species, *Aporrectodea longa* and *Aporrectodea rosea,* in relation to application dose of benomyl. The results obtained in this field study were compared with results from laboratory reproduction tests with other earthworm species. There was good agreement between effects of benomyl on reproduction in the laboratory and in the field. These results therefore suggest that standardized laboratory tests provide a reasonable prediction of the effect in the field. However, according to Van Gestel [55], results of field studies on the earthworm toxicity of pesticides are in agreement with those of laboratory studies when a homogeneous distribution of the pesticide dosage over the top 2.5-cm soil layer is chosen as a starting point. In field situations, earthworm exposure is strongly dependent on the degree of deposition of pesticides on the soil surface, on the behavior of the pesticide in the soil, and on the vertical distribution of earthworms in the soil. The soil ecosystem is very complex, where interaction occurs between abiotic and biotic factors. Therefore, extrapolation of effects of pesticides observed in laboratory studies to effects in the field studies may be impeded by various environmental variables (especially the soil characteristics and weather conditions) influencing exposure of earthworms to chemical [15]. Neuhauser and Callahan [49] suggested that more consideration should be given to evaluation of sublethal effects under field conditions. Ecotoxicological studies on soil fauna in laboratories usually involve single or a few species. For proper environmental risk assessment, three tiered studies should be conducted [70], that is (1) basic laboratory tests (mainly acute); (2) extended laboratory tests (mainly chronic); (3) tests using microcosms (model ecosystem tests) or even field tests. Although, the highest tier is most important for an ecotoxicological risk assessment, it is rarely performed due to its high complexity, costs and time needed [71]. De Silva [68] also indicates that linking of laboratory data to field may be possible and successful, but more research is required (especially w.r.t tropical conditions) on this aspect to state conclusively [15, 55, 69, 72, 73].

In conclusion, growth and reproductive parameters of earthworms exposed to agropesticides seem to be useful bioindicators of soil pollution. Such studies are simple to do and do not require great technical expertise. However, the studies conducted so far have focused on a few species of earthworms. Additional studies with different species of earthworm, including different endpoints, temperature regimes and soil types, are required. Research should be extended to ecologically relevant species of earthworms, as stated earlier [51], and also to other soil fauna to get a comprehensive knowledge on the malfunction in the soil biological processes due to pesticide pollution. All of the above-mentioned studies indicate negative impact of pesticides on earthworm growth and reproduction. Some studies also indicate that microorganisms in the soil help degrade the chemicals [74, 75]. So, there is a need to acquire more knowledge on the chemical nature, mode of action, and means of degradation of pesticides in soil, so that harm caused to soil fauna as well as to organisms higher up in the food chain can be minimized.

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