

A Field Study of Pesticide Pollution in Soil

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Abstract

The current work examines the sublethal effects of four widely used pesticides on the gut histology and enzymatic activity of the epigeic earthworm *Perionyx excavatus*: pendimethalin, pretilachlor, dimethoate, and cypermethrin. Various biochemical markers, such as acetylcholinesterase, alkaline phosphatase, and acid phosphatase, were evaluated during field research in Madhya Pradesh's Chhatarpur area to evaluate the physiological reactions of earthworms exposed to these substances. According to the data, the levels of enzyme activity in the treated samples were much lower than those in the control, with the effects of dimethoate and cypermethrin being the most noticeable. In earthworms exposed to pesticides, histological analysis of the chloragogen tissue lining the intestine also showed obvious damage and distortion, with cypermethrin causing the most tissue degradation. These results emphasise the need for more environmentally friendly pest control techniques and the harmful effects of agricultural pesticides on non-target soil species.

Keywords: Soil, Pesticides, Earthworms, *Perionyx excavates*, Enzyme.

INTRODUCTION

The xenobiotic assault by pesticides on ecosystems, non-target species, humans, and civilisation began with this unfortunate event. Written in the early 1960s by Rachel Carson, the fragile and melancholy "Silent Spring" set out to show how organochlorine insecticides like DDT had caused very negative consequences. In subsequent years, beginning in 1972, the use of pesticides containing organohlorine was outright banned in most nations. However, the usage of synthetic pesticides did not decrease. [1]

organophosphates, carbamates, blended formulations, and pyrethroids supplanted organochlorine as the primary pesticide. [2] Crop output, both food and cash crops, has to rise to keep up with the world's rapidly expanding population. Agriculture entered its modern age with the introduction of high-yielding crop varieties, community farming, and new technical equipment. Agrochemicals came into their own as a result of these innovations, which paved

the way for new farming techniques. [3]"Ecobichon" was published in 2001. Higher application rates and ever-increasing potency of insecticides were two of the many reasons that contributed to the pesticide industry's meteoric rise to new heights of expansion. The farmers saw a quick payoff for their efforts because of the abundant harvest. However, there were a lot of pesticides in the environment. [4]

No one can deny the importance of pesticides in reducing the harm that pests inflict to fruits and vegetables. [5] Ecosystems have suffered greatly due to the careless and chronic use of various pesticides over the last several decades. This is when environmental pollution became a critically important issue. Testing various foods and water sources for pesticide presence was one of the most important pieces of research. [6]

Pesticides in agricultural contexts disproportionately impact earthworms, the most visible and vulnerable non-target soil organisms. [7] They are particularly vulnerable to the pesticides used in agricultural production because they make up a significant portion of the soil's invertebrate biomass—up to 92%. Numerous studies have shown the crucial role of earthworms in nutrient cycling, litter decomposition, and soil formation. [8]

RESEARCH METHODOLOGY

Experimental investigation was conducted in Field studies.

- **Site Selection For Field Data Collection**

The Chhatarpur district in Madhya Pradesh, where SKU is located, was the site of the field tests.

A hot tropical monsoon pattern describes the area climate. Daytime highs may reach 40°C, and the summer season begins in April and continues until about the middle of June. The monsoon season, which begins in the middle of June and lasts well into September, is responsible for the majority of the annual rainfall of around 1500 millimetres. Winter, which begins in December and continues until the beginning of March, often has temperatures between 10 and 14 degrees.

- **Microorganism Used For Testing**

The species used for this investigation are the epigeic earthworms *Perionyx excavates*. Julika (1986, 1988) estimates that just over 500 species of earthworms call India home. According to Chanda et al. (2003), the Chhatarpur district in Madhya Pradesh is home to seventeen species that belong to two orders, six families, and thirteen genera. A widespread species in India, *Perionyx excavates* is native to the Chhatarpur area.

An overview of the chosen specimens' systematic placement and biology

Earthworms of the genus *Perionyx* and species *Perionyx excavatus* are members of the following taxonomic groups: phylum Annelida, class Oligochaeta, family Megascolicidae, order Haplotaxida.

The biology and distribution of *Perionyx excavatus*

Perionyx excavatus is the epigeic species of earthworm native to India. This species is widespread in India, from the southern plains to the northern Himalayas. Composite pits, locations for the disposal of organic waste from homes and businesses, and other such organically rich systems are perfect homes for *Perionyx excavatus*. Crop fields that get sewage also contain this. The length, width, and number of segments (125–180) of a *Perionyx excavatus* may vary from 25–185 mm, with a diameter of 2.7–7 mm. At 280–300 days, this species makes it through the year. Earthworms spin tuft-like extensions inside their S-shaped cocoons. "The juvenile," "non clitellate," and "clitellate" are the three noticeable morphological stages that earthworms go through after hatching.

- **Pesticides used**

This investigation made use of four pesticides in a variety of bioassays. The herbicides pendimethalin and pretilachlor, the organophosphate insecticide dimethoate, and the pyrethroid cypermethrin were the chemicals used. Indian farmers regularly apply all of these pesticides on their crops. In the acute toxicity bioassay, all four insecticides were used. To study the synergism between organophosphate insecticides and herbicides, researchers examined the combined effects of the herbicide pendimethalin and the organophosphate insecticide dimethoate on earthworm mortality. "We conducted chronic toxicity bioassays on all four pesticides after reviewing their acute toxicity data and considering their eco-

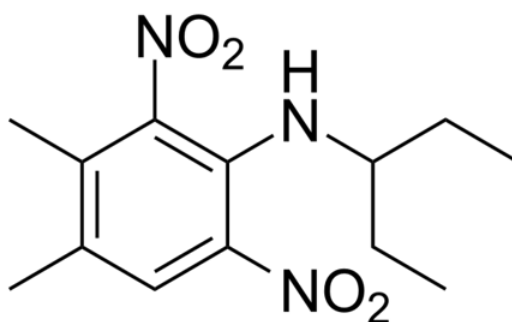
toxicological implications. The pesticides utilised in this investigation may be found in Table along with their sources of procurement.

Table 1: The study's pesticides

| Sl. No | Pesticide Group | Chemical Name | Commercial Name |
|--------|-----------------------------|------------------------|-----------------|
| 1 | Herbicides | Pendimethalin (30% EC) | DHANUTOP |
| | | Pretilachlor (50% EC) | RACER |
| 2 | Organophosphate Insecticide | Dimethoate (30% EC) | ROGORIN |
| 3 | Pyrethroid | Cypermethrin (10% EC) | USTAAD |

1. Pendimethalin

For the purpose of selective weed management in various crops, pendimethalin is used. Pendimethalin is a non-ionic dinitroalanine herbicide. According to Lee et al. (2000), it has a modest persistence in soil. The chemical structure of pendimethalin is shown below, and Table provides further technical information on the herbicide.



Pendimethalin

Table 2: Some characteristics of pendimethalin

| Parameters | Properties |
|----------------------|--|
| Chemical Name | 3,4-Dimethyl-2,6-dinitro-N-pentan-3-yl-alanine |

| | |
|-------------------------|---|
| Chemical Formula | C ₁₃ H ₁₉ N ₃ O ₄ |
| Melting Point | 47–58°C |
| Solubility | Soluble in water |
| Stability | Not degradable by microbes; strongly adsorbed to organic soil materials and clay. Ninety days is the half-life of soil. |

2. Pretilachlor

A selective herbicide belonging to the acetamide group, pretilachlor is used for the management of several annual grass and board leaf weed species (Dharumarajan, et al., 2008). According to Asokaraja and Ali (1995), Deepa (2002), and Tomoyoshi et al. (2004), it may be found in soil and water for an extended period of time and can also build up in plant components. The weather and the composition of the soil determine the residual characteristics. The photodecomposition and volatilisation processes disperse and deposit pretilachlor in the environment (Rai et al., 1999). The chemical structure of the herbicide is shown below, and Table provides further technical information regarding pretilachlor.

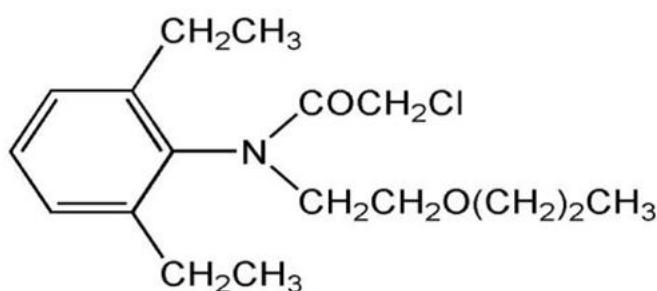


Table 3: Pretilachlor Properties

| Parameters | Properties |
|-------------------------|--|
| Chemical Name | 2-Chloro-N-(2,6-diethylphenyl)-N-(2-propoxyethyl)acetamide |
| Chemical Formula | C ₁₇ H ₂₆ ClNO ₂ |
| Melting Point | 47–58°C |
| Solubility | Soluble in water, benzene, hexane, methanol, etc. |
| Stability | Very stable in both water and dirt. About ten days is the half-life of soil. |

3. Dimethoate

An organophosphate pesticide and acaricide, dimethoate has several applications. As a class II moderately hazardous pesticide, dimethoate is quite mobile in dirt. Although it is not often detected in high concentrations in water, this pesticide is fairly persistent. But it's poisonous to birds in moderate to high doses, aquatic creatures in moderate doses, and honeybees in high doses (Gilbert, 2014). Although it degrades quickly, it is easily taken and disseminated by plants (Dauetrman, 1960). The chemical structure of dimethoate is shown below, and further technical information is provided in Table.

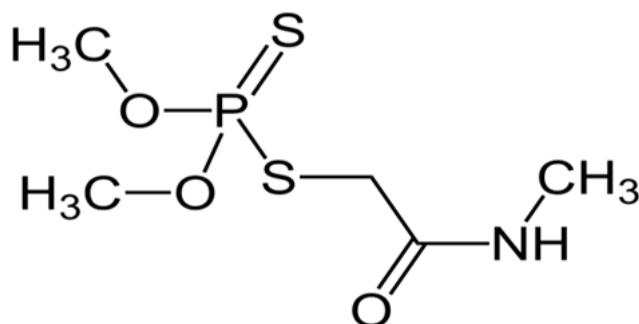
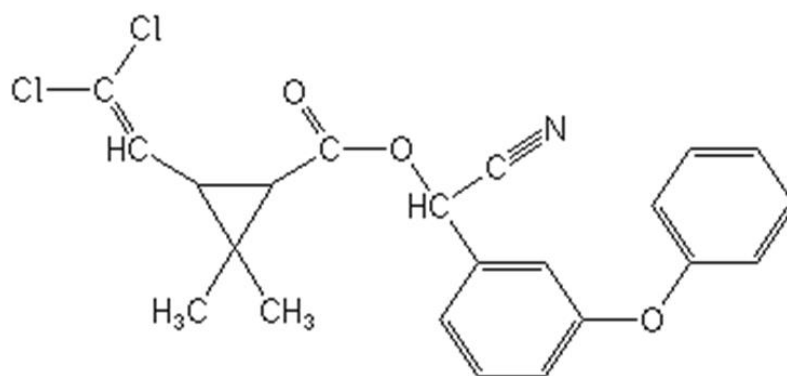


Table 4: Dimethoate Characteristics

| Parameters | Properties |
|------------------|--|
| Chemical Name | O,O-dimethyl S-[2-(methylamino)-2-oxoethyl] dithiophosphate |
| Chemical Formula | C ₅ H ₁₂ NO ₃ PS ₂ |
| Melting Point | 43–45°C |
| Solubility | Mixable with water |

4. Cypermethrin

Pyrethrum is an all-natural pesticide derived from dried Chrysanthemum flower heads; the active ingredient in pyrethrums is pyrethrin, an insecticide. The term "pyrethroid" describes a class of insecticides that include cypermethrin and other synthetic chemical compounds of pyrethrum. When it comes to controlling ectoparasites that infest livestock, sheep, and fowl, cypermethrin is a go-to herbicide. The broad-spectrum pesticide cypermethrin is useful against many different types of cotton, fruit, and vegetable crop pests. The assertion that cypermethrin is a wide range pesticide, meaning it kills both target and beneficial species, was made by Pascual and Perris in 1992. Frequent exposure to cypermethrin may cause insects to acquire a resistance, which makes the insecticide useless, according to Martinez-Cabrillo (1991). You may find the chemical structure of the pyrethroid cypermethrin below, along with detailed technical details in Table.



Cypermethrin

Table 5: The Cypermethrin Properties

| Parameters | Properties |
|-------------------------|---|
| Chemical Name | ((RS)- α -cyano-3-phenoxybenzyl (1RS)-cis, trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate) |
| Chemical Formula | C ₂₂ H ₁₉ Cl ₂ NO ₃ |
| Melting Point | 81–83°C |
| Solubility | Solubility in water: 0.009 mg/litre; soluble in organic solvents |
| Stability | Soil hydrolysis and photolysis break it down. Chemical half-life: 6-20 days; Biological half-life: less than 14 days. Hydrolysis of trans-isomers occurs 1.2-1.7 times more rapidly. (USDA, 1995) |

RESULTS AND DISCUSSION

- **Enzyme extrapolation in vivo utilising specific insecticides**

Acid phosphatase

Figure shows the effects of pretilachlor, cypermethrin, pendimethalin, and dimethoate on acid phosphatase activity. Enzyme levels of dimethoate, pendimethalin, pretilachlor, and cypermethrin were 11.6 ± 0.75 μ g PNP/mg protein/30 minutes in the pesticide treated set (T2), compared to 26.4 ± 1.25 μ g PNP/mg protein/30 mins in the control set (T1).

Table 6: Effects of Pendimethalin, Pretilachlor, Dimethoate, and Cypermethrin on the acid phosphatase activity of *Perionyx excavatus*: a one-factor analysis of variance

| Insecticide | Source of variation | df | Mean square | F | Significance |
|-------------|---------------------|----|-------------|--------|--------------|
| Dimethoate | Treatment | 2 | 207.07 | 207.07 | 0.000 |
| | Error | 6 | 1.00 | | |

| | | | | | |
|---------------|-----------|---|---------|---------|-------|
| Pendimethalin | Treatment | 2 | 149.97 | 164.802 | 0.000 |
| | Error | 6 | 0.910 | | |
| Pretilachlor | Treatment | 2 | 207.07 | 207.07 | 0.000 |
| | Error | 6 | 1.00 | | |
| Cypermethrin | Treatment | 2 | 308.280 | 308.280 | 0.000 |
| | Error | 6 | 1.000 | | |

Table 7: Acid phosphatase activity as a function of pesticide treatment: the least significant difference (LSD)

| Insecticides | Difference between | Mean difference | Significance |
|---------------------|---------------------------|------------------------|---------------------|
| Dimethoate | T1 & T2 | 11.30 | 0.000 |
| | T1 & T3 | 16.20 | 0.000 |
| | T2 & T3 | 4.90 | 0.001 |
| Pendimethalin | T1 & T2 | 9.20 | 0.000 |
| | T1 & T3 | 13.90 | 0.000 |
| | T2 & T3 | 4.70 | 0.001 |
| Pretilachlor | T1 & T2 | 11.30 | 0.000 |
| | T1 & T3 | 16.20 | 0.000 |
| | T2 & T3 | 4.90 | 0.001 |
| Cypermethrin | T1 & T2 | 14.80 | 0.000 |
| | T1 & T3 | 19.40 | 0.000 |
| | T2 & T3 | 4.60 | 0.001 |

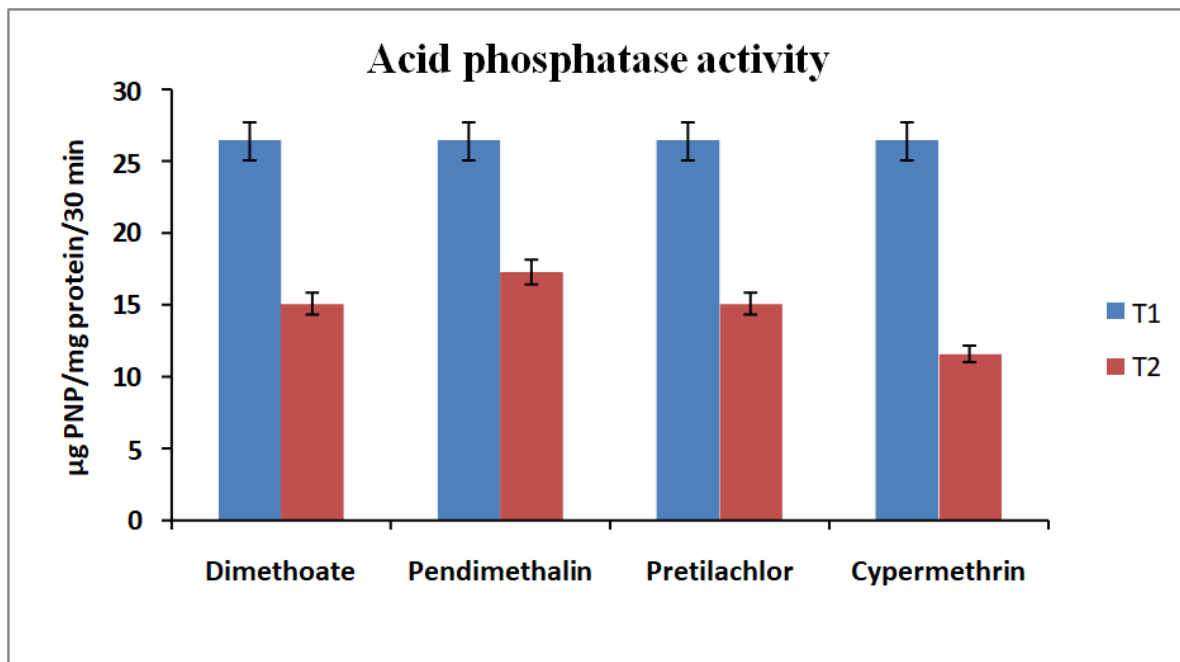


Figure 1: The acid phosphatase activity levels of *P. excavatus* were measured in two experiments: one with *P. excavatus* exposed to sublethal dosages (T2) of certain pesticides in an environment close to nature, and the other with no pesticide at all (T1).

Alkaline phosphatase

Figure displays the effects of dimethoate, pendimethalin, pretilachlor, and cypermethrin on the activity of alkaline phosphatase. The alkaline phosphatase level in the control set (T1) was 51.6 ± 2.25 $\mu\text{g PNP/mg protein/30 mins}$, whereas in the pesticide treated set (T2), the enzyme levels were 77.8 ± 1.50 , 57.1 ± 1.75 , 65.7 ± 1.10 μg , and 81.0 ± 2.75 $\mu\text{g PNP/mg protein/30 mins}$ for dimethoate, pendimethalin, pretilachlor, and cypermethrin, respectively.

Table 8: The alkaline phosphatase activity of Pendimethalin, Pretilachlor, Dimethoate, and Cypermethrin-exposed *Perionyx excavatus* was analysed using a one-factor ANOVA.

| Insecticide | Source of variation | df | Mean square | F | Significance |
|---------------|---------------------|----|-------------|---------|--------------|
| Dimethoate | Treatment | 2 | 1154.92 | 362.328 | 0.000 |
| | Error | 6 | 3.188 | | |
| Pendimethalin | Treatment | 2 | 89.11 | 23.895 | 0.001 |

| | | | | | |
|--------------|-----------|---|----------|---------|-------|
| | Error | 6 | 3.729 | | |
| Pretilachlor | Treatment | 2 | 355.030 | 142.344 | 0.000 |
| | Error | 6 | 2.494 | | |
| Cypermethrin | Treatment | 2 | 1443.510 | 214.515 | 0.000 |
| | Error | 6 | 6.729 | | |

Table 9: Alkaline phosphatase activity as a function of pesticide treatment: the least significant difference (LSD)

| Insecticides | Difference between | Mean difference | Significance |
|---------------|--------------------|-----------------|--------------|
| Dimethoate | T1 & T2 | -26.20 | 0.000 |
| | T1 & T3 | -38.40 | 0.000 |
| | T2 & T3 | -12.20 | 0.000 |
| Pendimethalin | T1 & T2 | -5.50 | 0.013 |
| | T1 & T3 | -10.90 | 0.000 |
| | T2 & T3 | -5.40 | 0.014 |
| Pretilachlor | T1 & T2 | -14.10 | 0.000 |
| | T1 & T3 | -21.40 | 0.000 |
| | T2 & T3 | -7.30 | 0.001 |
| Cypermethrin | T1 & T2 | -29.40 | 0.000 |
| | T1 & T3 | -42.90 | 0.000 |
| | T2 & T3 | -13.50 | 0.001 |

Acetylcholinesterase

Figure shows how the acetylcholinesterase activity is affected by dimethoate, pendimethalin, pretilachlor, and cypermethrin. Acetylcholinesterase levels were 160 ± 2.50 nmolesthiocholine/min/mg tissue in the control set (T1), and 60 ± 1.75 , 100 ± 3.10 , 120 ± 2.25 , and 40.8 ± 1.35 nmolesthiocholine/min/mg tissue for dimethoate, pendimethalin, pretilachlor,

and cypermethrin, respectively, in the pesticide treated set (T2). Cypermethrin exhibited the highest proportion of enzyme inhibition at 74.5 percent, whereas pretilachlor showed the lowest percentage of inhibition at 25%. Pendimethalin exhibited a 37.5% inhibition and dimethoate a 62.5% inhibition.

Table 10: Analysing the effects of pendimethalin, pretilachlor, dimethoate, and cypermethrin on the acetylcholinesterase activity of *Perionyx excavatus* using a single-factor ANOVA

| Insecticide | Source of variation | df | Mean square | F | Significance |
|---------------|---------------------|----|-------------|---------|--------------|
| Pendimethalin | Treatment | 2 | 11725.000 | 2.842E3 | 0.000 |
| | Error | 6 | 4.125 | | |
| Pretilachlor | Treatment | 2 | 5200.00 | 612.485 | 0.000 |
| | Error | 6 | 8.490 | | |
| Dimethoate | Treatment | 2 | 2800.00 | 512.977 | 0.000 |
| | Error | 6 | 5.458 | | |
| Cypermethrin | Treatment | 2 | 16341.64 | 4.955E3 | 0.000 |
| | Error | 6 | 3.298 | | |

Table 11: Acetylcholinesterase activity as a function of pesticide treatment: the least significant difference (LSD).

| Insecticides | Difference between | Mean difference | Significance |
|---------------|--------------------|-----------------|--------------|
| Pendimethalin | T1 & T2 | 100.00 | 0.000 |
| | T1 & T3 | 115.00 | 0.000 |
| | T2 & T3 | 15.00 | 0.000 |
| Pretilachlor | T1 & T2 | 60.00 | 0.000 |
| | T1 & T3 | 80.00 | 0.000 |
| | T2 & T3 | 20.00 | 0.000 |

| | | | |
|--------------|---------|--------|-------|
| Dimethoate | T1 & T2 | 40.00 | 0.000 |
| | T1 & T3 | 60.00 | 0.000 |
| | T2 & T3 | 20.00 | 0.000 |
| Cypermethrin | T1 & T2 | 119.20 | 0.000 |
| | T1 & T3 | 135.00 | 0.000 |
| | T2 & T3 | 15.80 | 0.000 |

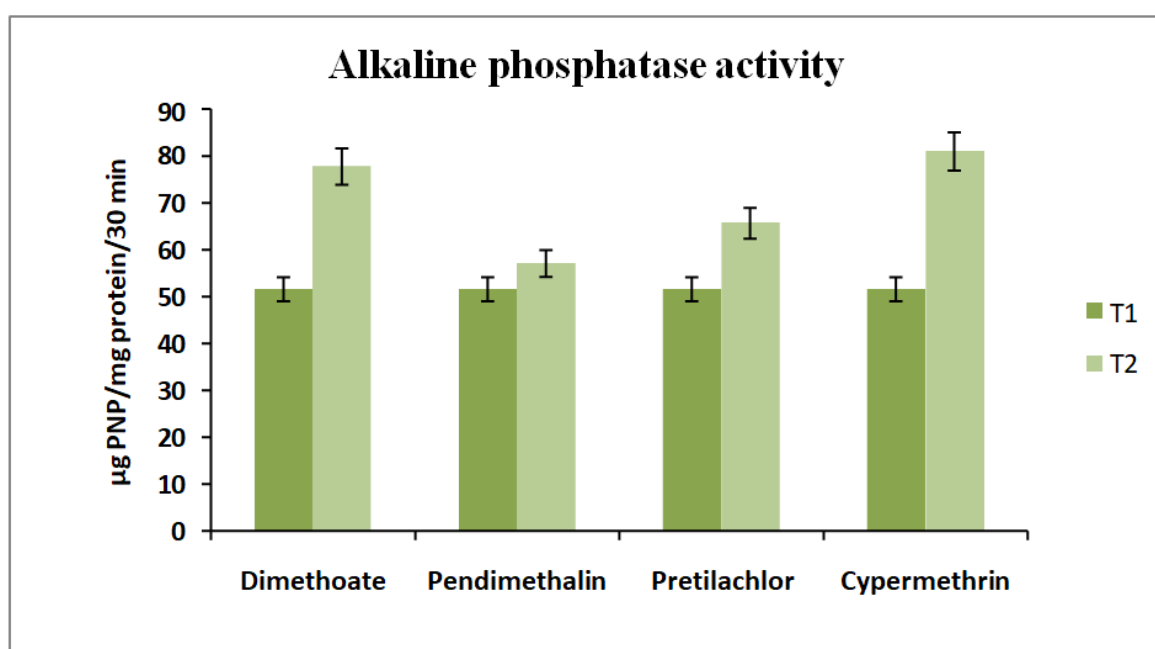


Figure 2: *P. excavatus* alkaline phosphatase activity levels in two groups: one subjected to sublethal dosages (T2) of certain pesticides in an environment mimicking nature, and the other without pesticide (T1).

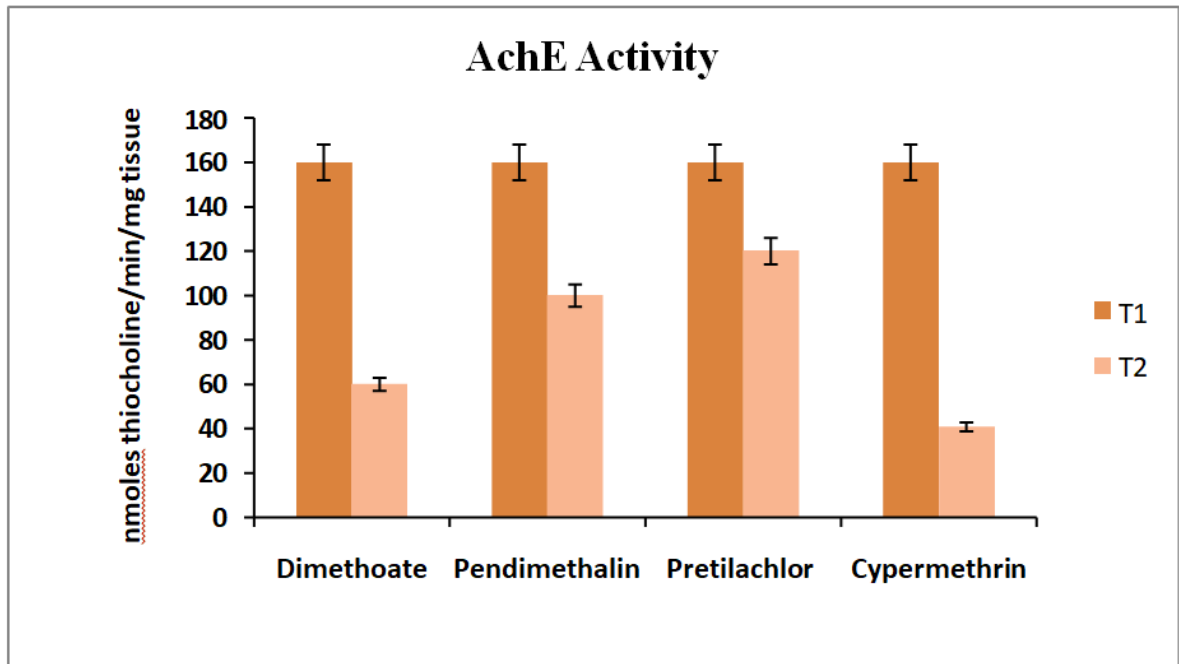


Figure 3: Comparing the acetylcholinesterase (AChE) activity of *P. excavatus* in two conditions: one with sublethal dosages (T2) of certain pesticides in an environment close to nature, and another with no pesticide (T1).

- **Pesticide effects on earthworm chloragogen cells in the gut**

Treatments with any of the three pesticides had a major impact on the earthworms' chloragogen cell tissue lining, which lines their digestive tracts. The earthworms in the control group had no damage to their guts or chloragogen tissue layer lining. However, in the instance of all the polluted sets of the chosen pesticides, the intestinal lining and the chloragogen tissue layer were both significantly deformed with vacuole-like structures.

The test specimens' chloragogen tissue layer and intestinal wall were the most severely affected by permethrin.

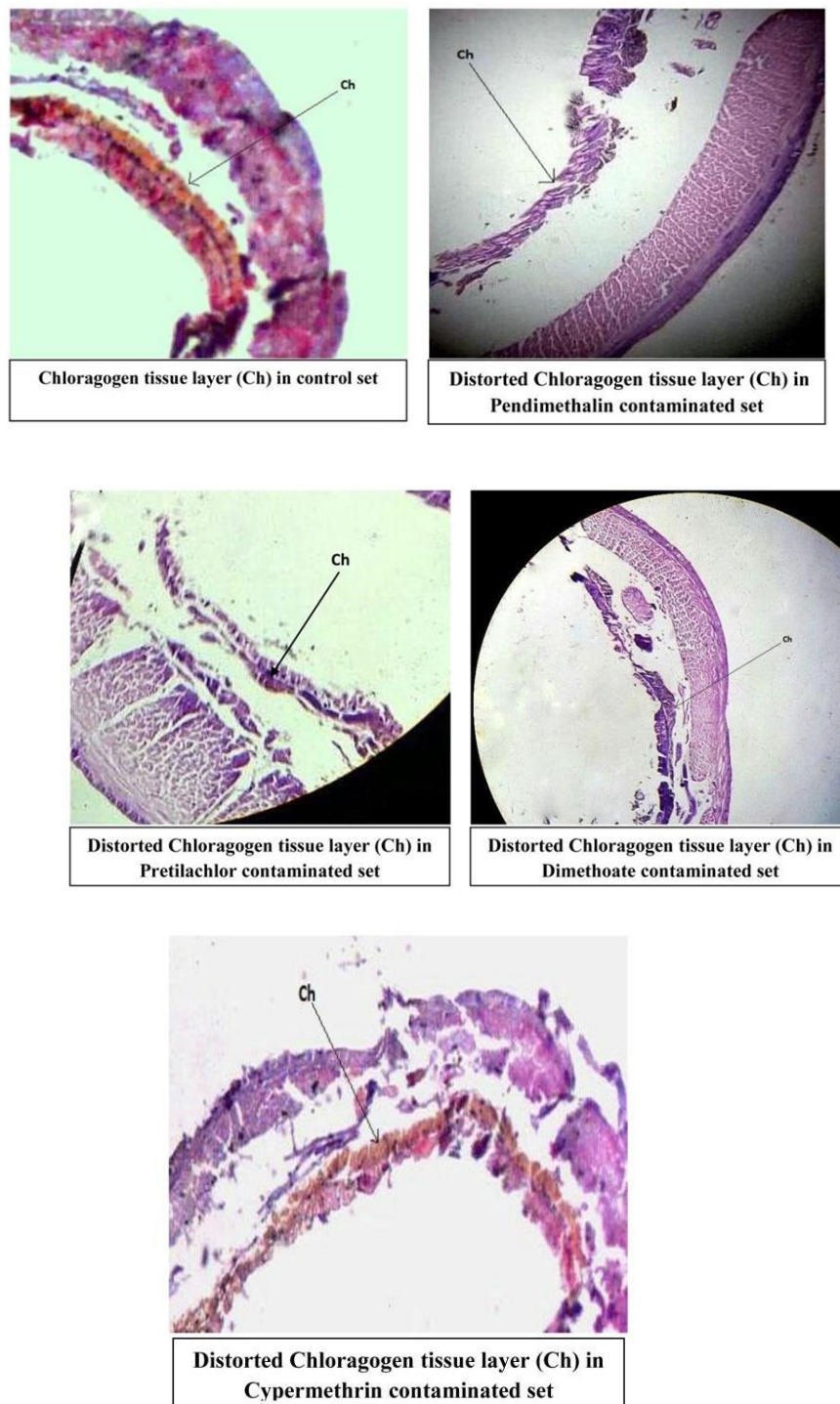


Figure 4: The chloragogen tissue layer (Ch) in both the control and pesticide-treated earthworm guts, as seen via histology.

CONCLUSION

The results of this research unequivocally show that the physiological and histological characteristics of *Perionyx excavatus* are adversely affected by sublethal quantities of common

pesticides, including dimethoate, cypermethrin, pretilachlor, and pendimethalin. A disturbance in metabolic and neurological processes is indicated by the noted decrease in vital enzyme activity, including acetylcholinesterase, acid phosphatase, and alkaline phosphatase. Significant structural damage to the chloragogen tissue and stomach lining was also found by histological investigation, particularly in samples exposed to cypermethrin. The health and ecological function of earthworms, which are essential for soil fertility and ecosystem balance, may be jeopardised by pesticide exposure, even at non-lethal levels, according to these impacts. As a result, the study highlights how crucial it is to reduce the use of pesticides and promotes further investigation into safe, ecologically friendly alternatives.

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