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AN ANALYSIS OF THE IMPACT OF SEVERAL PESTICIDES ON EARTHWORMS

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Abstract

India generates around 139,000 metric tons of malathion insecticide annually, demonstrating the pervasiveness of pesticides in modern farming practices. There are a lot of facilities in the country, including around 219 technical grade manufacturing units, almost 4000 formulation units, and more. There was a comprehensive earthworm survey done at five separate sites every month. Six different kinds of earthworms were gathered from various places, including gardens, paddy fields, sugarcane fields, ponds, and edible tubers. This study focuses on the toxicity assessment of the organophosphate pesticide malathion using the earthworm species *Eudrilus eugeniae* as a model organism. The mortality rate at different pesticide concentrations was measured over a 24- and 48-hour period using the filter paper technique to investigate contact toxicity. After 14 days of soil toxicity trials in artificial soil, the worms who made it through the ordeal had lost weight and, in some instances, transformed physically. The vermicast of pesticide-treated earthworms was tested for the enzyme activities of catalase, protease, and superoxide dismutase (SOD). In order to learn more about the negative effects of malathion on earthworms, this study compares worm populations in soils that have been polluted with pesticides versus unsoiled soils. Important information for determining earthworm-harming concentrations of malathion is provided by the results.

Keywords: Pesticides, Earthworms, Malathion, Contact toxicity, Enzyme activity.

Introduction

Contamination of soil and damage to large populations are common results of pesticide overuse and agricultural expansion [1]. Approximately $38 billion is spent on pesticides every year internationally [2]. Agricultural land should only be treated with pesticides that are toxic to the target species, partly biodegradable, and ecologically safe [3]. Unfortunately, most pesticides don't target specific organisms, meaning they kill harmless species that are essential to different ecosystems. Public awareness of environmental issues was sparked by the publication of Silent Spring by Rachel Carson in 1962, which brought this subject into the spotlight. Along the developmental scale, bioaccumulation affected humans and a number of other animals as a result of modern farming practices.

The reproductive, neurological, respiratory, and osmoregulatory systems of several animals are known to be affected by pesticides, which also change their appearance, behavior, and physiological composition [4]. To evaluate the impact of pesticides, earthworms are used as a model organism due to their susceptibility to these compounds [5]. As an important indicator of soil toxicity, contamination, and general metabolism, they

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constitute an integral component of soil biomass [6]. As a matter of fact, some species of earthworm have been used as bio-indicators, which means they are used to evaluate chemical pollution in the environment [7]. Ring testing and the standardization of toxicity tests using earthworms as the test species are two of the many studies that have substantially aided toxicology research. Environmental toxicity studies, risk assessments, and quality of the environment inspections have all made use of earthworms as indicator species [8]. Unfortunately, earthworms, which are essential to soil health, are frequently ignored when these chemicals are applied, which can lead to a reduction in their activity [9].

Soil is improved when earthworms decompose organic matter, releasing nutrients that plants need. You may find them all over soil, and they make up a big chunk of the invertebrate population. These play a significant role in nitrogen cycle and substantially boost soil ecosystem biological activity. The nitrogen cycle in soil is one of the many physical and biological properties impacted by earthworm activities. It should be noted, though, that the extent to which this effect is felt is determined by ancillary factors including weather, plant and animal life, and soil type. In addition, they make sure the soil stays healthy by digging and casting [10]. Soil permeability may be improved by adding costly minerals such as gypsum or calcium when earthworm populations are low. This is because earthworms boost soil water absorption by creating complex networks of tunnels. Soil pH is stabilized by the combined actions of carbonic acid as a buffer and the calciferous gland of earthworms. In terms of soil fertility and permeability, earthworms are well-known to play a major role [11].

Literature of Review

(Yasmin & D'Souza, 2010) [12] The impact of pesticides on earthworm life cycle stages and offspring have been extensively documented in scholarly literature. One possible explanation is that earthworms can provide valuable information on the chemical toxicity of soil organisms. The importance of earthworms as a food supply for many terrestrial vertebrate species, including birds and small mammals, and as a component in the biomagnification of numerous soil pollutants makes this issue worthy of research. Rather of more nuanced objectives like reproductive output, death has been the endpoint of most investigations. More sensitive testing methods are needed for polluted soils with lower (sub lethal) quantities of pollutants, in contrast to the acute (mortality) test, which can readily measure greater concentrations of pollutants.

(Pelosi et al., 2014) [13] Earthworms perform essential soil activities that keep many ecosystem functions running smoothly. However, these services are essential to the agro ecosystem's sustainability, even as intense cultural practices like pesticide usage may diminish them. Some studies have looked at the effects of pesticides on earthworms in the literature. We combed through those articles to find out how sensitive earthworms are to pesticides, how effective pesticide response indicators are, and where we still need knowledge. With the exception of naturally occurring chemicals and metals, our primary

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business is dealing with earthworm species from Europe and other legally permissible European goods. Physiology, gene expression, life-history features, population density, and behavior are some of the many levels of organization that are taken into account. At the community level, things are looked at in terms of biomass and density. According to our findings, pesticides have an impact on earthworms in every conceivable way. Examples of behavioral changes brought about by pesticide exposure include changes in feeding habits, disruption of enzyme systems, increased death rates, decreased fertility and growth, and overall decreases in population density and biomass. Fungicides and insecticides are the two most dangerous compounds because of their effects on reproduction and survival, respectively.

Materials and methods

Site description

Five different sites in India were visited at regular intervals from May to October 2024 to perform an earthworm research. The agricultural practices dictated the selection of the three primary fields: paddy (site 1), sugarcane (site 2), and edible tuber (site 3). Site 5 is a garden, and site 4 is a pond; both of these areas were devoid of pesticides so that we could compare the levels of contamination. By hand sorting, we were able to collect all of the earthworm species that were available.

Fig. 1. Sampling site

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Sampling protocol

Every month from May to October 2024, a total of five sites were surveyed for earthworms. At each site, eight quadrants measuring 0.50 m × 0.50 m × 0.30 m were used for the survey. Following the extraction of the soil core, the worms were manually sorted in accordance with the method described in reference [14]. The dirt was ground into lumps and then passed through fingers to separate the worms. The soil's pH, electrical conductivity (EC), organic carbon (OC), and total nitrogen (TN) were determined using standard methods. The carbon-to-nitrogen ratio, or C/N ratio, was calculated by dividing the predicted soil carbon percentage by the predicted soil nitrogen %. Polythene bags containing mother soil that had been withdrawn from the collection site were used to retain the worms, which were subsequently sorted according to species. Each quadrant was treated individually. The earthworms were preserved using a 5-10% formalin solution once they were delivered to the lab, following the suggested protocol [18].

Earthworm

In terms of earthworm test species, the oligochaete *E. eugeniae* is highly recommended by the OECD and is also considered one of the best worm species for composting [19-22]. G. T. Patil College's vermiculture unit in Nandurbar supplied adult earthworms with clitella that were well-developed, weighing 600-800 mg. The worms were subsequently raised in a controlled environment using synthetic soil, following the guidelines laid out by the OECD (OECD, 1984).

Pesticides

Chemically, malathion ($C_{10}H_{19}O_6PS_2$) is an organophosphorus insecticide that is derived from phospho-di-tuning acid. This pesticide is widely used in agricultural regions worldwide to protect a wide range of crops against sucking and eating insects, including fruits, vegetables, and flowers [23]. This is the first contact-based broad-spectrum pesticide with a continuous and rapid action. Almost every organ in the body is susceptible to this poison's entry points, which include the skin, lungs, gastrointestinal tract, and mucous membranes [24]. This study made use of commercial malathion, which is both dangerous and only 57% pure due to the introduction of certain impurities during manufacture. The US EPA has said that, due to the toxin's global consumption, the content in drinking water should not exceed 0.1-0.2 mg/L and that the proper technique should be followed to remove it from water sources [25, 26]. A litter of neutral pH distilled water was used to dissolve 1.639% malathion (a concentration of 1.07 kg/L) in order to make the solutions. We utilized a malathion toxin stock solution that was 1000 mg/L. The following concentrations of malathion were employed in the preparation and usage of samples: 1000 mg/L, 500 mg/L, and 100 mg/L.

Toxicity test methods

Contact toxicity test

Acute toxicity testing followed the procedures outlined in OECD standard for chemical testing no. 207 (1984) [27]. This is a quick screening method to determine if the

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chemical might be harmful to earthworms. A 14 cm diameter and 2 cm tall transparent plastic spherical container served as the test petri plate. Once the Whatman No. 1 round filter sheets were trimmed to size, the filter paper covered both sides. Four experimental plates were created and sprayed with 0.1 to 1 ml of the prepared test solution, respectively, using the solution that had been prepared. A single control test, consisting of one milliliter of de-ionized water, was produced. A single earthworm was placed on each circular plate for each treatment. The test was performed on adult earthworms that weighed between 650 and 750 mg when wet and were clitellum positive. After being rinsed with deionized water, the earthworms were blotted on filter paper to remove any residual stomach contents. Then, after three hours of setting, they were transferred to a test circular plate. To puncture the tiny holes in the round containers, a plastic sheet was inserted using needles. The trials were conducted over 48 hours in total darkness at a temperature of 28 ± 2^0 C. A little mechanical shock to the earthworm's head was used to check for death after 48 hours.

Soil toxicity test

A blend of 50% kaolinite, 10% ground sphagnum peat, and 70% fine sand made up the synthetic soil, as reported by the OECD (1984, 2004). We used a tiny bit of calcium carbonate to bring the pH level down to 6.0 ± 0.5. The toxicity tests required an adjustment of the water content to 35% of the dry weight. The insecticide, 10 mL of acetone, and a little amount of fine quartz sand were combined for each concentration that was evaluated. To remove the acetone, the sand and pre-moistened synthetic dirt were combined in a household mixer and left to mix for at least an hour. The synthetic soil was amended with distilled water until it reached the target final moisture content. Ten fully grown earthworms were placed in each of five 500 ml (63.6 cm2) glass jars together with 0.65 kg of dirt or half a kilogram of dry synthetic soil. A control group that was similarly prepared but did not use pesticide contained only 10 milliliters of acetone. To ensure proper air circulation, the jars were kept in an environment with a consistent light source ranging from 400 to 800 lx, a temperature of 20 ± 1 °C, and a relative humidity of 80 to 85%. The jars' polypropylene lids were left slightly ajar. At 7 and 14 days post-therapy, we assessed mortality. At the end of each experiment, the control group's mortality rate shouldn't be greater than 10%. In the preliminary experiments, several concentrations were used to determine the levels that caused 0-100% mortality, including 100, 500, and 1000 mg kg-1 dry soil.

Enzyme assays

Superoxide dismutase (SOD)

In order to determine superoxide dismutase activity, the method described in [28] was followed. Finding out how much adrenaline's autooxidation activity can be inhibited by SOD at 30 °C and pH 10.2 is part of this process. A single unit of superoxide activity is defined as the amount of SOD needed to prevent adrenaline auto-oxidation by 50%. The analysis required 3.0 ml of a 50 M Na_2CO_3 buffer in addition to 0.02 ml of the sample. The absorbance measurement was then kept at 480 nm for three to five minutes after adding 0.03

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ml of the epinephrine stock solution. To adjust the background, we used a blank that had all the chemicals but no sample.

Catalase (CAT)

We used a modified version of Beers and Sizer's approach to measure CAT activity. Each tube was then filled with 2.5 ml of pH 7.0 phosphate buffer, 2 ml of H_2O_2 solution, and 0.5 ml of sample. As a substrate, we used 30 mM of hydrogen peroxide (H_2O_2) and monitored the spectrophotometric drop in H_2O_2 concentration at 22 °C for 1 minute at 240 nm. One unit of enzyme activity degrades 1 mM H_2O_2 per minute, and this activity was represented as units per mg of protein.

Protease activities

To determine protease activity photometrically, the method described in [29] was employed. One millilitre of pH 7.0 phosphate buffer was added to a 50 millilitre Erlenmeyer flask holding one gram of substrate sample and left to incubate at 22° ± 2 °C for twenty-four hours. The mixture of 1% (w/v) skimmed milk and prepared agar was then put to petri dishes. Three millimetres-diameter holes were punched through the plates after they had solidified for thirty minutes. The prepared sample was added to 75 ml of wells that were cut to 6.3 mm in diameter after solidification. These plates were incubated at 37 °C overnight before imaging.

Result

Sampling site and soil parameters

Three sites of agricultural land poisoned with pesticides and two sites of unpolluted vegetation were sampled. Likewise, litter mass and soil characteristics differed greatly over the gradient.

In contrast to pesticide-contaminated areas, those without pollution had the highest C/N ratio. The trend of pH levels follows the same pattern as the C/N ratio. In contrast to the somewhat acidic pH of the pesticide-contaminated locations, the pH of the uncontaminated sites is slightly higher than neutral (Table 1).

Table-1. Physico-chemical parameters of the soil of study sites

Sr. No.	Parameters Observed	Study sites				
		Paddy field	Sugar cane	Edible tubers	Pond	Garden
1	pH	6.50 ± 0.30	6.20 ± 0.35	6.15 ± 0.22	7.12 ± 0.25	7.50 ± 0.26
2	Electrical conductivity (dS/m)	0.29 ± 0.04	0.17 ± 0.01	0.24 ± 0.02	0.27 ± 0.01	0.16 ± 0.01
3	C/N ratio (%)	22.5 ± 0.15	23.5 ± 0.45	22.5 ± 0.35	28.5 ± 0.25	28.00 ± 0.35
4	Organic matter content	65.30 ±	69.31 ±	66.74 ±	70.34 ±	74.33 ±

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	(%)	1.24	1.44	1.12	1.55	1.15
5	Soil moisture (%)	29.24 ± 1.11	30.45 ± 1.0	25.3 ± 1.15	22.4 ± 2.22	30.12 ± 0.11
6	Soil temperature (°C)	20.34 ± 6.63	18.3 ± 1.45	16.31 ± 5.65	21.45 ± 6.56	18.09 ± 6.43

Species density and richness

Table 2 shows the available earthworm species and families from all five sites. In all, six different earthworm species from three different families have been discovered thus far. All of these earthworms are native to the area. The pond and garden soil that were not polluted had a high species richness and population density of 42 and 6, respectively. The sites with the lowest species richness and population density indices were those that were impacted by pesticides, such as rice fields and edible tubers, with indices of 20 and 4, respectively.

Table 2. Different species of earthworm's population from the five different sites.

Earthworm species collected	**Sampling site**				
	1	**2**	**3**	**4**	**5**
FAMILY: ACANTHODRILIDAE					
Lumbricus rubellus (m2)	7	13	5	11	8
Eisenia fetida (m2)	0	0	5	5	10
Pheretima posthuma (m2)	4	6	4	10	0
FAMILY: OCTOCHAETIDAE					
Octochaetona sp. (m2)	6	4	0	6	8
FAMILY: MONILIGASTRIDAE					
Drawida chlorina (Bourne). (m2)	0	6	4	6	4
Drawida pellucida var. pallida Mich. (m2)	6	8	4	8	6
Species density (m2)	20	34	22	38	16
Species richness (m2)	6	7	6	4	5

Toxicity test methods

Contact toxicity test

Earthworms, or *E. eugeniae*, were subjected to a paper contact test to determine the contact toxicity of the pesticide malathion. Morphological abnormalities, including erectile dysfunction, coiling, and curling, were observed in all patients subjected to the highest dosages for 24 and 48 hours. At 24 and 48 hours post-exposure, Table 3 displays the fatality rates for concentrations of 0.1, 0.75, 0.5, and 1 ml. After 24 hours of exposure, the worms in the 0.75 and 1.0 ml concentrations died, and in all concentrations except 0.1 ml, they died after 48 hours.

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Table 3. The mortality rate of earthworm by contact toxicity test after exposure of 24 h and 48 h.

Concentration	After 24 h	After 48 h
Control	Alive	Alive
0.1 ml	Alive	Alive
0.25 ml	Alive	Dead
0.5 ml	Alive	Dead
0.75 ml	Dead	Dead
1.0 ml	Dead	Dead

Soil toxicity test

Figure 2 shows that no mortality occurred after exposure to the soil treated with insecticide at levels of 100, 500, and 1000 mg/kg. Lower body weight and, in some instances, morphological abnormalities were seen in earthworms that survived seven to fourteen days in soil treated with low and high rates of malathion (Fig. 2).

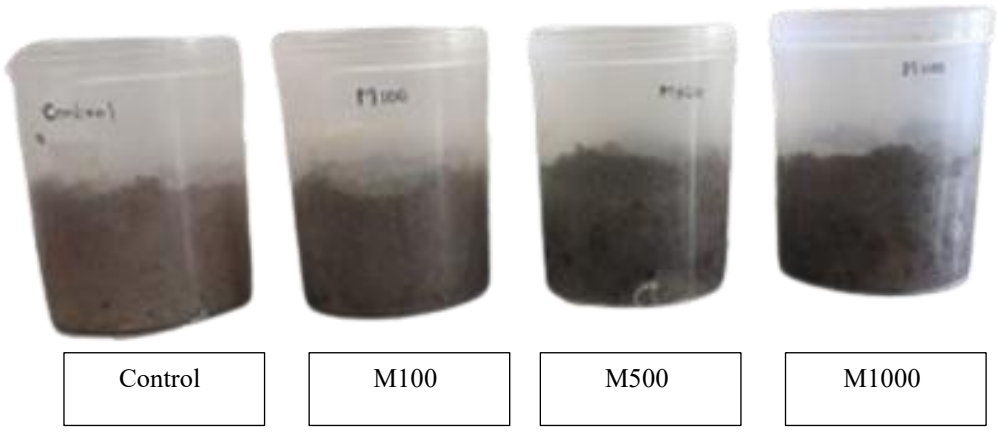

Fig. 2. Soil toxicity test for 7th and 14th day after exposure of pesticide.

Enzyme assay

Superoxide dismutase (SOD)

Table 4 shows that compared to the control group, M100, M500, and M1000 had significantly decreased SOD activity. This may occur if the earthworms' reduced activity on castings treated with pesticides is the cause.

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Table 4. SOD activity of vermicast of pesticide-treated earthworm.

Groups	Auto-oxidation rate (units/ 35 s)	SOD activity (mean ± SD)
Control	0.023	0.023 ± 0.00
	0.023	
	0.023	
	0.023	
	0.023	
M100	0.019	0.019 ± 0.00
	0.019	
	0.019	
	0.019	
	0.019	
M500	0.020	0.019 ± 0.69
	0.019	
	0.019	
	0.019	
	0.020	
M1000	0.022	0.021 ± 0.6
	0.021	
	0.021	
	0.021	
	0.021	

Catalase (CAT)

The catalase activities of M100, M500, and M1000 in Vermicast increased with increasing concentration. Table 5 shows that catalase activity increased when comparing the groups to the control.

Table 5. Catalase activity of vermicast of pesticide-treated earthworm.

Groups	Catalase activity (units/ 35 s)	Catalase activity (mean ± SD)
Control	1.015	0.983 ± 0.021
	0.992	
	0.973	
	0.968	
	0.966	
M100	1.022	1.006 ± 0.009
	1.007	
	1.002	
	0.999	
	1	
M500	1.601	1.516 ± 0.048

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	1.514	
	1.497	
	1.489	
	1.481	
M1000	1.876	1.759 ± 0.230
	1.784	
	1.733	
	1.708	
	1.693	

Protease activities

It was found that the casein/skimmed milk agar plates had a proteolysis zone. When compared to the control, the clear zone that developed in the plate indicated the protease activity. In comparison to the control, there is a decrease in zone formation and an increase in concentration."

Discussion

There is evidence of species aggregations in five different types of vegetation: gardens, ponds, paddy fields, edible tubers, and sugarcane plantations. This means that in each of these five types of vegetation, *L. rubellus and D. pellucida pallida* were detected. These results are good for unpolluted regions like gardens and ponds, but they are bad for pesticide-polluted areas like edible tubers, sugarcane fields, and paddy. Species richness and density values are available at all five sites. This finding was supported by [30], which asserted that the continuous use of synthetic pesticides had polluted the ecosystem, particularly the soil, and caused the extinction of useful species such as plants, bees, earthworms, and spiders. This experiment documented a decline in earthworm populations in the terrestrial ecosystem.

Due to the use of low input farming techniques, native species in eastern Gujrat were discovered to be well-suited to agroecosystems [31]. The research looked at 44 species, 36 of which were native and 8 of which were foreign. Of the native species, 25 were found exclusively in controlled environments. Reproduction biology of eight tropical earthworm species collected from rubber plantations in Madhya Pradesh has been examined [32]. Out of the eight species, three were native falcons and one was a peregrine falcon. The North Maharashtra region is home to ten distinct earthworm species, spanning seven taxa and six families [33]. Similarly, we found six different forms of earthworms in five different plant species throughout our experiment. Up to this point, studies have shown no correlation between soil temperature and earthworm population size, suggesting that soil temperature has a detrimental effect on ecosystem earthworm populations [34]. The distribution and survival of earthworm species were directly impacted by the availability of organic carbon, soil moisture, and pH neutrality, according to an analysis of the physico-chemical properties of the soil and weather in the research region. In zero-tillage soils treated with either annual or perennial residual mulch, the physicochemical properties of the castings were unchanged,

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including pH, EC, organic carbon, total nitrogen, accessible phosphorus, potassium, sodium, calcium, and magnesium [35]. Soil organic matter concentration is positively correlated with earthworm population density [36]. There is a positive correlation between soil organic carbon concentration and earthworm biomass and population density.

Unfortunately, there are still a lot of pesticide classes that lack lower-tier testing—specifically, toxicity studies on soil fauna. Such testing is also essential for the appropriate implementation of legislation that controls plant protection goods [37]. Pesticides can harm earthworms in two ways: either when they come into touch with them or when they eat soil that has been poisoned. The epidermis is the primary entry point for these toxins into the coelomic fluid, which carries them throughout the body. Previous studies have shown that earthworms absorb toxicants through their skin because of the frequent contact they have with contaminated soils [38–40]. Recent studies have shown that the skin of *E. eugeniae* became more sensitive to malathion as exposure time and concentration rose, causing contact toxicity. After 24 and 48 hours of applying Malathion, several morphological signs were seen, such as coiling, aberrant swelling, mucus secretion, bleeding, and fragmentation. In the aftermath of Garcia's (2004) development of the OECD's Tropical Artificial Soil (TAS) modified soil (OECD, 1984), ecotoxicological testing were conducted. As noted before, the soil humidity was adjusted by spraying pesticides into the soil in a way that evenly distributed the pesticide solutions or suspensions. The control group received just deionized water. An artificial soil test, which mimics earthworms' actual environment as closely as possible, allows for the absorption of insecticides mostly by the stomach [41]. Thus, a synthetic soil test is superior for determining the toxicity of a pesticide to earthworms. It is well-known that organochlorine pesticides interfere with acetylcholine breakdown, which changes cholinergic signalling by blocking the AChE enzyme's activity [42-44]. Earthworms are either not affected by or just slightly affected by some organophosphates, such as azinphosmethyl, diazinon, fenitrothion, and malathion [45]. Carbamates are more toxic to *E. fetida* than organophosphates, even though both chemicals inhibit AChE; this is due to the fact that carbamates' inhibitory pathway is reversible. This study confirmed the earlier findings that there was no death caused by contact with the pesticide-treated soil at any of the three dose levels tested (100, 500, and 1000 mg/kg).

Several factors, including microbial populations, organic carbon (OC), soil temperature, moisture content, total nitrogen (N), and potassium (K) levels, are positively correlated with enzyme activity [46, 47]. Research has shown that casts made by earthworms contain more enzymes than the soil around them, including carbohydrase, protease, phosphatase, and dehydrogenase. The enzyme content of earthworm castings produced from organic waste has not been studied beyond the limited amount of study on soil casts. Based on the existing results, the *E. eugeniae* vermicast showed elevated enzyme activity. The superoxide dismutase (SOD), the first line of defense in the body, converts superoxide to hydrogen peroxide (H2O2). With respect to the present study, the control group showed significantly higher SOD activity than M100, M500, and M1000. This study demonstrated

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that the earthworm's defense mechanism, catalase activity, which is essential for detoxifying H2O2, would be affected by an increase in pesticide concentration. It was also shown that this behavior become more intense as people got older. As an antioxidant defense system, it is one of the enzymes that prevents cell damage caused by free radicals. Hydrogen peroxide may be decomposed into oxygen and water by the enzyme peroxisomal hydroperoxide catalase [50]. In contrast to the control, the experimental groups exhibited concentration-dependent increases in catalase activity.

The castings of *Allolobophora caliginosa* exhibited an increase in protease activity [49]. The higher phosphatase activity of earthworm castings compared to un-ingested soil is likely due to the higher microbial biomass and high phosphate production by microorganisms, together with the increased inorganic P produced by the mineralization of organic P. This study found that as concentration increased, protease activity decreased, suggesting that the vermicast's microbial biomass had decreased.

Conclusion

According to this study, earthworm populations are much lower in pesticide-contaminated fields when contrasted with non-polluted areas. We found that the harmful levels of the insecticide malathion increased somewhat with increasing concentrations when we conducted toxicity tests. After being treated with pesticides, the vermicast of earthworms showed changes in enzyme activity, particularly in SOD, CAT, and protease. This suggests that the vermicast had less microbes.

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