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Ecological Risk of Pesticide Industry Waste Leachate: Insights from Earthworm Bioassays

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Abstract

A laboratory-based study was conducted to evaluate the acute toxicity of pesticide waste leachate using the earthworm *Eisenia fetida* as a test organism. Both lethal (mortality) and sublethal (behavioural and reproductive) endpoints were assessed to determine the ecotoxicological impact. The test media included direct leachate exposure as well as leachate-spiked compost (LSC) and leachate-spiked artificial soil (LSAS).

The median lethal concentration (LC_{50}) for direct leachate exposure was determined to be 12.4%. Sublethal effects were pronounced, with the median effective concentration (EC_{50}) for locomotion after 96 h exposure estimated at 50% in LSC and 10% in LSAS, indicating higher sensitivity in artificial soil. In LSAS, earthworms were unable to burrow at 50% concentration after 96 h and at 100% concentration after 48 h of exposure. Locomotory activity ceased completely beyond 72 h at 50% concentration and beyond 48 h at 100% concentration in LSAS. The reduction in locomotion and burrowing behaviour was more severe in LSAS compared to LSC.

Reproductive toxicity was also evident, as no cocoon production was observed at 100% leachate concentration after 96 h of exposure. The EC_{50} values for reproduction were calculated as 21.5% for LSC and 16% for LSAS. Overall, the results demonstrate that pesticide waste leachate exerts significant acute and sublethal toxic effects on *E. fetida*, with greater toxicity observed in artificial soil conditions. These findings highlight the potential ecological risks associated with improper disposal of pesticide wastes and underscore the importance of ecotoxicological evaluation in waste management practices.

Keywords - Pesticide leachate, Eisenia fetida, Acute and sublethal toxicity, Ecotoxicology, LC_{50} and EC_{50}, Soil contamination, Environmental risk assessment

Introduction

Rapid industrialization, urbanization, intensive agricultural practices, and improper disposal of hazardous wastes have resulted in widespread contamination of terrestrial ecosystems. Soil acts as a critical environmental sink and regulator of contaminants through adsorption, degradation, and transformation processes. However, excessive accumulation of hazardous substances can impair soil physicochemical properties, disrupt biological functions, reduce biodiversity, and facilitate the transfer of contaminants to groundwater and food chains. Such contamination threatens ecosystem services, agricultural productivity, and human health, making soil pollution a major global environmental concern (FAO, 2021; Rodríguez-Eugenio et al., 2018).

Among various industrial sectors, pesticide manufacturing and formulation industries are recognized as significant sources of hazardous waste. These wastes contain residual pesticides, intermediates, solvents, additives, and degradation products that may exhibit persistence, mobility, and toxicity in the environment. Improper disposal or landfilling of pesticide wastes can generate leachates containing complex mixtures of organic and inorganic contaminants capable of migrating into surrounding soils and groundwater systems. The occurrence of pesticide residues in subsurface environments has been widely reported, with leaching identified as one of the principal pathways for environmental dissemination of agrochemicals. Such contamination poses substantial risks to soil biota, groundwater quality, and ecological integrity (Rodríguez-Eugenio et al., 2018).

Conventional chemical characterization alone often fails to adequately predict the ecological hazards associated with complex waste matrices because interactions among contaminants may modify their bioavailability and toxicity. Consequently, ecotoxicological assessment has become an essential component of environmental risk evaluation and waste management. Toxicity bioassays provide an integrated measure of the biological effects of contaminants and are increasingly incorporated into regulatory frameworks for waste characterization, contaminated-site assessment, and landfill management. Both lethal and sublethal endpoints are considered important for understanding ecological impacts because adverse effects may occur at concentrations below those causing mortality. Acute mortality tests provide information on immediate toxic effects, whereas behavioural and reproductive endpoints are often more sensitive indicators of ecological disturbance and long-term population-level impacts.

Earthworms are among the most widely used bioindicators in soil ecotoxicology due to their ecological importance and close interaction with soil matrices. Through feeding, burrowing, and decomposition activities, earthworms improve soil structure, enhance nutrient cycling, stimulate microbial activity, and contribute significantly to ecosystem functioning. Their continuous exposure to soil contaminants through dermal contact and ingestion makes them particularly suitable for assessing soil pollution. Changes in earthworm survival, behaviour, growth, and reproduction can serve as sensitive indicators of soil contamination and may have cascading effects on terrestrial food webs and ecosystem processes (Hirano and Tamae, 2011; Tagliabue et al., 2023).

Among earthworm species, *Eisenia fetida* is the most extensively utilized model organism for terrestrial ecotoxicity testing. Its ease of culture, short life cycle, high reproductive capacity, and sensitivity to a broad range of pollutants have led to its widespread adoption in standardized toxicity protocols, including those developed by the Organisation for Economic Co-operation and Development (OECD) and the International Organization for Standardization (ISO). Numerous studies have demonstrated the suitability of *E. fetida* for evaluating the toxicity of contaminated soils, industrial wastes, sewage sludge, pesticides, and landfill leachates, making it a valuable organism for environmental risk assessment (OECD, 1984; OECD, 2016; Hirano and Tamae, 2011).

Recent investigations have highlighted that exposure of *E. fetida* to contaminated leachates can induce not only mortality but also behavioural alterations, oxidative stress, neurotoxicity, and reproductive impairment. Such sublethal responses are increasingly recognized as early-warning indicators of ecological stress and may provide a more realistic assessment of environmental risks than mortality alone . Furthermore, differences in contaminant bioavailability among soil matrices can significantly influence toxicity outcomes, emphasizing the need to evaluate waste-derived contaminants under different exposure conditions (Ellis et al., 2007).

In view of the environmental concerns associated with pesticide-manufacturing wastes and the limited information available regarding their ecotoxicological impacts on terrestrial organisms, the present study was undertaken to evaluate the acute and sublethal toxicity of pesticide-waste leachate using *Eisenia fetida* as a bioindicator species. Mortality, locomotory behaviour, burrowing activity, and reproductive performance were assessed in leachate-spiked compost and artificial soil systems. The findings are expected to improve understanding of the ecological risks associated with pesticide-waste disposal and provide scientific evidence for environmentally sound waste-management and regulatory decision-making.

Materials and Methods

Test Organism

The test organism, *Eisenia fetida*, was procured from a local vermiculture facility and maintained in earthen pots containing native soil under controlled laboratory conditions (20 ± 1 °C) in a BOD incubator. The worms were acclimatized for seven days prior to experimentation following standard ecotoxicological guidelines (OECD, 2016). Healthy, clitellate individuals (0.5–0.6 g; 8–9 cm) were selected and placed on moist filter paper for 3 h to allow gut clearance before exposure, as recommended for toxicity testing to minimize variability (ISO, 2012; OECD, 2016).

Test Media

Leachate Preparation

Pesticide solid waste was collected from a freshly deposited dumping site of a pesticide manufacturing unit and transported to the laboratory in airtight containers. Leachate was prepared using the standardized batch leaching procedure EN 12457-2, which is widely used for assessing the leaching behavior of solid wastes (European Commission, 2002; European Commission, 2018).

Leachate-Spiked Compost (LSC)

Compost used as test substrate was obtained from a local source and homogenized. It was subsequently spiked with predetermined concentrations of pesticide leachate to prepare the test media, following established protocols for spiked-soil ecotoxicity assessments (Domene et al., 2021).

Leachate-Spiked Artificial Soil (LSAS)

Artificial soil was prepared according to OECD Test Guideline 222 (OECD, 2016) with slight modifications. The composition included field soil (80%), peat (10%), and compost (10%). The prepared soil was thoroughly mixed and spiked with known concentrations of pesticide leachate prior to use in toxicity testing.

Determination of Median Lethal Concentration (LC_{50})

Acute toxicity was assessed using the filter paper contact test method in accordance with OECD guidelines (OECD, 2016). Groups of ten adult earthworms of uniform size were placed in glass Petri dishes (10 cm diameter) lined with Whatman No. 1 filter paper. Test concentrations of 5%, 10%, 15%, 25%, 50%, and 100% leachate were applied (5 mL per dish) to ensure uniform exposure. Control groups received distilled water only. Petri dishes were covered with perforated polyethylene sheets to prevent escape while allowing aeration. Mortality was recorded at 24 h and 48 h intervals. The LC_{50} values were calculated using probit analysis, a standard statistical method for toxicity evaluation (Finney, 1971; USEPA, 2012).

Behavioural Studies

Sublethal behavioural responses, including locomotion and geotaxis (burrowing behavior), were assessed in LSC and LSAS media. Earthworms were exposed in 250 mL beakers containing 200 g of test media. A volume of 25 mL of leachate (or distilled water for control) was added daily for up to 96 h, and the media was renewed every 24 h to maintain exposure consistency.

Locomotion was measured by placing worms on a rough surface and recording the distance traveled per minute using a thread method, as described in modified earthworm behavioural assays (Natal-da-Luz et al., 2018; García-Santos et al., 2020). Median effective concentration (EC_{50}) values for locomotion were determined.

For geotaxis, exposed worms were rinsed and placed on moist soil, and the time required for complete burrowing was recorded using a stopwatch. This method is widely used to assess sublethal stress responses in earthworms (OECD, 2016; Domene et al., 2021). After measurement, worms were returned to their respective test media for continued exposure.

Reproductive Studies

Reproductive toxicity was evaluated by assessing cocoon production in earthworms exposed to LSC, LSAS, and control conditions. A total of 20 worms were used per concentration. The number of cocoons produced was recorded, and EC_{50} values were calculated using the linear interpolation method (USEPA, 2012). Reproduction tests in *E. fetida* are recognized as sensitive indicators of chronic toxicity and are recommended in standard soil ecotoxicology protocols (OECD, 2016; ISO, 2012).

Results

Median Lethal Concentration (LC_{50})

Preliminary range-finding experiments were conducted to determine the appropriate concentration range for toxicity assessment. The results indicated 100% mortality of *Eisenia fetida* at concentrations ≥25%, which was therefore considered the upper limit for LC_{50} determination. No mortality or observable behavioural or morphological abnormalities were recorded in the control and 5% concentration groups throughout the exposure period.

The percentage mortality observed at different concentrations of the leachate is presented in Fig. 1. Based on probit analysis, the median lethal concentration (LC_{50}) for direct leachate exposure was calculated to be 12.4%, indicating a high level of acute toxicity.

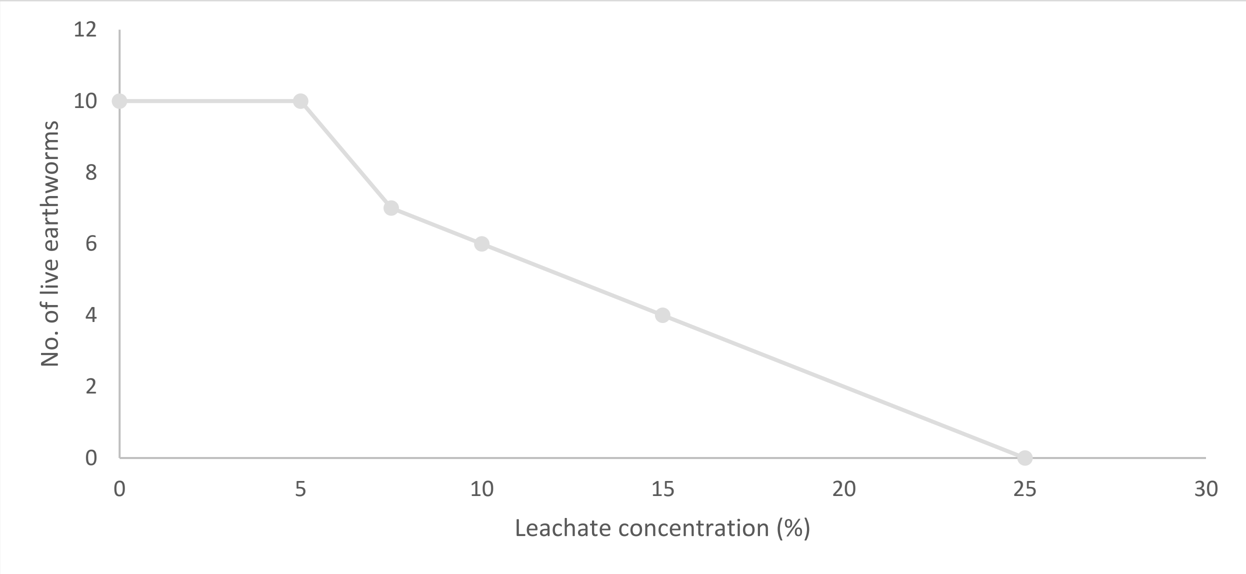

Fig. 1: Mortality of earthworm, *E. fetida*, exposed to different concentrations of pesticide waste leachate

Behavioural Studies

The effects of leachate-spiked compost (LSC) and leachate-spiked artificial soil (LSAS) on locomotion and geotaxis (burrowing behaviour) of *E. fetida* are presented in Tables 1 and 2, respectively.

Table 1: Effect of pesticide leachate-spiked compost (LSC) on the locomotion of *E. fetida* at different concentrations and time intervals.

S. No.	Leachate Concentration (%)	Exposure Time (hours)	Locomotion (cm/min)	Burrowing Time (seconds)
1	Control	24	65.4±9	110±6
		48	67.6±5	106±11
		72	68.1±10	111±9
		96	65.2±8	112±5
2	5	24	66.1±11	112±7
		48	60.2±6	111±10

		72	59.3±7*	117±8
		96	59.4±5	118±5
3	10	24	55.8±9	117±6
		48	52.5±6*	121±9
		72	49.2±10*	121±10*
		96	48.4±5	125±6*
4	15	24	42.3±8	127±7
		48	41.6±6*	127±9
		72	40.4±6	128±10*
		96	39.2±5*	128±10
5	25	24	40.3±12	127±5
		48	38.2±9	129±5
		72	39.7±8	130±9
		96	39.6±6	135±11
6	50	24	38.2±5	134±9*
		48	38.4±7	136±11*
		72	36.6±8*	139±11*
		96	33.4±4	139±6
7	100	24	35.2±9	138±10*
		48	34.6±9	140±11
		72	35.3±5*	142±9
		96	31.2±6*	144±5

Values are arithmetic mean ± S.D. of three replicates. Statistical significance: $p < 0.001$; $^*p < 0.01$

Table 2: Effect of pesticide leachate-spiked artificial soil (LSAS) on the locomotion of E. fetida at different concentrations and time intervals.

S. No.	**Leachate Concentration (%)**	**Exposure Time (hours)**	**Locomotion (cm/min)**	**Burrowing Time (seconds)**
1	Control	24	69.2±5	112±11
		48	69.4±6	115±2
		72	67.6±6	113±7
		96	68.2±8	115±2
2	5	24	65.8±9	120±9
		48	62.5±6*	122±10
		72	59.0±7	123±8
		96	53.4±5	126±8*
3	10	24	52.8±10*	132±9
		48	53.6±7	138±6*

		72	46.4±7	147±8
		96	34.8±4	150±12*
4	15	24	38.4±8*	145±7
		48	34.0±5	153±9
		72	28.6±6	164±9
		96	23.3±4	185±11
5	25	24	33.4±5*	155±10*
		48	27.6±9	186±10*
		72	20.5±4*	198±8
		96	17.2±7	204±12
6	50	24	16.7±5	173±12*
		48	10.0±2*	182±9
		72	No movement	210±8
		96	No movement	No Burrowing
7	100	24	7.6±4	202±10*
		48	No movement	No Burrowing
		72	No movement	No Burrowing
		96	No movement	No Burrowing

Values are arithmetic mean ± S.D. of three replicates. Statistical significance: $p < 0.001$; $*p < 0.01$

In LSC, locomotory activity decreased progressively with increasing leachate concentration and exposure duration. The highest locomotion rate (68.1 cm min^{-1}) was observed in the control group after 72 h, whereas the lowest value (31.2 cm min^{-1}) was recorded at 100% concentration after 96 h. The EC_{50} for locomotion at 96 h was estimated to be 50% leachate concentration. Similarly, burrowing time increased with both concentration and exposure duration. The minimum burrowing time (106 s) was observed in the control group after 24 h, while the maximum (144 s) was recorded at 100% concentration after 96 h.

In LSAS, behavioural responses were more pronounced compared to LSC. Locomotion decreased sharply with increasing concentration and exposure time. The maximum locomotion rate (69.2 cm min^{-1}) was observed in the control after 48 h, while the minimum (7.6 cm min^{-1}) occurred at 100% concentration after 24 h. Complete cessation of locomotion was observed at ≥50% concentration beyond 72 h and at 100% concentration beyond 48 h. The EC_{50} for locomotion at 96 h was determined to be 10% leachate concentration, indicating higher sensitivity in artificial soil.

Burrowing behaviour in LSAS followed a similar trend, with increasing burrowing time at higher concentrations and longer exposure durations. The minimum burrowing time (112 s) was recorded in the control group after 24 h, whereas the maximum (210 s) was observed at 50% concentration after 72 h. Earthworms failed to burrow at 50% concentration after 96 h and at 100% concentration after 48 h or longer exposure durations.

Reproductive Studies

The effects of pesticide waste leachate on cocoon production in LSC and LSAS are presented in Tables 3 and 4 and illustrated in Fig. 2. Maximum cocoon production was observed in the control groups for both media. A progressive decline in cocoon production was evident with increasing leachate concentration.

At 100% concentration, cocoon production was completely inhibited after 96 h of exposure. The EC_{50} values for reproductive output were calculated as 21.5% for LSC and 16% for LSAS, indicating comparatively higher toxicity in artificial soil. These results demonstrate that reproductive endpoints are highly sensitive indicators of sublethal toxicity in *E. fetida*.

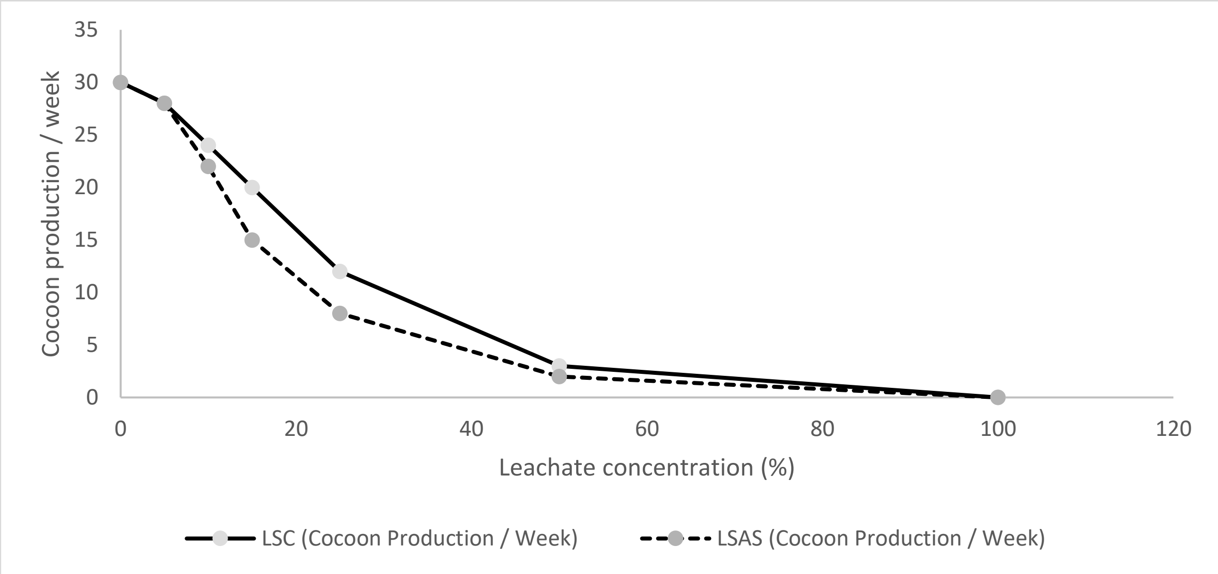

Fig. 1: Effect of pesticide waste leachate on the rate of cocoon production in earthworm (*E. fetida)* (LSC EC_{50} = 21.5%; LSAS EC_{50} = 16%)

Discussion

The present study demonstrates that pesticide waste leachate exerts significant acute and sublethal toxic effects on the earthworm *Eisenia fetida*, confirming its potential ecological risk when improperly managed. Soil contamination arising from industrial waste disposal, particularly pesticide residues, is increasingly recognized as a major environmental concern due to its persistence, mobility, and toxicity (FAO, 2021; Sharma et al., 2019). Toxicity bioassays using soil organisms are widely employed as rapid and integrative tools for assessing environmental hazards, as they capture the combined effects of complex contaminant mixtures (Natal-da-Luz et al., 2018; Domene et al., 2021).

In the present study, mortality of earthworms was observed at relatively low leachate concentrations (≥7.5%), with an LC_{50} value of 12.4%, indicating high acute toxicity. The absence of mortality in control and 5% treatments suggests that *E. fetida* can tolerate low levels of contamination; however, even slight increases in concentration resulted in significant adverse effects. Reported LC_{50} values for earthworms vary widely depending on the nature of the contaminant, exposure route, and test medium (Suthar et al., 2019; García-Santos et al., 2020), supporting the observed variability and highlighting the importance of matrix-specific toxicity evaluation.

Behavioural responses such as locomotion and burrowing are sensitive indicators of sublethal stress in earthworms. The progressive decline in locomotion and increase in burrowing time with increasing leachate concentration and exposure duration observed in this study are consistent with earlier findings that toxicants impair neuromuscular coordination and energy metabolism in earthworms (Liu et al., 2019; Zhang et al., 2021). Complete cessation of locomotion and burrowing at higher concentrations reflects severe physiological stress and loss of functional capacity. Notably, the toxic effects were more pronounced in leachate-spiked artificial soil (LSAS) than in leachate-spiked compost (LSC).

The comparatively lower toxicity observed in compost-amended media may be attributed to the higher organic matter content, which can adsorb and immobilize hydrophobic pesticide compounds, thereby reducing their bioavailability. Organic matter has been shown to significantly influence pesticide sorption, degradation, and toxicity in soils (Kaur et al., 2023; Silva et al., 2019). This supports the observation that compost acts as a mitigating matrix, whereas artificial soil, with lower binding capacity, allows greater exposure of organisms to toxicants.

Reproductive impairment observed in the present study further highlights the ecological significance of sublethal toxicity. The marked reduction in cocoon production, and its complete inhibition at higher concentrations, indicates disruption of reproductive physiology. Reproduction is a critical endpoint in ecotoxicology as it directly influences population sustainability and long-term ecosystem stability (OECD, 2016; van Gestel, 2012). Similar reductions in cocoon production under contaminant stress have been reported in several recent studies, demonstrating that reproductive endpoints are among the most sensitive indicators of soil pollution (García-Santos et al., 2020; Zhang et al., 2021).

The differences observed between LSC and LSAS in terms of reproductive output further emphasize the role of substrate characteristics in modulating toxicity. Organic amendments such as compost not only reduce contaminant bioavailability but may also provide nutritional support that partially offsets toxic stress (Suthar et al., 2019). Variability in cocoon production due to differences in substrate quality and food availability has also been widely documented, reinforcing the importance of environmental conditions in determining earthworm reproductive performance.

Overall, the findings of this study underscore the ecological risks associated with pesticide waste leachates, particularly in soils with low organic matter content. The combined effects on survival, behaviour, and reproduction of *E. fetida* highlight the need for comprehensive ecotoxicological evaluation of industrial wastes prior to disposal. Incorporation of bioassays alongside physicochemical analyses is essential for accurate environmental risk assessment and for developing sustainable waste management strategies.

Conclusion

The present study demonstrates that pesticide waste leachate exerts significant acute and sublethal toxic effects on the earthworm *Eisenia fetida*, highlighting its potential ecological risk in terrestrial environments. The relatively low LC_{50} value (12.4%) indicates high acute toxicity, while pronounced behavioural and reproductive impairments at sublethal concentrations confirm the sensitivity of earthworms as bioindicators of soil contamination.

Substrate-dependent variations in toxicity were evident, with leachate-spiked artificial soil exhibiting greater toxicity compared to compost-amended media. This difference can be attributed to the higher organic matter content in compost, which likely reduced the bioavailability of toxic compounds through adsorption mechanisms. The marked decline in locomotion, impaired burrowing behaviour, and complete inhibition of cocoon production at higher concentrations suggest severe physiological stress and potential disruption of population dynamics.

Overall, the study underscores the importance of integrating ecotoxicological bioassays with conventional chemical analyses for comprehensive environmental risk assessment of industrial wastes. The findings emphasize the need for stringent management and safe disposal of pesticide wastes to prevent soil degradation and protect soil biota. Future studies should focus on long-term field-based assessments and mechanistic investigations to better understand the fate, bioavailability, and ecological impacts of pesticide-derived contaminants.

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