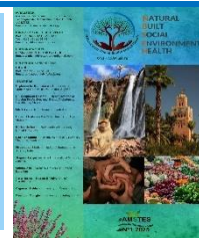




Natural Built Social Environment Health

ISSN 3085-461X

www.nbseh.org

A biomarker assessment of pesticide toxicity in endemic, native peregrine, and invasive earthworm species from the Muthalamada mango plantations, Kerala, India

Chandini Palakkunnel Kuttappan^{1a*}, Sasankan Prasanth Narayanan^{2b}, Syamkumar Reghu Nandan Pillai^{3c}, Rajathy Sivalingam^{1d}, Naveena Kannegowda^{4e}, Jayasooryan Kazhuthoottil Kochu^{4f}, Gayathry Olodathil Sadanandan^{3g}, Maneesh Kumar Shappumkunnath^{3h}

¹ Inter University Centre for Organic Farming and Sustainable Agriculture, Mahatma Gandhi University, Priyadarshini Hills P.O, Kottayam, Kerala, India -686 560

² Advanced Centre of Environmental Studies and Sustainable Development, Mahatma Gandhi University, Priyadarshini Hills, Kottayam - 686 560, Kerala, India

³ School of Environmental Studies, Cochin University of Science and Technology, Kochi, Kerala, India

⁴ Centre for Water Resource Development and Management, Kozhikode, Kerala, India

^aORCID: 0000-0001-6424-9452; Email: chandinipk2009@gmail.com

^bORCID: 0000-0002-7765-9570; Email: narayanakc@gmail.com

^cORCID: ****-****-****-****; Email: syamkumar4@gmail.com

^dORCID: ****-****-****-****; Email: rajkumar@cusat.ac.in

^eORCID: 0009-0008-0882-6233; Email: naveenak@cwrmdm.org

^fORCID: 0000-0002-6759-2093; Email: jkk@cwrmdm.org

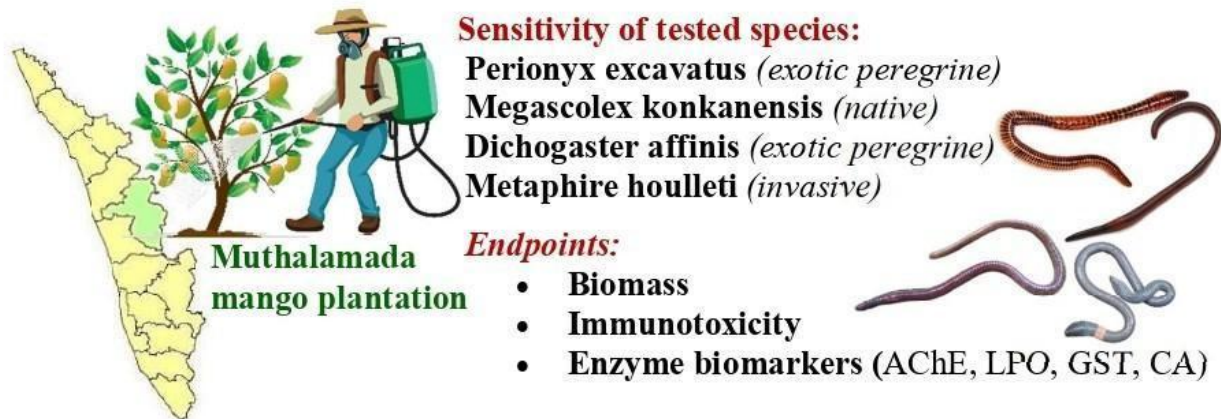
^gORCID: ****-****-****-****; Email: gayathri2196@gmail.com

^hORCID: ****-****-****-****; Email: maneesh005.sk@gmail.com

*Corresponding author: Chandini Palakkunnel Kuttappan; Email address: chandinipk2009@gmail.com

Graphical Abstract

This study examined the toxic effects of pesticide exposure on four earthworm species, using biomass reduction, immunotoxicity, and enzyme biomarkers as indicators. The native species *M. konkanensis* appeared more sensitive to pesticide exposure than the exotic species. *P. excavatus* showed reduced biomass and avoidance behaviour at certain concentrations. Enzyme activity varied across species, reflecting responses to pesticide stress. The results indicate that *P. excavatus*, *D. affinis*, and *M. houlleti* may be suitable indicators for assessing pesticide contamination in soil ecosystems.



Abstract

DOI: [10.63095/NBSEH.25.850535](https://doi.org/10.63095/NBSEH.25.850535)

Available Online 09 June 2025

Citation: Palakkunnel Kuttappan, C., Prasanth Narayanan, S., Reghu Nandan Pillai, S., Sivalingam, R., Kannegowda, N., Kazhuthoottil Kochu, J., Olodathil Sadanandan, G., & Shappumkunnath, M. K., 2025, A biomarker assessment of pesticide toxicity in endemic, native peregrine, and invasive earthworm species from the Muthalamada mango plantations, Kerala, India. *Natural Built Social Environment Health* 1(3). DOI: [10.63095/NBSEH.25.850535](https://doi.org/10.63095/NBSEH.25.850535)

This study assessed the toxic effects of pesticide exposure on four earthworm species: *Perionyx excavatus* Perrier, 1872; *Megascolex konkanensis* Fedarb, 1898; *Dichogaster affinis* (Michaelson, 1890); and *Metaphire houlleti* (Perrier, 1872). The organisms were exposed to pesticide-treated soil. Biomass reduction, immunotoxicity, and enzyme biomarkers were used as endpoints. Among the tested species, *M. konkanensis*, a native species, appeared more sensitive than the others. In biomass tests, chlorpyrifos showed no significant effect on final body mass ($p > 0.05$). *P. excavatus* showed a significant reduction in body mass between 0.001 and 0.02 ppm of the pesticide mixture ($p < 0.05$), and exhibited avoidance behaviour. *M. konkanensis* showed avoidance at 0.02 ppm of the mixture but not at lower concentrations. Changes in AChE, LPO, GST, and CAT activities indicated pesticide-related stress. These results support the use of *P. excavatus*, *D. affinis*, and *M. houlleti* as indicators for soil contamination by pesticides.

Keywords: Earthworms, Pesticides, Toxicity, Enzyme biomarkers, Mango plantations.

Introduction

Changes in ecological factors and environmental health can be monitored by soil invertebrates living in direct contact with the environment [1]. These organisms are exposed to xenobiotics through burrowing, dermal contact, ingestion, and the food chain [2,3]. Most invertebrates are sensitive to soil xenobiotics and are used as biomarkers and bioindicators [4–7]. As sentinel species, earthworms are considered effective indicators for monitoring the health of the terrestrial ecosystem because of their vital roles in pedogenesis, soil fertility, soil structure, and the terrestrial food chain [8–12]. They are therefore widely used to assess environmental risk and chemical toxicity in both laboratory and field conditions [13,14].

Monitoring gene or protein expression and enzyme or metabolite activity following exposure to a xenobiotic or stressor helps in understanding environmental stress. It also allows the evaluation of long-term exposure and inference of mechanisms of action [15]. The use of biomarkers in earthworms for ecological risk assessment has become well established over recent decades. A biomarker can be defined as a xenobiologically induced variation in molecular, cellular, or biochemical components, structures, or functions that is measurable in a biological system or sample [16,17].

In India, several studies have been conducted to examine the pesticide concentration and genotoxicity of model earthworm species, including *Eudrilus eugeniae* and *Eisenia fetida* [18,19]. In Kerala, studies on the ecotoxicological impacts of pesticides on endemic earthworms at the landscape level remain scarce, though they would provide useful information for ecological risk assessment and management of contaminated regions. The Muthalamada mango orchard (MMO) in Kerala is well known as the largest mango-producing area in the state. Soil samples collected from MMO revealed the presence of organochlorine, organophosphate, carbamate, and synthetic pyrethroid pesticides (see appendix).

This study aims to assess the ecotoxicological effects of pesticide exposure on earthworms collected from a contaminated site, using biochemical markers to evaluate toxicity.

Materials and Methods

Earthworm Collection and Identification

Earthworm samples were collected from four locations within the Muthalamada mango orchard in Kerala, India. Sampling sites were selected based on the presence of surface castings and soil moisture. Earthworms were collected from each site using three replicate plots measuring 40 × 50 cm. Sampling was conducted twice per year during July–September 2019 and July–September 2020.

Earthworms were extracted by hand-sorting using a 25 × 25 cm quadrat, to a soil depth of 30 cm [20,21]. Species identification was carried out using standard taxonomic references [22–25].

The dominant earthworm species collected from all sampling locations were *Megascolex konkanensis* Fedarb, 1898, *Dichogaster affinis* (Michaelsen, 1890), *Metaphire houlleti* (Perrier, 1872), and *Perionyx excavatus* Perrier, 1872. Among these, *M. konkanensis* is endemic, *P. excavatus* is a native peregrine species, and *D. affinis* and *M. houlleti* are exotic peregrine species [26]. Collected species were maintained under laboratory conditions at the School of Environmental Studies, CUSAT, Kerala. Samples were kept in a microcosm simulating natural conditions. Identification was performed using taxonomic keys and verified using standard procedures. To assess the impact of pesticides on non-target organisms, the four species were collected from selected stations within Muthalamada Panchayath (Figure 1). Sampling site coordinates and taxonomic details are provided in the appendix.

Artificial soil preparation

OECD artificial soil (10% organic matter, 20% kaolinite clay, and 0.5% calcium carbonate) [22] with decayed leaves of *Cassia fistula* as the organic matter source was used. Earthworms were maintained at 26 ± 3 °C in a substrate consisting of 30% cow dung and 70% coco, peat with a 12-hour photoperiod in the earthworm culture unit. Selected worms were transferred to an untreated test substrate 24 hours before the experiment for acclimatization.

Preparation of Pesticide Standards

Two pesticide standards, chlorpyrifos and a pesticide mixture, purchased from Merck, (India), were used in the toxicity study. Appropriate dilutions of chlorpyrifos and the mixture (lambda-cyhalothrin, imidacloprid, bifenthrin, quinalphos, diuron, malathion, chlorobenzilate, chlorpyrifos-methyl, and HCB) were prepared. Distilled water was used for the control treatment. The concentration of pesticides was calculated based on the total amount of test soil (1 kg of moist soil) in each container. Each concentration and control were tested in triplicate. Table 2 shows the list of pesticide standards.

From 100 ml of chlorpyrifos and 100 ml of the pesticide mixture, concentrations of 2, 1, 0.02, 0.001, and 0.002 mg/kg of dry soil were prepared. The test solutions in acetone were added to 50 g of soil. This portion of soil was treated with acetone and left overnight to allow the solvent to evaporate. The remaining 950 g of soil for each treatment were then added and thoroughly mixed with the spiked soil. Control soil was treated with acetone using the same procedure. Both control and treated soils were moistened with distilled water.

Voiding of Earthworm Gut

Two layers of filter paper were placed in a Petri dish and moistened with a small amount of distilled water. Small holes were pierced in the film using a needle. The Petri dishes were incubated in an artificial climate chamber at 25 ± 1 °C and 80–85% relative humidity for 12 h. During this process, the earthworms stopped feeding and voided their gut contents [27].

Exposure to Pesticides

Earthworm toxicity tests were performed according to the methods provided by OECD guidelines [28]. Earthworms ($n = 10$) were introduced into test chambers (plastic containers measuring 15×20 cm, in triplicate) filled with 800 g (moist weight) of test soil. The test chambers were closed with a moistened jute cloth with small perforations to allow air exchange. Before being introduced into the soil, the worms were rinsed with distilled water, blotted on Whatman No. 1 filter paper, and weighed to determine fresh body mass. The test chambers were maintained at room temperature under a 12-hour light/dark cycle. 10 g (dry weight) of finely ground cow dung, moistened to 50% (w/w), was used as feed. Moisture loss was monitored weekly and adjusted as needed [28, 29].

Artificial Soil Test

The artificial soil test was conducted according to OECD (1984) [28] to evaluate the median lethal concentration (LC₅₀—the concentration of the test substance that results in 50% mortality within the test period). The concentration was expressed in mg/kg (dry weight). Toxicity tests included both individual and combined exposure to the selected toxicants. Chlorpyrifos concentrations of 2, 1, 0.02, 0.001, 0.002, and 0.00 mg/kg were used, both individually and in pesticide mixtures. After 96 hours, earthworms that did not respond to gentle stimulation of a segment were recorded as dead. The number of deaths and cumulative mortality were determined by hand-sorting.

Biomass Test

The biomass test was performed according to Reinecke and Reinecke [30] in triplicate test chambers, each containing 10 ml of chlorpyrifos, applied both individually and as part of a pesticide mixture at concentrations of 2, 1, 0.02, 0.001, and 0.002 mg/kg, with 10 individuals per treatment. The containers were closed with moistened jute cloth to maintain appropriate humidity and temperature. Before the test, earthworms were washed, individually weighed, and placed in the chambers. They were fed with 5 g of ground cattle dung once a week during the 14 days experimental period. On the final day, worms were removed, sorted, rinsed with tap water, and blotted on Whatman No. 1 filter paper. Earthworms were then weighed using an electronic balance and returned to the soil. Only live

animals were washed and weighed. The change in body mass between initial and final measurement was recorded.

Avoidance Test

Avoidance tests were conducted according to the OECD [28,29]. Chlorpyrifos and the pesticide mixture were tested at various concentrations, with each treatment performed in triplicate. The test apparatus was a rectangular box (23.3 cm long, 16.7 cm wide, and 7.7 cm high) (see Appendix), divided into three equal elongated compartments (C1, C2, and C3) using plastic dividers (D1, D2, and D3) with 2 cm holes. Compartments C1 and C2 were filled with 950 g of soil mixed with chlorpyrifos and pesticide mixture. The third compartment (C3) served as a control. After introducing 10 earthworms per compartment, the dividers were removed within 10 minutes. The test chambers were covered with a moistened jute sack and placed under light to discourage the worms from escaping. After 48 hours, the plastic divider was reinserted and the number of earthworms in each compartment (treated or control) was recorded. Worms located along the divider zone (D1-D3), between the compartments (C1-C3), were counted separately using the equation number (1).

$$NR = \left[\frac{C-T}{N} \right] \times 100 \quad (1)$$

Where,

NR = the avoidance rate

C = the number of worms in control soils

T = the number of worms in the treated soils

N = the total number of earthworms introduced

An NR value of 0 is used as the threshold to assess avoidance behaviour.

A positive NR (>80 percent) indicates a strong avoidance reaction.

A negative NR indicates no avoidance behaviour.

Growth Inhibition Test

The growth inhibition test was conducted according to Shi *et al.* [31]. Earthworms were washed, dried on filter paper to remove faeces, weighed, and placed in a plastic container (10.5 × 9.6 × 7 cm), covered with a moistened jute sack to allow air circulation. The earthworms were fed with 5 gm of ground cattle dung and moist soil. Feeding was carried out daily throughout the test period. Body mass and mortality were monitored weekly. Before weighing, earthworms were washed, blotted with

filter paper, and weighed using an electronic balance, then returned to the soil. The final body weight of earthworms in each pesticide-treated container, recorded after 2 weeks of exposure, was used to calculate growth inhibition using the equation number (2).

$$GI_n = \frac{W_0 - W_t}{W_0} \times 100\% \quad (2)$$

Where GI_n is the growth inhibition for concentration n ; W_0 is the weight on the day (day 0), and W_t is the weight after t days of exposure.

Biochemical Analysis

The preparation of the extraction medium was performed according to Diogene [32]. After 14 days of exposure to the pesticides in the test chambers, coelomocytes were harvested from the earthworms using a modified, non-invasive extrusion technique [27,33].

Catalase Test

Catalase activity was determined following the method of Saint-Denis *et al.* [34]. The enzyme activity was measured using a Biotek microplate reader. Catalase activity was expressed in millimoles (mM) of H_2O_2 per minute per milligramme of protein.

Glutathione S-transferase (GST)

GST activity was determined following the method of Habig *et al.* [35]. Enzymatic activity was expressed as nanomoles of 1-chloro-2,4-dinitrobenzene conjugate formed per minute per milligram of protein.

Lipid Peroxidation (LPO)

Lipid peroxidation was measured using thiobarbituric acid reactive substances, following the methods of Ohkawa *et al.* [36]. Activity was expressed as malondialdehyde (MDA) equivalents in nanomoles per minute per milligramme of protein.

AChE Estimation

AChE activity in earthworms was assessed according to Ellman *et al.* [37]. Activity was expressed as micromoles of acetic acid released per minute per milligramme of protein. Enzyme activity was calculated by the equation number (3):

$$\text{Enzyme activity} = \frac{\text{Nano moles of acid liberated in 7.5 ml of reaction mixture}}{\text{Time in mins} \times \text{Protein concentration} \times \text{Enzyme Vol. used}} \quad (3)$$

Statistical Analyses

LC₅₀ (the concentration lethal to 50% of the population) and 95% confidence intervals were calculated. NOEC refers to the highest concentration of a toxicant that, under defined conditions of exposure, causes no observable change in the functional capacity, morphology, growth, development, or life span of the test organism [38]. Mortality and final biomass of earthworms were compared using analysis of variance (ANOVA), followed by Dunnett's post hoc test. Statistical analyses were performed using R software [39–41] and Microsoft Excel.

Results

The study identified five earthworm species from Muthalamada mango plantation (MMP), belonging to two families: Benhaminidae and Megascolecidae, over the course of the study. Figures 2 to 9 show the effects of chlorpyrifos and pesticide mixtures on *Dichogaster affinis*, *Megascolex konkanensis*, *Perionyx excavatus*, and *Metaphire houlleti*.

Effect of Chlorpyrifos and Pesticide Mixtures on the Mortality of Earthworms

Cumulative mortality at each exposure interval was analysed to determine the No Observed Effect Concentration (NOEC) for earthworm mortality. *Megascolex konkanensis* and *Metaphire houlleti* showed higher sensitivity (NOEC: 0.001 milligrammes per kilogram) to chlorpyrifos exposure at 96 h. *Perionyx excavatus* showed the least sensitive response (NOEC: 1 milligramme per kilogramme for 96 hours), with significant mortality ($p < 0.001$) observed at 2 mg/kg of chlorpyrifos compared to the control.

A similar study using pesticide mixture treatments showed significantly high mortality in all species after 96 hours of exposure ($p < 0.05$). The NOEC value for all the species was below 0.001 mg/kg. Cumulative mortality was analysed to determine the effects on earthworm survival. Figures 2 to 9 show the effects of chlorpyrifos and pesticide mixtures on *Dichogaster affinis*, *Megascolex konkanensis*, *Perionyx excavatus*, and *Metaphire houlleti*. From the analysis, *M. konkanensis*, *D. affinis*, and *M. houlleti* showed greater mortality variation to chlorpyrifos at 0.001 mg/kg after 48 and 72 hours (Figs 2-4).

Perionyx excavatus showed lower mortality after 96 hours at 2 mg/kg of chlorpyrifos compared to the control (Figure 5). In contrast, in the mortality test using the pesticide mixture, *Dichogaster affinis* showed the highest mortality across all concentrations after 96 hours compared to the control (Figure 6). *Megascolex konkanensis*, *Metaphire houlleti*, and *P. excavatus* showed reduced mortality at 0.001 mg/kg and 2 mg/kg of the pesticide mixture during both 48- and 96-hour intervals (Figs 7-9).

From the mortality analysis using the pesticide mixture, *Megascolex konkanensis* showed higher mortality than the other tested earthworm species. But, comparison of the survival rate with *Perionyx excavatus* showed no significant differences among replicates. In the case of *Dichogaster affinis*, a significant variation in survival was observed across the tested concentrations. *D. affinis* and *P. excavatus* showed tolerance to both pesticide mixtures and chlorpyrifos at 2 mg/kg. At concentration below 1 mg/mL, chlorpyrifos showed no toxic effect on earthworms during the mortality test.

Effect of Chlorpyrifos and Pesticide Mixtures on the Biomass of Earthworms

In the biomass test using chlorpyrifos treatments, none of the tested species showed a statistically significant reduction in fresh weight compared to the control (appendix). As with mortality, the pesticide mixture caused a greater reduction in biomass than chlorpyrifos alone ($p < 0.05$). All species showed NOEC below 0.001 mg/kg for the pesticide mixture, But the NOEC for chlorpyrifos was greater than 2 mg/kg. Although the effect of chlorpyrifos on *Megascolex konkanensis* was marginally significant ($p = 0.0467$), further analysis did not yield a statistically significant NOEC value.

Effect of Chlorpyrifos and Pesticide Mixtures on Growth Inhibition (GI)

The appendix shows the results of growth inhibition for the tested pesticides. Negative growth inhibition was observed at all chlorpyrifos concentrations. *Perionyx excavatus* (0.88 ± 0.2 to 1.51 ± 0.07) showed negative GI values. In contrast, *Megascolex konkanensis* exposed to 1 mg/kg and 0.1 mg/kg showed the highest growth inhibition during 0-28 days of exposure (0.77 ± 0.06 to 0.70 ± 0.06). In all treatments, the individual average weight of earthworms increased, indicating that 2 mg/kg of pesticide (both chlorpyrifos and mixture) was less toxic to *P. excavatus* (1.81 ± 0.34 to 2.15 ± 0.03) and *Metaphire houlleti* (0.27 ± 0.03 to 0.36 ± 0.02) at 2 to 0.002 mg/kg. But 2 mg/kg of the pesticide mixture resulted in lower growth inhibition in *M. houlleti*. Average growth responses across all concentrations were observed in *M. konkanensis* exposed to both chlorpyrifos and pesticide mixtures. The appendix contains graphs of growth inhibition.

Avoidance Test

The chlorpyrifos and pesticide mixture were tested to understand the toxicity behaviour of earthworms. During the avoidance test, *P. excavatus* and *D. affinis* showed positive avoidance to chlorpyrifos, which means they cannot sustain a low level of chlorpyrifos (Figure 10), whereas *D. affinis* and *M. houlleti* can sustain a high concentration of chlorpyrifos. In the avoidance test using a pesticide mixture, all the test species showed negative avoidance (Figure 11). Similar avoidance was observed in the field condition, where the endemic species migrates from pesticide-contaminated areas to safer zones.

Earthworm Enzyme Biomarkers

The earthworms in the control chamber were healthy and normal, and no mortality was observed during experimentation. After 28 days of exposure, statistically significant differences in AChE activity were observed between control and pesticide-treated earthworms. AChE activity was higher in *M. houlleti*, *Megascolex konkanensis*, and *Dichogaster affinis* at a concentration of 2 mg/kg of chlorpyrifos; in contrast, activity was lower in *P. excavatus* (Figures 12–15). Similar results were observed for AChE activity following exposure to the pesticide mixture (Figs 16–19). *D. affinis*, *M. houlleti*, *P. excavatus*, and *M. konkanensis* showed higher catalase (CAT) activity after exposure to 1 to 2 milligrammes per kilogramme of both chlorpyrifos and pesticide mixtures.

AChE, GST, and LPO activities showed no variation at a concentration of 2 mg/kg of the pesticide mixture or chlorpyrifos. In the case of *Metaphire houlleti*, CAT activity was higher after exposure to both pesticide mixture and chlorpyrifos. For *M. konkanensis*, LPO, AChE, and GST activities were elevated at 2 mg/kg of chlorpyrifos. For *P. excavatus*, CAT activity was also higher following chlorpyrifos exposure. Exposure to the pesticide mixture led to lower AChE, GST, and LPO activity in the same species. A similar trend was noted for the pesticide overall: CAT content was higher, whereas the other enzymes showed no significant variation at the lower concentrations (0.02 and 0.002 mg/kg). CAT and GST activities may be directly or indirectly involved in the biotransformation process. It was observed that, in all concentrations except the control, both chlorpyrifos and the pesticide mixture significantly inhibited CAT enzyme activity.

Discussion

Assessment of toxic effects on chlorpyrifos and selected widely used pesticides in Muthalamada Mango Orchards (MMOs) was conducted using OECD test methods. The results of this study reveal that native peregrine species such as *Perionyx excavatus* and exotic *Dichogaster affinis* showed no

significant mortality at lower concentrations of chlorpyrifos and the pesticide mixture. Earthworms exposed to lethal doses exhibited curling, bleeding, fragmentation, and mucus secretion. Previous studies have reported 50% mortality with chlorpyrifos in *Eisenia fetida*, with lower mortality observed in *Eudrilus eugeniae* [42]. The present results are consistent with those of Tiwari *et al.* [43], especially regarding morphological stress caused by pesticide exposure.

The effects of diazinon and chlorpyrifos on *A. caliginosa* were studied at three pesticide dosages (0.165, 0.066, and 0.020 $\mu\text{g}/\text{cm}^2$). It was found that the lowest concentration of chlorpyrifos causing adverse effects on growth and fecundity in adult earthworms was 28 mg/kg [44]. This confirms that chlorpyrifos can inhibit the development of earthworms. Gomez-Eyles *et al.* [45] reported effects in *E. fetida* and established an LC50 value of 2.36 mg/kg for synthetic pyrethroid pesticides. Other studies have shown 50% mortality in tropical earthworms such as *P. excavatus*, *M. peguana*, and *E. fetida* after 28 days of exposure to chlorpyrifos, indicating their relative tolerance to this compound [46].

These findings are consistent with the present study, showing that both *Perionyx excavatus* and *Dichogaster affinis* were more tolerant to chlorpyrifos than to the pesticide mixture. The lower NOEC values observed across all species in the pesticide mixture, compared to chlorpyrifos, suggest a synergistic effect. Such interactions, particularly in mixtures containing more than two active ingredients, often affect bioavailability outside the organism. Organophosphate and carbamate pesticides act synergistically because they share the same mode of action.

The impact of pesticides on earthworms under field conditions, however, depends on multiple factors, including the properties and persistence of the compound, application frequency, and site-specific characteristics such as organic carbon content, pH, soil texture, nutrient levels, and the composition of the soil fauna [47,48]. Because of the complexity of potential interactions between these factors, pesticide toxicity under laboratory conditions can vary widely. In this study, chlorpyrifos and pesticide mixtures did not cause mortality, even at the highest tested concentration (2 parts per million). This does not imply an absence of lethal effects on soil fauna; chlorpyrifos is known to affect non-target organisms such as oligochaetes and arthropods [49–52].

In general, microcosm studies have shown that pesticide exposure causes biomass loss in earthworms, indicating adverse effects [53–56]. There was a progressive weight loss from the beginning to the end of the trials, although high variability reduced the statistical significance of the results [57–59]. A similar trend was observed in the present study. The biomass of worms at the three highest concentrations was lower after the first chlorpyrifos application, and again lower after the second, suggesting that repeated pesticide exposure in close succession may amplify negative effects [60,61].

The observed increase in growth inhibition over time suggests that prolonged exposure weakens the self-repair mechanisms and stress resistance of *P. excavatus*, *M. houlleti*, *M. konkanensis*, and *D. affinis*, especially at 1 and 2 milligrammes per kilogramme of chlorpyrifos and pesticide mixtures.

The growth inhibition was negative in the control group, indicating that the earthworms gained weight. A similar observation was reported for *Eisenia fetida* by Yan *et al.* [62]. Li *et al.* [63] showed that earthworms exposed to tolclofos-methyl exhibited significant growth inhibition compared with the control, and that weight declined with increasing exposure time.

In line with those findings, the present study recorded weight loss in *Metaphire houlleti*. Previous research has also shown that earthworm responses to toxicants typically include growth inhibition and weight loss [64]. This loss may reflect the depletion of glycogen, proteins, and lipids triggered by chemical stress [65]. Detoxification plays a key role in eliminating exogenous compounds from the body. The avoidance test is a relatively recent method used to evaluate behavioural responses and provide rapid ecotoxicological screening for terrestrial species [66,67].

The avoidance behaviour of *Eisenia andrei* in pesticide-treated soils (carbofuran, chlorpyrifos, and metamidophos) was found to be similar under both laboratory and field conditions [68]. Comparable findings were also reported with pesticide mixtures containing imidacloprid, fipronil, thiamethoxam, captan, carboxim, and tiram [69]. According to Oi [70] and Capowiez and Bérard [71], the toxicity of pesticides to *Aporrectodea nocturna* and *Allobophora icterica* was evident, although these substances were not considered irritants.

Chlorpyrifos may not be an irritant for *Perionyx excavatus*, or the concentrations tested may have been too low to trigger avoidance. Similar results were recorded in *E. andrei* by Torkhani *et al.* [72]. There is still no clear explanation for the apparent preference for organochlorine-treated soils observed in some species. The absence of avoidance could be linked to the inability of chemoreceptors to detect toxicants.

In the present study, *M. konkanensis* appeared to prefer lower concentrations of pesticide mixtures, which may suggest a hormetic response – a beneficial effect at low doses of a stressor that would be harmful at higher levels. The rate of AChE activity increased with rising concentrations of organochlorine pesticides [73,74]. Similar trends were seen in *E. fetida* exposed to phenanthrene and pyrene [75]. The CAT enzyme plays a protective role against oxidative stress, supported by antioxidant systems [76].

In this study, exposure to chlorpyrifos and the pesticide mixture increased CAT activity, possibly because of reduced production of reactive oxygen species (ROS). Throughout the exposure period, all four earthworm species may have maintained stable ROS levels through enzymatic removal of

excess peroxide [77]. Organophosphates are known to inhibit AChE, leading to excessive accumulation of acetylcholine, which can result in muscular tetanus and mortality.

Conclusion

The exotic species of earthworms, such as *Perionyx excavatus*, *Metaphire houlleti*, and *Dichogaster affinis*, were dominating in the Muthalamada Mango Plantation (MMP). By contrast, the population of native species, such as *Megascolex konkanensis*, was declining. Based on the results, native peregrine *P. excavatus* and exotic species *D. affinis* and *M. houlleti* can serve as biomarkers for monitoring organophosphate and organochlorine pesticide contamination in agro-ecosystems. The irreversible inhibition of AChE by pesticide exposure causes an excess of acetylcholine, leading to hyperactivity in neuronal and muscular systems. Pesticide exposure altered the activity of AChE, LPO, GST, and CAT enzymes, and also caused morphological changes. This suggests a high level of toxicity in the exposed organisms. The effects were both dose-dependent and specific to the coelomic fluid. In contaminated environments, such changes may reduce the survival of non-target, ecologically beneficial organisms. In practice, agricultural land often contains a mixture of agrochemicals, including pesticides, resulting in complex interactions that may affect soil flora and fauna in unpredictable ways.

Acknowledgements

The authors are thankful to the University Grants Commission (UGC), Delhi, for the essential financial support from the UGC-SAP-DRS-II programme (2015-2020).

We thank Professor Dr V. Sivananandan Achari, School of Environmental Studies, CUSAT for, for his academic guidance and encouragement. We are also grateful to Mr Sudheesh and the farmers of Muthalmada for granting permission to collect earthworms for this experiment.

Disclosure statement

The authors declare that there are no conflicts of interest.

References

- [1] Gong, P., Guan, X., Pirooznia, M., Liang, C., & Perkins, E.J., 2012, Gene expression analysis of CL-20-induced reversible neurotoxicity reveals GABAA receptors as potential targets in the earthworm *Eisenia fetida*. *Environmental Science and Technology* **46**, 1223-1232. <https://doi.org/10.1021/es203642e>
- [2] Svendsen, C., Spurgeon, D.J., Hankard, P.K., & Weeks, J.M., 2004, A review of lysosomal membrane stability measured by neutral red retention: is it a workable earthworm biomarker?. *Ecotoxicology and Environmental Safety* **57**, 20-29. <https://doi.org/10.1016/j.ecoenv.2003.08.009>
- [3] Kammenga, J.E., Dallinger, R., Donker, M.H., Kohler, H.R., Simonsen, V., Triebkorn, R., & Weeks, J.M., 2000, Biomarkers in terrestrial invertebrates for ecotoxicological soil risk assessment. *Review of Environmental Contamination and Toxicology* **164**, 93-148. <https://europepmc.org/article/med/12587835>
- [4] Gong, P., & Perkins, E.J., 2016, Earthworm toxicogenomics: a renewed genome-wide quest for novel biomarkers and mechanistic insights. *Applied Soil Ecology* **104**, 12-24. <https://doi.org/10.1016/j.apsoil.2015.11.005>
- [5] 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. [10.5772/28265](https://doi.org/10.5772/28265)
- [6] Daewoo, M., Wahid, A., Hashmi, M.Z., Mukhtar, S., & Malik, Z. 2017, Use of earthworms in biomonitoring of soil xenobiotics. In : *Xenobiotics in the Soil Environment* (Springer), pp. 73-88. https://link.springer.com/chapter/10.1007/978-3-319-47744-2_6
- [7] Calisi, A., Latino, M.E., Corallo, A., Grimaldi, A., Ferronato, C., Antisari, L.V., & Dondero, F., 2019, Biomarkers in soil organisms: their potential use in the assessment of soil pollution and remediation. In: *Bioremediation of Agricultural Soils*, pp. 262-285. <https://irinsubria.uninsubria.it/handle/11383/2078820>
- [8] Hirano, T., & Tamae, K., 2011, Earthworms and soil pollutants. *Sensors* **11**, 11157-11167. <https://doi.org/10.3390/s111211157>
- [9] Devi, S.H., Vijayalakshmi, K., Jyotsna, K.P., Shaheen, S.K., Jyothi, K., & Rani, M.S., 2009, Comparative assessment in enzyme activities and microbial populations during normal and vermicomposting. *Journal of Environmental Biology* **30**, 1013-1017. Available online at : http://www.jeb.co.in/journal_issues/200911_nov09/paper_15.pdf (Consulted on 19 April 2025)
- [10] Liu, T., Wang, X., You, X., Chen, D., Li, Y., & Wang, F., 2017, Oxidative stress and gene expression of earthworm (*Eisenia fetida*) to clothianidin. *Ecotoxicology and Environmental Safety* **142**, 489-496. <https://doi.org/10.1016/j.ecoenv.2017.04.012>

- [11] Robidoux, P.Y., Hawari, J., Bardai, G., Paquet, L., Ampleman, G., Thiboutot, S., & Sunahara, G.I., 2002, TNT, RDX, and HMX decrease earthworm (*Eisenia andrei*) life-cycle responses in a spiked natural forest soil. *Archives of Environmental Contamination and Toxicology* **43**, 0379-0388. <https://link.springer.com/article/10.1007/s00244-002-1242-z>
- [12] Gautam, A., Ray, A., Manna, S., Sarkar, M.P., Ghosh, A.R., Ray, M., & Ray, S., 2020, Shift in phagocytosis, lysosomal stability, lysozyme activity, apoptosis and cell cycle profile in the coelomocytes of earthworm of polluted soil near a tannery field of India. *Ecotoxicology and Environmental Safety* **200**, 110713. <https://doi.org/10.1016/j.ecoenv.2020.110713>
- [13] Wang, Z., & Cui Z., 2016, Accumulation, biotransformation, and multi-biomarker responses after exposure to arsenic species in the earthworm *Eisenia fetida*. *Toxicology Research* **5**, 500-510. <https://doi.org/10.1039/c5tx00396b>
- [14] Irizar, A., Rivas, C., García-Velasco, N., de Cerio, F.G.J., Etxebarria, Marigómez, I., & Soto, M., 2015, Establishment of toxicity thresholds in subpopulations of coelomocytes (amoebocytes vs. eleocytes) of *Eisenia fetida* exposed in vitro to a variety of metals: implications for biomarker measurements. *Ecotoxicology* **24**, 1004-1013. <https://link.springer.com/article/10.1007/s10646-015-1441-9>
- [15] Mincarelli, L., Vischetti, C., Craft, J., & Tiano, L., 2016, DNA damage in different *Eisenia andrei* coelomocytes sub-populations after in vitro exposure to hydrogen peroxide. *Springer Plus* **5**, 1-7. <https://doi.org/10.1186/s40064-016-1950-x>
- [16] Li, W., Wang, C., & Sun, Z., 2011, Vermi pharmaceuticals and active proteins isolated from earthworms. *Pedobiology* **54**, S49-S56. <https://doi.org/10.1016/j.pedobi.2011.09.014>
- [17] Lourenço, J., Silva, A., Carvalho, F., Oliveira, J., Malta, M., Mendo, S., Gonçalves, F., & Pereira, R., 2011, Histopathological changes in the earthworm *Eisenia andrei* associated with the exposure to metals and radionuclides. *Chemosphere* **85**, 1630-1634. <https://doi.org/10.1016/j.chemosphere.2011.08.027>
- [18] Jeyaprakasam, A., Muniyandi, B., James, A.J.P., Karmegam, N., & Ponnuchamy, K., 2021. Assessment of earthworm diversity and pesticide toxicity in *Eudrilus eugeniae*. *Environmental Chemistry and Ecotoxicology* **3**, 23-30. <https://doi.org/10.1016/j.enceco.2020.11.001>
- [19] Reddy, N.C., & Rao, J.V., 2008. Biological response of earthworm, *Eisenia fetida* (Savigny) to an organophosphorous pesticide, profenophos. *Ecotoxicology and Environmental Safety* **71**, 574-582. <https://doi.org/10.1016/j.ecoenv.2008.01.003>
- [20] Blakemore R.J., 2009 Cosmopolitan earthworms: a global and historical perspective. *Annelids in modern biology* (ed. D.H. Shain), (Hoboken: John Wiley), pp. 257-283. <https://doi.org/10.1002/9780470455203.ch14>

- [21] Gates, G.E., 1973, Burmese earthworms, an introduction to the systematic and biology of the megadrile oligochaetes with special reference to Southeast Asia. *Transactions of the American Philosophical Society* **62**(7), 1–326. <https://doi.org/10.2307/2412107>
- [22] Julka, J.M., 1988, The Fauna of India and Adjacent Countries. Megadrile Oligochaeta (Earthworms). Haplotaxida: Lumbricina: Megascolecoidea: Octochaetidae, (Calcutta: Zoological Survey of India). <https://archive.org/details/dli.zoological.fi.028>
- [23] Stephenson, J. 1923. The Fauna of British India, including Ceylon and Burma, Oligochaeta. (London: Taylor and Francis, London). <https://www.nature.com/articles/113455b0#:~:text=Abstract,30s.>
- [24] Narayanan, S.P., Ahmed, S., & Thomas, A. P., 2025, Description of a new species of the earthworm genus *Moniligaster* Perrier, 1872 (Clitellata, Moniligastridae) from the Nilgiri Biosphere Reserve of Tamil Nadu State, India. *Zootaxa* **5589**(1), 142-154. <https://doi.org/10.11646/zootaxa.5589.1.12>
- [25] Narayanan, S.P., Kurien, V.T., Anuja, R., Hasyagar, V., Thomas, A.P., Paliwal, R., & Julka, J.M., 2023, Earthworm (Clitellata, Megadrili) fauna of Kuttanad wetland, southern part of Vembanad-Kol Ramsar site, India. *Opuscula Zoologica Budapest* **54**(1), 3-21. <https://doi.org/10.18348/opzool.2023.1.3>
- [26] Anderson, J.M., & Ingram, J.S.I., 1994, Tropical Soil Biology and Fertility: A Handbook of Methods. *Soil Science* **157**(4), 265. <https://dx.doi.org/10.2307/2261129>
- [27] Jhulka, J.M. (1993). Earthworm resources in India and their utilization in vermiculture. In: A.K., Ghosh (Ed.) *Earthworm Resource and Vermiculture*, Zoological Survey of India, Kolkata, India, pp. 51-56
- [28] OECD, 1984, Guideline for testing of chemicals, Earthworms: acute toxicity tests. (Paris: Organization for Economic Cooperation and Development) 207(OECD, Paris. <https://doi.org/10.1787/9789264070042-en>
- [29] OECD, 2004, *Test No. 222: Earthworm Reproduction Test (Eisenia fetida/Eisenia andrei)* (Paris: OECD Publishing). <https://doi.org/10.1787/9789264070325-en>.
- [30] Reinecke, S.A., & Reinecke, A.J., 2007. Biomarker response and biomass change of earthworms exposed to chlorpyrifos in microcosms. *Ecotoxicology and Environmental Safety* **66**, 92-101. <https://doi.org/10.1016/j.ecoenv.2005.10.007>
- [31] 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**, 31-38. <https://doi.org/10.1016/j.pestbp.2007.02.005>

- [32] Diogene, J., Dufour, M., Poirier, G.G., & Nadeau, D., 1997, Extrusion of earthworm coelomocytes: comparison of the cell populations recovered from the species *Lumbricus terrestris*, *Eisenia fetida* and *Octolasion tyrtaeum*. *Laboratory Animal* **31**, 326-336. <https://doi.org/10.1258/002367797780596068>
- [33] Eyambe, G.S., Goven, A.J., Fitzpatrick, L.C., Venables, B.J., & Cooper, E.L., 1991, A non-invasive technique for sequential collection of earthworm (*Lumbricus terrestris*) leukocytes during subchronic immunotoxicity studies. *Laboratory Animal* **25**, 61-67. <https://doi.org/10.1258/002367791780808095>
- [34] Saint-Denis, M., Labrot, F., Narbonne, J.F., & Ribera, D., 1998, Glutathione, glutathione-related enzymes, and catalase activities in the earthworm *Eisenia fetida/andrei*. *Archives of Environmental Contamination and Toxicology* **35**, 602-614. <https://link.springer.com/article/10.1007/s002449900422>
- [35] Habig, W.H., Pabst, M.J., & Jacoby, W.B., 1974, Glutathione S-transferase, the first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry* **249**, 321–336. [https://doi.org/10.1016/S0021-9258\(19\)42083-8](https://doi.org/10.1016/S0021-9258(19)42083-8)
- [36] Ohkawa, H., Ohishi, N., & Yagi, K., 1979, Assay for lipid peroxides in animal-tissues by thiobarbituric acid reaction. *Analytical Biochemistry* **95**, 351-358. [https://doi.org/10.1016/0003-2697\(79\)90738-3](https://doi.org/10.1016/0003-2697(79)90738-3)
- [37] Ellman, G.L., Courtney, K.D., Andres, V., & Featherstone, R.M., 1961, A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology* **7**, 88-95. [https://doi.org/10.1016/0006-2952\(61\)90145-9](https://doi.org/10.1016/0006-2952(61)90145-9)
- [38] Ghazali, A., & Inayat-Hussain, S., 2014, Encyclopedia of Toxicology (3rd Ed.) (Cambridge: Academic Press) 593-597. <https://doi.org/10.1016/B978-0-12-386454-3.01223-9>
- [39] R Core Team (2020) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.r-project.org/>
- [40] Ritz, C., Baty, F., Streibig, J.C., & Gerhard, D., 2015, Dose-Response Analysis Using R PLOS ONE. **10**(12), e014602. <https://doi.org/10.1371/journal.pone.0146021>
- [41] Torsten, H., Frank, B., & Peter, W., 2008, Simultaneous Inference in General Parametric Models. *Biometrical Journal* **50**(3), 346-363. <https://doi.org/10.1002/bimj.200810425>
- [42] Pawar, S., & Ahmad, S., 2013, Impact of Organophosphate (Chloropyriphose) on Weight and Growth of Earthworm, *Eisenia foetida*. *International Journal of Scientific Research* **4**(2), 2319–7064. <https://www.academia.edu/download/111363776/SUB151568.pdf>
- [43] Tiwari, K., Singh, S., & Pandey, R.S., 2019, Assessment of acute toxicity and biochemical responses to chlorpyrifos, cypermethrin and their combination exposed earthworm, *Eudrilus eugeniae*. *Toxicology Reports* **6**, 288-297. <https://doi.org/10.1016/j.toxrep.2019.03.007>

- [44] Booth, L.H., & O'Halloran, K., 2001, A comparison of biomarker responses in the earthworm *Aporrectodea caliginosa* to the organophosphorus insecticides diazinon and chlorpyrifos. *Environmental Toxicology and Chemistry* **20**, 2494-2502. <https://doi.org/10.1002/etc.5620201115>
- [45] Gomez-Eyles, J.L., Svendsen, C., Lister, L., Martin, H., Hodson, M.E., & Spurgeon, D.J., 2009, Measuring and modelling mixture toxicity of imidacloprid and thiacloprid on *Caenorhabditis elegans* and *Eisenia fetida*. *Ecotoxicology and Environmental Safety* **72**, 71-79. <https://doi.org/10.1016/j.ecoenv.2008.07.006>
- [46] Kreutzweiser, D.P., Good, K.P., Chartrand, D.T., Scarr, T.A., Holmes, S.B., & Thompson, D.G., 2008, Effects on litter dwelling earthworms and microbial decomposition of soil applied Imidacloprid for control of wood boring insects. *Pesticide Science* **64**, 112-118. <https://doi.org/10.1002/ps.1478>
- [47] De Silva, P.M.C., Pathiratne, A., & Van Gestel, C.A., 2010, Toxicity of chlorpyrifos, carbofuran, mancozeb and their formulations to the tropical earthworm *Perionyx excavatus*. *Applied Soil Ecology* **44**, 56-60. <https://doi.org/10.1016/j.apsoil.2009.09.005>
- [48] Muangphra, P., Sengsai, S., & Gooneratne, R., 2015, Earthworm biomarker responses on exposure to commercial cypermethrin. *Environmental Toxicology* **30**, 597-606. <https://doi.org/10.1002/tox.21937>
- [49] Belfroid, A., Seinen, W., Van Gestel, K., & Hermens, J., 1993, The acute toxicity of chlorobenzenes for earthworms (*Eisenia andrei*) in different exposure systems. *Chemosphere* **26**, 2265-2277. [https://doi.org/10.1016/0045-6535\(93\)90352-6](https://doi.org/10.1016/0045-6535(93)90352-6)
- [50] Kula, H., & Larink, O., 1997, Development and standardization of test methods for the prediction of sublethal effects of chemicals on earthworms. *Soil Biology and Biochemistry* **29**, 635-639. [https://doi.org/10.1016/S0038-0717\(96\)00030-2](https://doi.org/10.1016/S0038-0717(96)00030-2)
- [51] Acda, M.N., 2007, Toxicity of thiamethoxam against Philippine subterranean termites. *Journal of Insect Science* **7**, 1-26. <https://doi.org/10.1673/031.007.2601>
- [52] Rahmani, S., & Bandani, A.R., 2013, Sublethal concentrations of thiamethoxam adversely affect life table parameters of the aphid predator, *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae). *Crop Protection* **54**, 168-175. <https://doi.org/10.1016/j.cropro.2013.08.002>
- [53] Moser, S.E., & Obrycki, J.J., 2009, Non-target effects of neonicotinoid seed treatments; mortality of coccinellid larvae related to zoophytophagy. *Biological Control* **51**, 487-492. <https://doi.org/10.1016/j.biocontrol.2009.09.001>
- [54] Kilpatrick, A.L., Hagerty, A.M., Turnipseed, S.G., Sullivan, M.J., & Bridges Jr, W.C., 2005, Activity of selected neo-nicotinoids and dicotophos on non-target arthropods in cotton:

- implications in insect management. *Journal of Economic Entomology* **98**, 814-820. <https://doi.org/10.1603/0022-0493-98.3.814>
- [55] Viswanathan, R., 1997, Physiological basis in the assessment of ecotoxicity of pesticides to soil organisms, *Chemosphere* **35**, 323-334. [https://doi.org/10.1016/S0045-6535\(97\)00158-6](https://doi.org/10.1016/S0045-6535(97)00158-6)
- [56] Mostert, M.A., Schoeman, A.S., & Merwe, M.V.D., 2002, The relative toxicities of insecticides to earthworms of the *Pheretima* group (Oligochaeta). *Pest Management Science* **58**, 446-450. <https://doi.org/10.1002/ps.473>
- [57] Zhang, L., Dong, L., Shi, S., Zhou, L., Zhang, T., & Huang, Y., 2009, Organochlorine pesticides contamination in surface soils from two pesticide factories in Southeast China. *Chemosphere* **77**, 628-633. <https://doi.org/10.1016/j.chemosphere.2009.08.055>
- [58] Daam, M.A., Leitão, S., Cerejeira, M.J., & Sousa, J.P., 2011, Comparing the sensitivity of soil invertebrates to pesticides with that of *Eisenia fetida*. *Chemosphere* **85**, 1040-1047. <https://doi.org/10.1016/j.chemosphere.2011.07.032>
- [59] Wang, Y., Cang, T., Zhao, X., Yu, R., Chen, L., Wu, C., & Wang, Q., 2012, Comparative acute toxicity of twenty-four insecticides to earthworm, *Eisenia fetida*. *Ecotoxicology and Environmental Safety* **79**, 122-128. <https://doi.org/10.1016/j.ecoenv.2011.12.016>
- [60] Darling, C.T., & Thomas, V.G., 2005, Lead bioaccumulation in earthworms, *Lumbricus terrestris*, from exposure to lead compounds of differing solubility. *Science of the Total Environment* **346**(1-3), 0-80. <https://doi.org/10.1016/j.scitotenv.2004.11.011>
- [61] Wang, Y., Cang, T., Zhao, X., Yu, R., Chen, L., Wu, C., & Wang, Q., 2012, Comparative acute toxicity of twenty-four insecticides to earthworm, *Eisenia fetida*. *Ecotoxicology and Environmental Safety* **7**, 122-128. <https://doi.org/10.1016/j.ecoenv.2011.12.016>
- [62] Yan, X., Wang, J., Zhu, L., Wang, J., Li, S., & Kim, Y.M., 2021, Oxidative stress, growth inhibition, and DNA damage in earthworms induced by the combined pollution of typical neonicotinoid insecticides and heavy metals. *Science of the Total Environment* **754**, 141873. <https://doi.org/10.1016/j.scitotenv.2020.141873>
- [63] Li, M., Yang, Y., Xie, J., Xu, G., & Yu, Y., 2019, In-vivo and in-vitro tests to assess toxic mechanisms of nano ZnO to earthworms. *Science of the Total Environment* **687**, 71-76. <https://doi.org/10.1016/j.scitotenv.2019.05.476>
- [64] Liu, X., Hu, G., Chen, Y., Li, X., Xu, X., Li, S., Pei, F., & Wang, S., 2018, High-resolution multi-temporal mapping of global urban land using Landsat images based on the google earth engine platform. *Remote Sensing of Environment* **209**, 227-239. <https://doi.org/10.1016/j.rse.2018.02.055>
- [65] Ye, X., Xiong, K., & Liu, J., 2016, Comparative toxicity and bioaccumulation of fenvalerate and esfenvalerate to earthworm *Eisenia fetida*. *Journal of Hazardous Materials* **310**, 82-88. <https://doi.org/10.1016/j.jhazmat.2016.02.010>

- [66] Schreck, E., Geret, F., Gontier, L., & Treilhou, M., 2008, Neurotoxic effect and metabolic responses induced by a mixture of six pesticides on the earthworm *Aporrectodea caliginosa nocturna*. *Chemosphere* **71**, 1832-1839. <https://doi.org/10.1016/j.chemosphere.2008.02.003>
- [67] Andrade-Herrera, M., Escalona-Segura, G., González-Jáuregui, M., Reyna-Hurtado, R.A., Vargas-Contreras, J.A., & Rendón-von Osten, J., 2019, Presence of pesticides and toxicity assessment of agricultural soils in the Quintana Roo Mayan zone, Mexico using biomarkers in earthworms (*Eisenia fetida*). *Water, Air and Soil Pollution* **230**, 1-13. <https://doi.org/10.1007/s11270-019-4105-1>
- [68] Wu, F., Wan, J.H.C., Wu, S., & Wong, M., 2012, Effects of earthworms and plant growth-promoting rhizobacteria (PGPR) on availability of nitrogen, phosphorus, and potassium in soil. *Journal of Plant Nutrition and Soil Science* **175**, 423-433. <https://doi.org/10.1002/jpln.201100022>
- [69] Zelikoff, J.T., Wang, W., Islam, N., Twerdok, L.E., Curry, M., Beaman, J. & Flescher, E., 1996, Assays of reactive oxygen intermediates and antioxidant enzymes: potential biomarkers for predicting the effects of environmental pollution. *Aquatic Toxicology* **1**, 287-306. https://scholar.google.com/scholar_1
- [70] Oi, M., 1999, Time-dependent sorption of imidacloprid in two different soils. *Journal of Agricultural and Food Chemistry* **47**, 327-332. <https://doi.org/10.1021/jf980658k>
- [71] Capowiez, Y., & Bérard, A., 2006, Assessment of the effects of imidacloprid on the behaviour of two earthworm species (*Aporrectodea nocturna* and *Allolobophora icterica*) using 2D terraria. *Ecotoxicology and Environmental Safety* **64**, 198-206. <https://doi.org/10.1016/j.ecoenv.2005.02.013>
- [72] Torkhani, A.L., Eržen, N.K., Kolar, L., Celesetina, T.V., & Leštan, D., 2011, Does ivermectin attract earthworms? *Journal of Soils and Sediments* **11**, 124-128. <https://link.springer.com/article/10.1007/s11368-010-0284-5>
- [73] Yasmin, S., & D' Souza, D., 2010, Effects of pesticides on the growth and reproduction of earthworm: a review. *Applied and Environmental Soil Science* **1**, 678360. <https://doi.org/10.1155/2010/678360>
- [74] Rao, J.V., Pavan, Y.S., & Madhavendra, S.S., 2003, Toxic effects of chlorpyrifos on morphology and acetylcholinesterase activity in the earthworm, *Eisenia foetida*. *Ecotoxicology and Environment Safety* **54**(3), 296-301. [https://doi.org/10.1016/S0147-6513\(02\)00013-1](https://doi.org/10.1016/S0147-6513(02)00013-1)
- [75] Song, Y., Zhu, L.S., Wang, J., Wang, J.H., Liu, W., & Xie, H., 2009, DNA damage and effects on antioxidative enzymes in earthworm (*Eisenia fetida*) induced by atrazine. *Soil Biology and Biochemistry* **41**, 905-909. <https://doi.org/10.1016/j.soilbio.2008.09.009>

- [76] Madhavendra, S.S., 2003, Toxic effects of chlorpyrifos on morphology and acetylcholinesterase activity in the earthworm, *Eisenia foetida*. *Ecotoxicology and Environmental Safety* **54**(3), 296-301. [https://doi.org/10.1016/s0147-6513\(02\)00013-1](https://doi.org/10.1016/s0147-6513(02)00013-1)
- [77] Roberts, B.L. & Wyman Dorough, H., 1984, Relative toxicities of chemicals to the earthworm *Eisenia foetida*. *Environmental Toxicology and Chemistry* **3**(1), 67-78. <https://doi.org/10.1002/etc.5620030109>